# SUB-LETHAL EFFECTS OF PHARMACEUTICAL EFFLUENT ON TISSUES AND ORGANS OF *Clariasgariepinus* (Burchell, 1822)

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## DEDICATION

This research work is dedicated to the Almighty God, My dearly loving mother, Mrs. Helen Eleyele and Chief (Mrs.) Florence Ebun Oni.

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## ABSTRACT

This study assessed the physicochemical parameters of pharmaceutical effluent and investigated its sub-chronic toxicity on the tissues and organs of *Clariasgariepinus*. It also investigated the histopathological effects of the pharmaceutical effluent on tissues and organs of *C.gariepinus* and alterations in biochemical indices of the fish as a result of the effluent toxicity. This was with the view to providing information on potential adverse effects of the effluent on *C.gariepinus*.

Physicochemical parameters such as dissolved oxygen, temperature, pH, alkalinity, exchangeable anions and cations, total hardness and concentrations of five selected heavy metals (Cd, Cr, Cu, Fe and Pb) of the pharmaceutical effluent collected from a known pharmaceutical industry in Ilorin, Kwara State were evaluated. One hundred and eighty juveniles of*C. gariepinus*procured from the Fish Hatchery Unit, Department of Animal Science, ObafemiAwolowo University, Ile-Ife, which were acclimatized for one week in stock tanks were used for the toxicity assay. After acclimatization, a range finding test was carried out prior to the commencement of the definitive test. A static renewal bioassay procedure was adopted in which the test media was regularly renewed every 48 hrs at the set concentrations. Five juveniles of *C.gariepinus* were introduced into twelve different tanks containing gradient concentrations(0, 2, 4, 6, 8 and 10%) of the pharmaceutical effluent for the sub-chronic evaluation. This concentration was based on the median lethal concentration ($LC_{50}$) determined using Probit analysis. After 21 days of exposure, two fishes per concentration were randomly selected, the tissues were removed and analyzed for histopathological changes and antioxidant enzyme (Superoxide Dismutase (SOD), Catalase (CAT), and Glutathione-S-Transferase (GST))

activitiesfollowing standard protocol. One-way Analysis of Variance (ANOVA) was used to compare the means of results obtained.

The results of the physicochemical analysis of the effluent showed that the effluent was characterized by high alkalinity (118 mg/L), total hardness (131.34 mg/L), and dissolved oxygen (11.0 mg/L). However, the amount of Cd, Cr, Cu, Fe and Pb (0.01, 0.032, 0.10, 0.068 and 0.012 mg/L) respectively were below the national and international limits for fish culture.The $LC_{50}$ value for the acute toxicity was 18.32%.Histopathological examination of the liver showed major changes which included hyperemia, cytoplasmic vacuolation and cirrhosis. The fish muscle analysis showed thickening andnecrosis of the muscle bundle at the low effluent exposure concentrations, while hyperplasia, fibrosis and intramuscular edema were observed in the fish exposed to high concentrations of the effluent. The gills in the fish showed cellular necrosis, cellular infiltration by inflammatory cells, epithelial erosion and lifting with effluent exposure. Also, the fin tissues showed disruption of muscle bundles, osteonecrosis, cartilage and epithelial degeneration with erosion and ulceration.Highest values of antioxidant enzymesSOD, 0.01 µmol/min/mg protein, CAT, 24.57 µmol/min/mg protein and GST, 56.73 µmol/min/mg proteinobtained in the liver of the test organism exposed to the effluent were significantly ($p < 0.05$) higher from the values obtained in the gill, fin and muscle of the effluent exposed fish.

This study concluded that inspite of treatment, pharmaceutical effluent from a known pharmaceutical industry in Ilorin, was still a potent contaminant to juveniles of*C. gariepinus*.

# CHAPTER ONE

# INTRODUCTION

## 1.1 Background to the Study

The importance of water for sustenance of life cannot be overemphasized. Whether it is in use of running water in the homes, growing crops in the farms, or the increased uses in industry, remain immeasurable. It is important therefore, to note that depletion of this commodity either through contamination, or careless use, results in serious consequences (Owa, 2014). Water is considered polluted if some substances or condition is present to such a degree that the water cannot be used for a specific purpose (Anyata and Nwaiwu, 2000).

