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EFFECTS OF LEAD NITRATE ON GLUTATHIONE PRODUCTION LEVELS IN *Clarias gariepinus* (BURCHELL, 1822) POST JUVENILES

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ABSTRACT

The effects of lead nitrate on glutathione (GSH) production levels in *Clarias gariepinus* post juveniles were investigated. The 96 hours $LC_{50}$of the toxicant was determined. Six samples of the fish were exposed to sub-lethal concentrations of 0 mg/L, 28 mg/L, 43 mg/L and 57 mg/L with replicate in each case for a period of 28 days. Two samples of the fish were randomly selected from the treatments and control for the bioassay of GSH production levels in the kidney, liver and gill of the fish on the 14th and 28th day respectively. The results showed that 96 hours LC_{50} for *C. gariepinus* exposed to lethal concentrations of lead nitrate was 284.189 mg/L. The GSH production levels were significantly lower ($P \leq 0.05$) in the kidney of the fish exposed to treatments 28 mg/L and 43 mg/L and in the liver of the fish exposed in all the treatments on both days 14 and 28, respectively. The GSH production levels were also significantly lower in the gill of the fish exposed in all the treatments. The liver of *C. gariepinus* exhibited a better control of the toxicant and therefore, a better biomarker of oxidative stress due to lead nitrate.

Key Words: Glutathione, lead nitrate, 96 hours $LC_{50,}$ oxidative stress, *Clarias gariepinus*

INTRODUCTION

Environmental contamination and exposure to heavy metals such as lead is a serious problem throughout the world considering the fact that it causes a wide range of physiological and neurological problems in both the plant and animals (Dahiya *et al*., 2005). Fishes occupy top of the food chain and are one of the most susceptible aquatic organisms to toxic substances present in water and can therefore, accumulate large amount of toxicants (Alibabic *et al*., 2007; Yilmaz *et al*., 2007). Consumption of these toxicants especially lead by fish could be very deleterious to man, the ultimate consumer. As such, lead consumption has been associated with IQ decline, learning problems, slow growth, hyper activity and impaired hearing (Dahiya *et al*., 2005). Human exposure to heavy metals has risen dramatically in the last 50 years as a result of an exponential increase in the use of heavy metals in industrial processes and products (Ano *et al*., 2007).

Antioxidants are nutrients in food that protect cells from damage from free radicals. Free radicals are unstable molecules that are capable of damaging cells. This cell damage may increase the risk of cancer, heart disease, cataracts, diabetes, or infections. Glutathione (GSH) which is a non-enzymatic antioxidant is the body's essential health AID- (Antioxidant, Immune Booster and Detoxifier) (Gutman and Schettini, 2000). In fact, our lives depend on glutathione, without it, cells would disintegrate from unrestrained oxidation, the body would have little resistance to bacteria, viruses and cancer, and the liver would shrivel up from the eventual accumulation of toxins (Gutman and

Schettini, 2000). When levels of glutathione are sufficient or elevated, the body is better able to prevent illness, disease, and many of the degenerative processes of aging (Gutman and Schettini, 2000).

C. gariepinus or African sharp tooth catfish is a species of the family Claridae, the air breathing catfishes. *Clarias* is a widely distributed fish in Asia and Africa. In these areas, the fish is extremely popular on account of its tasty flesh, unparalleled hardness, rapid growth and somewhat acceptable market price (FAO, 2003). In Nigeria, *Clarias* is an indigenous fish occurring in freshwater throughout the country. It is suspected that apart from tilapia, *Clarias* is the most abundantly cultivated fish species in Nigeria (FAO, 2003). The common species found are *C. gariepinus*, *C. angullaris* and *C. buthupogon*.

