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Histopathologic lesions in the liver of African catfish *clarias gariepinus* exposed to sublethal concentration of ethanolic extract of *piper guineense*

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ABSTRACT
Post fingerings of *Clarias gariepinus* (size ranged and body weight, 8.0 – 10.5 cm and 12 – 16 g) respectively were exposed to sublethal dose of ethanolic extract of *Piper guineense* in a static bioassays to assess its histopathological effect on the fish liver. The findings revealed the destructive potential of *P. guineense* as an ichthyotoxic plant as exhibited by cell Hyperthrophy, Vascular degeneration, Vacuolisation, vascular congestion, granulation of eosinophilic cells and a remarkable degree of necrobiosis, the degeneration process leading to cell death.

Keywords: Histopathologic legions, liver tissue, *Clarias gariepinus*, Ethanolic Extract, *Piper guineense.*

1. Introduction
Ethanolic extract of *piper guineense* has been found to have insecticidal property to insect species including *Callosobruchus maculatus* and *Musca domestica* (Ivbijaro and Agbaje, 1986; Ivbijaro, 1990; Olaifa *et. al*, 1987). This botanical has also been found to be toxic to Cichlid fishes, *Oreochromis niloticus* and *Sarotherodon galilaeus* (Okorie *et al*, 1992; A.O. Okon, 2000).
However studies on its effects on catfish has is scanty. The potency of toxicants on fishes has been assessed using various parameters such as rate of opercular movement (Stoner et al, 1978; Holcome *et al*, 1982), Histopathological changes in tissues (Mathiessen and Brafield, 1973), Swimming ability (Spraque, 1971), and Feeding behaviour (Bardach *et al*, 1965).
P. guincense, commonly termed West African black pepper is an ichthyotoxic plant which belongs to the family Piperaceae. It is distributed throughout the tropical and subtropical regions of the world. It is sold locally as spice and medicine for currying stomach disorder (Dalzie, 1948).
The present investigation intends to give a fuller information on the impact of *P. guineense* on a species of fish that is most reared in Nigeria and also known to be very hardy in terms of adapting to stressful environments. The histopathologic implications of the botanical in the liver tissues are assessed.

2. Materials and Methods
2.1 Collection of Test Organisms
The post fingerlings of *Clarias gariepinus* (size ranged and body weight, 8.0 – 10.5 cm and 12 – 16 g) respectively were colleted from Vision park Farms, a reputable private fish farm in Uyo, Akwa Ibom State, Nigeria. They were transported to the laboratory in polythene bag containing aerated water. The fish were held in batches in aerated glass aquaria containing dechlorinated tap water to avoid stressful Condition. The fish were fed once a day with formulated feed. The faecal pellets and left over feed were siphoned out each day.
The water in each tank was replaced twice a week to avoid contamination. The fish were acclimatized for a minimum of seven days prior to treatment. They were accepted as being fully adapted where no death was observed for four consecutive days (FAO, 1986). Feeding was stopped 24 hrs prior to each set of experiment.

2.2 Preparation of Ethanolic Extract of *Piper guineense* (EEPG)

Some quantities of *Piper guineense* fruits were dried at 60 ^{0}C for 72 hrs, then ground using an electric blender. The powder form collected was stored in an air tight bottle until used. The homogenized sample of 450 g was later extracted with ethanol. The extract was evaporated to dryness in a rot evaporator at about 60 ^{0}C to obtain a crude brownish semi solid substance that weighed 150.5 g. The substance was wrapped with a black material to avoid light penetration and later stored in a refrigerator until needed.

2.3 Test Procedure

Ten fish were selected randomly and distributed in batches and placed in three aquaria containing test solution (0.4 g of extract free water only). Each set of experiment was replicated twice with a control. Temperature and pH were determined at the start of the experiment and maintained optimal levels.

The fish were exposed to 0.4 g (96 hr LC_{50}) for 21 days (Okorie, *et. al*; 1992). During the duration of the experiment, water in the tanks were replaced after every 48 hr with freshly prepared extract solution. After the exposure period, fish from the experimental and control aquaria were dissected. The liver tissues were collected and fixed in bouin's fluid embedded in paraffin and sectioned (7 microns thickness) and lator stain with haematoxylin/eosin stain. Histopathological changes due to treatment with the ethanolic extract of *P. guineense* were noted and photomicrographs taken.

3. Results

There were marked histological alterations in the liver tissue of *C. gariepinus* exposed to 0.4 g/l of ethanolic extract of *Piper guineense*. Plates 1a, 1b, and 2a, 2b illustrate the changes in liver controlled treated at different magnifications, x 100 and x 400.