Pollution is created by industrial activities, everyday human activities that man engages in, such as dumping of municipal waste in urban residential areas, oil spillage and agricultural practices and most notably, models of transportation. Also, Excess phosphorus – and nitrogen – containing fertilizer, herbicides and pesticides cause serious eutrophication when washed by rain into rivers and this endangers life (Owa, 2014). Thermal pollution which is the discharge of hot water from cooling engines in the industries is another source of water pollution. This increases water temperature and lowers the metabolic rate of organisms which then raises their oxygen demand (Evelyn and Tyav, 2013; Owa, 2014).

Pharmaceutical effluents are liquid wastes generated by pharmaceutical industries during the process of drug manufacturing (Agboola and Fawole, 2014). The occurrences of numerous pharmaceuticals in municipal waste water and in surface waters that receive waste water effluent have been reported (Hartmann *et al.,* 1998; Hirsch *etal*., 1999). Pharmaceutical plants generate a

wide variety of wastes during manufacturing, maintenance and housekeeping operations and they suffer from inadequate effluent treatment due to the presence of recalcitrant substance. (APHA, 1995).

The need to detect and determine the impact of contamination on the quality of the environment has prompted the study of, and research into, the biochemical and molecular markers of biological effects of pollutants on aquatic organisms (Faverney*et al.,* 2001). A biomarker, according to Peakall and Walker (1996), is "any biological response to an environmental chemical at the individual level demonstrating a departure from the normal status." Van der Oost*et al.* (2003) state that a biomarker should possess the following qualities: an assay that is reliable, cheap and easy; sensitivity to pollutant exposure and/or effects; mechanism of relationship between pollutant exposure and response should be understood; impacts of confounding factors to the biomarker response should be well established; and toxicological significance of biomarker and impact on organisms should be established. Biomarkers may also be defined as changes in the biological response that are linked to the exposure to, or toxic effects of, chemical substances in the environment, analysed in body fluids, cells or tissues. They are sensitive indicators demonstrating the penetration of a toxic substance into the organism and its distribution among tissues. They therefore are the decisive indicators of the toxic effect (Van der Oost*et al.,* 2003).

Fish have been used as aquatic contamination indicators for many years. In the case of an environmental disaster, they are unable to leave the site affected (Gadzala-Kopciuch*et al.,* 2004), bioaccumulate toxic substances (Andrade *et al.,* 2004) and because they are the last link in food chain in the aquatic environment, they may negatively influence the safety of food and raw materials of animal origin (fish and fish products) (Gadzala-Kopciuch*et al.,* 2004). Fish

exposure to chemical contaminants induce lesions in different target organs, especially in liver (Oliveira Ribeiro*et al*., 2005; Rabitto*et al*., 2005; Mela*et al*., 2007; Miranda *et al*. 2008) and gills (Tkatcheva*et al*., 2004; Oliveira *et al*., 2005; Benli*et al*., 2008). According to Lemes and Braccini (2004), and Ayas*et al*. (2007), liver is an important target organ related to important metabolic and detoxification mechanisms. In addition, gills are in permanent contact with water and represent an important target organ to pollutants dissolved in water, this organ is essential on gas exchanges and osmotic regulation. Pollutants can directly cause degeneration or necrosis (cell death) in gill tissues (Camargo and Martinez, 2007; Ayandiran*et al.*, 2009), 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 (Tietge*et al.,* 1988). Most of the gill injuries caused by sub-lethal exposure to pollutants affect the lamellar epithelium (Hinton and Laure´n, 1990); however, some alteration in the blood vessels can occur when fish are under severe stress.

Biochemical markers like enzymes are frequently used as an indicator of the general state of health and early warning of stress in fish under stressful conditions (Barnhorn and Van-vuren, 2004; Abou El-Naga *et al*., 2005; Osman *et al.*, 2010). Fish are therefore widely used to evaluate the health of aquatic ecosystem and their physiological changes serve as biomarkers of environmental pollution (Kock*et al.*, 1996).

This study was motivated to evaluate the acute and sub-lethal toxicity of pharmaceutical effluent on tissues and organs of *Clariasgariepinus*. *C. gariepinus*were used for this research because it is widely cultivated in Nigerian water bodies; it is hardy, it is able to tolerate both well and poorly oxygenated waters and it is highly relished in African dishes, hence used as biological indicators of ecotoxicological studies.

## 1.2 Justification of the Study

The increase in the use of various pharmaceutical products in Nigeria has resulted in the development of pharmaceutical manufacturing companies of which many of them are small scale and tend to exhibit improper discharge of their wastes(Velagaleti and Burns,