Histopathological Alterations In Gills Of Fingerlings Of Clarias Gariepinus (Burchell, 1822) Following Sublethal Acute Exposure To Hevea Brasiliensis

George, Ubong Uwem, Joseph Asor, Andy, John A.

Abstract: Studies on the toxic effects of water soluble fraction (WSF) of Hevea brasiliensis on the histology of the gills of Clarias gariepinus fingerlings showed severe impacts on the gills of the test organisms. Six concentrations were prepared from the water soluble fraction of the latex (0, 30, 40, 50, 60 and 70mg/l) for histopathological examination on the gills of the species. In the control group there was no histological change on the test organism. Gill samples for histological examination were prepared from the concentrations which toxic manifestation of the toxicant was observed. There was no toxic manifestation on the control (0mg/l), 30mg/l and in the 40mg/l of concentration, whereas the test organism in the 50mg/l, 60mg/l and 70mg/l concentration exhibited various reactions which included, erratic movement, vertical swimming position, colour changes, weaken swimming motions, and changes in opercula rate. In the 50mg/l concentration the gill showed disintegration of cells in gill, in the 60mg/l concentration it was observed to show Lamellae erosion and fusion of cells and in the 70mg/l concentration Pronounced lamellae erosion was observed in gill ray. Therefore, if latex obtained from Hevea brasiliensis finds its way into the aquatic environment as would be expected, it will cause deleterious ecological effects to both terrestrial and aquatic biota at the long term. Realizing the tremendous adverse effects associated with WSF of the latex from H. brasilensis, safety measures should be adhere by companies using rubber latex has their raw material.

Key words: Clarias gariepinus, Hevea brasiliensis, Gill, Fingerlings, Histopathology.

INTRODUCTION

Environmental pollution is a global problem and is common to both developed as well as developing countries. Environmental pollution is the result of urban industrial technological revolution and speedy exploitation of every bit of natural resources. The oraze of progress in agriculture, industry, transportation and technology is taken as the general criterion of development of any Nation. Such activities of man have created adverse effects on the survival of man himself and other living organisms in the biosphere (Pers.Com). Hevea brasiliensis contain substances like hydrocarbon, protein and phospholipids, carbohydrates, inorganic ions and metallic ions, with small amount of phytochemical compounds including hydrocyanic acid, phytic acid and alkaloids (George et.al., 2014). This toxic latex finds their way into the aquatic environment through effluent and surface run-off from industries that use Hevea brasiliensis as raw materials for the production of tyres, tubes and other household equipment's. The negative effects of Hevea brasiliensis on aquatic life have been reported (George et.al., 2013). However, despite their widespread use, little is known about their toxicity to fish and other aquatic invertebrates. Besides algae, macrophytes and benthic macro invertebrates, Fish are also considered good indicators of environmental quality and are therefore receiving special attention in eco-toxicological studies. These organisms can absorb contaminants in the water. Those that inhabit waters in the vicinities of urban areas may frequently be exposed to sub-lethal concentrations of pollutants. Clarias gariepinus is a freshwater fish and is the most cultured fish species in Nigeria and are generally strong fish. However, despite its broad range of distribution throughout Africa and its suitability for aquaculture, few studies have reported the effects of pollutants on the histopathology of the gills of Clarias gariepinus. Gills are the major organ for osmotic regulation, excretion and respiration in fish. The gills of fish are located on each side of the head beneath a gill-covering operculum and are composed of finger-like filaments attached to a cartilaginous gill bar. The lamellae, project from each filament and these consist of minute capillaries covered by a single layer of thin epithelial cells. The epithelium forms a barrier between the fish's blood and the surrounding water. Gills are generally considered a good tissue indicator of the water quality and are appropriate for the assessment of environmental impact (Mallatt 1985, Winkaler et al. 2001, Fanta et al. 2003). Histopathological studies are performed to evaluate the direct effects of contaminants on fish in laboratory bioassays (Schwaiger et al. 1992, 1997, Ortiz-Delgado et al., 2007). The aim of the present study was to determine the effects of Hevea brasiliensis on the gills of Clarias gariepinus in relation to concentration of the toxicant. To our knowledge, this is the first investigation on alterations of gill morphology in Clarias gariepinus exposed to sub-lethal concentrations of Hevea brasiliensis.

