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Acute Toxicity and Histopathological Changes in Gill and Liver of Catfish (Clarias Gariepinus) Juvenile Exposed to 2, 4-D Amine (Herbex D Sl®)

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Abstract
The acute toxicity and histopathological changes in the gill and liver of *Clarias gariepinus* juvenile exposed to Herbex-D SL ®, a herbicide containing 720g/l of 2, 4-D amine were investigated. In a static renewal bioassay, the fish were exposed to 0.00, 1.40, 1.44, 1.48 and 1.52mg/l of 2, 4-D amine. The 96 hours lethal concentration (LC_{50}) of 2, 4-D amine to juvenile of *Clarias gariepinus* was 2g/l. The fish exposed to 2, 4-D amine showed toxicological signs of erratic swimming, loss of reflexes and lethargy. Histopathological changes in the gill architecture were characterized by lifting of epithelia layer, vacuolization, inter-lamella hyperplasia and mild desquamation of the epithelia lining. There was an evidence of advancing phase of hepatic necrosis in the liver of fish exposed to 2, 4-D amine. 2, 4-D amine is highly toxic and its use in or near aquatic environment should be monitored.
Key words: 2,4- D amine (Herbex D SL®), *Clarias gariepinus*, Acute toxicity, Histopathology.

1.0 Introduction

Pollution caused by indiscriminate use of herbicides has been elucidated by many researchers in Nigeria (Obasohan, 2009). Herbicides have been reported to find their way into the aquatic environment through storm water run-off, drift, precipitation or direct application (Babatunde *et. al*., 2008 and; Perkins and Schlenk, 2005). Pollution induced physiological challenges in aquatic flora and fauna has been reported to cause their mortality and morbidity (Bennet *et al*., 1995). Herbicide causes damage to important vital organs and reduces the survival, growth and the reproduction performance of aquatic organisms when concentration exceeds tolerant level (Rahaman *et al*., 2002).

2, 4-D is a broadleaf herbicides recommended for use in both terrestrial and aquatic environment (Gervais *et al*., 2008). 2, 4-D exists in the form as amine, acid, butoxyethyl ester or several salts which vary in their chemical properties, environmental behaviour and toxicological characteristic (RED, 2005). The chemical has a half-life of 15 days (RED, 2005) and was reported by Tomlin (2006) to be highly toxic to aquatic organisms. The LC_{50} 2, 4-D amine to fish and aquatic invertebrate ranges from 80 – 2244 mg acid equivalents per litre (Gervais *et al*., 2008).

The toxicity of chemical to fish depends on size and species (Noga 2012, Craig, 2006). The 96 hours LC_{50} of 2, 4-D amine to fingerlings of *Clarias gariepinus* was reported by Gabriel *et al*. (2010) to be 165.36 mg/l. This study was therefore conducted to establish the 96 hours Lethal Concentration (LC_{50}) of juvenile of *Clarias gariepinus* to acute toxicity of 2, 4 D amine and establish the gill and liver response of *Clarias gariepinus* juvenile to acute toxicity of 2, 4-D amine.

2.0 Materials and Method

2.1 *Fish*

Juvenile of *Clarias gariepinus* with body weight of 19.3±0.33g obtained from a reputable fish farm were allotted into ten (10) aquaria tanks at 10 fish per tank. The fish were acclimatized for 2 weeks in which they were fed with commercial feed at 5% body weight. A daily photoperiodic of 12L: 12D was maintained during the acclimatisation and assaying.

2.2 Test Chemical

The test chemical was Herbex D-SL ® solution containing 720g/l of 2, 4-D amine manufactured by FISCO Nig. Ltd, Ibadan, Oyo-State, Nigeria. The chemical was an odourless, colourless liquid that did not change the colour of the water.

2.3 Acute toxicity test and histopathological analysis

The acute toxicity test was performed according to the static renewal method described by Sprague (1975) and APHA (1985). 10 fish per group were exposed in duplicate to five different concentrations of 2, 4-D amine (0.00,

1.40, 1.44, 1.48 and 1.52mg/l) in a 40 litres capacity aquarium. Mortality was recorded at experimental period of 12, 24, 48, 72 and 96 hours. The test solutions were renewed every 24 hours and the fish were not fed during the ninety-six hours (96hr) trial. After the 96 hours experiment, the gill and liver of the survived fish from each treatment were exercised and preserved in 10% formalin. The samples were processed for histological examination using standard histological techniques (Avwioro, 2002).

2.4 Statistical Analysis

Data obtained on mortality were analysed using probit analysis method (Finney, 1971) and the histological samples stained with haematoxylin-eosin were qualitatively analysed under a light microscope.

