ANTIOXIDANT ENZYMES AND LIPID PEROXIDATION RESPONSES IN TWO SIZES OF AFRICAN CATFISH (*Clarias gariepinus)* EXPOSED TO SODIUM BICARBONATE

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ABSTRACT

Some antioxidant enzymes which include catalase (CAT), Superoxide dismutase (SOD), Glutathione-S- Transferase (GST) and Lipid peroxidation (LPO) in the plasma of juvenile and adult life stages of Clarias gariepinus was investigated in this study. A total of 150 Clarias gariepinus which comprised of 75 juveniles (mean length 26.75± 5.98 and mean weight 265.06± 17.24) and 75 adults (mean length 46.88± 12.07 and mean weight 788.91± 45.70) were sourced from the center. After exposing the fish to different concentrations (0.00 - control; 50, 100, 150, and 200 mg/L) of sodium bicarbonate solution, blood samples were collected and the antioxidant enzymes activities were measured. Results from the analysis showed that the activities of SOD, LPO and GST increased significantly (P<0.05), while the values of CAT reduced in both sizes when compared to the control. Results indicate concentration dependent activation of oxidative stress and subsequent alterations in the activities of these antioxidant enzymes, which were more pronounced in the juveniles than the adult fish. The result from this study, therefore suggests that sodium bicarbonate may induce oxidative stress, which is capable of overwhelming the antioxidant system of this species, especially at higher concentrations of exposure to the chemicals.

Keywords: *African catfish, Sodium bicarbonate, Oxidative biomarkers, Aquaculture, Anaesthetics.*

INTRODUCTION

Aquaculture industry in Nigeria has expanded tremendously in recent times, making it one of the fastest food producing sector in the country [1]. As a result of this growth, fish in the cultured medium are being intensively manipulated from the hatchery to the final commercial stage. Consequent of this, the culture fish are subjected to handling stress on a regular basis [2]. Anaesthetics are widely used to reduce the incidence of stress in aquaculture [3]. Sodium bicarbonate, commonly referred to as baking soda, is a white substance that gives carbon-dioxide when dissolved in water [4]. Its main advantages lie in its low cost, wide availability and safety to both fish and humans [5]. Sodium bicarbonate has been effectively used as an anaesthetic in common carp (*Cyprinus carpio*) in both cold and warm water conditions [6], Rainbow trout (*Oncorhyncusmykiss*) [7,8], Nile tilapia (*Oreochromis niloticus*) [9] and in *Clarias gariepinus* [10].

The endogenous antioxidant system is important in animal metabolism as they are involved in defensive mechanisms against the impacts of xenobiotics, which may have significantly induced their alterations due to free radicals generated by reactive oxygen species (ROS). Banaee *et al.* [11] reported that the antioxidant capacity of tissues is critical in combating free radicals and ensuring normal metabolic functions in fish. Endogenous antioxidant enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GPx) have been reported to be of great importance in combating free radicals triggered by ROS [12]. SOD is one of the first major defensive steps against free radicals' damage. It catalyzes the conversion of super oxide radical (O_2^-) to hydrogen peroxide (H_2O_2) [13]. Glutathione peroxidase (GPx) is involved in the detoxification of hydrogen peroxide. These antioxidants (SOD and GPx) are used as biomarkers of toxicity of xenobiotics in fish since they defend the cells against cellular impairment [14]. Generally, the hazardous nature of nanoparticles and the risk of ZnO-NPs could particularly portend on aquatic organisms is connected with the induction of oxidative stress [15]. Oxidative stress induction could be determined by measuring the level of malondialdehyde, and by evaluating the alterations in the antioxidant system due to free radicals' disruption [16].

