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Effect of environmental pollution on oxidative stress in African catfish (*Clarias heterobranchus*)

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Abstract: Oxidative stress biomarkers: levels of Lipid peroxidation as well as changes in catalase and superoxide dismutase activities were investigated in tissues of African catfish, C heterobranchus inhabiting Warri River. Data were compared to those of reference hatchery. Lipid peroxidation products in fish from the midstream and downstream parts of the river were significantly ($P < 0.05$) different from fish collected from upstream. Similarly, lipid peroxidation products in tissues of fish from midstream and downstream parts of the river were significantly ($P<0.05$) different from fish in the reference hatchery. No significant difference was observed between fish in the upper part of the river and those from reference hatchery. Similar to lipid peroxidation, the activities of antioxidant enzymes, catalase and superoxde dismutases (SOD) were significantly ($P < 0.05$) different in fish from midstream and down stream parts of the river compared to fish collected from upstream and reference hatchery. The elevated levels of lipid peroxidation, catalase and superoxide dismutase activities in all tissues examined in C heterobranchus could be a reflection of oxidative stress on the fish.

Keywords: Catalase Activity, Fish, Lipid Peroxidation, Superoxide Dismutase Activity

1. Introduction

The River Warri is an important river in the Niger Delta of southern Nigeria. The economic importance of the river is predicated on its rich biota (Egborge 1986; Egborge and Tawarri 1987; Opute 1991, Tetsola and Egborge 1991; Ikomi 1995) that provide various kinds of fishes for human consumption, means of inland water transport for most communities in the region and the site of a large port at Forcados (Ikomi 2000). However, the river is highly polluted due to the presence of oil / petrochemical complex (Egborge 1991), enormous oil exploration activities in Warri and its environs and discharge of domestic and other industrial effluent into the river (Egborge, 1991).

The extent of pollution of Warri Rivers has been monitored by an array of the scientific communities (Atuma and Egborge, 1986 Egborge 1991; 1994)culminating in changes in water quality indices (Egborge and Benka – Coker, 1986; Ikomi 1993; 2000) and bioconcentratin of trace metals in fish (Kakulu et al 1997; Ezemonye 1992; Agada, 1994).

Fish in natural environment are often exposed to a variety of stressors that can adversely affect their health. Thus, there is a need to develop tools to assess environmental related stress in fish (Afonso et al 2003). Some of the biondicators of stress in fish include increase in levels of plasma cortisol, glucose and lactate (Barton and Iwama, 1991); induction of heat shock protein (Iwama et al 1999); glucose and drug metabolizing enzymes (Isamah and Asagba, 2004).

The induction of oxidative stress in fish by polluted environment is well documented (Bainy et al 1996; DiGuilio et al 1989; Hai 1997). This study reports on oxidative stress on Clarias heterobranchus from Warri River in southern Nigeria.

2. Materials and Method

2.1. Study Area

The study area is Warri River. It is located between latitude $5^{0}21^{1}$-$6^{0}.00^{1}$N and longitude $5^{0}25^{1}$E. The river took its source at Utagba – Uno and flow towards south west through Eziokpor, Amai, Otorho – Abraka, Warri and emptying into the sea at Forcados (Tetsola and Egborge, 1991).

The fishes, Clarias heterobranchus were obtained from the upper, middle and near the lower course of the Warri River between August and September, 2004. The fishes were caught using instrument made locally. They were transported alive to the laboratory and allowed to stabilize for one week before they were dissected to extract organs and tissues of interest. Similar fish of comparable size were obtained from a commercial fish pond located in Abraka and were used as control. The fishes were sorted and duly identified by the Department of Zoology, Delta State University, Abraka, Nigeria.

A total of 30 samples of mature size-matched male C heterobranchus (average wet weight 340±5g and average wet length 38.7±3.2cm) were collected from each site. Males were chosen for this study in order to be sex specific since biomarkers have been found to be sex related (Afonso et al 2003). All the reagents used were of analytical grade.

