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Histopathology of the Gills and Livers on African Catfish (*Clarias gariepinus*) Juvenile Exposed to Pentachlor

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Abstract: The histopathological effect of pentachlor, an organochlorine pesticide, on the gill and liver tissues of the African catfish (Clarias *gariepinus*) juvenile was carried out to investigate the toxicity of pentachlor when administered on the test sample within concentrations of 0.073 mg/L, 0.036 mg/L, 0.024 mg/L and 0.018 mg/L and a control in those replicates was used. The fish were acclimatized for 7 days under laboratory conditions before exposure. Sixteen (16) troughs, each of seven (7) juvenile samples of length 2.3 cm and average weight of 10-15 g, were used for the study. Twelve (12) troughs exposed to varying concentrations of pentachlor were used as treatment samples while four (4) troughs served as control. The juveniles were exposed to sub-lethal concentrations for 21 days with a break at every 48 h. The gills and livers were collected for histological examination. Histological data revealed that at 0.1 m/L pentachlor concentration, the gills showed epithelial hyperplasia with heamorrhage in the central venous of the cartilaginous core with the lifting of the epithelia and hypertrophy at 0.2 mg/L. At 0.3 mg/L exposure, epithelia hyperplasia, dilation of the secondary lamellae occurred and severe deformation of the secondary gill lamellae at 0.4 mg/L. However, the control presented normal mucous and epithelial cells with normal secondary and primary gill lamellae. The liver sample treated with 0.1 mg/L showed complete vascular degeneration. Control sample revealed normal blood vessels, sinusoid vessels and hypatocystes. This showed that pentachlor can accumulate in the liver and potentially cause damage to tissue over time.

Key words: Pentachlor, *Clarius gariepinus*, histopathology, sub-lethal, organochlorine.

1. Introduction

The degree of contamination in aquatic environment is frequently assessed by comparing contaminant concentration in associated biota [1].

In Nigeria, farmers often use agrochemicals containing pesticides made of polychlorinated hydrocarbons and organophosphates to protect crops and animals from insects, weeds and diseases [2]. However, the increasing use of pesticides to affect pest control in farms has let the extensive investigations on their harmful effects on aquatic flora and fauna [2]. Among these pesticides are abemection, carbonfuran, chlorpyrigos, dichlorvos, dimethoate, etc. and they are known to be commonly used in most urban areas in Nigeria.

Intensifications in agriculture have caused a lot of threats to marine life as a result of toxicity of pesticides, herbicides and other chemical which contains polychlorinated biphenyls which accumulate over time with the attendant dangers [3].

When pesticides enter aquatic systems, the environmental costs can be high eliminating thousands of fishes and other aquatic species with long term and irreversible consequences which manifest changes in the genetic structure of certain species and may even lead to their complete extinction [4].

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The assessment of the ecotoxicological risks and effects of pesticide preparations to non-target organisms has been studied Fishes are among the group of non-target aquatic organism [5]. Histological changes provide a rapid method to detect effect of irritants, especially chronic ones, in various tissues and organs [6]. Therefore, it was decided to determine the histopathological effect on gills and liver tissues in African catfish exposed to pentachlor.

PCBs are absorbed throgh the gastro-intestinal tract and distributed throughout the body of fishes and humans. Studies of individual PCBs congeners indicate in general that PCBs are readily absorbed, with an oral absorption efficiency of 75% to greater than 90%. because of their lipophylic nature, PCBs especially the more chlorinated congeners (tetra to hexachlorobiphenyl - 4 to 7 chlorine atoms) tend to accumulate in lipid tissues. Greater amount of PCBs are found in the liver, adipose tissue, lungs, skin and breast milk

