

*Full Length Research Paper*

# Histopathological responses of the gill and liver tissues of *Clarias gariepinus* fingerlings to the herbicide, glyphosate

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**African catfish, *Clarias gariepinus* fingerlings were exposed to sub lethal concentrations of herbicide, glyphosate (0, 0.05, 0.1%, v/v) over 42 days period. The gills showed marked alterations in the epithelia in response to glyphosate treatment. There was fusion in adjacent secondary lamellae resulting in hyperplasia, with profound oedematous changes, characterised by epithelial detachment. In the liver, the enlargement of the hepatocytes was related to the concentration and duration of exposure to glyphosate. There were also large vacuoles in the hepatocytes, with pyknotic nuclei, and cytolysis that increased with concentration. Focal necrosis was also observed in the hepatocytes. It was concluded that glyphosate has a deleterious effect on the organs of *C. gariepinus*.**

**Key words:** Histopathology, *Clarias gariepinus*, Glyphosate, gill, liver.

## INTRODUCTION

In recent times, there has been the invasion of Nigeria inland and costal waters with the obnoxious water hyacinth, *Eichhornia crassipes* (Mart.) Solms. This has led to the blockade of waterways, limited boat traffic, impairment of water quality, and reduced fishing activities. Its shading and crowding of native aquatic plants has also dramatically reduced biological diversity in aquatic ecosystems. Water hyacinth is prevalent in the coastal lagoons of West Africa during the wet season, but becomes reduced in those areas that become saline during the dry season. It does not tolerate brackish water, and salinity can limit its distribution.

Attempts have been made to control/eradicate this plant through mechanical (use of harvesters), biological (use of weevils), and chemical (use of herbicide) methods. The herbicide most commonly used for its control is glyphosate. It is a broad spectrum, non-selective systemic herbicide. It is usually formulated as an Iso-propyl amine salt. In using this chemical, there is the risk

of leaving residues that may affect non-target organisms. Various workers have reported on the effects of chemicals on aquatic organisms (Mitchell et al., 1987; Servizi et al., 1987; Abdelghani et al., 1997; Neskovic et al., 1996).

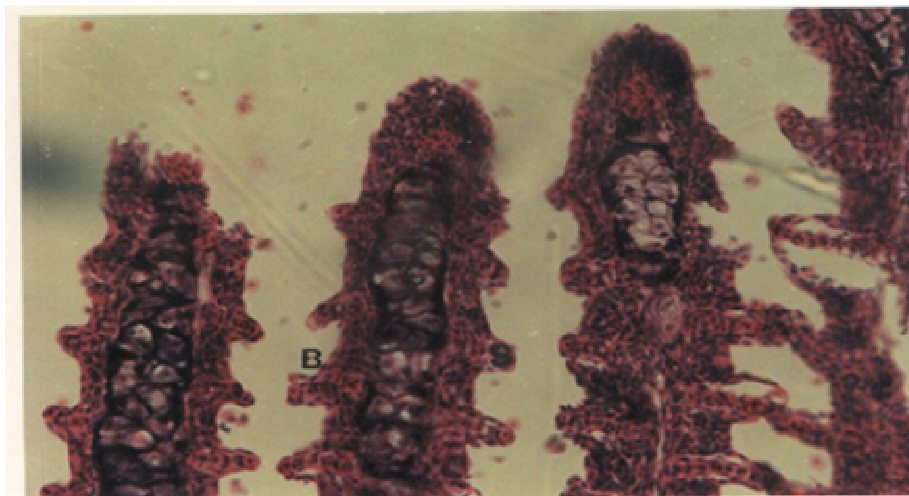
Jiraungkoorskul et al. (2002) reported histopathological changes in the liver and gills of Nile tilapia, *Oreochromis niloticus*, exposed to glyphosate herbicide. In the gills, filamentous cell proliferation, lamellar cell hyperplasia, lamellar fusion, epithelial lifting and aneurysm were observed. In the liver, there were vacuolations of hepatocytes and nuclear pyknosis.

