Chronic Toxicity of Pharmaceutical Effluent to *Clarias gariepinus* (Burchell, 1822)

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Abstract: The aim of this study was to investigate the chronic toxicity of pharmaceutical effluent on *Clarias gariepinus* gills and liver as biomarkers of environmental quality in toxicity testing of pharmaceutical effluent. The chronic toxicity test of pharmaceutical effluent was carried out on the histopathology of the gill and liver of juvenile African catfish (*C. gariepinus*) with mean weight of 10 ± 0.2g and standard length of 6.3 ± 0.3 cm. A range finding test was conducted prior to the experiment. The fish were later exposed to 0%, 6.25%, 12.5%, 25%, 50% and 100% concentrations of the effluent for 21 days in a static renewal bioassay procedure. The median lethal concentration (LC_{50}) value was 12.16%. Respiratory disturbance, erratic swimming, loss of equilibrium, lethargies and sudden death were observed in the exposed fish and these varied greatly with increase in concentration of the toxicant. This shows that mortality increases with increase in concentration. The differences observed in the mortalities of *C. gariepinus* at varying concentrations were significant ($p < 0.05$). The aim of this study was to investigate the suitability of *C. gariepinus* as potential biomarkers of environmental quality with respect to pharmaceutical effluent toxicity testing. The gill histopathological changes observed were epithelial lifting, interstitial oedema, leucocyte infiltration, hyperplasia of the epithelial cells, lamellar fusion, vasodilatation and necrosis. Abnormalies observed in the liver tissues of the treated fish were congestion of the central vein, vacoulation of hepatocyte, oedema, cellular infiltration and cellular necrosis (i.e cell death). Occurrence of the gill and liver anomalies in the test fish show their suitability for use in toxicity testing of pharmaceutical effluent.

Keywords: Gill histopathology, liver histopathology, chronic toxicity, pharmaceutical effluent, *Clarias gariepinus.*

INTRODUCTION

In Nigeria, and other developing nations, pollution of water resources is a very serious problem. Human and ecological disorders are being experienced in industrial settlements as a result of improper disposal of various chemicals. Only few chemicals have been ecologically tested in Nigeria for safety in spite of their environmental and ecological impacts. The Federal Government of Nigeria emphasizes the need for

adequate environmental protection in any technological and socio-economic development or endeavors by strictly asking industrial operators to sustainably manage the disposal of chemicals into the environment [1].

The presence of pharmaceutical chemicals in the environment is a matter of concern due to their lipophilic and non-biodegradable nature, as well as their biological activities [2]. Currently, there is scarce measurable evidence of the environmental impact of pharmaceutical chemicals on human health [3]. In recent times, a wide range of pharmaceuticals have been found in fresh and marine waters, and it has been shown that even in reduced quantities, some of these compounds are potentially capable of causing harm to both aquatic and terrestrial life forms [4].

The increasing use of various pharmaceuticals in Nigeria has resulted in an astronomical rise of pharmaceutical manufacturing companies. Many of these companies are small scale and tend to exhibit improper discharge of their wastes. Pharmaceutical chemicals have potential long term effects on the environmental species [2, 5]. During the last few years, the interest to assess the presence of Pharmaceutical compounds in the environment has been on the increase [6]. However, there were reports concerning the occurrence of Pharmaceutical residue in the environment even during the 1970s [7]. Preliminary results from studies conducted in North America and some European countries have confirmed the presence of many pharmaceuticals in the environment [8].

Pharmaceutical effluents are liquid wastes generated by pharmaceutical industries during the process of drugs manufacturing. The steps involved in compounding of drugs (which include extraction, processing, purification and packaging) generate air emissions, liquid and solid wastes. The occurrences of numerous pharmaceuticals in municipal waste water and in surface waters that receive waste water effluent have been reported [9-10]. Liquid effluents resulting from equipment cleaning after batch operation contain toxic organic residues. Their composition vary depending on the product manufactured, the materials used in the process and other process details. Thus, the Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), suspended solids as well as phenols and pH of pharmaceutical effluents is not consistent depending on the product manufactured, materials used and the processing details [6].

Generally, pharmaceutical industries do not generate uniform waste streams, due to the variety of medicines produced during any given processing period. There are risks associated with pharmaceutical effluents to human health and environmental species; hence there is a great need for this to be established. The US Food Drug and Administration (US FDA) has recommended that toxicity test (LC50, EC50) using both aquatic and terrestrial organisms, algae species bioassay test and other necessary tests should always be carried out on pharmaceutical effluents [11].

