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Comparative evaluation of the histopathological effects of florfenicol at a fixed therapeutic dose on the gill tissue of Oscar fish *(Astronotus ocellatus)* following oral and immersion administration

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Abstract

This study compared the histopathological effects of the antibiotic florfenicol on the gills of Oscar fish *(Astronotus ocellatus)* following oral and immersion administration. Thirty fish weighing 50 ± 5 g were divided into three groups (n = 10): an immersion group (florfenicol 10 mg/l for 60 minutes daily), an oral group (florfenicol 10 mg/kg body weight in feed for 7 days), and a control group without treatment. On day seven, gills were fixed in 10% buffered formalin. Sections were stained with hematoxylin–eosin (H&E)and examined microscopically. Histopathological lesions such as epithelial hyperplasia, edema, lamellar fusion, vascular congestion, necrosis, and structural changes were assessed using a semi-quantitative scoring system (0–3 for each lesion). Statistical analysis was performed using Chi-square and Kruskal–Wallis tests ($p < 0.05$). Results showed that florfenicol induced observable histopathological alterations in gill tissues at therapeutic doses, and lesion severity depended on the route of administration ($p < 0.01$). Controls showed no abnormalities. Oral treatment caused mild changes, including slight epithelial thickening and a moderate reduction of interlamellar space (score = 2). Immersion caused severe extensive epithelial hyperplasia, edema, vascular congestion, lamellar fusion, and structural disruption (score = 8). These findings suggested that oral administration of florfenicol may be safer than immersion for gill health in ornamental fish.

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Introduction

Aquaculture has emerged as one of the fastest-growing sectors of global animal food production and plays an increasingly important role in economic development, food security, international trade, and sustainable production systems (Pradeepkiran, 2019; Naylor *et al.,* 2021). Parallel to food-fish aquaculture, the ornamental fish industry has developed into a specialized and high-value sector that significantly contributes to the global bioeconomy. However, intensive husbandry practices, frequent transportation, water quality fluctuations, environmental stressors, and pathogen introduction have made ornamental fish highly vulnerable to infectious diseases, particularly bacterial infections (Sedeño-Díaz and López-López, 2012**;** Wannas and Mohammed, 2025). Within this context, the Oscar fish (*Astronotus ocellatus*), due to its commercial value, popularity in home aquaria, and sensitivity to environmental changes, is considered an important model species in ornamental fish health and therapeutic studies (Peyghan *et al.,* 2019).

The increasing prevalence of bacterial diseases in ornamental aquaculture has led to extensive use of antibiotics as a primary therapeutic measure. Although antibiotics effectively reduce mortality and disease severity, their improper or repeated use may contribute to antimicrobial resistance, tissue residues, alteration of the normal microbiota, and unintended damage to non-target organs (Thanner *et al.,* 2016; Mallik *et al*., 2023; Au-Yeung *et al.,* 2025). Within the One Health framework, antimicrobial resistance in aquaculture is considered a global concern because aquatic environments can serve as reservoirs and transmission pathways for resistance genes across ecological and food webs (Cabello, 2006; Jasovský *et al.,* 2016). In ornamental species, unsupervised or empirical antibiotic use has similarly been identified as an overlooked source of resistance development (Au-Yeung *et al.,* 2025).

Florfenicol, a synthetic broad-spectrum phenicol antibiotic, is widely used in veterinary medicine and aquaculture. It acts by binding to the 50S ribosomal subunit and inhibiting bacterial protein synthesis, resulting in a concentration-dependent bacteriostatic effect against numerous Gram-positive and Gram-negative organisms (Cannon *et al*., 1990; Gaikowski *et al.,* 2003). Due to its favorable bioavailability and therapeutic performance in multiple fish species, florfenicol is routinely applied in the treatment of bacterial infections such as furunculosis and columnaris disease (Jangaran Nejad *et al.,* 2017; Trif *et al.*, 2023). Nevertheless, its increasing use has raised concerns regarding the emergence of resistant strains and potential adverse or off-target tissue effects (Mallik *et al*., 2023; Sumithra *et al.,* 2023).

Although florfenicol's therapeutic efficacy is well documented, its tissue safety-especially in ornamental fish-remains insufficiently clarified. Several studies have reported that florfenicol exposure may induce histological alterations, modulate immune responses, or disturb physiological processes in some fish species (Abu Zahra *et al*., 2024; Bardhan *et al.,* 2024). In tilapia, florfenicol-associated changes in tissue histoarchitecture have been suggested as indicators of biosafety concerns (Bardhan *et al*., 2024). Furthermore, reviews have emphasized the need to reassess the safety profile, usage patterns, and unintended effects of florfenicol across species (Trif *et al.,* 2023).

