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Enzimas plasmáticas na brânquia de juvenis de Clarias gariepinus expostos a níveis crônicos de alquil benzeno sulfonato linear

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A B S T R A C T

Linear alkyl benzene Sulphonate (LAS) inhibited all the enzymes in the organ examined except alanine aminotransferase (ALT) and Acid phosphatase (ACP) on exposure of one hundred and fifty *C. gariepinus* juveniles (mean weight 246.30±14.12g SD/mean length 16.15±1.40cm SD) to chronic levels of 10.00, 20.00, 30.00, 40.00 and 50.00 mg/l for 30 days. Generally, the impact of LAS on the selected enzymes in the gill caused remarkable increase in Aspartate aminotransferase (AST) and significant decrease ($P<0.05$) in Alkaline phosphatase (ALP), except at 10.00mg/l with increase in detergent concentration when compared to control.

Key words: *Aspartate aminotransferase* (AST), Alanin aminotrasminase (ALT), Acid phosphatase (ACP) and Alkaline phosphatase(ALP) , Linear alkyl benzene Sulphonate (LAS)

Introduction

The increasingly obvious effects of pollution of the biosphere in general and aquatic ecosystems in particular are known to everyone and are the subject of daily accounts in the popular press as well as textbooks (Rand and Petroceili (1985) and scientific monographs Conneil and Miller (1984) and Nurnberg, (1985). The gill epithelium is the site of gas swap, ionic regulation, acid-base balance, and nitrogenous waste excretion by fishes. The last three processes are restricted by passive and active transport of various solutes across the epithelium. Various environmental pollutants (e.g., heavy metals, detergents, acid rain, and organic xenobiotics) have been found to affect the morphology of the gill epithelium. Associated with these morphological pathologies, one finds alterations in blood ionic levels, as well as gill Na, K-activated ATPase activity and ionic fluxes. Such physiological disturbances may underlie the toxicities of these pollutants. In addition, the epithelial transport steps which are affected in the fish gill model resemble that in the human gut and kidney, sites of action of a variety of ecological toxins.

Materials and methods

One hundred and fifty *C. gariepinus* juveniles (mean weight, 246.30± 14.12g SD; mean length 16.15±1.40cm SD) obtained from Abduls Fish Farm Rukpakulusi, Port Harcourt, Rivers State were transported by car in a 20L trough covered with a perforated cover to Fisheries laboratory in the Department of Fisheries and Aquatic Environment of Rivers State University of Science and Technology, Port Harcourt. On arrival, five specimen per aquarium were acclimated in thirty rectangular plastic aquaria containing twenty litre of water each for 7 days. The aquaria were washed with a piece of foam and fish fed once daily with a 42% crude protein diet at 3% body weight. A range finding test (trial test) was carried out using local detergent (Linear alkyl benzene Sulphonate (LAS) obtained from Rivers State Vegetable Oil Company (RIVOC), Port Harcourt. Five fish were exposed to each concentration (0.00, 10.00, 18.00, 25.00, 37.00, 50.00 mg/l) of detergent for 10 days. Sub-lethal concentration for the definitive test was done based on the range finding test. Five graded concentrations - 10.00, 20.00, 30.00, 40.00 and 50.00 mg/l of the solutions were prepared and thoroughly mixed to avoid hot spot in five replicates each. Five fish were introduced into each aquarium and covered with a perforated plastic lid to prevent escape of the fish. To avoid injuries or bruises on experimental fish, a scoop net was used daily to collect and transfer fish to empty buckets without water until aquaria with solution were ready. Ammonia-nitrogen, alkalinity, conductivity, dissolved oxygen, water pH, temperature and turbidity were the physicochemical parameters considered in this study. At the end of the investigation period, fish were killed with a blow on the head and dissected in order to collect 0.5g of gill with the aid of penknife. Sample was macerated with pestle and mortar. Alkaline phosphatase, Alanineaminotransferase (ALT), Aspartate aminotransferase (AST), Acid Phosphatase (ACP) were determined in the gill using standard methods.

Results

The mean values of alkalinity, conductivity and turbidity differed significantly ($P \leq 0.05$) while ammonia, temperature and pH did not with increase in detergent concentration. Dissolved oxygen decreased with increase in detergent concentration (Table 1). Linear alkyl benzene Sulphonate (LAS) elicited the production of aspartate aminotransferase (AST) in the gill (Table 2). At 10.00, 20.00, 30.00, 40.00 and 50. 00mg/l, LAS respectively raised AST activities in the gill by 62.50% (81.25±26.57IU/L), 70. 00% (85.00±27.39IU/L), 15.00% (57.50±19 .36IU/L), 87 .50% (93.75±30.65IU/L) and 24.00% (62.50±35.71 IU/L). ALT activities in the gill was also elevated by 25.00% (25.00IU/L) in all the concentrations when compared with control (20.00±0.00). LAS also raised ACP activities by 20.00% (15.00±0.0IU/L) at 20.00 and 30.00mg/l and ALP activities by 51.86% (155.00±11.55IU/L) at 10.00mg/l when compared with their respective controls. The impact of LAS caused a 20% decline in ACP at 40.00 and 50.00mg/l when compared with control and also respectively decreased ALP at 20.00, 30.00, 40.00 and 50.00mg/l by 17.17% (85.00±00IU/L), 41.46% (60.00±00IU/L), 29.27% (72.50±14.43IU/L) and 34.13% (67.50±5.00IU/L) when compared with controls.

