

## EFFECTS OF ETHANOLIC EXTRACT OF *Piper guineense* ON THE GILLS HISTOLOGY OF *Clarias gariepinus*



ISSN: 2141 – 3290  
www.wojast.com

**OKON, A. O. AND ISANGEDIHI, A.I.**

*Department of Fisheries and Aquaculture*

*Faculty of Agriculture*

*University of Uyo, Uyo, Nigeria*

[anivisionpark@yahoo.com](mailto:anivisionpark@yahoo.com)

**ABSTRACT:** The effect of the sub-lethal dose (0.4g/l) of dechlorinated water of *P. guineense* on the histology of gills of *Clarias gariepinus* exposed for 21 days in a static bioassay revealed, serious pathological changes in the fish gills. The changes include abnormal area of cell proliferation and hypertrophy of ceratobranchial arch, lamella, muscle fibre, connective tissue distortion, disruption of gill, epithelial cells and vacuolization, inflammation, lesion, vascular congestion and cellular degeneration as compared. These findings revealed the desstructure properties of *Piper guineense*.

### INTRODUCTION

The sub-lethal effects of toxicants on fish tissues have been documented. For example Sastry and Malik (1979) revealed the sub-lethal effect of Demecron on digestive system of freshwater fish; *Channa Punctatus* exposed for 20days caused most conspicuous pathological changes in the liver such as vacuolation of the cytoplasm of hepatocytes, enlargement of the nuclei, rupture of the cell membrane, liver cord disarray and damage of connection tissue.

Mattiessen and Brafield, (1973) reported that the liver of mercury-exposed fish was congested and that mercury also caused serious histological effect on Stickle back, *Gasterosteus, aculeatus* (L) such as detachment and sloughing of epithelial cells, coalescing of adjacent secondary lamella epithelia. The cytoplasmic abnormalities included extensive vacuolation, followed by swelling of nuclei and mitochondria leading to cellular disintegration.

*Piper guineense* (the West Africa black pepper) is an ichthyotoxic plant which belongs to the family Piperaceae. It is widely distributed in the tropical and subtropical regions of the world. *Piper* is sold locally as spice and medicine for curing stomach disorder (Dalzie, 1948). The toxicity of *Piper guineense* to some invertebrates such as nymph adult and grasshopper (*Zonocerus variegatus*) (L) was attributed to Piperine, the active ingredient acting with guineensine (Ivbijaro and Agbaje, 1986). The present investigation is aimed at revealing the effect of sub-lethal concentration of ethanolic extract of *piper guineense* on the histology of *Clarias gariepinus* gills

### MATERIALS AND METHOD

#### Collection of Test Organisms

The post fingerlings of *Clarias gariepinus* (size ranged and body weight, 8.0 – 10.5cm and 12 – 16g respectively) were collected from Visionpark Frams in Uyo, Akwa Ibom State, Nigeria. The samples 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 and fed once a day with formulated feed. The fecal 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. They were considered fully adapted when no death was observed for four consecutive days (FAO, 1986). Feeding was stopped 24 hrs prior to treatments.

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

Some quantities of *Piper guineense* fruits were dried at 60°C for 72 hrs, and then milled in an electric blender. The powder substance collected was stored in an air tight bottle until used. Thereafter 450g of the homogenized sample was extracted with ethanol. The extract was evaporated to dryness in a rotary evaporator at about 60°C to obtain a crude brownish semi solid substance that weighed 150.5g. The extract was preserved in the refrigerator until needed.

### **Test Procedure**

Ten fish samples were selected randomly and distributed in batches and placed in three aquaria containing test solution (0.4g 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 at optimal levels.

The samples were then exposed to 0.4g (96hr LC 50) for 21 days (Okorie, *et.al*, 1992). During the duration of the experiment, water in the tanks were replaced after every 48hrs 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) for staining with haematoxylin/eosin stain. Histopathological changes due to treatment with the ethanolic extract of *P. guineense* were noted and photomicrographs taken.

## **RESULTS**

Treatment of *Clarias gariepinus* with 0.4g/l of ethanolic extract of *Piper guineense* produced marked histological alterations in the gills tissues examined.

Figure 1 shows the control gills tissue. It shows a normal area of filament, gill arch, ceratobranchial arch, lamella and cartilage. The connective tissue and interstitium are well displayed, no cellular abnormality. However, the treated gills (Fig, 2) revealed abnormal area of cell proliferation and hypertrophy of ceratobranchial arch, lamella and connective tissue distortion. It also revealed the disruption of gill, epithelial cells, filament in addition to fusion of primary lamellae and vacuolization. Vascular congestion and cellular degeneration were also noticeable.

## **DISCUSSION**

The presence of toxic substance in the environment causes alterations in the vital functions carried out by the gills and in morphological structure of the organ. Thus the histopathological analysis of fish gills has been used as a tool that is extremely important in the evaluation of the quality of aquatic ecosystems. The results, obtained in this study revealed the occurrence of alternation such as change in structure of epithelium, vacuolization and hyperplasia of the epithelium of the primary lamella. The secondary lamella showed hypertrophy of the secondary secretory cells, epithelial lifting, alteration of structure, Pyknotic nucleus of the pillar cells. Klontz, (1972) has reported that reactions of the gills due to irritants includes inflammation, hyperplasia, lamellar fusion, excessive production of mucus, epithelial lifting, flattening of the secondary lamella and formation of aneurysms. These are in accordance with the observation in this study. Ferrandes and Mazon, (2003) went further to show that the epithelial lifting observed led to an increase in the distance between water and blood thus impairing oxygen uptake in to the organism. Similar results were observed by Thophon *et al* 2003; Stentiford *et al* 2003; Mazon *et al* 2002; Teh *et al*, 1997.

