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Gill Damage in *Clarias Gariepinus* Exposed to Cypermethrin

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Abstract

Cypermethrin is a synthetic pyrethroid insecticide and is used against a wide range of pests in agriculture, public health and animal husbandry. Following the exposure, cypermethrin was administered at concentration of 0.01mg/L, 0.05 mg/L and 0.1 mg/L (A-C) and the control (0.0 mg/L) in a renewal bioassay system for a period of 21 days and standard histological techniques was used for the study. Gill histological section of the 0.01mg/L treated fish showed pathological changes; proliferation of epithelial cell at the secondary lamellae, severe destruction and degeneration of the gill filament, with prominent acidophil cell. Rapid cell lysis throughout the epithelium with chronic degenerated secondary lamella (SL) and disintegrating of epithelial lining, regeneration of mucous cell in the deeper layers of epithelial lining, hyperplasia was evident in 0.05mg/L treated groups. Uncontrolled regeneration of epithelial cells (ECs) hyperplasia,and proliferation of epithelial cell at the base secondary lamella was prominent in 0.1mg/L group. The degree of distortion of the gills was proportional to the exposure periods and concentration of the cypermethrin was found to be dose and time dependent which possibly led to asphyxiation and stress to fish

Key word: *Clarias gariepinus*, gill damage, cypermethrin,

Introduction

Cypermethrin is a synthetic pyrethroid insecticide first synthesized in 1974 and first marketed in 1977 (Gordon 2004). It is used against a wide range of pests in agriculture, public health and animal husbandry. In agriculture, cypermethrin control many pests like moth pests of cotton, fruit and vegetable crops. Cypermethrin is also used for crack, crevice and spot treatment to control insect pests in store, warehouse, industrial buildings, on ships, railcar, buses, trucks and aircraft. In animal husbandry it can serve as insect repellent for horses.

Cypermethrin is very toxic to fishes and aquatic invertebrates. The LC_{50} (96hour) for cypermethrin in rainbow trout is 0.0082 mg/L and in bluegill sunfish is 0.0018 mg/L when leached into the aquatic environment as a result of runoff etc. It is metabolized and eliminated significantly more slowly by fishes than by birds or mammals, which may explain this compound as been highly toxic in fishes than other organisms (Bradburg & Coats, 1989b). Saxena & Seth (2002) suggested that cypermethrin is readily absorbed by the gills of the fish even from very low concentration in water. Shires (1983), pointed out that fish mortality rate may occur because of the use of cypermethrin in normal agricultural practice. Haya (1988), reported that exposure of fish to sublethal concentration of cypermethrin resulted in decreased growth and impaired swimming performance especially on the juvenile. He also asserted that acute exposure of fishes to cypermethrin cause nervous system, respiratory surface and renal ion irregularity. The gills are not only for gaseous exchange in fish, they also perform several other physiological functions including osmoregulation, respiration and excretion of nitrogenous wastes. The fish gill is the primary target of toxicant dissolved in water. Gill damage is actually the direct cause of death in major situations of toxicity to fish. The gill serves as a major route for uptake of xenobiotics from water. Changes in the environmental parameters often damage this vital organ because of its delicate structure. Mallat (1985) reviewed gill structural changes in fish exposed to chemicals and concluded that most lesion were non-specific and represented a condition that would eventually be lethal. Chemically-induced gill damage is divided into; indicator of direct toxic effects, such as necrosis, epithelial desquamation and separation of the branchial epithelium from the basement membrane and indicators of defence responses to toxic effects such as mucous hypersecretion, lamella fusion and hyperplasia of the gill epithelium. Review article (Dutta 1997) have clearly demonstrated that increased concentration of several heavy metal seriously damage the gills of teleostean fish. Hemalatha & Baerjee(1997) reported histopathological changes due to the toxic impact of zinc($ZnCl_2$) on the gills and accessory respiratory organs of *Heteropneustes fossilis.* Therefore, there are reasons to focus on gills when trying to understand the impact of pollutant in fish.

Clarias gariepinus respires in and out of the water. It is very hardy since it tolerate both well and poorly oxygenated waters. It is widely cultivated and found in water bodies in Nigeria hence used as biological indicators in ecotoxicological studies (Wekker 2000).

