Some Effects of Mercury Chloride on the Liver and Gills of *Clarias gariepinus*.

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Abstract: This study was carried out to investigate the effects of mercury chloride on *Clarias gariepinus*. A static renewal bioassay was adopted in which the test media was renewed at the same concentration once every 48 hours. Based on the LC_{50} (0.5mg/L), three concentrations, 2.5mg/L, 1.25mg/L, 0.625mg/L mercury chloride in 25 liters water, representing $^1/_5$, $^1/_{10}$, $^1/_{20}$ of the LC_{50} respectively were administered on *C. gariepinus* juveniles. The control was not given any concentration of the mercury chloride. Six fishes were sacrificed from each treatment group and the control group at five days intervals. The gills and livers were removed and prepared for histopathological observation. The histological examination of the liver and gill tissues showed changes which include locally extensive vacuolar degeneration and variably sized cytoplasmic vacuoles of the hepatocytes and swelling of epithelium in the treated groups. Gills and liver of fishes in the control group did not exhibit any of these changes i.e. the histology of these organs showed no alteration in the control. The study shows that mercury chloride exhibits toxic effects on *Clarias gariepinus*.

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1. Introduction

Various activities by man in recent years have increased the quantity and distribution of heavy metals in the atmosphere, land and water bodies. The extent of this wide spread but diffused contamination has raised concern about their hazards on plants, animals and humans.

Human existence on earth is almost impossible without chemicals. Chemicals and their products are very important to mankind due to the benefits they accrue. However, exposure to them during production, usage and their uncontrolled discharge into the environment has caused lots of hazards to man, other organisms and the environment itself. Over 100,000 of chemicals used by man are maintained in the ecosystem and several of these have been reported to exhibit toxic effects on lives (Nowierski et al. 2006; Herrera-Silveira et al. 2004; Malallah et al. 1998; Parsons et al. 1984).

Mercury is a highly toxic element found both naturally and as an introduced contaminant in the environment. It has been shown that mercury can be of high threat and risk to the health in which the risk to health is determined by the exposure mechanism, the form of mercury present because the forms are more toxic than one another and geochemical and ecological factor that changes the forms. Once it's released to the environment it travels a long distance depositing into water depending on their density (Njoroge, 2007).

The indiscriminate use, careless handling, accidental spillage, or discharges of product containing mercury chloride $HgCl_2$ have harmful effects on the fish population and other forms of aquatic life and may contribute long term effects in the environment (Akhtar, 1986). The direct effect of mercury chloride $HgCl_2$ leads to death of fish through loss of habitat and food supply (Ervnest, 2004). The disposition of mercury chloride in fishes has been characterized after water and oral administration, with the pattern of tissue distribution varying, depending on the administrative route. Regardless of the exposure route, the liver, gills and kidney tended to accumulate the highest quantities of these metals.

African catfish are widely used to evaluate the health of aquatic ecosystem, physiological changes and also serve as biomarkers of environmental pollution. To stabilize the normal growth of catfish it is very essential to maintain the living things in their natural environment. Environmental Protection Agency (EPA) banned the use of mercury chloride or any product containing mercury chloride in 1990. But still, mercuric chloride is being used as a disinfectant and pesticide. Mercury chloride is still in use in Nigeria despite its diverse effect on fish and the aquatic environment. Once mercury chloride dissolves into the water, aquatic organism i.e. fish absorbs it in (Croteau et al. 2005). It accumulates in the fish and depending on the concentration of mercury chloride mortality may occur. The fish starts different abnormal behavior and this leads to the damage of vital organs in fish and also suffocation. There are different factors that affect the bioaccumulation of mercury chloride in fish.

The bioaccumulation of trace elements in living organisms and biomagnification in them describes the processes and pathways of these (possible) pollutants from one trophic level to another, exhibiting the higher bioaccumulation ability in the organisms concerned. Increasing concentration through the food chain caused higher retention time of toxic substances than that of the other normal food components. Therefore, various fish species are widely used as bio-indicators of metal contamination (Svobodova et al. 2004).

In its natural geographical areas of occurrence, *Clarias gariepinus* feeds on plankton, arthropods, mollusks, vegetables, fish, reptiles, and amphibians, showing a very wide and generalist diet (Myers et al. 2000). This catfish is a top food chain predator, and being a voracious feeder, could deeply modify preexisting biotic interactions in the community. The impact of this large invasive predator species in a native food web of a community with many endemic species of short size is imminent.