Conversely, oxidative stress is an imbalance between the production of reactive oxygen species and antioxidant mechanism in cellular systems that results in damaging of the cells [16]. Chemical applications induce reactive oxygen species through several biochemical mechanisms which results in lipid peroxidation, alterations of cellular redox status and certain aging disease conditions [17]. Exposure of fish species to anaesthetics by sodium carbonate have been reported to cause changes in internal physiology of fish [18]. A study by Akinrotimi *et al.* [19], demonstrated changes in the haematological parameters of catfish (*Clarias gariepinus*) exposed to sodium carbonate. Also, in an extensive study by Akinrotimi *et al.* [20], alterations were observed in some plasma enzymes, of fish exposed to sodium carbonate anaesthetics, Changes in antioxidants in fish exposed to anaesthetics, sodium chloride inclusive were limited, thus necessitating the need for this work. The aim of the current study is to determine the effects of sodium bicarbonate on antioxidant enzymes in different sizes of *C. gariepinus* which hitherto has not been reported.

MATERIALS AND METHODS

Experimental Location and Fish

The study was carried out in African Regional Aquaculture Center, an outstation of Nigerian Institute for Oceanography and Marine Research, Aluu, Rivers State, Nigeria. A total of 150 *Clarias gariepinus* which comprised of 75 juvenile (mean length 26.75± 5.98 and mean weight 265.06± 17.24) and 75 adult (mean length 46.88± 12.07 and mean weight 788.91± 45.70) were sourced from the center. The fishes were transported in six open 50 litre plastic containers to the laboratory and acclimated for a period of seven days.

Source of anaesthetics agents

The test chemical, sodium bicarbonate was purchased off shelf from a chemical shop in Port Harcourt. It is manufactured by Hunan Chembird Industrial Company Limited, Changhsha, China, for use in fish transportation. The concentrate is prepared at 9 g/100 ml of water following the method of Booke *et al.* (1998). And it is administered by immersion.

Preparation of test solution

A stock solution of the anaesthetics was prepared by adding 1 ml of the anaesthetic concentrate to 1 litre of water. Exposure concentration of anaesthetics were 0.00 (control); 50, 100, 150, and 200 ml/L. Thirty, (15 for each size) of 50 L plastic containers were labeled and each filled with water from the borehole to the 30 L mark. The different concentrations were prepared by serial dilution by measuring 50, 100, 150 and 200 of the stock solutions (×30) that was made into 30L with the borehole water that gave the desired concentrations.

Experimental Design and procedure

The experimental design is a 1x2x3 complete randomized design. The chemical is hydrophilic in nature and the anaesthetic solution was then stirred with a glass rod (50 cm in length) for homogeneous mixture. Within 10 minutes, the tanks were randomly stocked with five juveniles per tank and five adults per tank, using a scoop net. Three tanks were used for each concentration as well as the control for each of the fish sizes. The tanks were not aerated during the experimental period. Water quality parameters were also determined using the methods APHA [21]. Duration of fish exposure to various anaesthetics at different concentrations depends on the induction and recovery time.

Antioxidants Enzymes Assay

At the end of each experimental period (90 minutes), 2ml of fresh blood sample was collected from the caudal region with a fine needle and poured into heparinized sample bottles.Blood samples were centrifuged immediately for 15

minutes at 5000 rpm.Plasma specimens were separated, pipetted into eppendorf tubes and stored in a refrigerator at -20°C until assayed [22]. The results were read using a universal microplate reader on a Jenway visible spectrophotometer (Model 6405, Shanghai, China). The activity of antioxidants in centrifuged plasma was determined spectrophotometrically using the method of Beechey *et al.* (1975). LPO was estimated by measuring TBARS (thiobarbituric acid-reactive substances) in serum samples according to a modified method of Jentzsch [23]. Briefly, 0.2 ml of serum was added to the reaction mixture containing 1 ml of 1% ortho-phosphoric acid, 0.25 ml alkaline solution of thiobarbituric acid-TBA (final volume 2.0 ml) followed by 45 min heating at 95 . The results were expressed as nmol MDA per milliliter of plasma.

Statistical Analysis

All the data were expressed as mean and standard deviation of mean. The statistical package, SPSS Version 22 was used for the data analysis. The means were separated using tukey multiple comparasion test and the two means were considered significant at 5 % ($P<0.05$).

RESULTS

The water quality parameters (Table 1) were within the same range except in DO and pH.. The effects of Sodium bicarbonate on the antioxidants in the plasma of *C.gariepinus* juveniles are presented in Table 2. It was observed that the values of SOD, GST and LPO increased with increasing concentrations of the chemical. While CAT decreased significantly when compared to the control values. The same trend was observed in the antioxidants of adult fish exposed to the chemical (Table 3).

