

Alkaline Phosphatase (ALP) Activity in Selected Tissues & Organs of *Clarias gariepinus* Exposed to Different Levels of Paraquat

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Abstract

Analyzing the activities of alkaline phosphatase (ALP) in tissues can help detect tissue damage cause by toxicants such as paraquat. The activities of alkaline phosphatase (ALP) in some tissues and organs of *Clarias gariepinus* exposed to various levels of paraquat (2, 4, 6 and 8ppm) were studied for a period of thirty (30) days. The tissues and organs investigated include blood plasma, kidney, liver, gills and muscle. Results showed changes in ALP activity in the treatment group as compared to the control group except in the muscle and plasma where there were no significant changes. ALP activity was highest in the kidney in the control group (511.25 ± 291.76 IU/L) while the lowest level of enzyme activity in the control group was observed in the muscle (1146.67 ± 173.52 IU/L). Peak enzyme activity in the kidney was observed at 4ppm of paraquat. This later declined at higher treatment concentrations. There was no significant increase in ALP activity in the liver and gills at lower paraquat concentrations but a significant spike in enzyme activity was observed at 8ppm. Analyzing the activities of ALP in tissues can help detect tissue damage cause by toxicants such as paraquat. It can consequently be inferred that ALP activity can be used as a reliable biomarker for diseased condition in the kidney, liver and gills but not the plasma or muscle of *Clarias gariepinus*.

Keywords: Alkaline phosphatase, pollution, *Clarias gariepinus*

1. Introduction

Ichthyofauna and other aquatic biota are very susceptible to the adverse effects of contaminants that pollute aquatic environments. These contaminants or toxicants affect the biochemistry of aquatic biota; fishes inclusive which could result in some physiological changes in these organisms (Van Varen, 1986).

An example of such contaminants includes the herbicide paraquat used in weed control in both terrestrial and aquatic environments. It is generally believed that the mechanism of action of paraquat is associated with the production of reactive oxygen species, and the superoxide ions. This theory has been demonstrated in-vivo where it was observed that the toxicity of paraquat is increased by organisms' exposure to elevated oxygen pressure (Autor 1974, Bus et al, 1975, Bauman et al, 1992).

Clarias gariepinus, (African Catfish) belongs to the family Claridae and occupies a vital position in the fish industry of the Niger Delta region of Nigeria in particular as well as the world over. This is probably because of its good taste, high value and cultivability (Sikoki et al, 1998; Eliot, 1995, Richter, 1979; and Hogeendorn, 1979).

The enzyme alkaline phosphatase (ALP) is present in the liver in appreciable amounts. It is also found in the small intestines, kidney, placental tissues and osteoblasts. Under stressful situations such as the exposure of fish to toxicants like paraquat, enzyme activity is bound to be affected.

2. Materials and Methods

5 groups of fishes (20 in each group) weighing between 270 and 300g were aquarium bred for 3 months. It was ensured that the water in the aquaria containing fishes of different groups was pure and pollutant free. The water was kept at room temperature and at a pH of 7.0. The first group was the control group placed in an aquarium containing no paraquat. The second to fifth groups of fishes were placed in aquaria containing 2ppm, 4ppm, 6ppm and 8ppm of paraquat respectively. The fishes were fed with 30% of crude protein at 1% body weight. After thirty (30) days, 0.5g of each of the organs (Kidneys, livers, gills and muscles) was macerated and analyzed for ALP using the Bessey, Lowry and Brock (1946) method. ALP analysis was done by the determination of a color change of a buffered phenolphthalein substrate. Alkaline phosphate was incubated at 37^oc for 30 minutes at a pH of 10.5 of buffered substrate which contains 4-nitrophenolphosphate. The enzyme hydrolyses the substrate liberating 4-nitrophenol and organic phosphate. NaOH was added to stop the reaction. The absorbance of the yellow color produced was measured with a colorimeter using a deep violet filter paper

and confirmed at a spectrophotometric wave length of 400mm.

Statistical analysis of the data obtained was subjected to one-way analysis of variance (ANOVA) to test if the exposure to paraquat produced significant change in enzyme activity in these tissues and organs. Where differences were observed, Duncan's multiple range test (DMRT) was used to compare or separate differences between means.