The importance of the gills in respiration and ionic regulation of fish has prompted many investigations of the effects caused to this organ by changes in environmental factors [12-14]. The gills are efficient tools for biomonitoring potential impacts [15], because of their large area in contact with the water and high permeability [16-18], and environmental impact caused by pollutants may affect fish gills [19-20].

Pollutants can directly cause degeneration or necrosis (cell death) in gill tissues [21-22], but fish can develop mechanisms to react to pollutants that can result in cell hyperplasia, with increased density of the cells of the secondary lamellae [23]. Most of the gill injuries caused by chronic (sublethal) exposure to pollutants affect the lamellar epithelium [24]; however, some alteration in the blood vessels can occur when fish are under severe stress [21].

Metals in effluents can either increase or decrease histopathological changes, depending on the additive effects of the reacting metals in such an effluent, concentration, fish species type, physiological status of the fish species, length of exposure and other factors [25-26]. Histological alterations in fish liver is a highly sensitive and accurate way to assess the effects of xenobiotic compounds both on field and in the laboratory.

Xenobiotic compounds usually concentrate in the tissues of aquatic biotas and are known to produce cumulative deleterious effects [27-28]. Therefore, the application of environmental toxicology studies on non-mammalian vertebrates is rapidly expanding for the evaluation of the effects of noxious compounds [29-30]. Indiscriminate discharge of such compounds that contains mixtures of heavy metals such as herbicide, pesticides, detergent etc, their careless handling, accidental spillage or discharged of treated effluents into natural waterways have harmful effects on the fish population and other forms of aquatic life and may

contribute long term effects in the environment [31-32].

Pollutants build up in the food chain and are responsible for the adverse effects and death in aquatic organisms [33]. Fishes are widely used to evaluate the health of aquatic ecosystem and their physiological changes serve as biomarkers of environmental pollution [34]. *C. gariepinus* is most widely used because; it is hardy since it is able to tolerate both well and poorly oxygenated waters. It is widely cultivated in Nigerian water bodies, hence used as biological indicators of ecotoxicological studies. Thus, the aim of this study is to investigate the chronic toxicity of pharmaceutical effluent on *C. gariepinus* gills and liver as biomarkers of environmental quality in rivers, because environmental impacts caused by pollutants may affect these fish tissues.

MATERIALS AND METHODS

Effluent Sampling

The test solution (pharmaceutical effluent) used in this toxicity test was collected from the discharge point of an indigenous Pharmaceutical Industry in Oyo State, Nigeria. The company produces analgesics, anti-malarias, anesthetics, multivitamins, antibiotics, and human vaccines. The effluent samples were collected using plastic kegs. The pH readings of the samples were taken at the point of collection and then kept at 4 °C until use.

Fish Samples

Juveniles of African catfish (*C. gariepinus*) with mean weight 10 ± 0.2g and standard length range of 6.3 ± 0.3 cm, procured from Oyo State Fish Hatchery, Ogbomoso, Nigeria, were used for this toxicity assay. The test organisms were acclimatized to laboratory conditions for two weeks, in stock tanks to avoid overcrowding. During acclimatization and experimental periods, the test organisms were fed once daily with commercial feed pellets. The remnants containing unconsumed feeds and faecal particles were removed from the stock tanks and the test solutions were replenished every 24 hours [35].

Physicochemical Properties

The water temperature was 30.02 ± 0.09^0 C, pH was 6.72 ± 0.08 [(The pH was determined using a pH meter (model E512). The pH meter was standardized by buffer of pH 7 and 9 just before use, each time it was engaged in pH determinations [36], total dissolved solids (TDS) was 152.0mg/L, (this was determined to measure the dissolved oxygen consumed by microorganisms [37], biological oxygen demand (BOD) was calculated using the formula:

BOD = a – b x 4ppm

Where: a = Titration of distilled water; b = Titration of water samples; PPM = Parts per Million and, the dissolved oxygen was 11.02 ± 1.22 mg/l (this was taken using the method proposed by A.P.H.A., 1985) [36], the formula used was,

$$\text{Dissolved Oxygen (mg/L)} = \frac{\text{Titrant (N)(8)(1000)}}{100}$$

Heavy Metals Determination

Concentrations of eight (8) heavy metals were measured in the experimental effluent. These are Cadmium (Cd), Chromium (Cr), Copper (Cu), Iron (Fe), Manganese (Mn), Nickel (Ni), Lead (Pb), Zinc (Zn)

Toxicity Testing

A static renewal bioassay procedure [38] was adopted in which the test media was regularly renewed at every 24 hours at the set concentrations. A preliminary investigation (range finding test) was carried out prior to the commencement of the research to determine the definitive concentrations suitable for the testing chemical [39]. The definitive concentrations used for this toxicity tests were 6.25%, 12.50%, 25.00%, 50.00% and 100.00%, as well as the control (0.00%) in two replicates [39-40]. Ten fully acclimatized fish were exposed to each concentration of the effluent. The toxicity test was conducted for 21 days. This permitted the monitoring of the behavioral and mortality responses of the test organisms to varying concentrations of the pharmaceutical effluent.