Table1: Physico-Chemical Parameters of Test Media

Concentration (mg/l)	DO (mg/l)	Temperature (°C)	pH	NH_3 (mg/l)
0.00	5.92±0.39 ^{b}	29.54±2.09 ^{a}	6.66±1.21 ^{a}	0.01±0.01 ^{a}
50.00	5.71±0.54 ^{b}	29.33±3.48 ^{a}	6.71±1.44 ^{a}	0.02±0.01^{a}
100.00	5.43±0.29 ^{b}	29.74±1.79 ^{a}	6.92±1.82 ^{a}	0.02±0.01 ^{a}
150.00	5.08±0.69 ^{b}	29.65±3.32 ^{a}	7.99±0.32^{b}	0.03±0.01^{b}
200.00	4.39±0.44 ^{a}	29.88±5.54 ^{b}	8.32±0.66^{c}	0.03±0.01^{b}

Means within the same column with different super scripts are significantly different ($P<0.05$)

Table 2: Antioxidants Enzymes Activities in *C. gariepinus* Juveniles Exposed to Sodium bicarbonate

Concentration (mg/l)	Antioxidants Enzymes (IU/L)			
	CAT(IU/L)	**GST(IU/L)**	**SOD(IU/L)**	**LPO(nmol/ml)**
0.00	0.69±0.02 ^{b}	0.33±0.01^{a}	0.43±0.07^{a}	6.06±0.78 ^{a}
50.00	0.62±0.15 ^{b}	0.36±0.01 ^{a}	0.49±0.07 ^{a}	7.99±0.89 ^{a}
100.00	0.55±0.03 ^{b}	0.53±0.01 ^{b}	0.77±0.00^{b}	10.00±1.62 ^{b}
150.00	0.30±0.02 ^{a}	0.63±0.02 ^{b}	0.89±0.01^{b}	12.00±1.71 ^{b}
200.00	0.18±0.06 ^{a}	0.86±0.01 ^{c}	1.41±0.02^{c}	14.32±2.57 ^{b}

Means within the same column with different super scripts are significantly different ($P<0.05$)

Table 3: Antioxidants Enzymes Activities in *C.gariepinus* Adults Exposed to Sodium bicarbonate .

Concentration (mg/l)	CAT (IU/L)	GST(IU/L)	SOD(IU/L)	LPO(nmol/ml)
0.00	0.77±0.05 ^{b}	0.40±0.05 ^{a}	0.51±0.04 ^{a}	8.81±1.32 ^{a}
50.00	0.71±0.03 ^{b}	0.43±0.01 ^{a}	0.59±0.01 ^{a}	10.66±1.52 ^{b}
100.00	0.69±0.01 ^{b}	0.60±0.20^{b}	0.99±0.07 ^{a}	12.00±1.00 ^{b}
150.00	0.54±0.05 ^{a}	0.69±0.10^{b}	1.43±0.02^{b}	13.66±2.88 ^{b}
200.00	0.35±0.08 ^{a}	0.96±0.07^{c}	1.96±0.09^{b}	19.09±2.03 ^{b}

Means within the same column with different super scripts are significantly different ($P<0.05$)

DISCUSSION

Reactive oxygen species (ROS) which include hydrogen peroxide (H_2O_2), superoxide anion and hydroxyl radicals are generated during biochemical reactions and the antioxidant enzymatic systems protects organisms from the toxic effects of the free radicals and help to maintain cellular homeostasis by neutralizing the ROS [24]. When there is an imbalance between the ROS and the antioxidant system due to excessive generation of the free radicals, cellular oxidative stress develops [25]. Free radicals generated reacts with biological macromolecules causing increase in lipid peroxidation (LPO), deoxyribonucleic acid damage and protein oxidation with ultimate disturbance in the physiological processes [26]. In this study, the data showed that lipid peroxidation significantly increased in a concentration dependent manner. The elevated values of lipid peroxidation obtained in this study agree with previous reports in fish exposed to different herbicides and other toxicants [27].