Among fish organs, the gill is one of the most sensitive structures due to its extensive contact with the aquatic environment and its roles in gas exchange, osmoregulation, ammonia excretion, and acid–base balance. The delicate epithelial layers make the gill highly vulnerable to chemical substances, pollutants, and pharmaceuticals. Consequently, histopathological examination of gill tissue is widely recognized as a reliable biomarker in aquatic toxicology and drug safety assessments (Bernet *et al*., 1999). Gill lesions such as epithelial hyperplasia, lamellar fusion, edema, congestion, necrosis, and degenerative changes are commonly observed responses to chemical or pharmacological stressors (Almeida *et al.,* 2019; Li and Zhang, 2020) and may impair respiratory efficiency if persistent (Ariyaee *et al.,* 2015).

An important but less explored aspect of florfenicol safety involves the influence of the administration route on the extent and pattern of tissue injury. In aquaculture, florfenicol is commonly administered either orally or through immersion baths. These methods differ substantially in absorption dynamics, pharmacokinetics, tissue distribution, and exposure duration of both target and non-target organs (Jangaran Nejad *et al.,* 2017; Gaikowski *et al*., 2003). During immersion treatment, the gills

are directly exposed to the drug dissolved in water, potentially increasing the likelihood of local accumulation and structural injury. In contrast, oral administration involves gastrointestinal absorption and metabolic processing, resulting in a different distribution profile and possibly reduced direct exposure of the branchial tissue.

Despite the practical importance of this distinction, most previous studies have focused on therapeutic efficacy or pharmacokinetic parameters, while direct comparative assessments of florfenicol-induced histopathological effects via different administration routes in ornamental fish-particularly *A. ocellatus*-remain limited (Trif *et al.,* 2023). This gap is especially relevant given the frequent empirical treatment of ornamental species in aquarium settings, where evidence-based guidelines for safe antibiotic administration are often lacking.

Therefore, the present study was designed to compare the histopathological effects of florfenicol on the gill tissue of Oscar fish following immersion and oral administration. By clarifying potential route-dependent differences in tissue response, this study aims to contribute to the identification of safer therapeutic strategies, reduction of off-target tissue damage, and optimization of antibiotic use in ornamental aquaculture.

Materials and Methods

Study design

The present study was conducted as an experimental investigation under controlled laboratory conditions using a completely randomized design (CRD). The aim of this design was to evaluate and compare the histopathological effects of florfenicol on the gill tissue of Oscar fish (*A. ocellatus*) following two routes of administration, namely immersion and oral treatment. The experimental setup was designed to minimize the influence of environmental confounding factors and to ensure accurate comparison among treatments. All experimental procedures were carried out in accordance with ethical principles for the use of laboratory animals, with particular attention to minimizing stress to the fish. The use of laboratory models in aquatic toxicology and veterinary drug safety studies is a common and reliable approach for evaluating tissue-level effects of pharmaceuticals and provides valuable information regarding drug safety under culture conditions (Mallik *et al*., 2023; Trif *et al.,* 2023).

Experimental fish and husbandry conditions

A total of 30 Oscar fish (*A. ocellatus*) with a mean body weight of 550 ± 5 g were sourced from a reputable ornamental fish breeding center in Ahvaz, Khuzestan, Iran. Upon arrival, the fish were distributed into 50-l glass aquaria, each equipped with an internal mechanical-biological filtration system (Sunsun JP 024F, China) and an aeration pump (Resun AC 9904, China) to ensure optimal water quality.

To mitigate transport-related stress, a 10-day acclimation and quarantine period was observed. During this phase, water temperature was maintained at 26–28°C using digital heaters (Atman AT 200, China). Water pH and dissolved oxygen (DO) were monitored daily and kept at 7.0–7.5 and 6–8 mg/l, respectively, using a Hanna HI 2211 pH meter (Italy) and a YSI Pro20 DO meter (USA). Approximately 30% of the water volume was replaced every two days with dechlorinated water. Fish were fed twice daily with a commercial pellet diet (Tetra Cichlid Sticks, Germany) at a rate of 2–3% of their body weight. These stable conditions were maintained to prevent stress-induced physiological or histological alterations (Ruben *et al.,* 2025).

