Aquaculture Studies, 19(1), 01-07
http://doi.org/10.4194/2618-6381-v19_1_01

PROOF
RESEARCH PAPER

Evaluation of Histopathological Effects of Sub-lethal Concentrations of Chloramphenicol Antibiotic on the Gill of *Clarias gariepinus* Juvenile (Burchell, 1822)

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Article History
Received 27 November 2018
Accepted 28 January 2019
First Online 28 January 2019

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Keywords
Bioconcentration
Bioaccumulation
Congestion
Bioassay
Edema
Capillary

Abstract

An investigation on the effect of Chloramphenicol on the gill tissues of *Clarias gariepinus* juveniles was carried out in the laboratory using static renewal bioassay techniques. Two hundred and seventy fish with length 7.39±39cm and weight 12.37±65g were exposed to sub lethal concentrations of chloramphenicol for 70 days. The gills were removed for histopathological examination. The observed histopathological changes in the gills were occlusion of tubular lumen, congestion of blood vessels, degeneration of primary and secondary gill lamellar, fragmentation of central axis, lamellar fusion and degeneration of epithelial and marginal cells in the test organs. The severity of the lesions along the concentration gradients was observed to be proportional to dosage. The effect was most severe at the highest concentration (12.5 mg/l). The observed abnormalities in the gills of *Clarias gariepinus* juveniles exposed to chloramphenicol shows the suitability of *Clarias gariepinus* in the toxicity testing of antibiotics.

Introduction

Chloramphenicol is commonly used as a fish health promoter in aquaculture and often included in fish feeds (Amravuawho, Akinyemi, Oyewusi, Bankole, & Ezeri, 2016). It is bactericidal in action through the inhibition of the growth of pathogenic bacteria. At times, antibiotics are used to check diseases by treating the water or fish before disease occurs (Alderman & Hastings, 1998). In these prophylactic and therapeutic methods of treating fish disease, it is always profitable because it prevents loss and allows fish to grow faster but there are several drawbacks. However, the overuse of antibiotics in treatment of fish aquaculture could create public health issues (Manoharan, 2014). Antibiotics are theoretically not different from other common aquatic contaminants since some of them possess non-biodegradable properties. They can bioacumulate in the tissues of aquatic animals. Bioconcentration, bioacumulation and biomagnification factors have been known for long to be useful factors in retrospective and prospective assessments of commonly known pollutants in the aquatic ecosystems. This is because relevant information obtained in these factors can provide relationship between exposure and threshold toxicity (Archer, Petrie, Kasprzk-Harden & Wolfaardt, 2017). This antibiotic has inherent toxicity resulting in immunotoxicity and carcinogenicity (Shalaby, Khattab & Abdel-Rahman, 2006).

Fish gills are prone to contamination due to their regular contact with aquatic environment (Perry & Laurent, 1993). Mostly, fish exchange gases through the gills, which consist of tissues called filaments. These filaments have multiple functions which include the transfer of ions and water, exchange of oxygen, carbon dioxide, acids and ammonia (Ferguson, 1989). A

filament consists of a capillary network that provides a large surface area for gaseous exchange (Pane, Haque & Wood, 2004). The monitoring and assessment of histopathological distortion in fish organs is a good method for determining the side-effects of antibiotic drugs in the field and laboratory experiments. Therefore, the use of antibiotic toxicology studies on aquatic organisms is imperative to enable the farmers evaluate its noxious effect mostly in the essential organs. However, there is dearth of information on the evaluations of toxicological effect of chloramphenicol on the histopathology of the vital organs of *Clarias gariepinus* juveniles. This present study was carried out to determine the effect of sub lethal concentrations of chloramphenicol on the histopathological samples of the gills of African catfish *Clarias gariepinus* juveniles.

Materials and Methods

Experimental Fish

A total of two hundred and seventy African catfish *Clarias gariepinus (*juveniles) were procured from Kennedy Fish Farm Abakaliki and transported in plastic container to the Wet Laboratory of the Department of Fisheries and Aquaculture, Ebonyi State University, Abakaliki (Latitude 6°, 20, 49, N, Longitude 8°, 06, 11 E) Ebonyi state, Nigeria. The fish were acclimated for 3weeks (21days) under laboratory conditions in 70 liters capacity plastic vats before the commencement of the study.