- *George, Ubong Uwem, Joseph Asor, Andy, John A.*
- *Department of Fisheries and Aquaculture, Institute of Oceanography University of Calabar, Calabar.*
- *Department of Zoology and Environmental biology, university of Calabar, Calabar.*
- *Correspoding Author: gboy4jesus@yahoo.com.*

MATERIALS AND METHODS

Study Area

Samples of Hevea brasiliensis was collected from rubber research instititute, Calabar, Nigeria located within longitude (08°22'10.583'E) and latitude (05°8'2.85''N). The climate of the area is tropical and is characterized by distinct wet and dry seasons. The study area is generally rainforest and is surrounded by two major rivers, the Calabar River and Great Kwa River. Human activities in the area include farming, hunting, fishing and sand mining. (Pers. Com). (Figure 1).

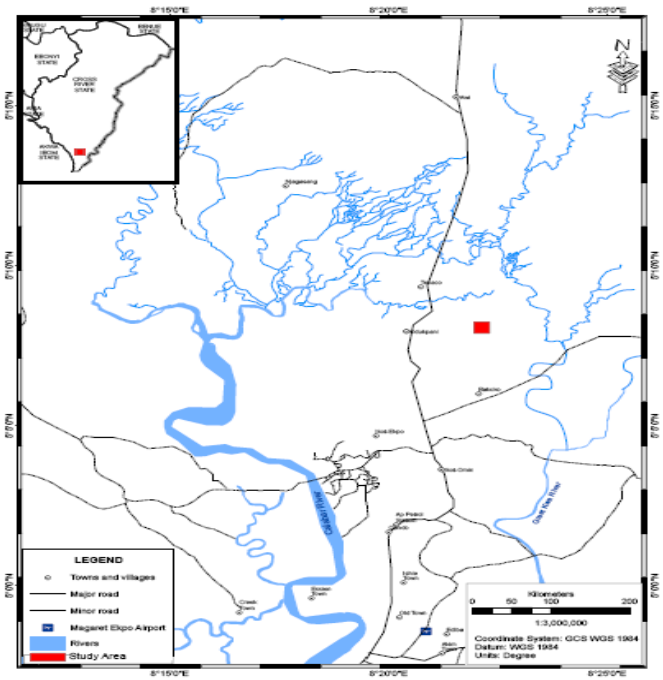

Figure 1: Map of the Study Area

Collection and Transportation of Test Organism

A total of 120 healthy Clarias gariepinus fingerlings were used for this study. The fingerlings were in the range of 2.5 – 4cm in size. The fish were bought from the University of Calabar Fish Farm, Cross River State located within the University of Calabar at latitude $04^0$5, 020'N and longitude $008^0$20' 450' E, respectively. (Asuquo and Bassey, 1999 and Akpan et al., 2002), and was transported to the Research Laboratory of the Institute of Oceanography (IOC), University of Calabar, where they were acclimatized.

Acclimatization and Maintenance of Study Organisms

In the laboratory, Clarias gariepinus fingerlings were allowed to acclimatize to laboratory conditions for 24 hours in the glass tank and aerated with air stone connected to electrically powered aquarium pumps.

PREPARATION OF TOXICANT SOLUTION

The water soluble fraction (WSF) of Hevea brasiliensis latex was obtained by vigorously shaking rubber extract with flittered habitat water in a separatory funnel. The system was allowed to stand for six hours to effects complete phase separation, after which the lower aqueous layer containing the WSF was collected for the toxicity test.

Stocking of Specimens

Ten (10) Clarias gariepinus fingerlings were gently caught using a hand net in order to avoid stress, into glass tanks measuring 25 X 10 X 15cm from an acclimatized tank. The glass tank was filled with 2 liters of dechlorinated water.

Preliminary Test

The concentration ranges chosen for the preliminary test of WSF of Hevea brasiliensis latex on Clarias gariepinus were 0, 10, 20, 30, 40 and 50mg/l. Ten fish were randomly introduced into each of the reconstituted latex and each concentration was set in duplicate with control containing dechlorinated water without the addition of WSF of Hevea brasiliensis latex.