African catfish, *Clarias gariepinus* used in this study is a common aquaculture species in Nigeria and is of commercial importance. This paper reports on the effects of glyphosate on the gills and liver of *C. gariepinus*.

## MATERIALS AND METHODS

Juvenile *C. gariepinus* fish (mean body length = 7.61 cm; mean body weight = 3.25 g) belonging to the same cohort were obtained from CHI farms in Lagos. Fish were transported in oxygenated polythene bags to the laboratory where they were acclimatized in glass aquaria for seven days before the commencement of the experiment. Fish were fed with formulated diet at 3% body weight

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**Figure 1.** The photomicrograph of gill tissue of *Clarias gariepinus* (control), showing the primary (P) lamella, the secondary (S) lamella and the choroide cell (B). The cartilaginous core (C) forms the frame of the primary lamella with channels of blood sinusoids between them. Magnification is X40.

daily. Eight fish per tank were exposed to two sub-lethal concentrations of glyphosate (0.05% v/v; 0.1% v/v) and a control (non-chlorinated water, without glyphosate). These concentrations were selected based on earlier results of acute toxicity tests in which 100% mortality occurred within 14 days in stronger glyphosate concentrations. Each experimental condition was replicated twice over 42 days period. The glyphosate solutions were prepared by diluting isopropyl amine salt in water.

Water in the aquaria were analysed frequently for the exact concentration of glyphosate. Fish were killed and the tissues of the gill and liver were fixed in 10% formal saline, and were analysed histologically every 14th day. The fixed tissues were dehydrated in an increasing gradient of alcohol (70, 80, 90 and 100%) for 30 min each. The tissues were eventually dried in acetone, and cleared in xylene for 30 min. The tissues were then infiltrated by embedding in molten wax and sectioned at 8  $\mu$ m. Each section was then mounted on a slide, stained with haematoxylin and counterstained with eosin.

## RESULTS

### Gill Tissue

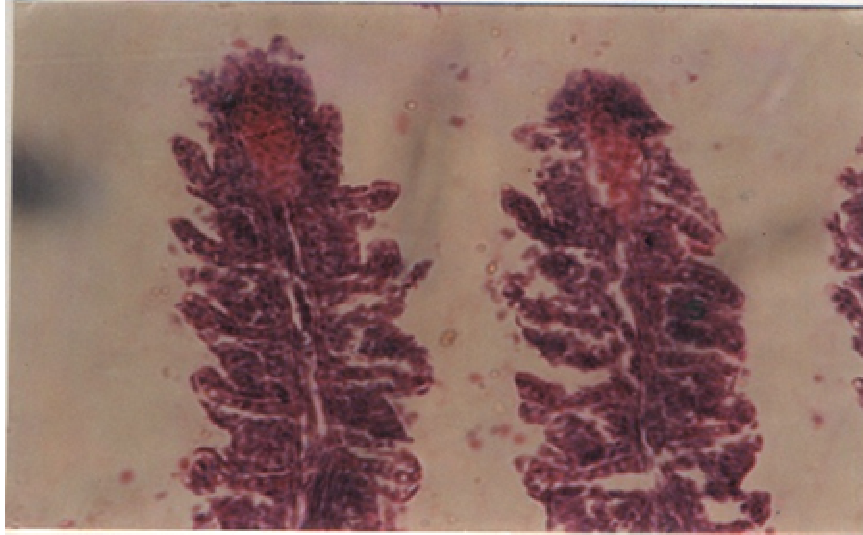
The gill arches of *C. gariepinus* in the control group show normal arrangement pattern. The arches contain primary lamellae (Figure 1). Projecting on the lateral sides of primary lamellae are the secondary lamellae (respiratory lamellae). The entire mass of primary lamellae is covered by stratified squamous epithelium. The surface of the secondary lamellae is covered with a delicate layer of a simple squamous epithelium that is the active exchange pillar cells. In the core of the primary lamellae is a rigid mass of cartilaginous tissues around which are traces of vascular channels. The chloride cells are more frequent at the base of the secondary lamellae.

