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Effects of Sub-Lethal Concentrations of Diazinon on Total Protein and Transaminase Activities in *Clarias gariepinus*

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Abstract: Diazinon-induced changes in the total protein and transaminase activities of *Clarias gariepinus*, a common Niger Delta wetland fish were assessed. Adult fish (mean length 35.24±2.80 cm) were acclimatized to laboratory conditions for 7 days and then exposed to varying sub-lethal concentrations of diazinon (1.0, 2.5, 5.0, 7.5 and 10.0 mg/L) in semi-static bioassays for 30 days. Total protein and the activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined in plasma, muscle, liver, gills and kidney. The levels of total protein in plasma and muscle were significantly lower ($p<0.05$) in all test concentrations in comparison with the control; but no concentration-dependent in depletions were observed. On the other hand, protein concentrations in liver, kidney and gills decreased with increased concentration of diazinon. In addition to significant differences in ALT and AST in liver, kidney, gills and muscle when compared with the control, concentration dependent activities in ALT was observed to in the liver and gills. It is concluded that protein concentration as well as ALT activities in gills and liver of *Clarias gariepinus* are more useful biomarkers of sub-lethal effects of diazinon than total protein, ALT and AST activities in plasma.

Key words: Alanine aminotransferase, aspartate aminotransferase, bioassay, *Clarias gariepinus*, diazinon, plasma

INTRODUCTION

The frequent use of pesticides for various industrial, agricultural and domestic purposes are veritable sources of pesticide introduction into the environment. These pesticides, even when applied in restricted areas are washed and carried away by rains and floods to large water bodies like ponds and rivers and alter the physico-chemical properties of water (Behalchandra *et al*., 2001). These are proving to be highly toxic, not only to fishes but also to other organisms, which form food of the fishes (Madhab *et al*., 2002). Sub-lethal pollution, which results in chronic stress conditions also have negative effect on aquatic life (Adedeji *et al*., 2008).

The effects of toxicants on the enzyme activity and protein content of fresh water fish have been observed by a number of investigators (Ramalingam and Ramalingam, 1982; Rashatwar and Hyas, 1983; Verma *et al*., 1984). Transamination represents one of the principal pathways for the synthesis and deamination of amino acids, thereby allowing an interplay between carbohydrate and protein metabolism during fluctuating energy demands of the organisms in various adaptive situations (Waarde and Henegaurajen, 1982). Therefore, attention has been focused on the changes in the amino transferees; alanine amino transferases (ALT) and aspartate amino transferase (AST), which promote gluconeogenesis from amino acids, as well as the effect of changes in amino transferase activities on the liver condition (Hilmy *et al*., 1981; Rashatwar and Hyas, 1983).

Diazinon is a common active substance of organophosphorous pesticides (Roberts and Hutson, 1998), and is an anticholinesterase, which causes loss of functional co-ordination that results in immobilization of organisms (Brooks, 1976). The objective of this work was to study the effect of diazinon on total protein content and transaminase activities in plasma, muscle, gill, liver and kidney of *Clarias gariepinus* and to determine which of these could best serve as a biomarker using this species.

MATERIALS AND METHODS

Fish samples for this study were obtained from a private fish farm at Abuloma road, Port Harcourt, Rivers State of 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 assays

