Histopathological changes and tissue residue concentrations of monosex Nile tilapia (*Oreochromis niloticus*, L) fries exposed to oxytetracycline

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Abstract
The current study evaluated the biosafety of oxytetracycline (OTC) exposure for 30 days in monosex *Oreochromis niloticus* fries. The fries were exposed to OTC for 3 h/day for 30 days at 350 (0.5X), 700 (1X), 2100 (3X), 3500 (5X), and 7000 (10X) mg/L and compared with control (0X). The OTC exposure at 5X and 10X concentrations caused 100% mortality within 4 days and 5 min, respectively. The mortalities recorded in 0.5X, 1X, and 3X groups were 3.33 ± 1.15%, 14.67 ± 1.15%, and 47.33 ± 11.37% on day 30, respectively. The feed intake was decreased up to 23.33% in the 3X group during the exposure period. The OTC residue levels on 30-day exposure were 216.53 ± 14.71, 450.56 ± 44.31, and 1141.26 ± 63.64 μg/kg, which reduced to 40.40 ± 3.25, 76.68 ± 2.77, and 95.61 ± 5.13 μg/kg after 15 days of termination of exposure in the 0.5X, 1X, and 3X groups, respectively. The histopathological changes observed in the 1X group were epithelial detachment, desquamation of secondary lamellar epithelium, lamellar fusion, and inflamed cartilaginous core in the gills, alteration in the integrity of gut mucosa, degeneration of muscularis mucosae and necrosis in the intestine, the disintegration of the nephritic tubule, necrosis, and gloomerulopathy in the kidney, and dilated vascular duct, necrotized hepatic tissue, diffused hepatic parenchyma, vacuolation, and fatty changes in the liver. The OTC exposure induced marked tissue changes histologically in a dose- and time-dependent manner, which undoubtedly reduced the growth of tilapia.

Keywords Nile tilapia · Oxytetracycline exposure · Cumulative mortalities · Histopathology · OTC residues
Introduction

World aquaculture production attained 114.5 million tonnes in live weight in 2018. Inland aquaculture produced 51.3 million tonnes of aquatic animals in 2018, which contributes 62.5% of the total world’s farmed food fish production (FAO 2020). Tilapia is known as ‘aquatic chicken’ due to its wide range of adaptability to the adverse environment and high growth rates. Global tilapia production increased by 3.3% in 2020 (6 million tonnes) for the first time, despite the impact of COVID-19 (Fletcher 2020). Among tilapia species, Nile tilapia (Oreochromis niloticus) is the most important species. Tilapias are well-adapted to prevailing conditions and can tolerate a wide range of environmental factors such as water temperature, salinity, dissolved oxygen, and ammonia (El-Sayed 2006). However, stressful situations adversely affect tilapia and make them more susceptible to different diseases (El-Sayed 2006). Antibiotics usually are non-toxic to the host and are used for the treatment of bacterial infections of animals and humans (Leal et al. 2019; Limbu et al. 2021). Several antibiotics are used for the prevention and treatment of fish diseases in most fish farming areas (Austin and Austin 2016; Limbu et al. 2021). To date, only three antibiotics, namely sulfadimethoxine + ormetoprim, oxytetracycline, and florfenicol are approved by the USFDA as feed additives for the treatment of bacterial diseases in fish (USFWS 2015; FDA 2021). Worldwide, oxytetracycline (OTC) is one of the most commonly used antibacterial agents to control bacterial infections in aquaculture (Leal et al. 2019; Limbu et al. 2021). Antibiotic application methods for fish can be divided into six different categories comprised of oral (via medicated feed), bath, dip, flush, injection, and topical application (Austin and Austin 2016). Besides, the FDA also approved the use of OTC for the marking of skeletal tissues in finfish fry and fingerlings as an aid in identification at a recommendable dose of 200–700 mg/L of water for a 2–6-h duration (FDA 2004).