Varied morphologic changes occurred in the gill tissue of the treated aquaria. This was more evident in higher

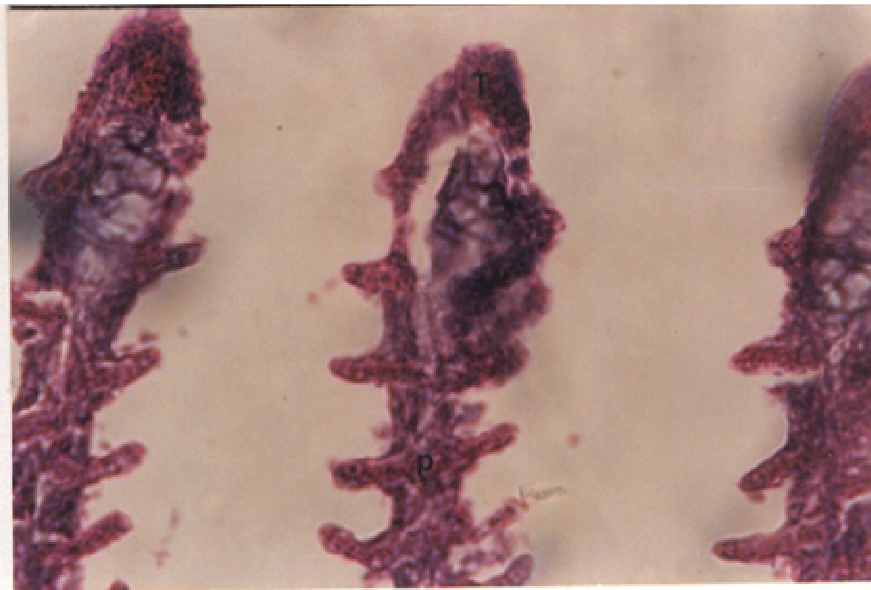
concentrations of glyphosate compound as well as duration of exposure. At 0.05% v/v concentration on the 14th day (Figure 2), the gills exhibited marked alterations in their epithelia. The epithelia is no longer continuous particularly the more delicate respiratory lamellae. Thus, there is fusion at adjacent secondary lamella as a result of hyperplasia. Oedema at the secondary lamellae and swelling of the epithelia cells were observed with epithelia detachment. At concentration of 0.1% v/v at 14th day interval (Figure 3), the primary and secondary lamellae show significant reduction in size. The oedematous separation of primary and secondary lamellae leads to sloughing at the tip. The pillar cells have been altered and blood spaces expanded. At 28th day (Figure 4, 0.05% v/v), following supervening distress, the elongated secondary lamella appears club-like. A measure of fusion is equally noticed at the tips. Severe epithelial disintegration occurs after 42nd day (0.05% v/v) of exposure (Figure 5). This is followed by a necrosis of lamellae epithelial cells (primary and secondary). There is severe hyperplasia with profound oedematous changes characterized by epithelial detachment.

### Liver tissue

A section of the liver seen in the light microscope (LM) at low magnification reveals the general histology of the organ in the control group (Figure 6). It exhibited the typical parenchymatous appearance. Each tube is surrounded by a very thin connective tissue capsule, extends as trabeculae into the body of the lobes and dividing them into irregularly shaped lobules. The liver hepatocytes are polygonally shaped with central spherical nucleus. The cells are arranged as irregular cord-like



**Figure 2.** The 14<sup>th</sup> day exposure (0.05% v/v) showing fusion of *Clarias gariepinus* adjacent secondary lamella (S), edematous changes and discontinuous epithelia lining. Magnification is X40.