Histopathological Preparation

After 21 days, the gills and livers were removed and prepared for probable histopathological degradations [41]. The organisms were decapitated, dissected and assessed individually by separating the experimental fish from the control fish. After proper dissection, the gills were carefully removed and small pieces were fixed in 10% formalin for 24hrs: After which, the liver were dehydrated through a series of graded alcohol, cleared in xylene, infiltrated with paraffin in a vacuum oven at 56 ^{0}C, then embedded in paraffin wax. Sections of 6 microns thickness were cut, mounted and stained with heamatoxylin and eosin. Each section was then used to make slides of tissue and was observed under the microscope for proper description of their histological structures, appearance, and cell arrangement. The respective photomicrographs of the slides were also taken for proper observations and interpretations.

RESULTS

Physicochemical Analysis

To evaluate the pollution load in the pharmaceutical effluent before

use, it was analyzed for various physico-chemical parameters. The physicochemical parameters measured were temperature (tempt. ^{0}C), hydrogen ion concentration (pH), total suspended solids (TSS), total dissolved solids (TDS) and biological oxygen concentration (BOD). The results were compared with the specifications of the Federal Environmental Protection Agency (FEPA) [42], as shown in Table 1

Table 1: The physico-chemical analysis of the pharmaceutical effluent

Parameters	Pharmaceutical Effluent	FEPA (1991) Specifications
Tempt ^{0}C	23	Less than 40
pH	6	6-9
TSS (mg/l)	80	25
TDS (mg/l)	384	2000
BOD	98	30

Heavy Metals Analysis

The results obtained from the heavy metal contents (Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Zn) detected in the pharmaceutical effluent showed that Cu, Mn and Ni which were slightly above the permissive limits. The results of the heavy metals analysis was also compared with FEPA specifications. See Table 2.

Table 2: Heavy Metals Analysis of the Pharmaceutical Effluent

Heavy Metals (mg/l)	Pharmaceutical Effluent	FEPA (1991) Specifications
Cadmium	0.01	0.01
Chromium	0.05	0.05
Copper	0.22	0.10
Iron	0.07	0.30
Manganese	0.20	0.05
Nickel	0.56	0.05
Lead	0.12	0.12
Zinc	0.04	5.00

Fish Mortality

The result of the mortality observed during the experiment is shown in Table 3.

Table 3: Mortality rate of *Clarias gariepinus* exposed to pharmaceutical effluent

Concentration (%)	Total Mortality	% Mortality
Control	0	0
6.25	2	20
12.50	5	50
25.00	8	80
50.00	10	100
100.00	10	100

Determination of LC_{50}

The LC_{50} is the effective concentration at which fifty percent (50%) of the test organisms are killed. This was determined using a probit analysis (SPSS 15 software). The LC_{50} at 21 days was 12.16% (Figure 1).

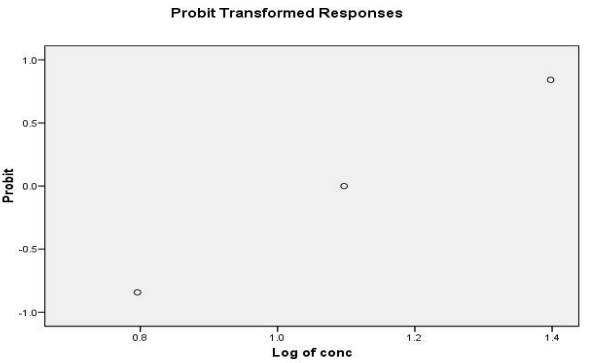

Figure 1: the probit analysis of the LC_{50} after the chronic toxicity test (LC_{50} = 12.16%).

Histopathology of the Gills

Various histopathological abnormalies were observed in the test organisms. These include epithelial lifting, cellular infiltration and vacuolation in the gills. The histological degradations in the tissues were classified [43]. Four major histological changes (epithelial lifting, cellular/leucocyte infiltration, vacuolation, and necrosis) were considered.