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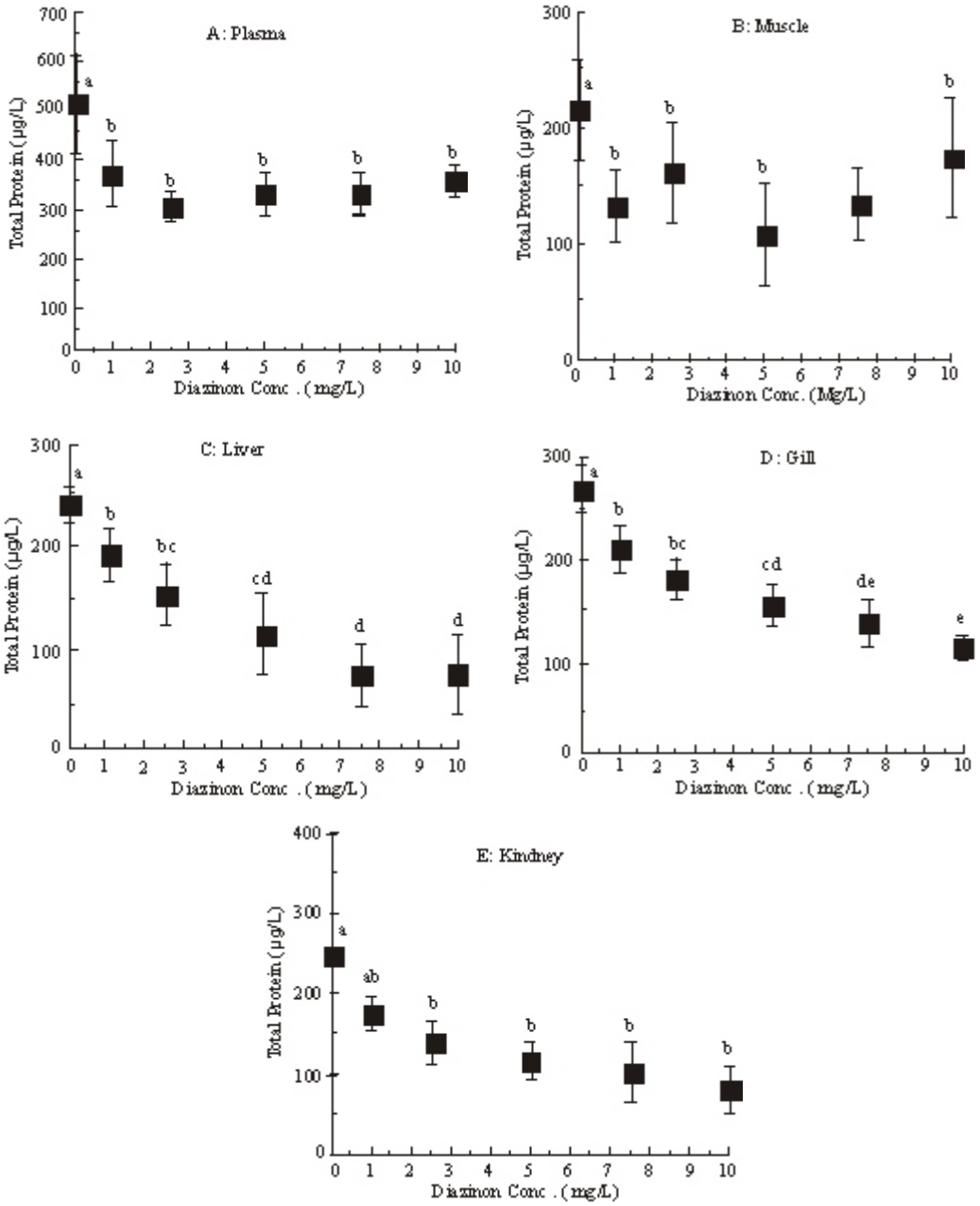

Fig. 1: Total protein in concentrations (mean±S.D, n = 4) in plasma and organs of *Clarias gariepinus* exposed to sub-lethal concentrations of diazinon for 30 days. Means with different letters are significantly different ($p<0.05$)

were conducted from May to October 2007. Fifty-eight adult *Clarias gariepinus* (mean weight 275±53.12 g; mean length 35.24±2.80 cm) were acclimated individually in rectangular aquaria for seven days during which they were fed once a day (9.00-11.00 h) with 35% crude protein diet at 1% biomass).

Sublethal concentrations of diazinon for the assay (1.0, 2.5, 5.0, 7.5 and 10.0 mg/L) were determined based on the range finding test (Inyang *et al*., 2010). These were prepared by transferring 0.02, 0.13, 0.25, 0.37 and 0.5 mL, respectively of the original concentration of diazinon and making it up to 30 L with borehole water in the test acquaria; 30 L of the diluent water was used as control. Four replications of each treatment level (concentration) and control were set up by introducing fishes individually into each acquarium. The exposure period lasted for 30 days during which the exposure media were renewed daily. The physicochemical characterization of the water