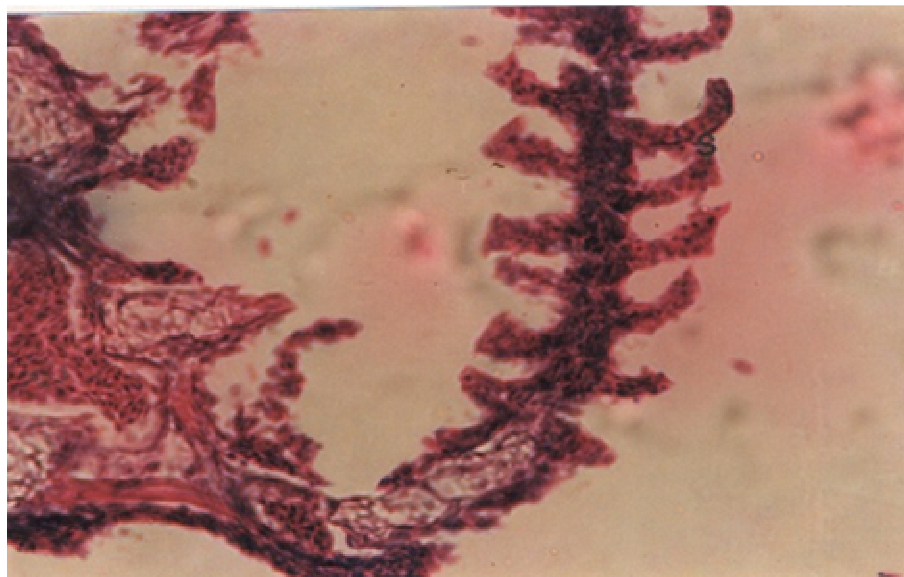


**Figure 3.** At increased concentration (0.1% v/v), there was sloughing at the distal part of primary lamella of *Clarias gariepinus* with irregular epithelia thickening (T) at the tip. Magnification is X40.

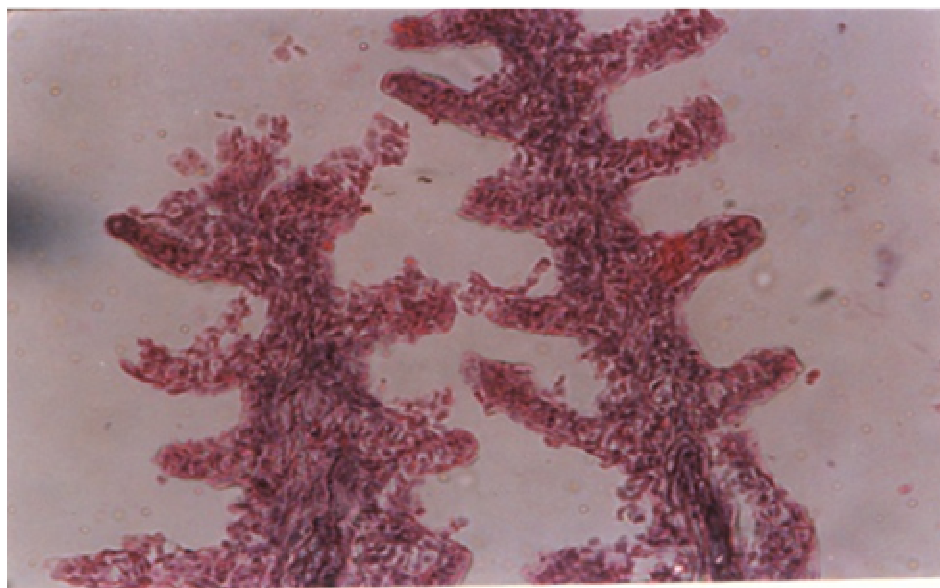
structure in the section separated by sinusoids. The radial arrangements of the cells show normal convergence towards the central vein.

Histomorphology of liver specimens exposed to glyphosate compound show diffuse changes in the hepatic parenchyma. More significantly is the enlargement of hepatocytes in proportion to the increase in size of the nuclei. At day 14 (Figure 7, 0.05% v/v), the specimen is characterized by an increase in density of connective tissue with imminent congestion at the sinusoidal spaces.