3.0 Result

3.1 Acute Bioassy

Agitated swimming, loss of reflexes, discolouration and lethargy are some of the toxicological signs observed in *C. gariepinus* exposed to 96 h acute toxicity of 2, 4-D amine. There was an observable increase in behavioural response as concentration increased. The mortality pattern and probit mortality of *C. gariepinus* exposed to the test chemical are presented in Table 1. No mortality was recorded in the control group and the fish exposed to 1.40g/l of 2, 4-D amine. The 96 h LC50 was estimated to be 2g/l (Figure 1).

Table 1: Percentage mortality and Probit mortality of *C. gariepinus* juvenile exposed to acute toxicity of 2, 4-D amine

Concentration (g/l)	Total mortality	Percentage mortality	Log of concentration (x)	Probit mortality (y)
0.00	0.00	0.00	0.00	0.00
1.40	0.00	0.00	0.00	0.00
1.44	1.00	10.00	0.15	3.72
1.48	2.00	20.00	0.17	4.16
1.52	4.00	40.00	0.18	4.75

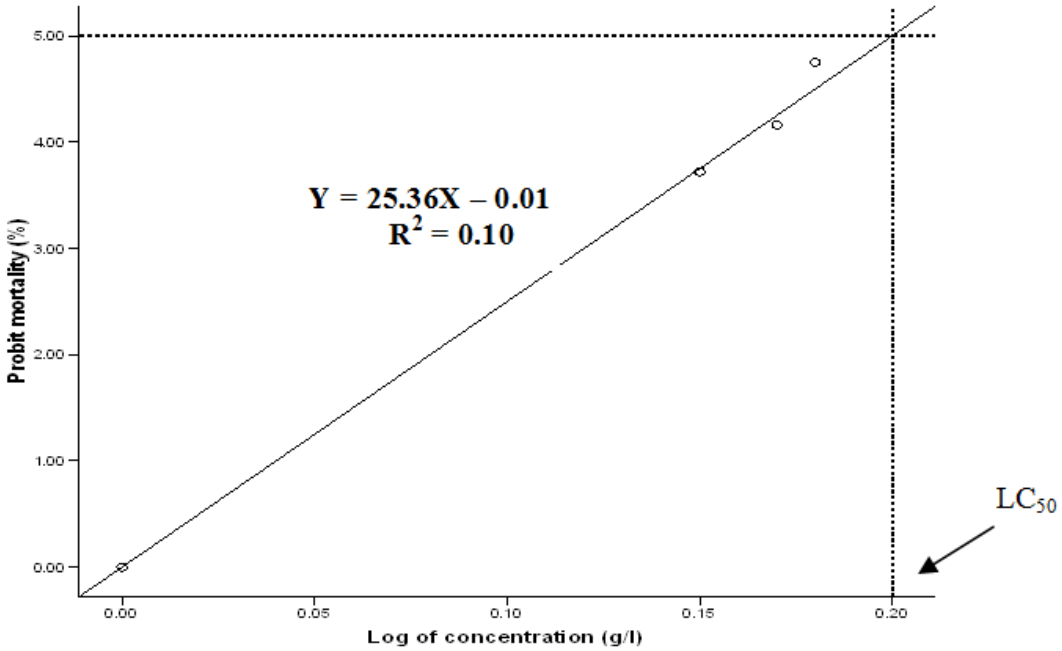

Figure 1: 96 h LC50 of *Clarias gariepinus* juveniles exposed to acute concentration of 2, 4-D amine.

3.2 Histopathological Findings

The photomicrograph showing the histological sections of gill filament and liver of *Clarias gariepinus* juvenile assessed are presented in figure 1 below. Figure 2 (A) shows the gill filament from the control group that was not exposed to 2, 4-D amine while Figure 2 (B) shows the gill filament from *C. gariepinus* exposed to 96 hours acute toxicity of 1.4 g/l, 1.44 g/l, 1.48 g/l or 1.52 g/l of the test chemical. There was an evidence of lifting of epithelia layer, vacuolization, inter-lamella hyperplasia and mild desquamation of the epithelia lining. The alterations in the gill architecture were observed in all fish exposed to the acute concentrations of 2, 4-D amine except the control

group.

Figure 2 (C) represents the photomicrograph of the histological section of liver from the control group showing no visible pathological lesion. Figure 2 (D) shows the liver histological section of *Clarias gariepinus* after 96 hours exposure to acute concentrations of 2, 4 D-amine. Advancing phase of hepatitis was observe in *C. gariepinus* exposed to the acute concentrations of 2, 4 D-amine. There was also an infiltration of inflammatory cells (macrophage) and lymphocyte in the hepatocyte.

