TOXIC EFFECTS OF SUBLETHAL CONCENTRATIONS OF DIETHYL PHTHALATE ON THE GILLS OF AFRICAN CATFISH (*Clarias gariepinus*) JUVENILES

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ABSTRACT

An investigation on the effect of Diethyl phthalate (DEP) on the gill of the African catfish Clarias gariepinus was carried out in the laboratory. Seventy-five (75) catfish fingerlings were subjected to continuous exposure to sublethal concentrations of DEP (30, 40, 60 and 80 µg/L) for a period of four weeks. The gills of the catfish were removed every 15 days for histological examination. The degree of distortion of the gills (heamorrahging of the gill, continuous degeneration of the gill filaments, disruption of epithelium owing to rapid cell lysis, severe destruction of the lamellae) was proportional to the exposure periods (0, 15 and 30 days) and concentration of the DEP was found to be dose and time dependent which led to asphyxiation and stress in the catfish.

Keywords: *Clarias gariepinus*, Gills, Toxicopathology, Diethyl phthalate

INTRODUCTION

About 80,000 chemicals have been introduced into the environment within the last 50 years (Curtis and Skaar, 2002). There is mounting evidence that some of these chemicals may pose extensive, even global threats to wildlife and humans (Vos *et al*., 2000; Fox, 2001; Curtis and Skaar, 2002). Diethyl phthalate (DEP) is an industrial chemical used in products such as insecticides, mosquito repellants, camphor substitute, plasticizers for cellulose, bathing soaps, cosmetics, pharmaceutical coatings, after shave-lotion, detergent, esterplastic film and sheet etc (Kamrin and Mayor, 1991; Huang *et al*., 2008). DEP in aquatic environment originates from a variety of compounds of anthropogenic origin such as pesticides, detergent and plasticizers (Fatoki *et al*., 2010). The continual use of these compounds (DEP) has prompted some concern on the effects of these chemicals on the early life stages of fish (ATSDR, 1999). The indiscriminate disposal of DEP made products and careless handling have harmful effects on the fish population and other forms of aquatic life when leached into the water bodies and may contribute long term toxic effects in the environment.

The gills are not only for gaseous exchange in fish, they also perform several other physiological functions including osmoregulation and excretion. 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. The review article by Dutta (1997) have clearly demonstrated that increased concentration of several heavy metals seriously damage the gills of teleostean fish. Hemalatha and 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 and 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 sub-lethal concentration of DEP on gill histology, impaired respiration and stress in *Clarias gariepinus* were studied.

MATERIALS AND METHODS

Seventy-five fingerlings of *Clarias gariepinus* (13.13 ± 2.27g body weight and 7.0 ± 0.1 cm standard length) were used for the experiment. They were purchased from Aquafish Limited, Awka, Anambra State, Nigeria and transported to Fisheries Laboratory, University of Nigeria, Nsukka where the fish were 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 (Oluah and Didigwu, 2008).

The fish were randomly divided into five treatments group (A – E) in 25 litre glass aquarium filled to 20 litre mark with aerated deep well water. Each group was further replicated thrice, with each replicate having 5 fishes. The stock solution of DEP was made by dissolving 1 gram of DEP (Ohio, USA) in 1000ml of water and serial dilution made. The fish in groups A and B were exposed to 30 µg/L and 40 µg/L of DEP respectively. Similarly, the fish in groups C and D were exposed to 60 µg/L and 80 µg/L of DEP respectively, and group E (control) fishes were kept in separate aquaria containing aerated water. After each of the exposure periods of 0, 15 and 30 days, catfish 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 6 µm thickness, stained with heamatoxylin and eosin (H&E), and examined using light microscopy (Nikon TE 3000). Photomicrographs were taken at x40 magnification with a digital camera (Nikon, 9000).