The concerns about the consequences of antibiotic use on public health have encouraged the development of strict regulations on the controlled use of antibiotics in aquaculture and animal husbandry. Biosafety evaluation of a drug is a major preliminary step for estimation of the level of dangers associated with any drug. Studies to estimate the residual level of drugs in the body are also an important parameter in biosafety analysis. The USFDA has established a tolerance level of 2.0 µg/g of OTC in the muscle and skin of fish (FDA 2021). The maximum residue limit (MRL) of OTC is 200 µg/kg (Codex Alimentarius 2018). Pharmaceuticals have been receiving increasing attention regarding their potentially harmful effects on the environment mainly because they induce biological effects and persist for a long time in the water, soil, and organisms (Bojarski et al. 2020). In this study, we evaluated the effect of the recommended exposure doses of OTC (350 and 700 mg/L) as well as the toxic effects at the higher doses (2100, 3500, and 7000 mg/L) in monosex O. niloticus fries when exposed for 3 h daily for 30 consecutive days. The residual OTC concentrations in exposed fish were also quantified after providing a sufficient withdrawal period.

Materials and methods

Animal ethics and biosafety statement

The guidelines of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals, Ministry of Environment and Forests (Animal Welfare Division),
Government of India) on care and use of animals in scientific research were followed during the experiment. The experimental protocols were approved by the ICAR, Government of India, New Delhi, under the All-India Network Project on Fish Health (F. No. CIBA/AINP-FH/2015–16 dated 16.7.2015).

**Experimental fish and feed**

The experimental fish monosex Nile tilapia *Oreochromis niloticus* fries of size 1.50±0.50 cm and 0.180±0.001 g were procured from M/S Biswas Hatchery, Bathatala, Naihati (22° 53′ 42.7848″ N; 88° 26′ 6.1224″ E), North 24 Parganas district, West Bengal, India, and brought to the laboratory in oxygen-packed double-layered polythene bags. After proper water temperature acclimatization in the tanks for 3 h and 2 ppm potassium permanganate dip treatment for 3 min, the fries were maintained in the fibreglass-reinforced plastic (FRP) tanks containing 400-L aerated bore-well water at 500 fries/tank. The fries were fed with crumbled commercial pellet feed (CP 9910S, CPF India Pvt. Ltd., India) on demand during the 7 days of acclimatization before the experiment.

**Study design for oxytetracycline exposure**

The FDA-approved dose of OTC for the marking of skeletal tissues in finfish fry and fingerlings as an aid in identification is 200–700 mg/L of water for a 2–6-h duration (FDA 2004). The maximum dose of 700 mg OTC/L of water as ‘X’ was purposively selected for the biosafety experiment. To evaluate the biosafety of OTC, 3-h exposure (immersion treatment) was carried out at five different concentrations, viz., 350 (0.5X), 700 (1X), 2100 (3X), 3500 (5X), and 7000 (10X) mg/L and compared with control (0X) in glass aquaria (L: 60 cm×H: 30 cm×W: 30 cm), in triplicate. The acclimatized population was allocated into any of 18 glass aquaria at 50 fries each, protected by nylon cover and continuously aerated. The fries were again acclimatized in the aquaria condition for 4 days with continuous aeration. The water quality parameters of the rearing tanks such as dissolved oxygen (4.70–5.60 mg/L), pH (7.40–7.80), temperature (28.00–32.00 °C), ammonia (0.0030–0.0084 mg/L), nitrate (0.14–0.25 mg/L), and nitrite (0.17–0.32 mg/L) were maintained within the optimum limits throughout the experiment. The suspension containing OTC at the test concentrations was prepared by thoroughly mixing the required quantity of oxytetracycline hydrochloride powder (HiMedia, India) in 1 L of water separately with a stirrer to get a clear solution. The fries from all the aquaria were collected carefully and placed in the respective OTC suspensions in 15 containers for 3 h/day for consecutive 30 days. Fresh OTC suspensions were prepared daily and the used OTC suspensions were disposed of safely as per the guidelines (WHO 1999). The control group was not exposed to OTC but subjected to a similar treatment in 3 containers containing the medium used for the preparation of OTC suspension. After 3 h of exposure, the fries were transferred to their respective aquaria. The fries were fed with commercial pellet feed powder at 3% of the body weight twice daily. About 50% of the water was replaced thrice weekly to avoid the accumulation of waste and excreta. After 30 days of exposure treatment, the fries were kept in the respective aquaria for another 15 days for observation. The pH of the OTC suspensions used for the immersion treatment was measured with a digital pH meter (Thermo Fisher Scientific WD-35634–10 PHTESTR 10 OAKTON-EA). Observations on mortality, external gross lesions, and behavioural changes were recorded daily.