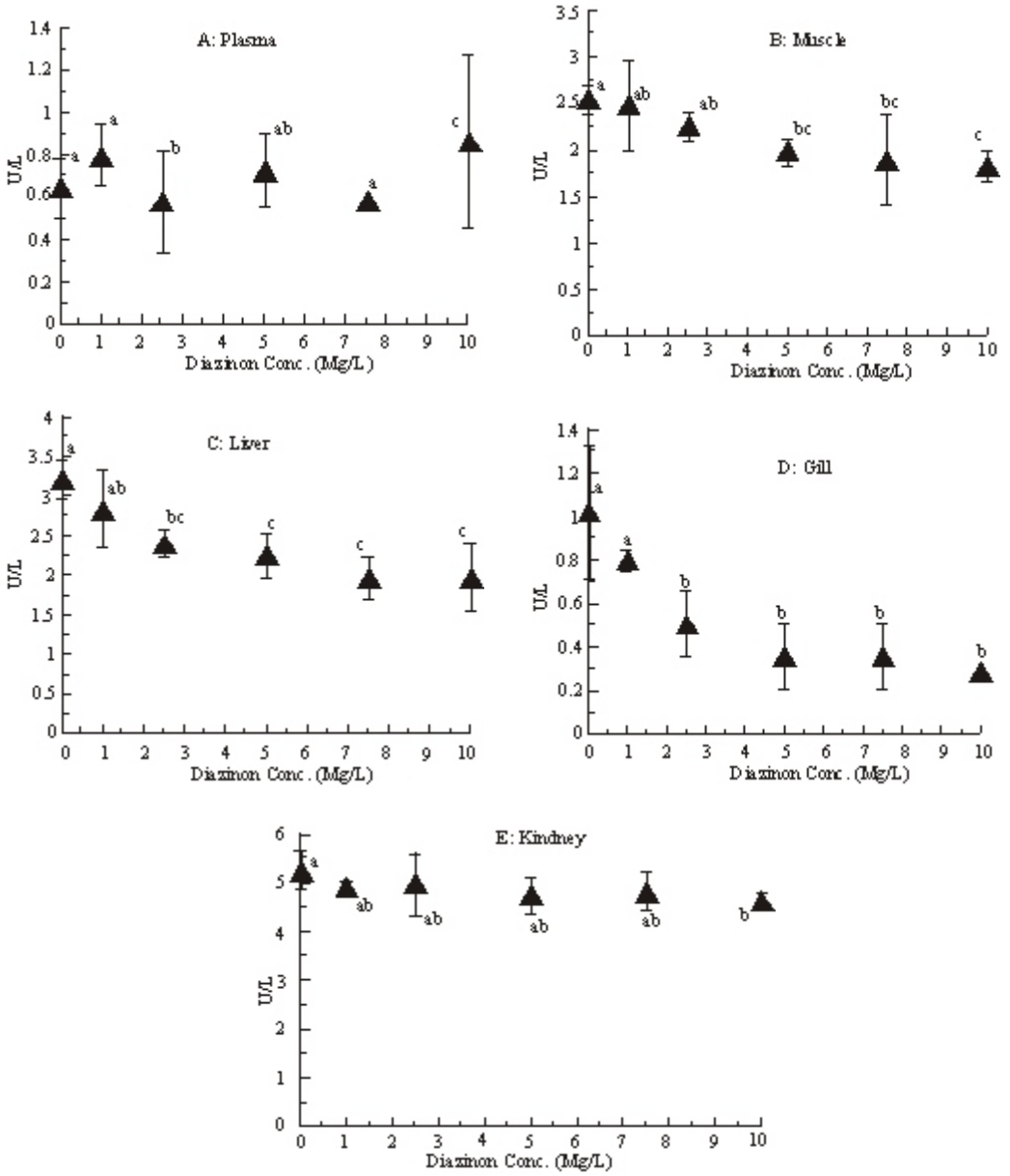

Fig. 2: Alanine aminotransferase (ALT) activities (mean±S.D, n = 4) in plasma and organs of *Clarias gariepinus* exposed to sub-lethal concentrations of diazinon for 30 days. Means with different letters are significantly different ($p<0.05$)

used for fish bioassay was carried out using standard methods (APHA, 1998) and the following values were obtained: temperature 26.0ºC, pH 6.20-6.37, dissolved oxygen 5.38-7.21 mg/L, alkalinity 15.25-17.0 9 mg/L, conductivity 99.50-136.12 µS/cm and turbidity 0.42-0.50 NTU (Inyang *et al*., 2010).

