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Biomarker of Exposure: Alterations in GIT of Post Juvenile Africa Cat Fish (*Clarias gariepinus*) Exposed to sub-lethal concentrations of Glyphosate Herbicide (IPA 360g/L)

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ABSTRACT: The study investigates the effect of a commonly and widely used herbicide 'IPA Glyphosate' on the histopathology of gastrointestinal tract of exposed post juvenile catfish (*Clarias gariepinus*) under laboratory condition. Results showed that alterations in GIT were both dose and time dependent and include: hyperplasia of gastric mucosa, necrotic hyperplasia, severe degenerative and necrotic changes in the intestinal mucosa and submucosa. The alterations further buttress the advantage of histopathological markers for detecting rapid response of organisms to toxins in its early stage. This study has shown that the use of glyphosate herbicide can act as a stressor on non-target organisms such as *C. gariepinus* and with the alterations observed can go a long way to affect the absorption of food in the intestine, hence, the growth of the fish.

KEYWORDS: Biomarker, GIT, Glyphosate, *Clarias gariepinus*.

1 BACKGROUND

The effects of toxicant on the gastro-intestinal tract of fish may ranged from slight changes in mortality, secretion and absorptive functions to more severe effects associated with mucosal integrity, blood flow or neuromuscular control. These effects could ultimately influence the ability of organism to thrive [1]. Some studies indicated that high levels of some metals in diet may cause increased apoptosis of intestinal cells [2]. Only a few studies have documented the histomorphological alterations of intestine in the fish exposed to heavy metals [1], [3]. Reference [4], [5] demonstrated some alterations in the gut of *Channa punctatus* and *Heteropneustes fossils* following mercury intoxication. Reference [6] observed that there was no histological alteration in the GIT of *Salvelinus ailpinus* exposed to dietary intake of inorganic and methyl mercury. In Nigeria today, farmland close to water bodies are constantly sprayed with herbicide. Experiments have documented how chemical herbicides are more cost effective and give better weed control than hand weeding include a study in which maize yields doubled and production costs fell by 61% when Nigerian farmers used atrazine [7]. More recently, chemical control decreased costs by as much as 50% and increased yields up to 55% on Nigerian cassava, yam, and soybean plots [8]. Constant spraying of farmland close to water bodies may pose a great risk to the aquatic fauna of such water bodies including fishes. In this investigation, glyphosate herbicide was considered alongside catfish as such fish farms are cited close to many farms in southern part of Nigeria.

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2 MATERIALS AND METHOD

2.1 EXPERIMENTAL FISH SPECIMEN AND CHEMICALS

One hundred and twenty normal post juvenile *Clarias gariepinus* of both sexes with a mean weight of 135.44±1.99 g and mean length of 28.32±0.844 cm were purchased from Osayi farms in Benin City, Edo state. They were kept in 60 L aquaria at 27.5±0.4 ◦C, pH 7.3, with 12:12 h photoperiod. They were left unfed in the first 2 days to adapt to a change in environment before feeding them with the fish diet. Laboratory aquaria were well aerated and provided with external filtration and a layer of gravel on the bottom. Fish were normally fed once a day with pelleted commercial food (Durante Aquaculture fish concentration-2mm). They were allowed to acclimate to captivity conditions for two month prior to taking the blood samples. Careful netting and handling was implemented to minimize stress. The commercial formulation of glyphosate (360 g/l-41 w.wt IPA) at five nominal concentrations 72, 54, 32 and 18 mg/L were used. These concentrations were defined taking into account: the result of the range finding test.

2.2 THE SUB-LETHAL TEST

The sub-lethal concentrations were used to perform the experiment according to reference [9] procedure for the static renewal technique. The tests consisted of a control and four concentration groups, three replicates per group, with ten fish in each replicate.

2.3 TISSUE PREPARATION

The GIT of the test animals (fish) were excised keeping the structure intact, rinsed in normal saline, fixed in 10% formalin for about 24 h at 4°C, dehydrated through series of graded alcohol, cleared in xylene, infiltrated with paraffin at 56°C, then embedded in paraffin wax [10]. Thin section of the selected gill tissues of about 6 – 7 Nμm) was cut by means of a rotatory microtone, dehydrated and stained with haematoxylin and eosin. The sections were examined and photomicrographs using an Olympus BH2 microscope fitted with photographic attachment were taken. The prepared slides were used to describe the histological structure observed under light microscopy.