There are large vacuoles within the cytoplasm resulting from cell membrane degeneration. Many of the nuclei have become pyknotic with gradual process of cytolysis. At 0.1% v/v (Figure 8) the effect becomes more profound around the hepatic parenchyma. The cytoplasm is highly vacuolated while the nuclei continue to be pyknotic, Focal necrosis are noticed in many regions with increase in cytolysis. Exposure at 28th day (Figure 9) shows similar appearance with more lethal effect. However, following the exposure for 42nd day (Figure 10), haemorrhage occ-



**Figure 4.** At 28<sup>th</sup> day exposure (0.05% v/v), the secondary (S) epithelia of *Clarias gariepinus* show club-like appearance with overlapping at the tip. Magnification is X40.



**Figure 5.** At 42<sup>nd</sup> day exposure (0.05% v/v), there was thickening of *Clarias gariepinus* primary lamella with severe edematous changes at the secondary lamella. Magnification is X40.

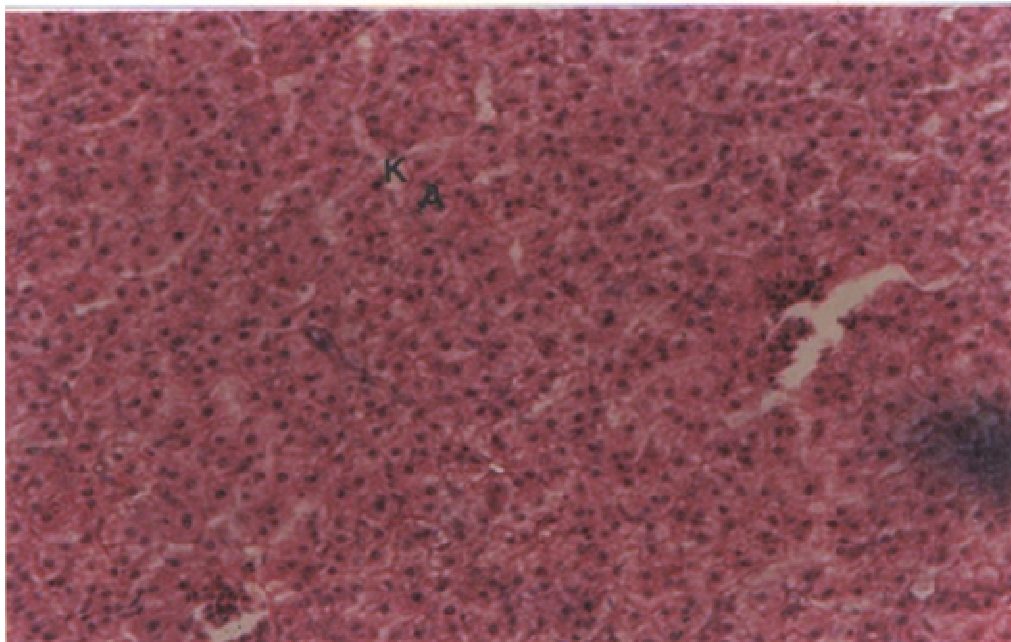
urs leading to extensive necrosis of hepatic cells.