Experimental design and grouping

Following acclimation, the fish were randomly assigned to three experimental groups (n = 10 per group):

1. Immersion group: florfenicol administered via water.

2. Oral group: florfenicol administered via the diet.

3. Control group**:** no medication.

The experimental trial lasted seven days, during which environmental parameters and feeding regimes remained identical across all treatments to minimize confounding variables. At the end of the trial, five fish per group (n = 15 in total) were randomly selected for histopathological examination to ensure unbiased representation.

Florfenicol administration

The florfenicol used in this study was the commercial formulation Aquaflor® (Florfenicol 50%; Merck Animal Health, USA). This antibiotic is among the most widely used agents in aquaculture for the control of bacterial infections because of its broad-spectrum activity against both Gram-positive and Gram-negative bacteria (Trif *et al.,* 2023).

Immersion treatment

In the immersion group, florfenicol was administered at a dose of 10 mg/l, calculated according to the aquarium water volume, and completely dissolved in the water. Fish in this group were exposed to the drug solution for 60 minutes daily. At the end of each immersion period, the fish were transferred to aquaria containing fresh, drug-free water to prevent excessive drug accumulation in the environment. This route allows direct contact of the drug with the gill surface, and absorption occurs primarily through the branchial epithelium (Sumithra *et al.,* 2023).

Oral treatment

In the oral treatment group, florfenicol was administered at a dose of 10 mg/kg body weight. The calculated amount of the drug was mixed with a measured quantity of commercial pellet feed. A small amount of fish oil was added to improve adhesion of the drug to the feed, and the medicated diet was then air-dried at room temperature for 24 hours. The medicated feed was offered daily for seven consecutive days. This method resembles the conventional use of florfenicol in aquaculture farms and allows the evaluation of its systemic effects (Gaikowski *et al.,* 2003).

Control group

Fish in the control group were maintained under the same conditions of temperature, water quality, and feeding, but they received no drug treatment. This group served as the baseline for comparison in evaluating treatment-related histopathological alterations.

Anesthesia and sampling

At the end of day seven, the fish were anesthetized using clove oil at a concentration of 100 mg/l. Clove oil is a commonly used and safe anesthetic in aquatic laboratory studies and is widely applied during tissue sampling procedures (Fujimoto *et al.,* 2018). After deep anesthesia was achieved, the gill arches were carefully removed from the branchial cavity, and tissue samples were collected for histopathological examination. The tissue samples were immediately fixed in 10% buffered formalin to preserve cellular architecture and prevent tissue degradation or autolysis.

Tissue processing and histological sectioning

After fixation for 24–48 hours, the samples were processed for histological preparation. First, the tissues were dehydrated through a graded ethanol series of 70%, 80%, 90%, 96%, and 100%. They were then cleared in xylene and subsequently embedded in molten paraffin at 56–60°C.

The paraffin blocks were sectioned using a rotary microtome (Leica RM2235 Rotary Microtome, Leica Biosystems, Germany), and sections of 4–5 µm thickness were obtained. The prepared sections were mounted on glass slides and, after drying in a laboratory oven (Memmert UF55 Oven, Memmert GmbH, Germany), were processed for staining.

Histopathological staining

To visualize cellular and tissue structures, the sections were stained using the standard hematoxylin and eosin (H&E) method. In this technique, cell nuclei are stained blue to dark purple by hematoxylin, whereas the cytoplasm and extracellular components are stained pink to red by eosin. This method is one of the most widely used staining techniques in histopathology and enables detailed assessment of structural alterations (Suvarna *et al*., 2018).

Microscopic examination

The stained slides were examined using a light microscope (Olympus CX23 Light Microscope, Olympus Corporation, Japan) under different magnifications, particularly at × 400. Several microscopic fields were randomly selected and evaluated on each slide. The histopathological

changes assessed included lamellar epithelial hyperplasia, interlamellar edema, secondary lamellar fusion, cellular necrosis, vascular dilation, and inflammatory cell infiltration.

Histopathological lesion scoring

To compare the severity of gill histopathological alterations among the experimental groups, a four-grade semi-quantitative scoring system was applied based on the method described by Bernet *et al*. (1999). In each fish, the main histopathological lesions observed in the gill tissue were evaluated individually, including epithelial hyperplasia (EH), lamellar fusion (LF), and necrosis (N).

For each lesion, severity was scored according to microscopic observations using the following scale: 0 = no lesion, 1 = mild lesion, 2 = moderate lesion, and 3 = severe lesion. The score for each fish was calculated by summing the individual scores of the evaluated lesions according to the following formula:

Total histopathological score = EH+LF+N

Accordingly, the minimum possible total score was 0 and the maximum possible total score was 9. Based on the total score, gill tissue condition was classified into four categories: 0–2, normal tissue; 3–5, mild damage; 6–7, moderate damage; and 8–9, severe damage.