RESULTS AND DISCUSSION

Gill histology: The histological structure of the normal gill (control) of *Clarias gariepinus* (Figure 1) was characterized by the presence of primary lamellae (PL). The PL was rounded at the apices along with projecting secondary lamellae shaft which are clearly inter spaced wi9ith the gill rakers, thus confirming the proper architecture of the normal gill. 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 feeding rate which may be as a result of collapse of the gill blood vessels which affected the survival of the catfish. In fish exposed to 30 µg/L of DEP after 15 days, the gill architecture (Figure 2) showed prominent lamellae with acidophil cells evident and degeneration of gill lamellae with loss of original shape. At day 30, haemorrhaging of the gill filament (Figure 3) was evident. The present study have shown that DEP affected the gill structures which lead to severe lamella destruction, haemorrhaging of the gill filament and intensification of hyperplasia of the primary and secondary lamellae. This indicates that due to exposure of the fish to toxic DEP, the protective role of the thin layer of slime collapsed and failed to prevent the penetration of DEP, subjecting the cellular constituents lining the extensive surface area of the gills to the toxicity of the DEP. This led to various degrees of wear and tear, which caused damage to the delicate protective device of the gill epithelia of *Clarias gariepinus.* The gill sections of the fish exposed to 40 µg/LDEP for 15 days showed deposits of fatty cells that were prominent compared to fish exposed to 40 µg/L DEP for 30 day showed enlargement of filament with a thick coat of mucus covering the entire gill filament (Figures 4 and 5), 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)

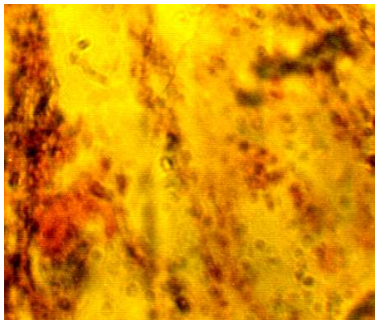

Figure 1: Gill section of *Clarias gariepinus* (control) showing no damage to gill structural architecture. (H&E, Mag. x40)

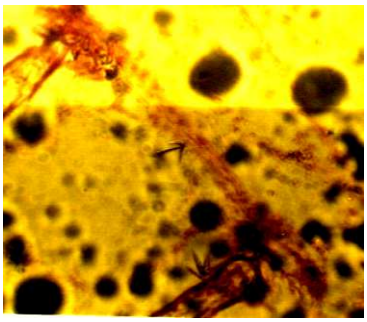

Figure 2: Gill section of *Clarias gariepinus* exposed to 30 µg/L of diethyl phthalate for 15 days. Prominent lamellar showing acidophil cells. (H&E, Mag. x40)

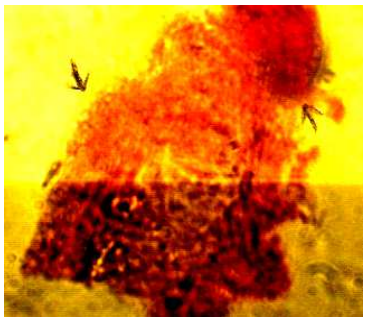

Figure 3: Gill section of *Clarias gariepinus* exposed to 30 µg/L diethyl phthalate for 30 days. Haemorrhaging of the gill filament. (H&E, Mag. x40)

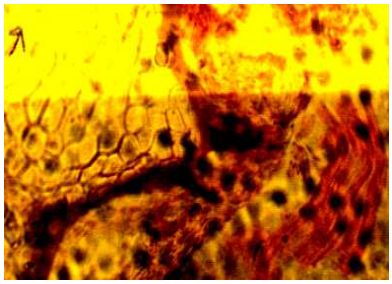

Figure 4: Gill section of *Clarias gariepinus* exposed to 40µg/L diethyl phthalate for 15 days. Fatty cells are prominent. (H&E, Mag. x40)