The gill sections of the control showed normal cell arrangements (Plate 1). The gill sections of the treated fish showed cellular infiltration, vacuolation and high epithelial lifting (Plate 2).

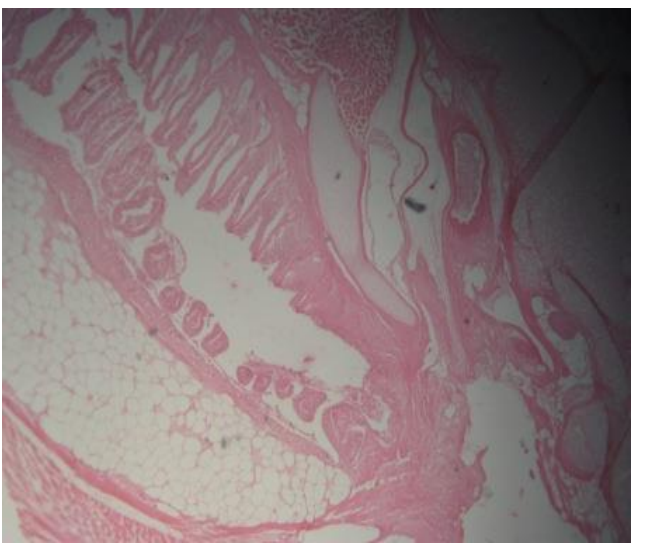

Plate 1: Photomicrograph of the gill of the fish in the control group (Mag. x100)

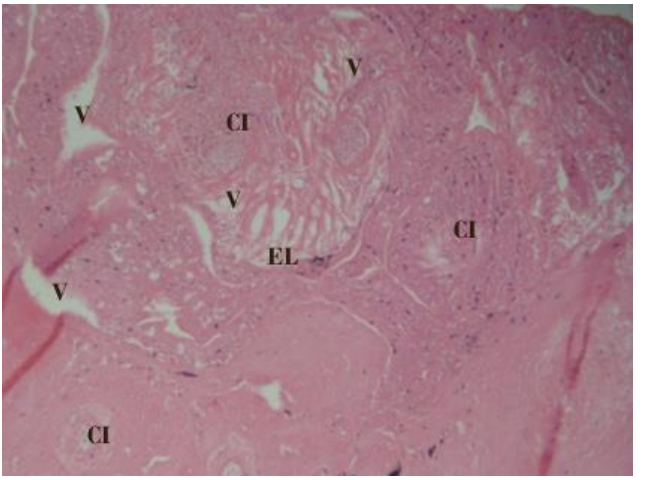

Plate 2: Photomicrograph of the gill of the fish in effluent treatment showing cellular infiltration (CI), vacoulation (V) and high level of epithelial lifting (EL) (Mag. x100)

Histology of the Liver

Histological investigations of the liver tissues showed a typical structural organization of the parenchymatous cell appearance of the hepatocytes in the untreated fishes (Plate 3). However in the fishes treated with the pharmaceutical effluent (Plate 4), the major histological abnormalities observed were oedema, cellular infiltration, congestion of central vein and cellular necrosis, which showed a progressive architectural distortion at varied concentrations.

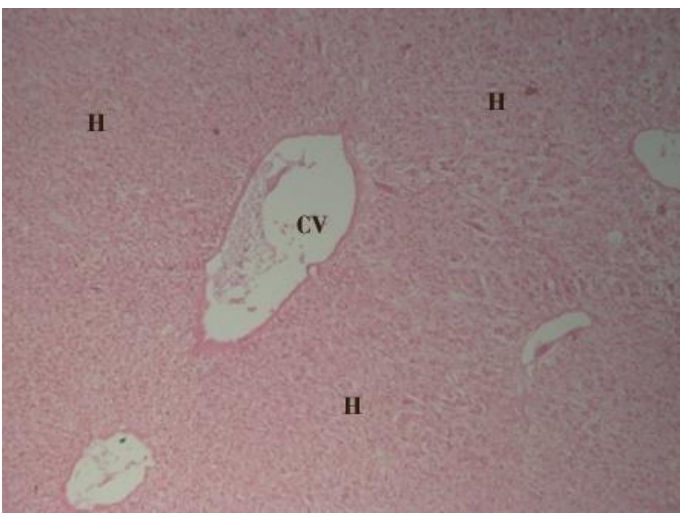

Plate 3: Photomicrograph of the liver of the fish in the

control group, showing normal Central Vacuole (CV) and well arranged Hepatocytes (H). (Mag. x100)