Aquatic organisms are being decimated due to the accumulation of chemical agents which may have been washed into their habitats and thus, suffer from tissue damage as a result of physiological and biochemical alterations [28, 29]. To counteract the effects of these toxic chemicals, fish have evolved complex defensive mechanisms in form of antioxidants. The induction or inhibition of these antioxidants are widely accepted as biological indicator of xenobiotic-induced peroxidative injury in fish tissues, and have been used in diagnosing the negative impacts of xenobiotics in aquatic environment [30]. SOD and GST are used as biomarkers of lipid peroxidation in organisms exposed to chemical perturbations. These enzymes provide the first line of defense against formation of oxyradicals by converting superoxide radicals into hydrogen peroxide and subsequently into water and molecular oxygen. The induction of these enzymes in the tissues of sodium carbonate exposed fish at 150 and 200 mg/l is an indication of oxidative stress through the production of reactive oxygen species (ROS). Accordingly, the elevated SOD and GST activities in this study is suggestive of sodium carbonate induced adaptive response to eliminate the formation of lipid peroxidation for polyunsaturated fatty acids which might have damaged the cell membrane. Several reports have earlier indicated that exposure to sodium carbonate and other chemicals in aquatic organisms can exacerbate the liberation of ROS which could cause oxidative damage to biological systems [31, 32]. The formation of ROS could be ascribed to the accumulations of the sodium carbonate in the tissues of *C.gariepinus*. Previous results had earlier confirmed that fish accumulates toxicants in different tissues from the surrounding environment [33, 34, 35]. Elevated levels of SOD and GST in the tissues after 15 days exposure, particularly at the highest concentrations of the toxicant, may be associated with the tolerance and/or detoxification ability of *C.gariepinus* especially under sodium carbonate exposure regime.

Increased activities of the CAT within the exposure time indicate that the rate of reactive oxygen species production may have increased with change in concentration from control to 200mg/l of sodium bicarbonate. Increased production of the free radicals may result to oxidative stress as the antioxidant enzyme system is overwhelmed. Dabas *et al.* [36] reported that fish has limited capacity of the antioxidants to neutralize the effects of the free radicals. The decreased CAT when compared with the control may be due to free radical damage on the macromolecules of the fish. Puerto *et al.* [37] reported that decreased CAT activity may be attributed to direct damage of protein structure and an increased production of hydrogen peroxide. This result suggests the onset of oxidative damage of macromolecules due to the overwhelming presence of reactive oxygen species generated from the exposure of catfish to different concentrations of sodium bicarbonate.

Lipid peroxidation has been adduced as the main reason for the loss of functional integrity in organisms during the formation of oxyradicals [38] and has been used in the assessment of oxidative damage [40, 41]. In this study, the induction of lipid peroxidation in the tissues of Sodium bicarbonate exposed fish as reflected by the high LPO levels is an indication that the fish is under oxidative stress. However, this seemed not to have manifested during the early days of exposure due to the similarities observed in LPO value between the exposed fish and the control until after 15 days at higher toxicant concentrations. Increased levels of LPO are indicative of lipid components' vulnerabilities to the reaction of free radicals, thereby increasing lipid peroxidation. The increase in LPO levels may be explained by the disproportionate generation of ROS, and could be linked to the diminution of the antioxidants owing to their leakage into the blood circulation [42, 43], Similar to this result, increased LPO have also been observed in the tissues of fish [44, 45].

CONCLUSION

The results of the current study highlight the importance of considering all potential physiological and biochemical effects of a prospective anaesthetic agents used in aquaculture. It is clear that different anaesthetics can have marked effects upon blood chemistry of fish. Thus, careful consideration should be taken when selecting an anaesthetic for use in aquaculture. In conclusion, this study showed that Sodium bicarbonate at varying concentrations induced detrimental effects on the antioxidant system of *C.gariepinus*. However, the elevated activities of SOD and GST indicate resistance to the toxic effects even at higher concentrations and longer period of exposure. The concentrations of Sodium bicarbonate used in this study may, therefore, be considered as ecologically relevant; suggesting that concentrations greater than these under short-term or long-term exposure may produce deleterious effects on the antioxidant system, and thus, weakens the adaptive threshold of the fish.

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