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ORIGINAL ARTICLE

Histopathological Changes in Liver of *Clarias gariepinus* Fish exposed to sub-lethal concentrations of Lead Nitrate and Crude Oil

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ABSTRACT
Structure and function of normal liver of fishes make the liver a target for toxicants such as heavy metals and crude oil. Pathological effects on liver of juvenile Clarias gariepinus fish (mean weight 138±12g and length 28±1.5cm) in aquaria exposed to varying concentrations of lead nitrate and crude oil. The experimental fishes were divided into 5 groups, with 20 fish samples each. Group A served as adequate Control. Groups B and C were exposed to 20mg/l and 35mg/l lead nitrate while Groups D and E where treated with 300ppm and 600ppm crude oil, respectively. Treatment lasted for 70 days after which liver tissues from control and exposed groups were examined histologically. Photomicrographs of liver sections from control group showed no evidence of histopathological changes but those from treated groups indicated varying levels of hyperemia, leukocyte infiltration, extensive necrosis, fibrosis, ballooning degeneration and loss of liver architecture.
Keywords: *Lead nitrate, crude oil, Clarias gariepinus liver, histopathology, photomicrographs.*

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INTRODUCTION

Liver in fishes is a major site for amino acid homeostasis, glucose regeneration, lipid processing, hormone metabolisms and the production of numerous plasma constituents and fibrinogen. The liver also plays an important role in the excretion of xenobiotic compounds which targets the liver because of its strategic location, enzyme complements, and dual blood supply and enterohepatic circulation [1].Fresh water fishes are often subjected to pollution especially near industrial or populated areas [2, 3, and 4]. Heavy metals have been shown to exert a wide range of effects on metabolism, physiology, behavior and ecology of fishes. Some of these effects are disturbances in osmo-regulation and respiration [5], tissue damage [5], reduced energetic resources [6] and poor performance [7]. Contamination of water by heavy metals and crude oil may directly or indirectly lead to fish kills, reduced fish productivity or elevated concentrations of undesirable chemicals in edible fish tissues which can affect the health of humans eating these fishes [8, 9].The African catfish *Clarias gariepinus* is commonly found in the Nigerian inland waters [10]. Fresh water contamination with crude oil and heavy metals such as lead is increasingly becoming a subject of great concern over the past decades not only because of their threat to pollute water supplies but also because of the damage caused to aquatic life especially fishes [11] in which bioaccumulation of heavy metals make the consumption of fish a potential danger to public health [2].

The objective of this study was to investigate the histopathological changes in the liver of *Clarias gariepinus*, experimentally exposed to sub-lethal concentrations of inorganic pollutant lead nitrate, and organic pollutant crude oil.

MATERIALS AND METHODS

Juvenile *Clarias gariepinus* fish numbering 100, procured from Rehoboth Farms Nkwelle-Ezunaka near Onitsha Nigeria, were acclimatized for 14 days before being separated into the five experimental groups (A, B, C, D and E) in aquaria. Each group comprised 20 fishes and was fed with pellets of Skretting® fish feeds at 1% biomass; half at 900 hours and 1700 hours respectively. Group A served as adequate control. Groups B and C were treated with 20mg/l and 35mg/l lead nitrate respectively while Groups D and E were exposed to 300ppm and 600ppm crude oil respectively Representative samples of live *Clarias gariepinus* fishes from each group were euthanized, dissected and the livers eviscerated for histological examination according to [3] as follows: The organs were labeled and fixed separately in small plastic containers in 10% Formal-Saline solution at room temperature for 24hrs to prevent autolysis and putrefaction. 1000mls of Formal-Saline solution was formulated with 100ml of concentrated formaldehyde, 900ml of tap water, and 8.5g of sodium chloride crystals. The preserved tissues were left in running water for thirty minutes to remove all traces of fixative before being sectioned into pieces of 2mm diameter and then dehydrated for 30 minutes in ascending series70, 90, and100%concentration of ethanol respectively. The tissues were cleared of ethanol overnight in chloroform and then immersed in molten paraffin wax for infiltration at 60ºC in an electric oven for one hour. The tissues were then embedded in fresh molten paraffin wax at room temperature. The embedded tissues were sectioned, each 5µm in thickness, with a microtome knife with a manual rotary microtome (American optical comp. No. 800). The serial sections were mounted on clean microscope glass slides previously subdued with egg albumen to enhance proper adhesion of the tissue on the slides. The slides were properly labeled, cleared of paraffin in two changes of Xylene for thirty minutes; and hydrated in descending grades 90, 70, and 50% concentration of ethanol for ten minutes respectively. Staining was done using Harris H&E stain in a glass staining trough. The stained sections were dehydrated in ascending grades 50, 70 and 90% concentration of ethanol, cleared in chloroform solution, and covered with cover slides using Canada balsam for proper adhesion. The permanent slides were produced and photomicrographs of the sections taken with photomicrograph microscope Model-Gallen III No. Bm 600 at varying magnifications [12].