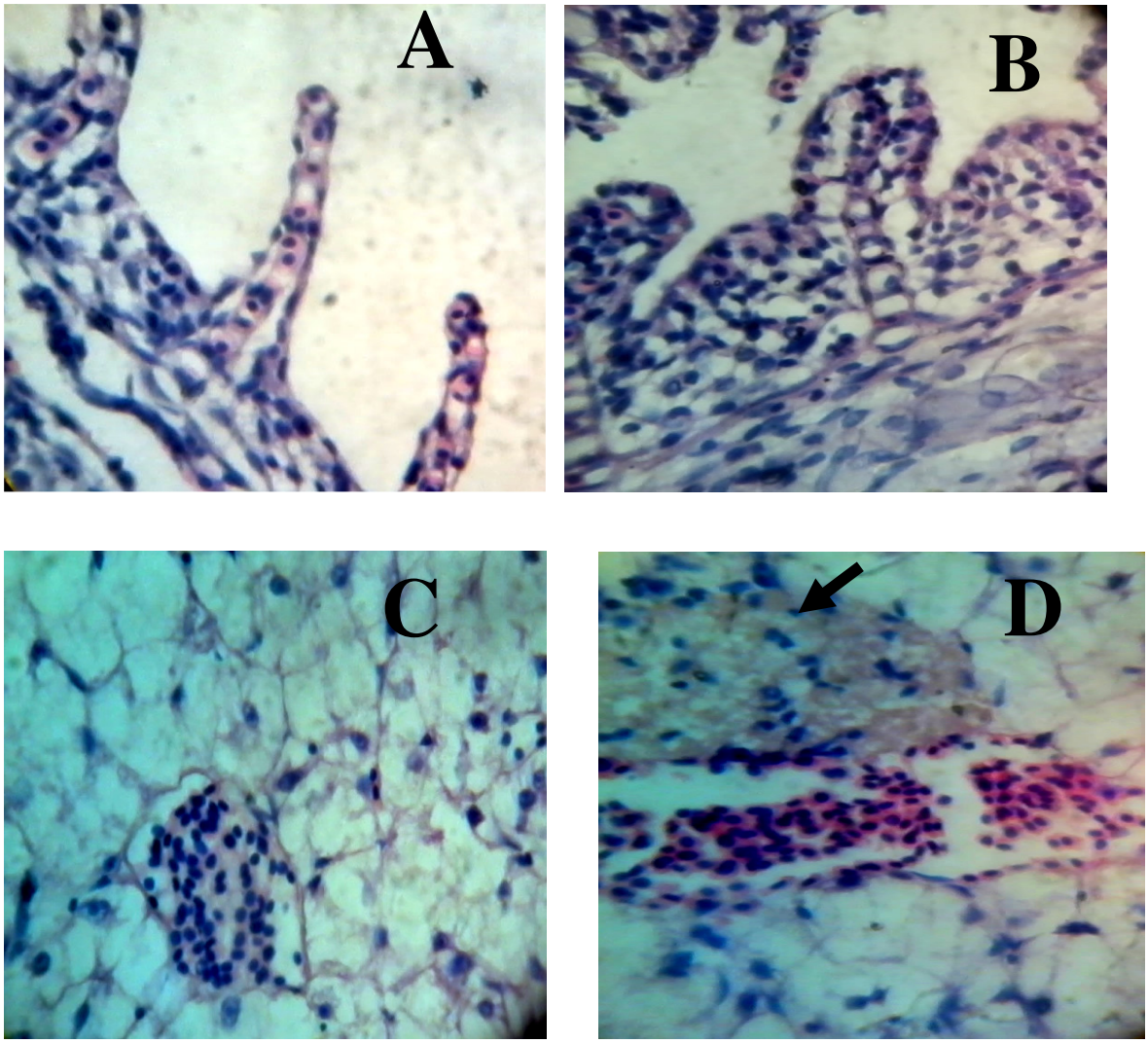

Figure 2: Histological sections of gill filament **(A & B)** and liver **(C & D)** from *Clarias gariepinus* juvenile. **A**- Normal gill filament of fish from the control group. **B** – filament from *C. gariepinus* juvenile exposed to acute concentrations of 2,4 D-amine showing lifting in epithelia layer and inter-lamella hypertrophy. **C** – Normal liver of *C. gariepinus* from control group. **D** – Liver of *C. garepinus* exposed to acute concentration of 2, 4 D-amine showing advancing phase of hepatitis (arrow) with infiltration of lymphocytes. H & E. x100.

