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Chronic Exposure to No-Effect Concentration of Diazinon Induced Histological Lesions in Organs of *Clarias gariepinus*

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Abstract

In all parts of the world pesticides have been found in the aquatic ecosystem and scientific evidence has also shown that they can enter the food chain. Diazinon is an organophosphate pesticide, widely used in agriculture to control a wide variety of suckling and leaf eating insects and recently in fish culture to suppress some parasitic diseases; nevertheless, there is little study on its adverse effect on fish. In this study, seventy-two (72) apparently healthy catfish comprising adult and juvenile of both sexes were used to set up triplicate experimental groups of those exposed to culture water alone (control group), fish exposed to pre-determined no-effect concentration (0.405 ppm) of Diazinon (test group). The fish were exposed for 28 days and fish were sacrificed and organs harvested on days 21 and 28 to determine the effect of long-term exposure to diazinon on organ histology. Histological changes observed in diazinon-exposed catfish were hyperplasia and fusion of the gill epithelium, hyperplasia of mucoid producing cells and aggregation of melanin pigment in the skin. Histological lesions were also seen observed in other organs, including severe diffuse cellular swelling and fatty degeneration of the liver, interstitial congestion of the kidney, carbon deposit on the wall of the heart and multifocal haemorrhage. The water quality of the control was not significantly different from that of the test group throughout the experiment. The lesions detected in cells, tissue, or organs represent an integration of cumulative effects of physiological and biochemical stressors. The histological alterations observed in vital organ of fish show that exposure to "no-effect"

concentration of diazinon induced structural damage in fish organs and are likely to affect the functionality of the organs. For example, the adverse effect on the gill might disrupt its feeding and oxygen uptake.

Keywords

Diazinon, Histopathology, *Clarias gariepinus*

1. Introduction

The end consequences of leaching and rain runoff, pesticides or pesticide residues are added continuously to the aquatic environment, thereby polluting fish and water bodies [1]. The natural aquatic systems may extensively be contaminated with indiscriminate use of pesticides, careless handling, accidental spillage, or discharges of untreated effluents into natural waterways and these will have harmful effects on fish population and other forms of aquatic life and may contribute long-term effects in the environment [2]. Water pollution by pesticides is a serious problem to all aquatic fauna, flora and to a considerable extent man [3]. Unfortunately, fish and water bodies are in grave danger because of bioaccumulation of pesticides. Many types of fish and different aquatic ecosystems have been adversely affected by pesticides which end up being suspended in water thereby making the water unfit for aquatic life [1].

In all parts of the world, pesticides have been found in the aquatic ecosystem and they also enter the food chain. Organophosphate pesticides are also used in fish culture in order to suppress some parasitic diseases nevertheless, the pesticide preparations are considered harmful to fish in most cases [4]. Fish are notorious for their ability to concentrate pollutants in their body tissues and since they play an important role in human nutrition, necessitating the need for screening to ensure the unduly high level of some toxic metals is not being transferred to man through fish consumption [5].

Clarias species is a widely distributed fish in Asia and Africa. In these areas, the fish is extremely popular on account of its tasty flesh, its unparalleled hardness, its rapid growth and high market price [6]. *Clarias gariepinus* is one of the choice fish distributed widely in Africa and other tropical countries of the world where they inhabit calm lakes, ponds, rivers and swamps in areas that are seasonally flooded. Catfish have inhabited all continents at one time or another [7] Due to the residual effects of pesticides, important organs are damaged. Acute and sublethal bioassays are carried out to check the short- and long-term effects of these organophosphates on animal life [8]. Among the common organophosphate used is Diazinon with the chemical formula (0,0-diethyl 0-[6-methyl-2(1-methylethyl)4-pyrimidinyl] is an organophosphate pesticide, is extensively used in agriculture and domestic pest control. This pesticide is also used to control a variety of insects including: aphids,

beetles, scales and pill bugs, primarily in household environment and in agriculture crops [9].

