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Research Article

Arsenic Accumulation in *Clarias gariepinus* and Effects on Catalase Activity

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Abstract

Heavy metals are common environmental contaminants probably due to increase in discharge of pesticides, sewage and untreated industrial effluents. One of such heavy metals is arsenic which is found in seawater, warm springs, groundwater, rivers, and lakes. In aquatic environments, arsenic occurs as a mixture of arsenate and arsenate, with arsenate usually predominating. The unrestricted application of arsenic pesticides, industrial activities, and mining operations has led to global occurrence of soluble arsenic above permissible level of 0.01mg/L. Continuous exposure of freshwater organisms including fish to low concentrations of arsenic results in bioaccumulation. The present study was designed to investigate the effect of arsenic accumulation on the catalase activity in the muscle tissues of African catfish *Clarias gariepinus* fingerlings. The fishes were exposed to 1 mgL^{-1}, 2 mgL^{-1}, 3 mgL^{-1} and 4 $mgL^{-1}$of sub - lethal doses of arsenic (As_2O_3). Two fishes were randomly selected and sampled after seven days of exposure and the muscle (flesh) extracted for determination of arsenic accumulation and assay of catalase activity. The result of the catalase activity was 86.9 u mol/min, 13.3 u mol/min, 14.2 u mol/min and 15.1u mol/min for 1, 2, 3, 4 mgL-1 respectively in all the fishes sampled. This indicates a concentration dependent significant increase ($p < 0.05$) in the values of catalase activity in all the fishes sampled when compared to the control. The results suggest that in the muscle tissue of *Clarias gariepinus* arsenic accumulation is quantity dependent, and that activity except for the sample exposed to 1mg/L which recorded the highest value in catalase activity 86.9 u mol/min is accumulation/concentration dependent. Thus even at sub-lethal concentration arsenic can induce oxidative stress resulting in alterations in catalase activity in the muscle of the fishes.

Keywords: Arsenic; Heavy Metals; Catalase; Catfish; Oxidative Stress; Reactive Oxygen Species (ROS)

Introduction

Metabolic activity of animals is one of the most important factors that play a significant role in heavy metal accumulation (Jorgenson., *et al.* 2010). Recently, the rise of advance technology has brought people to the exploration and discovery of many other purposes of heavy metals, not only for civilization, but also for their own benefits (Zaki., *et al.* 2015). In the industrialized world that we live in today, heavy metals are significant environmental pollutants and their toxicity is a problem of increasing significance for ecological, evolutionary, nutritional and environmental reasons (Nagajyoti., *et al.*, 2010; Jaishankar., *et al.* 2013). This is due to the outstanding physiological and chemical properties of heavy metals, which has widened their range of usage in industrial processes; in fertilizer and biocides production. Contamination of aquatic system by heavy metals has for long been a source of concern, since metals accumulate in aquatic organisms such as fish and transform into persistent metallic compounds (Taweel., *et al.* 2011; Sercikova., *et al.* 2013). The accumulated toxic metal components can have adverse effects on aquatic life, which further disrupt the biological food web and eventually threaten mankind as the ultimately consumer (Mashifane and Moyo, 2014; Yuswir., *et al.* 2015). Aquatic ecosystem is the natural habitat of fish and water pollution directly affects the underwater fish, and human beings are indirectly at high risk by consuming these fishes (Mendil., *et al*. 2010; Monferran., *et al*. 2016). The study of bio - accumulation of heavy metal in the living tissues of aquatic animals is a significant method to monitor the pollution level of water bodies and at the same time can prove to be a helpful method in studying the biological role of heavy metals present at an increased level in fish and other aquatic organisms [1].

Materials and Methods

Fish samples used were procured from the Applied Biology and Biotechnology, Enugu State University of Science and Technology (ESUT) fish farm on 26th June, 2018. Deionized water was procured from the University of Nigeria Teaching Hospital Enugu (UNTH), on 26th June, 2018. The collected fish samples were allowed to acclimatize under laboratory conditions for seven (7) days prior to

experimentations. The fingerlings were subjected to graded doses of the heavy metals to determine safe doses for exposure. After determining the safe dose, the fingerlings were exposed to sub-lethal concentrations ranging from 1 - 4 $mgkg^{-1}$. Two fish samples were collected from each concentration for heavy metal determination at and catalase assay at the end of seven (7) day period.

Statistical analysis

Data obtained in this study were analyzed as Mean ± Standard Deviation (M ± SD). The data obtained were analyzed using one way analysis of variance (ANOVA) using statistical package and service solution (SPSS) and where significant ($P < 0.05$) difference were obtained, Scheme multiple comparison were used to detect the source of difference.

Results

Concentration Introduced (mg/kg)	Accumulation (mg/kg) M±SD
1	0.000 ± 0.000
2	0.018 ± 0.002
3	0.021 ± 0.001
4	0.031 ± 0.000
Control	0.000 ± 0.000

Table 1: Concentration of arsenic accumulated in the muscle after 7 days of exposure.

* The result is significant at $p<0.05$.

Concentration (mg/kg)	Catalase Activity (µmol/min)
1	86.900
2	13.300
3	14.200
4	15.100

Table 2: Concentration of arsenic introduced and the corresponding catalase activity.