The exposure of fish and different aquatic organism to mercury chloride ($HgCl_2$) is not just a problem from the past; it remains a problem of today due to the great damage it confers on the aquatic life (Mozaffarian and Rimm, 2006). This study was undertaken to analyse the effects of sublethal concentrations of mercury chloride ($HgCl_2$) on histological parameters in African cat fish *Clarias gariepinus*. The choice of *Clarias gariepinus* was informed and its high commercial value in Nigeria.

2. Materials and Method

120 samples of *C. gariepinus* of mean weight 33.4 $\pm$ 10g were used for the experiment. They were purchased from a fish farm (Flourish fish) in Ota, Ogun State and transported in an oxygen bag to the laboratory. The fish were held in 25-litre capacity plastic bowls containing water, during which they were allowed to acclimatize for about two weeks and were fed with commercial floating pellet feed at 5% of their body weight, unconsumed feed was removed and the water was changed every 48 hours.

A range finding toxicity test was carried out prior to the definitive experiment to determine the LC_{50} which was found to be 0.5mg/L. A static renewal bioassay was adopted in which the test media was renewed at the same concentration once every 48 hours. The juveniles were randomly divided into four groups of twenty-four fish and each group subdivided into a set of three subgroups consisting of eight fish each. The twenty-four subgroups were kept in twelve different tanks in 25 liters of water. Based on the LC_{50}, three concentrations, milligrams per liter mercury chloride in water were used. The four groups were respectively exposed to 0.625 mg/L, 1.25 mg/L, 2.5 mg/L and 0 mg/L of mercury chloride ($HgCl_2$), representing groups A to D. In other words, there were three experimental groups and one control group, each group having three replicates. Six fishes were sacrificed from each treatment group (two each from each subgroup) and the control group at five days intervals. The experiment was run for fifteen days.

The organs (gills, livers) were removed and prepared for histopathological observation. They were fixed in bouin's fluid for 24 hours, washed with 70% ethanol and dehydrated through a graded series of ethanol (Kelly, 1979; Schalm et al. 1995). They were embedded in paraffin, sectioned using a microtome at 4 – 5 μm thickness, stained with hematoxylin and eosin and examined using light microscope and photomicrography (Keneko, 1989).

3. Results

The liver (fig 1-9) and gills (fig 10-18) of treatment group fishes show pathological changes. Changes ranging from vacuolar degeneration in the hepatocytes, multiple foci of hepatocytes and aggregates of heterophils in hepatic parenchyma containing several variably-sized and large-sized cytoplasmic vacuoles in the hepatocytes, aggregations of inflammatory, dissociated hepatic cords, black necrotic spots and perivascular accumulation of lymphocytes were observed in liver cells. Liver of fishes in group A is observed to show progressive damage as diffuse severe vacuolar degeneration of hepatocytes with variably-sized vacuoles (Fig 1-3). The hepatic tissue of the treatment group B (Fig 4-6) also showed a similar pattern as multiple foci of hepatocytes containing variably-sized cytoplasmic vacuoles, several foci of aggregates of heterophils and lymphocytes in the hepatic parenchyma with the damage increasing as the treatment time prolonged. Slight locally extensive vacuolar degeneration of hepatocytes can also be seen here. The hepatic tissues of group C (Fig 7-9) shows variably-sized cytoplasmic vacuoles in the hepatocytes giving rise to a moderate random vacuolar degeneration of the hepatocytes and also perivascular accumulation of lymphocytes. The control group, (Fig 19) showed no visible lesions, no vacuolation and no necrosis throughout the treatment period.

Gills of fishes in group A is observed to show progressive damage as, severe loss of the gill lamellae (Fig 10-12). The gills of the treatment group B (Fig 13-15) also showed a similar pattern as slight thickening of the secondary gill lamellae, numerous basophilic (blueish) walled structures within the epithelium of the gills and thickening of the gills with fibrous metaplasia of the covering epithelium with the damage increasing as the treatment time prolonged. The gills of group C (Fig 16-18) shows that the gill

capillaries are markedly congested and autolysis. The control group, (Fig 20) showed no visible lesion.