Preparation of Chloramphenicol Solution

Chloramphenicol capsules (250mg) containing chloramphenicol sodium succinate as the active ingredient (manufactured by Jiangsu Huayang Pharmaceutical Company, China) was used to prepare the stock solution. Six hundred and fifty (650) capsules of chloramphenicol were decapsulated and stored in an air tight container. The chloramphenicol powder was thereafter dissolved in dechlorinated water to prepare the stock solution. Later on, the calculated amount of stock solution was added to the experimental units by serial dilution method so as to get the desired sub lethal test concentrations.

Experimental Design

Completely randomized design (CRD) was used for the experiment. The experimental fish, *Clarias gariepinus* (juveniles) with mean weight and length of 12.37±.65g and 7.39±.39cm respectively were used for the experiment. The fish were randomly distributed into the plastic vats containing dechlorinated water. During the period of acclimation and the experiment, the fish were fed on 42% crude protein diet at 4% body weight twice daily throughout the period of acclimation and experiment. It is from the acclimated fish that the test fish were selected for bioassay studies. Mortality did not exceed 5% during acclimation period. Sub lethal concentrations of chloramphenicol used were T_1 (2.5 mg/l), T_2 (5.0 mg/l), T_3 (7.5 mg/l), T_4 (10.0 mg/l) and T_5 (12.5 mg/l). During the exposure period, 10 fish each was introduced into the 70 capacity plastic vats containing 30L of well aerated dechlorinated tap water. The already prepared concentrations were introduced into the first five plastic vats serially, the sixth served as the control (devoid of the chloramphenicol solution). Each concentration was replicated three times. Water in each tank was replaced every three days throughout the period to prevent fouling resultant from fecal matter and feed remnants. Chloramphenicol powder was reintroduced in the culture water at the same varying concentrations along side. The experiment lasted for the period of 70 days, during which methods of renewal bioassay test were employed in this investigation. No mortality was recorded throughout the research. The buccal cavities of sacrificed fish were dissected open to collect the gills for histopathological analysis. The organs collected were fixed in 10% formalin to avoid autolysis and preserve cells in conditions identical to that during life.

Water Quality Analysis

Physico-chemical characteristics of experimental tank water were closely monitored and measured daily throughout the duration of the study. Water in experimental tanks was siphoned out and replaced with fresh water every three days to remove faecal waste and maintain desired water quality. The following parameters were tested for water temperature using a thermometer, hydrogen ion concentration (pH), ammonia, nitrate, nitrite, dissolved oxygen (DO) and hardness using methods described by APHA (2002).

Histopathology Analysis

The buccal cavities of sacrificed fish were dissected open and the gills removed for histopathological analysis. The gills collected were fixed in 10% formalin to avoid autolysis and preserve cells in conditions identical to that during life.

Tissue Processing (Dehydration, Dealcoholization and Infiltration)

The tissues were processed in an automatic tissue processor (Model-Leica AP). The tissues were first processed by putting it in tissue cassette and dehydrated in graded alcohol of 70 - 100% and the alcohol was removed with xylene (Bancroft and Stephen, 1990). The clearing agents were replaced by passing the tissues through molten paraffin wax maintained at temperature higher than the melting

point of wax, to fill the intercellular spaces for easy microtomy.

Embedding (Casting) and Mounting on Wooden Block

The tissues were embedded in the molten paraffin wax poured inside the Leuckhart embedding metal box and allowed for some minutes to solidify before it was detached. The detached paraffin blocks were mounted on the wooden block with the aid of hot spatula, which melted the portion of the paraffin to be placed on wooden block. The melted part quickly solidified to the wooden block on removing the spatula.

Microtomy, Sectioning and Microphotography

The wooden blocks were screwed to the microtome chuck and paraffin block trimmed to expose embedded tissues and then sectioned at 6 µm with a rotator microtome (Leica Rm2135) Sectioned tissues were stained with heamatoxylin and eosin using the method of Bancroft and Stephen (1990). Microphotography of the sectioned tissues produced photomicrograph of cells of chloramphenicol exposed tissues with control with the use of microscope. This procedure was conducted at the histopathology laboratory, Federal Teaching Hospital (FETHA) Abakaliki Ebonyi State, Nigeria.

Statistical Analysis

Results obtained from investigation were subjected to one way analysis of variance (ANOVA) using the Statistical package for social sciences (SPSS version 20) to determine the significant difference between the various treatments and control. Duncan Multiple Range Test was used to compare the differences between means at ($P<0.05$). Data were presented as mean ± SE.