Lead is known to cause oxidative damage in several tissues by bringing about an imbalance in generation and removal of reactive oxygen species (ROS) (Nagaraja *et al., 2*011). The main sources of heavy metal pollution in fresh water ecosystem are the agriculture, industry and mining activities (Kumar *et al*., 2007); which are usually brought in through run offs and seepages. A disturbance in the balance between the pro-oxidants and antioxidants leading to detrimental biochemical and physiological effects is known as oxidative stress. Oxidative stress is the result of an imbalance between the production of reactive oxygen species (ROS) and antioxidant defenses in living organisms (Nishida, 2011). This is a harmful condition in which increase in free radical production, and/or decreases in antioxidant levels can lead to potential damage of lipids, proteins and DNA (Padmini *et al*., 2004). Oxidative and nitrative stress results from increased production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) mediated by pollutants (Scoullos *et al*., 2007). Research addressing the usage of antioxidants (especially glutathione) as natural endowment of fish in dealing with xenobiotics in their environment, with special reference to lead is scarce and needs urgent attention especially in this part of the world. The presence of heavy metals ($PbNO_3$) in aquatic environment is one of the major concerns of the environmental agencies in most parts of the world (Tay *et al*., 2009*).* The roles played by antioxidants such as GSH is not well espoused. This paucity of knowledge has led to the neglect of the crucial roles played by glutathione in counteracting the effects of oxidative stress generated as result of the presence of heavy metals and other toxicants in their environment. Fish accumulates toxic chemicals (such as lead) directly from water and diet (Tchonwou *et al.,* 2014). This research was aimed at determining the physiological effects taking place within the *Clarias gariepinus* post juveniles due to the varying concentrations of the Lead Nitrate.

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MATERIALS AND METHODS

Sample collection/acclimatization

A total 250 post juveniles of *C. gariepinus* were purchased from a commercial fish farm, and transported in large plastic container filled with water to the laboratory. The fish were then placed in plastic fish tanks (Aquaria) for acclimatization. The fish were fed twice daily (morning and afternoon) with vital feed (2 mm) for 2 weeks.

Chronic exposure of *C. gariepinus* to sub-lethal concentrations of lead nitrate

The LC_{50} value that was obtained from the 96 hours lethal concentration experiment was used to derive the sub-lethal concentrations. The treatments were 0.00 mg/L, 28.40 mg/L, 43.00 mg/L and 57.00 mg/L concentrations of Lead Nitrate. Each concentration had a replicate tank. The experiment ran for 28 days. The water in the tanks were replaced after every 72 hours and Lead Nitrate was freshly dissolved in the water each time it was changed asides from the control set-up which was kept Lead Nitrate free (Non-static renewal method) (OECD, 1993). Feeding process was carried out as usual.

Sample harvesting and preparation

On the 14th day 2 fish samples were selected at random from each tank for organ extraction. In each case the extracted organs (gills, liver and kidney) were homogenized in Sodium Phosphate buffer (0.2M, P_H 8.0) using ceramic mortar and pestle. The buffer solution was prepared from mixture of sodium di-hydrogen orthophosphate with 0.1M and disodium hydrogen orthophosphates with 0.1M. The pH of the solution was adjusted to 8.0.

GSH Bioassay

The following reagents were used for the analysis: 0.2 M phosphate buffer (8.40g of NaH_2PO_4 and 9.94 g of Na_2HPO_4 was dissolved in distilled water and made up to 1000 ml mark in a volumetric flask. The buffer was adjusted to pH 8.0); 10% Trichloroacetic acid (10g of TCA was dissolved in distilled water and made up to 100 ml in the volumetric flask); and Ellman's reagent (19.8 mg of 5,5'-dithiobis nitro benzoic acid (DTNB) in 100 ml of 0.1% sodium nitrate). To 150 µL of the tissue homogenate (in phosphate-saline pH 7.4), 1.5 ml of 10% TCA was added, and centrifuge at 1500 g for 5 min. 1.0 ml of the supernatant was treated with 0.5 ml of Ellman's reagent and 3.0 ml of phosphate buffer (2.0 m pH 8.0). The absorbance was read at 412 nm. Estimation of reduced glutathione was determined by the method of Ellman (1959) as described by Rajagopalan *et al*. (2004). The amount of glutathione was calculated using a GSH standard curve and expressed as microngrams of GSH formed/mg protein in each case.