All histopathological evaluations were performed in a semi-quantitative manner to allow comparison of lesion severity among groups. This scoring approach is widely used as a standard method for assessing histopathological changes in aquatic toxicology studies (Bernet *et al.,* 1999).

Table 1. Criteria for histopathological scoring of gill tissue.

Score	Description	Severity	Percentage of involvement
0	Normal tissue architecture; no detectable lesions.	None	0%
1	Focal or localized lesions; minor changes in lamellae.	Mild	< 25%
2	Frequent lesions; clearly visible in multiple areas.	Moderate	25 – 50%
3	Diffuse/extensive lesions; severe tissue disintegration.	Severe	> 50%

Data collection

The study data were collected from two main sources. Primary data included direct examination of tissue sections, microscopic observations, and recording of the type and severity of histopathological lesions. In addition, library resources and published scientific articles were used to interpret the findings and compare them with previous studies.

Statistical analysis

The data obtained from histopathological examination were recorded as semi-quantitative scores. The frequency of lesion occurrence among the experimental groups was analyzed using the chi-square test. To compare the severity of histopathological lesions between groups, the non-parametric Kruskal–Wallis test was applied because the scoring data were ordinal in nature. When significant differences were detected, Dunn's post hoc test was performed for pairwise multiple comparisons between groups.

Histopathological scores were expressed as median values with interquartile ranges (Median [IQR]). Statistical significance was considered at $p < 0.05$. All statistical analyses were performed using SPSS Statistics version 26 (IBM Corp., Armonk, NY, USA).

Results

Descriptive findings of histopathological lesions

Histopathological examination of the gills of Oscar fish (*A. ocellatus*) revealed that both the extent and severity of tissue lesions were markedly influenced by the route of florfenicol administration. Overall, evaluation of tissue

samples collected from 15 experimental fish across the control, oral treatment, and waterborne exposure (immersion) groups showed distinct patterns of epithelial, vascular, and structural lesions among the groups, with more pronounced damage observed in the treated fish.

Descriptive analysis of the data indicated that the highest proportion of lesions fell within the severe category. With respect to epithelial alterations, 46.7% of the samples exhibited severe lesions, 13.3% showed moderate lesions, 6.7% had mild lesions, and 33.3% showed no pathological change. In the assessment of vascular changes, 46.7% of the samples displayed moderate lesions and 13.3% mild lesions, whereas 40% showed no vascular abnormality. A similar distribution was observed for structural changes, with 46.7% of the samples presenting severe damage, 13.3% moderate damage, 6.7% mild damage, and 33.3% no detectable lesion (Table 2).

Assessment of the overall histopathological lesion severity index further showed that 46.7% of the fish exhibited severe lesions, 13.3% mild lesions, and 40% no observable lesions. These findings suggest that exposure to florfenicol, particularly through waterborne administration, may be associated with a substantial increase in gill tissue damage.

Table 2. Frequency and severity of histopathological alterations in the gills of Oscar fish exposed to a fixed dose of florfenicol.

Histopathological parameter	Normal, n (%)	Mild, n (%)	Moderate, n (%)	Severe, n (%)
Epithelial changes	5 (33.3)	1 (6.7)	2 (13.3)	7 (46.7)
Vascular changes	6 (40.0)	2 (13.3)	7 (46.7)	0 (0.0)
Structural changes	5 (33.3)	1 (6.7)	2 (13.3)	7 (46.7)
Overall lesion status	6 (40.0)	2 (13.3)	0 (0.0)	7 (46.7)

Relationship between florfenicol administration route and epithelial alterations

Statistical analysis demonstrated a significant association between the route of florfenicol administration and the occurrence of epithelial changes in the gill tissue ($p < 0.001$). In the control group, all samples retained the normal epithelial architecture of the secondary lamellae, and no evidence of hyperplasia, epithelial thickening, or epithelial lifting was observed. In contrast, the oral treatment group showed epithelial alterations in a proportion of the samples, with approximately 40% of the fish exhibiting severe epithelial damage. The highest degree of epithelial injury was recorded in the immersion group, in which all samples (100%) showed severe epithelial lesions.

These findings indicated that direct exposure of the gill tissue to the drug solution may intensify epithelial cellular responses and enhance the severity of hyperplasia and epithelial thickening.

Relationship between drug administration route and vascular changes

Data analysis also revealed a significant relationship between the route of florfenicol administration and vascular alterations in the gills ($p = 0.002$). In the control group, the vascular structure of the secondary lamellae was entirely normal, with no evidence of congestion, hemorrhage, or abnormal vascular dilation. In the oral treatment group, some samples showed mild to moderate vascular changes, mainly manifested as increased erythrocyte accumulation within the lamellar capillaries and mild vascular congestion.

