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Effect of Diazinon on Acid and Alkaline Phosphatase Activities in Plasma and Organs of *Clarias gariepinus*

[1]I.R. Inyang, [2]E.R. Daka and [1]E.N. Ogamba
[1]Department of Biological Sciences, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria
[2]Department of Applied and Environmental Biology, Rivers State University of Science and Technology, Port Harcourt, Nigeria

Abstract: The aim of this study was to determine the effect of the pesticide, diazinon, on phosphatases in the plasma and organs on *Clarias gariepinus*. Adult *Clarias gariepinus* were exposed in four replicates to varying sublethal concentrations diazinon (ranging from 1.00 to 10.0 mg/L) in 30-day semi-static bioassays. Alkaline phoshatase (ALP) and acid phosphate (ACP) were determined in plasma and other organs (gastrointestinal tract - GIT, kidney, muscle, gill and liver) of the fish after the experimental exposures. Dizinon did not cause any statistically significant difference on plasma ALP over the concentrations tested ($p>0.05$), but ACP showed significantly higher mean value at 10 mg/L compared to the control. ALP and ACP values in all the organs (GIT, intestinal tract, kidney, muscle, gill, liver) decreased with increasing concentration of diazion. This indicates an evidence of inhibition of these enzymes in the organs by the toxicant, and therefore alteration of biochemical processes in *C. gariepinus* which can be used as bio-indicators of the effects of diazinon in the Niger Delta environment.

Key words: *Clarias gariepinus*, diazinon, phoshatases, enzymes, plasma, inhibition

INTRODUCTION

Pesticides in the aquatic environment can negatively affect the ecosystem. Pesticides could contaminate land and water from production sites and storage tanks, run-offs from fields; discarded, or sprayed especially into water to kill algae. Diazinon [0,0-diethyl-0(2-150proye-6-methepyrimidin4yl) phosphorothioate] is a contact organophosphate pesticide and extensively used both in agriculture and households to control insects in soil, plants, fruits and vegetable crops (Banaee *et al*., 2008). Diazinon is easily washed into surface waters and because of its aquatic distribution, it affects a wide range of non-target organism like invertebrates, mammals, birds and fishes, especially those inhabiting aquatic environment (Burkepile *et al*., 2000). Fish may be good indicators of contamination by pollutants because their biochemical responses are quite similar to those found in mammals (Banaee *et al*., 2008),

According to Hassel (1990), biochemical changes occurs in fishes that are exposed to environmental contaminants, such changes which may include pesticides and their metabolites have necessitated a number of studies to determine their effects in aquatic environment on biochemical parameters in fish (Luskova *et al*., 2002). Several authors have investigated the effect of pesticide in fish (Luskova *et al*., 2002; Almeida *et al*., 2005; Simeon, 2007; Banaee *et al*., 2008; Prashanth and Neelagund, 2008). The aim of this study was to evaluate the chronic toxicity of diazinon on acid phosphatase (ACP) and alkaline phosphatase (ALP) of *clarias gariepinus*, a common Niger Delta wetland fish.

MATERIALS AND METHODS

Sixty adult *clarias gariepinus* (mean weight, 268±52.10 g; mean length, 34.55±1.23 cm) were obtained from a private fish farm at Abuloma Road, Abuloma, Port Harcourt, Nigeria. They were transported to the wet laboratory of the Department of Fisheries and Aquatic Environment, Rivers State University of Science and Technology, Port Harcourt, Nigeria where the study was conducted from May to October 2007.

The fish were acclimated individually in rectangular aquaria for seven days during which they were fed once a day with 35% crude protein at 1% biomass (9.00-11.00 h). A range finding test (trial test) was carried out in which five concentrations of diazinon were prepared from the original solution (600 mg/L). Fish were grouped into six groups of 0.0, 0.1, 0.5, 10.0, 12.5 and 15.0 mg/L. The test solution was renewed daily immediately after washing the aquaria and fish was fed

Corresponding Author: Erema R. Daka, Department of Applied and Environmental Biology, Rivers State University of Science and Technology, Port Harcourt, Nigeria. Tel: +234(0)8033385665

Table 1: ALP and ACP in Plasma and GIT of *Clarias gariepinus* exposed to diazinon for 30 days (Mean±S.D., n = 4)

	Plasma				GIT			
Conc. of diazinon (mg/L)	ALP (U/L)	Control (%)	ACP (U/L)	Control (%)	ALP (U/L)	Control (%)	ACP (U/L)	Control (%)
0	8.60±0.46^{a}		7.37±0.38^{a}		4.72±0.24^{a}		4.15±0.37^{a}	
1.0	8.16±0.23^{a}	101.2	7.37±0.65^{a}	100	4.26±0.23^{b}	90.3	3.92±0.27^{a}	94.5
2.5	7.37±0.50^{a}	91.4	7.03±0.65^{a}	95.4	3.81±0.23^{c}	80.7	3.35±0.23^{b}	80.7
5.0	8.18±0.44^{a}	101.5	7.256±0.44^{a}	98.4	3.58±0.44^{c}	75.8	2.93±0.51^{b}	70.6
7.5	8.60±0.27^{a}	100	6.91±0.38^{a}	93.7	2.67±0.00^{d}	56.6	2.39±0.19^{b}	57.6
10.0	8.52±0.27^{a}	105.7	8.06±0.27^{b}	109.3	2.39±0.19^{d}	50.6	1.50±0.44^{c}	36.1

