

CHANGES IN HISTOLOGY OF JUVENILE FRESHWATER FISH *Clarias gariepinus* (BURCHELL) EXPOSED TO PRETILACHOR + PYRIBENZOXIM HERBICIDE

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ABSTRACT

Environmental contamination by agricultural chemicals, particularly herbicides, poses a significant threat to aquatic ecosystems and their inhabitants, including fish. This study aimed to evaluate the acute histological and behavioral alterations induced by exposure to a pretilachlor + pyribenzoxim herbicide mixture in *Clarias gariepinus* juveniles. Two hundred and fifty healthy *C. gariepinus* juveniles were acclimatized for two weeks. Batches of 10 fish per tank were randomly distributed into 18 experimental tanks. Following range-finding tests, a definitive acute toxicity test was conducted over 96 hours using various concentrations (0.40, 0.425, 0.45, 0.475, and 0.5 ml) of the herbicide. Throughout the experiment, water quality parameters, behavioral patterns, and mortality were meticulously monitored. Post-exposure, samples were collected for comprehensive histological analysis. Behavioral observations revealed significant stress indicators, including loss of reflex, air gulping, erratic swimming, barbell deformation, and molting. Histological examination of the gills showed infiltration of the mucous membrane of the secondary lamellae, which were also overlaid with detritus. In the kidneys, mild degeneration of the renal tubules accompanied by diffuse cellular infiltration was evident. Furthermore, the epidermis of the skin appeared detached from the underlying dermis. These pronounced histological and behavioral changes underscore the acute toxic effects of pretilachlor + pyribenzoxim on *C. gariepinus*. The study suggests that histological analysis is a highly useful method for assessing the acute impacts of herbicides on cultured fish. Consequently, the potential toxic effects of pretilachlor + pyribenzoxim must be carefully considered during its application near aquatic environments to mitigate ecological harm.

Keywords: *Clarias gariepinus*, Pretilachlor + pyribenzoxim herbicide, Histological alterations, Acute toxicity, Aquatic environment.

1. INTRODUCTION

The African catfish is a fresh water fish that is widely distributed in Africa. In addition to being significant in capture fisheries, it is a key specie in aquaculture. It is widely available and valued by farmers due to its quick development, large size tolerance to poor water quality parameters, omnivores feeding behavior, adaptability to crowding and high market value. It has also been successfully propagated artificially. Yet, its wellbeing and species' survival are in danger due to aquatic environments being polluted by toxicants (Agbebi and Owoeye 2012).

Herbicides enter aquatic ecosystems primarily through runoff from precipitation, application near surface water resources, or direct disposal of empty containers. This contamination alters water quality and impacts associated aquatic flora and fauna (Aghoghovwia *et al.*, 2019). Fish are frequently employed as bioindicators to evaluate herbicide effects, which include disruptions to reproduction, food conversion efficiency, growth, and increased mortality rates (Aghoghovwia *et al.*, 2019).

Histopathological studies provide crucial data by detecting cellular and subcellular changes in organs much earlier than the manifestation of external symptoms. A key advantage of histopathological biomarkers in environmental toxicology is their ability to reveal toxicant-induced damage in target organs like gills, kidneys, and liver, where structural alterations are often more discernible than functional impairments (Fanta *et al.*, 2003). These structural changes serve as early indicators of organismal health compromise. Histopathology is a sensitive tool for establishing causal links between contaminant exposure and biological responses, effectively identifying direct chemical effects within target organs in laboratory settings (Boran *et al.*, 2012). Therefore, this study aims to investigate the histological alterations in *C. gariepinus* juveniles exposed to Pretilachlor + Pyribenzoxim herbicide.

2. MATERIALS AND METHODS

Fish collection and acclimatization

Two hundred and fifty (250) healthy and active *C. gariepinus* juveniles were collected from Research Farm of Federal University of Technology Akure, Ondo State, Nigeria; and transported in a plastic container filled with pond water to the Central Laboratory of Fisheries and Aquaculture Department, Faculty of Agriculture, Adekunle Ajasin University Akungba-Akoko, Ondo State. Fish collected were acclimatized under laboratory conditions for two weeks (14 days) prior to the commencement of the experiment.