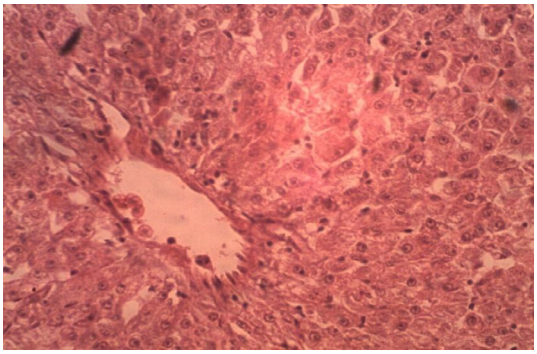

Fig 1 Liver of group A fishes after 5 days shows diffuse severe vacuolar degeneration of hepatocytes

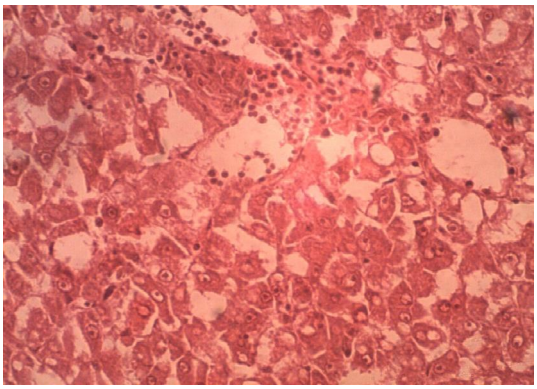

Fig 2 Liver of group A fishes after 10 days show diffuse severe vacuolar degeneration of hepatocytes

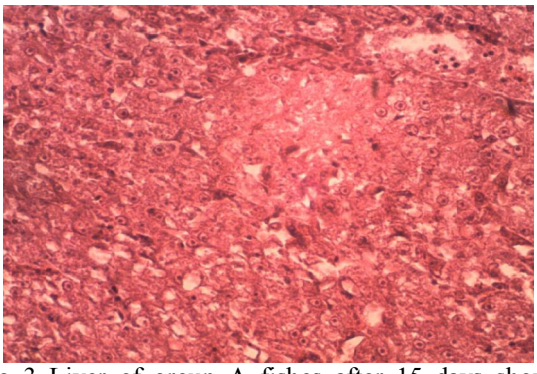

Fig 3 Liver of group A fishes after 15 days shows random vacuolar degeneration of the hepatocytes; as they contain variably-sized vacuoles; there is moderate dissociation of hepatic cords

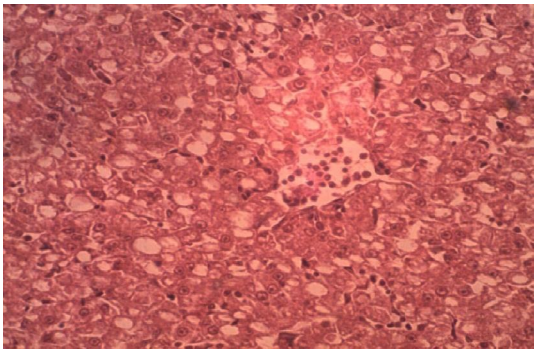

Fig 4 Liver of Group B fishes after 5 days shows multiple foci of hepatocytes containing variably-sized cytoplasmic vacuole

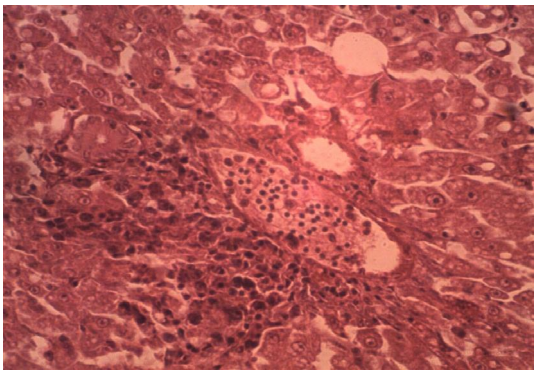

Fig 5 Liver of group B fishes after 10 days shows slight locally extensive vacuolar degeneration of hepatocytes

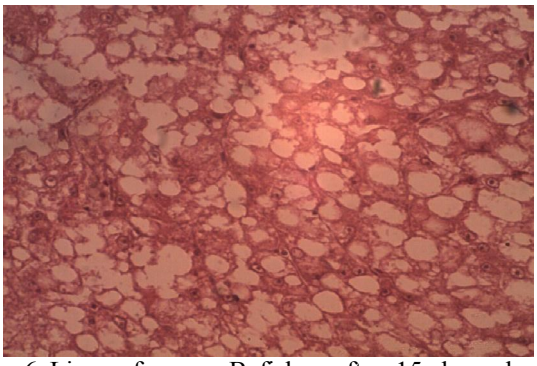

Fig 6 Liver of group B fishes after 15 days shows multiple foci of hepatocytes containing variably-sized cytoplasmic vacuoles and several foci of aggregates of heterophils in the hepatic parenchyma.