Means with the same superscripts in a column are not significantly different ($p<0.05$)

Table 2: ALP and ACP in gill and liver of C*larias gariepinus* exposed to diazino exposed to diazinon for 30 days (Mean±S.D., n = 4)

	GILL				Liver			
Conc. of diazinon (mg/L)	ALP (U/L)	Control (%)	ACP (U/L)	Control (%)	ALP (U/L)	Control (%)	ACP (U/L)	Control (%)
0	2.88±0.24^{a}		2.28±0.34^{b}		3.78±0.24^{a}		5.15±0.44^{ab}	
1.0	2.53±0.27^{ab}	87.9	1.61±0.59^{ab}	70.6	3.49±0.00^{ab}	92.3	5.99±0.38^{a}	106.0
2.5	2.30±0.00^{b}	79.9	2.16±0.55^{ab}	94.7	3.06±0.37^{b}	80.7	4.84±0.80^{b}	80.8
5.0	2.19±0.23^{b}	76.0	1.50±0.69^{bc}	65.8	2.84±0.64^{bc}	75.1	3.92±0.80^{b}	69.4
7.5	2.16±0.47^{b}	75.0	1.61±0.27^{ab}	70.6	2.04±0.71^{d}	54.0	3.06±0.49^{c}	54.2
10.0	1.73±0.23^{c}	60.1	1.04±0.23^{c}	45.6	2.19±0.37^{cd}	57.9	3.55±0.63^{c}	62.8

Means with the same superscripts in a column are not significantly different ($p<0.05$)

once daily as in the acclimation period for seven days (Inyang *et al*., 2010a). Sublethal concentrations for the main test (definitive test) were decided based on the range finding test. The concentrations were prepared by placing 0.02, 0.13, 0.25, 0.37 and 0.5 mL, respectively of the original concentration of diazinon and making up to 30 L with borehole water in the aquaria; these gave test concentrations of 1.0, 2.5, 5.0, 7.5 and 10.0 mg/L.

There were six treatment levels (including control) with four replicates each. Four fishes were introduced individually into each aquarium containing the various concentrations of the toxicant for a period of 30 days. Toxicity was determined by renewal static bioassay. The physicochemical characterization of the water used for fish bioassay was carried out according to the method described by APHA (1998). Mean values and standard Temperature 26.00ºC, pH, 6.30-6.37, dissolved oxygen, 5.40-7.30 mg/L. Alkalinity, 15.25-17.09 mg/L, Conductivity 99.50-136.12 mS/cm and turbidity, 0.42-0.58 NTU (Inyang *et al*., 2010b). Blood samples were collected from the fish (behind the anal fin) with 23G size needle and syringe for enzyme analysis and was preserved in heparinsed bottles, the fish were killed after blood collection and dissected in order to collect samples of the liver, kidney gill muscle and Gastrointestinal tract (GIT), then 0.5 g each of the organs were macerated (grounded) with pestle and mortar. The fish were not fed prior to blood collection (Reish and Oshida, 1986). Deionized water was used for preservation and stabilization, and samples were centrifuged at the rate of 300 rpm for 10 min. The supernatants were then removed and stored in plain bottles at -20ºC for analysis.

All enzymes were assayed spectrophotmetrically. The method of Hafkenscheid and Kohler (1986) was used for ALP analysis while that of Andersch and Szcypinski (1947) was used for ACP analysis. The data were subjected to Analyses of Variance (ANOVA). Where significant difference exist, Ducan Multiple Range Test (DMRT) were used to test for pair-wise significant differences ($p<0.05$) between treatments (Wahua, 1999).

RESULTS AND DISCUSSION

The levels of ALP in plasma of *C. gareipinus* did not show any clear trend with increase in concentration of diazinon (Table 1) and there was no significant difference in mean ALP value when compared to values in control fish. On the other hand ACP values in plasma decreased with increase in concentration of diazinon; at the lower concentrations of diazinon no significant difference was recorded, but there was significant difference between the ACP value at 10.0 mg/L compared to the control ($p<0.01$). In the gastrointestinal tract (GIT), ALP and ACT values were depressed with increase in concentration of diazinon. ALP values in all treatments were significantly lower than the controls and amongst the treatments levels significant differences were found except for 2.5 and 5 mg/L. For ACP, no significant difference was found between 1.0 mg/L but all other concentrations have significantly lower mean values than the control ($p<0.05$) (Table 1).

