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# Variations in Lactate Dehydrogenase and Creatine Kinase Activities in the Plasma of the African Catfish: *Clarias gariepinus* (Burchell, 1822) exposed to Sublethal Concentrations of Potassium Permanganate

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## ABSTRACT

*Changes in lactate dehydrogenase and creatine kinase in the plasma of the African catfish Clarias gariepinus (Burchell, 1822) subjected to sublethal concentrations (2.0; 6.0and 10.0 mg/L) of potassium permanganate over a period of 192 hours were studied in a semistatic (renewal) system. Potassium permanganate exposure caused significant ($P < 0.05$) increases in alanine aminotransferase and aspartate aminotransferase. Increased activities of both aminotransferases indicated amplified transamination processes and were used as stress indicators.*

**Keywords:** Potassium permanganate, lactate dehydrogenase, creatine kinase, *Clarias gariepinus*, Nigeria.

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## INTRODUCTION

Enzymes are increasingly used not only as indicators of pathological processes [1 - 4] but they also play an important role in toxicology [5] and as indicators of stress [6]. Lactate dehydrogenase and creatine kinase are primarily used as indicators of stress. Biochemical and physiological indicators such as enzymes could be used (as biomarkers) to identify possible environment contamination before the health of aquatic organisms is seriously affected [7] and to develop water quality indices [8 - 11]. Such a biochemical approach has been advocated to provide an early warning of potentially damaging changes in stressed fish [12].

Changes in plasma enzyme activity are used as indicators of tissue injury, environmental stress, or a diseased condition. The rate of increase of plasma enzyme activity depends on the concentration of an enzyme in cells, the rate of leakage caused by injury, and the rate of clearance of the enzyme from plasma [13].

Valarmathi and Azariah [14] state that the increase or decrease in the levels of plasma enzymes may be sufficient to provide information of diagnostic value. Verma *et al.*,[15] reported on the toxic effect of some toxicants on certain biologically important enzymes.

Nowadays, enzymogram plays an important role in diagnosis and prognosis of animal disease [16]. 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 [17]. Lactate dehydrogenase (LDH) is released to liver, lung, muscle, heart and kidney tissue after cellular damage. Skeletal and cardiac muscle damage results in great increase of plasma creatine phosphokinase (CPK).

This research work is conducted with the objective of investigating the effect of sublethal concentration of potassium permanganate ($KMnO_4$) a commonly used disinfectant and chemotherapeutic agent in aquaculture management of diseases and parasites on lactate dehydrogenase and creatine kinase activities in the plasma of the widely consumed African catfish *Clarias gariepinus* with particular reference to the concentration of the agent and duration of exposure.

## MATERIALS AND METHODS

Apparently healthy live specimens of *Clarias gariepinus* (mean weight, 165.15$\pm$3.45g; mean length 29.42$\pm$6.56cm) 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 1g standard AnalaR grade granules in 1 litre of deionised water to form 100% concentration. From this stock solution, various concentrations used in the investigations were prepared by dilution.

Three concentrations of $KMnO_4$ **(**2mg$L^{-1}$, 6mg$L^{-1}$ and 10mg$L^{-1}$) as well as a control with no $KMnO_4$ were used for the experiment. Each treatment was triplicated. The experimental tanks consisted of large plastic containers of 150L capacity, filled to half their capacities and covered with a lid made of fine polyethylene gauze screen of 1mm mesh size, to prevent the fish from jumping out of the containers. Experimental fish were fed daily with Catfish feed (Dizengoff; 4.5mm; Protein 42%, Fat 13%, Fibre 1.9% and Ash 1.2%) at 3% of their body weights. The fish were not fed 24hours prior to the experimental period, as well as during the experimental period, which lasted 192 hours. 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 [18] procedures. The water quality parameters measured included pH 6.48 $\pm$ 0.32, temperature 28.4 $\pm$ 1.2$^oC$, dissolved oxygen 7.36 $\pm$ 1.12mg$L^{-1}$, free carbon dioxide 4.85 $\pm$ 0.06 mg$L^{-1}$ and total alkalinity 34.6 $\pm$ 1.54 mg$L^{-1}$.