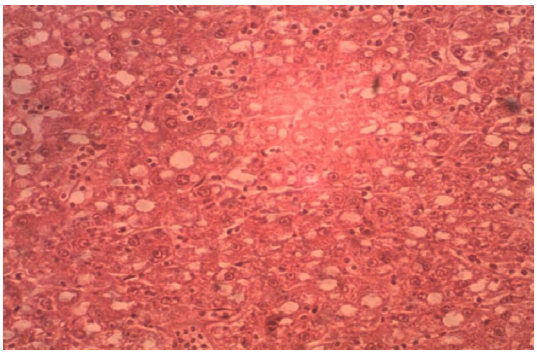

Fig 7 Liver of group C fishes after 5 days shows variably-sized cytoplasmic vacuoles in the hepatocytes.

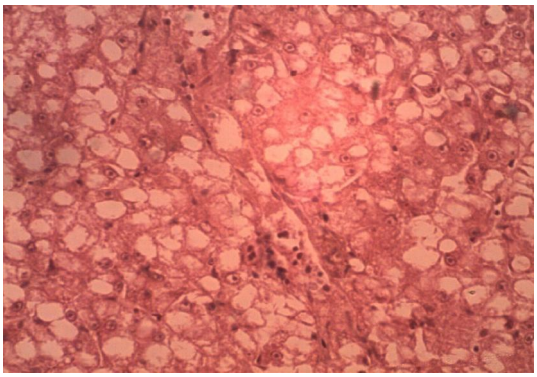

Fig 8 Liver of group C fishes after ten days shows large-sized cytoplasmic vacuoles in the hepatocytes, it also shows perivascular accumulation of lymphocytes

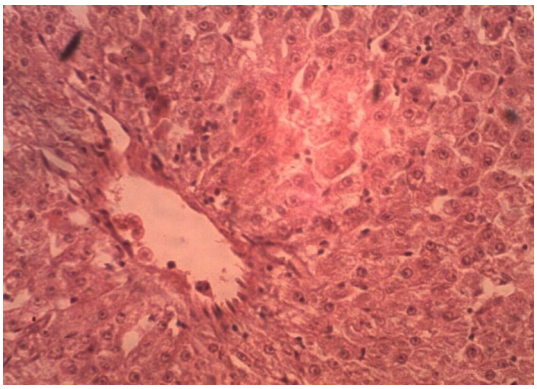

Fig 9 Liver of group C fishes after 15 days shows slight locally extensive vacuolar degeneration of hepatocytes

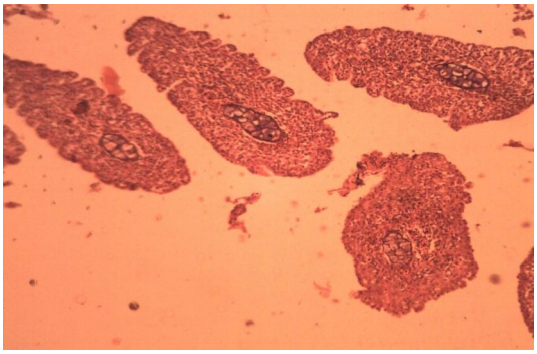

Fig 10 Gills of group A fishes after 5 days shows moderate swelling of epithelium of secondary gill lamellae.