By contrast, vascular lesions were considerably more pronounced in the immersion group. In this group, all samples exhibited clear signs of vascular disturbance, including capillary congestion, extensive erythrocyte accumulation within the lamellae, and increased vascular pressure in the branchial tissue.

Relationship between florfenicol administration route and structural alterations

The results further demonstrated a significant association between the route of florfenicol administration and the development of structural changes in the gills ($p < 0.001$). In the control

group, the architecture of the filaments and secondary lamellae appeared completely normal, and the interlamellar spaces were well preserved. No lamellar adhesion, deformation, or disruption of the normal gill architecture was observed in this group.

In the oral treatment group, mild structural alterations were detected in some samples, including a relative reduction in interlamellar spacing and limited adhesion of secondary lamellae. Although these changes suggested the onset of a tissue response to the drug, their severity was clearly lower than that observed in the immersion group.

In the immersion group, structural lesions were extensive and pronounced. These changes included widespread fusion of the secondary lamellae, shortening of lamellae, marked reduction of interlamellar spaces, and disorganization of filament architecture, ultimately disrupting the normal structural arrangement of the gills.

Relationship between drug administration route and overall lesion severity

Evaluation of the overall histopathological lesion index also showed that lesion severity was significantly affected by the route of florfenicol administration (p = 0.002). In the control group, all samples exhibited normal gill structure, and the overall lesion score was zero. In the oral treatment group, some samples showed mild to moderate lesions, whereas in the immersion group all samples exhibited severe tissue damage.

These findings suggested that direct contact between the gill tissue and the drug solution can markedly intensify histopathological injury.

Table 3. Quantitative comparison of gill histopathological scores in Oscar fish (*A. ocellatus*) across experimental groups.

Lesion Category	Control (n = 5)	Oral group (n = 5)	Immersion group (n = 5)	*H*-value	*p*-value
Epithelial Changes (EC)	0 (0–0)[a]	1.0 (1.0–2.0)[b]	3.0 (3.0–3.0)[c]	12.45	< 0.001
Vascular Alterations (VA)	0 (0–0)[a]	1.0 (0–1.0)[a]	2.0 (2.0–3.0)[b]	10.12	0.002
Structural Damage (SD)	0 (0–0)[a]	1.0 (1.0–2.0)[b]	3.0 (3.0–3.0)[c]	12.45	< 0.001
Total Score	0 (0–0)[a]	2.0 (2.0–3.5)[b]	8.0 (7.0–9.0)[c]	13.18	< 0.001

Note: Data were presented as Median (Interquartile Range). Different superscript letters (a, b, c) within a row indicated significant differences between groups (Dunn's post hoc test, $p < 0.05$). H-value represents the Kruskal–Wallis test statistic.

Statistical analysis using the Kruskal–Wallis test revealed significant differences in the severity of all histopathological categories among the groups ($p < 0.01$, Table 2). The immersion group exhibited the highest total score (Median = 8.0), which was significantly higher than both the oral group (p = 0.008) and the control group ($p < 0.001$). Post hoc comparisons showed that while oral administration induced only mild-to-moderate changes, waterborne exposure led to severe diffuse lesions in 100% of the sampled fish (n = 5 per group).

Histopathological findings in gill tissue

Microscopic examination of tissue sections showed that the gill structure in the control group was entirely normal. The filaments and secondary lamellae were regularly arranged and displayed normal architecture. The epithelial lining of the lamellae was thin and uniform, and no epithelial, vascular, or structural abnormalities were detected. According to the histopathological scoring system, the scores for epithelial, vascular, and structural changes in this group were all zero, resulting in a total lesion score of zero (Table 3).