From the foregoing, the effects of sublethal concentration of cypermethrin on gill histology and impaired respiration and stress in *Clarias gariepinus* was studied.

Materials and Methods

One hundred and twenty fishes of mean weight 19.14 $\pm$ 2.27g were obtained from Aquafish Limited, Awka, Anambra State, and transported to our laboratory where the fish was acclimatized for three weeks before the commencement of the study. During the period of acclimation and the experiment, the fish were fed *ad libitum* on 55% crude protein diet.

The fish were randomly divided into five treatments groups (A - D) of thirty fish. Each group was further randomized into three replicate experiments containing twelve fish each. The fish in group A, B and C were exposed to 0.01 mg/L, 0.05 mg/L, and 0.1 mg/L respectively. The fourth group (Control) was exposed to tap water as the control. Commercial sample of cypermethrin contains 100g/L and 1ml of commercial sample containing 0.1g of cypermethrin was dissolved in 999.9ml of water and serial dilutions were made.

The temperature, pH and the dissolved Oxygen of the tap water used in the study were 27.5^{o}C, 7.2 mg/L and 6.4 mg/L respectively, while the total hardness was 100 mg/L $CaCO_3$. The tap water contained (mg/L) Ca^{2+}, 4.01;Mg^{2+}, 9.73; Na^{+}, 4.9; Cl, 7.5; SO_4^{2-}, 15.6; NO^{-}_3, 0.96 and total phosphorus 0.04.

After each of the exposure periods of 7, 14 and 21 days, fish from the respective experimental groups as well as control aquaria were sacrificed and the gills collected and fixed in 10% formal saline, processed routinely, embedded in paraffin, sectioned at 4-6 µm thickness, stained with heamatoxylin and eosin (H&E), and examined using light microscopy (NIKON TE 3000).Photomicrograph was taken at x10, x20, or x40 magnification with a digital camera (Nikon, 9000)

Results and Discussion

Gills are the major respiratory organs and all metabolic pathways depend upon the efficiency of the gills for their energy supply and damage to these vital organs causes a chain of destructive events which ultimately lead to respiratory distress (Magare, & Partil 2000).

The histological structure of the normal gill (control) of *Clarias gariepinus* (Fig. 1) characterized by the presence of primary lamellae (PL). The PL is rounded at the apices along with projecting secondary lamellae shaft which are clearly inter spaced and gill rakers confirming the proper architecture of the tissue. In the treated aquaria there was a drastic reduction in the activity of the fishes. The swimming became slower and there was reduction in their rate of feeding habits which may be as a result of collapse of the gill blood vessels which affected the survival of the cat fish. In fish exposed to 0.01 mg/L of Cypermethrin after 7 days, the gill architecture (Fig 2) showed prominent lamellae with acidophil cells evident and degeneration of gill lamellae with loss of original shape. The present study has shown that Cypermethrin affected the gill structure which leads to severe lamella destruction, and intensification of hyperplasia of the primary and secondary lamellae (Fig 6 and 8). This indicates that due to exposure of the fish to toxic cypermethrin, the protective role of the thin layer of slime collapsed and failed to prevent the penetration of cypermethrin, subjecting the cellular constituents lining the extensive surface area of the gills to the toxicity of the cypermethrin. This had led to various degrees of wear and tear, which caused damage and disease state to the delicate protective device of the gill epithelia of *Clarias gariepinus.* The gill sections of the fish exposed to 0.05 mg/L cypermethrin for 14 days showed regeneration of mucous cells in the deeper layers of the epithelial lining and disjointed lamella with disintegrating of epithelial lining (Figs 5 and 6), respectively. Secretion of mucus over the gill curtails the diffusion of oxygen (David *et al.* 2002), which may ultimately reduce the oxygen uptake by the fish. Kalavathy *et al.* (2001) reported that the dimethoate was efficiently absorbed across the gill and diffuse into the blood stream leading to toxicity in *Sarotherodon mossambicus.*

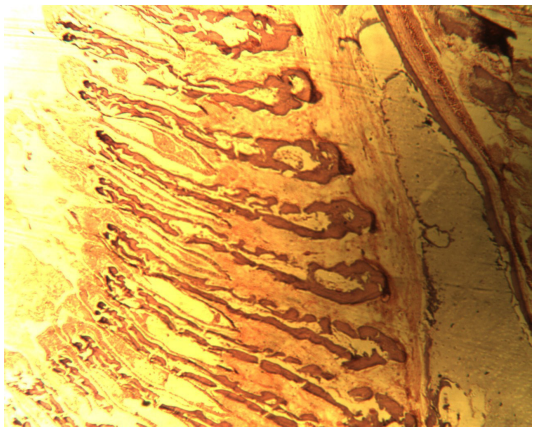

Fig 1. Gill section (magnification (Mag.) (H&E) x40) of control African catfish (*Clarias gariepinus*) showing no changes.