Results

Water quality analysis

Result from water quality analysis shows that the mean water quality parameters obtained in the treatment tanks did not vary significantly (*P>0.05*) from those of the control tanks (Table 1). All were within the suggested tolerance ranges. No mortality was recorded during the experimental period.

Histopathology of Gills

Results on histological analysis of gills of *Clarias gariepinus* juveniles exposed to Sub-lethal concentration of chloramphenicol for 70 days are presented in Figure 1 to 6. Figure 1 (control) shows that the primary gill lamellar, secondary gill lamellar, gill epithelia cell, central axis, pillar cell and marginal cell are well arranged in normal position. Figure 2 (12.5mg/l) revealed that the gills of *Clarias gariepinus* has sustained various degree of damages which includes, blood congestion (BC), degeneration of primary gill lamellar (DPGL), fragmentation of central axis (FCA) and degeneration of epithelia cell (DEC). Figure 3 (10.0mg/l) shows various degree of damages on *Clarias gariepinus* gills which includes, blood congestion (BC), degeneration of primary gill lamellar (DPGL), degeneration of secondary gill lamellar (DSGL), fragmentation of central axis (FCA), lamellar fusion (LF) and degeneration of epithelia cell (DEC). Figure 4 to 6 (7.5mg/l, 5.0mg/l, 2.5mg/l) also indicates various degree of damages to the gill of *Clarias gariepinus* which includes, dilation (D) of the blood vessels, blood congestion (BC), degeneration of primary gill lamellar (DPGL) hyperplasia (Hy), degeneration of secondary gill lamellar (DSGL), fragmentation of central axis (FCA), lamellar fusion (LF) and degeneration of epithelia cell.

Discussion

Physico-chemical water parameters such as temperature, hydrogen ion concentration (pH), nitrate, nitrite, ammonia and hardness were measured every 24 hours (Table 1). The mean water quality parameters obtained in the treatment tanks did not vary significantly (*P>0.05*) from those of the control tanks. All were within the suggested tolerance ranges. No mortality was recorded during the experimental period.

There were eight gill arches on both side of the buccal cavities of *Clarias gariepinus* and consists of

Table 1. Mean values of Water Quality Parameters during Exposure of *Clarias gariepinus* Juveniles to Sub lethal Concentrations of Chloramphenicol for 70 days.

	Concentrations (mg/l)					
	Control	T1	T2	T3	T4	T5
Parameters		2.5mg/l	5.0mg/l	7.5mg/l	10.0mg/l	12.5mg/l
Temperatures(˚C)	24.39±32[a]	24.18±2[a]	23.24±4[a]	23.21±41[a]	25.13±0[a]	25.54±30[a]
pH	6.75±19[a]	7.47±16[a]	7.10±13[a]	7.01±13[a]	6.75±17[a]	7.05±13[a]
DO(mg/l)	6.95±19[a]	6.85±23[a]	6.52±19[a]	6.65±18[a]	7.30±08[a]	6.89±16[a]
Nitrite (mg/l)	0.03±001[a]	0.03±001[a]	0.03±001[a]	0.03±001[a]	0.03±00[a]	0.03±001[a]
Nitrate (mg/l)	0.01±06[a]	0.01±05[a]	0.01±04[a]	0.01±004[a]	0.01±00[a]	0.01±005[a]
Ammonia (mg/l)	0.25±03[a]	0.25±03[a]	0.25±03[a]	0.25±02[a]	0.25±02[a]	0.25±02[a]
Hardness (mg/l)	28.0±02[a]	28.0±02[a]	28.0±02[a]	28.0±02[a]	28.0±02[a]	28.0±02[a]

***** Means (±SE) of water quality parameters obtained in the treatment tanks did not vary significantly (*P*>0.05)

multiple gill filaments lying side by side in opposite direction. Each arch consisted of multiple lamellae, arranged in two rows perpendicularly to each filament. Secondary gill lamellae are composed of cells demarcating the capillary channel in the cells. Histopathology of the gills of fish in control groups shows normal organizational structure of the cells in the lamellae and filaments (Figure 1). However,

