EVALUATION OF BIOCHEMICAL PARAMETERS OF *Clarias gariepinus* EXPOSED TO SUBLETHAL CONCENTRATION OF CYPERMETHRIN

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ABSTRACT

Cypermethrin has being seen to be a contaminant of freshwater and marine ecosystem. Therefore, a study was designed to determine the sublethal effects of cypermethrin on freshwater fish, Clarias gariepinus. The fish was treated with 0.01 mg/L, 0.05 mg/L and 0.1 mg/L cypermethrin dissolved in distilled water. Fish were killed by cold narcosis on an ice block and dissected to obtain liver and muscle samples; 10% homogenates in an ice-cold saline were prepared. Liver aspartate aminotransferase (AST), alanine aminotransferase (ALT), liver and muscle acid phosphatase (ACP) were measured. There was a significant increase in liver and muscle ACP in cypermethrin treated catfish compared to the control. Liver AST level significantly increased at all concentrations for experimental duration (P<0.05) compared to the control. These alterations in enzyme activity may have long-term effects on organisms (fish) that are continuously exposed to low doses of cypermethrin in an aquatic ecosystem.

Keyword: Cypermethrin, *Clarias gariepinus*; Aspartate aminotransferase, Alanine aminotransferase, Acid phosphatase

INTRODUCTION

In recent years many research articles have appeared discussing the impact of man made xenosterogenic compounds on wild life. The suspect chemical is a synthetic pyrethroid insecticides used in the agriculture, public health and animal husbandry (Gordon, 2004). It can enter into the aquatic environment following agricultural use through runoff of dissolved chemicals or soil bound particles into water.

Cells naturally contain enzymes for their functions such that damages to cellular membrane lead to their escape into the blood where their presence or activities can easily be measured as an index of cell integrity (Coles, 1974; Coppo *et al.,* 2002). Serum chemistry could be used to identify tissue damage (Patti and Kulkarni, 1993). Aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ACP) are normally found within the cells of the liver, heart, gills, kidneys and muscles (Shalaby, 2004), but their increase in the plasma indicate tissue injury or organ dysfunction (Wells *et al.,* 1986). However, changes in plasma glucose, total proteins and cholesterol concentrations can be indicative of a classical general adaptive response to stress in fishes exposed to pollutants (Martinez *et al.,* 2004). This is because fish blood is very sensitive to pollution-induced stress (Patti and Kulkarni, 1993). Biochemical changes in fishes exposed to various pollutants have been documented (Attar, 2005; Ogueji and Auta, 2007; Kori-Siakpere and Ubogu, 2008; Mousa *et al.,* 2008; Shalaby, 2004). Despite the insecticidal use of cypermethrin in Nigeria, its biochemical effects on *Clarias gariepinus* has not

been studied for a fish that is widely cultivated (FAO, 1977) and naturally abounds in our waters where it is of commercial importance (Fagbenro, 1992).

This study is therefore, aimed at evaluating biochemical changes in *C. gariepinus* adults exposed to cypermethrin.

MATERIALS AND METHODS

Fish: One hundred and twenty fingerlings *C. gariepinus* (19.14 $\pm$ 2.27g and 27.23 ± 0.19 cm) purchased from Kune Integrated Farms Limited, Katsina, Nigeria were acclimatized at the Fisheries Research Wet Laboratory, Department of Zoology and Environmental Biology, University of Nigeria Nsukka for two weeks. The catfishes were fed *ad libitum* with 4 mm 45% crude protein Vital Pelleted Catfish Feed. Water in the aquaria was changed thrice weekly with dechlorinated water to maintain an environment free from toxicant arising from fish excrement and uneaten feed.

Treatments: A pilot study was conducted to determine three graded concentrations (Omitoyin *et al.,* 1999; Fafioye, 2001) prior to the actual experiment that was based on renewal bioassay (APHA, 1981). Ten adult *C. gariepinus* were introduced at random into each of these aquaria A – D and replicated into three before the preparation and administration of cypermethrin. The fish in groups A, B and C were exposed to 0.01 mg/L, 0.05 mg/L, and 0.1 mg/L, respectively. The fourth group (Control) was exposed to tap water as the control. Water quality characteristics of temperature, pH, dissolved oxygen (DO) and total hardness as equivalent of calcium carbonate were determined. The temperature was 27.1° C, pH 7.9, the DO 6.4 mg/L and total hardness 100 mg/L equivalent of $CaCO_3$. The water contained (mg/L) Ca^{2+}, 4.01; Mg^{2+}, 9.73; Na^{+}, 4.9; Cl^{-}, 7.5; SO_4^{2-}, 15.6; NO_3^{-}, 0.96, and total phosphorus, 0.04 (Ozioko, 1988).

Assay: The liver and muscle tissues were assayed for enzymatic activity. The fish were stunned by being placed on a block of ice and then dissected to obtain liver and muscle samples; 10% homogenates in ice cold saline were prepared. The homogenates were used for estimating enzyme activity. The liver and muscle homogenates were used for estimating the activity of acid phosphatase activity (ACP) (Bassey *et al*, 1946) and aspartate and alanine aminotransferase (AST and ALT) (Frankel and Reitman, 1957).

Analysis: the data were analysed using one-way analysis of variance (ANOVA) and Duncan new multiple range test at $P < 0.05$. Values were expressed as mean ± SD.

RESULTS

There was a significant increase in liver and muscle ACP level in cypermethrin treated groups compared to control (Figure 1). Interestingly, there was a high acid phosphatase levels in the liver tissues which was seen to be statistically significant ($P \leq 0.05$) at day 21 compared to the control and all cypermethrin treated groups. However, at day 7 and day 14, the acid phosphatase level of the treated groups was not statistically significant ($P > 0.05$) compared to the control. Acid phosphatase level in the muscle tissues were not statistically significant ($P > 0.05$) between the control and all cypermethrin treated groups (Figure 2). Liver AST level significantly increased and the mean increase was not significant ($P > 0.05$) in day 21 cypermethrin treated fish compared to the control (Figure 3). On the other hand, liver ALT was significantly different ($P < 0.05$) in cypermethrin-treated group with that of the control (Figure 4) with increase in toxicant concentration.