In the control liver tissue Plates 1a and 1b at magnifications x100 and x400 respectively, the hepatocytes (H) are arranged in a definite chord like pattern with prominent lumen (L) and sinusoidal layer (SL). The nucleolus of each hepatocyte is either sphericall or lightly ovoid with regular surfaces scattered chromatin granules and one or more nucleoli.

The Hepatic tissue from treated fish at magnifications x 100 and x400, (Figs 2a and 2b respectively) revealed cellular disorganization as seen by the breakdown of the chord – like arrangement of hepatocytes (H) (compared with Figs 1a and 1b) and a remarkable degree of necrobiosis; the degenerative process leading to cell death. There is vascular degeneration (VD) and congestion, evidence of granulated eosinophilic cells, rupture of cell (GEC) membrane and thickening of connective tissue (CT).

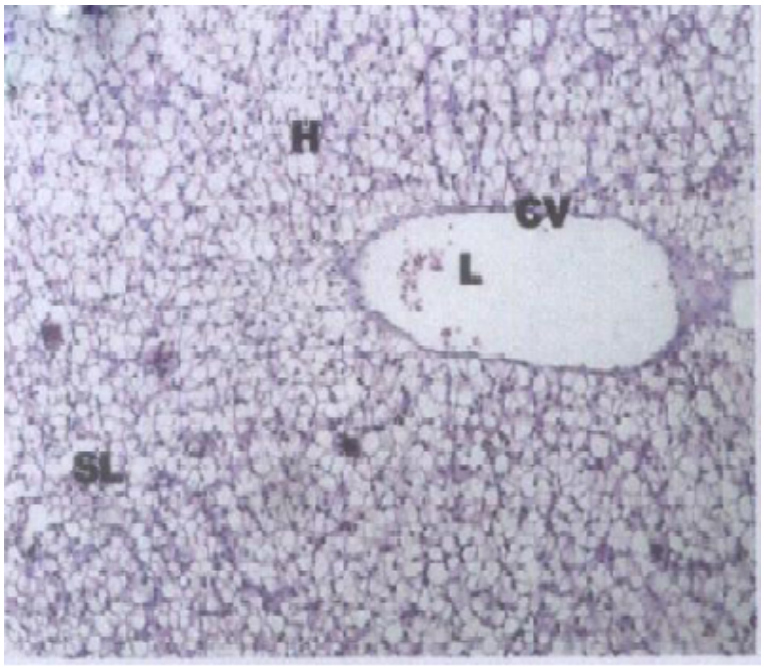

Plate 1a: Histologic photomicrograph of control liver tissue at magnification x100.
H – Hepatocyte, CV – Cytoplasmic Vacuolation,
SL – Sinusoidal Layer, E – Epithelial Lining

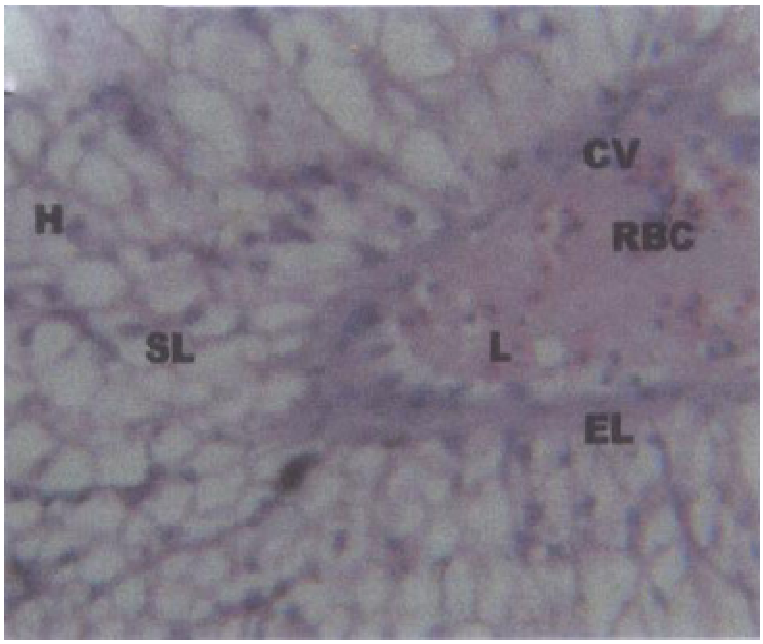

Plate 1b: Histologic photomicrograph of control liver tissue at magnification x400.
H – Hepatocyte, CV – Cytoplasmic Vacuolation,
SL – Sinusoidal Layer, E – Epithelial Lining,
RBC – Red Blood Cell