2.2. Methods

2.2.1. Preparation of Extract for the Determination of Lipid Peroxidation

Of the isolated organs 0.5g were separated and homogenized with 10ml of ice-cold 0.05M phosphate butter pH 7.0 containing 1% (w/v) Triton X-100, excess butylated hydroxyl toluene (BHT) and a few crystals of protease inhibitor, phenylmethylsulfonyl fluoride using an MSE blender immersed in ice. Triton X-100 solubilizes membrane-enclosed organells while BHT prevents in vitro oxidation of lipid during homogenization. The extract was centrifuged at 7000g for 20 min (4^0C). The supernatant (S_1) was used for the determination of lipid peroxidation by the method of Hunter et al (1963) as modified by Gutteridge and Wilkins (1982).

2.2.2. Extraction and Assay of Catalase

Catalase was measured with a similar (S_1) fraction after addition of 1% (V/V) of ethanol and incubation at 4^0C for 15 min. This treatment is reported to reverse the inactivation of catalase, which takes place, by the formation of compound 11 (Cohen et al, 1970). Catalase activity was determined according to Beers and Sizer (1952) by measuring the decrease in the H_20_2 concentration, at an absorbance of 240nm. An extinction coefficient for H_20_2 of $40M^{-1}$ cm^{-1} (Abei, 1974) was used in the calculation.

2.2.3. Extraction and Assay of Superoxide Dismutase

An aliquot of the supernatant (S_1) was precipitated on ice with 0.30 volume of chloroform/ methanol (3:5v/v) stirred for 20min and centrifuged at 7000g at 4^0C. The obtained supernatant (S_2) was used for the assay of superoxide dismutase (SOD) activity, which was based on its ability to inhibit the oxidation of epinephrine by superoxide anion (Aksnes and Njaa, 1981). One unit of superoxide dismutase activity is defined as the amount of enzyme required for 50% inhibition of the oxidation of epinephrine to adrenochrome at 480nm per min (Misra and Fridovich, 1972). Manganese dependent SOD was analyzed in the presence of 1mM NaCN to suppress Cu-ZnSOD activity and the cytosolic Cu-ZnSOD activity was determined as the difference between total and cyanide – sensitive enzyme activity (Crapo et al, 1978). The enzyme activities were assayed with an SP 1800 UV/VIS Spectrophotometer.

3. Statistical Analysis

All the results were expressed as means ±SE and all data were analyzed using Analysis of variance (ANOVA). Significant difference between the control and polluted sites means were determined at 5% ($P < 0.05$) confidence level using Duncan's Multiple Range Test.

4. Results and Discussion

Oxidative stress biomarkers were studied in the muscle, liver, kidney, heart and intestinal tract of African catfish, C heterobranchus from Warri River and comparable fish from a local fish hatchery which served as control. Oxidative stress biomarkers were lipid peroxidation, catalase and superoxide dismutase activities. The results showed that lipid peroxidation was significantly ($P<0.05$) higher in all the organs/tissues from Warri River compared to fish from reference hatchery (table 1). Similarly, the level of lipid peroxidation products in fishes collected in the lower sector of the river were significantly ($P<0.05$) higher than those of fishes collected from the upper sector (table 2). This observation is consistent with the report of Fatima et al (2000) and Achuba (2002).

Table 1. *Levels of lipid peroxidation in organs of catfish from Warri River and control. Level of lipid peroxidation (μmolml^{-1}).*

Organ/tissue	Control	Upper	Middle	Lower
Liver	6.6±2.21 ^{a}	6.7±1.4 ^{a}	10.5±2.5 ^{a}	13.6±1.8a
Gills	3.38±0.28 ^{b}	3.21±1.1 ^{b}	8.80±1.5 ^{a}	16.7±2.3 ^{c}
Muscle	4.38±0.34 ^{b}	3.86±1.3 ^{b}	5.32±1.2 ^{b}	5.89±1.6 ^{b}
Brain	6.56±0.42 ^{a}	5.11±2.1 ^{b}	23.2±1.5 ^{c}	26.14±1.5 ^{c}
Kidney	4.49±078 ^{b}	4.12±0.3 ^{b}	6.3±1.4 ^{b}	9.13±2.2 ^{b}
Intestinal tract	11.13±0.48 ^{c}	8.11±1.8 ^{c}	17.21±1.5 ^{c}	24.4±1.6 ^{c}
Heart	4.38±0.83 ^{b}	5.13±1.6 ^{b}	7.55±1.7 ^{b}	12.6±1.9 ^{a}

