Toxicological Pathology of Juvenilecatfish Exposed to Petrol

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Abstract— *Heterobranchuslongifilis juveniles weighing* $22.2 \pm 0.1g$, *length* $16..2 \pm 0.1cm$ *were acclimatized for seven (7) days with daily renewal of water to maintain good physico-chemical quality of water suitable for the fish. Acute toxicity test of refined petroleum product (petrol) was carried out in the laboratory to determine its effect on juveniles of Heterobranchus longifilis. Different concentration were obtained through exploratory test of 1ml of toxicant in 9ml of water,1ml of toxicant in 99ml of water, 1ml of toxicant in 999ml of water, The research was carried out using five (5) concentrations, (4.0ppm, 8.0ppm, 10.0ppm and 12.0ppm) of petrol, and a control, replicated three (3) times. Results showed behavioral changes, organ abnormalities and that petrol was toxic to the test fish even at the lowest tested concentration. Percentage mortality of 90%, 70%, 50%, 30% and 10% was recorded in 12.0ppm, 10.0ppm, 8.0ppm, 6.0ppm and 4.0ppm concentration of petrol at 96 hours exposure respectively. The result also showed that the mortality of Heterobranchus longifilis juveniles exposed to petrol in water increased with increase in concentration and time of exposure. The liver showed vacuolar degeneration in the hepatocytes, focal areas of necrosis and fibrosis, aggregations of inflammatory cells between the hepatocytes, dilation and congestion in blood sinusoids and thrombosis formation in the central veins, while the muscle tissues were moderately affected after the exposure. The entire test organisms in the control showed no histological abnormalities, while their staining patterns and cellular arrangement remained unaffected. The tissue was viewed under digital light microscope at the magnification of (X100 and 400).*

Keywords - *Toxicology, Pathology, Heterobranchus longifilis, Petrol.*

I. INTRODUCTION

The Niger Delta is characterized by exploration and exploitation and exportation of crude oil and it related activities. These activities have impacted negatively on the aquatic fauna in the Niger Delta environment and constitute a threat to the biodiversity of the aquatic organisms.Petroleum hydrocarbons and their products can enter the aquatic environment via different sources such as petroleum extraction, transportation, urban runoff; biogenic hydrocarbons produced naturally, accidental discharges and oil spills (Botello *et al.*, 1997). Petroleum hydrocarbons are lipophilic in nature and can extremely be taken up by a wide spectrum of marine and fresh water animals and accumulate in useful lipid compartments like cellular membranes (Neff, 1979, De la Torre *et al*, 2000), disturb the physiological and physico-chemical membrane properties and can integrate into biological system (Sikkema, *et al.*, 1994.).

Many physiological processes such as enzyme function, muscle contraction and osmoregulation, are directly dependent on the unique properties of biological membranes (Neff, 1979). Some petroleum compounds have the potentials to be biomagnified through the food chain (Neff, 1979). Petroleum pollutants tend to accumulate more in the organism than the environment, therefore fish are largely being used for the assessment of the quality of the aquatic environment and can be used as bio-indicators to evaluate the environmental contaminant levels (Lopes *et al.*, 2000; Ankyakora *et al*., 2005).

Petrol is the mixture of hydrocarbons obtained from crude oil through the process of fractional distillation. Fractional distillation basically involves heating a mixture at different temperature to obtain individual compound. It is a complex mixture of volatile hydrocarbons containing paraffins (alkanes), naphthenes (cycloalkanes), olefins (alkenes) and aromatics with carbon numbers predominantly between C4 and C12. Itah and Essien (2005). The major component of premium motor spirit are hydrocarbons and they are known to be oxygen demanding wastes which create a biological oxygen demand (BOD) that could completely deplete the oxygen resources in a given body of water (Helm, 1980).

Histology represents a useful tool to assess the degree of pollution. The description and assessment of histological changes in different organs represent a very sensitive and crucial parameter in determining cellular changes that occur in target organs such as gills, liver and gonads (Dutta, 1996). The harmful effect of pollutants on fish liver, gill, and muscle histology may depend upon the duration of the exposure (chronic or acute), and the concentration level of the specific pollutants as well as other factors such as temperature, age of fish, interaction with other pollutants, water chemistry and metabolic activity of the fish (Heath, 1995) .

Although literatures abound on the effects of crude oil on *Clarias gariepinus*, there is paucity of such information on *Heterobranhus longifilis.* Though a common catfish in Nigerian waters, its toxicity responses have received little investigative studies in

comparison to other local species. Its high fecundity, high level of disease resistance and good flavor (Erondu *et al*., 1993 and Nwadukwe, 1993) makes it an important food item worthy of indebt research attention. It is against this background that the need to determine the acute toxic concentration of refined petroleum product on *H. longifilis* arose.

