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Oil Injection of *Heterobranchus bidorsalis* Adults and its Effects on Aspartate Transaminase Activity

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ABSTRACT: The activity of aspartate transaminase enzyme in *Heterobranchus bidorsalis* adults (mean weight, 135.42 ±0.42g) when injected with different concentrations of Bony-light crude oil (BLCO) was studied within 4 days toxicity and 42 days recovery periods. Significant decreases in the values of aspartate transaminase enzyme concentrations (AST) ($mg100ml^{-1}$) were recorded in the fish liver supernatant as the BLCO concentrations increased from 10.00 to 50.00 $\mu l.g^{-1}$. Fish samples injected with 10.00 $\mu l.g^{-1}$ BLCO recorded highest values of AST than those injected with 20.00 – 50.00 $\mu l.g^{-1}$ BLCO. Increases in AST values during the recovery period at day 14 (25%), day 28(15%) and day 42 (5%) suggested some measure of relief on the liver tissues form oil toxicity and were a reflection of the tremendous effect of the oil injection on the activities of the aspartate transaminase enzymes within the liver. This result is consistent with the suggestions of other workers on the necessity for a comparative monitoring of biomarkers and pathological changes in the liver tissues in order to use good enzymatic markers as indicators of organ dysfunction. The AST values in this study: whether on decreases due to fish recovery from the toxic effect of oil, suggested that aspartate transaminase enzyme activity in *H. bidorsalis* adults was dose-dependent. Hence, this enzyme could be used as a biomarker in the fish to monitor pollution levels.

Key Words: *Heterobranchus bidorsalis*, Aspartate transaminase, Biomarker, Pollution level, Crude oil injection.

Introduction

The effects of xenobiotic contamination in an ecosystem can be estimated through analysis of biochemical changes in organisms inhabiting that region (Tuvikene *et al.*, 1996; Norris *et al*., 2000; Brewer *et al*., 2001). The response of aquatic organism to pollution is given by changes through expression of several key enzymes, especially those of biotransformation system (Ozmen *et al*., 2005). These biomarkers may be sensitive and specific early warning signs for aquatic pollution (Strmac and Braunbeck, 2000).

Polyaromatic and halogenated hydrocarbons (PAHs and HAHs), heavy metals, polychlorinated biphenyls (PCBs), crude oil and pesticides may enter fresh water systems from industrial waste-waters, oil spill accidents, urbanal discharges and agricultural activities. All these pollutants may in the long-term, result in ecotoxicotogical effects. Persistent organochlorine (OC) pesticides accumulate in the adipose tissues of non-target organisms and biomagnifies in the food chain (Henriksen *et al*., 2000).

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The aquatic ecosystem, like the terrestrial environment is continuously subjected to changes in quality following the introduction of substances of diverse characteristics arising from man's cultural activities (Oluah, 2001). The author stated that alterations in water quality usually predispose the fish to stress and disease which as a result, provoke quick response in the physiology of the fish, especially the haematological parameters. The potential utility of biomarkers for monitoring both environmental quality and health of organisms inhabiting polluted ecosystems has received increasing attention in recent times (Lopes *et al*., 2001; Samecka-Cymerman and Kempers, 2003; Gauthier *et al*., 2004). Many enzymatical markers have been applied to determine the degree of exposure of animals to pollutants. Several specific enzymes have been proposed for monitoring purposes of water pollution (Agradi *et al*., 2000). Such enzymes as esterases can be used as a biomarker for the random use of insecticides in an aquatic system; especially when the risk of contamination of non-target organisms is involved (Ozmen *et al*., 1999; Brewer *et al.*, 2001). Carboxylesterase, lactate dehydrogenase, alkaline and aspartate aminotransferase; as well as alkaline and acid phosphatase have been considered as useful biomarkers to determine pollution levels (Asztalos *et al*., 1990; Raberg and Lipsky, 1997, Baron *et al.*, 1999; Basagalia, 2000).

Scarcity of published information in Nigeria on the effect of crude oil pollution on enzyme activities in indigenous fish species informed this study. This research was therefore designed to investigate the effect of injecting *H. bidorsalis* adults with different concentrations of Bonny-light crude oil on aspartate transaminase enzyme activity in the fish. The essence was to inject doses of this pollutant into the fish and record the responses of the enzyme over a toxicity period and a recovery period.