3. Results

The results showed changes in ALP activity in the treatment group as compared to the control group except in the muscle and plasma where there were no significant changes. ALP activity was highest in the kidney in the control group (511.25 ± 291.76 IU/L) while the lowest level of enzyme activity in the control group was observed in the muscle (1146.67 ± 173.52 IU/L). Peak enzyme activity in the kidney was observed at 4ppm of paraquat. This later declined at higher treatment concentrations. There was no significant increase in ALP activity in the liver and gills at lower paraquat concentrations but a significant spike in enzyme activity was observed at 8ppm. All these are shown in the figure below.

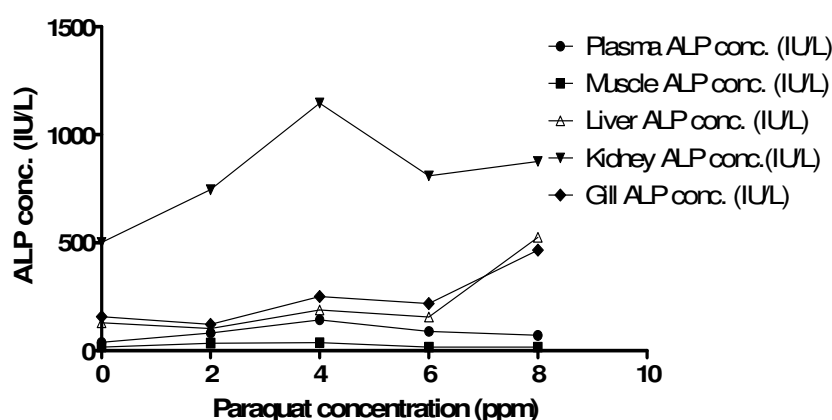


Figure1. Alkaline Phosphatase (ALT) activity in organs of *Clarias gariepinus* exposed to various levels of paraquat for 30 days.

4. Discussion

Enzymes perform catalytic activities under normal conditions as effective biochemical functioning. In the fish, under toxicant or pollutant induced stress, transamination and body metabolism are conditioned to maintain physiological equilibrium. When *Clarias gariepinus* was exposed to various levels of paraquat there was an increase in ALP activity in the fish which is an indication of inactive or ineffective transamination and oxidative deamination. It could also indicate a defect in the promotion of gluconeogenesis from amino acids due to changes in amino transferase activities in the organs investigated. Increase in ALP activity is one of the most sensitive biomarkers employed in the diagnosis of hepatic damage because they are cytoplasmic in nature and are released into the circulation after cellular damage. Mayne, 2002; Leelavinothan et al, 2005 and Luskova et al. 2002 worked on *Cyprinus carpio* exposed to 32.5mg/l of diazinon for 96 hours and reported that it produced depressed activities of some enzymes; ALP inclusive in the plasma of the fish.

Chetty et al (1980) attributed the increase in ALP activities of fishes exposed to toxicants to disturbance in the Krebs cycle. Significant increase in the activities of ALP in the liver of albino rats exposed to monocrotophos methyl parathion and dimethoate administered orally for 90days was reported by Kaur et al (2002). The inference was that such increase is an indication of cellular toxicity of these organophosphates causing a release of the enzyme into the circulation. Ayalogu et al (2001); Gabriel et al (2005) have also reported elevations in ALP activities of organisms exposed to different toxicant. Hogue et al (1993) and Fafioye et al (2007) also attributed an increase in ALP activity in the liver of fish to cellular damage. A change in ALP activity reflects a change in endoplasmic reticulum mass (Edquist, 1992). ALP also functions in the conversion of nicotine adenine dinucleotide phosphate to nicotine adenine dinucleotide (Morton, 1955). Therefore an increase in ALP activity after exposure will eventually result in shift in biosynthesis and energy metabolism pathway of the exposed organism (Ovuru et al 2000).

Analyzing the activities of ALP in tissues can help detect tissue damage cause by toxicants such as paraquat. It can consequently be inferred from this study that ALP activity can be used as a reliable biomarker for cellular

damage and hence diseased condition in the kidney, liver and gills but not the plasma or muscle of *Clarias gariepinus*.

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