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Histopathological analysis

As the test fries were very small, the whole fries were used for histology. Samples for histopathology were collected on day 0, day 30 of OTC exposure, and the last day of the experiment (day 45). Two fries from each aquarium were collected arbitrarily and euthanized by immersion in water containing clove oil at 20 μL/L. The fries were then fixed in Bouin’s solution for 24 h and then transferred to 70% ethyl alcohol overnight. The fixed samples were processed by standard techniques and embedded in paraffin wax. Thin sections (5 μm) were prepared and stained with haematoxylin and eosin (Roberts 2012). The histopathological alterations were identified under the advanced trinocular research microscope (Olympus, Japan: Model BX51).

Oxytetracycline residue analysis by LC–MS/MS

Samples for OTC-residue analysis by LC–MS/MS were collected from all the tanks on day 0 and 30 OTC exposure and day 15 post-OTC exposure. Three fries from each aquarium were collected arbitrarily, euthanized by immersion in water containing clove oil at 20 μL/L (AVMA 2020; Roy et al. 2021) for 5 min. The fries were dissected, beheaded, degutted, washed thoroughly, and stored at −20°C until analysis. The OTC residues from 2 g of whole fry homogenates were extracted, purified as described by our network partners (Manna et al. 2021; Sharma et al. 2021), and quantified by LC–MS/MS analysis (AB Sciex 4000 QTRAP Mass spectrometry coupled with Exion HPLC system) at the ICAR-Central Institute of Fisheries Technology, Kochi, India. The European Commission’s (EC) directives for the validation of the method on the specific parameters were followed according to 2002/657/EC (European Commission 2002).

Statistical analysis

The experimental results were expressed as the mean ± standard deviation. The data on fry mortalities and feed intake were analyzed by one-way analysis of variance (ANOVA) followed by Duncan’s multiple range test using the Statistical Package for Social Sciences (IBM-SPSS) Version: 22.0, considering a probability level of $p < 0.05$.

Results

Evaluation of biosafety of oxytetracycline

Oxytetracycline exposure

During the treatment regime, no gross lesions on the skin and gills were found in any of the lower OTC concentrations (0.5X, 1X, and 3X). Behaviours like swimming to the surface during feeding, aggressive feeding, and distribution throughout the water column were considered normal. No abnormal behaviour was observed throughout the experimental period. The feeding behaviour was normal and all feeds were consumed in the 0X, 0.5X, and 1X groups. In the 3X group, the feed intake was decreased significantly by about
23.33% during the exposure period \((p < 0.05)\). Post-exposure, the feed intake was improved significantly \((96.33 \pm 1.53\%)\) and reached almost the normal level. The mortalities \((3.33 \pm 1.15\%)\) were the lowest in the 0.5X group followed by 1X \((14.67 \pm 1.15\%)\) and 3X \((47.33 \pm 11.37\%)\) groups during the exposure period (Table 1). In fries exposed to 5X and 10X OTC concentrations, 100% mortalities were noted within 4 days and 5 min, respectively. Significant differences in fry mortalities were noted among the treatment groups during the exposure period \((p < 0.05)\). The control group was normal (Fig. 1A). The mouth was wide open in the 3X group (Fig. 1B). The lethargic fries of the 5X group gasped for air, exhibited flared opercula, and experienced mortalities in the range of 61% on the first day to 100% on the fourth day of OTC exposure (Fig. 1C). On the other hand, all the fries died in 5 min of exposure with burst belly in the 10X concentration (Fig. 1D). No mortalities were noted during the post-exposure period.

Oxytetracycline residues

The whole fries were taken for the analysis of OTC residues. As the 5X and 10X groups experienced 100% mortalities, they were not included in this study. No residue was found in the control group. The OTC residues recorded in the exposed fries of the 0.5X, 1X, and 3X groups were 216.53 \(\pm 14.71\), 450.56 \(\pm 44.31\), and 1141.26 \(\pm 63.64\) μg/kg on day 30, respectively. After 15 days of termination of exposure, the residue levels reduced to 40.40 \(\pm 3.25\), 76.68 \(\pm 2.77\), and 95.61 \(\pm 5.13\) μg/kg in the respective groups (Table 1; Fig. S1).