Discussion.

It was observed in this experiment that LAS had no impact on ammonia, temperature and pH. This corroborates the findings of Onusiriuka and Ufodike (1994) in *C. gariepinus* exposed to Akee apple and sausage plants extract. Often times, water quality parameter values in bioassays are within tolerance ranges in tropical waters (EIFAC, 1977 and EPA, 1976) agrees with conductivity and alkalinity values recorded in this work. The major biochemical response to the effect of detergents in fishes is the inhibition of the activities of a number of enzymes such as AST, ALT, ACP, and ALP (Abel, 2006). Detergents disrupts the nervous structure of fish by plummeting the activity of enzymes at nicotinic and musculanic receptors (Misra *et al.*, 1991). Dropping the activity of these enzymes allows a protracted effect of acetylcholine, a neurotransmitter, on the receptors. The disabling of enzymes can have a toxic effect leading to harmful physiological effects or even fatality at a certain concentrations. Detergent surfactants restrain acetylcholine esterase in the brain of *Clarias gariepinus* (SETAC, 1997) and also 80% inhibition in the neural tissue of *Tilapia guineensis* following acute effect of detergent (Ezemonye *et al*., 2007). Zaccone *et al*. (1985) evaluated the toxic effects of ionic active detergent, LAS on fish for some days and observed a decrease in the activity of the enzymes in the plasma. In this study, the activities of AST and ALT increased significantly in the gill which indicated cellular toxicity of the detergent even at low doses under prolonged period of exposure. This agrees with the findings of Swisher (1975) who reported that the activity levels of AST, ALT, and total adenosine triphosphate (ATPase) in muscles, gills, liver and brain of *T. mossambica* exposed to detergent, showed that transaminases were elevated in all the tissues in addition to a shift in aminotransferases reaction under the impact of surfactants. Uedeme-Naa and Erondu, (2016) noted that changes in fish enzymes profile are important pollution indicators in any aquatic system with living organisms. Transamination is one of the principal pathways for the synthesis and deamination of amino acids, enabling carbohydrate and protein metabolism during fluctuating energy demands under various adaptive conditions (Chetty *et al*., 1980). Maintenance of internal homeostasis through biochemical processes in the Krebs cycle may be reflected in varying levels of enzymes AST, ALT, ALP in the serum (plasma) occasioned by cellular damage in the functional organs such as liver, heart, gill, muscles and kidney as they are generally found in the tissues of these organs (Heath, 1991). This work partially agrees with the work done by Gabriel and George (2005) who noted that serum AST and ALT are raised when disease process affects cell integrity in that LAS raised serum AST, decreased ALP and did not affect ALT activities in the gill when compared with control. Giboney, (2005) observed that Phosphates (ALP and ACP) and transferases (AST and ALT) tests are part of standard laboratory tests to detect health abnormalities in animals. Alterations in these enzymes (protein that regulate the rate of a chemical reaction in the body) activities of fish resulting from toxicant or contaminant effects in various organs of fish have been reported (Begun, 2004). Importantly, biochemical changes in fishes are aimed at maintaining equilibrium in the presence of toxicants, which are known to disrupt physiological and biological processes (Wedemeyer and Mcleay, 1981). The transaminases are a group of enzymes catalyzing interconversion of amino acids and α - ketoacids by transfer of amino groups and elevated activity of these tissues – specific enzyme have been used to diagnose damage to liver. The exposure of gill AST, ALT, ACP and ALP enzymes to detergent in this work resulted in alterations in their activities which agree with the findings of Ozer*et al.,* 2008, who reported that when hepatocytes are damaged, enzymes normally located in cytosol are liberated into the extra cellular space and enter the circulation due to membrane defects causing increased permeability. Detergent induced alterations in aspartate aminotransferase and alkaline phosphates activities have been reported in fish and this elevation was directly attributed to toxic action of detergent on gill (Agrahari *et al.,* 2007). Soufy *et al.* (2007) noted that following chronic carbofuran treatment, in monosex *O. niloticus*, initial increase and then sharp decrease in ALT, AST, and ALP enzyme activities were observed and these findings were confirmed by severe hepatic necrosis preceded by exposure period whereas in this work, it was observed that toxicant declined ALP, raised AST and had no significant impact on ALT and ACP enzyme activities in the gill of *C.gariepinus* juvenile with increase in concentrtion. Datta *et al.,* (2009) observed elevated level of AST and ALT in *C. batrachus* exposed to detergent at sublethal concentration. This is also in partial agreement with this work, where AST was raised and ALT was as control with increase in detergent concentration. Detergent can inhibit the activities of many enzymes especially those involved in the cellular glucose uptake, glucogenesis, fatty acid oxidation and production of glutathione due to its sulfhydryl group