Long-term persistence of polychlorinated biphenyls (PCBs) in aquatic environments poses significant threats to fish and human health, worldwide. Although PCBs have not been causally linked to declines in fish populations, PCB exposure is associated with acute and chronic immune-suppression [7] and can increase disease susceptibility [8], thereby reducing long-term population fitness and survival. Coplanar PCBs are thought to be the most toxic due to their affinity to the aryl hydrocarbon receptor (AhR), which results in dioxin-like effects via up-regulation of the *CYP1A1* gene [9]. However, recent evidence suggests that non-coplanar PCBs can pose endocrine and immunosuppressant effects that are independent of AhR activation [10, 11]. Consequently, the PCB mixtures that were widely manufactured and distributed and that contained several different coplanar and non-coplanar PCB congeners can influence immune health via interacting physiological pathways. Previous studies have largely focused on congener-specific immune response, particularly of coplanar PCBs [11], and less is known about the immune response of fish to PCB mixtures. The use of pesticides in agriculture has risen significantly to meet human demands for food, clothing and medicine. Causes of pollution in water bodies are recognized globally as a serious issue affecting both human and no-human population. The extent of tissue damage in aquatic life is related to the degree of environmental contamination. The level of toxicant and the duration of exposure are additional factors that influence the degree of distortion.

2. Methodology

2.1 Study Area

This research was conducted at the laboratory of the Department of Environmental Management and Toxicology in Michael Okpara University of Agriculture, Umudike. Umudike is about 5 km from Umuahia, the capital city of Abia State. It is located along Umuahia Ikot-Ekpene road in Abia State.

2.2 Sample Collection and Acclimatization

Juvenile of *Clarias gariepinus* with body weight 10 ± 0.33 g obtained from MOUAU fish farm were allotted into ten (10) troughs with ten fish (10) per trough. The fish were acclimatized for seven (7) days in which they were fed with commercial feed at 5% body twice a day under laboratory condition. Dead and abnormal ones were removed leaving the healthy ones for the analysis. Excess feed and faeces were siphoned out on daily basis.

2.3 Experimental Setup

The acclimatized juvenile fish were collected using scoop net. Sixteen (16) troughs each containing 7 juvenile fish of 2-3 cm length with average weight of 10-15 g were used. The test samples were exposed to four different treatments and these were conducted in two replicates. Twelve (12) troughs served as treatment samples while 4 troughs served as control. Whereas eighty-four of the samples were exposed to treatment water of known amount of PCB of pentachlor, the other

twenty-eight served as a control and were exposed to water free from pentachlor. The working samples were exposed to the water for twenty-one days at interval of seven days. After each interval, the fish samples were randomly selected, sacrificed and their blood was taken for analysis. After the 21 days the fish samples were also selected and sacrificed with their tissues taken for histological test.

2.4 Preparation of Test Solution

A total of 1.34 mL of pentachlor (pentachlorobiphenol) was introduced into a beaker using a micropipette and made up to 1,000 mL with distilled water. This gives exactly a concentration of 1.0 g/dm^3 or 1,000 mg/L.

2.5 Chronic Pulse Toxicity Procedure

Chronic toxicity test was carried out by following standard acute static fish toxicity test procedure [12] to evaluate the lethal concentrations of pentachlor. Five samples of *C. gariepinus* juveniles were exposed in duplicates to four low concentrations of the PCB and a control in a set of completely randomized design. Stock solution of 1 g/L was prepared as described above after which 4 mg/L, 6 mg/L and 8 mg/L pentachlor test solutions were prepared. Seventy-five juveniles were then exposed to each test solution in a plastic aquarium. Fifteen juveniles were kept in fresh water as control. Temperature, pH, dissolved oxygen, electrical conductivity and total dissolved solids of the test solution were monitored during the experiment.

2.6 Sub-lethal Test Procedure

Stock solution of 1 g/L was prepared as described above after which test solutions of 0.073 mg/L, 0.036 mg/L, 0.024 mg/L and 0.018 mg/L were prepared. A semi-static toxicity test procedure was used in which 112 juvenile *C. gariepinus* will be randomly distributed to 5 experimental groups; group A—control (no exposure), group B—exposed to 0.073 mg/L of pentachlor for 4 h and transferred to fresh water, while group C—exposed to 0.036 mg/L of pentachlor for 4 h and transferred to fresh water; Group D was exposed to a solution containing 0.024 mg/L of pentachlor while group E was exposed to a solution containing 0.018 mg/L of pentachlor. The test solutions were renewed every 48 h after changing the water. After 24 h, 7 days, 14 days and 21 days respectively, three samples from each test group waere randomly selected and blood was collected for haematological analysis. Fish were feed throughout the experiment.