Range finding and definitive tests

A range-finding test experiment was carried out to determine the effective concentrations of pretilachlor + pyribenzoxim to be used. 96h definitive tests was carried out based on the results from the range finding test, following static bioassay procedures described by Parish (1985). Batches of 10 juvenile *C. gariepinus* were distributed into a set of 18 rectangular plastic tanks (75x45x45 cm) each filled with 30L of unchlorinated water. Six test solutions of pretilachlor + pyribenzoxim (0.00, 0.40, 0.425, 0.45, 0.475 and 0.5ml) determined from the range finding test was introduced in a single dose directly into the treatment tanks containing the fish in triplicate and covered with net and perforated lid. The test fish were not fed throughout the 96 hours test. The behavioral patterns and mortality of the test fish in each treatment tank was monitored and recorded every 15min for the first one hour, every hour for 4 hours, every 4 hours for 24hours and every 24hours for 96hours. Dead fish was removed immediately with scoop net to avoid contamination due to rotting.

Histopathological examination

Histopathological examinations were carried out to assess possible alterations in the skin, gills, and kidneys of test fish for each treatment. The organs and tissues were preserved in sampling bottles containing 10% formalin before examination. The organs and tissues were removed from the fish with the use of new and clean razor blade. The examinations were carried out at the

Department of Veterinary Pathology Laboratory, Faculty of Veterinary Medicine, University of Ibadan, Nigeria, following Lynch's medical laboratory procedures.

The organs were dehydrated in periodic acid Schiff's reagent (PAS) following the method of Hughes and Perry (1976) in graded levels of 50%, 70%, 90% and 100% alcohol for 3 days, to allow paraffin wax to penetrate the tissue during embedding. The organs were cleaned and embedded in melted wax and carefully sliced into thin sections with a rotatory microtome (5µm thick). The cut sections were again cleaned by placing them in warm water (38 °C) from where they were transferred into clean slides and oven-dried at 58 °C for 30 minutes to melt the wax and stained with Harris' haematoxylin-eosin (H and E) stain (Bancroft and Cook, 1994). The slides containing sectioned tissues were cleaned using xylene and graded levels of 50%, 70%, 90%, 95% and 100% alcohol for two minutes each. The sections were again stained in haematoxylin-eosin for ten minutes and mounted in dipterox on glass slides. To obtain their photomicrography, the stained sections were examined and photographed at different magnifications (x40, x100 and x400) by means of a binocular light microscope (Olympus Japan 312545) fitted with a digital camera (Olympus CH XSZ-107BBN), a photographic attachment (Olympus C35 AD4) and an automatic light exposure unit (Olympus PM CS5P). The photomicrograph organs were read and recorded accordingly.

3. RESULTS AND DISCUSSION

The most sensitive indicators of potentially fatal pesticide/herbicide effects in fish are behavioral changes (Banaee *et al.*, 2011; Rauf and Arain, 2013). The behavioral responses observed during *C. gariepinus* exposure to pretilachlor + pyribenzoxim herbicide in this study is similar to what was observed by Alkahemal-Balawi *et al.*, 2011 on the responses of *Cyprinus carpio* exposed to different concentrations of fenthion. Fish in the experimental groups exhibited abnormal behavior such as loss of reflex, air gulping, erratic swimming, barbell deformation, and molting. This abnormal behavior was caused by the inhibition of acetyl cholinesterase activity, which led to the accumulation of acetylcholine in cholinergic synapses and caused hyper stimulation of the toxicants. As a result, swimming ability is one of the metrics thought to be a potential sensitive indication of toxicant exposure. According to reports of Alkahemal-Balawi *et al.*, 2011, when fish are under stress, they become hyperactive, possibly to escape the unpleasant environment, hence, they require more oxygen to meet their energy needs.