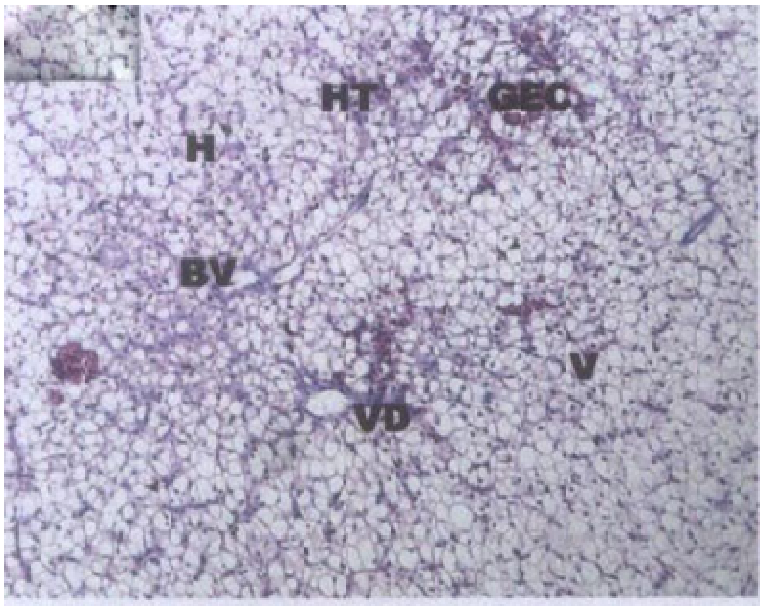

Plate 2A: Histologic photomicrograph of treated liver tissue at magnification x100
HT – Hyperthrophy, VD – Vascular Degeneration,
V – Vacuolization, GEC – Eosinophilic Cells

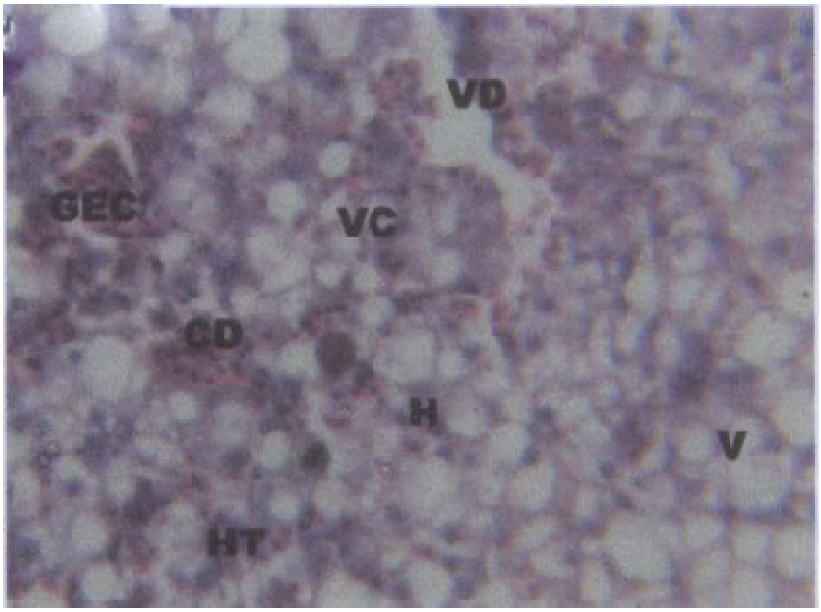

Plate 2b: Histologic photomicrograph of treated liver tissue at magnification x400
HT – Hyperthrophy, VD – Vascular Degeneration,
V – Vacuolization, GEC – Eosinophilic Cells,
VC – Vascular Congestion

4. Discussion

The histopathological legions observed in this study are non-specific alterations induced by exposure of the fish to the toxicant. Similar effects have been reported by various workers in different fish tissues (Eller 1971; George *et. al*, 1981; king, 1962; kazuo, T and Kentaro, K (1979).

George and gerardi, (1981) revealed the histopathological examination of the liver as one of the most sensitive and significant indicators of chronic Cyanide poisoning in rainbow trout. It is established that liver is the site of synthesis for the protein portion of the egg yolk (Ho and Vanstone, 1961) and it is undoubtable that a liver tissue impacted by *Piper guineense* extract may not be able to exhibit those functions. Moreso, considering the complex role played by liver, it is not surprising that the toxified fish could show such substantial damage.

Ruby *et. al*; (1979) equally observed substantial abnormalities similar to this study but in gonads of the rainbow trout exposed to various concentrations of Hydrogen cyanide.

Similar alterations is the liver tissue of fishes have been reported to be responsible for the inability of the liver tissue to supply the products of metabolism for optimal growth and also the elimination of the required waste products generated from metabolic activities (Sastry and Malik, 1979; Arunahalam, *et al*, 1980).

Based on the fact that *P. guineense* is a staple spice in the tropics, there is an urgent need for further research with this botanical to ascertain the condition for its safe use in various organisms in the environments.

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