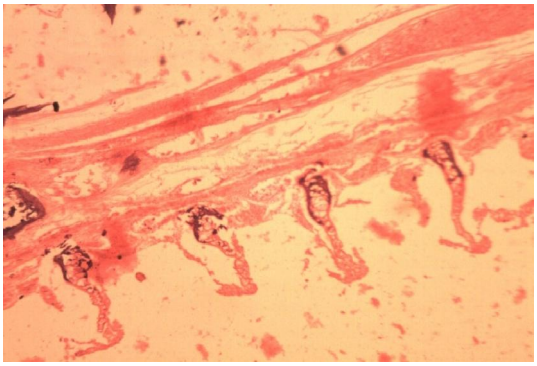

Fig 11 Gill of group A fishes after 10 days shows severe loss of the gill lamellae OR possible autolysis

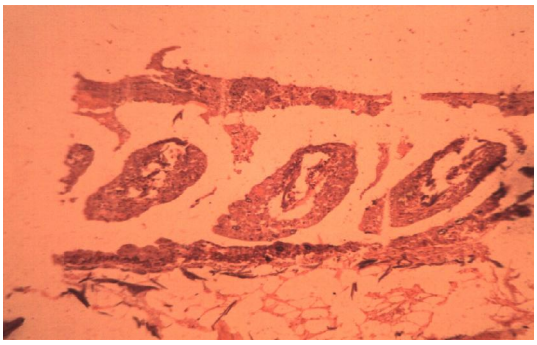

Fig 12 Gills of Group A fishes after 15 days shows severe loss of the gill lamellae

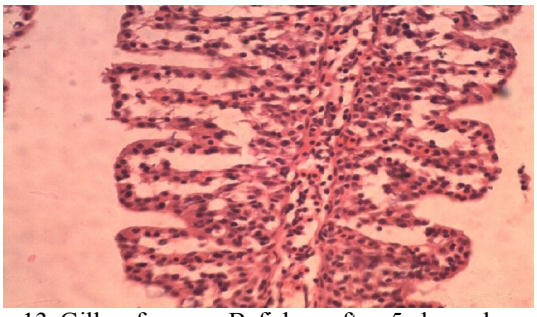

Fig 13 Gills of group B fishes after 5 days shows a slight thickening of the secondary gill lamellae.

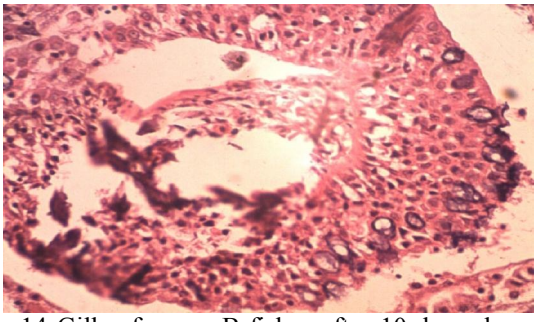

Fig 14 Gills of group B fishes after 10 days shows a numerous basophilic (blueish) walled structures within the epithelium of the gills

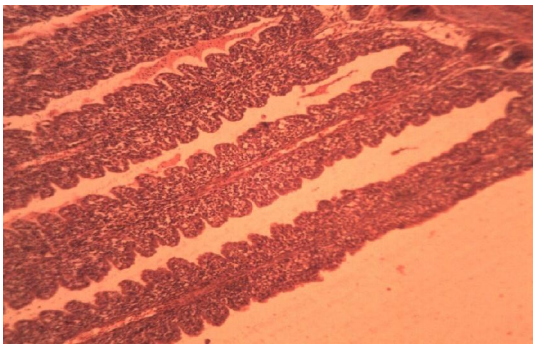

Fig 15 Gills of group B fishes after 15 days shows marked thickening of the gills with fibrous metaplasia of the covering epithelium.

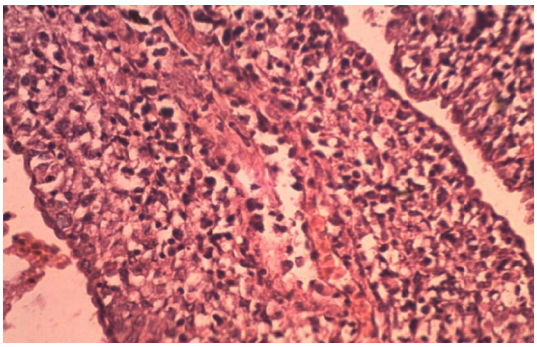

Fig 16 Gills of group C fishes after 5 days shows markedly congested at the capillaries

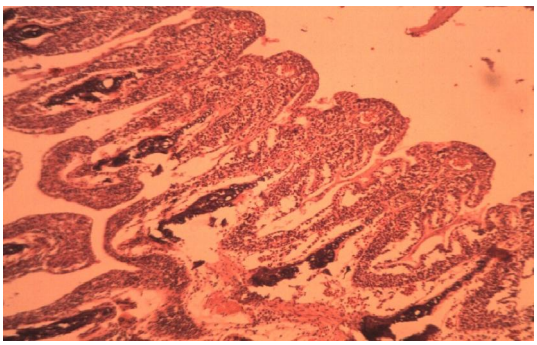

Fig 17 Gills of group C fishes after 10 day shows severe loss of gill lamellae or possible artefacts.