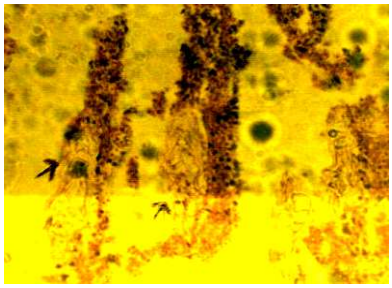

Figure 5: Gill section of *Clarias gariepinus* exposed to 40µg/L diethyl phthalate for 30 days. Arrows point to enlarged filament. (H&E, Mag. x40)

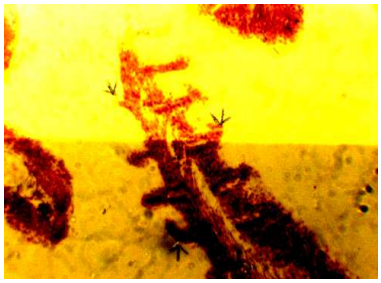

Figure 6: Gill section of *Clarias gariepinus* exposed to 60µg/L diethyl phthalate for 15 days. Un-enlarged filaments are narrow with disjointed lamella and disintegrating epithelial lining. (H&E, Mag. x40)

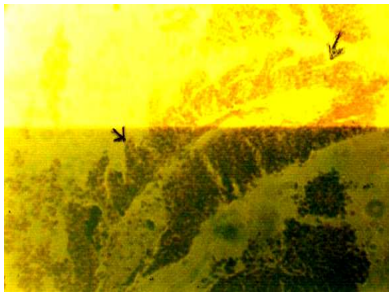

Figure 7: Gill section of *Clarias gariepinus* exposed to 60 µg/L diethyl phthalate for 30 days. Gill tissue with marked decrease in size following rapid cell lysis throughout the epithelium (H&E, Mag. x40)

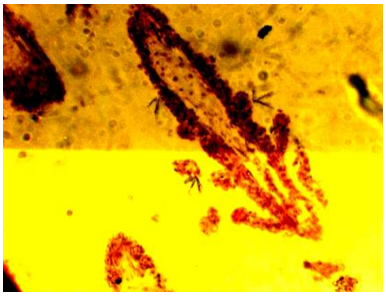

Figure 8: Gill section of *Clarias gariepinus* exposed to 80 µg/L diethyl phthalate for 15 days. Severe destruction of the lamella is shown with arrows. (H&E, Mag. x40)

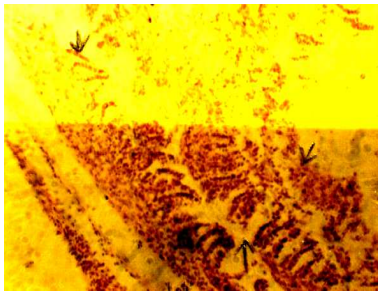

Figure 9: Gill section of *Clarias gariepinus* exposed to 80µg/L diethyl phthalate for 30 days. Extensive lamellar fusion and intensification of hyperplasia of the primary and secondary lamella (H&E, Mag. x40)

reported that the dimethoate was efficiently absorbed across the gill and diffuse into the blood stream leading to toxicity in *Sarotherodon mossambicus.* Fish gills exposed to 60 µg/L DEP for 15 days showed destruction of the lamellae which were disjointed (Figure 6). Destruction of lamellae was also observed in the fish exposed to 60 µg/L DEP fish for 30 days. Extensive damage in the lamellar configuration and reduction in the number of the lamella (Figure 7) was indicative of impaired respiratory function of the fish due to reduced gill surface area. Extensive lamellae fusion and intensification of hyperplasia of primary and secondary lamella was evident in 80 µg/L DEP-treated fish on day 30 (Figures 8 and 9). Mallat (1985) also observed alteration in gill histology which partially represents the damage and the compensatory response of fish. Karlson *et al*. (1986) and Hemalatha and Baerjee (1997) also observed gill epithelial damage and detachment of the respiratory epithelium from the basement membrane due to exposure of *Salmon gairdneri* and *Heteropneustes fossilis* to cadmium and zinc chloride respectively.

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