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Histopathological lesion in the gill of *Clarias gariepinus* exposed to sublethal dose of insecticidal fruit extract of *Dinnettia tripetala*

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Abstract

Fingerlings of *Clarias gariepinus* (size range and body weight of 3.9-6.0cm and 0.08-3.75g respectively) were exposed to sublethal dose (0.7g/l) of ethanolic extract of *Dinnettia tripetala* fruit in a static bioassay to examine its histopathological effects on the fish gill. The deleterious effects of *D. tripetala* as an ichthyotoxic plant was depicted by condensed and diminished clara and pilaster cells accompanied by mucous cell proliferation in the treated gill tissue. These structural alterations hindered gaseous exchange in the gill leading to histotoxic hypoxia.

Keywords: Histopathology, Gill, *Clarias gariepinus*, *Dinnettia tripetala*.

1. Introduction

Natural insecticides are increasingly being used to combat insect pests that compete for food and affect public health (Chiyvareejja *et al.*, 1997) [1]. They are preferred to their synthetic counterpart largely because they are common, less harmful to the environment and are biodegradable. Several studies have however shown that these plant toxins at low concentrations are very toxic to all groups of aquatic fauna (Tiwari and Singh, 2003) [2]. Phytochemical analysis indicate that such piscidal or ichthyotoxic plants contain active ingredients that are deleterious to fish when discharged into small rivers and streams, without proper treatment. Other than mortality, the increased contamination of aquatic ecosystems causes severe morphological and physiological alterations in fish and other aquatic organisms (Mazon *et al.*, 2002) [3].

D. tripetala popularly known as pepper fruit is widely consumed in some parts of West Africa especially Western Cameroons, Ivory coast and Southern Nigeria on account of its nutritional and medicinal properties (Keay *et al.*, 1960) [4]. It belongs to the family *Annonaceae* and is widely distributed in the tropical rainforest and savanna regions (Okwu *et al.*, 2005) [5]. The insecticidal potential of the constituents of the plant have been documented (Egwyunyenga *et al.*, 1998; Ukeh *et al.*, 2012.) [6,7]. Consequently, it is used as a pesticide to protect smoked fish from insect pests to guard against post-harvest losses (Olayinka – Olagunju, 2014) [8] and may ultimately be washed into the aquatic habitat. Despite its widespread use both for nutritional and medicinal purposes, the toxicity of this plant to aquatic organisms, particularly fish is scanty. The present study was conducted to investigate the effect of sub-lethal dose of *D. tripetala* fruit extract on the histology of the gill of *C. gariepinus* fingerlings in order to assess the ichthyotoxic potential of the plant.

C. gariepinus is the most commercially important aquaculture species in Nigeria. It is hardy, grows rapidly, and is desirable as food. It is a valuable fish species worldwide. Its potential for intensive culture with relative poor water quality (FAO, 2000) [9] made it the choice for this study.

2. Materials and Methods

2.1 Collection of Test Organisms

The fingerlings of *C. gariepinus* (weight range 0.08g – 3.75g, total length 3.9-6.0cm) were collected from Safe Food Farms in Uyo, Akwa Ibom State, South-south Nigeria. They were transported to the laboratory in a polythene bag containing aerated water.

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The fish were acclimatized to laboratory condition using transparent plastic tanks of length 22.6cm and width of 18.5cm with 6 liters of water for two (2) weeks as recommended by F.A.O (1986) [10]. The water was changed twice a week and the fishes were fed once daily with 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.

2.2 Preparation of Ethanolic Extract of *D. tripetala*

Fruits of *D. tripetala* was dried at 60 °C 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.

2.3 Test Procedure

Ten randomly selected fish were distributed in batches and placed in three aquaria containing test solution (0.7g/l of extract) 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 0.7g /l (96hr LC₅₀) 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.