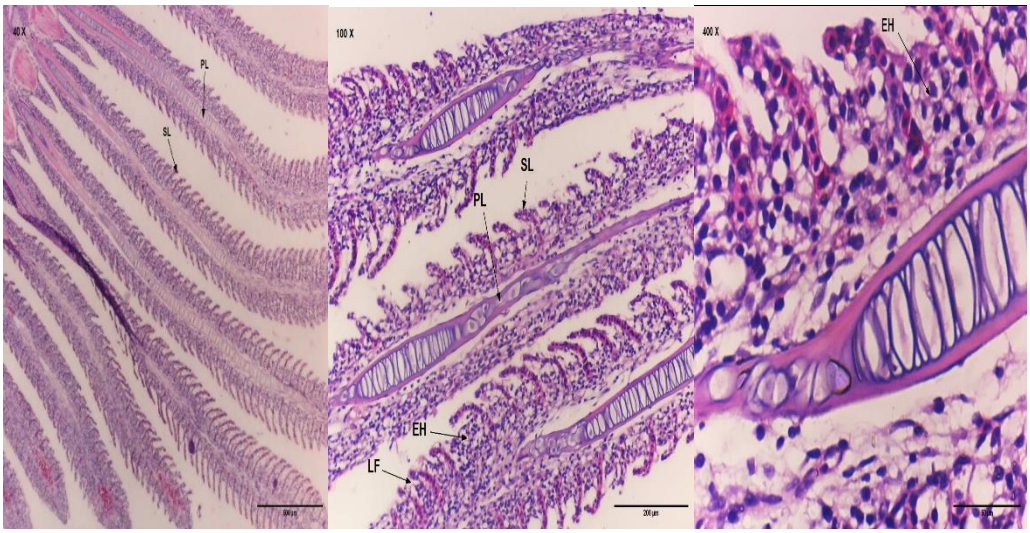


Fig. 1. Representative hematoxylin and eosin (H&E)-stained photomicrographs of gill tissue from the control group. Gill filaments exhibited normal histological architecture with well-organized primary lamellae (PL) and secondary lamellae (SL). No histopathological alterations were observed. Scale bars = 500 µm (40×), 200 µm (100×), and 50 µm (400×).

In the oral florfenicol group, the overall gill architecture was largely preserved, although mild histopathological alterations were observed. These changes included slight epithelial hyperplasia in the secondary lamellae, accompanied by mild epithelial thickening and increased cellular density. A relative reduction in interlamellar spacing was also evident, and focal lamellar adhesion (lamellar fusion) was observed in some areas. No significant vascular lesions, such as congestion or hemorrhage, were detected in this group, and the vascular structures remained within normal histological limits. According to the scoring system, the epithelial change score was 1, the vascular change score was 0, and the structural change score was 1, resulting in a total histopathological lesion score of 2, which was classified as mild gill damage.

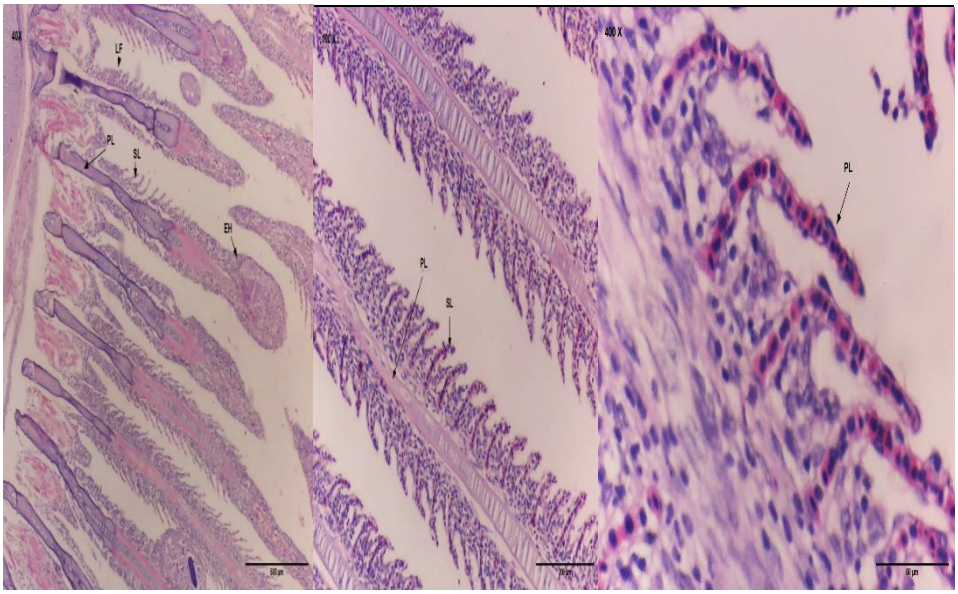


Fig. 2. Representative hematoxylin and eosin (H&E)-stained photomicrographs of gill tissue from fish treated with the drug via feed. Mild histopathological alterations were observed, including epithelial hyperplasia (EH), focal lamellar fusion (LF), and slight vascular congestion (VC). The overall lamellar architecture remained largely preserved. PL, primary lamella; SL, secondary lamella. Scale bars = 500 µm (40×), 200 µm (100×), and 50 µm (400×).

