

Liver and Kidney Histopathology: Biomarkers of No. 1 Fuel Toxicosis in African Catfish, *Clarias gariepinus*

¹Gabriel, U.U., ²Ezeri, G.N.O. and ¹E.U. Amakiri,

¹Department of Fisheries and Aquatic Environment,

Rivers State University of Science and Technology, P.M. B. 5080, Port Harcourt, Nigeria

²Department of Aquaculture and Fisheries Management, University of Agriculture, Abeokuta, Nigeria

Abstract: African catfish, *C. gariepinus* (mean total length, 31.49±5.45cm, sem; total length, 18.50±2.42g, sem) were exposed to grade levels of light fuel oil-No.1 fuel (0.75, 150 and 300 $\mu\text{L L}^{-1}$) in triplicates for 14 days. The histology of the kidneys from the normal showed uniform morphology of the nuclei and areas with mild to moderate steatosis. Exposed fish showed an increased glomerular cellularity, degeneration of kidney tubules with infiltrate of few neutrophils. The interstitia were infiltrated by inflammatory cells (neutrophils and lymphocytes) which was concentration-dependent. There was extensive necrosis with the majority of the neoplastic tubules in the nephroblastoma stage at the highest concentration. The tubular cells were also hypertrophic and the lumina contained amorphous eosinophilic materials. The sections of the livers from the control fish had normal tubules, haematopoietic tissues and portal triad that contained dilated portal vein. There were some areas showing mild to moderate steatosis. In exposed fish, there was degeneration of cords of hepatocytes and severe necrosis of hepatocytes, pyknosis and karyolysis of hepatocellular nuclei as well as hyalination of hepatocytes with narrowing of the liver sinusoids channels. The central veins were congested with nucleated erythrocytes, centrilobular necrosis with areas of ballooning degenerative vacuolation and steatosis particularly in the higher concentrations. There were focal areas of infiltration of the liver by inflammatory cells. Results from this study indicate that the pathology of the liver and kidney of *C. gariepinus* could be a good biomarker for the assessment of light fuel toxicosis.

Key words: Liver, kidney, histopathology, fuel toxicosis, necrosis, hepatocytes

INTRODUCTION

Pollution from industrial and oil-related activities is common in many parts of the world. In Nigeria many industrial and urban centres are contaminated with a variety of pollutants that are potentially harmful to aquatic organisms. Contamination from crude oil and petroleum products are more rampant in the Niger Delta with highest concentration of crude exploration, exploitation, transporting and refining activities. According to Dublin-Green *et al.* (1998) there were 5334 reported cases of oil spills between 1976-1990 releasing about 2.8 million barrels of oil into the land swamps, estuaries and coastal waters of Nigeria. The trend continues unabated.

Water Soluble Fractions (WSF) of crude oil were reported to be toxic to various aquatic organisms such as *Menidia beryllina* (Al-Yakoob *et al.*, 1996) *M. beryllina* and *Palaemonetes pugio* (Gundersen *et al.*, 1996) crude oil to pink salmon embryo (Brannon *et al.*, 2006) and Gulf killifish (Liu *et al.*, 2006). Remedial agents such as

dispersants alone or in combination with crude oils were shown to be toxic to Pacific salmon, *Fundulus heteroclitus* hatchlings (Couillard *et al.* 2005) Pacific herring eggs and larvae (Barron *et al.*, 2003) and Australian bass, *Macquaria novemaculeata* (Cohen and Nuggeoda, 2000). Exposure of fish to crude, WSFs and light fuel oils produced varying degrees of pathological changes in the gill, liver and kidney of exposed organisms (Prasad 1991; Brannon, *et al.*, 2006; Cohen and Nuggeoda 2000; Barron *et al.*, 2003; Couillard *et al.*, 2005). The polychaetes, *Neanthes arenaceodentata* were shown to be resistant to WSFs of No. 2 fuel (Rossi and Anderson, 1976, 1978). There are no reports on the effects of crude or refined oil on the histopathological changes in the liver and kidney of *Clarias gariepinus*, an important aquaculture species in many parts of the world. The present investigation was conducted to provide information on the histological changes in the liver and kidney of *C. gariepinus* under exposure to light fuel oil, No.1 fuel as biomarkers of refined petroleum oil toxicosis.

