

# HISTOPATHOLOGICAL ALTERATIONS IN THE GUT OF *Clarias gariepinus* EXPOSED TO SUBLETHAL DOSE OF ETHANOLIC EXTRACT OF *Piper guineense*



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## ABSTRACT

Fingerlings of *Clarias gariepinus* (size range and body weight, 5.9-13.0cm and 1.88-12.12g respectively) were exposed to sublethal dose of ethanolic extract of *Piper guineense* in a static bioassay to examine its histopathological effects on the fish gut. The findings reveal the destructive potential of *P. guineense* as an ichthyotoxic plant depicted by abnormal area of mucosa necrosis, lamina propria degeneration, gastric pit, gastric erosion, hypertrophy and epithelial lining degeneration. These alterations are bound to negatively affect the metabolism of the fish leading to poor growth and survival.

## INTRODUCTION

Agricultural, industrial and domestic effluents generally contain a wide variety of organic and inorganic pollutants, such as solvents, heavy metals, pesticides, fertilizers and suspended solids (Pandey *et al.*, 2003) which are, invariably, discharged into small rivers and streams, without proper treatment. The increased contamination of aquatic ecosystems causes severe morphological and physiological alterations in aquatic organism (Mazon *et al.*, 2002). In a degraded environment, particularly where pollutants occur in chronic and sub lethal concentrations, changes in the structure and function of aquatic organism are more frequent than mass mortality. One of the possible methods of evaluating the effect of pollutants in fish is to examine their organs morphological changes (Polesksic and Mitrovic – Tutundzic, 1994).

*Piper guineense* popularly known as West African black pepper is widely consumed in some parts of West African especially Nigeria and Ghana on account of its nutritional and medicinal properties (Negbenebor *et al.*, 1999). It belongs to the family *Piperaceae* and is widely distributed in the tropical and subtropical regions of the world (Negbenebor *et al.*, 1999., Dalzie, 1994). Several studies have confirmed the insecticidal potential of the constituents of the plant (Okonkwo and Okoye, 1996, Olaiya *et al.*, 1987., Ekesi, 2000). Consequently, it is used as a pesticide to boost agricultural production and is ultimately washed into the aquatic habitat. This study was conducted to investigate the effect of sub lethal dose of *P. guineense* ethanolic extract on the histopathology of the gut of *Clarias gariepinus* fingerlings.

## MATERIALS AND METHOD

### Collection of Test Organisms

The fingerlings of *C. gariepinus* (weight range 1.88g – 12.12g, total length 5.9cm-13.0cm) were collected from Vision Park Farms in Uyo, Akwa Ibom State. They were transported to the laboratory in a polythene bag containing aerated water. They were acclimatized to laboratory condition using transparent plastic tanks of length 22.6cm and width of 18.5cm in 6 liters' of water for two weeks as recommended by FAO, (1986). The water was changed twice a week and the fishes were fed once daily with multi feed containing 45% protein, 12g of fat, 2.2g of calcium (Ca), 1.2 of phosphors (P) and 8.5g of ash. The tanks were covered with netting material to prevent the fish from jumping out.

### Preparation of Ethanolic Extract of *Piper guineense* by Soxhlet Extraction

Fruits of *P. guineense* were dried at 60 degree centigrade for 72 hrs and ground using an electric blender. The powder form was stored in an air tight bottle until used. The homogenized sample was later extracted with ethanol.

The extract was then evaporated to dryness in a water bath at a temperature of 45°C to obtain a crude brownish substance. The substance was wrapped with a black material to avoid light penetration and stored in a refrigerator until needed.

### Test Procedure

Ten randomly selected fish were distributed in batches and placed in three aquaria containing test solution (0.4g of extract per litre of water) and a control tank containing extract free water only. Each set of experiment was replicated twice with a control. The samples were exposed to the sub lethal dose of 0.4g/l (96hr LC<sub>50</sub>) of the extract solution for 4 days. Within the duration of the experiment, water in the tank was replaced after every 48hr with fresh extract solution.