Materials and Methods

Eighteen (18) plastic containers (25litre capacity) were randomly stocked with 360 adults of *Heterobranchus bidorsalis* {mean weight ± standard error of mean (s.e.m.), 135.40 ± 0.42g} at 20 fish per container. The experiment was designed to have 15 plastic containers (5 x 3) each with 24 litre dechlorinated tap water and stocked with fish injected with 10.00, 20.00, 30.00, 40.00 and 50.00 $\mu 1g^{-1}$ Bonny-light crude oil (BLCO). Three (3) plastic containers had fish samples that were not injected with crude oil and served as the controls (0.00 μlg^{-1}). The injection of fish with graded concentrations of BLCO was carried out with the aid of 2.50ml disposable hypodermic syringes, just below the dorsal fin.

Two study periods were adopted for the research namely: the toxicity period and the recovery period. Four (4) days was adopted as the toxicity period of the injected BLCO concentrations on the basis that the lethal concentrations (LC_{50}) of many pollutants are assessed within 4 days (96 hours) 96h $LC_{50.}$ The recovery period lasted for 42 days and was monitored fortnightly. At the end of the toxicity period, the surviving fish and plastic containers were washed and replenished with 24l dechlorinated tap water. A 38% crude protein diet (Table 1a) was fed to the fish at 3% body weight per day ($bw.d^{-1}$) during the toxicity period and at 5% $bw.d^{-1}$) during the recovery period. The proximate composition of the test diet (Table 1b) was carried out as described by Windham (1996). Records of the water temperature (27 ± 0.02 ^{o}C) and the pH (6.60 ±0.20) were taken with the aid of a maximum and minimum mercury-in-glass thermometer and a pH meter (Model Ph-l-201-L) respectively. The percent mortality (PM) and the percent survival (PS) of the fish were estimated during the toxicity and recovery periods of the study.

Liver samples of fish from each triplicate treatment of BLCO and the control were dissected with sharp surgical blades and scissors and washed in distilled water to remove traces of blood. The liver samples were macerated and homogenized as described by Devi *et al*. (1993) and placed in ice-cold 0.25M sucrose (Oluah *et al*., 2005). The liver homogenate was centrifuged at 5000rpm for 15 minutes at 4 ^{0}C and the supernatant transferred to clean microfuge tubes. The samples were then stored at -80 ^{0}C until enzymatic assays were carried out (Ozmen *et al*., 2005).

The estimation of serum aspartate transaminase activity, as a measure of liver function, was carried out at the Cynbald Diagonistic Laboratory, Abakpa-Nike, Enugu, Nigeria, using triplicate samples of the preserved liver supernatant (serum). Three test-tube tests were carried out thus: the serum-aspartate substrate reagent test, the serum blank test and the reagent blank test.

For the serum-aspartate substrate-reagent test, 0.50ml of the substrate was pipetted into a test tube and warmed to 37 ^{0}C in a bath for 15 minutes. 0.10ml liver serum was added and incubated for 60 minutes, after which 0.50ml dinitropheny hydrazine reagent was added. The test tube was then removed from the bath, the content thoroughly mixed and allowed to stand at room temperature for 20 minutes. Next was the addition of 5ml 0.4 N NaOH, thorough mixing by immersion and allowing to stand for 10 minutes. The mixture was then read in a colorimeter at 505nm against distilled water. The unit of activity of the aspartate

transaminase enzyme was obtained from a standard calibration curve. The serum blank and the reagent blank tests were also carried out for purposes of comparison with the serum-aspartate substrate reagent test on the calibration curve.

All the data obtained were subjected to analysis of variance (ANOVA) (Steel and Torrie, 1990) to determine statistical differences between treatment means ($P < 0.05$). Simple percentages were also used where appropriate to explain the analyzed data.

Results

The gross and proximate compositions of the diet fed to *H. bidorsalis* adults during the experimental periods are shown on Tables 1a and 1b respectively.