Definitive Test

The concentration ranges chosen for the WSF of Hevea brasiliensis latex for the toxicity test on Clarias gariepinus after the preliminary tests were 0, 30, 40, 50, 60 and 70mg/l. The duration of the experiment was 96hours. The fish were starved in order to minimize waste production. The distress behavior were closely monitored and recorded from the onset of the experiment 6h, 12h, 24h, 48h, 72h and 96h respectively. The initial water parameter was taken prior to stocking of test organism. Dissolved oxygen, temperature, pH, nitrite and ammonia were monitored dialy using mercury – in – glass thermometer, Lurton Do and pH meters. The battery operated meters were calibrated according to manufacturer's instructions before being used for measurement (Boyd, 1989, 1990).

Histopathology

The gills tissues isolated from the test animal were fixed in formal-saline for 48 hours. The fixed tissue was processed manually through graded ethanol, cleared in xylene, impregnated and embedded in paraffin wax. These sections were cut with a rotary microtome, stained by haematoxylin and eosin technique, examined microscopically for pathological changes.

Statistical Analysis

The difference between control and Hevea brasiliensis treated groups were compared by students t- test using (SPSS Inc; Chicago, USA). The results were considered significant only if the p value was less than 0.05.

Results

Clarias gariepinus exhibited various reactions which included, erratic movement, vertical swimming position, colour changes, weaken swimming motions, and changes in opercula rate. All fish in the control, 30mg/l and 40mg/l concentration did not show any toxic manifestation to toxic effects of WSF of Hevea brasiliensis. Plate 1 shows the normal distribution of gill lamella and rakers of the fish at 0 mg/l of toxicant. Cell disintegration is shown in Plate 2 at 50 mg/l of toxicant, while Plate 3 shows lamellae erosion and fusion of cells at 60 mg/l of toxicant. In Plate 4, the gill of the fish shows lamellae erosion at 70 mg/l of the toxicant. (Table 1 and Plate 1-4).

Table 1:Histological Changes in gills of Clarias gariepinus fingerlings

Concentrations (mg/l)	Histological Changes
0	No visible alteration on gill ray
50	Disintegration of cells in gill ray
60	Lamellae erosion and fusion of cells in gill ray
70	Lamellae erosion of gill ray

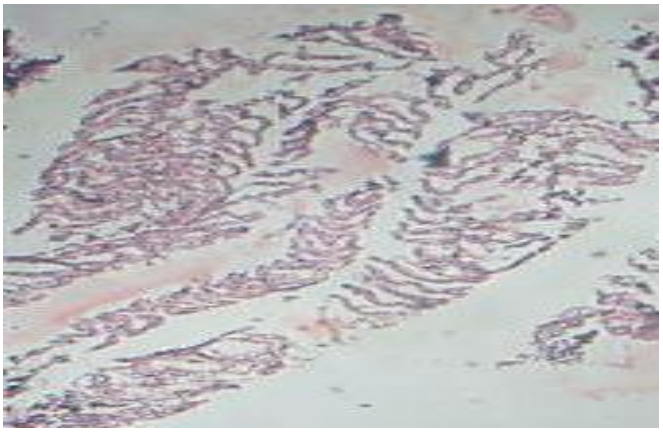

PLATE 1: Normal distribution of gill lamellae in *C. gariepinus* (Control)

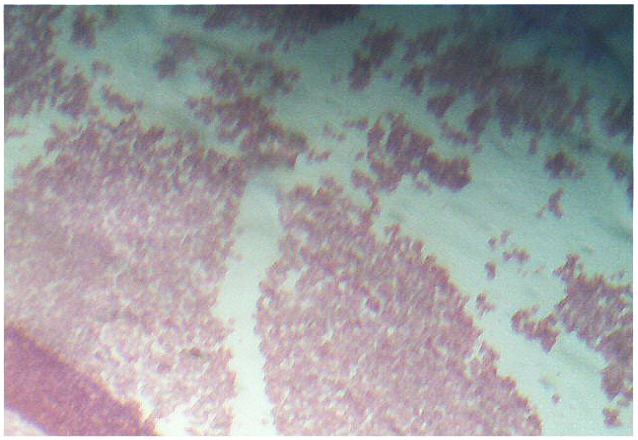

PLATE 2: Gill of C. gariepinus showing cell disintegration at 50 mg/l WSF of H. brasiliensis at the end of the experiment (96 hours).