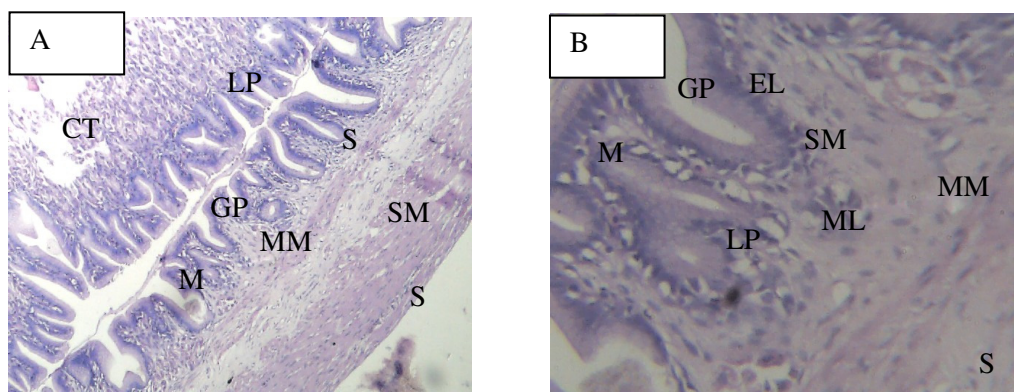
### Preservation of Fish Organs for Histopathological Analysis

The samples used for histological examination were randomly selected and dissected to remove the gut. The gut tissues were fixed in Bouin's fluids embedded in paraffin and sectioned (7 microns thickness). They were then stored with haematoxylin/eosin stain. Histopathological alterations due to treatment with the ethanolic extract of *P. guineense* were noted and photomicrographs taken.

## RESULTS AND DISCUSSION

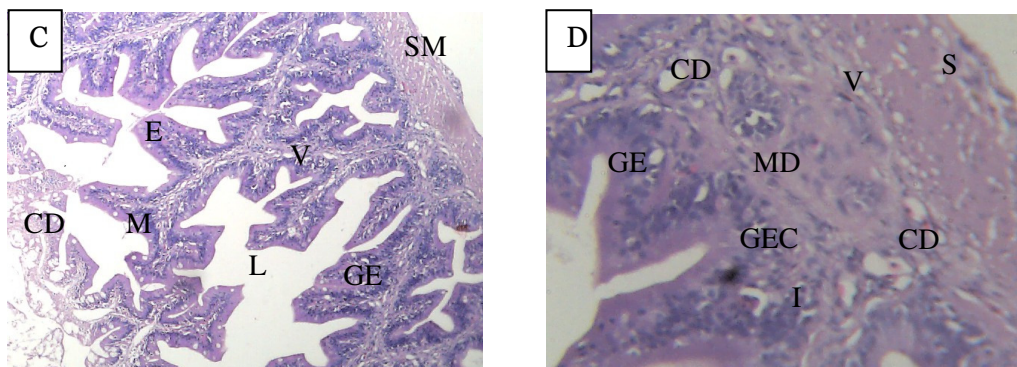
Marked histological changes in the gut tissues of *C. gariepinus* were observed after treatment with ethanolic extract of *P. guineense*. Plate A(100x) and B(400x) of gut without extract of *P. guineense* show normal area of mucosa, lamina propria, submucosa, muscularis and serosa with the area of connective tissue well displayed. No recognizable cellular abnormality was seen in the gut of the control fish. Plate C (100x) and D(400x) of gut treated with extract of *P. guineense* show abnormal area of mucosa necrosis, lamina propria degeneration, gastric pit, gastric erosion, hypertrophy and epithelial lining degeneration as compared to control. The results show that the histology of the gut of *C. gariepinus* fingerlings can be altered by the ethanolic extract of *P. guineense*.

The result is similar to the works of Gardener and Yevich (1970). Gutierrez *et. al.*, (1978) and Newman and MacLean (1974).The gut is responsible for storage, digestion and absorption of food. The observed alterations are bound to hamper these functions.



GUT-CONTROL

Histologic photomicrographs of Gut without extract at magnification A (x100) and B(x400) stained with H & E technique.



Histologic photomicrographs of Gut treated with Piper guineense at magnification C (x100) and D(x400) stained with H & E technique.

**KEY:**

CD: Cellular degeneration

E: Epithelium

L: Lumen

GE: Gastric Epithelium

M: Mucosa

V: Vacuolation

S: Submucosa

MD: Myofilament degeneration

GEC: Granulated Eosinophilic Cells

SM: Submucosa

I: Interstitium

**CONCLUSION AND RECOMMENDATION**

There is no doubt that the histological changes observed in the gut of *C. gariepinus* are due to the toxicity of *P. guineense*. The toxicant has deleterious effect on the gut of *C. gariepinus* and consequently has the potential to significantly alter the normal metabolism, growth and the survival of the fish. Its release into the aquatic habitat must be discouraged.

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