2.7 Assessment of Behavioral Changes

After exposure of the fish to various concentrations of the toxicant, observations were carried out on the behavioural and morphological responses of the fish. Control fish were also monitored along with the toxicant concentrations to provide a reference for assessing any behavioural or morphological changes. Responses were recorded on their differed from the control. The behavioural and morphological characteristics monitored were erratic swimming, loss of equilibrium, general activity, increased excitability, mortality, vertical suspension, mucous secretion, startle response and deformities. Each container was observed for 10 to 15 min which allowed sufficient time for an accurate evaluation of each fish.

2.8 Histological Study Procedure

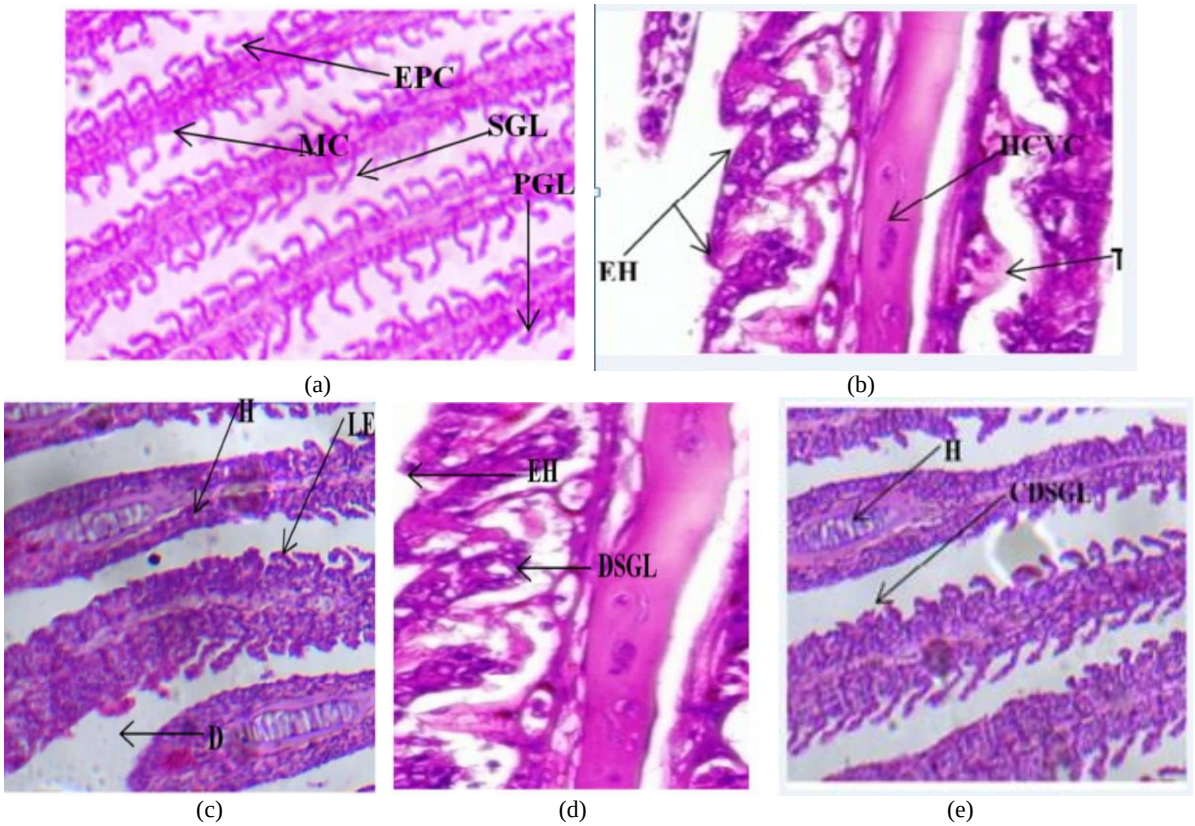
The liver and gills of fish from the control and exposed groups were studied and compared for histological changes. These organs were collected from fish and immediately fixed in 10% formalin for 24 h according to Velisek, Svobadova and Machova [13] and Khoshnood, Jamili, Khodabandeh, Mashinchian and Motallebi [14]. Specimens were processed further by passing through graded series of alcohol (30%, 50%, 70%, 95% and absolute ethanol) for 2 h each. This is the dehydration process. Specimens were later passed through xylene (clearing agent) and embedded in paraffin wax of 60 ℃ melting point. From the preserved paraffin blocks, 3 μm thick sections were