## DISCUSSION

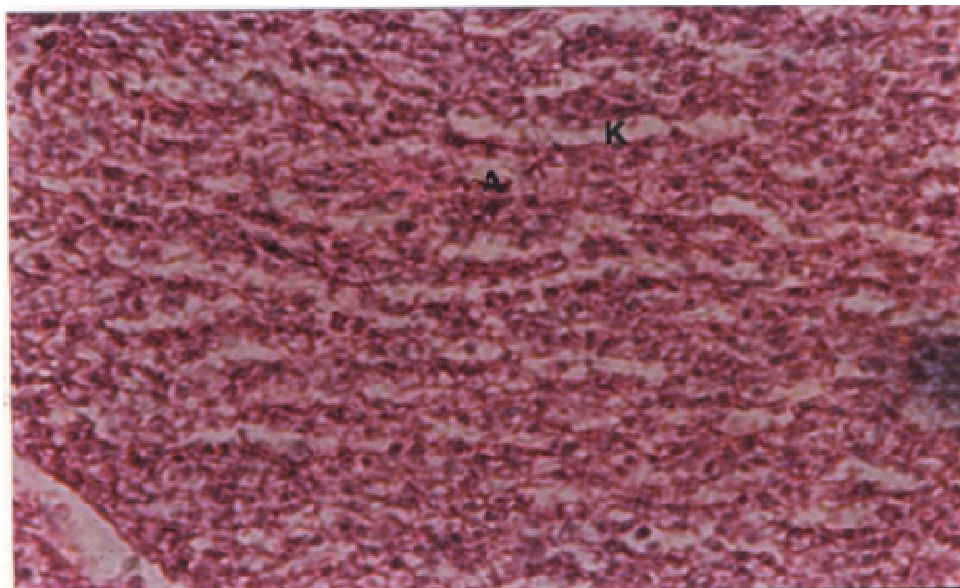
Glyphosate is most widely used as herbicides. It is said to be persistent and mobile in soil and water constituting most common terrestrial and aquatic contaminant (Cox, 1998). Glyphosate can be detected in most aquatic systems including streams, ponds and surface water (WHO, 1994). Thus, Glyphosate concentrations measured in the

field may exceed the values recommended for drinking water by the European Community (EC) guideline (98/83/EC) as well as WHO guideline (Jiraungkoorskul et al., 2002). In cognisance of these facts, it therefore raises concern to monitor and to assess their impacts in the environment.

Fish species are most sensitive to aquatic pollutants during their early life stages (Jiraungkoorskul et al., 2002). Folmar et al. (1979) reported that the exposure of early life stages of rainbow trout and channel catfish to



**Figure 6.** The liver tissue of juvenile fish (control) showing *Clarias gariepinus* irregular cords of hepatocytes (A). Between them are the sinusoids (K) with perlobular capsule indistinct. Magnification is X40.

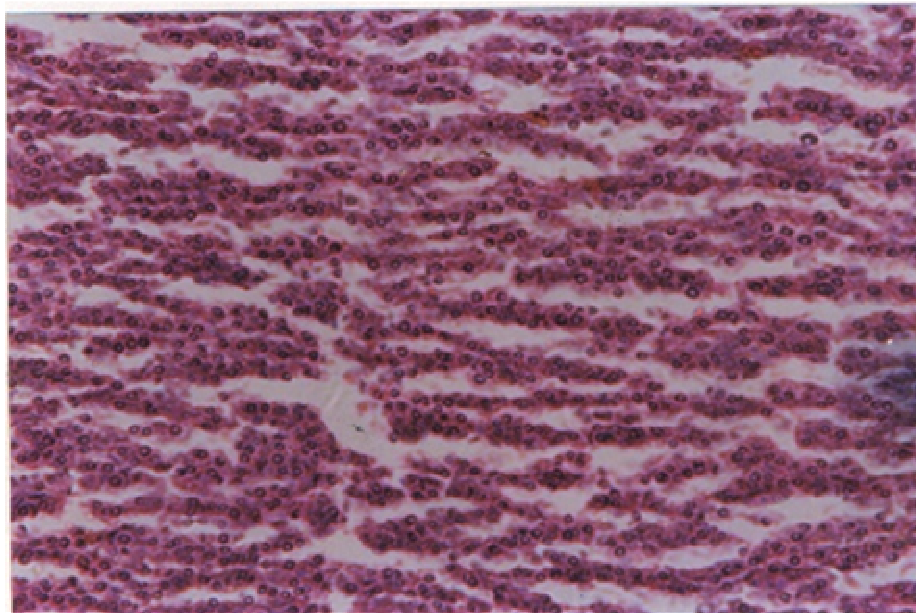


**Figure 7.** At 14<sup>th</sup> day exposure (0.05% v/v), the density of fibres of *Clarias gariepinus* increased significantly within the sinusoids (K). The hepatocytes (A) enlarge with some pyknotic nuclei. Magnification is X40.