RESULTS

On the 14th day the GSH production levels in the kidney of the fish exposed in treatment C3 were significantly lower than the control. However, the production levels were significantly higher in C1 and C2. On the 28th day the GSH production levels were significantly higher than the control, while there was no significant difference in GSH production levels between treatment C1 and C3. Treatment C2 had the lowest GSH production levels (Table 1). On the 14th day GSH production levels in the gills of the fish exposed to treatment C1 and C3 were significantly higher than the control, while GSH production level was significantly lower than control in treatment C2. On the 28th day on the other hand, only treatment C2 and C3 was observed to have GSH production levels significantly higher than the control (Table 2).

In the liver of the fish there was an increase in the GSH production levels in treatment C2 (89.40µg/mL-121.10 µg/mL) from day14 to day 28. However, in treatment C1 and C3 there were significant decrease in the GSH production levels. On the 14th day GSH production levels in the liver of the fish exposed in treatment C2 was significantly lower than the control. However, on the 28thday the GSH production levels in all treatments were significantly lower than the control. There was a general decline in GSH production levels in the treatments on the 28th day (Table 3).

Table 1: Glutathione production levels in the kidney from the 14th and 28th day of chronic exposure of *C. gariepinus* juveniles to sub-lethal concentrations of Lead Nitrate

Concentration (Mg/L)	Day 14 (µg/Ml)	Day 28 (µg/Ml)
Control	44.5 ± 0.00^{b}	83.00 ± 0.00^{c}
C1	78.90 ± 41.40^{d}	60.00 ± 9.50^{b}
C2	73.40 ± 41.40^{c}	40.15 ± 10.35^{a}
C3	25.65 ± 0.85^{a}	56.10 ± 13.65^{b}

Mean values with different alphabet are significantly different ($P\leq0.05$) from each other; C1 =28.4mg/L, C2 =43mg/L, C3 =57mg/L, µg/ml=unit of GSH

Table 2: Glutathione production levels in the gills from the 14th and 28th day of chronic exposure of *C. gariepinus* juveniles exposed to sub-lethal concentrations of Lead Nitrate

Concentration (mg/L)	Day 14 (µg/ml)	Day 28 (µg/ml)
control	40.75±0.25[a]	128±2.50[d]
C1	45.15±7.35.00[b]	66.00±3.50[c]
C2	40.65±6.85[a]	43.15±16.35[b]
C3	45.40±5.40[b]	38.75±4.25[a]

Mean values with different alphabet are significantly different (P≤0.05) from each other; C1 =28.4mg/L, C2 =43mg/L, C3 =57mg/L, µg/ml=unit of GSH

Table 3: Glutathione production levels in the liver from the 14th and 28th day of chronic exposure of *C. gariepinus* juveniles exposed to sub-lethal concentrations of Lead Nitrate

Concentration(mg/L)	Day 14 GSH (µg/ml)	Day 28 GSH (µg/ml)
Control	126.5±0.50[b]	136.20±0.40[d]
C1	63.90±0.60[b]	112.8±82.50[c]
C2	63.65±13.85[a]	87.15±46.65[b]
C3	48.50±12. 00[b]	45.00±1.85[a]

Mean values with different alphabet are significantly different (P≤0.05) from each other; C1 =28.4mg/L, C2 =43mg/L, C3 =57mg/L, µg/ml=unit of GSH

DISCUSSION

A variety of environmental pollutants are known to change the GSH level in aquatic organisms, including heavy metals (Olaifa *et al*., 2004). There were initial increases in the GSH production levels in the kidneys of the fish exposed in C1 and C2 on day 14. While the GSH production levels in C3 were significantly lower than the control on the same date. The initial increases in the GSH production levels in lower concentrations of lead nitrate as well as the production levels in the control treatments may be due to the fact that at these concentration levels the effects of the xenobiotic on the fish are not yet felt to have triggered the usage of the antioxidant in the defence of the body. This is probably why at the highest concentration level the toxic effects of the pollutant elicited considerable decline in the production levels of the GSH. However, the production levels were significantly lower relative to control and the values obtained in the C1 and C2 decreased considerably on the 28th day. With increase in the duration of exposure of the fish to the toxicant there was probably the necessity for the usage of the glutathione in order to counteract the effects of bioaccumulation of the toxicant. The kidney being an organ of excretion (amongst other functions) may have been actively engaged in dealing with the xenobiotics in the environment of the fish (Samuel *et al.*, 2015). Similar findings on the utilization of GSH due to toxicants were reported by Moniuszko-Jakoniuk *et al*. (2005); Michalak (2006) and Jurczuk *et al*. (2006). The values obtained in C3 increased from day 14 to 28 probably because the fish has now undergone some level of adaptation and the rate of usage of the antioxidant to the oxidative stress may have reduced.