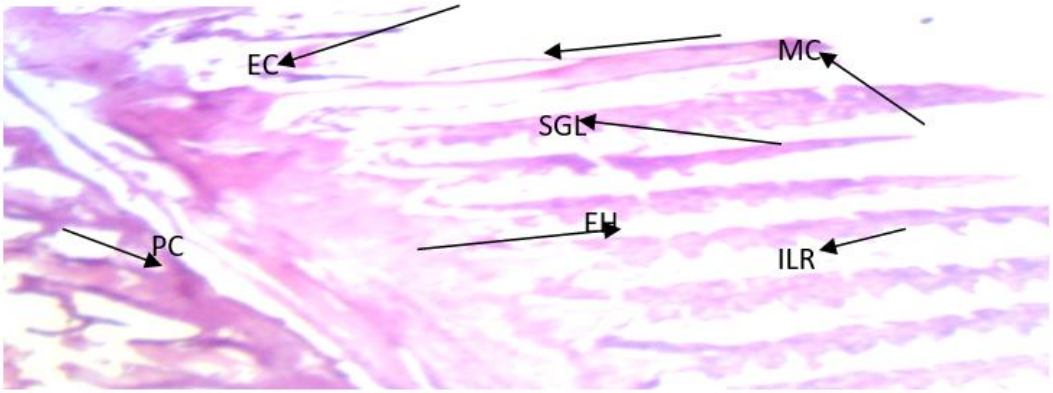

Figure 1. Photomicrograph of gill in control group of fish showing primary gill lamellar, secondary gill lamellar, gill epithelia cell, central axis, pillar cell and marginal cell well arranged and gills exposed to Sub lethal concentration of chloramphenicol for 70 days.

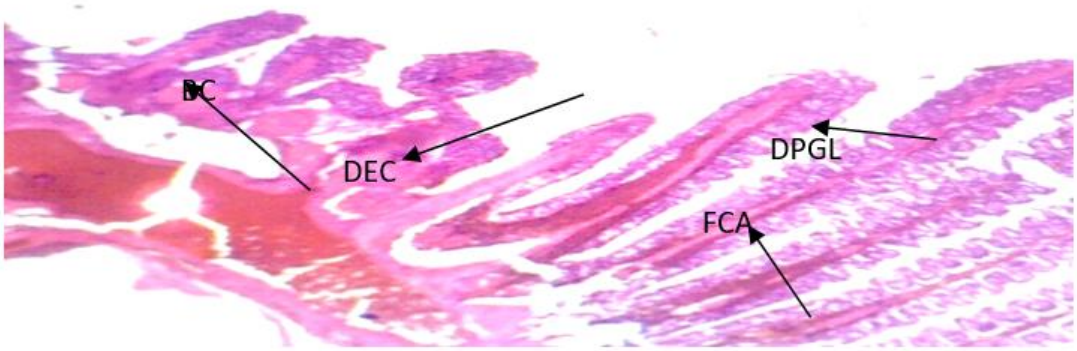

Figure 2. Photomicrograph of gill exposed to Sub lethal concentrations of Chloramphenicol for 70 days at 12.5 mg/l showing various degree of damages which includes: Blood congestion (BC), degeneration of primary gill lamellar (DPGL), fragmentation of central axis (FCA) and Degeneration of epithelia cell (DEC) (Mag.x60).

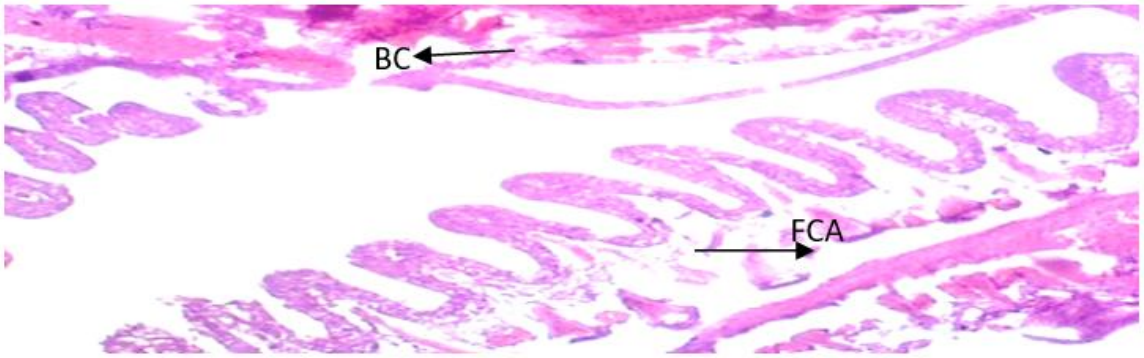

Figure 3. Photomicrograph of gill exposed to Sub lethal concentrations of Chloramphenicol for 70 days at 10.0 mg/l showing various degree of damages which includes: Blood congestion (BC), degeneration of primary gill lamellar (DPGL), degeneration of secondary gill lamellar (DSGL), fragmentation of central axis (FCA), lamellar fusion (LF) and Degeneration of epithelia cell (DEC) (Mag.x60).