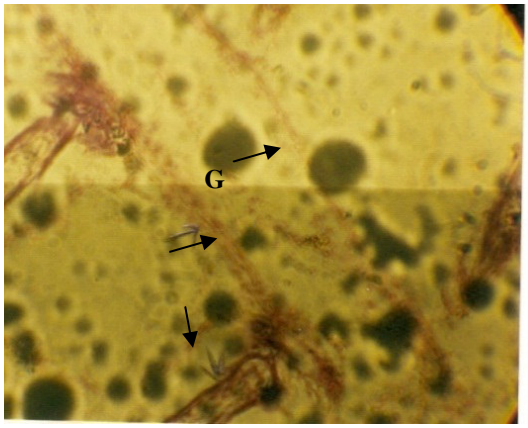

Fig 2. Gill section (Mag. (H&E) x40) of *Clarias gariepinus* exposed to 0.01 mg/L of cypermethrin for 14 days. Prominent lamellar(PL) showing the acidophil cells. G. granulocytes

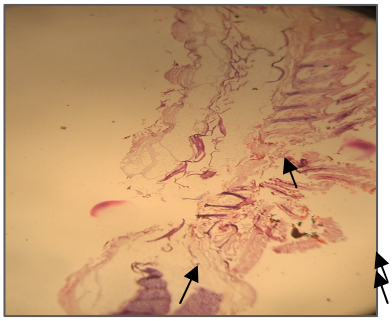

Fig 3: Gill section (Mag (H&E)x40) of Clarias gariepinus exposed to 0.01mg/L of cypermethrin for 21days showing severe deformation and degeneration

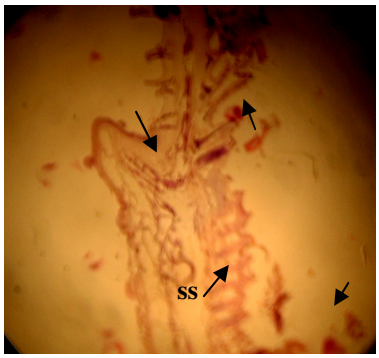

Fig 4. Gill section (Mag. (H&E) x40) of *Clarias gariepinus* exposed to 0.05mg/L cypermethrin for 7 days showing degeneration of the gill filament, epithelial hyperplasia resulted in complete fusion of secondary lamellae and large subepithelial space

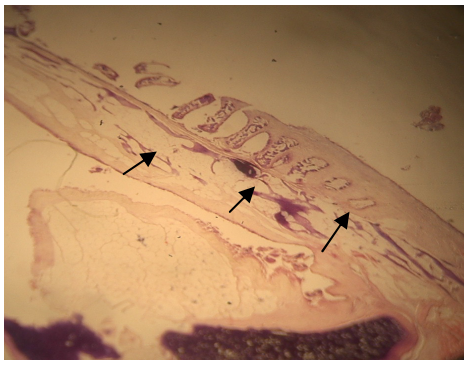

Fig 5. Gill section (Mag. (H&E) x40) of *Clarias gariepinus* exposed to 0.05mg/L showing regeneration of mucous cells (Mcs) in the deeper layers of epithelial lining of primary lamellae (PL) and hyperplasia after 14 days exposure of cypermethrin (0.05mg/L).

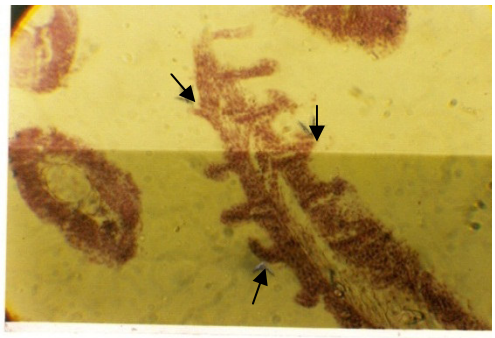

Fig 6. Gill section (Mag. (H&E) x40) of *Clarias gariepinus* exposed to 0.05mg/L cypermethrin for 15 days showing marked decrease in size following rapid cell lysis throughout the epithelium with chronic degenerated secondary lamellae (SL).