MATERIALS AND METHODS

Juveniles *C. gariepinus* (mean total length, 31.49±5.45 cm, sem; total length, 18.50±2.42 cm, sem) were acclimated in six circular aquaria in 20l water for seven days. The fish were fed a 25% crude protein diet at 1% biomass half at 800 and 1600 h, respectively. The low boiling point No. 2 fuel was obtained from a gas station. Water-fuel dispersion of the various concentrations of No.1 fuel (75, 150 and 300 $\mu\text{L L}^{-1}$) was prepared by adding determined amounts of the fuel to water determined amount of borehole water (dissolved oxygen, 4.43±1.4 mg L^{-1} ; pH, 6.78±1.24; alkalinity, 28.32±3.04) and mixed vigorously. The control had no fuel oil. Each aquarium was stocked with three fish. Water fuel dispersion in the various test aquaria and the bore water in the control were renewed daily during the 14 day experimental period. At the end of the period, samples of kidneys and liver were obtained from both exposed and control fish, preserved in 10% formol saline, processed for histological studies (stained with eosin and haematoxylin) using standard methods. Permanent sections were read under the microscope.

RESULTS

Kidneys from the control fish had glomeruli with cellular glomerular tuft. The interstitia appeared slightly infiltrated by neutrophils (Fig. 1a). Fish exposed to 75 $\mu\text{L L}^{-1}$ toxicant showed an increased glomerular cellularity and degeneration of kidney tubules with infiltrate of few neutrophils (Fig. 1b). The interstitia were infiltrated by neutrophils and occasionally lymphocytes. The vessels were essentially unremarkable. Sections from fish exposed to 150 $\mu\text{L L}^{-1}$ toxicant showed increased glomerular tuft and the interstitia was also infiltrated by neutrophils. There was mild to moderate necrosis of the glomeruli (Fig. 1c). In fish exposed to 300 $\mu\text{L L}^{-1}$ fuel oil; the kidney there had marked cellularity of tubules with infiltrations by lymphocytes and neutrophils. There was also extensive necrosis with the majority of the neoplastic tubules in the nephroblastoma stage (Fig. 1d). The interstitia were heavily infiltrated by inflammatory cells. The tubular cells were hypertrophic and the lumina contained amorphous eosinophilic materials.

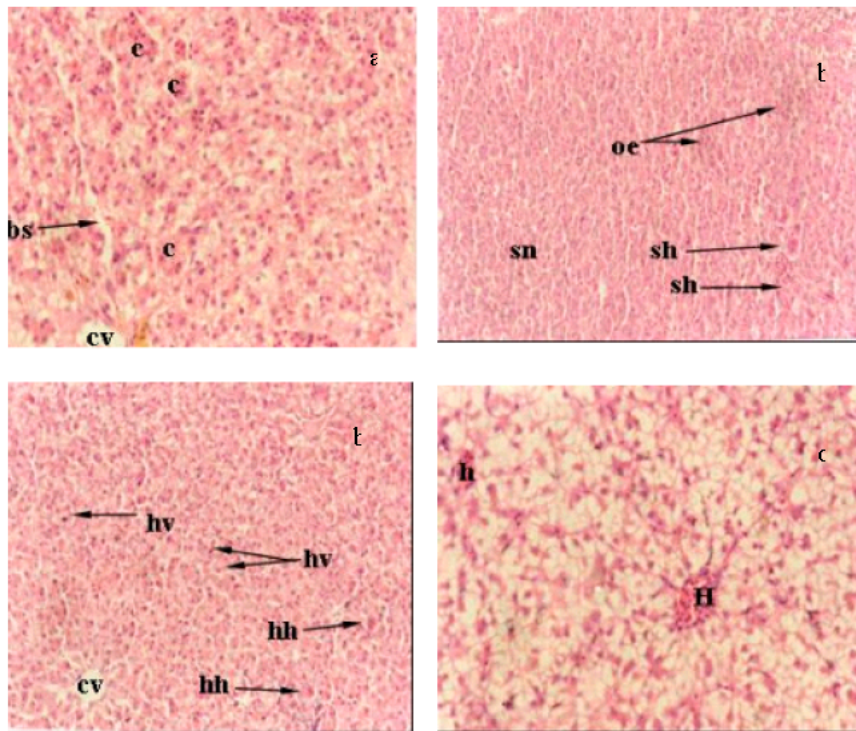


Fig. 1: Cross sections of liver from *Clarias gariepinus* exposed to various levels of kerosine for two weeks. (a) Control, (b) 75 $\mu\text{L L}^{-1}$, (c) 150 $\mu\text{L L}^{-1}$ and (d) 300 $\mu\text{L L}^{-1}$. A (Control)- hepatocytes in cords (c), blood sinus (bs) and central vein (cv). Note the uniform morphology of the nuclei and areas with mild to moderate steatosis. B- Degeneration of cords of hepatocytes and severe necrosis of hepatocytes (sn), pyknosis and karyolysis of nuclei and as well as hyalination of hepatocytes (sh). Note narrowing of sinusoid channels. C-Hepatocellular vacuolation (hv) and hypertrophy (hh). D-Hepatocellular steatosis, hyperaemia of the central vein (H) and sinusoid (h). Note the rounded vacuoles and peripherally displaced nuclei. H and E 200x