histopathology of the of the gills of fish treated with chloramphenicol resulted in different degrees of ailments which included hypertrophic condition in the epithelial tissues of primary filament in the gills, fusion of secondary gill lamellae, and degeneration of primary and secondary gill lamellae. Other observed changes during the experiment included lifting of epithelial; edema in interstitial cells and congestion of blood in the

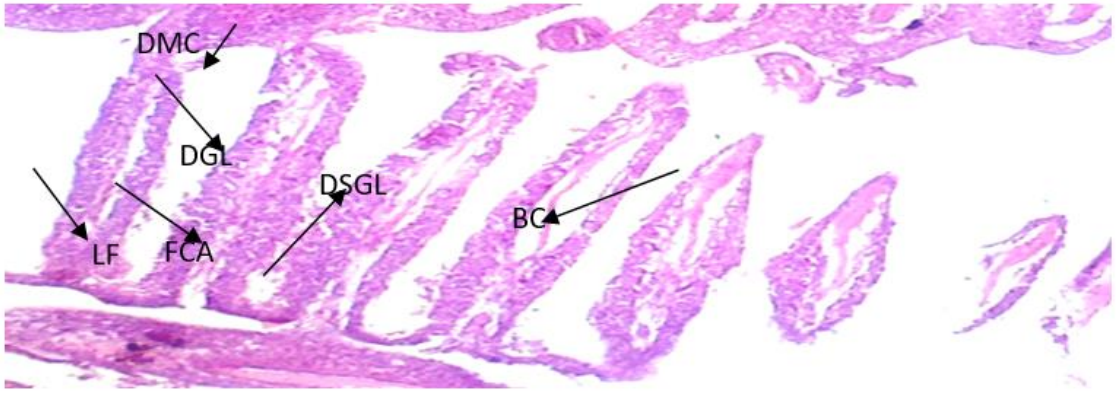

Figure 4. Photomicrograph of gill exposed to Sub lethal concentrations of Chloramphenicol for 70 days at 7.5 mg/l, showing various degree of damages which includes: dilation (D) of the blood vessels, blood congestion (BC), degeneration of primary gill lamellar (DPGL) hyperplasia (Hy), degeneration of secondary gill lamellar (DSGL), fragmentation of central axis (FCA), lamellar fusion (LF) and degeneration of epithelia cell (DEC) (Mag. × 60).

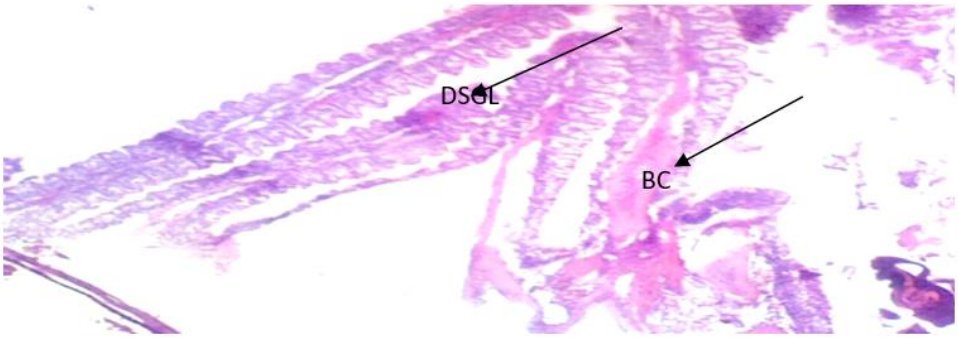

Figure 5. Photomicrograph of gill exposed to Sub lethal concentrations of Chloramphenicol for 70 days at 5.0 mg/l showing various degree of damages which includes: blood congestion (BC), degeneration of primary gill lamellar (DPGL), hyperplasia (Hy), degeneration of secondary gill lamellar (DSGL), fragmentation of central axis (FCA) and degeneration of epithelia cell (DEC) (Mag. × 60).

