EFFECTS OF LEAD ON THE HISTOPATHOLOGY OF GASTROINTESTINAL TRACT OF *Clariasgariepinus*JUVENILES

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Abstract: The acute toxicity test of lead to *Clariasgariepinus* juveniles and the rate of bioaccumulation in the intestine of *Clariasgariepinus* juveniles was investigated by Standard Methods by APHA (2000). At the end of the 96 hours the gastrointestinal tract were rinsed in 10% formalin and preserved for further analysis. In this study, the LC_{50} values of *Clariasgariepinus* at 96 h period was determined to be 50.118 ppm or 50.12 mg/ L. Results obtained from this study showed that lead ($Pb(NO_3)_2$) has extensive histological changes in the gastrointestinal tract exposed to the various lead concentrations of 100 mgL^{-1}, 75 mgL^{-1} and 50 mgL^{-1}. The lead concentration disturbed the homeostasis and led to physiological disorders in their functions and subsequently to the death of the juveniles *Clariasgariepinus*. The effects of toxicant on the gastrointestinal tract of fish on this study ranged from slight changes in motility, secretion and absorptive functions to more severe effects associated with mucosal integrity, blood flow and impacted neuromuscular control.

Keywords: *Clariasgariepinus*, degenerative inflammation, gastrointestinal tract, hemorrhage, necrosis, toxicant

Introduction

The contamination of freshwater with a wide range of pollutants has become a matter of concern over the last few decades (Otitoloju and Pedro, 2002, Babatunde, 2008 &Babatunde, 2014). Metal contamination in the environment is an ongoing problem, particularly in aquatic environments, and there has been extensive investigation of metal effects on aquatic organisms (Niyogi and Wood, 2004). A great variety of pollutants affect the majority of water course which receive domestic, industrial and agricultural effluents (Reglero*et al.*, 2009; Abdallah*et al.*, 2010; Mirhashemi*et al.*, 2010).

Lead is a naturally occurring element in the Carbon group with symbol Pb (from Latin: plumbum) and atomic number 82 (Nevin, 2000). It is dense, bluish-gray metallic element that was one of the first known metals. It has a low melting point of 327.5°C and a high density of 11.34 g/cm^3(Sanderson and Bellrose, 1986). Lead is found in nature as a component of various minerals (McCulley*et al*., 1991). Some, such as galena (PbS), cerussite ($PbCO_3$), and anglesite ($PbSO_4$), are economically important sources of lead (Chandravathy and Reddy, 1996). A normal constituent of the earth's crust, trace amounts occur naturally in soil, plants and water, and if it is left undisturbed, lead is nearly immobile (Ordija, 1993). Lead (Pb) is a soft, malleable and heavy post-transition metal with bluish-white color after being freshly cut, but it soon tarnishes to dull grayish color when exposed to air (DeFrancisco*et al*., 2003).

The effects of toxicant on the fish may range from slight changes in mortality, secretion and absorptive functions to more severe effects associated with mucosal integrity, blood flow or neuromuscular control. These effects could ultimately influence the ability of organisms to thrive (Schlenk Benson, 2005). Some studies indicated that high levels of some metals in diet may cause increased apoptosis of intestinal cells (Berntssen*et al.*, 1999).High concentrations of heavy metals have been discovered and bioaccumulation also has been reported over the years (Nevin, 2000). This research was to carry out the toxicity of such metals to common fish food in Nigeria and research the effects on our protein source.

Materials and Methods

The method employed in this experiment is based on recommended method for the test of acute toxicity of pollutant to fish described by Standard Method of Examination of Water and Waste Water (APHA, 2000).

Selection of fish

It has been suggested by Fish Toxicity Testing Framework (2012) that economically local fish should be used in toxicity assay. *Clariasgariepinus*(Burchell, 1822)also called African catfish belongs to the Phylum; Chordata, Class; *Osteichthyes*, Order; *Siluriformes*, Family; *Clariidae* (air breathing fishes). They are black on the dorsal surface with dark green or olive color and white on the ventral surface. The head is dorso-ventrally flattened, with skin usually smooth in the young and coarsely granulated in adult.

African catfish is of high commercial value and are very common in Nigerian fresh waters. They are of high nutritive value and data of their nutritive value have been well documented by Otitoloju (2001). People shows much interest in fish farming and farmers are being encouraged to set up both small and large scale fish farms the most common fish which can be kept in captivity and grow to table size within a short period of time is the African catfish. On the basis of availability, commercial and ecological important *Clariasgariepinus* was chosen for this study.

