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Full Length Research Paper

Variations in alanine aminotransferase and aspartate aminotransferase activities in African catfish: *Clarias gariepinus* (Burchell, 1822) at different sublethal concentrations of potassium permanganate

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Potassium permanganate ($KMnO_4$) is a widely used freshwater aquaculture chemotherapeutant for the treatment and prevention of waterborne parasitic and fungal diseases. The specific objective of this research is to determine its effects on alanine aminotransferase and aspartate aminotransferase activities in the plasma of the widely consumed African catfish, *Clarias gariepinus*. *C. gariepinus* were exposed to sublethal concentrations (0.0, 2.0, 6.0 and 10.0 mg/L) of potassium permanganate for 12, 24, 48, 96 and 192 h adopting the static renewal bioassay technique and subjected to blood and plasma analyses. Empirical data of the results obtained were subjected to statistical analysis using two-way analysis of variance (ANOVA) to test the level of significance between the various sublethal concentrations of $KMnO_4$ and the exposure periods. Exposure to Potassium permanganate caused significant ($p < 0.05$) increase in alanine aminotransferase and aspartate aminotransferase. Increased activities of both aminotransferases indicated amplified transamination processes and were used as stress indicators.

Key words: Potassium permanganate, aspartate aminotransferase, alanine aminotransferase, *Clarias gariepinus*, Nigeria.

INTRODUCTION

The effect of toxicants on enzymatic activity is one of the most important biochemical parameters, which is affected under stress. When an organ is diseased due to the effect of a toxicant, enzyme activity appears to be increased or it may be inhibited due to the active site being either denatured or disturbed. Since some enzymes catalyze a few steps in the metabolism of carbohydrates and protein, they are present in most tissues. The increase or decrease in their level may be sufficient to provide information of diagnostic value (Valarmathi and Azariah, 2003).

Demonstrations of increase or decrease in specific plasma activity with disease encourage researchers to evaluate a variety of enzyme systems looking for those which are organs or tissue specific. Nowadays, enzymogram plays an important role in diagnosis and prognosis of animal disease (Coles, 1989). Changes in plasma enzyme activity are used as indicators of tissue injury, environmental stress, or a diseased condition. The increase in enzyme activity depends on the enzyme concentration in cells, rate of leakage caused by injury and rate of clearance of the enzyme from plasma (Boyd, 1983).

Modifications in enzyme activity occur by cell death, increase or decrease enzyme production, obstruction of normal excretory route, increase cell membrane permeability, or impaired circulation (Kaneko, 1989).

Increase plasma aspartate aminotransferase (AST) is associated with cell necrosis of liver and skeletal or cardiac muscle, starvation, and lacking vitamin E. Plasma alanine aminotransferase (ALT) is an acute hepatic damage

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good marker (James et al., 1991; Svoboda, 2001).

The variation in enzyme activities in the freshwater fish exposed to various pollutants have been reported (Nemcsok et al., 1981; Sjobeck et al., 1984; Begum and Vijayaraghavan, 1995; Desmet et al., 2001; Humtsoe et al., 2007). It has also been reported that the variation in enzyme activities in heavy metal treated fish is due to increased permeability of the cell as well as the direct effect of the heavy metal on the tissues (Roy, 2002).

The research work is conducted with the objective of investigating the effect of sublethal concentration of potassium permanganate ($KMnO_4$), a commonly used chemotherapeutant in aquaculture management of diseases and parasites; on alanine aminotransferase and aspartate aminotransferase activities in the plasma of the widely consumed African catfish *Clarias gariepinus* with particular reference to the concentration of the therapeutant and duration of exposure.

MATERIALS AND METHODS

Apparently healthy live specimens of *C. gariepinus* (mean weight, 165.15 ± 3.45 g; mean length 29.42 ± 6.56 cm) were purchased from Tomab Fish Farms, Obiaruku, Delta State, Nigeria; and transported to the Animal and Environmental Biology Research Laboratory, Delta State University, Abraka where they were kept in large plastic drums supplied with clean borehole water. Fish were acclimatized to the experimental conditions for two weeks. Mortality during the period of acclimatization was less than 2%.

