ACTA SCIENTIFIC MICROBIOLOGY (ISSN: 2581-3226)

Volume 2 Issue 10 October 2019

Research Article

Induction of Catalase Response in *Clarias* Exposed to Graded Concentration of Iron as Fish's Body Defence Mechanism Against Environmental Pollutants

Ujah II[1]*, Nsude CA[1], Okpako IO[2] and Ugwu CM[1]

[1]*Department of Applied Biochemistry, Enugu State University of Science and Technology Enugu State, Nigeria*

[2]*Delta State University Abraka, Delta State, Nigeria*

***Corresponding Author:** Ujah II, Department of Applied Biochemistry,Enugu State University of Science and Technology Enugu State, Nigeria.

Received: August 20, 2019; **Published:** September 10, 2019

DOI: 10.31080/ASMI.2019.02.0371

Abstract

Heavy metals and their salts are considered as very important group of environmental pollutant. In this study, induction of catalase response in *Clarias* exposed to four different concentration iron ((0.01 mg, 0.02 mg and 0.03 mg and 0.04 mg/Kg) was investigated over a seven day period. At the end of the exposure period, two fishes were randomly selected from the different concentrations to assess for heavy metal accumulation in the muscles of *Clarias gariepinus* and the assay of the corresponding catalase activity. The catalase of activity of the *Clarias* in the control when compared with those exposed to graded concentrations of iron shows an induction of catalase upon exposure of the *Clarias* to the graded concentrations of iron which could confer a measure of protection against iron accumulation.

Keywords: Heavy Metals; Catalase; Iron; Catfish; Toxicity

Introduction

Fish are particularly vulnerable and heavily exposed to pollution due to their habitat and feeding habits which exposes them to the detrimental effects of water pollutants [1]. Fish, in comparison with invertebrates, are more sensitive to many toxicants and are a convenient test subject for indication of ecosystem health [2]. Heavy metals are released from a variety of natural and human sources [3]. In aquatic environments, heavy metal pollution may be due to direct atmospheric deposition, geologic weathering, through the discharge of agricultural, municipal, residential or industrial waste products and from wastewater treatment plants [4]. The contamination of water and sediments at high concentrations of these metals is a serious threat because of their toxicity, long persistence, bioaccumulation and bio magnification in the food chain [5]. Fishes are considered to be most significant bio monitors in aquatic systems for the estimation of metal pollution level [6], possessing several specific advantages in describing the natural characteristics of aquatic systems and in assessing changes to habitats [7]. Bioaccumulation of heavy metals in fish could occur from ingestion of contaminated food via the alimentary tract or as well as absorption through the gills and skin [8]. Distribution to other organs is through the blood stream and subsequent accumulation takes place after absorption [9]. Trace metallic elements such as copper, zinc, chromium, nickel, cobalt, molybdenum Mo and iron have a known important biological roles [10], especially in their low concentrations and toxicity occurs either at metabolic deficiencies or at high concentrations, while others such as lead and mercury are toxic even in low concentrations. The toxicity of these metals could result in typical biological oxidative processes which could be dealt with through the body's antioxidant defense mechanism; one of such antioxidant is catalase. Catalase catalyzes the decomposition of hydrogen to water and oxygen and It is an important enzyme which protects the cell from oxidative damage [11]. An examination of the catalase response elicited in fingerlings of fish upon exposure to iron will help to know the possible damages that iron could cause in the aquatic contaminated organisms and the protections offered by catalase.

Materials and Methods

Materials

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, while the deionized water was procured from the University of Nigeria Teaching Hospital Enugu (UNTH), on 26th June, 2018.

Methodology

Exposure of the fish to concentration of heavy metals

Fingerlings of *Clarias gariepinus* of similar age (4 weeks old) obtained were allowed to acclimatize to laboratory conditions for a period of seven days before they were used for bioassay. During the acclimatization, the fingerlings were fed with 0.5 mm fish feed. Water in the plastic tanks was changed once every two days to prevent accumulation of wastes and decaying food particles. Feeding was discontinued 12 hours prior to commencement of bio - assay.

After acclimatization active catfishes, *Clarias gariepinus* (fingerlings) were randomly assigned to bioassay containers already holding treated or untreated test media. For the series of bioassay, five *Clarias gariepinus* were exposed per treatment as well as the untreated control. In the procedure adopted, the test media was changed into a fresh solution at exactly the same concentration every three days. At the end of the seven days experimental period, two live juveniles were randomly selected from the treated and the untreated media and dissected. The muscle was carefully extracted for heavy metal determination and enzyme assay.