Table 1: Behavioral response of *C. gariepinus* exposed to Pretilachlor + Pyribenzoxim herbicide

Behavior	24 hours exposure					48 hours of exposure					72 hours of exposure					96 hours of exposure				
	(ml)	(ml)	(ml)	(ml)	(ml)	(ml)	(ml)	(ml)	(ml)	(ml)	(ml)	(ml)	(ml)	(ml)	(ml)	(ml)	(ml)	(ml)	(ml)	(ml)
Loss of reflex	-	-	-	-	-	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+

Air gulping	-	-	-	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+
Erratic swimming	-	-	-	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+
Hyperactive	-	-	-	-	+	+	-	-	+	+	+	+	-	-	+	+	+	+	-	+	+	+	+	+
Motionless state	-	-	-	+	+	+	-	-	-	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+

Key: - represent absent, + represent present

Histological alterations have been used as significant indicators in order to examine specific target organs during environmental monitoring. The histological results observed in the tissues of *C. gariepinus* in this study indicate that pretilachlor + pyribenzoxim herbicide caused moderate to severe alteration in the gill and kidney which are important organs performing vital functions such as osmoregulation, respiration and acid base balance. The alterations observed in the gill of juvenile *C. gariepinus* in this research shows mucous membrane of the secondary lamellae appeared infiltrated and overlaid with detritus. The concentrations of pretilachlor + pyribenzoxim used as well as the different exposure periods showed different degrees of histological changes. Histological results indicated that gill was the primary target tissue affected by pretilachlor + pyribenzoxim. Since the gills are the main point of entry for pesticides, they are often regarded as an excellent indicator of water quality. Gills are essential for fish's respiratory, osmoregulation, and excretory processes (Adewumi *et al.*, 2018). The mucous membrane of the secondary lamellae overlaid with detritus can be attributed to increased capillary permeability (Olurin *et al.*, 2006). Damages observed in the gills indicated that acute toxicity of pretilachlor + pyribenzoxim caused impairment in gaseous exchange efficiency of the gills and this is similar to the findings reported by Boran *et al.*, 2012; and Patnaik *et al.*, 2011.

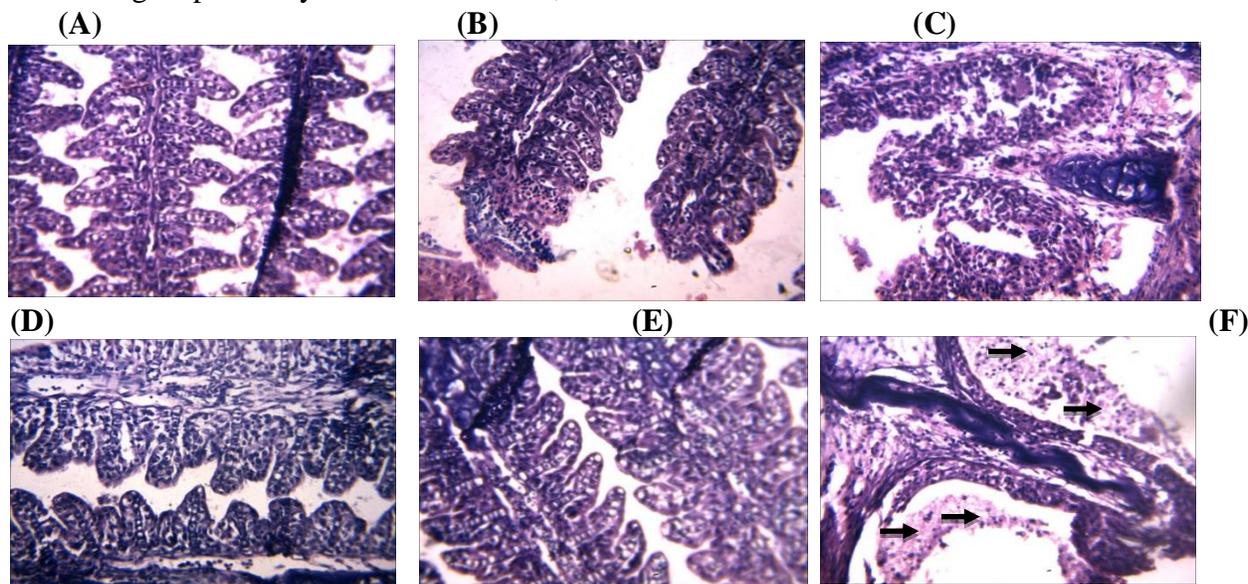


Fig 1: Gill of *C. gariepinus* juvenile exposed to different concentrations of pretilachlor + pyribenzoxim (HE x400) (A) Control fish showing normal gill. (B) Fish exposed to 0.40 ml

showing no visible lesions. (C) Fish exposed to 0.425 ml showing no visible lesions (D) Fish exposed to 0.45 ml showing no visible lesion. (E) Fish exposed to 0.475 ml showing no visible lesion. (F) Fish exposed to 0.50 ml showing mucous membrane of the secondary lamellae infiltrated and overlaid with detritus.