Stock solution of potassium permanganate ($KMnO_4$) was prepared from 1 g standard AnalaR grade granules in 1 L of deionised water to form 100% concentration. From this stock solution, various concentrations used in the investigations were prepared by dilution.

Triplicates of the same experimental concentration design were conducted. For each triplicate, a set of four tanks were stocked with 20 randomly selected fish. At the end of the acclimatization period, each tank was randomly assigned to one of three treatments, plus control. Three tanks were dosed for each testing concentration of potassium permanganate: 2, 6, 10 mg/L of $KMnO_4$ and one is keep without $KMnO_4$ (0 mg/L).

The experimental tanks consisted of large plastic containers of 150 L capacity, filled to half their capacities and covered with a lid made of fine polyethylene gauze screen of 1 mm mesh size, to prevent the fish from jumping out of the containers. Experimental fish were fed daily with Catfish feed (Dizengoff; 4.5 mm; Protein 42%, Fat 13%, Fiber 1.9% and Ash 1.2%) at 3% of their body weights. The fish were not fed 24 h prior to the experimental period, as well as during the experimental period, which lasted 192 h. Natural photoperiod was maintained during the acclimation and experimental period.

The water quality parameters of the experimental set up bioassay, with $KMnO_4$ toxicant and control, were conducted at every sampling time according to APHA (1998) procedures. The water quality parameters measured included pH 6.48 ± 0.32, temperature 28.4 ± 1.2°C, dissolved oxygen 7.36 ± 1.12 mgL^{-1}, free carbon dioxide 4.85 ± 0.06 mgL^{-1} and total alkalinity 34.6 ± 1.54 mgL^{-1}.

The test was performed using a semi-static renewal method in which the exposure medium was exchanged for every sampling time to maintain toxicant strength and level of dissolved oxygen as well as minimizing the level of ammonia excretion during this experiment.

Two fish were randomly caught individually using a small hand net from each experimental tank at each sampling time. The experiments were conducted three times, yielding a total of six fish for each treatment at each sampling time. The sampling was done just before the initial addition of $KMnO_4$ (0 h = start) and then at 12, 24, 48, 96 and 192 h.

Blood from the selected fish was drawn from the caudal vessels with a heparinised disposable plastic syringes and a hypodermic needle. The use of plastic syringe is a necessary precaution with fish blood, because contact with glass results in decreased coagulation time (Smith et al., 1952).

Plasma was obtained by centrifugation and diluted 1:20 with deionised water. The diluted plasma was then stored in a refrigerator at -4°C and later analysis were conducted for enzyme activities: Plasma alanine aminotransferase and aspartate aminotransferase. All determinations were carried out in duplicates for each sample.

Plasma alanine aminotransferase and aspartate aminotransferase were determined colorimetrically using commercial diagnostic kits (Dialab Producktion, Austria) using a spectrophotometer (Spectrumlab 21A, Lenjguang Tech, China).

Plasma alanine aminotransferase and aspartate aminotransferase were measured based upon International Federation of Clinical Chemistry (IFCC) recommendations by kinetic decreasing methods.

Plasma aspartate aminotransferase was measured based on the oxido-reductive process of $NADH/NAD^+$ in the presence of oxaloacetate and the resulting decrease in absorbance at 340 nm being directly proportional to the aspartate aminotransferase in the sample (U/L).

Plasma alanine aminotransferase was similarly measured based on the oxido-reductive process of $NADH/NAD^+$ in the presence of pyruvate and the resulting decrease in absorbance at 340 nm being directly proportional to the alanine aminotransferase in the sample (U/L).

Data analysis

Results obtained for the triplicates from all three experiments were combined and subjected to statistical analysis using two-way analysis of variance (ANOVA) to test differences between the various levels of sublethal concentrations of $KMnO_4$ and the exposure periods. Multiple comparisons of the means were analyzed by the Bonferroni tests. All analyses were performed using the software programme (GraphPads Prism® Software version 5.0, San Diego, CA). Results were considered significant at the 95% confidence level ($p < 0.05$).