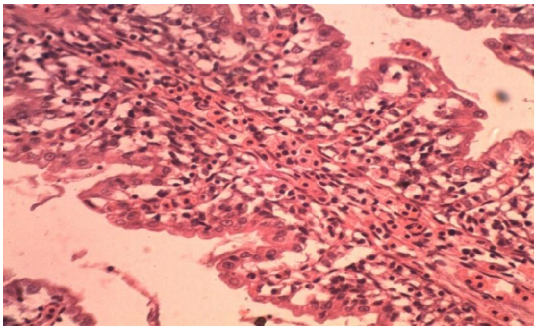

Fig 18 Gills of group C fishes after 15 days showing autolysis.

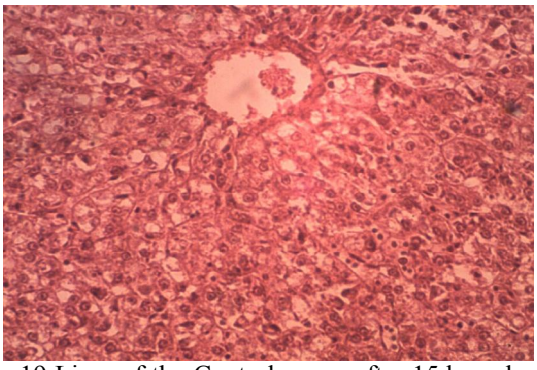

Fig 19 Liver of the Control group after 15days shows slight artifact with no visible lesion.

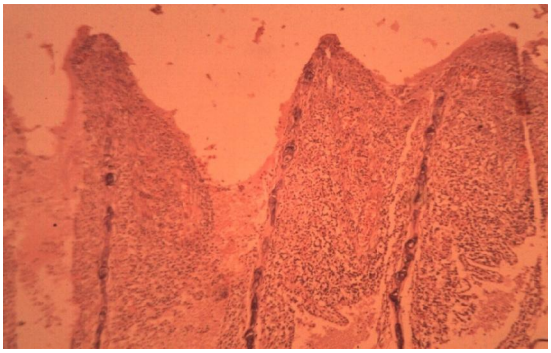

Fig 20 Gills of control group after 15 days shows no visible lesion no visible lesion

4. Discussions

Results of this present study revealed that *Clarias gariepinus* manifest changes in the liver and gills. It is likely that the pathological alterations in the tissues of the fish could be as a direct result of the heavy metal used in treating the fish. The histopathological alterations in the gills and liver of the fish are in agreement with those observed by many investigators (Fatma 2009; Atif et al. 2009; Ogundiran et al. 2010.) who have studied the effects of different pollutants on fish tissues. Histopathology is the microscopic examination of tissue in order to study the manifestations of a disease.

The liver of fish in group A shows diffuse severe vacuolar degeneration of hepatocytes unto the 5th and 10th day (Fig 1 & 2), while the liver of group B (Fig 4) shows multiple foci of hepatocytes containing variably-sized cytoplasmic vacuole unto the 5th day. Several foci of aggregates of heterophils in the hepatic parenchyma are observed in group B (Fig 5) onto the 10th day. Also moderate degeneration of the hepatocyte was observed with prolonged treatment (Fig 6). The liver of group C (Fig 7) shows variably-sized cytoplasmic vacuoles in the hepatocytes unto the 5th day. The liver of the control group show no visible lesion except for slight artifact (Fig 19).