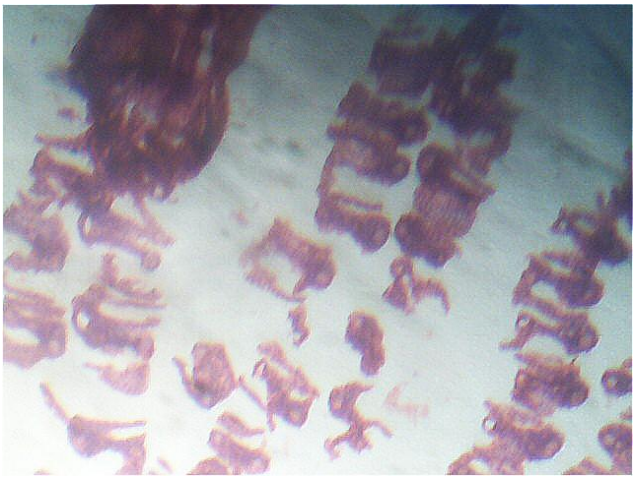

PLATE 3: Gill of C. gariepinus showing lamellae erosion and fusion of cells at 60 mg/l WSF of H. brasiliensis at the end of the experiment (96 hours).

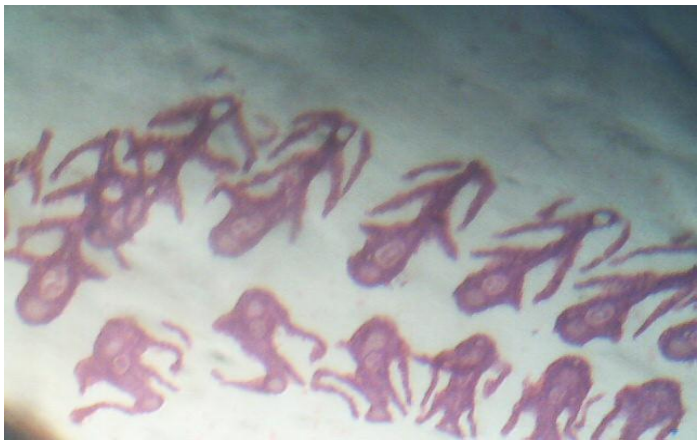

PLATE 4: Gill of C. gariepinus showing lamellae erosion at 70 mg/l WSF of H. brasiliensis at the end of the experiment (96 hours)..

Discussion

The effects of the water soluble fraction of H. brasiliensis latex showed severe destruction of the gill lamellae in Clarias gariepinus. However, the gill lamellae of the organism in the control were not affected. In the 50 mg/l concentration of the toxicant it was observed to cause cell disintegration in the gill. In the 60 mg/l concentration of the toxicant, the gill lamellae of Clarias gariepinus were observed to have become eroded and fused. Pronounced lamellae erosion was observed in 70 mg/l concentration of the toxicant. Cell disintegration in gill has been reported by Diana et al., (2007) in Carassius auratus gibelio when investigating biochemical and histological effects of deltamethrim on the species with different effects such as lamellae cells hypertrophy and nuclear pycnosis in the basal cells. Hypertrophic, necrotic, atrophy and dystrophy of secondary lamellae have also been reported in Clarias gariepinus juveniles exposed to refined petroleum oil and kerosene under laboratory conditions. The changes in the gills of Clarias gariepinus exposed to the water soluble fraction of H. brasilensis fall within the general responses of fish organs to environmental pollutants. Fernandes and Mazon (2003) observed that fish gills are the prime target organ of all pollutants due to their extensive surface in contact with the external medium and the reduced distance between the external medium. Gill morphology is an important biomarker providing a rapid method of detection of the effect of pollutants (Gabriel et al., 2007; Banerjee, 1993). The general morphological changes in the gills recorded in this study have been reported in Astyanax sp. After 96hrs exposure to water soluble fraction of crude oil (Akaishi et al., (2004), Gabriel et al., (2007) also reported similar changes in Clarias gariepinus exposed to petroleum oil and kerosene.

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