The recent and notable work published on the effects of diazinon on various aspects of fish such as on the structure of the testis of bluegill [10]; on the common carp (*Cyprinus carpio*) embryos and larvae [11]; on expression and activity of intestinal P-glycoprotein [12]; on acetylcholinesterase activity and lipid peroxidation in the brain of *Oreochromis niloticus*. [13]; immunotoxicity and hepatic function evaluation in Nile tilapia (*Oreochromis niloticus* [14]; on acute toxicity of African catfish [15]; on the clinical and biochemical alterations associated with toxicity *in Clarias gariepinus*. [16]; on total protein and transaminase activities in *Clarias gariepinus* [17]; sublethal concentrations on total protein in tilapia fish (*Oreochromis Niloticus*) [18]; toxicological effect on African catfish (*Clarias anguillaris*) [19]; induced clastogenity and pathological changes in ovaries and testes of *Clarias gariepinus* [20] and on blood profile, histology of liver, gills and kidney of catfish, *Clarias gariepinus* [21].

It is imperative to find out the detrimental effects of pollutants specially pesticides on fish since they form an important link in food chain and their contamination by pesticides imbalance the aquatic system. Pesticides severely affect the fish in different ways mostly affecting the vital organs, therefore the present study was carried out to determine the histological changes of organs induced by chronic exposure of catfish (*Clarias gariepinus*) to diazinon.

2. Methodology

Seventy-two (72) apparently healthy adult and juvenile catfish (*Clarias gariepinus*) of both sexes were set up in triplicate, adults and juveniles were exposed separately to avoid cannibalism. Four (4) catfish each (adult & Juvenile) male and female were exposed to different treatment which includes pre-determined no effect concentration 0.405 ppm of diazinon [20] and water (control group). Route of exposure was via culture water. For each group, samples were collected on days 21 and 28 for histology which is the specify days for most chronic effects of fish in aquaculture (organs collected were skin, gills, kidney, hearts and liver) fixed in 10% neutral buffered formalin. Tissue specimens were processed routinely for paraffin sections of 4 - 5 μm thickness, stained with hematoxylin and eosin [22].

3. Histopathology

For histopathological study, specimens from the skin, gills, kidneys, heart, and liver were collected from each fish on day 21 and 28, fixed in 10% neutral-buffered formalin. Tissue specimens were processed routinely for paraffin sections of 4 - 5 μm thickness, stained with hematoxylin and eosin [22]. The water quality like temperature, pH and dissolved oxygen were analyzed weekly 28.35˚C ± 0.75˚C, 7.50 ± 0.00 and 4.16 ± 0.28 mg/L respectively.

4. Results

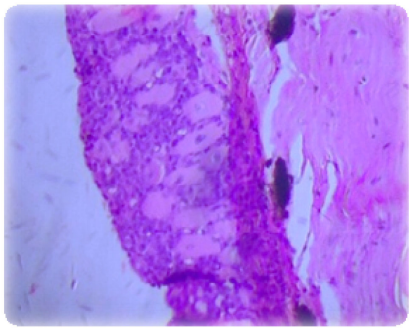

Figure 1. Photomicrograph of a normal skin (H & E) M: ×400.

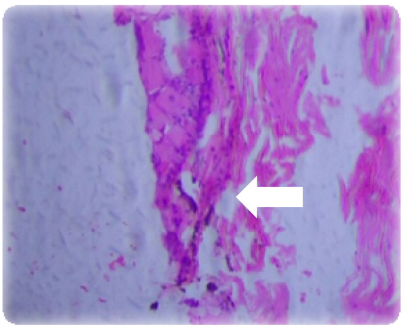

Figure 2. Photomicrograph of the skin showing hyperplasia of mucoid producing cells and aggregration of melanin pigment at 28 days of exposure (H & E) M: ×400.

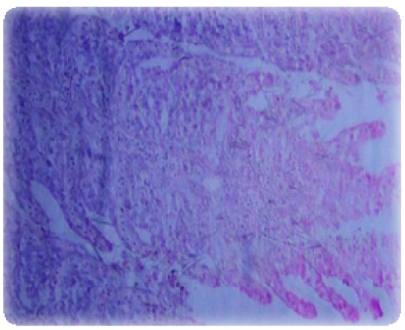

Figure 3. Photomicrograph of a normal gills (H & E) M: ×400.