Liver of fish is responsible for the digestion, filtration and storage of glucose. It is found in the anterior part of the body cavity as a brownish red mass. The liver also produces many enzymes that stored in the gall bladder. These enzymes assist in the breakdown of food. The liver functions to store food energy (Tayel et al. 2008). The present study suggests a strong link between heavy metals and lesions in the liver. Sorensen (1991) cited that heavy metals in Elbe might cause liver damage. Aly et al. (2003) obtained similar results after exposure to lead pollution. They found that the vacuolar degeneration and necrosis of hepatocytes may appear after 3 days but after 2 weeks, hemolysis of red blood corpuscles and vacuolar degeneration as well as necrosis of hepatocytes was observed. The liver is the main organ for detoxification that suffers serious morphological alterations in fish exposed to heavy metals (Verma and Tonk, 1983). Alterations in the liver may be useful as markers that indicate prior exposure to environmental stressors. The liver of the exposed fish had vacuolated cells showing evidence of fatty degeneration. Necrosis of some portions of the liver tissue that were observed resulted from the excessive work required by the fish to get rid of the toxicant from its body during the process of detoxification, and this is similar to the observation of Rahmn et al. (2002).

In present study, moderate swelling of epithelium of secondary gill lamellae is observed in group A (Fig 10) unto the 5th day and also severe loss of gill lamellae is observed (Fig 11) unto the 10th day. The secondary gill lamellae of group B (Fig 13) shows a slight thickening unto the 5th day. On the 10th day it shows numerous basophilic (blueish) walled structures within the epithelium of the gills (Fig 14) and as treatment prolonged the covering epithelium shows marked thickening of the gills with fibrous metaplasia (Fig 15). The capillaries of fishes in group C (Fig 16) shows markedly congestion unto the 5th day. Also the gill lamellae of fishes in this group show severe loss and possible artifacts (Fig 17) unto the 10th day. Also Autolysis was observed (Fig 18) as treatment prolonged. In the control group no possible lesion is observed unto the last day of treatment (Fig 20).

There are many routes for the entry of heavy metals into the body of fish, namely oral ingestion, absorption through gills, general body surface and gastrointestinal tract. After absorption the metal makes its way into the target organ, where it produces various types of disturbances. The gills carry out the functions of respiration, osmoregulation and excretion; remain in contact with external environment, is particularly sensitive to changes in the quality of water and considered to be the primary target of the contamination. The gill is made up of filaments of primary lamellae arranged in double rows. Secondary lamellae arise from these filaments. The secondary lamellae are lined by a squamous epithelium (Dutta et al., 1993).

According to Leino et al. (1997) the exposure of fish to the toxicant Atrazine resulted in several pathological alterations in different tissues of fish. According to the studies by Handy and Penrice (1999) on the effect of mercuric chloride on the gills of in rainbow trout, *Oncorhynchus mykiss* showed histological damage in the gills such as tissue necrosis, chloride cells hypertrophy and emergence of blood. Severe gill damage of fish exposed to high levels of water-borne organic and inorganic Mercury (Hg) was described. In addition, Gregory *et al.* (2002) reported the effects of dissolved mercury on the gills of mollusca (*Perna perna*), reinforcing the physiological importance of this respiratory structure to aquatic organisms and its vulnerability to dissolved toxicants once this organ is in direct and constant contact with the surrounding water. According to Gregory *et al*. (2002), the effects of sub-lethal and more realistic doses of mercury on fish have not yet been explored in detail, but only the effects of low and moderate doses although alteration to these vital organs have been used as a morphological biomarker to assess environmental pollution. Many toxicants can be associated with pathological gill events, and it becomes difficult to relate gill damages to a particular pollutant in a contaminated environment (Takashima and Hibiya, 2008). However, alterations to the gill tissues described in this work may be said to be as a result of exposure to mercury since mercury is the only contaminant used. Further proof of this is that no form of damage was observed in the untreated fishes.

Some of the effects of this exposure to mercuric chloride may be respiratory and osmoregulatory disorders. These alterations also may play a defensive role against contamination rather than have an irreversible toxic effect. However, these modifications can produce adverse effects on fish health, and may increase their susceptibility to secondary infectious diseases and even death (Hawkins et al. 2008).

Conclusively, this study has been able to establish the fact that, exposure of *C. gariepinus* juvenile to even low concentrations of mercuric chloride can induce various toxicological effects and histological degradation, which dependent on the period of exposure and concentration of toxicant. Hence pathology of these organs could serve as an important biomarker of mercury toxicity. Asides, changes in the main organs involved in xenobiotic metabolism could have serious consequences on the overall physiology of the exposed fish.

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