obtained using a rotary microtome and sections were further processed. The sections were floated on a water bath which maintained at 2 to 3 ℃ below the melting point of paraffin wax. They were placed on a hot plate thermostatically and maintained at a temperature of 2 to 3 ℃ above the melting point of paraffin wax. When properly dried (15 to 30 min), they were stained with haematoxylin and eosin (H and E), dehydrated, cleared and mounted on clean glass slides, avoiding air bubbles and observed under a light microscope [15].

Haematoxylin and eosin stainings were used for the demonstration of general tissue structures in various colours. The nuclei as well as some calcium salts and urea take up blue colour. Other tissue structures appear red, pink or orange in color (eosinophilic) [15]. These slides were subsequently made into permanent slides. The permanent slides prepared were mounted one after the other and viewed at different magnifications of the microscope. Photo-micrographs of the slides were taken and the results presented in plates as seen in Figure 1.

3. Results

The histopathological effect of pentachlor, an organochlorine pesticide, on the gills and livers tissue of the African catfish (*Clarias gariepinus*) juvenile was carried out to investigate the toxicity of pentachlor when administered on the test sample of *C. gariepinus* and results as presented.



(a) (b) (c) (d) (e)

Fig. 1 Histopathological Test Result of Gills Exposed to Pentachlor

(a): Control Gill, showing Mucous Cell (MC), Epithelial Cell (EC), Secondary Gill Lamella (SGL) and Primary Gill Lamellae (PGL). (b): Gill exposed to 0.1 mg/L pentachlor showing Epithelial Hyperplasia (EH), Haemorrhage in the central venous of cartilagenous core (HCVC) and Telengiectasia (T). (c): Gill exposed to 0.2 mg/L pentachlor showing Lifting of the Epithelial (LE), Desquamation (D) and Hypertrophy. (d): Gill exposed to 0.3 mg/L pentachlor, showing Epithelial Hyperplasia (EH), Dilation of the Secondary Gill Lamellae (DSGL). (e): Gill exposed to 0.4 mg/L pentachlor, showing Hypertrophy (H), Congestion and Deformed Secondary Gill Lamellae (CDSGL).