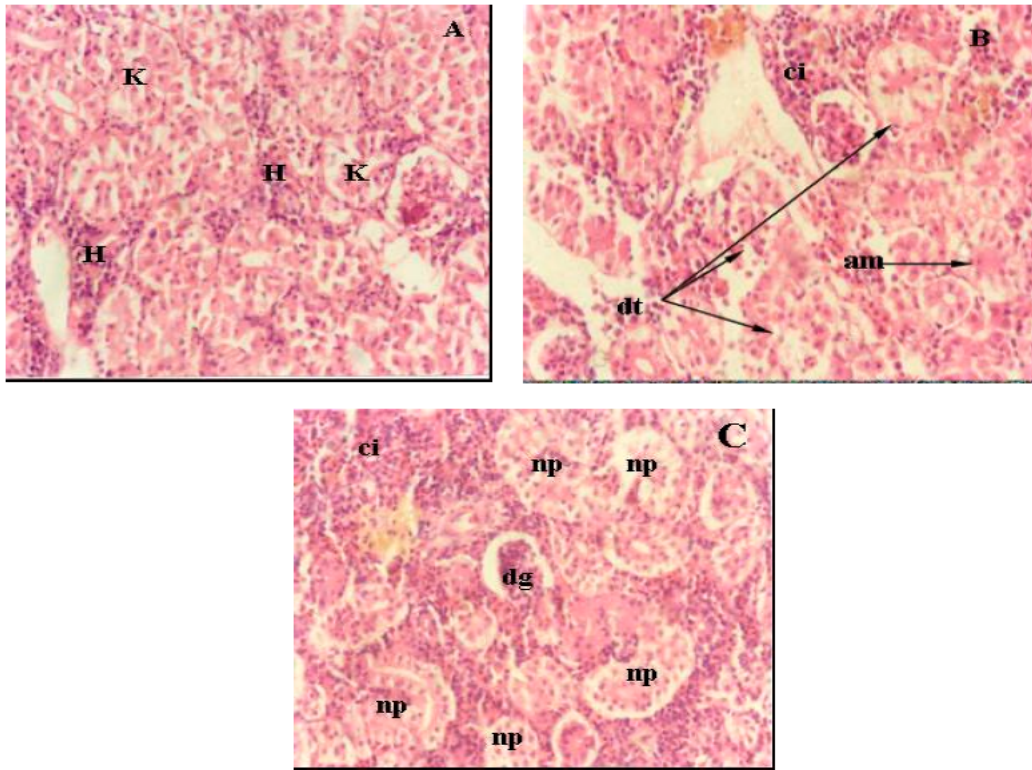


Fig. 2: Sections of kidney from *Clarias gariepinus* exposed to various levels of kerosene for two weeks. (a) Control, (b) $75 \mu\text{L L}^{-1}$ and (c) $300 \mu\text{L L}^{-1}$. Normal sections from the kidney of *Clarias gariepinus* (A). H= haematopoietic tissue, K=kidney tubule. (b)- degenerating kidney tubule (dg), cell infiltration, erythrocytes (ci), amorphous materials in kidney tubule (am). C- neoplastic kidney tubules (np). Note that majority of the tubules were in the advanced stage of neoplasia (nephroblastoma). H and E 200x

The sections of the livers from the control fish had normal tubules, haematopoietic tissues and portal triad that contained dilated portal vein. The hepatocytes were arranged in plates separated by narrow or collapsed sinusoidal channels. The histology of the livers showed uniform morphology of the nuclei and areas with mild to moderate steatosis (Fig. 2a). In the lowest concentration, there was degeneration of cords of hepatocytes and severe necrosis of hepatocytes, pyknosis and karyolysis of hepatocellular nuclei as well as hepatocellular hyalination with narrowing of the liver sinusoids channels (Fig. 2b). The central veins were congested with nucleated erythrocytes. Additional disruptive changes such as areas of centrilobular necrosis with areas of ballooning degenerative vacuolation were intense in fish exposed to $150 \mu\text{L L}^{-1}$ of the toxicant. There was also congestion of the central vein and focal areas of infiltration of the liver by neutrophils and lymphocytes. Sections from fish exposed to 300 mg L^{-1} had similar but more intense pathological changes such as necrosis of hepatocytes

compared to those in $150 \mu\text{L L}^{-1}$. There was hepatocellular steatosis, characterized by the rounded vacuoles and peripherally displaced nuclei and hyperaemia of the central vein and sinusoid (Fig. 2c). The central veins were congested with inflammatory cells. There were also areas of infiltration of the liver by neutrophils and lymphocytes.