Histopathological alterations

Kidney

The control group had normal kidney architecture (Fig. 2A). Mild to marked damages in the kidney tissues were observed in the 0.5X and 1X groups. Moderate to marked kidney tissue damages were observed in the 3X group. The major histopathological changes found in the kidney of fries were loss of nephritic tubular epithelial cells, dilated Bowman’s space, and glomerulopathy in the 0.5X group (Fig. 2B). The changes like the disintegration

| Parameters              | Period | Treatment groups*          |
|-------------------------|--------|---------------------------|
|                         |       | Control (0X) | 350 mg/L (0.5X) | 700 mg/L (1X) | 2100 mg/L (3X) |
| Feed intake (%)         | A      | 100.00 ± 0.00 | 100.00 ± 0.00 | 100.00 ± 0.00 | 76.67 ± 2.87*# |
|                         | B      | 100.00 ± 0.00 | 100.00 ± 0.00 | 100.00 ± 0.00 | 96.33 ± 1.53#   |
| Mortality (%)           | A      | 0              | 3.33 ± 1.15   | 14.67 ± 1.15* | 47.33 ± 11.37*  |
|                         | B      | 0              | 0             | 0             | 0             |
| Oxytetracycline residues (μg/kg) | A       | 0              | 216.53 ± 14.71 | 450.56 ± 44.31 | 1141.26 ± 63.64 |
|                         | B      | 0              | 40.40 ± 3.25  | 76.68 ± 2.77  | 95.61 ± 5.13   |

*, 5X (3500 mg/L) and 10X (7000 mg/L) exposure caused 100% mortalities in 4 days and 5 min, respectively; A, OTC exposure period (30 days); B, post-OTC exposure period (15 days). *Values with asterisk (*) within a row differed significantly from other treatment groups \((p < 0.05)\). #Values sharing hash (#) differed significantly \((p < 0.05)\).
of the nephritic tubule, necrosis, and glomerulopathy were observed in the kidney of the 1X group. Degeneration of the nephritic epithelial lining, constriction of the lumen, thickening of the epithelial lining, and vacuolation of the nephritic tubule was observed in fries exposed to 3X concentration (Fig. 2C). After 15 days of the termination of exposure, widened lumen, inflamed nephritic tubule, degeneration of the epithelial lining, and necrosis were observed in the kidney of tilapia fries exposed to 0.5X concentration (Fig. 2D). The tilapia fries exposed to 3X concentration had vacuolation in the nephritic tubule, necrosis, degeneration of the epithelial lining, and hypertrophied nephritic cells (Fig. 2E).

Liver

The liver tissues of the control group were normal throughout the experiment (Fig. 3A). Mild to marked liver tissue damages were observed in the 0.5X and 1X groups. The 3X group had moderate to marked liver tissue damages. The fries exposed to 0.5X concentration showed fatty changes in the hepatic tissues (Fig. 3B). The major histopathological changes found in the liver of fries on the 30th-day exposure to 1X concentration were necrosis and fatty changes in the hepatic tissue (Fig. 3C). The 1X group also had dilated vascular duct, necrotised hepatic tissue, diffused hepatic parenchyma, and vacuolation and fatty changes in the hepatic tissue. In the 3X group, vascular duct without blood cells, fatty changes in the hepatic tissue, loosely and densely packed hepatic parenchyma, vacuolation
in the pancreas, and necrotised hepatic tissue were noted (Fig. 3D). After 15 days of the termination of exposure, the persistence of fatty changes in the hepatic tissue was observed in fries of the 0.5X group (Fig. 3E). The fries exposed to 1X concentration had loosely as well as densely packed hepatic parenchyma along with fatty changes in the hepatic tissue (Fig. 3F). The 3X group had fatty changes in the hepatic tissue and erythrocyte infiltration into blood sinusoids (Fig. 3G).