Table1a. Gross Composition of Experimental Diet

Ingredients	% Composition
Yellow maize	9.29
Soyabean meal	54.84
Fishmeal	16.65
Blood meal	10.97
Salt	0.25
Vitamin mix [1]	0.63
Mineral mix[2]	2.40
Total	100.00

[1]Vitamin mix provided the following constituents diluted in cellulose ($mgKg^{-1}$ of diet): thiamin, 10; riboflavin, 20; pyridoxine, 10; folacin, 5; pantothenic acid, 40; choline chloride, 3000; niacin, 150 vitamin $B_{12,}$ 0.06; retinyl acetate (500,000 $I\mu g^{-1}$), 6; nenadione-Na-bisulphate, 80; inositol, 400; biotin, 2; Vitamin C, 200; alpha tocopherol, 50; cholecalcipherol (1,000,000 $I\mu g^{-1}$).

[2] Contained as gKg^{-1} of premix: $FeSO_4.7H_2O$, 5; $MgSO_4.7H_2O$, 132; K_2SO_4, 329.90; KI, 0.15; $MnSO_4.H_2O$, 0.7; and cellulose, 380.97.

Table 1b. Proximate Composition of Experimental Diet.

Nutrient	% Composition
Crude protein	37.58
Ether extract	5.18
Ash	10.48
Dry matter	11.80
Nitrogen free extract	36.46
Total	100.00

Table 2 shows the aspartate transaminase enzyme concentrations (AST) ($1\mu L^{-1}$) in the fish injected with 10.00-50.00 μlg^{-1} BLCO and the control (0.00 μlg^{-1}) during the toxicity and recovery periods. Table 3 shows the percent mortality and survival of the fish. The control fish recorded significantly ($P < 0.01$) higher values of AST in the livers than those injected with the various concentrations of BLCO (Table 2). This result is exemplified both at the toxicity and the recovery periods of the study. The AST values in the control fish livers were relatively of the same magnitude; ranging between 6.54 to 6.82 $1\mu L^{-1}$ within both study periods (Table 2).

Table 2. Aspartate Transaminase Enzyme Concentration IμL^{-1} in *H. bidorsalis* Adults Exposed to Different Concentrations of Bonny-Light Crude Oil

Study period	Duration (Days)	Crude Oil type	BLCO[1] Concentration (μlg^{-1})					Control	Overall mean
			10.00	20.00	30.00	40.00	50.00	0.00	$\bar{x}$
Toxicity period	4	BLCO	3.92^{a} ±0.03	2.35^{b} ±0.02	1.41^{c} ±0.01	0.85^{d} ±0.01	0.50^{e} ±0.02	6.54^{f} ±0.04	2.60 ±0.02
Recovery period	14	BLCO	4.90^{a} ±0.03	2.94^{a} ±0.02	1.76^{c} ±0.02	1.06^{d} ±0.01	0.63^{e} ±0.01	6.82^{f} ±0.03	3.02 ±0.02
	28	BLCO	6.26^{a} ±0.04	3.38^{b} ±0.03	2.02^{c} ±0.02	1.22^{d} ±0.01	0.72^{e} ±0.01	6.70^{f} ±0.04	3.38 ±0.03
	42	BLCO	6.57^{a} ±0.04	3.55^{b} ±0.02	2.12^{c} ±0.02	1.28^{d} ±0.01	0.76^{e} ±0.02	6.76^{f} ±0.04	3.51 ±0.03

[1] Bonny-light crude oil, Numbers in the same row with different superscripts differ significantly ($P < 0.05$; $P < 0.01$).

Table 3. Percent Mortality and Survival of *H. bidorsalis* Adults Exposed to Different Concentrations of Bonny-light Crude Oil for 4 Days (Toxicity) and 42 Days (Recovery) Periods

Study Period	Duration (Days)	% Mortality						% Survival					
		BLCO[1] Concentratio μlg^{-1})					Control	BLCO[1] Concentration μlg^{-1})					Control
		10.00	20.00	30.00	40.00	50.00	0.00	10.00	20.00	30.00	40.00	50.00	0.00
Toxicity period	4	2.00	5.00	5.00	40.00	50.00	0.00	98.00	95.00	95.00	60.00	50.00	100.00
Recovery period	14	2.00	3.00	4.00	32.00	40.00	0.00	98.00	97.00	96.00	68.00	60.00	100.00
	28	1.00	2.00	2.00	24.00	36.00	0.00	99.00	98.00	98.00	76.00	64.00	100.00
	42	0.00	1.00	1.00	16.00	26.00	0.00	100.00	99.00	99.00	84.00	74.00	100.00