Accumulation(mg/kg)	Activity(µmol/min)
0.000 ± 0.000	86.900
0.018 ± 0.002	13.300
0.021 ± 0.001	14.200
0.031 ± 0.000	15.100
0.000 ± 0.000	0.000

Table 3: Accumulation of arsenic in the fish tissues and the corresponding catalase activity.

* Result significant at $p<0.05$.

Discussion

This research work was done to determine the effect of arsenic accumulation on the catalase activity in the muscle tissues of *Clarias gariepinus* and the results are as indicated in Tables 1-3. Fish liver, kidney and muscle tissues are highly endowed with antioxidant enzymes including catalase (CAT), glutathione peroxidase (GPX), superoxide dismutase (SOD), glutathione S - transferase (GST) and glutathione reductase (GR) to protect them from oxidative stress. Variation in *Clarias gariepinus* muscle catalase (CAT) activity was recorded in this study. After seven (7) days of experimentation, it was observed that the control and 1 $mgkg^{-1}$ arsenic exposed fishes had undetected level of arsenic. However the concentration accumulated by the other fishes was in respect to quantity introduced, as the fishes exposed to 2 $mgkg^{-1}$, 3 $mgkg^{-1}$, and 4 $mgkg^{-1}$ of arsenic all accumulated significant amount of arsenic 0.018, 0.021, 0.031mg/kg respectively. But the catalase activity did not follow this trend. As higher catalase (CAT) activity 86.9 µ mol/min was recorded in the fishes exposed to 1 $mgkg^{-1}$ arsenic, while the rest of the fishes exposed to 2, 3, and 4 mg/kg all record a gradual increase in catalase activity, compared to control fish group which showed no accumulation and consequently no activity was recorded. The elevated catalase (CAT) activity in 1 $mgkg^{-1}$ arsenic exposed fishes could be due to increase in production of reactive oxygen species (ROS) which consequently led to increased catalase activity in an attempt to eliminate or cushion this effect. This is in agreement with the findings made by Avci., *et al*. [2] in muscle and hepatic tissues of *Silurus glanis*, Atli and Canlis [3] in gills, liver and muscle tissues of *Oreochromis niloticus*. The lack of activity in control fish sample could be attributed to fact that in all the organs of fish the muscle has the lowest accumulation and activity when tests are conducted, as is emphatically published in the research by Tyokumbur., *et al*. [4]. The research by Nwani., *et al*. (2014) on *Clarias gariepinus* further suggests the same. There was a sharp decrease in muscle catalase (CAT) activity 13.3 µ mol/min in fishes treated with 2 $mgkg^{-1}$ of arsenic. This sharp decrease in activity might be associated with the direct binding of A_2O_3 to the catalase (CAT) thiol (-SH) group as quantity was increased, which rendered active catalase (CAT) less active. This is similar to the findings by Palace., *et al*. (1992) who exposed rainbow trout to cadmium and reported lower CAT activity in the hepatic tissues and concluded that reduction in catalase (CAT) level is due to direct binding of metals that alter its structure. There was a gradual increase in activity 14.2 µmol/min and 15.1µmol/min in fishes treated with 3mg/kg and 4 $mgkg^{-1}$ arsenic respectively. This increase in activity from 13.3 µmol/min, 14.2 µmol/min and 15.1µmol/min can be attributed to gradual adaptability to the toxicity posed by arsenic as oxidative stress abounded. This is in agreement with the findings made by Firat and Kargin [5], suggesting that as a result of oxidative stress, fish adapted to either increase or decrease in antioxidants level. This is also true to the report made by Sunaini., *et al.* (2016) reporting that catalase activity was decreased after exposure to sub - lethal dose of arsenic. Com-

paring all the results to the control, it is safe to say that there was an increase in catalase activity across all the fish samples assayed; which is in contrast with the research conducted by Sunaini., *et al.* (2016) who recorded lower catalase activity in muscle of fish after exposure to sub - lethal doses of arsenic. Their findings was largely due to the fact that the fish they used in their researches was from rivers which had a good chance of getting contaminated by arsenic, which explains why they recorded a significant accumulation, and consequently record catalase activity in the control. With this in mind, it vital to know that variation in responses of the antioxidant enzymes to metal exposures, depends upon body tissues, metals and exposure types (lethal or sub - lethal) (Pandey., *et al*. 2011). Toxicity of compounds to organisms has however been known to be dependent on concentration, sex, developmental stages, and exposure periods (Pandey., *et al*. 2011).This study suggest that catalase which is antioxidant in function is highly sensitive to metal pollution as its activities change significantly, suggesting it could be helpful in predicting sub - lethal metal toxicity and useful as an early warning tool in biomonitoring studies [6-25].

Conclusions

The present findings suggest that in the muscle tissue of *Clarias gariepinus*, arsenic activity is dependent on quantity or concentration introduced, as well as concentration accumulated; as activity increased with increased accumulation. This suggests that even at sub-lethal concentration arsenic can induce oxidative stress resulting in alterations in catalase activity in the muscle of the fishes. On the basis of results obtained in this study and previous studies, it can be concluded that antioxidant enzymes like catalase (CAT) and others are helpful in preventing the harmful effects of heavy metals. Moreover, they are cautionary indicators for severe damage to organisms living in aquatic environment. The consequence of this research work further reveals that catalase (CAT) is a susceptible bio-indicator of an organisms' antioxidant defense system. However, it is still essential to study further antioxidant enzymes in different aquatic animal models for better understanding.

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