**Gills**

The control group had normal gill architecture throughout the experiment (Fig. 4A). Mild to moderate damages on the gill tissues of *O. niloticus* fries were observed in the 0.5X and 1X groups, while moderate to marked gill damages were observed in the 3X group. Desquamation of secondary lamellar epithelium, shortening of secondary lamellae, inflammation of secondary lamellar tip, inflamed cartilaginous core, and epithelial lifting were observed in the fries exposed to 0.5X concentration (Fig. 4B). Changes like epithelial detachment, desquamation of secondary lamellar epithelium, lamellar fusion, and inflamed cartilaginous core were observed in the gills of fries immersed in 1X concentration (Fig. 4C). The gills of fries exposed to 3X concentration exhibited shortening and curling of secondary lamellae, inflammation of secondary lamellar tip, inflamed cartilaginous core, desquamation of secondary lamellar epithelium, and necrotized primary lamellae (Fig. 4D). After 15 days of the termination of exposure, shortening and curling of secondary lamellae, inflamed cartilaginous core, and desquamation of secondary lamellar
epithelium were observed in fries exposed to 0.5X concentration (Fig. 4E). In addition, inflammation of the secondary lamellar tip was observed in fries exposed to 1X concentration (Fig. 4F). The fries exposed to 3X concentration were observed to have non-nucleated pillar cells, necrosis, inflammation of secondary lamellar tip, inflamed cartilaginous core, shortening of secondary lamellae, and desquamation of secondary lamellar epithelium (Fig. 4G).

**Intestine**

The intestine of the control group had normal architecture (Fig. 5A). Meagre to mild damages in the intestine tissues were observed in the 0.5X and 1X groups. The 3X group had moderate intestinal tissue damages. The major histopathological changes found in the intestine of fries exposed to 0.5X concentration were disintegrated muscularis mucosae (Fig. 5B). The integrity of the gut mucosa was altered along with degeneration of muscularis mucosae and necrosis in fries exposed to 1X and 3X concentrations (Fig. 5C). After 15 days of the termination of exposure, the intestine of the fries had degenerated epithelial lining in 1X (Fig. 5D) and 3X concentrations (Fig. 5E).

Fig. 3  Histopathological alterations in the liver of monosex *Oreochromis niloticus* fries exposed to varying concentrations of oxytetracycline on day 30. [A] Untreated control at ×200; [B] 350 mg/L (0.5X group) at ×400; [C] 700 mg/L (1X group) at ×400; and [D] 2100 mg/L (3X group) at ×400; and on day 15 post-exposure [E] 0.5X group at ×200; [F] 1X group at ×200; and [G] 3X group at ×200 showing fatty changes in the hepatic tissue (F), necrotized hepatic tissue (N), vacuolation in the pancreas (V), loosely packed hepatic parenchyma (LPH), densely packed hepatic parenchyma (DPH), and erythrocyte infiltration into blood sinusoids (EI); H&E staining
The antibacterial agents are of particular concern for aquatic ecosystems and public health since they are extensively used in aquaculture (Limbu et al. 2021). In this study, the fries exposed to OTC at the higher concentrations (5X and 10X) resulted in 100% mortality within 4 days and 5 min, respectively, due to the toxic effect of OTC than its permissible limit (USFWS 2015; Limbu et al. 2021). The fries of the 0.5X, 1X, and 3X groups recorded a dose- and time-dependent increase in mortalities upon exposure. The mortalities were increased by 1.74 and 5.49 folds between 10 and 20 days, as well as between 10 and 30 days of exposure periods, respectively. The increase in mortalities was about 3.14 folds between 20 and 30 days of the exposure. A similar trend was observed by Roy et al. (2021), where the increase in OTC concentrations and exposure time resulted in increased mortalities in *O. niloticus* fries due to increased toxicity. Significant differences in the mortalities were noted with increasing concentrations of OTC and exposure time, which can be attributed to the OTC toxicity due to the hydrochloride formulations, and lowering the pH of the water (Marking et al. 1988). Low water pH is considered toxic to fish, thus contributing to water toxicity (Marking et al. 1988). In the present study, it was found that the pH of the OTC solutions used for exposure was 3.3, 3.9, 4.2, 5.2, and 6.4 in 10X, 5X, 3X,