The production levels of GSH in the gills of all the fishes exposed in all the treatments including the control exposed to sub-lethal concentration of lead nitrate were relatively the same on the 14th day. However, the GSH production levels were significantly lower than the control on the 28th day of the exposure. At these sub-lethal concentrations, the effects of the xenobiotic may not have been felt on the 14th day since the gill is the first point of contact. But with the increase in the

duration of the exposure and probably increase in the bioaccumulation of the toxicant there was the need for the utilization of the antioxidant in combating the oxidative stress generated due to the presence of the toxicant. This was particularly evident at the highest concentration where the mean value of glutathione production was drastically reduced in comparison to the mean value obtained in the control. Ayoola *et al*. (2014) recorded significant differences in GSH, MDA, SOD and total protein in the gills of *Hemichromis fasciatus* and *Chrysichthys nigrodigitatus* collected from polluted Lagos lagoon. Olojo *et al*. (2005) attributed the degree of distortion of the gills to the duration of exposure periods.

The GSH production levels in the liver of *C. gariepinus* exposed to sub-lethal concentration of lead nitrate in all the treatments were significantly lower than the control but the usage of the antioxidant was more in the highest treatment (57mgL) on day 14 of the exposure. Similar scenario played out on the 28th day. The liver of the fish may have been actively engaged in dealing with the oxidative stress generated at each stage of the exposure since liver is the key organ and the principal site where the metabolism of carbohydrates, lipids and proteins take place (Vikramjit, 2012). It is also known that liver possesses high potential for ROS generation, which seems to be efficiently counterbalanced by powerful protective mechanisms to detoxify and repair damaged lipid and proteins (Oliveira *et al*., 2008; Lattuca *et al*., 2009; Nahrgang *et al*., 2010). The out-come of this research is in conformity with the findings of Sisein *et al*. (2014) who attributed the significantly lower GSH value in the liver of *C. gariepinus* from Gbarantoru Swamp in comparison with Niger Delta University Agricultural Farm (control) partly to the increased accumulation of heavy metals which led to more utilization of GSH to detoxify metals and ROS. The out-come of this research was also in agreement with Saliu *et al*. (2012) who reported that GST and GSH reduced after exposure of post juvenile *C. gariepinus* to Lead Nitrate in comparison to the control.

CONCLUSIONS AND RECOMMENDATIONS

The 96 hours LC_{50} of *C. gariepinus* exposed to lethal concentrations of Lead Nitrate $(PbNO_3)_2$ under laboratory conditions was 284.189 mg/L. The GSH production levels in the various organs of *C. gariepinus* exposed to sub-lethal concentrations of lead nitrate varied from concentration to concentration. The GSH production levels were significantly lower in the kidney of the fish exposed to sub-lethal concentration of 28mg/L and 43mg/L; and the values obtained in these treatments decreased considerably on day 28 respectively in comparison with the control. The GSH production levels were significantly lower in the gill of the fish exposed in all the treatment on day 28 relative to control. The GSH production levels were significantly lower in the liver of the fish exposed in all the treatment on both day 14 and 28 respectively relative to the control. These organs were actively engaged in dealing with the effects oxidative stress generated due to the presence of the toxicant.

The liver of *C. gariepinus* exhibited a better control of the oxidative stress and therefore, can be used as an indicator organ in the evaluation of GSH production levels as biomarkers of oxidative stress due to lead nitrate. More research that would indicate glutathione production levels at the 7th, 21st and 42nd days should be carried-out for better appreciation of the importance of the non-enzymatic antioxidant at these stages in the exposure of the fish to the toxicant.

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