After the 30-day exposure period, blood samples for biochemical analysis were collected from each fish (behind the anal fin) with 23 G size needle and syringe. Fish were not fed prior to blood collection. Samples were preserved in heparinsed bottles and fish were sacrificed after blood collection and dissected for the collection of the gills, liver, kidney and muscle. 0.5 g of each organ was macerated (ground) with pestle and mortar. Physiological saline was used for preservation and stabilization. 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.

The activities of aspartate amino transferase (AST) and alanine amino transferase (ALT) in plasma, gills, kidney, liver and muscle were assayed using the colorimetric method of Reitman and Frankel (1957),

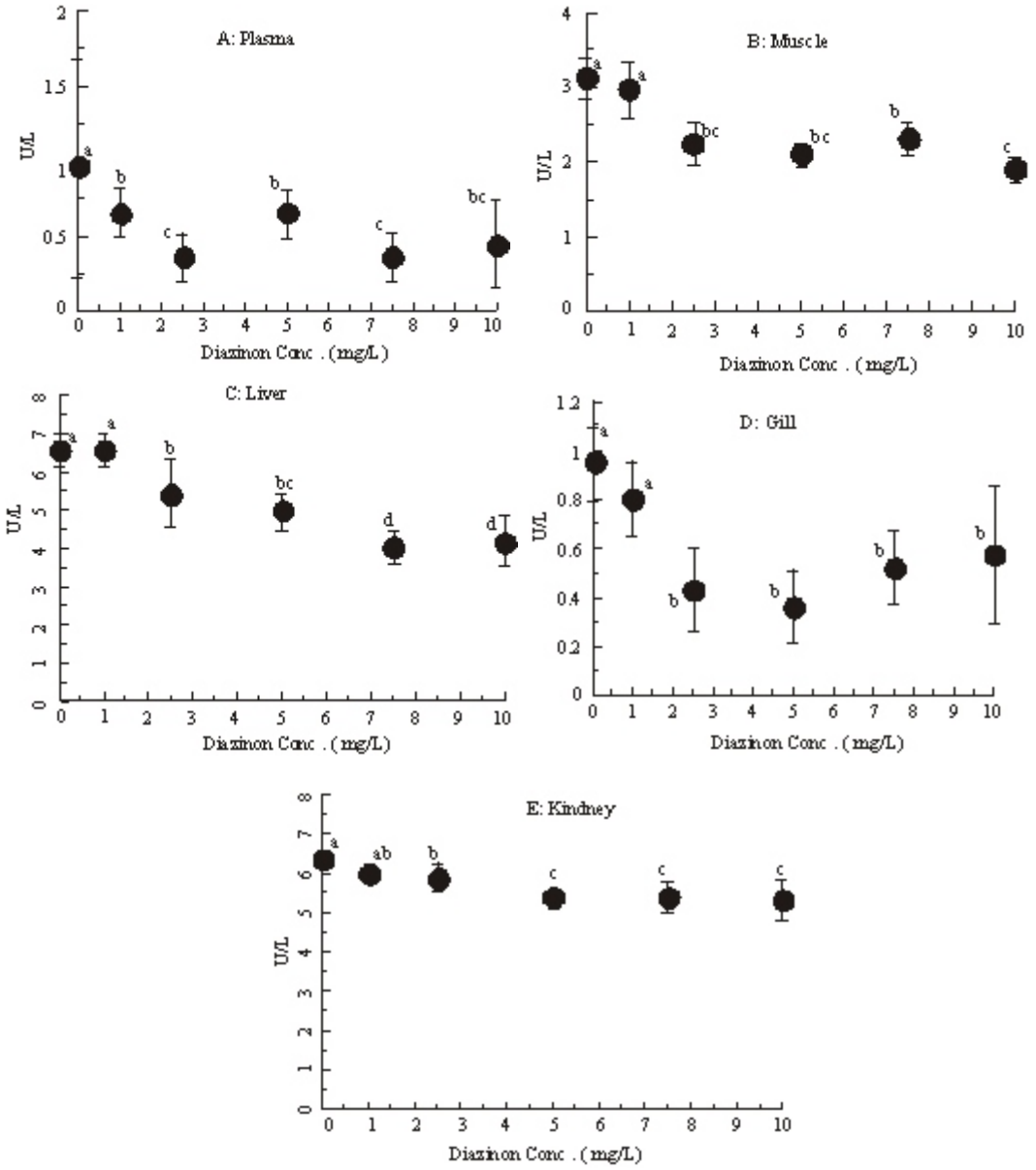

Fig. 3: Aspartate aminotransferase (AST) activities (mean±S.D, n = 4) in plasma and organs of *Clarias gariepinus* exposed to sub-lethal concentrations of diazinon for 30 days. Means with different letters are significantly different ($p<0.05$)

while total protein levels in supernatants were determined by the method described by Lowery *et al.* (1951).