An essential part of the kidney's job is to keep the body's homeostasis in check. In addition to removing waste from blood, it is also in charge of selective reabsorption, which aids in preserving the volume, pH, and erythropoiesis of blood and bodily fluids (Iqbal *et al.*, 2004). The alterations observed in the kidney of *C. gariepinus* exposed to acute concentrations of pretilachlor + pyribenzoxim in this study includes mild degeneration of the renal tubules and diffuse cellular infiltration which are in accordance with the findings of Oulmi *et al.*, 2005 and Ayoola 2008. The acquired data showed that changes in water quality were most detrimental to the kidney. This might be as a result of the fact that the kidney is one of the main detoxifying sites in fish bodies.

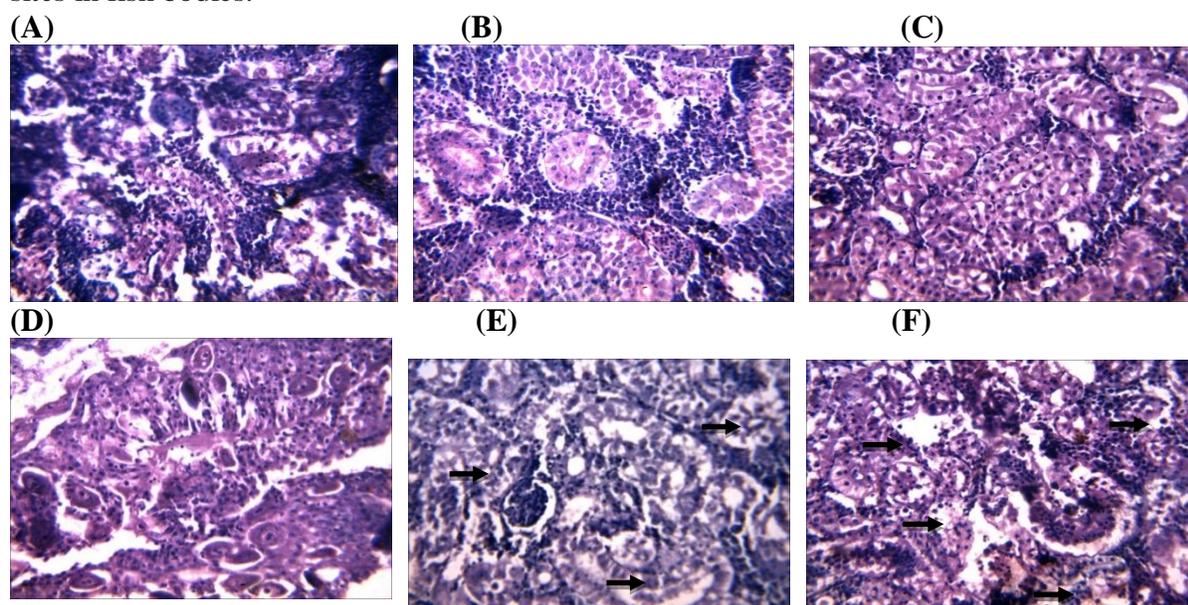


Fig.2: Kidney of *C. gariepinus* juvenile exposed to different concentrations of pretilachlor + pyribenzoxim (HE x400). (a) Control fish showing normal kidney with no lesion. (b) Fish exposed to 0.40 ml showing no observable lesion. (c) Fish exposed to 0.425 ml of showing no observable lesion (d) Fish exposed to 0.45 ml showing no observable lesion (e) Fish exposed to 0.475 ml showing no observable lesion. (f) Fish exposed to 0.50 ml showing mild degeneration of the renal tubules and diffused cellular infiltration

Skin and gills are highly sensitive to pollutants due to their direct contact to aquatic environment. It has been reported that the skin is sensitive to steroid hormone activity (Pottinger and Pickering, 1985). Severe alteration was observed in the treatment exposed to highest concentration of pretilachlor + pyribenzoxim showing that the epidermis appeared detached from the underlying dermis. This can be pointed to a physiological response such as, detached pavement cells, vacuolation of the cytoplasm and severely deformed cell nuclei stated by Sayed *et al.*, 2012. Generally, the lesions detected in cells, tissues or organs represent an integration of cumulative effects of physiological and biochemical stressors and therefore, can be linked to the exposure and subsequent metabolism of chemical contaminants (Adeyemo, 2008).