II. MATERIALS AND METHODS

A. *Source and Collection of Test Fish*

A total of one hundred and eighty (180) juvenile of *Heterobranchus longifilis* (Valenciennes, 1840) of mean weight (22.2±0.1g) were collected from Department of Fisheries, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. The juveniles were weighed and transferred into an experimental tank for acclimation for seven (7) days with daily renewal of water to maintain good physico-chemical quality of water suitable for the fish. The fish were fed twice a day at 8.00am and 5.00pm with commercial fish feed daily at 3% body weight for 96 hours (4 days). The dissolved oxygen (DO) content of water was maintained by aeration pumps connected to the experimental aquarium. It is from the acclimated fish that test fish were selected at random for the study.

B. *Experimental Design*

For experimental set-up a total of eighteen aquaria of 25 liters each was used for five (5) concentrations, replicated three (3) times and control. Ten (10) juvenile (22.2g±0.1g) were randomly allotted to each aquaria and filled with 20 litres of borehole water. The aquarium were labeled, control, 4.0ppm, 6.0ppm, 8.0ppm,10.0ppm,and 12.0ppm respectively. The aquariums were covered with nylon mesh screen. This was to prevent the fish from jumping out of aquarium. The artificial aerating of water was stop because according to Sprague (1972) aeration degrades toxicant in the aquarium. The experiment was a continuous flow through auto gravimetric system. The experiment which lasted for 96 hours (4 days) involved three phases.

C. *Preparation of Petrol Concentrations*

The refined petroleum (petrol) was obtained from Nigeria National Petroleum Cooperation (NNPC) Mega Station at Umuahia along Enugu – Port Harcourt express road, about 15km north of Abia Tower. This is to get the purest petrol as many independent filling stations within Umuahia town may be dealing on adulterated petrol. Exploratory test was conducted in which the experimental fish were exposed to the toxicant prepared as follows:

1 ml of toxicant in 9ml of water = 0.1
1ml of toxicant in 99ml of water = 0.01
1ml of toxicant in 999ml of water = 0.001ppt

Screening test was carried out in 0.1, 0.01 and 0.001 of the petrol concentration in a 1L beaker with single fish. Concentration of petrol that caused death within 30 minutes was omitted from the definitive test (Gurure, 1987). Test solutions were prepared by serial dilutions from the petrol sample of 0.001ppt which was the stock solution. 1ml of the stock solution in another 1000ml of dilution water gave the concentration of one part per million (1ppm). (OECD, 1992; FAO, 1984). From the stock solution, the following dilutions were made 0.08, 0.12, 0.16, 0.20, and 0.24 to obtain test concentration of 4ppm, 6ppm, 8ppm, 10ppm and 12ppm in each aquarium containing 20 litres of borehole water. The safe concentration value was calculated by multiplying the 96 hrs LC_{50} by an application factor of 0.1 (EIFAC, 1983).

D. Sample Collection and Histopathological Analysis

Twenty four hours after last exposure, the animal were anesthetized with chloroform vapour and dissected. The harvested tissues were carefully dissected out, trimmed of all fat and connective tissue blotted dry to remove any blood. The tissues were fixed in 10% formal saline, and then transferred to a graded series of ethanol. On day 1, they were placed in 70% alcohol for 7 hours, then transferred to 90% alcohol and left in the latter overnight. On day 2, the tissues were passed through three changes of absolute alcohol for an hour each then cleared in xylene. Once cleared, the tissues were infiltrated in molten paraffin wax in the oven at 58°C. Three changes of molten paraffin wax at one-hour intervals were made, after which the tissues were embedded in wax and blocked out. Prior to embedding, it was ensured that the mounted sections to be cut by the rotary microtome were orientated perpendicularly to the long axis of the Tissues. The sections were designated "vertical sections". Serial sections of 5 µm thick were obtained from a solid block of tissue, fixed on clean albuminized slides to prevent sections coming off the slides and later stained with haematoxylin and Eosin staining techniques, after which they were passed through ascending grade of alcohol, cleared in xylene and mount in DPX mountant, allowed to dry at room temperature and observed histopathologically under digital light microscope.

III. Results

The liver histology of the test fish in the control appeared relatively normal (plate A).

Compared to the control specimens, various histological changes were identified in the livers of juvenile *H. longifilis* exposed to the petroleum hydrocarbons (PHC). PHCs induced alterations in the histoarchitecture of the liver. The relevant histological alterations observed were: vascular and cellular

degeneration, hyperplastic hepatocyte, cytoplasmic vacuolation and inflamation

The blood sinusoids were observed to be dilated between the cords of hepatocytes. According to Nabila *et al*., (2009) and Van Dyk *et al*., (2007), vacuolations of hepatocytes are associated with the inhibition of protein synthesis, energy depletion, disaggregation of microtubules, or shifts in substrate utilization. In this study, all treatments led to hepatocytic necrosis compared to control groups. Hepatocytic necrosis changes were observed in fish at different concentration of the refined petroleum.