Site determination

This Experiment was carried out in the zoology garden of the Department of Biological Sciences, Faculty of Science, Kaduna State University, (Coordinates: 10º31N; 7º26E) and 6.14m above sea level, Nigeria, 2015.

Collection of fish

Two hundred live active juveniles (8 weeks old) of *Clariasgariepinus* were purchased from united Patry fish farm in Barakalahu, Kaduna East, Kaduna Nigeria and transported in plastic bucket containing half filled with pond water to local fish pond (length: 84.00 cm, height: 115.00 cm, bottom diameter: 95.00 cm and top diameter: 102.00 cm) in the Zoological garden of the Department of Biological Sciences Kaduna State University, Kaduna. The fish were kept in the transparent (plastic) aquaria to observe any visible pathological symptoms. Before introducing in the pond fish were treated with 0.1% of $KMnO_4$ solution to serve any dermal infection.

Acclimatization

The juvenile fish were kept in the fish pond, containing dechlorinated water for a period of fourteen days to acclimatize to the environment before they were used in the bioassays.

Feeding

The juveniles were fed with fish feed (Coppens 2 mm) in broadcasting method at 3% of body weight twice daily, and

the water was changed once every 48h to enhance oxygen content in the water. Feeding was stopped one day prior to the bioassay to avoid contamination of the toxicity of the water due to their excretory product.

$$\frac{weight\ of\ lead\ required\ x\ molecular\ weight\ of\ lead}{atomic\ weight\ of\ lead}$$

$$\frac{2\ x\ 331.21\ g}{207.2} = 3.179\ g$$

Preliminary screening (pilot test)

Thirty fish of 8.70-9.60cm size and weight 5.15-6.02g, ten to each aquarium were exposed to higher lead and lower concentration of 5g L^{-1}, 3g L^{-1} and 2g L^{-1} respectively to determine the appropriate concentration range for testing chemical before the start of actual experiment.

General bioassay techniques

Bioassay containers

Eight rectangular plastic bowls (volume: 30.00 liters, height: 25cm, bottom diameter: 24.00cm and top diameter: 34.00cm) were used as bioassay containers.

Experimental design

Two hundred active juveniles of African catfish of 5.15-6.02g weight and size 8.70-9.60cm were acclimatized for two weeks in fish pond of 50.00cm by 68.00cm size using underground water (borehole).

Lead test and preparation

Lead as $Pb(NO_3)_2$ with molecular weight 331.21 g, and purity of 99.5%. The metal was of analytical grade and manufactured by BDH laboratory supplies Poole, a division of BH151TD England. Stock solutions of lead metal was prepared by taking computed amount (2g) and made up to the desired volume (1 liter) using distilled water to achieve a stock of 2g L^{-1}. The stock solutions were serially diluted to obtain solutions with desired concentrations selected after range finding experiments. The different concentrations required were calculated as follows:

Selection of animal for bioassay

Hundred and ten active juveniles of *Clariasgariepinus*of similar age and size (age: 8 weeks old, mean snout to tail length: 8.70-9.60cm, mean weight: 5.15-6.02g) were taken from ponds and randomly assigned to experimental containers.

Randomization

The juveniles were properly distributed into the eight aquariums at random as described by Bano (1999) and Babatunde (2008).

Bioassays

Acute toxicity test of lead

10 juveniles of *Clariasgariepinus*of similar age and size were taken from pond, using a scoop net and randomly assigned to bioassay containers already with test media or untreated control. Each treatment was replicated twice, giving a total of 80 juveniles exposed per treatment. The fish sample and replicate were exposed to three lead concentrations; Pb^{3+} at; 100mg L^{-1}, 75mg L^{-1}, 50mg L^{-1} with an untreated control (0.00).

Quantalresponse

After the introduction of lead, the fish were observed at pre-determined intervals; (0,10,20, 30,40,50,60 min; 2,4,8,16,24,32,36,48, 72 and 96 h) validity period. Juveniles were taken to be dead if no body movements including the operculum were observed, even when prodded with a blunt glass rod. Mortality was assessed once every 24h for a period of 4 days as recommended by Babatunde*et al.*(2014).