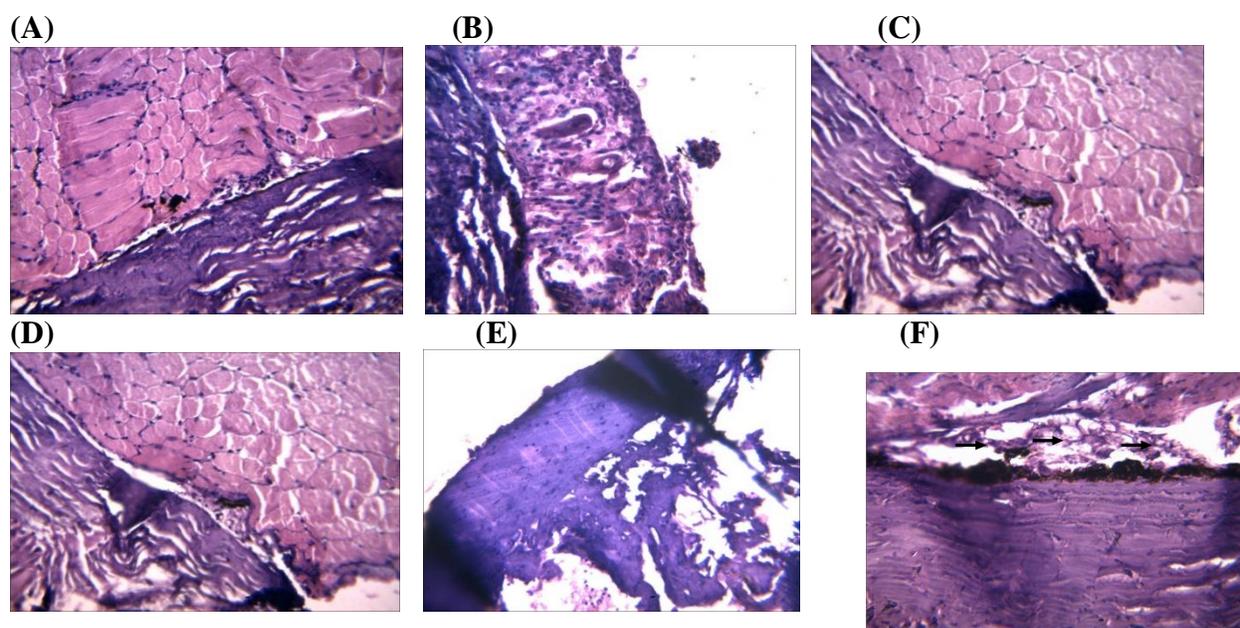


Fig. 3: Skin of *C. gariepinus* juvenile exposed to different concentrations of pretilachlor + pyribenzoxim (HE x400). (a) Control fish showing normal skin. (b) Skin exposed to 0.40 ml of showing no observable lesion. (c) Skin exposed to 0.425 ml of showing no observable lesion. (d) Skin exposed to 0.45 ml of showing no observable lesion. (e) Skin exposed to 0.475 ml of showing no observable lesion. (f) Fish exposed to 0.50 ml of showing epidermis detached from the underlying dermis.

4. CONCLUSION

The histological and behavioral changes that were observed in *C. gariepinus* juveniles exposed to acute concentrations of pretilachlor + pyribenzoxim herbicide caused moderate and severe damage to the organs. These adverse effects on the skin, gills and kidney were simultaneously correlated with severe biochemical, physiological changes. This suggest that histological analysis may be a useful method for observing the effects of herbicide on cultured fish. Therefore, the toxic effects of pretilachlor + pyribenzoxim should be taken into concern during its use near the aquatic environment.

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