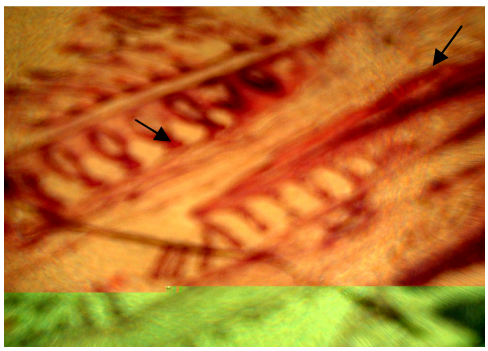

Fig. 7. Gill section (Mag. (H&E) x40) of *Clarias gariepinus* exposed to 0.1mg/L cypermethrin for 7 days. Gill structure with epithelial cell proliferation at the base of secondary lamellae (SL),thin layer of slime covering the surface of the SL

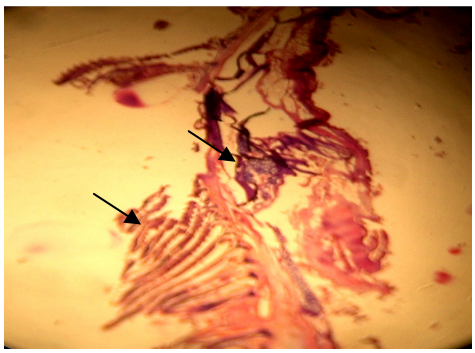

Fig. 8. Gill section (Mag. (H&E) x40) of *Clarias gariepinus* exposed to 0.1mg/L cypermethrin for 14 days showing gill epithelial hyperplasia

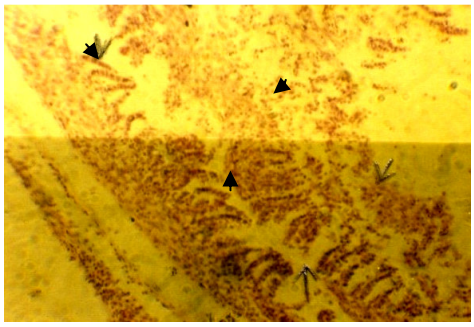

Fig 9. Extensive lamella fusion and intensification of hyperplasia of the primary lamella (PL) and secondary lamella(SL) after 21days exposure of cypermethrin (0.1mg/L). (Mag. (H&E) x40)

Fish gills exposed to 0.01 mg/L cypermethrin for 14 days showed prominent lamellar showing acidophil cells and severe deformation and degeneration of the gill (Fig 4). Destruction of lamellae was also observed in the fish exposed to 0.01 mg/L cypermethrin fish for 21 days. Extensive damage in the lamellar configuration and reduction in the number of the lamella (Fig 9) was indicative of impaired respiratory function of the fish due to reduced gill surface area. Uncontrolled regeneration of epithelial cells (ECs) causes hyperplasia of PL, resulting in their increased thickness respiratory distress as a consequence of the impairment of oxidative metabolism. Disturbance in oxidative metabolism was reported earlier under cypermethrin toxicity in *Tilapia mossambicus* (David *et al.* 2003). Respiratory distress caused by the degeneration of gills is a sign that the total surface area of the gill has been drastically reduced. The changes in the gills were adaptation by the fish to cope with challenge of the toxicant. For example fusion of the secondary lamellae was an attempt by the fish to reduce available surface area of cypermethrin. But this may result in the reduction of available surface for respiration and ionic exchange, consequently resulting in an internal hypoxic and toxic environment. According to Evans *et al*. (2005) the gill of fish is a multipurpose organ that in addition to providing for aquatic gaseous exchange plays a dominant role in osmotic and ionic regulation, acid-base regulation and excretion of nitrogenous waste. Hence, impairment of the gill functions by the overall effect of the pathological changes in the gill of exposed fish will have grave consequences for the fish with respect to the normal function of the gill.

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