2.4 Preservation of Fish Organs for Histopathological Analysis

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

3. Results and Discussion

Distinct histological changes in the gill tissues of *C. gariiepinus* were observed after treatment with ethanolic extract of *D. tripetala*. Plate A shows histologic section through the gill that had not been treated with extract of *D. tripetala*. Normal cellular pattern with area of gill arch, filament, ceratobranchial arch and cartilage, pilaster and clara cells, extracellular matrix and area of connective tissue are well displayed. No recognizable cellular abnormality was seen in the gill of the control fish. Plate B shows gill treated with extract of *D. tripetala* revealing condensed and diminished clara and pilaster cells and mucous cell proliferation as compared to control group. Mucous cell proliferation observed in the gill histology is a protective feature leading to increase production of mucus aimed at preventing further damage to the gill tissues. Increased amount of mucus production is also known to have some detoxification properties (Benda and Westman, 1985) [11]. However, hyper secretion of mucus may impede gaseous exchange and further predispose fish to hypoxia (Adeogun *et al.*, 2012) [12].

Toxicant introduced into aquatic systems can cause structural changes in tissues and organs of fish leading to obstruction of physiological functions. The histologic changes in the gill observed in the present study implicate *D. tripetala* extract as a fish toxicant. The alterations may have compromised the process of gaseous exchange resulting in histotoxic hypoxia.

Several reports have indicated that gill lesions do not only indicate possibilities of impaired respiratory functions but impaired osmo-regulatory functions as well (Mallat, 1985; Au, 2004; Tang and Au, 2004.) [13, 14, 15]. Even slight structural damage can render a fish vulnerable to osmo-regulatory as well as respiratory difficulties (Hughes and Morgan, 1973) [16] thereby affecting the overall metabolism and survival of the fish.

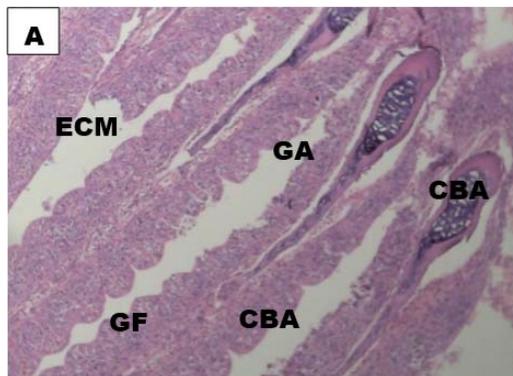


Plate A: Histologic section through the Control Gill without treatment stained with H and E technique

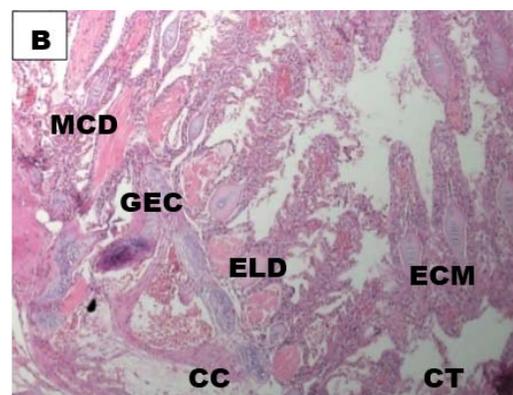


Plate B: Histologic section through the Gill treated with 0.7g/l of *D. tripetala* stained with H and E technique

Keys

Eld	-	Epithelial Lining Degeneration
Cba	-	Ceratobranchial Arch
Gec	-	Granulated Eosinophilic Cells
Cc	-	Clara Cells
Ct	-	Cartilage
Ga	-	Gill Arch
Ecm	-	Extracellular Matrix
Gf	-	Gill Filament
Mcd	-	Muscular Cell Degeneration

4. Conclusion

The results of the present study show that the histology of the gill of *C. gariiepinus* can be distorted on exposure to fruit extracts of *D. tripetala* resulting in respiratory impairment. Consequently, the botanical is ichthyotoxic and should be kept away from the aquatic environment as much as possible.

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