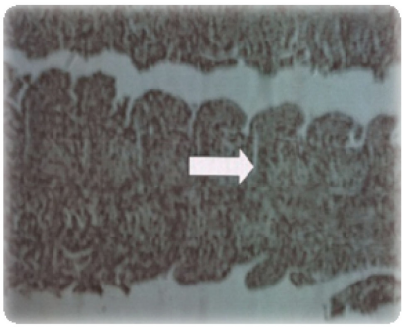

Figure 4. Photomicrograph of the gills showing Epithelial hyperplasia and fusion of the secondary epithelia (arrows) at 28 days of exposure (H & E) M: ×400.

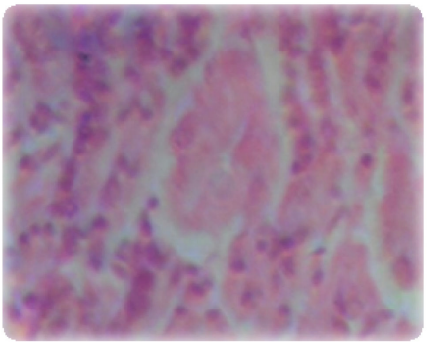

Figure 5. Photomicrograph of a normal kidney (H & E) M: ×400.

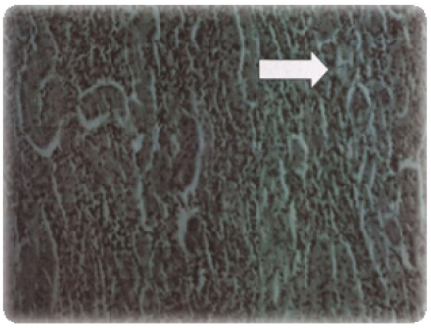

Figure 6. Photomicrograph of the kidney showing interstitial congestion at 28 days of exposure (H & E) M: ×400.

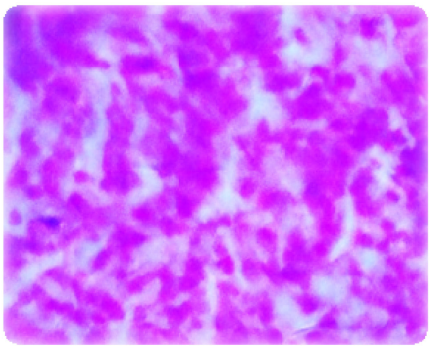

Figure 7. Photomicrograph of a normal heart (H & E) M: ×400.

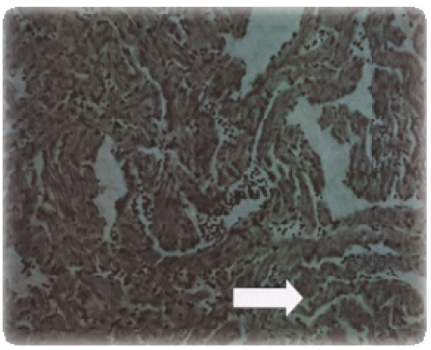

Figure 8. Photomicrograph of the heart showing carbon deposits on the wall at 28 days of exposure (H & E) M: ×400.

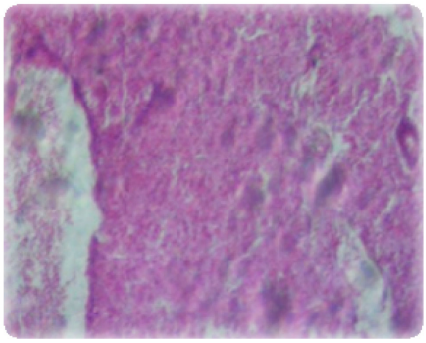

Figure 9. Photomicrograph of a normal liver (H & E) M: ×400.

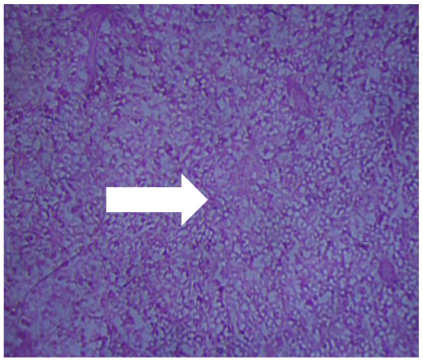

Figure 10. Photomicrograph of the liver showing massive hepatic degeneration at 28 days of exposure (H & E) M: ×400.