The profile of ALP in the gill showed variations between 87% of control values at 1.0 mg/L to 60% at 10.0 mg/L. No significant difference was found in mean values at 1.0 mg/L in contrast with control, but values in 2.5 to 10.0 mg/L were significantly lower than control (Table 2). ACP values in gill were significantly lower than controls in 5.0 and 10.0 mg/L with values ranging overall from 45

Table 3: ALP and ACP in Kidney and Muscle of *Clarias gariepinus* exposed to diazinon for 30 days (Mean±S.D., n = 4)

	Kidney				Muscle			
Conc. of diazinon (mg/L)	ALP (U/L)	Control (%)	ACP (U/L)	Control (%)	ALP (U/L)	Control (%)	ACP (U/L)	Control (%)
0.0	10.94±0.44^{a}		10.14±0.50^{a}		5.65±0.23^{a}		5.30±0.27^{a}	
1.0	10.37±0.80^{a}	94.9	9.45±0.96^{a}	93.2	5.07±0.85^{ab}	89.7	4.88±0.32^{a}	92.1
2.5	11.06±1.89^{a}	101.1	9.91±1.86^{a}	97.7	4.49±0.58^{bc}	79.5	3.97±0.37^{b}	75.1
5.0	7.49±058^{b}	68.5	7.83±0.53^{b}	77.2	4.15±0.84^{bcd}	73.5	3.66±0.87^{c}	69.1
7.5	7.60±0.59^{b}	69.5	7.95±0.44^{b}	78.4	4.03±0.44^{cd}	71.3	3.44±0.63^{c}	64.9
10.0	6.37±1.73^{b}	58.1	7.49±0.58^{b}	73.8	3.32±0.48^{d}	58.8	2.95±0.32^{c}	55.7

Means with the same superscripts in a column are not significantly different ($p<0.05$)

to 70% of control. ACP values in the liver were slightly higher at 1.0 mg/L than control levels, but in all other concentrations the values were lower than control (62 to 80 % of control); mean values at 5 and 10.0 mg/L were significantly lower than control (Table 2). The levels of ALP in liver exhibited monotonic decrease with increase in concentration of diazinon with mean values ranging from 92 to 57% of the control.

ALP and ACP levels in kidney show similar patterns in response to diazinon exposure. At the lower exposure doses of 1 and 2.5 mg/L no significant differences were found between the values of these enzymes in the kidney of exposed fish and controls. However, at the higher concentrations (5, 7 and 10 mg/L) there were significant reductions in the kidney enzyme levels of exposed fishes (Table 3). Similarly ALP and ACP levels in the fish muscles were significantly lower in concentrations of 2.5 mg/L and above in comparison with controls, but no significant difference was observed between values at 1.0 mg/L and control ($p<0.05$).

Test for phosphatases (ALP and ACP) are part of laboratory test investigations to detect health abnormalities in animals (Ayalogu *et al*., 2001, Giboney, 2005). Biochemical alteration in these enzyme activities of fish resulting from toxicant or contaminant effects in various organs of fish have been reported (Gill *et al*., 1991, Begum, 2004). Significantly, such biochemical changes in fish are aimed at maintaining equilibrium in the presence of these toxicants which are known to disrupt physiological and biochemical process (Wedemeyer and McLeay, 1981). Plasma ALP was not significantly different between treatment concentrations and control while ACP values gave significant difference at the highest diazinon exposure of 10.0 mg/L. Some workers have illustrated that enzyme pattern in the serum reflects the physiological state of the organ (Ayalogu *et al*., 2001). According to Krishan and Veena (1980), increase in plasma levels of ALP was observed in fish exposed to 2,3,4 - triaminoazo benzene resulting in the hepatocellular damage. The result of this study is in conformity to these findings.

ALP is a microsomal enzyme implicated in membrane transport because of its high level in vertebrate kidney and its hydrolytic action on a number of phosphomonoesters of organic materials like glucose (Edqvist *et al*., 1992). This enzyme decreased significantly ($p<0.05$) in the organs following exposure of C*larias gariepinus* to diazinon. The reason for this decrease is probably due to inhibition by the toxicant. ALP is known to occur in the cell membrane and may be involved in metabolic transport (Edqvist *et al*., 1992). This decrease may denote a decrease in membrane transport. In this study, a significant decrease ($p<0.05$) was also recorded in ACP in all the organs, also indicating inhibitions by the toxicant. A significant inhibition level was observed in GIT (36.1%) at the highest concentration of the toxicant, while the rest of the organs recorded between 45.6-73.8% at 10.0 mg/L which revealed diazinon toxicity for *Clarias gariepinus*. The inhibition differences between treatment groups as reasoned by Almeida *et al*. (2005) may be due to differences in weight, rate of uptake, detoxification and activation of the pesticide in the fish. According Gabriel and George (2005), enzyme activities under toxicant exposure could be influence by concentration and mode of action of the toxicant, mode of exposure, duration of exposure and species specific response.

CONCLUSION

We conclude that phosphatase levels the plasma do not represent good indices of the effect of diazinon in *C. gariepinus*. However, the activities of ALP and ACP in the organs (GIT, kidney, muscle, gill and liver) of *C. gariepinus* potentiate useful bio-indictors in the Niger Delta environment with respect to the sub-lethal effects of organophosphate pesticides in fish. For cost-effectiveness, monitoring programmes could include ACP and ALP levels in muscle and/or gills of *C. gariepinus*.

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