DISCUSSION

Cypermethrin has been found to be very toxic to fishes and aquatic invertebrates. In this present study it was evident that liver and muscle ACP levels were significantly increased in the cypermethrin treated fish. It was evident that liver and muscle acid phosphatase level were significantly increased in cypermethrin treated fish at various concentrations and also decreased significantly at other groups.

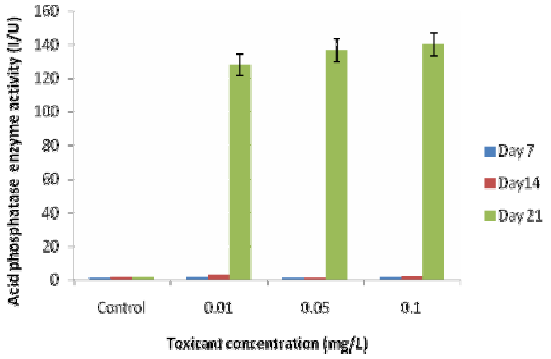

Figure 1: Alkaline phosphatase activity in liver of *Clarias gariepinus* fingerlings exposed to cypermethrin

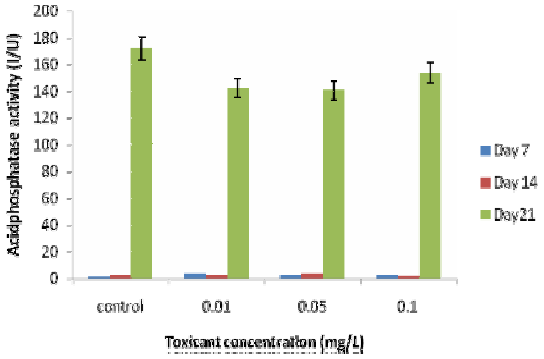

Figure 2: Alkaline phosphatase activity in muscle of *Clarias gariepinus* fingerlings exposed to cypermethrin

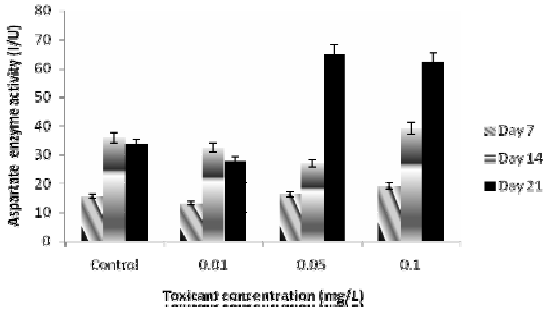

Figure 3: Aspartate aminotransferase activity in liver of *Clarias gariepinus* fingerlings exposed to cypermethrin

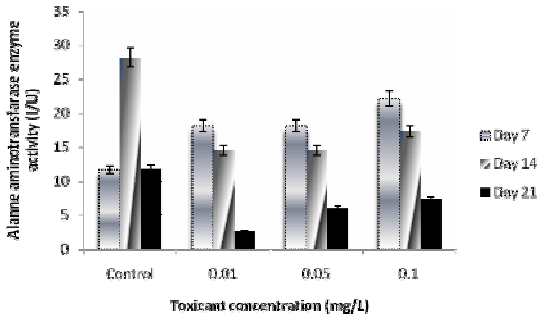

Figure 4: Alanine aminotransferase activity in liver of *Clarias gariepinus* fingerlings exposed to cypermethrin

This increase was probably due to increased lysosomal activity in the liver and muscle tissues. Similar increased lysosomal activity in the liver and muscle tissues has been reported (Nivedita *et al*., 2002). Furthermore, ACP is an inducible enzyme because its activity goes up when there is a toxic impact and the enzyme begins to counteract the toxic effect. Subsequently, the enzyme may begin to drop either as a result of having partly or fully encountered the toxin or as a result of cell damage. In a study of male Sprague-dawley rats treated with 50 ppm (w/v) cypermethrin in drinking water for four months, there was significant increase in liver ACP (Sonde *et al*., 2000). It is apparent that cypermethrin causes increased ACP activity in the liver and muscle by interacting with lysosome. Lowe *et al*. (1992) reported that alteration in the membrane permeability can have severe consequences such as leakage of hydrolytic enzyme including ACP, which could have detrimental effect on the cell.

Liver AST levels were significantly increased in cypermethrin treated fish though not statistically significant compared with the control. This indicates that cypermethrin stimulates glutamate transaminase activity in the liver which could be due to toxic injury caused by cypermethrin, which may stimulate tissue repair through protein turn over and increased respiration. AST levels were comparatively lower in cypermethrin treated group indicating that cypermethrin does affect mitochondrial function. This agrees with Nivedita *et al.* (2002). This correlates well with increased AST activity in the liver of cypermethrin treated fish. In this regard, it can be said that cypermethrin toxicity leads to enhanced AST activity, which is indicative of high protein turnover and amino acid metabolism. This was in line with the study of Nivedita *et al.* (2002) and Muthuviveganandavel *et al*. (2007). Liver ALT level were significantly increased in day 7 and decreased with increase in concentration throughout the study period in cypermethrin treated fish compared to the

control indicating that transaminase activity is enhanced in the liver due to cypermethrin toxic insult. In this regard, it can be said that cypermethrin toxicity leads to enhanced AST and ALT activity, which is indicative of high protein turnover and amino acid metabolism.

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