[1] Bonny-light crude oil

The AST values in the livers of fishes injected with 10.00- 50.00 μlg^{-1} BLCO decreased with increasing concentrations of oil injection (Table 2). Both at the toxicity and recovery periods of the study, 10.00 μlg^{-1} BLCO concentration was associated with the highest aspartate transaminase enzyme concentration in the fish livers when compared to the values recorded with the BLCO concentrations (20.00-50.00 μlg^{-1}) (Table 2). However, there were significant differences in the values of AST in the fish livers as a result of fish injection with different concentrations of BLCO and the control ($P < 0.05$; $P < 0.01$) (Table 2).

Increases in the AST values in fish livers were obtained from day 14 of the recovery period: irrespective of the BLCO doses to which the fishes were previously injected (Table 2). Significantly, AST values were increased by 25% at day 14, 15% at day 28 and 5% at day 42. Despite these improvements of aspartic transaminase enzyme activities during the recovery period and up to day 42, the highest AST value recorded with the fish injected with 10.00 μlg^{-1} BLCO (6.57 ±0.4 $mg100ml^{-1}$) was still lower than the AST value of the control fish at day 42 (6.76 ±0.4 $mg100ml^{-1}$) (Table 2).

The percent mortality (PM) and the percent survival (PS) of the fish both at the toxicity and recovery periods (Table 3), indicated that the fish injected with between 40.00 μlg^{-1} and 50.00 μlg^{-1} BLCO concentrations recorded highest fish mortality and lowest fish survivals. Comparatively, the least fish mortality was recorded when the fish was injected with 10.00μlg^{-1} BLCO (Table 3).

Discussion

The decrease in the concentration of aspartate transaminase (AST) in *H. bidorsalis* adults of this study with increasing concentrations of injected BLCO agrees with the report of Ozmen *et al.* (2005) for other pollutants. The workers reported that the activities of lactate dehydrogenases, aspartate transaminase, carboxyl esterase, and acid phosphatase decreased with increasing concentrations of such metals as cadmium, copper and lead in water. Oluah and Njoku (2001) observed a linear relationship between tissue glucose levels in *Clarias gariepinus* caused by increased enzymatic activities and paraquat (herbicide) concentration in water. Additionally, Simon *et al* (1983) obtained similar results when *Cyprinus carpio* was exposed to paraquat. All these results infer that enzymatic responses to *in vivo* or *in vitro* concentrations of pollutants is dose-dependent.

This assertion was exemplified in this study by the significant decreases ($P < 0.05$; $P < 0.01$) (Table 2) in the AST values in fish livers as the doses of BLCO increased from 10 μlg^{-1} to 50.00 μlg^{-1}. Increases in AST values during the recovery period at day 14 (25%), day 28(15%) and day 42 (5%) (Table2) imply that the previous injections of the fish with BLCO had tremendous effect on the activities of aspartate transaminase enzyme within the liver. This result supports the suggestions made by Ozmen *et al.* (2005) that there is need for a comparative monitoring of biomarkers and pathological changes in liver tissues in order to identify the best enzymatic markers that would serve as indicators of organ dysfunction. The recorded percent increases in AST values within the 14-42 days recovery period (Table 2) were also influenced by the concentrations of BLCO previously injected into the fish. This result is also consistent with Ozmen *et al.* (2005) which stated that cholinesterase (AChE) activity of *Cyprinus carpio* during recovery from their exposure to pesticides might be influenced by the concentration of previous exposure to the pollutants. Sancho *et al*. (2000) also reported that AChE concentration was reduced in animals previously exposed to pesticides in their environment and later transferred or moved to clean water environment. Ozmen *et al.* (2005), however, reported that although some biomarkers notably: carboxyl esterase, lactate dehydrogenase, acid phosphatase, aspartic transaminase and cholinesterase are being used world-wide in several pollution monitoring programmes, some enzymes activities still require further research before they can be used routinely in pollution monitoring. From this study, aspartate transaminase enzyme could be used as a biomarker in *H. bidorsalis* adults for monitoring crude oil pollution of Nigerian waters.

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