Values are means ± SE of determinations for five fishes. Mean with different superscript letters in the same row are significantly different at $P < 0.05$

Lipid peroxidation has been used as a measure of xenobiotic-induced oxidative stress in fish and these include lipid perroxidation in Atlantic croacks (Thomas et al 1993); Indian catfish (Parihar and Dubey, 1995) and Channel catfish (DiGuilio et al 1993). Moreover, increase in lipid peroxidation has been reported in fish exposed to polluted environment (Munkittrick et al 1998; 2000. Fatima et al 2000).Besides acting as a mediator in oxidative stress, higher levels of lipid peroxidation products can adversely affects cellular functions (Munkittrick et al 1998; 2000) and adduct with proteins and DNA which may predispose the cell to mutagenesis and carcinogenesis (Bailey et al, 1992; 1996).

The activities of superoxide dismutase and catalase were

higher in fish from Warri River relative to fish from reference hatchery (Table 2 and 3). Like lipid peroxidation, the activities of these antioxidant enzymes were higher in fishes collected from downstream compared to those obtained from upstream. This result is consistent with earlier observations (Achuba 2002; Fatima et al 2000; Livingstone, 2001).A Previous report indicated that in response to increased levels of reactive oxygen species and oxidative damage, cells will usually increase the accumulation of a number of enzymatic antioxidants (Downs et al, 2002). Cu/ZnSOD and MnSOD are some of the markers of cellular responses to increased reactive species and have been found to accumulate in response to oxidative stress (Downs et al 2002). Similar responses have been reported in aquatic species in an environment with a history of exposure to xenobiotic causing oxidative stress (Rodriquez-Ariza et al 1995; Otto and Moon, 1996). Exposure to xerobiotics has been reported to greatly induce production of reactive oxygen species (Gokaoyr and Husay 1998; Livingstone 2001).

Table 2. *Superoxide dismutase activities in organs of catfish from Warri River and control. Superoxide dismutase activities (Unitsg ^{1}wet wt).*

Organ	Total SOD		Cu/2nSOD	MnSOD
Liver	Control	669.3±30 ^{a}	568.9±24 ^{a}	100.4±13 ^{a}
	Upper	652.8±60 ^{a}	556.1±22 ^{a}	96.8±33 ^{a}
	Middle	875.9±29 ^{c}	744.6±25 ^{c}	131.4±40 ^{c}
	Lower	946.1±41 ^{c}	798.2±35 ^{c}	146.7±50 ^{c}
Gills	Control	437.4±60 ^{a}	374.0±50 ^{a}	63.4±10 ^{a}
	Upper	421±3.100 ^{a}	360.1±63 ^{a}	61.3±32 ^{a}
	Middle	591.1±101 ^{c}	508.6±84 ^{c}	82.5±17 ^{c}
	Lower	682.6±35 ^{c}	591.1±63 ^{c}	913±33 ^{c}
Muscle	Control	247.4±35 ^{a}	207±80 ^{a}	39.0±40 ^{a}
	Upper	251.5±30 ^{a}	201±70 ^{a}	48.3±65 ^{a}
	Middle	343.3±29 ^{c}	288±24 ^{c}	54.9±50 ^{c}
	Lower	351.1±53 ^{c}	292±12 ^{c}	58.8±34 ^{c}
Brain	Control	328.7+135 ^{a}	269.5+111 ^{a}	59.2+24 ^{a}
	Upper	316.5+77 ^{a}	258.1+126 ^{a}	58.2+33 ^{a}
	Middle	467.2+169 ^{c}	383.1+139 ^{c}	84.1+30 ^{c}
	Lower	482+121 ^{c}	398.3+101 ^{c}	84.8+22 ^{c}
Kidney	Control	740.2+145 ^{a}	621.8+122 ^{a}	118.4+23 ^{a}
	Upper	673.3+102 ^{b}	578.3+89 ^{b}	103+44 ^{b}
	Middle	839.3+222 ^{c}	705.0+10.6 ^{c}	134.3+36 ^{c}
	Lower	878.8+156 ^{c}	746.8+117 ^{c}	138.0+22 ^{c}
Intestinal tract	Control	217.4+33 ^{a}	180.5±27 ^{a}	36.95±60 ^{a}
	Upper	214.6+26 ^{a}	173.4±18 ^{a}	41.30±11 ^{a}
	Middle	371.8±35 ^{c}	308.6±29 ^{c}	63.21±60 ^{c}
	Lower	383.9±77 ^{c}	311.7±38 ^{c}	71.11±35 ^{c}
Heart	Control	1589.1±38 ^{a} ^{b}	1318.95±32 ^{a} ^{b}	270.15±60 ^{a}
	Upper	1504.0±44 ^{c}	1238.0±66 ^{c}	266.1±80 ^{c}
	Middle	3258.5±57 ^{c}	2704.55±47 ^{c}	553±100 ^{c}
	Lower	3867.4±67 ^{c}	3285.6±101 ^{c}	581±110 ^{c}