**Discussion**

Fig. 4 Histopathological alterations in the gills of monosex *Oreochromis niloticus* fries exposed to varying concentrations of oxytetracycline on day 30. [A] Untreated control at ×200; [B] 350 mg/L (0.5X group) at ×200; [C] 700 mg/L (1X group) at ×200; and [D] 2100 mg/L (3X group) at ×200; and on day 15 post-exposure [E] 0.5X group at ×200; [F] 1X group at ×400; and [G] 3X group at ×200 showing desquamation of secondary lamellar epithelium (DS), epithelial lifting (LE), shortening of secondary lamellae (SHL), inflammation of secondary lamellar tip (I), inflamed cartilaginous core (ICC), epithelial detachment (ED), lamellar fusion (LF), necrotized primary lamellae (N), curling of secondary lamella (C), and non-nucleated pillar cells (NNP); H&E staining.
Fig. 5  Histopathological alterations in the intestine of monosex *Oreochromis niloticus* fries exposed to varying concentrations of oxytetracycline on day 30. [A] Untreated control at ×200; [B] 350 mg/L (0.5X group) at ×200; [C] 2100 mg/L (3X group) at ×200; and on day 15 post-exposure [D] 1X group at ×200; and [E] 3X group at ×400 showing deeply stained mucoid epithelial cells and disintegrated muscularis mucosae (DM), altered integrity of the gut mucosa with degeneration of the muscularis mucosae (D) and necrosis (N), and degeneration of the epithelial lining (DE); H&E staining.
1X, and 0.5X doses, respectively. It has been reported that the suitable water pH for rearing *O. niloticus* is 5.5–9.0 and it can survive in a pH as low as 4 (Rebouças et al. 2015), which corroborate the findings of the present study as all the fries died when exposed to 5X and 10X concentrations. The present study also observed that the survival in *O. niloticus* fries was above 96% in 0.5X and 100% in control groups, where the pH was 6.4 and 7.6, respectively. Due to lowered pH, the fish may be on to a state of stress and the osmoregulation gets altered ultimately leading to mortality (Zahangir et al. 2015). It is known that lamellar degeneration decreases respiratory efficiency and this damage may cause changes in gill epithelium–like desquamation (Smart 1976). In this study, desquamation of secondary lamellar epithelium was noted in the fries exposed to all OTC concentrations. The higher concentrations of OTC might have caused severe lamellar degeneration and other toxic changes in the gills resulting in decreased respiratory efficiency causing suffocation and ultimately leading to mortality. Applications of higher dosages of OTC greatly impair the liver regeneration and decrease the mitochondrial protein synthesis after causing deficiency in cytochrome oxidase-C and ATP synthetase enzymes (Den-Bogert et al. 1983) and immune functions (Sanchez-Martinez et al. 2008), thereby making the fish more susceptible.

The use of OTC may result in residues in exposed animals, especially if proper withdrawal times for treated animals have not been used. The treated fish should not be harvested for human consumption until a specified withdrawal period has elapsed (Limbu et al. 2021). According to the Codex Alimentarius (2018) and FDA (2021), the MRL of OTC is 200 μg/kg. The OTC residues quantified in *O. niloticus* fries exposed to 0.5X, 1X, and 3X OTC concentrations were 216.53 ± 14.71, 450.56 ± 44.31, and 1141.26 ± 63.64 μg/kg on day 30 of exposure, respectively. On day 15 post-exposure, the residue levels reduced to 40.40 ± 3.25, 76.68 ± 2.77, and 95.61 ± 5.13 μg/kg, which conform the regulations of the Codex Alimentarius (2018). Likewise, it was noted that the OTC residues in fish muscle did not decline until 6 weeks (Abraham et al. 2021a). Also, Reda et al. (2013) recorded OTC residues in tilapia muscle samples at a level of 0.05 μg/g muscle after 15 days of feeding cessation. The results of this experiment indicated the retention of the drug in the tissues without elimination even on day 15 post-OTC exposure under tropical conditions.