Heavy metal determination

The total fish were dried at 70°C for 24 hours. The dried samples were then ground in homogenizer. The crucibles and the caps were washed in 10% HNO_3 and ached at 750°C for 2 hours in a muffle furnace. The crucibles were then labeled with graphite pencil and 0.5 g of the dried samples weighed into the crucibles. The ash was then scraped into vials and the crucibles rinsed with 10ml acid with the aid of a repippetor. The vials were then capped and shaken thoroughly. The extract was diluted to 1 L with distilled water and the diluted extract was measured by atomic absorption spectrophotometer spectra AA model number 220FS.

Determination of catalase activity

This was determined following the method of Luck (1974). The absorption of hydrogen peroxide at 240 nm decreases with upon the degrading of catalase. From the decrease in absorbance the catalase activity can be calculated. A volume, 3.0 ml of H_2O_2 - phosphate buffer was taken in an experimental cuvette, followed by rapid addition of 40 µL of the enzyme extract, the time required for the decrease in absorbance by 0.05 units was recorded at 240 nM. The enzyme activity was calculated using the equation:

$$Activity = \frac{\text{Change in abs x total reaction volume}}{sample\ volume\ x\ extinction\ coefficient}$$

Total reaction volume = 3.0 mL, sample volume 0.1 mL, extinction coefficient = 40 $m^{-1}cm^{-1}$

Results

Concentration Introduced(mg/Kg)	Concentration Accumulated(mg/Kg) (M±SD)
0.010	0.923 ± 0.004
0.020	0.756 ± 0.013
0.030	0.549 ± 0.055
0.040	0.101 ± 0.001
Control	0.011 ± 0.001

Table 1: Concentration of iron accumulated in the fish after 7days of exposure.

Concentration (mg/Kg)	Catalase Activity (µmol/min)
0.010	13.100
0.020	15.900
0.040	16.300
Control	11.900

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

Accumulation(mg/Kg)	Activity(umol/min)
0.923 ± 0.004	13.100
0.756 ± 0.013	15.900
0.549 ± 0.054	16.300
0.101 ± 0.001	11.900

Table 3: Accumulation of iron in the fish muscle and the corresponding catalase activity.

Discussion

Industrial and mining effluents produce large quantities of iron that are often discharged into the aquatic environment. According to Singh., *et al*. (2011) Iron II (Fe^{2+}) is more toxic than the ferric (Fe^{3+}). As an essential element iron (Fe) is known for its presence in proteins such as hemoglobin, cytochrome and several redox enzymes (Galvin, 1996). Human's ingestion of high amounts of iron (Fe) cause irreversible disruptions in several tissues (Galvin, 1996). It can also be toxic to fishes when it alters the routine oxygen consumption (Grobler., *et al.* 1989). In this research the muscle tissue of *Clarias gariepinus* was dissected and assayed for iron accumulation and catalase activity after exposing the fish to sub-lethal dose of ferrous iron for a period of seven (7) days. The result of the assay showed that there was an increase in accumulation in all the fishes assayed. The fishes exposed to 4 mg/Kg had the lowest accumulation 1mg/kg. This is significant when compared to the control

which accumulated 1mg/kg. The other fishes exposed to 1, 2, and 3 mg of all accumulated significant amount of the iron 0.923, 0.756 and 0.550 mg/Kg respectively. The control accumulated 0.011 mg/Kg which may come from the feed used in the study. From the results, the accumulation of iron in the muscle of Clarias gariepinus is concentration dependent, except the fish exposed to 4 mg/Kg. This lower accumulation could be due to depuration upon accumulation of higher doses of the metal. The result of catalase activity suggests an increase in catalase activity across all the fishes assayed compared to the control. In the control, catalase activity recorded was 11.93 u mol/min. The fishes exposed to 1mg of iron recorded the lowest catalase activity 13.1 u mol/min. This could be attributed to the fact that iron being an essential element needed to be in a higher quantity to induce toxicity (Etero – muras., *et al.* 2010). The fishes exposed to 2 and 4 mg also recorded increasing catalase activity 15.9 and 16.3 u mol/min respectively. Even though an increase in catalase activity was recorded in this research, it is important to note that metal accumulation in fish and the corresponding catalase activity is affected by several factors such as specificity to chemical environmental conditions, exposure route and the species of fish (Etero – muras., et al. 2010). Thus from the study, there was induction of catalase response to the graded concentrations of iron salt. This is a cell's defense mechanism which could confer protection against these stressors; however in higher concentration the antioxidant role of catalase could be overwhelmed leading to a possible oxidative stress [12-18].