RESULTSAND DISCUSSIONS

Plate 1 is a photomicrograph of a normal liver section (from control group). Plates 2&3 are photomicrographs showing alterations in the histology of the liver tissues due to lead nitrate while Plates 4&5are photomicrographs showing alterations in the histology of the liver tissues due to crude oil. It was nearly impossible to attribute these histopathological changes to other pollutants since juvenile fishes used in the study were not exposed to any toxicant before procurement. Since toxicity of any chemical may alter the physiological state of affected animals, thereby impairing their various metabolic activities, it is therefore to clearly understood that these chemicals, lead nitrate and crude oil used in this study caused injury to the tissues observed in liver tissues exposed to them. Currently liver histopathology is used mainly as a descriptive tool to assess the health status of fish exposed to toxicants [13], and histopathological alterations of fish liver are also frequently used in monitoring programs as markers of fish health. Numerous field studies reported liver histopathological changes in fish from contaminated environments [3] and liver histopathology has been found to be a sensitive indicator of pollutions stress and impaired fish health [14, 15].

Histopathological changes observed in liver tissues in the present study included hyperemia, leukocyte infiltrations, extensive necrosis, fibrosis, ballooning degenerations and varying degrees of destruction of histological architecture of the liver. These histopathological alterations are similar to the changes reported in fishes exposed to sub-lethal concentrations of metals [3, 16, 17 & 18]. The necrotic degeneration of the hepatocytes, vacuolation and leukocyte infiltration observed in the liver sections of the fish were in line with the report on the liver of fish exposed to metal from Elbe Estuary by [19]. Acute and extensive necrosis, generalized swelling and pyknosis of hepatocytes nuclei with cytoplasmic vacuolation on liver cells of fish exposed to metal toxicity were also reported by [20] while [3] observed extensive hyperemia, edematous sinusoids, apoptotic hepatocytes with pyknotic nuclei and widespread necrotic hepatocytes with mononuclear leucocytes infiltrations and pigment deposits in liver tissues of *Chrysichthys nigrodigitatus* due to heavy metals in the River Niger. We recall that the concentrations of Pb in the fish from Niger River studied by [3] was found to be higher than the WHO Standard of 0.05mg/L in aquatic foods, which indicated that Niger River at Onitsha was already experiencing some significant impairment in relation to Pb ion. The implication is that consuming fish from the River could cause Pb-induced health problems since lead is a cumulative poison and a potent enzyme inhibitor which is easily incorporated into enzyme structures. Lead (Pb) inhibits the synthesis of haeme in organisms and thus interferes with the effective utilization of iron [21, 22]. Elevated concentrations of Pb cause cytological

degenerations in fish organs as well as heart, liver and kidney dysfunction in man [23, 24]. Lead in the River must have come chiefly from automobile and power generating plants' exhaust pipes as well as lead deposits in soils, especially from Nkwelle-Ezunaka industrial site, which were washed into the River Niger by runoffs during the rains. It is important to note that Rehoboth Farms where fish samples for this study were procured is located in Nkwelle-Ezunaka. Thus fish farms in the study area are potentially at risk of contamination with heavy metal, which may accumulate in tissues of fishes and pose dander to public health [2].