The test was performed using a semi-static renewal method in which the exposure medium was exchanged every sampling time to maintain toxicant strength and level of dissolved oxygen as well as minimizing the level of ammonia excreted 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 $KMnO_4$ (0hr = start) and then at 12, 24, 48, 96 and 192 hours.

Blood from the selected fish was drawn from the caudal vessels with a heparinised disposable plastic syringe and hypodermic needle. The use of plastic syringe is a necessary precaution with fish blood, because contact with glass results in decreased coagulation time [19].

Plasma was obtained by centrifugation and diluted 1:20 with deionised water. The diluted plasma was then stored in a refrigerator at -4$^{o}$C and later analysis were conducted for enzyme activities: plasma lactate dehydrogenase and creatine kinase. All determinations were carried out in duplicates for each sample.

The plasma lactate dehydrogenase was measured by using the optimized kinetic method which is based on the fact that reduced nicotinamide adenine dinucleotide is oxidized to nicotinamide adenine dinucleotide, the resulting decrease in absorbance using a spectrophotometer at 340 nm being directly proportional to the lactate dehydrogenase in the sample [20 - 22].

The plasma creatine kinase was measured using the optimized standard colorimetric method [22] according to the recommendations of the Deutsche Geselischaft für Klinische Chemie (DGKC) using a commercial kit (Randox Laboratories Limited, U.K., using a spectrophotometer at a wavelength of 340 nm

Results obtained for the triplicates from all three experiments were combined, 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

Plasma lactate dehydrogenase levels observed in *C. gariepinus* following exposure to the various concentrations of potassium permanganate over the period of 192 hours is graphically shown in Fig.1.; while the percentage variation with respect to the control values are listed in Table 1. The mean control values of the plasma lactate dehydrogenase ranged from 39.86 U/L to 41.77 U/L. Increased activities of lactate dehydrogenase were recorded in the plasma of the *C. gariepinus* exposed to the three concentrations (2, 6 and 10mg/L) of potassium permanganate over the exposure period of 192 hours. Statistical analysis using ANOVA showed that there was significant difference exposed fish with increase in concentration of the $KMnO_4$ and exposure time. However, multiple comparison of means using Bonferroni test showed that the means of plasma lactate dehydrogenase in the treated fish at the start of the experiment (zero time) did not differ significantly ($P<0.05$) from the increased values in all treatments following 12 hours exposure. At higher exposure periods of 24, 48, 96 and 192 hours, the activities of the plasma lactate dehydrogenase were statistically ($P<0.05$) different from the zero time values. The maximum elevation (14.01) was recorded in the 10mg/L $KMnO_4$ exposed fish at 192 hours. The observed increase was also time-dependent.