Histopathology is a well-established tool to figure out the qualitative changes in the affected organs and the patterns of recovery. The teleostean kidney is one of the first internal organs to be affected by the contaminants in the water (Roberts 2012; Bojarski et al. 2020). The fries exposed to 700 mg OTC/L of water (1X) for 30 days showed changes such as necrosis and disintegration of the nephritic tubule with 20% deviations from the normal architecture. These changes were, however, not prominent on day 15 post-exposure. The severity of epithelial degeneration was increased to the extent of 15% damages in the tissues on day 30 OTC exposure, which subsequently decreased to < 10% after 15 days of cessation of the exposure. On day 30, the OTC-exposed fries also showed necrosis with 30% of the tissue damages from the normal architecture. The extent of damage was decreased to 15% on day 15 post-exposure, suggesting a recovery process. On the last day of the experiment, the fries immersed in 3X concentration showed 10% renal tissue damages due to vacuolation of the nephritic tubules. The increase in the frequency of new nephrons and regenerated tubules is an indication of the process of recovery of the damaged kidney in fish (Cormier et al. 1995). The changes found in the kidney tissues of the present study were also observed in the kidney of OTC fed *O. niloticus* juveniles (Julinta et al. 2017; Abraham et al. 2021b) and fries (Roy et al. 2021) with glomerular expansion and alteration of Bowman’s space. Likewise, the most common alterations found in the kidney of fish exposed to water contaminants are tubule degeneration (cloudy swelling and hyaline
droplets) and changes in the corpuscle, such as dilation of capillaries in the glomerulus and alteration of Bowman’s space (Sharma et al. 2021).

The histopathological changes are generally associated with the response of hepatocytes to toxicants. The liver of OTC-exposed *O. niloticus* fries exhibited diffused, necrotised tissue with fatty changes in the hepatic parenchyma (Rodrigues et al. 2017) and vacuolation of hepatic parenchyma (Sharma et al. 2021). The liver and pancreatic tissues showed necrosis and fatty changes in the hepatic parenchyma, which corroborate the earlier observations due to ampicillin in *C. gariepinus* (Laith and Najiah 2013) and OTC in *O. niloticus* (Abraham et al. 2021b; Roy et al. 2021). The hepatic tissue was damaged by vacuolation and degeneration of the inner epithelial layer ranging from mild (<5%) to moderate with 20% deviations from the normal architecture, which is relatable to the study of Rodrigues et al. (2017) in *O. mykiss* and Roy et al. (2021) in *O. niloticus* fries. However, such changes were not seen on day 15 post-exposure. Moderately severe necrotic conditions with 35% deviations from the typical architecture were observed on the 30th day of OTC exposure. The intensity of damage was decreased to about 15% upon discontinuance of exposure. The histological alterations represented the response of fries to the direct effect of higher OTC-doses for an extended exposure period.

The gills are an important multifunctional organ in fish because they have important roles including respiration, osmoregulatory functions, acid–base regulation, and nitrogenous waste excretion (Roberts 2012). In the present study, the OTC exposure caused systemic changes in the gills, which corroborate the observations of Rodrigues et al. (2017), who reported progressive lesions such as hypertrophy, lamellar fusion, shortening and curling of secondary lamella, and epithelial lifting in the gills of *Oncorhynchus mykiss* juveniles due to acute and chronic exposure to OTC. Lamellar degeneration and desquamation may result in loss of respiratory efficiency and/or function. The observed histological alterations in the gills of fries could be attributed to the OTC-induced toxicity (Marking et al. 1988) and disruption of the osmoregulatory function and reduce oxygen consumption (Peebua et al. 2008). The desquamation of the secondary lamellae was most pronounced on day 30 of exposure of the fish to OTC. The deviation in epithelial detachment was about 10% from its normal condition, which was, however, undetected on day 15 post-exposure. The intensity of inflammation in the lamellar tip was moderate on day 30 OTC exposure, i.e. 20% from its normal condition. The intensity was, however, decreased to <15% with the termination of exposure, thus indicating the recovery process. The degeneration of secondary lamellae to the tune of 10% from its normal condition was observed on day 30 of OTC exposure, but it became almost normal on day 15 post-exposure. The intensity of shortening of length and curling of secondary lamellae was also reduced, thus suggesting recovery of the fries. These results suggested that the inflammatory changes may last longer, which has an impact on the growth of fish. The curling of secondary lamellae with a deviation of 15% from the normal architecture was noted on day 30, but it was absent thereafter. Necrosis was the most common change in the gill tissues at the increased intensity on day 30 of exposure. Interestingly, on day 15 post-exposure, non-nucleated pillar cells were observed with a <5% deviation from the normal architecture. Mallatt (1985) gave comprehensive information on the structural changes in fish gills in response to toxicant exposure. The observations on the persistence of changes like shortening, desquamation, and curling of secondary lamellae even after 15 days of cessation of exposure together with retardation of fish growth suggested the adverse effect of OTC exposure on the gills as well as on the growth, which may be long-lasting. Similarly, changes like hyperplasia, desquamation, necrosis of epithelium, epithelial lifting, oedema, lamellar fusion, collapsed secondary lamellae, and curling of secondary lamellae have been reported in *O. niloticus*
(Jiraungkoorskul et al. 2002) and *Cyprinus carpio* (Cengiz 2006) exposed to pesticides or herbicides.