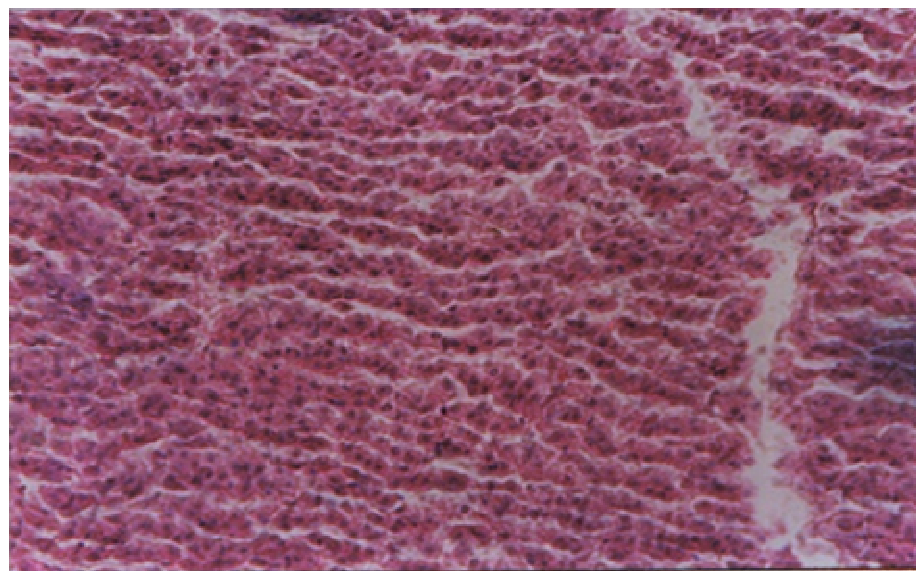
glyphosate shows that egg stage was least sensitive for both species. The toxicity of glyphosate increases in the early swim-up stages but decreases in the fingerlings stage as the catfish grows. The gills of *C. gariepinus* exposed to glyphosate (14th day) show secondary lamellae fusion, hyperplasia and oedema. This was in agreement with previous report that glyphosate causes varying

degrees of histopathological changes in the gills filament (Jiraungkoorskul et al., 2003). Kumaraguru et al. (1982) reported fusion of adjacent secondary lamellae as a result of hyperplasia in the gill of rainbow trout *Salmo gairdneri* exposed to permethrin.

Hyperplasia in some situations represents adaptations by the organism to protect underlying tissues from any



**Figure 8.** At increased concentration (0.1% v/v) at 14<sup>th</sup> day interval, the hepatocytes of *Clarias gariepinus* showed further enlargement with vacuoles and pyknotic nuclei. The sinusoidal congestion was more apparent. Magnification is X40.

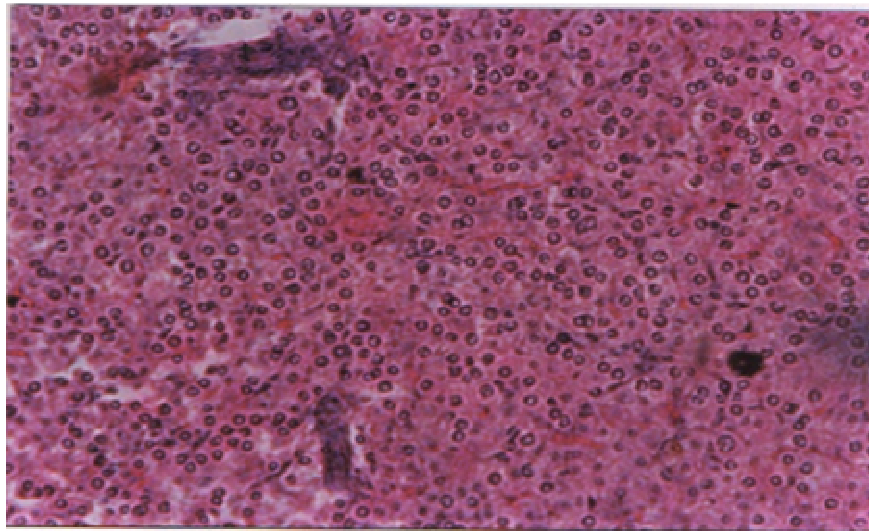


**Figure 9.** Exposure at 28<sup>th</sup> day (0.05% v/v) of *Clarias gariepinus* showing enlarged hepatocytes, vacuoles, pyknotic nuclei and hepatic sinusoids. Magnification is X40.