3 RESULT AND DISCUSSION

Pathology has been a crucial and vigorous method in established routine toxicology studies carried out for the purpose of risk assessment. In aquatic toxicology, application of the histopathology techniques in assessing toxicological pathology of organisms has many advantages [11]. The gastro-intestinal tract is one of the main routes for the uptake of xenobiotics present in the diet or in the water that the fish inhabit [12], [13]. The effects of toxicant on the gastro-intestinal tract of fish may range from slight changes in motility, secretion and absorptive functions to more severe effects associated with mucosal integrity, blood flow or neuromuscular control. These effects could ultimately influence the ability of the organism to thrive [1]. The main changes reported in gastro-intestinal tract included hydropic degeneration of the digestive gland [14], proliferation of mucous cells, hyperaemia, atrophy and metaplasia. Some studies have indicated that high levels of some metals in diet may cause increased apoptosis of intestinal cells [2]. Only a few studies have documented the histomorphological alterations of intestine in the fish exposed to heavy metals [13], [3].

The GIT of *C. gariepinus* exposed to glyphosate herbicide in this current experiment showed severe degenerative and necrotic changes in the intestinal mucosa and submucosa, atrophy in the muscularis and submucosa and aggregations of inflammatory cells in the mucosa and submucosa with edema between them.

According to reference [15], the observed irritation and destruction of the mucosa membrane of the intestine, hampered absorption. The pathological alterations in the intestine of the studied fish are in agreement with those observed by many investigators about the effects of different toxicants on fish intestine [16], [17], [18]. Epithelial degeneration, inflammatory cells infiltration in the submucosa as well as submucosa edema was seen in the intestine of tilapia fish exposed to carbofuran [19]. Reference [19] investigated the histological alteration in the intestine of *Tilapia zilli* and *Solea vulgaris* obtained from Like Victoria and observed some lesions in the intestine, degenerative and necrotic changes in submucosa and mucosa with edema between them, dilation in the blood vessels of serosa and atrophy in the muscularis and sub mucosa.

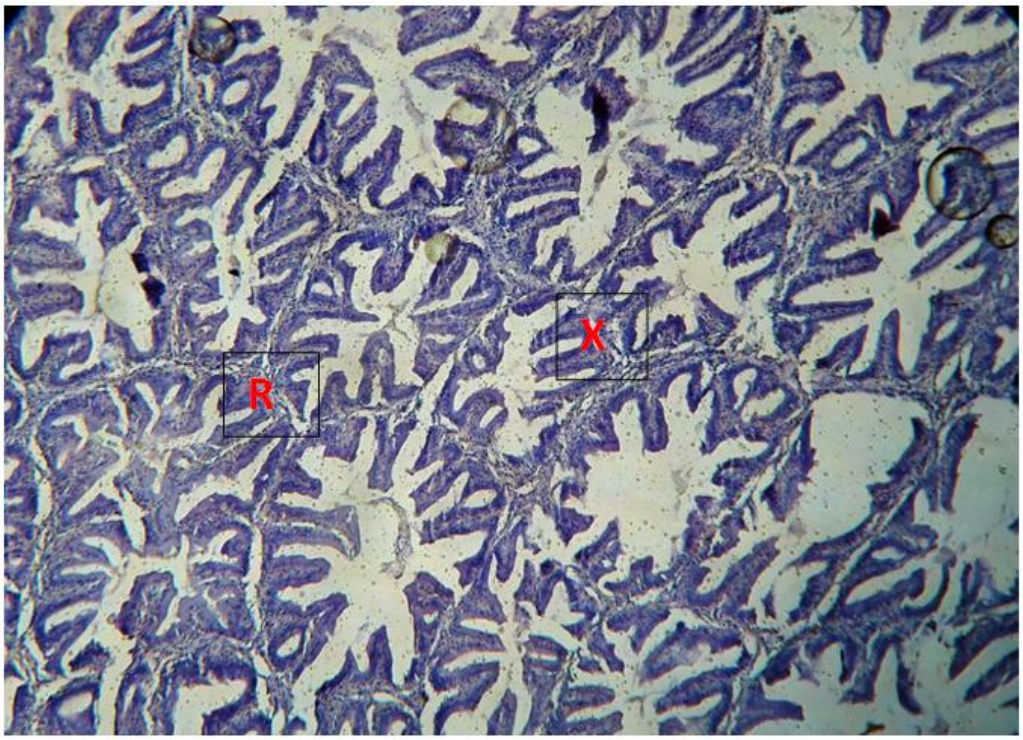

Plate 1a: Photomicrograph of C. gariepinus Gastrointestinal Tract (Control) showing the normal GIT structure. R and X (Muscularis) (H and E stain x100)