4.0 Discussion

Increase in swimming, gasping for air, loss of balance and reflexes are some of the behavioural characteristics which can serve as biosensor in fish under the influence of a toxicant (Chindah *et al*., 2004; Bobmanuel 2006 and; Auta *et al*., 2005). These toxicological signs were observed in this study and may result from the adaptive response of the fish to the toxic stress imposed by 2, 4-D amine. Similar behavioural responses were reported by Gabriel and Kparobo (2002) when post fingerlings of *Clarias gariepinus* was exposed to acute toxicity of Cypermethrin. Macleod and Pessah (1973) also reported similar responses in Rainbow trout under the toxic influence of mercury. The LC_{50} value obtained in this study fell within the range of 0.08 – 0.22g/l reported by Gervais *et al*. (2008) for fish and aquatic invertebrate. The LC_{50} of 0.20g/l of 2, 4- D amine to *C. gariepinus* juvenile recorded in this study is close to 0.17g/l reported by Gabriel *et al*. (2010) when fingerlings of *C.*

gariepinus were exposed to amine salt of 2, 4-D. Toxicity of fish to toxicant depends on size and species (Noga 2012 and Craig, 2006). Kousar and Javed (2012) reported an age dependent increase in the tolerance of *Labeo rohita, Cirrhina mrigala, Catla catla and Ctenopharyngodon idella* to acute toxicity of copper. The result of this study revealed similarity in tolerance between juvenile and fingerlings of *Clarias gariepinus* to acute toxicity of 2, 4-D amine.

Fish gill is an organ with a large surface area, highly sensitive to the effect of toxicant (Reiser *et al*., 2010). Ferguson (2006), Wood (2001) and Evans *et al*. (2003) described the interaction of the gill lamella epithelium with the water-borne pollutants as one of the routes of gill damage. The gill surfaces have a direct contact with aquatic toxicants (Simonato *et al*., 2008) and have been shown to display various structural alterations in the branchial respiratory epithelia surface upon exposure (Rosety-Rodriguez *et al*., 2002 and; Ferguson, 2006). The gill lesions found in *Clarias gariepinus* exposed to acute concentrations of 2, 4- D amine in this study were also reported by Simonato *et al*. (2008) and Akaishi *et al*. (2004) when fish were exposed to acute concentrations of crude oil. The degenerative lesions (vacoulization, epithelia lifting and desquamation of the eipthelia lining) and proliferative response (hypertrophy) in this study were severe in the fish exposed to the highest concentration of 2, 4 D - amine. Similar dose-response degenerative lesions were recorded by Olufayo and Alade (2012) when *Heterobranchus bidorsalis* was exposed to acute toxicity of Cypermethrin. The alterations in the gill architecture are adaptive, necessary to reduce the rate of absorption of toxic substances. Epithelia hypertrophy increases the water-blood distance thereby reducing the rate of absorption of xenobiotics (Agamy, 2013). However, Epithelia hypertrophy decreases the respiratory surface area thus, reducing the effectiveness of gas exchange and also leading to osmoregulatory dysfunction (Sakuragui *et al*., 2003).

The liver is prone to damage by toxic chemicals due to its role as a vital organ in breaking down chemicals (Ladipo *et al*., 2011). The histological section of the liver of *Clarias gariepinus* juvenile exposed to acute toxicity of 2, 4 - D amine for 96 hours in this study did not show conspicuous abnormal pattern when qualitatively compared with the control group. Ladipo *et al*, (2011) reported similar result when *C. gariepinus* was exposed to 96 hours acute toxicity of paraquat dichloride. However, there was evidence of advancing phase of hepatocyte necrosis in *C. gariepinus* exposed to 2, 4 D-amine in this study. Osmoregulatory dysfunction from toxin-mediated diseases has been implicated by Ferguson (2006) to be responsible for necrosis of hepatocyte. Length of exposure of toxicant to an organism determines the severity of injury on the organs of the fish (Ferguson, 2006). Exposure of *C. gariepinus* juvenile to acute concentration of 2, 4 D-amine beyond 96 hours may therefore inflict noticeable pathological changes in the hepatocyte.

5.0 Conclusion

In conclusion, the amine salt of 2, 4 – D amine is highly toxic to juvenile of *Clarias gariepinus*. The gill and liver of *C. gariepinus* juvenile when exposed to acute concentration of 2, 4-D amine would cause maladaptive histopathological changes which could lead to their death. Monitoring the use of Herbex D- SL ® (720g/l of 2, 4 D- amine) in controlling weed is therefore advocated.

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