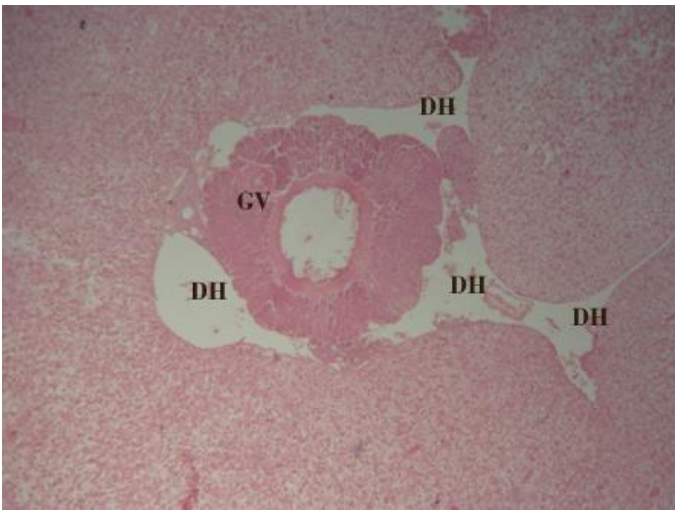

Plate 4: Photomicrograph of the liver of the fish in the effluent treatment showing a necrotic condition (cell death), with total hepatocyte degeneration (DH) and glycogen vacuolation (GV) (Mag. x100)

DISCUSSION

At the control experiment, no adverse behavioral response or any record of mortality was recorded throughout the period of the bioassay. However, the fish exposed to the pharmaceutical effluent concentrations showed some signs of stress, lack of balance, erratic movement, gasping for breath, over secretion of mucus and loss of equilibrium. The stressful and erratic behavior of the fish in this investigation gives a signal to respiratory impairment, and this may be as a result of the effect of the pharmaceutical effluent on the gills [44].

Indiscriminate deposition of effluent into an aquatic system might decrease the dissolved oxygen concentration, which could impair respiration leading to asphyxiation (an indication of unconsciousness or death produced by failure of the blood to become properly oxygenated in the lungs) and may ultimately result into organ architectural degradation such as liver dysfunction [26].

Previous histopathological studies of fish exposed to pollutants have shown that fish gills are efficient

indicators of water quality. Fish gills are vulnerable to pollutants in water because of their large surface area and location. However, the gills perform numerous functions, which include respiration, excretion of nitrogenous waste products and acid-base balance. Functional impairments of gills caused by pollutants cause significant damages to the health of the fish, and fish gills are considered to be the most appropriate indicator of water pollution levels [45]. Also, there are increasing evidence that toxic compounds have the potential to cause the most harm to tissues and organs that are contacted first [46].

The sections of the liver obtained from the treatment group had disrupted histological organization compared with the control group. Some of the deleterious effects seen in the section of the liver obtained from the treatment group include degeneration and disruption of the hepatocytes (liver cells), degeneration of the cells lining the bile ducts and occlusion of the central portal vein. With these histological abnormalities, the anatomical, physiological and biochemical functions of the liver could be compromised.

The hepatocytes frequently contain glycogen and the hepatocytes maintain a steady level of blood glucose. This is one of the main sources of energy for use by the body [47-48]. A compromise in the integrity of the hepatocytes could lead to improper functioning of the liver.

The concentrations of Cd, Cr, Fe and Pb in the effluent fell within FEPA specifications, their residual effects which may impair organs like the gills, liver, brain, kidney and genital organs should not be ruled out. Zinc that fell below tolerable limit could also by way of its additive effect become toxic to all forms of aquatic life. Even if concentrations of metals fall within or below FEPA specifications, they could also be biomagnified in a water body; the resultant effect could be gradual accumulation of the metals in water which in turn become toxic to aquatic organisms [44].

CONCLUSION

Fish are intimately associated with their aqueous environment, therefore physical and chemical changes in their ecosystems are rapidly reflected as quantifiable physiological measurements.

Even though individual concentrations of any drug or toxicant (e.g heavy metals) might be low, the combined concentrations from different drugs or toxicants could be fatal to aquatic or human health. The study provides indications of potential

adverse environmental impacts of pharmaceutical effluent in the receiving environment. It helps to provide and encourage awareness among the general public that pharmaceutical effluent is toxic to the environment. This study has shown that gills and liver of C. gariepinus are sensitive to and suitable for toxicological studies on pharmaceutical effluent.

NOTE: The authors have no conflict of interest.

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