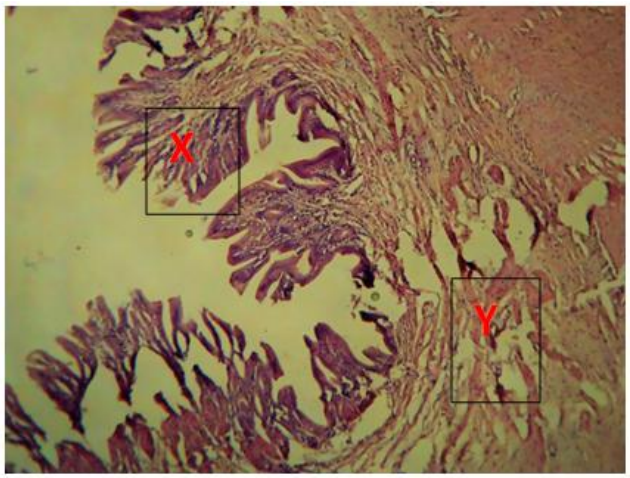

Plate 1b: Photomicrograph of GIT of C. gariepinus exposed to 72 mg/L glyphosate severe degenerative and necrotic changes in the intestinal mucosa and submucosa (X and Y) (H and E Stain x400).

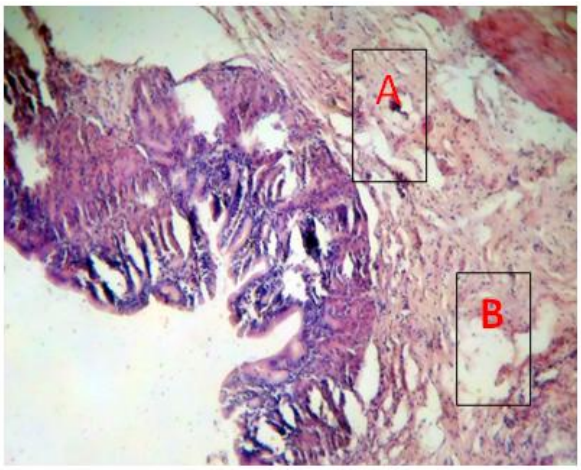

Plate 1c: Photomicrograph of GIT of C. gariepinus exposed to 54 mg/L glyphosate severe degenerative and necrotic changes in the intestinal mucosa and submucosa (A and B) (H and E Stain x400).

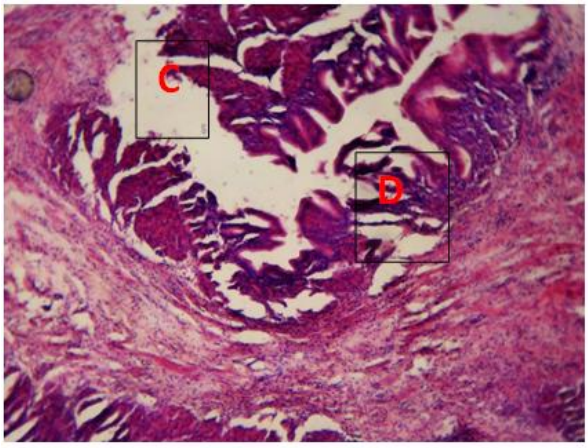

Plate 1d: Photomicrograph of GIT of C. gariepinus exposed to 32 mg/L glyphosate. Haemorrhage in the submucosa and aggregations of inflammatory cells in the mucosa and submucosa (C and D) (H and E Stain x400)

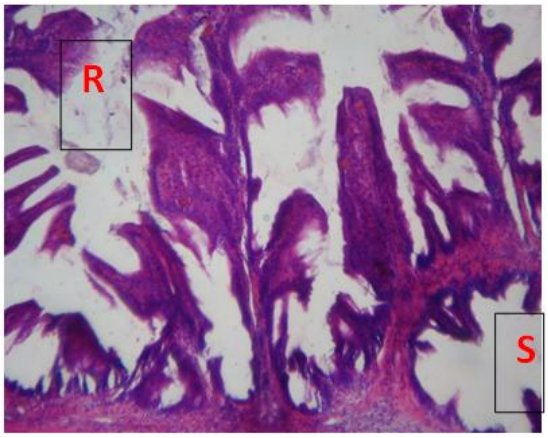

Plate 1e: Photomicrograph of GIT of C. gariepinus exposed to 18 mg/L glyphosate Severe degenerative and necrotic changes in the intestinal mucosa and submucosa (R and S) (H and E Stain x400)

4 CONCLUSION

In conclusion, pollution from pesticide usage has shown to cause damage to the health of organisms in water and indirectly on humans when they consume these fish due to the accumulation of toxic substances. This study has shown that the use of glyphosate herbicide can act as a stressor on non-target organisms such as *C. gariepinus* and with the alterations observed can go a long way to affect the absorption of food in the intestine, hence, the growth of the fish.

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