RESULTS

Mean plasma aspartate aminotransferase activities in the *C. gariepinus* exposed to the various toxicant concentrations and at different exposure periods is shown in Figure 1; while the percentage variation with respect to the control values are presented in Table 1. The activities of the plasma aspartate aminotransferase in the control group ranged from 15.65 - 16.90 U/L. There was generally a gradual increase in the plasma aspartate aminotransferase levels in the treated fish with increase in both $KMnO_4$ concentration and exposure time. Similarly, there was significant difference ($p < 0.05$) in the means of plasma aspartate aminotransferase levels in the treated fish with increase in the concentration and exposure time.

Multiple comparisons using Bonferroni test fail to show any significant differences between mean levels of

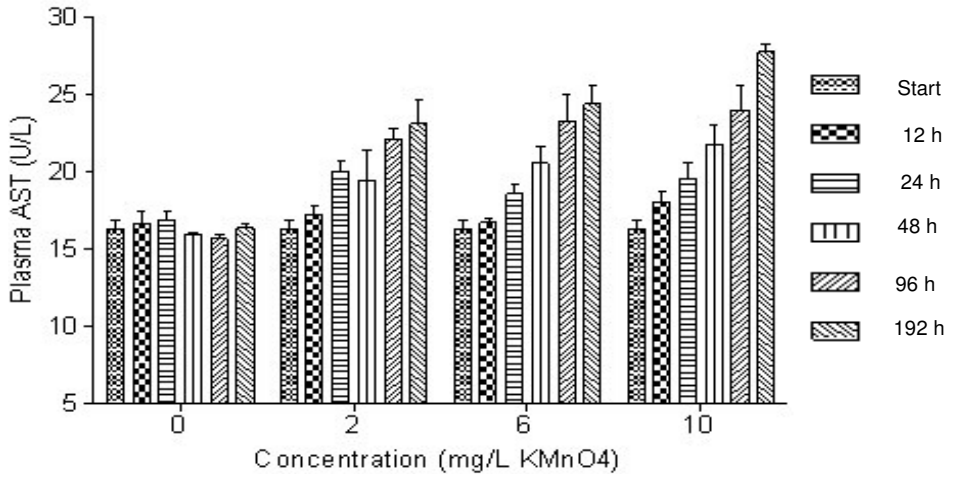

Figure 1. Mean values of activities of plasma aspartate aminotransferase in *C. gariepinus* exposed to the various sublethal concentrations of potassium permanganate over a period of 192 h. Each column represents the mean value and vertical bars indicate the standard error of the mean.

Table 1. Percentage variation of activities of plasma aspartate aminotransferase of *C. gariepinus* exposed to the various sublethal concentrations of $KMnO_4$ over a period of 192 h.

Concentration ($mg/L\ KMnO_4$)	Exposure period (h)					
	Start	**12**	**24**	**48**	**96**	**192**
2	0.00	3.67	18.11	22.36*	40.96*	41.31*
6	0.00	0.42	9.70	29.28*	48.82*	49.02*
10	0.00	8.31	15.44	36.90*	53.16*	69.83*

* Indicates significant difference ($p < 0.05$) from the zero time (start) values.

plasma aspartate aminotransferase for zero time and mean values following 12 and 24 h exposure all treatments of $KMnO_4$. On the other hand, significant ($p < 0.05$) differences were observed in fish exposed to all the treatments of $KMnO_4$ at 48, 96 and 192 h. Activities of plasma aspartate aminotransferase levels in the treated fish showed a seemingly dose- and time-dependent increase with the maximum elevation percentage (69.83) being recorded in 10 mg/L $KMnO_4$ exposed fish at 192 h.