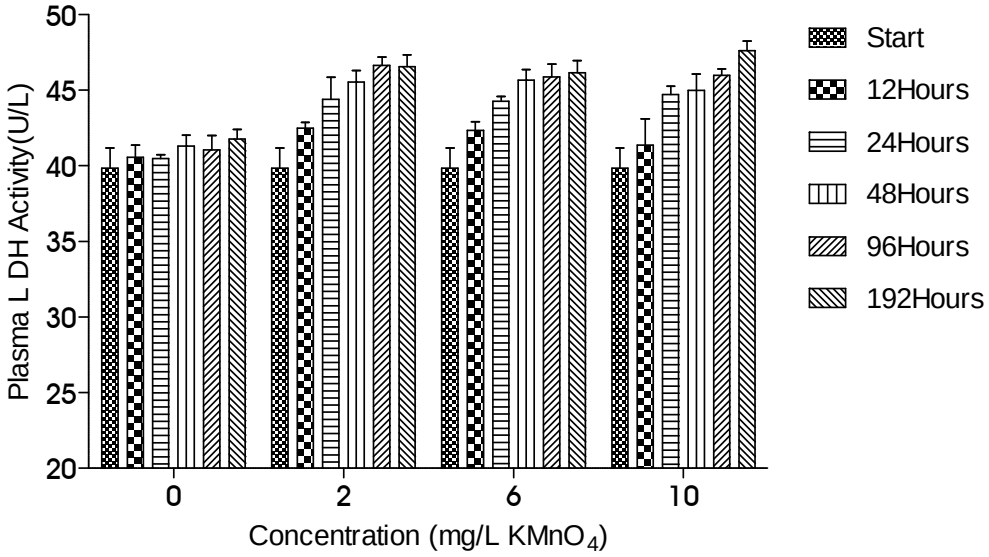


**Fig. 1: Mean values of activities of plasma lactate dehydrogenase in *C. gariepinus* exposed to the various sublethal concentrations of potassium permanganate over a period of 192 hours. Each column represents the mean value and vertical bars indicate the standard error of the mean.**

**Table 1: Percentage variation of activities of plasma lactate dehydrogenase of *C. gariepinus* exposed to the various sublethal concentrations of $KMnO_4$ over a period of 192hours.**

| Concentration ($mg/L\ KMnO_4$) | Exposure Period (Hours) | | | | | |
|---|---|---|---|---|---|---|
| | START | 12 | 24 | 48 | 96 | 192 |
| 2 | 0.00 | 4.71 | 9.63* | 10.23* | 13.62* | 11.47* |
| 6 | 0.00 | 4.36 | 9.34* | 10.53* | 11.81* | 10.49* |
| 10 | 0.00 | 1.95 | 10.40* | 8.86* | 10.82* | 14.01* |

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

The levels of plasma creatinine kinase observed at different concentrations and during different exposure period of the test organism is given in Fig. 2.; while the percentage variation with respect to the control values are presented in Table 2. The activities of the plasma creatinine kinase in the control group varied between 65.87 U/L and 68.56 U/L. There was a steady increase in the mean values of plasma creatinine kinase with increase in concentration and exposure time with the exception of a slight decrease of – 0.66% in the fish exposed to 2mg/L $KMnO_4$ after 24 hours exposure. There was however no significant difference (P>0.05) observed statistically (ANOVA) in the change of plasma creatinine kinase levels with concentration and however an aposteriori comparison using Bonferroni test showed that the means of plasma creatinine kinase levels of the zero time was statistically (P<0.05) different from the values of the fish exposed to 10mg/L $KMnO_4$ at 96 hours and all treatment of $KMnO_4$ at 192 hours. The maximum elevation percentage (18.21) was recorded in the fish exposed to 2mg/L $KMnO_4$ for 192 hours.