Degeneration was observed at all concentrations, and it mostly occurred as granular degeneration (plate B-G). There were a lot of small grains of powder in hepatocytes and this gave a blurred appearance of the observed parenchymal tissue. The parenchymal degeneration in the samples were combined with vacuolar (watery) degeneration (plate B-G). Aggregations of inflammatory cells between the hepatocytes were observed as well. The present result agrees with toxicological impact of diesel fuel on the vital organs of Oreochromis mossambicus and Saxena *et al* (2008) who observed these alterations in Tilapia zillii and Solea vulgaris under the influence of different pollutants from Lake Qarun, Egypt.

HISTOLOGICAL SECTION THROUGH THE LIVER

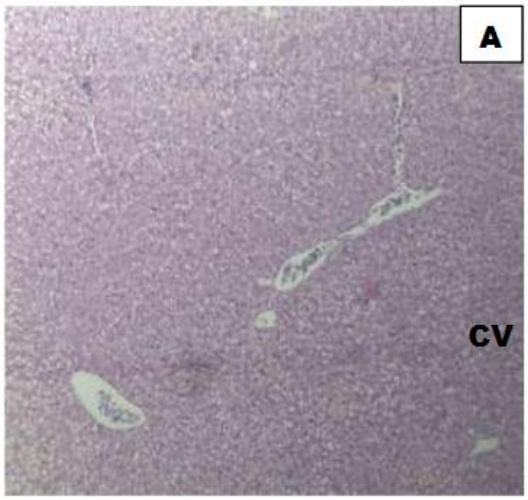

Plate A(Control)
Normal cellular pattern of portal triad, sinusoidal lining radiating outwardly from the central vein,the hepatocytes are conspicuously seen, no abnormality seen.

C**onclusion:**Not affected.

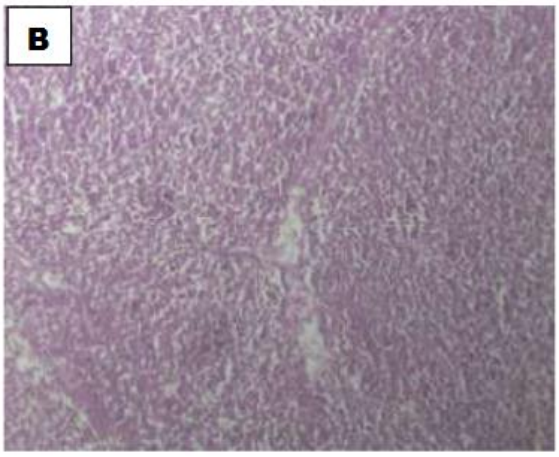

Plate 5: **Treatment 2 of the liver (4.0ppm)**
Abnormal cellular pattern of portal triad, vascular degeneration and hyplastic hepatocyte with cytoplasmic vacuolation.

C**onclusion:** Moderately affected.

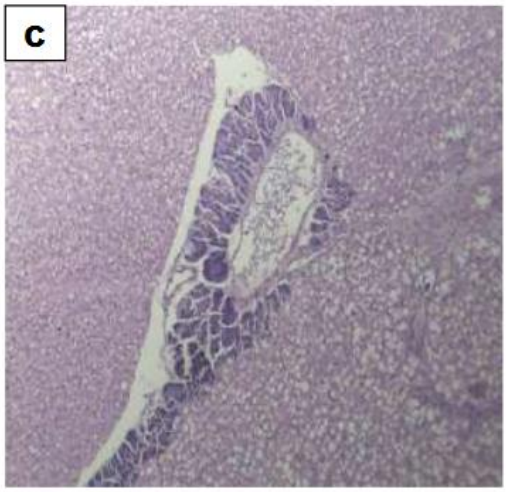

Plate C (6.0ppm)
Abnormal cellular pattern of portal triad, vascular and cellular degeneration and hyplastic hepatocyte, cytoplasmic vacuolation and inflammation.