Severe degeneration of lamina propria with about 30% deviation from the normal architecture was noted in the intestine of OTC-exposed *O. niloticus* fries on the 30th day. The severity was reduced to 15% upon discontinuation of the exposure, which indicated that the OTC exposure had a degenerative effect on the lamina propria of the fish intestine. The histopathological changes observed were similar to Lawal et al. (2012), who observed focal degenerative epithelium along with glandular necrosis, mucoid cell deposition, and vacuole formation in OTC fed *Clarias gariepinus* juveniles along with degeneration of epithelial lining, necrosis, and vacuolation of lamina propria. The degenerative and necrotic changes observed in the different intestinal layers of the studied fish may be due to the direct effect of OTC or toxic metabolites on the cells or to a reduction in oxygen supply to the tissues (Desai et al. 1984). These histopathological alterations in the intestine of fries are in agreement with those observed by many investigators in the fish intestine upon exposure to different toxicants (Cengiz 2006; Limbu et al. 2021; Roy et al. 2021). Overall, OTC exposure has some damaging effects on the vital organs of the body. Histopathologically, the gills were the most damaged organ, which suggested that the drug was absorbed by the gills and circulated throughout the body by the blood circulation system. The fish gills are the major entry point of pollutants that take them from the water and diffuse across the membrane (Limbu et al. 2021). The route of drug excretion was the kidney, which was also severely affected by high doses of OTC. Contaminants present in water are known to affect kidney tissue (Limbu et al. 2021). The oral entry was the possible secondary entry point of the drug which affected the intestine and liver. Alike, relatively mild to moderate histopathological lesions were observed in the kidney, liver, and intestine of OTC fed *O. niloticus* fries (Roy et al. 2021).

**Conclusion**

In general, there is a lack of systematic study on biosafety, tissue-level changes in the vital organs, and tissue residues in OTC fish muscle in Indian conditions, which necessitated the exploration of such information through this study. The OTC exposure had a deleterious effect on monosex *O. niloticus* fries and reduced the survival and growth in a time- and dose-dependent manner by making the environment toxic and unfavourable by decreasing the pH of the water up to 3.3 at the highest concentration. The OTC exposure also elicited severe histopathological changes in the vital organs. The gill tissues were the most affected at an increased intensity with dose and time. The histopathological alterations resulting from OTC exposure may lead to a reduction in the functional efficiency and/or malfunctioning of several organ systems of the fries. Nevertheless, the observations on the decrease in the extent of tissue damage suggested that the fries can recuperate upon the termination of exposure. Also, the results confirmed that histopathological alterations are good to evaluate the early effects and the responses of fries to acute or chronic exposure to drug stressors. The detectable levels of OTC residues were noted in the tissues of fries even after 15 days of cessation of exposure. The results of the present study may provide probable baseline data on the safety of OTC exposure to the policymakers and regulatory authorities for responsible fisheries and aquaculture.

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Data availability The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy restrictions.

Code availability Not applicable.

Declarations

Ethics approval The current study was performed in compliance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India. The experimental protocols were approved by the ICAR, Government of India, New Delhi, under the All-India Network Project on Fish Health (F. No. CIBA/AINP-FH/2015–16 dated 16.7.2015).

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