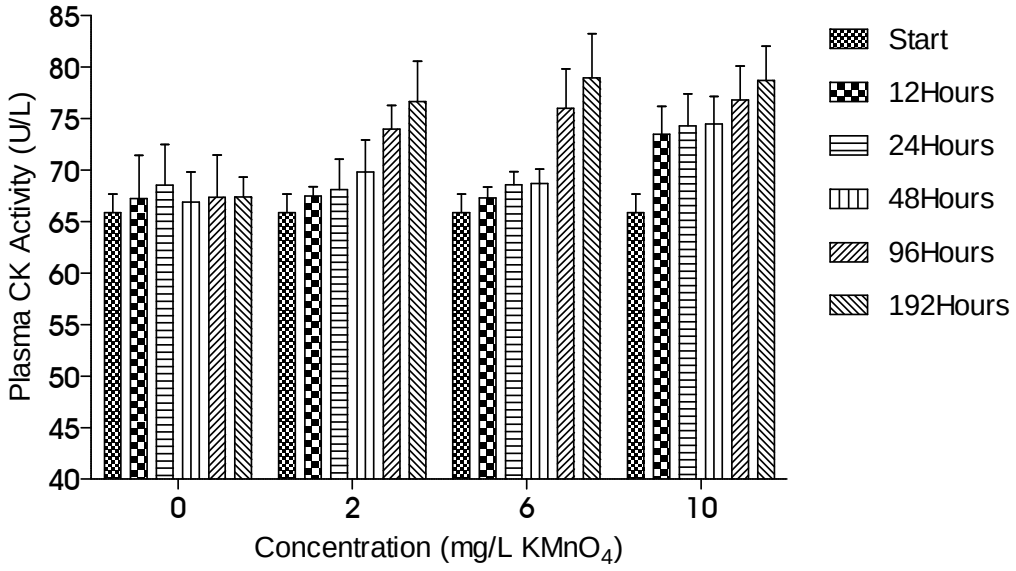


**Fig. 2: Mean values of activities of plasma creatine kinase in *C. gariepinus* exposed to the various sublethal concentrations of potassium permanganate over a period of 192 hours. Symbols as in Fig. 1.**

**Table 2: Percentage variation of activities of plasma creatine kinase of *C. gariepinus* exposed to the various sublethal concentrations of $KMnO_4$ over a period of 192hours.**

| Concentration (mg/L $KMnO_4$) | Exposure Period (Hours) | | | | | |
|---|---|---|---|---|---|---|
| | START | 12 | 24 | 48 | 96 | 192 |
| 2 | 0.00 | 0.37 | -0.66 | 4.38 | 9.80 | 18.21* |
| 6 | 0.00 | 0.09 | 0.01 | 2.75 | 12.87 | 17.19* |
| 10 | 0.00 | 9.27 | 8.36 | 11.35 | 14.06* | 16.83* |

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

## DISCUSSION

Plasma enzymes levels in fish have been proposed to be good indicators of extreme stress and provide information of organ dysfunction [23]. 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 [14]. 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.

The activity of LDH showed a marked elevation in the plasma of *C. gariepinus* exposed to the various concentrations of potassium permanganate over the 192h exposure period. The increase in plasma LDH activity may therefore reflect an increased dependence on anaerobic carbohydrate metabolism of the exposed fish. Thus, sublethal levels of potassium permanganate affect the efficiency of tissue metabolites and cause pathological changes possibly in the gills, kidney and liver as seen in the histopathological findings below. The increase in LDH level indicated metabolic changes i.e. the glycogen catabolism and glucose shift towards the formation of lactate in the stressed fish, primarily in the muscle tissue [24].

Supporting evidence that LDH activity may be enhanced due to sublethal effect of aquatic pollutants (11 in number) in the case of the African sharptooth catfish, *Clarias gariepinus* inhabiting Lake Maryut in Egypt is provided in the work of Adham [25].

Creatine kinase catalyzes the reversible transfer of the phosphoryl group from phosphocreatine to ADP, regenerating ATP. CK participates in an ubiquitous role to meet the energy demand for homeostasis during environmental changes. The phosphocreatine/creatine kinase is present in some excitable tissues, such as *Narcine brasiliensis* electric organ [26], and in nonexcitable tissues, such as *Squalus acanthias* rectal gland [27], *Gillichthys mirabilis* gills [28], and *Oreochromis mossambicus* gills [29], with high and fluctuating energy demand. Plasma CK exhibits the physiological stress responses in big game fish after capture, perhaps because of muscle damage and subsequent release of cytosolic soluble CK in the plasma [23]. Total CK activity significantly declined 20% in the fish (*O. mossambicus*) brain after exposure to hypergravity for 7 days [30].

The serum levels of CK showed significant variation in the presence of environmental stressors (acute handling and transport stress) in channel catfish [31].

The result obtained in the activities of CK in the current study is indicative of the fact that potassium permanganate caused damage to the skeletal musculature causing the release of CK into blood plasma possibly resulting from a disturbed permeability and integrity of the cell membranes.