The mean plasma alanine aminotransferase activities in fish exposed to different concentrations of potassium permanganate under different exposure period is graphically presented in Figure 2; while the percentage variation with respect to the control values are presented in Table 2. The plasma alanine aminotransferase activeties in the control fish varied between 22.87 and 27.44 U/L. There was an initial decrease (-4.47%) in the mean level of plasma alanine aminotransferase observed in the test fish as the concentration increased from 0 - 2 mg/L $KMnO_4$ at 12 h, it increased as the exposure time increased to 192 h. In 6 and 10 mg/L $KMnO_4$ exposed fish, enzyme activities increased following 12 - 192 h of exposure. There was significant difference in the mean levels of plasma alanine aminotransferase of the treated fish with increase in concentration of the toxicant and exposure time. Comparison of means using Bonferroni test revealed that the only observed decrease in the mean levels of plasma alanine aminotransferase in the fish exposed to 2 mg/L $KMnO_4$ at 12 h was not significantly different ($p < 0.05$) from the zero time (start) values. In all the other treatments and exposure time, there was significant difference between them and the zero time values. However, the maximum percentage elevation (53.88) of enzyme activity was recorded in the 10 mg/L $KMnO_4$ exposed group following 96 h exposure.

DISCUSSION

Plasma enzymes levels in fish have been proposed to be good indicators of extreme stress and provide information of organ dysfunction (Wells et al., 1986). Toxicants cause a disturbance in the physiological state of the animal which affects enzyme activity. Toxicants bring about distortions in the cell organelles, which may bring about elevation or inhibition in the activities of the enzymes

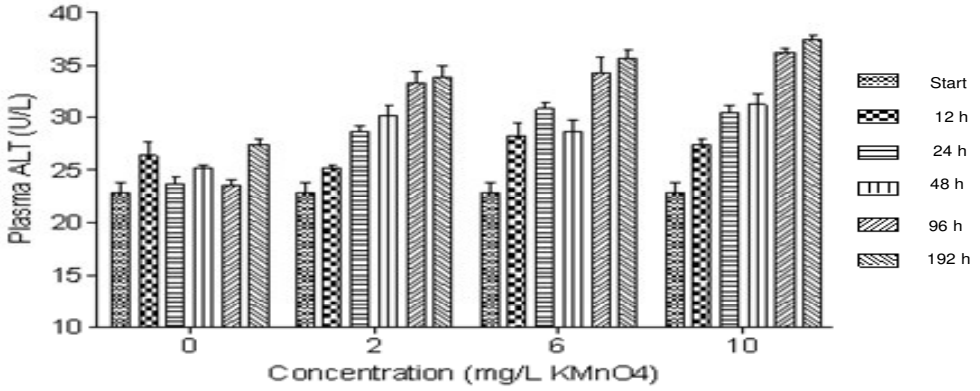

Figure 2. Mean values of activities of plasma alanine aminotransferase in *C. gariepinus* exposed to the various sublethal concentrations of potassium permanganate over a period of 192 h. Symbols as in Figure 1.

Table 2. Percentage variation of activities of plasma alanine aminotransferase acid of *C. gariepinus* exposed to the various sublethal concentrations of $KMnO_4$ over a period of 192 h.

Concentration (mg/L $KMnO_4$)	Exposure period (h)					
	Start	12	24	48	96	192
2	0.00	-4.47	20.83*	19.60*	41.20*	23.43*
6	0.00	7.21*	29.72*	14.04*	45.52*	29.99*
10	0.00	4.06*	28.33*	24.06*	53.88*	36.33*

* Indicates significant difference ($p < 0.05$) from the zero time (start) values.

(Valarmathi and Azariah, 2003). Plasma enzyme levels depend on the rate of release of enzymes from damaged cells, which in turn depend on the rate at which damage is occurring and at the extent of cell damage.

Aminotransferases are intracellular enzymes which are normally localized within the cells of the liver, heart, gills, kidney, muscle and other organs. Aspartate aminotransferase (AST) is present in high concentrations in the heart, liver, skeletal muscle, kidney and erythrocytes while alanine aminotransferase (ALT) is present in high concentrations in liver and to a lesser extent in skeletal muscle, kidney and heart (Zilva et al., 1992). The levels of these enzymes increase in the plasma when the cells are damaged or their membranes disrupted, allowing the enzymes to leak out of the cells. These enzymes are therefore of major importance in assessing and monitoring liver cytolysis (Wada and Snell, 1962). The significant changes in the activities of AST and ALT enzymes in blood plasma indicate tissue impairment caused by stress (James et al., 1991; Svoboda, 2001).