In the immersion group, tissue lesions were considerably more severe compared to other groups. This group exhibited extensive hyperplasia of the secondary lamellar epithelium, accompanied by marked epithelial thickening. In many areas, epithelial lifting and widening of the subepithelial space were evident, indicating pronounced tissue edema. Regarding vascular changes, moderate to severe capillary congestion and extensive hemorrhage (erythrocyte accumulation) within the lamellae were observed. Structural lesions were severe, characterized by widespread fusion of secondary lamellae, significant narrowing of the interlamellar spaces, and complete disorganization of the lamellar arrangement. In several regions, these alterations progressed to focal necrosis and lamellar deformation.

Based on the scoring system, the epithelial change score was 3, the vascular change score was 2, and the structural change score was 3, yielding a total histopathological lesion score of 8. This high score underscores the severe gill damage and potential respiratory failure resulting from direct exposure to the florfenicol solution.

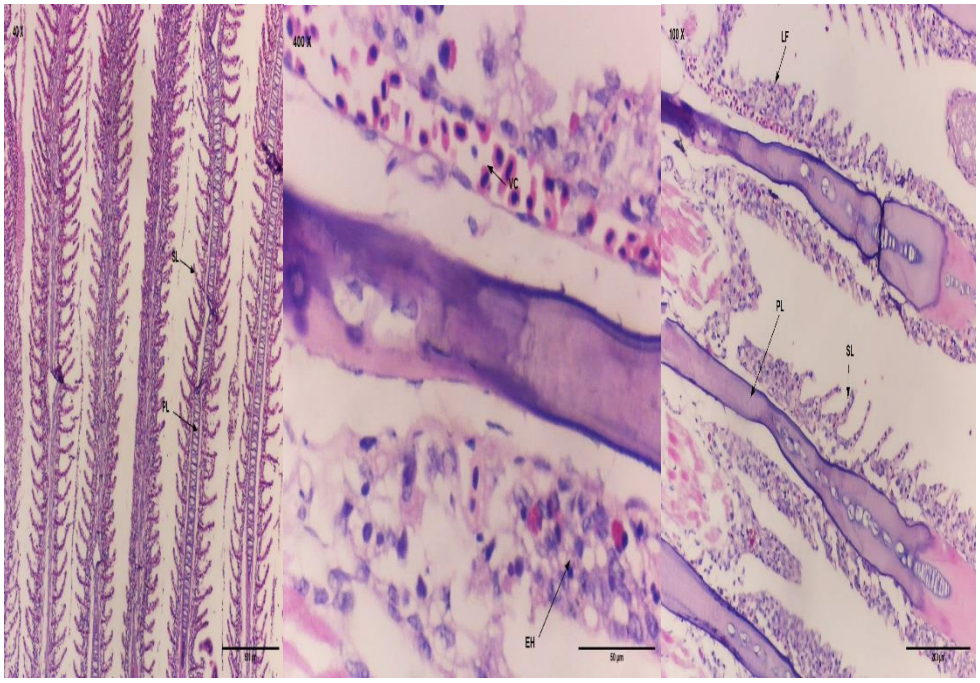


Fig. 3. Representative hematoxylin and eosin (H&E)-stained photomicrographs of gill tissue from fish treated with the drug via water. Marked histopathological alterations were observed, including epithelial hyperplasia (EH), lamellar fusion (LF), and vascular congestion (VC). The lesions were more severe than those observed in the feed-treated group. PL, primary lamella; SL, secondary lamella. Scale bars = 500 µm (40×), 200 µm (100×), and 50 µm (400×).

Overall, the findings of the present study demonstrated that the severity of gill histopathological lesions in Oscar fish was strongly influenced by the route of florfenicol administration. While the control group maintained normal gill architecture, the orally treated group exhibited only mild alterations, such as focal epithelial hyperplasia. In contrast, the immersion group displayed severe and extensive lesions, including epithelial lifting, edema, hemorrhage, and widespread lamellar fusion leading to necrosis. These structural and vascular damages highlighted the high sensitivity of gill tissue to direct contact with the drug and the potential for impaired respiratory function in exposed fish (Figs 1, 2, and 3).

Discussion

The present study demonstrated that the administration of florfenicol, even at therapeutic doses and over a short duration, is associated with histopathological alterations in the gill tissue of Oscar fish (*A. ocellatus*). Statistical analysis confirmed that the severity and pattern of these lesions were significantly influenced by the route of administration ($p < 0.01$). Specifically, the immersion-treated group exhibited more

pronounced lesions compared to the orally treated group. These observations suggest a possible link between the route of exposure and the degree of tissue response, potentially due to the direct interaction between the gill epithelium and the waterborne medication.