Tissue preparation

At the end of the 96 h Acute toxicity test, live *Clariasgariepinus*per replicate was randomly selected, dissected keeping the structure intact, the muscles and the gastrointestinal tract were rinsed in normal saline, fixed in 10% formalin and preserved for about 24 h at 4°C, till further analysis.

Elemental analysis

The test organs were carried in a sterile container to the laboratory for Chemical and elemental analysis at the National Research Institute for Chemical Technology (NARICT) at the end of the 96 h of the experiment.

Results and Discussion

Histopathology

Histology has been used as a test for evaluating toxic effects of water pollutants in fish (EIFAC 1983; Murty, 1986 and Tayel*et al*., 2007). Results from histological studies are useful in establishing water quality criteria (FAO 1980; Mahmoud and El-Naggar, 2007).

The Gastrointestinal tract

The gastrointestinal tract of *Clariasgariepinus* exposed to various concentrations of lead in this current experiment showed severe degenerative and necrotic changes in the intestinal mucosa and submucosa, atrophy in the muscularis and submucosa and aggregations of inflammatory cells in the mucosa and submucosa with edema between them (Plate II & III). The control sample and its replicate show normal structure of the villi, columnar epithelium, muscularis mucosa and the submucosa of the gastrointestinal tract (Plate I).

Internal anatomy of the gastrointestinaltracts

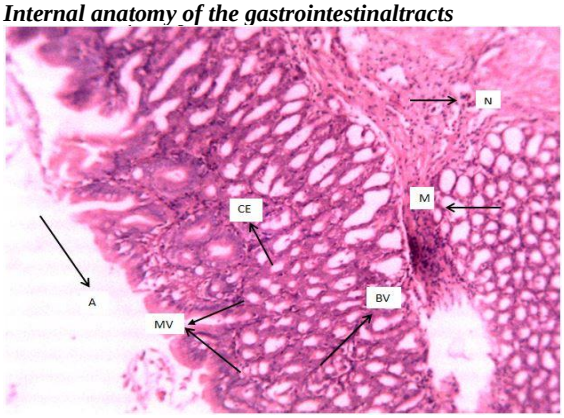

Plate I: Microphotograph of the control GIT of *Clariasgariepinus*in xylene; The mucosa can be seen, including microvilli (MV), columnar epithelium (CE), muscularis mucosa (M); nucleus (N); and adipose tissue(A); fatty tissue in the body cavity, surrounding the intestine submucosa (s) x100

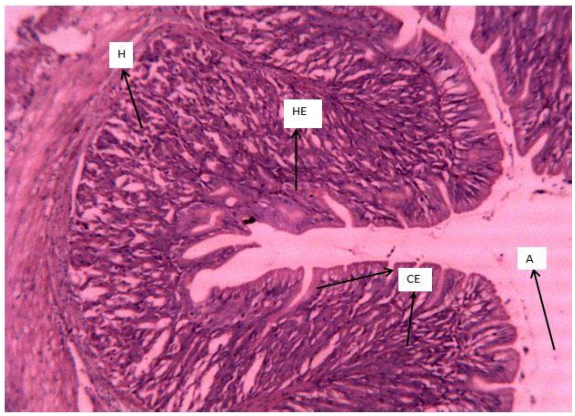

Plate II: Microphotograph of the GIT of *Clariasgariepinus* in xylene exposed to 50 mg L^{-1} of lead; Hemorrhage (H) in the intestinal wall and severe necrosis with Hemosiderin (HE) of the mucosa and submucosa (Ne) x100

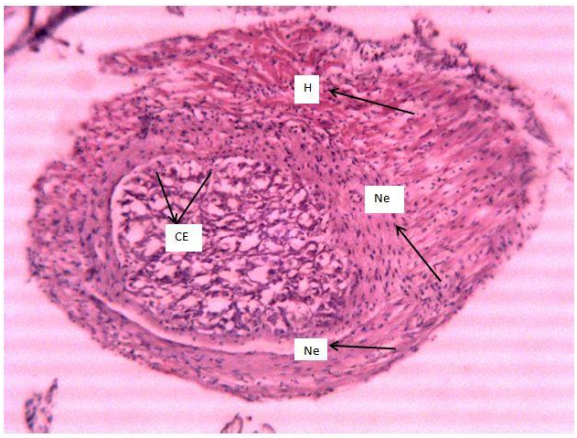

Plate III: Microphotograph of the Orange shape of the GIT of *Clariasgariepinus*in xylene exposed to 75 mg L^{-1} of lead; severe Hemorrhage (H) in the intestinal wall and columnar epithelium (CE), degenerative necrosis of the mucosa and submucosa (Ne). x100