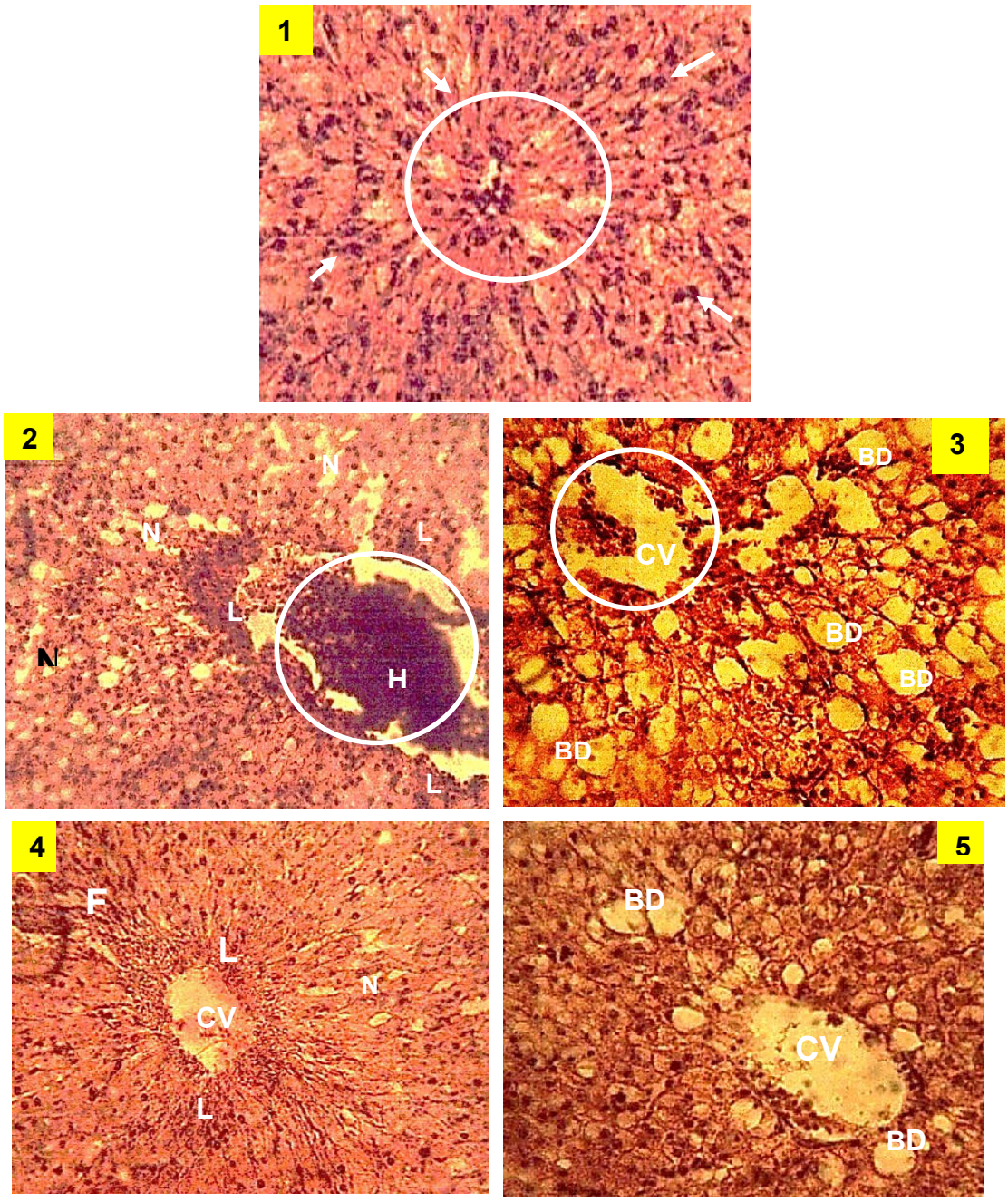

Plate 1: Photomicrograph of the histological section of normal liver tissue from fish in control group showing central vein (circled) and hepatocytes (arrow head). H&E stain x 400.

Plate 2: Photomicrograph of the histopathological section of liver tissue treated with 20mg/l of lead nitrate showing hyperemia (H) of the central vein, leukocyte infiltration (L) around the central vein and necrosis (N) of hepatocytes. H&E stain x 400.

Plate 3: Photomicrograph of the histopathological section of liver tissue treated with 35mg/l of lead nitrate showing complete destruction of liver parenchyma, with ballooning degeneration (BD) and extensive necrosis of hepatocytes with loss of liver histological architecture. H&E stain x 400.

Plate 4: Photomicrograph of the histopathological section of liver tissue treated with 300ppm crude oil showing cellular infiltration (L), necrosis and fibrosis (F) of hepatocytes. H&E stain x 400.

Plate 5: Photomicrograph of the histopathological section of liver tissue treated with 600ppm crude oil showing necrosis, cellular infiltration and ballooning degeneration of hepatocytes with complete loss of liver histological architecture. H&E stain x 400.

CONCLUSION

From this study, it is evident that sub-lethal concentrations of lead nitrate and crude oil had significant histopathological alterations in the liver tissues of the fish studied. Therefore prolonged exposure of fish to chemical pollutants may lead to increased morbidity and mortality, hence decrease in productivity. The consumption of contaminated fish and other aquatic foods may also pose serious public health risks. In view of the ever-increasing importance of fish as a source of high quality animal protein in Nigeria, it is necessary to make a comprehensive assessment of the hazards posed by lead (Pb) and crude oil spillage in Nigeria's water bodies and elsewhere in the world.

DISCLOSURE OF INTEREST

All authors declare that they have no conflicts to report.

ANIMAL STUDIES

All institutional and national guidelines for the care, use and euthanasia of live *Clarias gariepinus* fishes before they were dissected and the gills eviscerated for histological examination in the laboratory were followed.

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