Under immersion, the gills serve as a primary interface for drug uptake. It is hypothesized that this direct contact might contribute to a higher local drug burden within the branchial tissues compared to systemic delivery. However, as tissue drug concentrations were not quantified in this study, this remains a speculative explanation that warrants further pharmacokinetic investigation.

The structural complexity and thin epithelial barrier of fish gills, while optimized for gas exchange (Evans *et al*., 2005), render them highly susceptible to external chemical agents. In the present study, the control group maintained normal lamellar architecture, confirming that husbandry conditions were not a confounding factor. In contrast, the treatment groups exhibited classical patterns of injury, including epithelial hyperplasia, lamellar fusion, and edema (Mallatt, 1985; Roberts, 2012).

From a pathophysiological standpoint, these alterations could be interpreted as part of a non-specific adaptive or protective response. For instance, the observed epithelial thickening may represent a biological attempt to increase the diffusion distance, thereby potentially limiting the influx of the drug into the circulatory system. While this might serve a protective role, it likely occurs at the expense of respiratory efficiency, potentially leading to localized tissue hypoxia (Bernet *et al*., 1999). The higher severity of lesions in the immersion group, particularly the fusion of secondary lamellae, might be related to the continuous exposure of the epithelium to the antibiotic in the water column. In theory, this persistent contact could lead to local physiological stress. Similarly, the observed vascular congestion and capillary dilatation may suggest a disruption in local hemodynamics or a mild inflammatory response. While previous studies have linked antibiotic exposure to the production of reactive oxygen species (ROS) and oxidative stress (Abu Zahra *et al.,* 2024), further biochemical assays are required to confirm whether such mechanisms were active in the present experimental model.

The observed differences between the two administration routes could potentially be explained through the lens of comparative pharmacokinetics, although such parameters were not directly measured here. In immersion treatment, the drug is absorbed across the large surface area of the gills. In contrast, during oral administration, the drug undergoes gastrointestinal absorption and potentially first-pass hepatic metabolism (Plumb and Hanson, 2010), which might result in a different distribution profile and a lower effective concentration reaching the branchial tissue.

Our findings regarding the impact of florfenicol on gill morphology align with observations in other species, such as Nile tilapia (Bardhan *et al*., 2024) and catfish (Abu Zahra *et al.,* 2024).

Furthermore, while the current study relied on manual semi-quantitative scoring, the integration of emerging technologies such as digital pathology and artificial intelligence could be considered a future perspective. Utilizing deep-learning algorithms may enhance the objectivity and efficiency of quantifying gill lesions in aquatic toxicological research.

Despite providing valuable insights into the histopathological effects of florfenicol on the gill tissue of *A. ocellatus*, this study has several limitations that should be considered when interpreting and generalizing the findings. First, the sample size in each experimental group was limited. Although sufficient for an initial histopathological assessment, it may reduce the generalizability of the results to larger populations or to different aquaculture conditions.

Second, the duration of the study was relatively short and mainly focused on the acute effects of florfenicol exposure. As a result, subacute and chronic effects of the drug, as well as the potential reversibility of the observed lesions after treatment withdrawal, could not be evaluated.

Third, only a single dose of florfenicol was tested in two administration routes. Therefore, a detailed dose–response analysis and the determination of safe or toxic thresholds were beyond the scope of the present study. Another limitation is that the evaluations were based primarily on histological observations, while complementary markers such as respiratory physiological parameters, oxidative stress biomarkers, blood biochemical changes, or molecular indicators of inflammation were not

assessed. Consequently, interpretation of the precise cellular and functional mechanisms underlying florfenicol-induced injury remains limited.

Finally, this study was conducted on a single ornamental fish species. Accordingly, extrapolation of the findings to other fish species, particularly farmed species with different physiological characteristics, should be made with caution. Nevertheless, despite these limitations, the present findings provide a useful foundation for future studies aimed at evaluating the safety of antibiotic therapies in aquatic animals.

In conclusion, while florfenicol is an essential tool in aquaculture, its administration-particularly via immersion-appears to be associated with significant, albeit potentially transient, structural changes in the gills of Oscar fish. These results highlight the importance of considering the route of administration in safety assessments. Since the current study focused primarily on histopathological endpoints, future research incorporating tissue residue analysis, pharmacokinetic modeling, and oxidative stress markers is necessary to provide a more definitive mechanistic understanding of florfenicol-induced tissue alterations.

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Conflict of Interest

The authors declare that there are no conflict of interest related to this study.

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