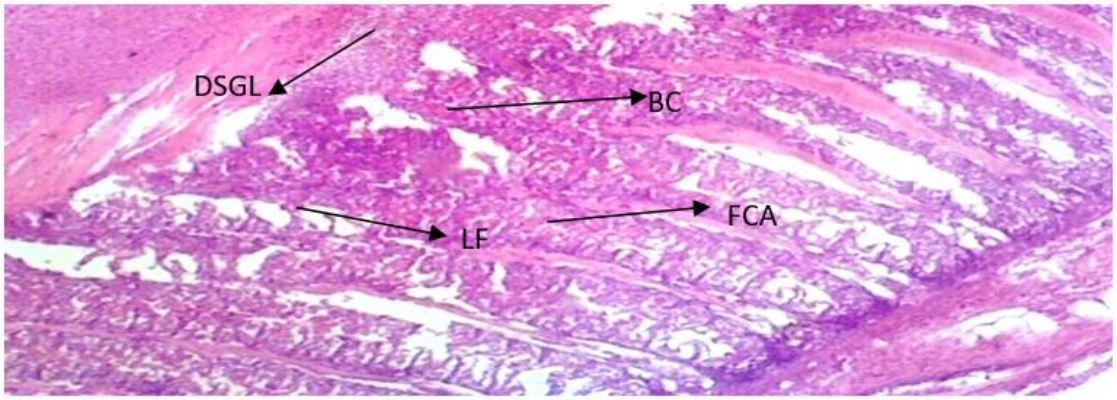

Figure 6. Photomicrograph of gill exposed to Sub lethal concentrations of Chloramphenicol for 70 days at 2.5 mg/l showing various degree of damages which includes: Blood congestion (BC), hyperplasia (Hy), degeneration of secondary gill lamellar (DSGL), lamella fussion (LF), fragmentation of central axis (FCA) (Mag. × 60).

central axis of primary filaments were also detected at gill lamellae (Figures 3 - 6). The evaluation revealed that concentration of 12.5 mg/l of chloramphenicol induced degenerative condition in the cell which consequently resulted in necrotic disease of the epithelial tissues of the gill (Figure 2).

The gills are essential organs for respiration, osmoregulation, acid-base balance and elimination of nitrogenous waste (Heath, 1987). Fish gills are known to be prone to damage in contaminated environment because of their direct contact with water in the aquatic environment. As a result, they are susceptible to destruction by any contaminant in the water bodies (Roberts, 1978). The proliferation of cells and widening of the epithelium in gill filaments can result to fusion in the lamellar (Figueiredo-Fernandes *et al*., 2007). The fusion and hyperplasia of gill lamellae is caused by the effect of the toxicants which changes the mucus covering the cell in the glycoprotein and can as well effect the negative charges of the epithelial thereby favoring adhesion between the lamellae (Ferguson, 1989). The lamellar epithelium lifting is another histopathological anomaly that occurred, possibly caused by edematous condition (Pane *et al*., 2004).

The experiment further revealed that edema within the cells is one of the most common injuries observed in epithelium of fish gills exposed to toxicants (Mallatt, 1985). In this case, damages in the pillar cells can be as a result of an increased rate of blood flowing inside the lamellae, causing marginal channel dilation, congestion of blood and aneurysm (Rosety-Rodriguez *et al.*, 2002). Changes like lifting of epithelial cells, hypertrophy of the cells and fusion of secondary gill lamellae are signs of defensive mechanisms to barricade the entrance of toxic substances into the blood. As a result of the increasing gap between water and blood, the intake of oxygen is impeded. These histopathological impairment of the gills possibly resulted in hypoxic condition, failure in respiratory system with ionic and acid-base balance (Alazemi, Lewis & Andrews, 1996).

Conclusion

The outcome of this research has revealed that the use of antibiotics in farming aquacultural species has toxic effects. The escalating use of a variety of antibiotics in fish farms has resulted in an increase in bioconcentration and bioaccumulation of these chemicals in the tissues of aquatic animals. The observed histopathological changes in the gill were congestion of blood vessels, degeneration of primary and secondary gill lamellar, fragmentation of central axis, lamellar fusion and degeneration of epithelial and marginal cells of the gill. The severity of the lesions along the concentration gradients was observed to be proportional to dosage. The monitoring and evaluation of histopathological alteration in fish organs is a good method for determining the side-effects of antibiotic drugs in the field and laboratory experiments. Consequently, the use of antibiotic toxicology studies on aquatic organisms is very important to enable the farmers assess its deleterious effect mostly in the vital organs.

Acknowledgement

Authors are grateful to Mrs. Chioma Awoke for type-setting the manuscript.

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