5. Discussion

Fish and aquatic invertebrates have been considered to be an efficient and cost effective model system for studying the toxic, mutagenic, and carcinogenic potential of pollutants [23] [24] [25] [26] due to their ability to metabolise, concentrate, and store water-borne pollutants [27]. The exposure of fish to chemical contaminants induced a number of lesions in different organs [28]. Gills [29], kidney [30], and liver [31] are suitable organs for histological examination in order to determine the effect of pollution.

Histological changes when compared with the normal organs which was only exposed to cultured water made on skin, gills, kidneys, heart and liver of the diazinon exposed fish are represented in **Figures 1-10**. Showing hyperplasia of mucoid producing cells and aggregation of melanin pigment, Epithelial hyperplasia and fusion of the secondary epithelia, hyperplasia of hematopoietic cells and interstitial congestion, carbon deposits on the wall and severe diffuse cellular swelling and fatty degeneration showing by a massive hepatic degeneration respectively after 28 days' expose to Diazinon respectively.

Gills and skin of fishes is the primary initial target of toxic chemicals, pesticides inclusive and cytological changes in gill morphology. Gills are mainly to be respiratory organs and any damage to them would cause disturbance in all physiological and metabolic activities by reduction in the supply of oxygen. It is

quite evident that the damage caused to the gills results in impairments in gaseous exchange capacity of the gills, which ultimately lead to respiratory distress. The gills showing hyperplasia and fusion in this study, similar alterations in the gills have also been reported in the fishes exposed to metals [32] [33] [34] and organic contaminants [35] [36] [37] for the damage of gills in the fish treated with toxicants. The kidneys of fish exposed to diazinon showed histological changes after long time exposure of 28 days of treatment such as hyperplasia of hematopoietic cells and interstitial congestion. [38] has also reported that exposure of cypermethrin causes pathological effects on gills, liver and kidneys of catfish. Fusion of tubules and condensation of glomeruli content were reported by them. Also Pyknotic nucleus, destruction of fusion of tubules, condensation of nuclei was observed in the kidney of *Clarias gariepinus* exposed to diethyl phthalate [39] as seen in the present study.

The organ most associated with the detoxification and biotransformation process is the liver, and due to its function, position and blood supply [40]. Liver is an important organ for assessing the pollutant's effects as the chemicals are stored in it. Severe diffuse cellular swelling and fatty degeneration of the liver (massive hepatic degeneration), hepatocytes necrosis may be the results of inability of the fish to regenerate new hepatocytes [37]. Necrosis occurred in the liver of exposed fish can also be related to the excessive burden of work for the detoxification of insecticide from the body. Similar line of reasoning for the liver necrosis was presented by [41] in fish treated with toxicant. [39] [42] [43] have reported necrosis of tubular epithelium and pyknotic nuclei in the liver of fish exposed to chemicals. In this study, the heart presented with carbon deposits on the wall which can also be indicative of reduction of oxygen for the circulation of blood to all part of system leading anaemia which can be injurious to the fish wellbeing.

6. Conclusions

The histopathological changes observed in the skin, gills, kidney, heart and liver of the *Clarias gariepinus* in the present study indicate that the fish were responding to the direct effects of the contaminants. Such information confirms that histopathological alterations are good biomarkers for field assessment, in particular in tropical areas that are naturally subject to a multiplicity of environmental variations. As a result, this study showed histopathological changes in different organs of the catfish exposed to prolonged dose of diazinon, giving an affirmatory of diazinon having a detrimental effect on the cellular integrity of the organs. Therefore, in culturing, these could lead to poor performance, reduced productivity, compromised immune status and may eventually lead to death of the fish which at large can result in economic detune of the fish industry.

Therefore, strict and monitory control of the use of diazinon is hereby recommended to prevent harmful contact on feral and cultured fish.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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