Conclusion

In view of these results, accumulation of iron in *Clarias gariepinus* muscle is dependent on the concentration exposed. Comparing the accumulation to the catalase activity as seen in this study: 0.923 mg/Kg -13 u mol/min, 0.756 mg/Kg - 15.9 u mol/min, 0.549 mg/Kg -16.3 u mol/min, 0.101 mg/Kg -11.9 u mol/min, there was an increase in catalase activity at moderate concentrations of iron but the catalase activity decreased at higher concentrations. This suggests that at certain concentration, the catalase activity could be overwhelmed. There is the need therefore to establish this threshold and constantly do monitoring of our aquatic ecosystem to ascertain the level of aquatic degradation and enforcing some policies to protect the aquatic environment from these degradations.

Bibliography

1. Yarsan E and Yipel M. "The important terms of marine pollution, biomarkers, biomonitoring, bioaccumulation, bioconcentration and biomagnifications". *Journal of Molecular Biomarkers and Diagnosis* (2013): 1-4.

2. Authman MMN., *et al*. "Heavy metals pollution and their effects on gills and liver of the Nile Catfish Clariasgariepinus inhabiting El-Rahawy Drain, Egypt". *Article in Global Veterinaria* 10 (2013): 103-115.

3. Bauvais C., *et al*. "Sponging up metals: Bacteria associated with the marine sponge Spongia officinalis". *Marine Environmental Research* 104 (2015): 20-30.

4. Garcia JC., *et al*. "Ecotoxicological effects of carbofuran and oxidized multiwalled carbon nanotubes on the freshwater fish Nile tilapia: Nanotubes enhance pesticide". *Ecotoxicology and Environmental Safety* 111 (2015): 131-137.

5. Has-Schön E., *et al*. "Heavy metal profile in five fish species included in human diet, domiciled in the end flow of River Neretva". *Archives of Environmental Contamination and Toxicology* 50.4 (2006): 545-551.

6. Rashed MN. "Monitoring of environmental heavy metals in fish from Nasser Lake". *Environment International* 27.1 (2001): 27-33.

7. Lamas S., *et al*. "Testing the use of juvenile Salmotrutta L. as biomonitors of heavy metal pollution in freshwater". *Chemosphere* 67.2 (2007): 221-228.

8. Sfakianakis DG., *et al*. "Effect of heavy metals on fish larvae deformities". *Environmental Research* 137 (2015): 246-255.

9. Fazio F., *et al*. "Bioaccumulation of heavy metals in blood and tissue of striped mullet in two Italian Lakes, Italy". *Journal of Aquatic Animal Health* 26.4 (2014): 278-284.

10. Abadi DRV., *et al*. "Comparative investigation of heavy metal, trace, and macro element contents in commercially valuable fish species harvested off from the Persian Gulf". *Environmental Science and Pollution Research* 5 (2014): 45-49.

11. Chelikani P., *et al*. "Diversity of structures and properties among catalases". *Cellular and Molecular Life Sciences* 61.2 (2004): 192-208.

12. Frazer DM and Anderson GJ. "Iron imports. I. Intestinal iron absorption and its regulation". *American Journal of Physiology, Gastrointestinal and Liver Physiology* 289.4 (2005): 631-651.

13. Mahboob S., *et al*. "Tissue metal distribution and risk assessment for important fish species from Saudi Arabia". *Bulletin of Environmental Contamination and Toxicology* 92.1 (2014): 61-66.

14. Nadadur SS., *et al*. "Iron transport and homeostasis mechanisms: Their role in health and disease, Indian". *The Indian Journal of Medical Research* 128.4 (2008): 533-544.

15. Omar WA., *et al*. "Integrating multiple fish biomarkers and risk assessment as indicators of metal pollution along the Red Sea coast of Hodeida, Yemen". *Ecotoxicology and Environmental Safety* 110 (2014): 221-231.

16. Polat F., *et al*. "The effects of point pollutants- originated heavy metals (lead, copper, iron, and cadmium) on fish living in Yeşilırmak River, Turkey". *Toxicology and Industrial Health* 32.8 (2015): 1438-1449.

17. Theil EC., *et al*. "Absorption of iron from ferritin is independent of heme iron and ferrous salts in women and rat intestinal segments". *The Journal of Nutrition* 142.3 (2012): 478-483.

18. Wang J and Pantopoulos K. "Regulation of cellular iron metabolism". *The Biochemical Journal* 434.3 (2011): 365-381.

Volume 2 Issue 10 October 2019