## REFERENCES

[1] Bell, R.G. *J Fish Res Board Can* **1968,** 26(6), 1247 – 1268.
[2] Racicot, J. C. Gaudet, M. and Leary, C. *Journal of Fish Biology* **1975**, 7, 825 -835.
[3] Shieh, R.G, *Journal of Fish Biology* **1978**, 12, 13 - 18.
[4] Rehulka, *Czech J Anim Sci* **2002**, 47, 30 - 44.
[5] Luskova, V., Svoboda, M., and Kolarova J. *Acta. Vet Brno* **2002**, 71,17 - 123.
[6] Jiminez, B. D. and Stegeman, J, J. *Am. Fish Soc. Symp.* **1990** 8,69 - 79.
[7] Barnhoorn, I. E. J. and Van Vuren, J. H. J. *Ecotox, Environ, Safe* **2004**, 59,180 - 185
[8] Powers, D. A. *Science* **1989**, 246, 3352 - 3358.
[9]Gayet, J. C., Haouz, A. and Burstein, C. *Biosens. Bioelectron* **1993**, 8, 177 - 183.
[10] Melancon, M. J., Bioindicator used in aquatic and terrestrial monitoring. Lewis, Boca Raton, FL**. 1995.**
[11] Pickering. A. and Pottinger, T. Biochemical effects of stress. Elsevier Science, New York. **1995.**
[12] Casillas, E. Myers, M. and Ames, W. E. *Aquat toxicol* **1983,** 3, 61 - 78.
[13] Boyd, J. W. *Veterinary Clinical Pathology.* **1983,** 12, 9 – 24.
[14] Valarmathi, S. and Azariah, J. *Turkish Journal of Zoology* **2003,** 27, 253 – 256.
[15] Verma, S. G., Rani, S. and Dalela, R. C. (1981), *Toxicology Letters* **1981**, 9**,** 327 - 337.
[16] Cole, E. H. *Veterinary Clinical Pathology* (4th Ed); W. B. Saunders Co. Philadelphia, **1989.**
[17] Kaneko, J. J. Clinical Biochemistry of Domestic Animals. 4th Edn., Academic Press, San Diego. **1989.**
[18] APHA, Standard methods for examination of water and wastewater. 20th Edn. American Public Health Association Washington D C. **1998**, 1976p
[19] Smith G. G., Lewis W. M. and Kaplan H. M, *The Progressive Fish-Culturist* **1952**, 14: 168 - 197.

[20] Wacker, W. E. C., Ulmer, D. D. and Valu, D. D. *New England Journal of Medicine* **1956**, 225, 449 – 454.
[21] Cobaud, P. A. and Wroblewski, F. *American Journal of Pathology*. **1958,** 10: 234 - 236.
[22] Tietz, N. W. *Clinical Guide to Laboratory Tests*. 3ed. W. B. Saunders Co, Philadelphia. **1995.**
[23] Wells, R. M. G., Mcintyre, R. H., Morgan, A. K. and Davie, P. S. *Comparative Biochemistry and Physiology* **1986**, 84A, 565 – 571.
[24] Simon, L. M., Nemcsok, J. and Boross, L. *Comparative Biochemistry and Physiology* **1983**, 75C, 167 – 169.
[25] Adham, K. G. *Journal of Applied Ichthyology* **2002**, 18(2): 87 – 94.
[26] Blum, H., Balschi, J. A., and Johnson, R. G. *Journal Biological Chemistry* **1991,** 266, 10254-10259.
[27] Friedman, D. L. and Robert, R. *Journal of Biological Chemistry* **1992**, 267, 4270 - 4276.
[28] Kultz, D. and Somero, G. N. *American Journal of Physiology Regulatory Integrative Comparative Physiology* **1995**, 268: R1003-R1012.
[29] Weng, C. F., Chiang, C. C., Gong, H. Y., Chen Mark, H. C., Lin Cliff, J.F., Cheng, C. Y., Hwang, P. P. and Wu, J. L. *Physiological and Biochemical Zoology* **2002**, 75, 29-36.
[30] Slenzka, K., Appel, R. and Rahmann, H. *Neurochemistry International* **1993**, 22, 405 - 411.
[31] Ellsaesser, C. F. and Clem, L. W. *Comparative Biochemistry and Physiology* **1987,** A 88, 589 - 594.