DISCUSSION

Vacuolation of hepatocytes with pyknotic (condensed) nuclei in the liver was most likely due to deposition of glycogen and lipids (Myers *et al.*, 1987) as a result of hepatotoxicity induced by the presence of fuel oil and/ or reduced food intake (Khan and Kiceniuk, 1984). Ferguson (1989) observed that a common morphologic response of fish liver to toxicity is the loss of hepatic glycogen and/or lipid. This condition according to Wolfe and Wolfe (2005) may have occurred by direct intoxication or it may have occurred secondary to decreased body condition caused by inanition, stress or concurrent

disease. Furthermore, the authors noted paradoxically that toxic exposure may also result in the accumulation of fats as recorded in this study. Similar observations were made in *C. gariepinus* exposed to plant extracts (Fafioye *et al.*, 2004) and lead (Olojo *et al.*, 2005) *Onchorhynchus gorbuscha* exposed to water soluble/accommodated fractions of crude oil (Brand *et al.*, 2001), *O. mykiss* exposed to water accommodated trace petroleum residues (Rudolph *et al.*, 2001) and demersal rockfish exposed to crude oil (Marty *et al.*, 2003).

Fatty degeneration of the liver (steatosis) recorded in the liver of exposed fish may be suggestive of metabolic disorders and it is commonly associated with dietary deficiency in response to xenobiotic (Myers *et al.*, 1987). These changes are normally reported in diseased organisms or those exposed to toxicants [Khan and Kiceniuk, 1988; Hawkins *et al.*, 1988; Ogbulie and Okpkwasili, 1999]. It may have resulted from disturbances in any of the steps in the sequence of the events from fatty acid entry to lipoprotein exit (Mitchell and Cotran, 2004). Petroleum hydrocarbon has been shown to disrupt lipid metabolism leading to accumulation in lobsters (Capuzzo and Lancaster, 1982; Capuzzo *et al.*, 1982). The degree of fatty change in exposed *C. gariepinus* especially at 300 $\mu\text{L L}^{-1}$ far exceeded that in the control implicating the oil as being responsible for the change. Lipid or glycogen vacuolation suggests the accumulation of triglycerides usually within the hepatocytes and may have been responsible for the hepatocyte enlargement/ hyalination (Wolfe and Wolfe, 2005). Steatosis has been correlated with neoplasms, however, its role in the progression of lesions towards neoplasm formation in fish is not well understood (McCain *et al.*, 1982).

Inflammatory cell infiltration response accompanied by severe necrotic change, pyknotic and interstiti nuclei, represent the necrogenic action of the fuel on the liver and will greatly impair liver functions. Similar changes have been reported in the liver of *Astyanax* sp. Exposed to WSFs of crude oil (Akaishi *et al.*, 2004) *Perca fluviatilis* and gold fish exposed to oil sand process affected water (Nero *et al.*, 2006). Centrolobular necrotic change was the main change in *C. gariepinus* exposed to fuel oil and agrees with the observation of Popp (1991) who suggested that the distribution of the interstitial system in the liver resulting in a higher concentration of the toxicant in the centrolobular region account for the occurrence and frequency of centrolobular toxicity.

The changes recorded in the kidney section from the exposed fish such as increased glomerular cellularity, interstitial being infiltrated with blood cells, distortion and disarray of the glomeruli that were concentration-dependent were similar to those caused by petroleum in English sole (Mc Cairn *et al.*, 1978) and rats (Dede and

Kagbo, 2001) and *C. gariepinus* exposed to plant extracts (Onusiriuka and Ufodike 2000, Fafioye *et al.*, 2004) for various periods. Peripheral blood and cephalic kidney of turbot, *Scophthalmus maximus* and Atlantic cod, *Gadus morua* had micronuclei and severe nuclei abnormality such as nuclear buds, binucleated and fragmented-apoptotic cells) induced by crude oil and nonylphenol (Barsiene *et al.*, 2006). exposure. Such changes and neoplasia of kidney tubules particularly at the highest concentration recorded in this study may greatly impair the filtration functions of the kidney with grave consequences for the exposed fish. However, the onset development and extent of these changes may be toxicant, concentration and exposure time dependent.

Results from this study strongly suggest that exposure to sublethal concentrations of No.1 fuel can cause remarkable changes in the kidney and liver of exposed *C. gariepinus*. Hence, the pathology of these organs could serve as important biomarkers of No.1 fuel toxicosis. Asides, disruptive 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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