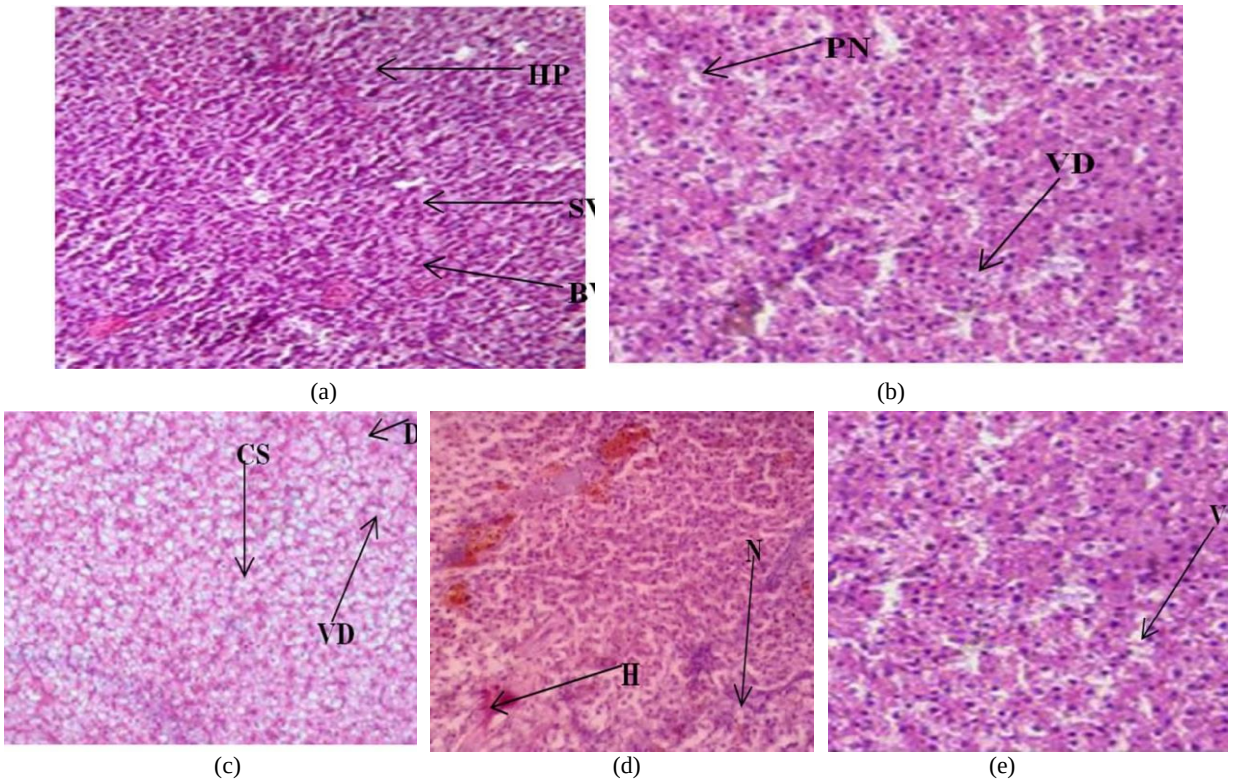


Fig. 2

(a): Control Liver, showing Hypertocytes (HP), Sinusoid Vessels (SV) and Blood Vessels (BV). (b): Liver exposed to 0.1 mg/L pentachlor, showing Psychotic Nuclei (PC), and Vascular Degeneration (VD). (c): Liver exposed to 0.2 mg/L pentachlor, showing Vascular Degeneration (VD), Degeneration of Hypertocyte (DH) and Congestion of Sinusoids (CS). (d): Liver exposed to 0.3 mg/L pentachlor, showing Hemosiderin (H) and Necrosis (N). (e): Liver exposed to 0.4 mg/L, showing Vascular Degeneration (VD).

4. Discussion

Results of histopathological study on the gills and liver of *C. gariepinus* exposed to varying concentrations of pentachlor revealed that at 0.1 mg/L pentachlor concentration, the gills showed epithelial hyperplasia with heamorrhage in the central venous of the cartilaginous core with the lifting of the epithelia and hypertrophy at 0.2 mg/L and 0.3 mg/L exposure, epithelia hyperplasia occurred with dilation of the secondary lamellae and severe deformation of the secondary gill lamellae at 0.4 mg/L; however, the control presented normal mucous and epithelial cells with normal secondary and primary gill lamellae. The liver sample treated with 0.1 mg/L showed complete vascular degeneration. Control sample revealed normal blood vessels, sinusoid vessels and hypatocystes.

Gills serve as a protective barrier against toxicant, reducing the absorption and accumulation of harmful substances in the organs. However, because they are constantly in contact with water, gills are directly exposed to pollution. At low concentration of 0.1 mg/L, epithelial hyperplasia, haemorrhage in the central venous of the cartilagenous core occurred which worsened as the concentration increased to 0.2 mg/L and above. There was a significant difference between the gills of the control and those of the samples exposed to pentachlor. The liver samples also showed significant changes ranging from psychotic nuclei to vascular degeneration and necrosis as the concentration of pentachlor increases. The liver is a major organ that performs various metabolic functions, playing a crucial role in processing nutrients and removal of harmful substances. Pentachlor can accumulate in the liver and lead to various anomalies affecting its normal metabolic function and potentially causing damage to tissue over time.

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