irritant (Meissner and Diamandopoulous, 1977). However, increase in thickness of epithelial layers and fusion of adjacent secondary lamellae as a result of hyperplasia would not only decrease the surface area available for oxygen extraction, but also would increase the oxygen diffusion distance between water and blood (Skidmore and Tovell, 1972). Thus, while hyperplasia may indeed be having a protective function it may also inhibit the res-

piratory, secretory and excretory functions of the gills. It was observed to lower circulation at the gills, widen the blood spaces and contract the pillar cells (Fanta et al., 2003). Hyperplasia and epithelial lifting are part of the pathological features observed by Cardoso et al. (1996) in Pacama, *Lophiosilurus alexandri*.

Variations in the epithelial surface of gills show important physiological adaptations relating to the area availa-



**Figure 10.** Exposure at 42<sup>nd</sup> day (0.05% v/v) of *Clarias gariepinus* showing hemorrhage, enlarged hepatocytes, vacuoles, pyknotic nuclei and cytolysis. Magnification is X40.

ble for increased gaseous exchange (Kendall and Dale, 1979). Their vulnerability is thus considered because of their external location and for the fact that they are in intimate contact with water and are thus liable to damage by irritant materials. The lamellar reduction of the treated fish must have been caused by respiratory stress originally defined by Selye (1950) as the sum of all the physiological responses by which an animal tries to maintain or re-establish a normal metabolism in the face of a physical or chemical force. Haemorrhage and sloughing of the branchial arteries at the opercula end of the primary lamellae can disrupt the circulation of the deoxygenated blood via the branchial arteries into the secondary lamellae in a direction opposite to that of water flow. As a result, oxygen uptake is hampered. This can cause asphyxiation, tissue necrosis and finally death. There were apparent lamellae oedematous changes probably due to increase capillary permeability (Roberts, 1978). When severe this could lead to difficulty in respiration and osmo regulatory stress may result (Skidmore and Tovell, 1972).

The liver of fish does not show the diversity of pathology seen in higher animals, probably as a result of lack of Kupffer cells in the liver sinusoid (Ellis et al., 1976). However, its susceptibility to a number of toxic and the consequential metabolic disturbances cannot be overemphasized (Roberts, 1980; Olojo et al., 2005). The high proportion of fibrotic tissue within the lobules and peribiliary connective tissue of the treated specimens indicate hepatic cirrhosis. It is thus believed that the most dramatic cirrhosis found in fish is the peribiliary cirrhosis of the hepato-renal syndrome associated with dietary toxicity (Anderson et al., 1976). The most frequent of the degeneration was hepatocytes enlargement with large vacuoles and sinusoid conjection, pyknosis and karyolysis obser-

ved in cases of severe intoxication with pollutants (Jiraungkoorskul et al., 2003).

The shrinkage of the hepatic cells can result in cirrhosis - the contracting of the blood vessels thereby greatly impeding the portal flow through the liver. The functions of the liver such as the conversion of glucose to glycogen for storage, regulation of lipids and deamination of amino acids are impaired.

The blockage of the sinusoids makes the blood flow from the hepatic artery and veins into the central vein rather difficult. The sinusoids widened to make up the right volume of blood in the central vein. The function of the canaliculi that forms the bile duct is hampered and as such, bile secreted from gall bladder cannot adequately get into them.

In conclusion, the exposure of *C. gariepinus* to glyphosate caused histopathological alterations both in gills and liver tissues. Because its concentration in streams is often at sub-lethal levels, the histopathological effects are gradual. This may not result in fish kill immediately but definitely represents a health hazard to human consumers.

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