binding capability (Arthur, 1970). Homtsoe *et al.* (2007) reported significant decrease in gill AST and ALT in *Labeorohita* exposed to detergent which reflects significant decrease in structure and function of cell organelles like endoplasmic reticulum and membrane transport system. This is in agreement with gill ALP of this work as activities decreased with detergent concentration when compared with control. Luskova *et al*. (2001) reported that *Cyprinuscarpio* exposed to 32.5mg/l of detergent for 96 hours produced depressed activities in the enzymes (AST, ACP and ALP) in the plasma of the fish and it is in line with enzyme like ALP which was raised at 10.00mg/l and later decreased as concentration increased. Breth and Grooves (1979), reported that increases in serum ACP, AST and bilirubin generally point to some sort of hepatic damage. Chamber (1978) posited that all the serum enzymes in shrimps were raised when exposed to varied levels of crude oil. This only agrees with AST activity in this work.

Table 1: Water Quality Variables (Mean ± S.D) in the Experimental tanks during the exposure period.

Variables	Concentrations (mg/l)					
	0.00	10.00	20.00	30.00	40.00	50.00
Ammonia. (mg/l)	1.76±0.2^{a}	1.59±0.37^{a}	1.44±0.43^{a}	1.54±0.62^{a}	1.49±0.20^{a}	1.44±0.40^{a}
Alkalinity(mg/l)	77.00±12.76^{a}	87.00±21.64^{a}	96.00±21.14^{ab}	115.00±21.66^{b}	133.00±21.12^{b}	141.12±21.11^{b}
Temperature (ºC)	30.42±2.33^{a}	30.27±0.48^{a}	30.45±0.63^{a}	30.55±0.59^{a}	30.70±0.73^{a}	30.71±0.84^{a}
P^{H}	6.39±0.57^{a}	6.45±0.65^{a}	6.68±0.30^{a}	6.60±0.14^{a}	6.60±0.16^{a}	6.40±0.21^{a}
Conductivity(S/m)	211.50±16.71^{a}	270.25±18.61^{ab}	303.75±28.11^{b}	343.25±19.621^{b}	345.25±14.11^{b}	360.11±28.11^{c}
Turbidity (mg/l)	21.50±6.23^{a}	25.00±7.16^{b}	27.00±6.21^{b}	26.11±7.11^{b}	45.00±7.11^{c}	73.00±8.11^{d}
D/O (mg/l)	5.59±0.98^{c}	3.81±0.68^{ab}	3.2±0.72^{ab}	2.95±0.64^{b}	2.55±0.33^{b}	2.33±0.13^{b}

Means within the same column with different super scripts differ significantly (P<0.05).

Table 2: Activities of selected enymes in the gill of *C. gariepinus* juveniles exposed to jumbo detergent for 30 days (Mean ± S.D)

Concentration (mg/l)	AST (IU/L)	% Control	ALT (IU/L)	% control	ACP (IU/L)	% Control	ALP (IU/L)	% Control
0.00	50.00±17.32^{a}	100	20.00±0.00^{a}	100	12.50±5.00^{a}	100	102.50±20.21^{c}	100
10.00	81.25±26.57^{c}	+62.50	25.00±10.00^{a}	+25.00	12.50±5.00^{a}	0.00	155.00±11.55^{d}	+51.86
20.00	85.00±27.39^{c}	+70.00	25.00±10.00^{a}	+25.00	15.00±0.00^{a}	+20.00	85.00±0.00^{ab}	-17.17
30.00	57.50±19.36^{ab}	+15.00	25.00±10.00^{a}	+25.00	15.00±0.00^{a}	+20.00	60.00±0.00^{a}	-41.46
40.00	93.75±30.65^{d}	+87.50	25.00±10.00^{a}	+25.00	10.00±0.00^{a}	-20.00	72.50±14.43^{b}	-29.27
50.00	62.50±35.71^{b}	+24.00	25.00±10.00^{a}	+25.00	10.00±0.00^{a}	-20.00	67.50±5.00^{a}	-34.13

Means within the row with different superscripts (a,b,ab,c,d) are significantly different (P<0.05).

Conclusion

It is clear that LAS can produce a gross enzymic instabilities in fish gill epithelium, which is often associated with osmoregulatory, acid-base, or hemodynamic malfunction (David, 1987, Nkpondion et al., 2016). So, government must put in place regulatory bodies to check on industries discharging detergent based effluents into our water bodies.

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