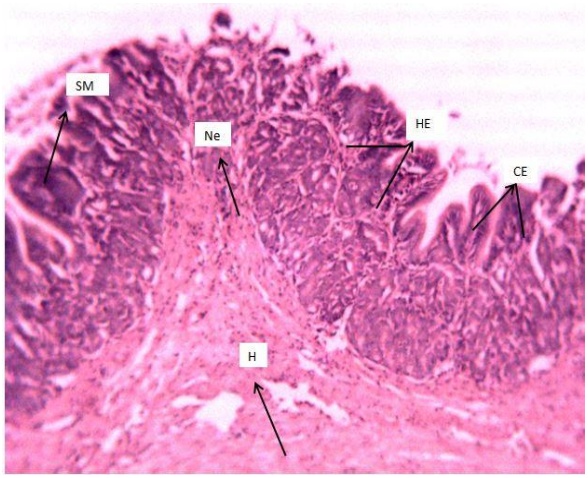

Plate IV: Microphotograph of the GIT of *Clariasgariepinus*in xylene exposed to 100 mg L^{-1} of lead; severe Hemorrhage (H) in the intestinal wall and severe necrosis (Ne) of the mucosa (M) and submucosa (SM), Hemosiderin (HE) in the columnar epithelium (CE) x100

The Gastrointestinal tract

The gastro-intestinal tract is one of the main routes for the uptake of xenobiotics present in the diet or in the water that the fish inhabit. In this study the control sample and its replicate showed a normal structure of the gastrointestinal tract, whereas the fish exposed to lead showed severe degenerative and necrotic changes in the intestinal mucosa and submucosa, atrophy in the muscularis and submucosa and aggregations of inflammatory cells in the mucosa and submucosa with edema between them.

The histology of the intestine in many teleosts has also been studied (Ezeasor and Stokoe 1981; Park and Kim 2001; Cinar and Senol 2006; Diaz *et al*., 2008). The structure of the stratum compactum seen in the submucosa has been reported in teleost intestine. The cells of the gut associated lymphoid tissues which include eosinophilic granular cells, intraepithelial lymphocytes and wandering leukocytes have been documented as part of intestinal local defense mechanism (Ezeasor and Stokoe, 1981; Dorin*et al*., 1993; Powell *et al*., 1993; Delashoud*et al*., 2010). The nature and form of teleost mucosal folds have also been reported (Ribeiro*et al*., 1999), even their role in nutrient absorption (Ezeasor and Stokoe, 1981).

According to Bhatnagar*et al*. (2007) the observed irritation and destruction of the mucosa membrane of the intestine, hampered absorption. The pathological alterations in the intestine of the studied fish are in agreement with those observed by many investigators about the effects of different toxicants on fish intestine (Hanna *et al*., 2005; Cengiz*et al*., 2006; Tongo, 2010).

Epithelial degeneration, inflammatory cells infiltration in the submucosa as well as submucosa edema was seen in the intestine of tilapia fish exposed to carbofuran (Soufy*et al*., 2007). Soufy*et al*. (2007) investigated the histological alteration in the intestine of *Tilapia zilli* and *Solea vulgaris* obtained from Like Victoria and observed some lesions in the intestine, degenerative and necrotic changes in submucosa and mucosa with edema between them, dilation in the blood vessels of serosa and atrophy in the muscularis and sub mucosa Heavy metals can be said to be dangerous to fish and other aquatic organisms and man that feed on them.

Conclusion

The effects of toxicant on the gastrointestinal tract of fish on this study ranged from slight changes in motility, secretion and absorptive functions to more severe effects associated with mucosal integrity, blood flow or neuromuscular control. These effects could ultimately influence the ability of the organism to thrive. The main changes reported in gastro - intestinal tract included hydropic degeneration of the digestive gland, proliferation of mucous cells, hyperaemia, atrophy and metaplasia. Some studies have indicated that high levels of some metals in diet may cause increase damages to the gastrointestinal organs (GIT) of fish and indirectly to other organisms, including man that feed on them. This calls for care and regular monitoring of our water bodies against heavy metals

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