The data were subjected to Analyses of Variance (ANOVA). Where 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

Total protein values in plasma were significantly higher in the control fish than all the treatment concentrations of diazion ($p<0.05$), but no concentration-dependent pattern was observed in the plasma protein levels (Fig 1A). A similar profile was found for total protein in fish muscle (Fig. 1B). However a concentration-dependent effect was found in the protein profile in liver, gills and kidney with a progressive decrease in the concentration of proteins in these organs with increase in the concentration of diazinon (Fig. 1C to E).

No clear trends in ALT activities were found in the plasma of fish exposed to different concentrations of diazinon (Fig. 2A). In the other organs (muscle, liver, gills and kidney), significant inhibition ($p<0.05$) of ALT activities was observed in all the sub-lethal concentrations

of diazinon tested (Fig. 2B to E). Significant concentration-dependent inhibitions were recorded in the liver (Fig. 2C) and gills (Fig. 2D) but in the kidney, only the highest concentration elicited ALT inhibition compared to the control (Fig. 2E).

Significant inhibitions of AST activities were recorded in the plasma at all test concentrations of diazinon in comparison with the control values, but there was no clear trend with increase in concentration (Fig. 3A). Concentration-dependent inhibition of AST activities were observed in the muscle, liver, kidney and gills (Fig. 3B to E). In all of these organs, no significant difference was found in the AST activity between the lowest test concentration of diazinon (1.0 and 2.5 mg/L) and the control; however, at the higher concentrations significant differences were found in a monotonic manner.

Plasma proteins which include globulins, fibrinogens and albumins, serve as a vital function in carrying materials from one part of the fish to another via circulation. They have nutritive, transporting, protective, buffering and energetic functions. When compared with fish in controls, significantly lower plasma protein levels were observed in all diazinon concentrations, indicating that at sublethal levels, the synthesis of protein is inhibited. Similar observations have been made for DDT and malathion in Sarotherodon melanotheron (Ramalingam and Ramalingam, 1982), diquat in carp (Magdy *et al*., 1993), and cypermethrin iin the Koren Rockfish, *Sebastes schlegeli*, (Jee *et al*., 2005). Our study, however, did not show concentration-dependent effects thus posing problems for the application of this index in *Clarias* a biomarker. According to Das and Mukherjee (2000), exposure of fish for a longtime to most toxicants including pesticides interferes with protein metabolism. After 96 h of action, diazinon produced a significant decrease ($p<0.05$) in protein concentration in the blood plasma of the experimental carp, as compared with the control group (Luskova *et al*., 2002). Decrease in total protein in fish exposed of toxic levels of toxicants could be attributed to either a state of hydration and change in water equilibrium in the fish or a disturbance in liver protein synthesis, or both (Gluth and Hanke, 1984).

All biological activities are regulated by enzymes and hormones, which are also proteins. Assessment of protein and enzymes activities can be considered as a diagnostic tool to determine the physiological status of cells or tissues (Manoj, 1999). Alterations of ALT and AST activities of fish resulting from toxicant or contaminant effect in various organs of fish have been reported (Sastry and Subhadra, 1985; Gill *et al*., 1991; Begum, 2004). Such biochemical changes in fish are aimed at maintaining equilibrium in the presence of these toxicants, which are known to disrupt physiological and biochemical processes (Wedemeyer and McLeay, 1981). Results of this study show that the activities of ALT and AST decreased as the concentration of diazinon increased in all the organs tested in a dose-dependent pattern. Similar result was also reported by Luskova *et al*. (2002) when they exposed *Cyprinus carpio* to 32.3 mg/L of diazinon for 96 h which produced depressed activities in the enzymes (AST, ALT and ALP).

We conclude that protein levels in plasma and muscle of *Clarias gariepinus* could be a diagnostic tool but are not necessarily good biomarkers of xenobiotics. However, protein levels in liver, gill, kidney as well as the activities of ALT and AST in these organs could serve as useful biomarkers of sub-lethal effects of diazinon in the aquatic environment.

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