The pattern of AST and ALT activities recorded in the present study show that cellular damage could arise from the exposure of the experimental fish to potassium permanganate resulted in the leakage of these enzymes to the extracellular fluid. Increased plasma AST and ALT activity, an obvious biochemical symptom of liver cytolysis may thus be attributed to increase in liver AST and ALT and contribution from other organs of the body. Aminotransferases play vital roles in carbohydrate-protein metabolism in fish and other organisms' tissues (Eze, 1983). The aminotransferases occupy a central position in the amino acid metabolism as they help in retaining amino groups (to form a new amino acid) during the degradation of amino acid and also involved in the biochemical regulation of intracellular amino acid pool. They help in providing necessary intermediates for gluconeogenesis. The observed alterations in their activities in the exposed fish may therefore have adverse effect on the amino acid metabolism of the tissues and consequently the intermediates required for gluconeogenesis.

Increased activities of both aminotransferases indicated amplified transamination processes. An increased in transamination occurs due to amino acid input into the TCA cycle in order to cope with the energy crisis during toxicant-based stress (Philip et al., 1995).

REFERENCES

APHA (1998). Standard methods for examination of water and wastewater. 20th Edn. American Public Health Association Washington D C., p. 1976.

Begum G, Vijayaraghavan S (1995*). In vivo* toxicity of dimethoate on protein and transaminases in the liver tissue of freshwater fish Clarias batrachus (Linn.). Bull. Environ. Contamination Toxicol., 54: 370-375.

Boyd JW (1983). The mechanisms relating to increases in plasma enzymes and isoenzymes in diseases of animals. Vet. Clin. Pathol., 12: 9-24.

Coles EH (1989). Veterinary Clinical Pathology (4th Ed); W. B. Saunders Co. Philadelphia

Eze LC (1983). Isoniazid inhibition of liver glutamic oxaloacetic transaminase from goat (Carpa hercus). Inter J. Biochem., 15: 13-16.

Humtsoe N, Davoodi R, Kulkarni BG, Chavan B (2007). Effect of arsenic on the enzymes of rohu carp Labeo rohita (Hamilton, 1822). Raffles Bull. Zool., 14: 17-19.

James R, Sivakumar V, Sampath K, Rajendran P (1991): Individual and combined effects of zinc, cadmium and copper on growth of *Oreochromis mossambicus*. Indian J. Fish., 38: 198-200.

Kaneko JJ (1989). Clinical Biochemistry of Domestic Animals. 4th Edn., Academic Press, San Diego.

Philip GH, Reddy PM, Sridevi G (1995). Cypermethrin-induced *in vivo* alterations in the carbohydrate metabolism of freshwater fish, *Labeo rohita*. Ecotoxicol. Environ. Safety, 31(2): 173-178.

Roy SS (2002.) Some toxicological aspects of chloropyrifos to the intertidal fish *Boleopthalmus dussumieri*. Ph.D. Thesis, University of Mumbai, India. pp. 52-71.

Smith CG, Lewis WM, Kaplan HM **(**1952). A comparative morphologic and physiologic study of fish blood. Progressive Fish-Culturist, 14: 169-172.

Svoboda M (2001). Stress in fish – review. Bul. VURH Vodnany, 37: 69 -191.

Valarmathi S, Azariah J (2003). Effect of copper chloride on the enzyme activities of the crab *Sesarma quadratum* (Fabricus) Turk. J. Zool., 27: 253-256.

Wada H, Snell EE (1962). Enzymatic transamination of pyridoxamine. II. Crystalline pyridoxamine-pyruvate transaminase.. J. Biolo. Chem. 237: 133-137.

Wells RMG, Mcintyre RH, Morgan AK, Davie PS (1986). Physiological stress responses in big gamefish after capture: observation on plasma chemistry and blood factors. Comparative Biochem. Physiol., 84A: 565-571.

Zilva JF, Pannall PR, Mayne PD (1992). Liver Disease and Gall Stones In: Clin. Chem. Diagnosis Treat. (Eds) Edward Arnold, London. pp. 287-306.