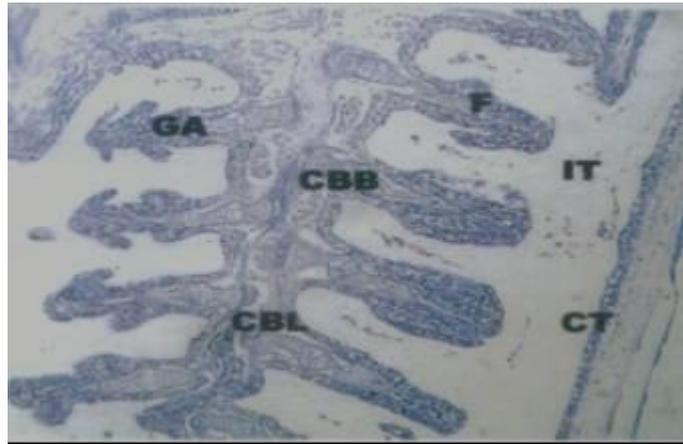


Figure 1: Histologic Photomicrograph of Control gill tissue at magnification x100 GA – Gill arch, F – Filament, CBB – Ceratobranchial Bone, IT – Intertitium CBL – Ceratobranchial lamella, CT – Connective Tissue

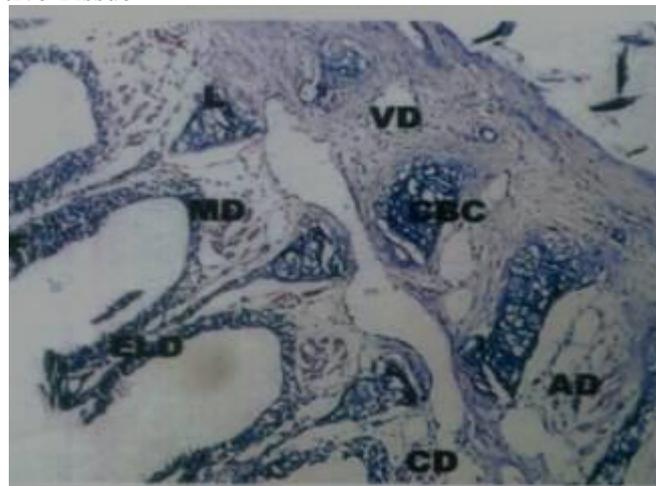


Figure 2: Histologic Photomicrograph of treated liver tissue at magnification x100, L – Lumen, MD – Muscular degeneration, VD – Vascular degeneration, CBC – Ceratobranchial Cartilage, AD – Adipocytes, ELD – Epithelial Lining degeneration, CD – Cellular degeneration.

### CONCLUSION AND RECOMMENDATION

Numerous toxicants are known to cause deleterious effects on fishes, and it is obvious that the lesions observed in the gill tissues of the treated fish samples were due to the toxic effects of the ethanolic extract of *Piper guineense*. The denatured fish may be dangerous to human health. Therefore the use of this ichthyotoxic plant for fishing should be highly discouraged.

### REFERENCES

- Dalzie, J. M. (1948). *The Use of Plants of West Tropical Africa: The Crown Agents for Colonies* 612pp.
- FAO (1986). *Manual of Methods in Aquatic Environment Research*, Part 4: Bases for selecting Biological Tests to Evaluate Marine Organisms, FAO, Rome, 56p.
- Ivbijaro, M. F. and Agboje, M. (1986) Insecticidal activities of *Piper guineense* (Schurn and Thonn) and *Capsicum maculatus* F. *Insect Sci. Appl.* 7 (4): 521 – 524.
- Klonntz, G. W. (1972). Haematological techniques and the immune response in rainbow trout. *Symposium Zoological Society of London*, 30: 89-99.

- Mathiessen, P. and Brafield, A. E. (1973). The effects of dissolved zinc on the gills of the Stickleback, *Gasterosteus aculeatus* (L), *J. Fish Biol.* 6: 607 – 613.
- Mazon, A. F., Monteiro, E. A. S., Piaheiro, G. H.D and Fernandes, M. N. (2002). Haematological and physiological changes induced by short term exposure to copper in the freshwater fish, Teleost, *Prochilus scrofa*. *Brazilian Journal of Biology*, 62 (4A): 621-631
- Okorie, T.G.,A.O. Ugwumba and Okon, A.O., (1992). Toxic and sublethal effects of ethanolic extract of *Piper guineense* on *Oreochromis niloticus* (Linn). (Pisces: Cichlidae). *Journal of Experimental and Applied Biology*, 4(1-4):34-52.
- Sastry, K. V and P. K. Gupta. (1978). Histopathological and enzymological studies on the effect of Chronic Lead nitrate intoxication in the digestive system of freshwater Teleost, *Channa Punctatus*. *Environmental Resources*, 17:472 – 479.
- Stentiford, D. Longshaw, M., Lyons, B. P., Jones, G., Green, M. and Feist, S. W., (2003). Histopathological biomarkers in estuarine fish species for the assessment of biological effects of containments. *Marine Environmental Research*. 55
- Teh, S.J; Adams, S.M and Hinton, D. E., (1997) Histopathologic biomarkers in freshwater fish Populations exposed to different types of contaminants stress. *Aquatic Toxicology*, 37: 51 – 70.
- Thophon, S., Kruatrachue, M., Upatham, E. S., Pokethitiyook, P., Sahaphong, S. and Jarit Khuang S., (2003). Histological alterations of white seabass, *Lates calcarifer*, in acute and sub-chronic cadmium exposure *Environmental Pollution*, 121: 307 - 320