C**onclusion:** Moderately affected

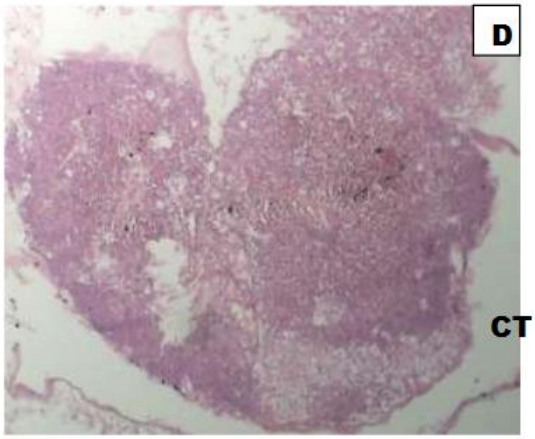

Plate D (8.0ppm)

Abnormal cellular pattern of portal triad, vascular and cellular degeneration and hyplastic hepatocyte, cytoplasmic vacuolation, and inflammation .

Conclusion: Moderately affected.

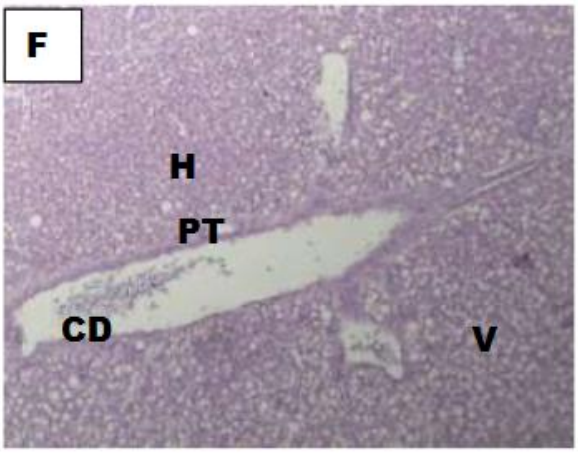

Plate F(10.0ppm)
Abnormal cellular pattern of portal triad, vascular and cellular degeneration and hyplastic hepatocyte, cytoplasmic vacuolation. and inflammation .

Conclusion: Strongly affected

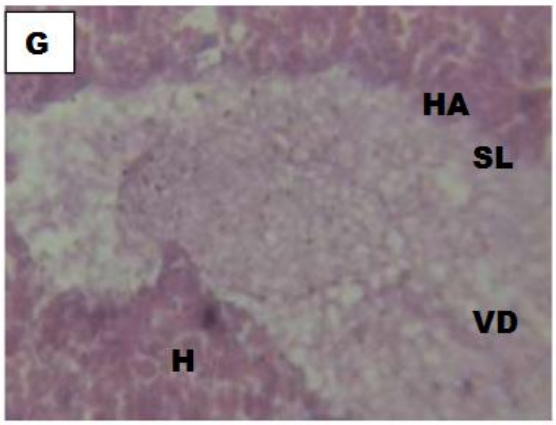

PLATE 9: TREATMENT 6 OF THE LIVER (12.0PPM)
Abnormal cellular pattern of portal triad, vascular and cellular degeneration and hyplastic hepatocyte, cytoplasmic vacuolation and inflammation.

Conclusion: Strongly affected

IV. CONCLUSION

The present study has shown the toxicity of petrol on liver, gill and muscle in fresh water fish *Heterobranchus longifilis*. The findings of the present histological investigation demonstrate a direct correlation between petroleum hydrocarbons (PHCs) lethal concentrations and exposure time with histopathological disorders and lesions observed in liver tissue.

Therefore, histopathology is a useful biomarker for environmental contamination, since it discriminated between control and test groups. The liver was observed to be affected by petrol to which the fish was subjected. *H. longifilis* is shown to be appropriate for environmental monitoring and *in situ* because of its hardy nature.

The vital component of petrol, hydrocarbon may get accumulated in fish tissue and cause cancer in consumers. Hence, the environmental manager should act appropriately in time to prevent the spilling of petrol in the aquatic environment.

V. RECOMMENDATION

From the report of this study, it is recommended that aqua culturists should ensure that ponds are located in places isolated from petrol pollution source. In other to achieve this environmental impact, assessment / feasibility studies must be carried out before siting fish ponds so that all sources of pollution will be identified. Periodic checks of the pond for oxygen deficiency must be carried out because lack of oxygen in the pond has a negative effect on the fish survival and reproduction. The best resistant species of fish must be selected for aqua culture practice.

VI. LIST OF ABBREVIATIONS

H=Hepatocytes, PT= Portal triat, CV= Central vein, HH= Hapatocitic hyperplesia, VD= Vascuolation, A= Atrophy, CT=Connective tissue, CD=Cellular degeneration, HA=Hepatic artery, SL=Sinusoidal linings.

VI. ACKNOWLEGEMENT

I wish to express my profound gratitude to my supervisor Professor G . C . Onuoha for her encouragement, guidance, suggestions and correction during the experiment.

I am also grateful to Dr Samson Oyebadejo of gross anatomy laboratory, Department of Human Anatomy, College of Health Sciences, University of Uyo.

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