Values as means ± SE of determinations from five fishes. Mean with different superscript letters in the same row are significantly different at P < 0.05.

Previous reports have implicated SOD and catalase as working in tandem to dismutate oxygen radicals at physiological conditions (Achuba and Osakwe 2003). SOD converts superoxide anions to hydrogen peroxide which is broken down to oxygen and water by catalase (Voet and Voet, 1990). It is, therefore, no surprise for the observed increase in catalase activities in all the studied organs (Table 2). Pollution-induced increase in the activity of catalase had been reported earliar by some investigators (Rodriquez-Ariaze et al 1993; Hasspieler et al, 1994, Isamah et al, 2000).

In generally, total superoxide dismutase and catalase activities have been reported as a potent mediator in chemical stress in fish (Achuba and Osakwe, 2003). Simirnoff (1993) found that an increase in the capacity of antioxidant defense in response to an increased level of reactive oxygen represents an indirect measure of oxidative stress. Fatima et al (2003) reported a significant increase in extra-hepatic oxidative stress in tissues such as kidney and gill of fish exposed to pulp and paper mill effluents. The higher value of antioxidant enzyme activities in fishes collected from the middle sector of the river relative to fish from reference hatchery and from the upper sector predicts that the fish collected in these regions of Warri Rivers are experiencing oxidative stress. The difference in the activity of these enzymes is a function of its environment and this has been established by Izokun-Etoibhio et al (1990).

It is relevant to conclude that a polluted environment could result in increase lipid peroxidation, superoxide dismutase and catalase activities in tissues of *C heterobranchus*. On the whole, the results presented suggest that environmental pollution could act as a mediator in the induction of oxidative stress in *C heterobranchus.*

Table 3. *Catalase activities in organs of catfish from Warri River and control. Catalase activities (μmolmin $^{-1}g^{-1}$ tissue).*

Organ/tissue	Control	Upper	Middle	Lower
Liver	83.±2.0 ^{a}	78.6±4.1 ^{a}	272.3±2.3 ^{c}	361±5.6 ^{c}
Gills	73.0±1.5 ^{a}	75.1±3.3 ^{a}	178.0±10.8 ^{c}	182±4.3 ^{c}
Muscle	46.1±1.9 ^{a}	48.3±2.5 ^{a}	138±9.3 ^{c}	153. ±5.0 ^{c}
Brain	21.4±0.5 ^{a}	23.0±1.1 ^{a}	47.47±5.1 ^{c}	52.3±2.1 ^{c}
Kidney	64.0±1.7 ^{a}	62.1±3.2 ^{a}	146.0±11.3 ^{c}	167.8±6.6 ^{c}
Intestinal tract	72.0±1.3 ^{a}	76.3±5.4 ^{a}	183.4±1.7 ^{c}	203±12 ^{c}
Heart	93.4±1.5 ^{a}	89.6±3.7 ^{a}	205±1.9 ^{c}	242±6.6 ^{c}

Values are means ± SE of determination from five fishes. Means with different superscript letters in the same row are significantly different from each other at P<0.05.

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