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Full Length Research Paper

Changes in liver and plasma enzymes of *Clarias gariepinus* exposed to sublethal concentration of diesel

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This study investigated the effects of graded levels of sub-lethal concentrations of diesel on the biochemistry profile of *Clarias gariepinus* after exposure for a period of 28 days. 118 (male and female) *C. gariepinus* were used for the study. They were divided into four groups. Group 1 served as the untreated control (0) while Groups 2, 3 and 4 were treated with graded levels of diesel 0.3, 0.6 and 0.9 part per millon (ppm) for the study of liver enzymes. For plama enzymes study, Group 1 served as the untreated control (0), while Groups 2, 3 and 4 were treated with graded levels of diesel of 2.0, 3.0, 4.0 ml stock solutions for 28 days. The liver samples were collected and analysed for liver enzyme. Alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) were assessed on days 14, 21, and 28. Liver enzymes showed that exposure to diesel led to significant reduction ($p<0.05$) in ALT, AST and ALP activities after 14, 21 and 28 days of assessment. Evalution of the plasma enzymes showed that exposure to diesel led to significant increases ($p<0.05$) in plasma ALT, AST and ALP activity all through the studies except for Group B (0.4 ml) in which ALP was significantly higher ($p<0.05$) when compared to the control after 14 days of exposure. In plasma enzymes, a low significant relationship was observed between enzyme ALP and AST ($r=0.338$, $P<0.05$), low significant relationship was observed between enzyme ALT and ALP ($r=0.485$, $P<0.05$) also a low significant relationship was observed between enzyme ALT and AST ($r=0.868$, $P<0.01$).

Key words: Liver and plasma enzymes, *Clarias gariepinus*, diesel, clinical chemistry.

INTRODUCTION

Fish plays an important role not only in human diets but also in livestock nutrition. The African catfish, *C. gariepinus* of the family *Clariidae* is an important aquaculture species in many parts of Africa. They are highly resistant to muddy water and can survive extremes of aquatic deoxygenation and dessication (Bok and Jongbloed, 1984). It is also among the most common freshwater fish widely consumed in Nigeria. The African catfish is advocated for aquaculture because it grows fast and feeds on a large variety of agricultural by-product. It is also being raised in high densities resulting in high net yields (6 to 16t/half/year) (Omoergie et al., 1994). It matures fast and reproduces easily in captivity. *C. gariepinus* can therefore be a good model to study responses to various environmental contaminants.

Diesel fuel in general is any liquid fuel used in diesel engines. Petroleum-derived diesel is increasingly called petrodiesel. Ultra-low sulfur diesel (ULSD) is a standard for defining diesel fuel with substantially lowered sulfur contents (Chris, 2007). Exposure to combustion-derived fine particulate air pollution is a recognized risk factor for cardiorespiratory mortality and morbidity(Miller et al., 2007; Dockery et al., 1993). Inhalation of diesel exhaust, a major component of fine particulate air pollution in urban environments, impairs vasomotor function and endogenous fibrinolysis (Mills et al., 2007). Rats raised with daily exposure to diesel exhaust particles and urban particulate matter have increased blood pressure, plasma endothelium (ET-1) concentrations (Vincent et al., 2001), and ET-1 expression in cardiac tissue. Oil pollution, one of the environmental consequences of crude oil exploration activities produces aqua-toxicological effects,

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which are harmful to aquatic life (Karl-Siakpere, 2000; Agbogidi et al., 2005; Ajon et al., 1981). In Nigeria, oil industry operation are both onshore and offshore and all the oil terminals and most refineries in the country are located in the Niger Delta region and hence more than 90% of oil-related activities take place in this region (Imevbore and Adeyemi, 1981).

Spills incident in various scales involving different kinds of oil reported more rampant and endemic in the coastal area, because this is the site of most oil refining and terminal operations. The presence of crude and refined oils in the environment is often due to sabotage. The situation is quite different in the case of this used oil called “spent oil” where its disposal in the environment is deliberate and indiscriminate with disregard to its pollution effect. Petroleum hydrocarbon in its crude, refined or spent form has negative impact on both the human, animal and plant species. Oil in the environment constitute hazard to organisms which range from impairment of functions to death. The impacts of such oil spills on different aquatic organism need to be assessed on a regular basis in order to ascertain its immediate effect and also to predict its possible effects on physiological characteristic of fish (*C. gariepinus*) and other aquatic organisms hence the need for this study.

MATERIALS AND METHODS

The *C. gariepinus* used for the study were obtained from Anambra River Basin, Otuocha, Anambra state and acclimated to laboratory conditions. The catfish juveniles were divided into 15 plastic flow-through (60 L) plastic fish culture tanks containing twenty - five litres of water each and acclimated to laboratory conditions for 14 days, aerated and covered with mosquito nets to prevent the fish from jumping out. Each plastic tank had five catfish. During acclimatization period, fish were fed with standard feed twice daily. The feeding rates expressed as percent of body weight in *C. gariepinus* fingerlings at different body weight.The optimum recommended feeding rate was 4% body weight from 19.5 to 31 g and 3% of body weight for an increase of body weight from 33 to 47 g. For an increase in body weight from 47 to 90 g, the recommended feeding rate was 3% of body weight.

The recommended doses are subject to alteration with variation of water temperature. Water was replaced every 48 h after feeding in order to maintain a healthy environment for the fish. This helps circulate the oxygen supply for the fish and removal of any accumulated waste. After acclimatization, 50 post fingerlings of *C. gariepinus* with an average length 13 cm and average weight of 35 g were selected for liver enzymes study. The physico-chemical parameters of the water estimated as APHA (1998) and are as follows: dissolved oxygen, 6.2 ± 0.08 mg/L; pH, 7.2 ±12.00; water temperature, 26.0 ± 20°C.

Petroleum diesel also known as diesel-fuel obtained from Shell Company, Warri Nigeria was used for evaluation of its toxicity to fish.

The determination of the sub-lethal concentration was carried out by using separate circular plastic tubes of 60 L of water capacity were taken and different concentration of diesel. Four stock solution 0.3 part per millon (ppm), 0.6 and 0.9 ppm for each experimental tank (Keller, 1989). A control tube with 60 L of water and five fish were also maintained (no toxicant) for liver enzymes.

Liver enzyme analysis

At the end of 14, 21 and 28 days of exposure, the fishes were decapitated and dissected. The liver was dissected out and washed in distilled water to remove traces of blood. The liver samples were macerated and homogenized using 0.1 normal saline and then placed in ice-cold 0.25 M sucrose (Oluah et al., 2005). The liver homogenate was centrifuged for 15 min at 4°C and the supernatant was transferred into clean microplate. The samples were stored at 80°C until enzymatic assays were carried out (Oluah et al., 2005).

Plama enzyme

The stock solutions were 2.0, 3.0 and 4.0 ml/L, respectively for each flow tank. The flow tanks were properly sealed with nylon nets and a rubber to prevent the fish from jumping. 68 *C. gariepinus* were subjected to different concentrations (0, 2.0, 3.0 and 4.0 ml/L) of diesel - fuel. Each of these toxicants where introduced in triplicate: Group 1(untreated control) treatment Groups 2, 3 and 4). Each of the experimental tanks contain four groups and five fishes each. The experimental tanks contained 60 L of water and labeled according to treatment.

Plasma enzymes analysis

At the end of the exposure period of 14, 21 and 28 days, blood sample from fish were collected by cardic puncture. The blood was collected in labeled sample bottles for the analysis. The aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were determined according to Reitman and Frankel colometric method using Randox kits, while Alkaline phosphatase activities was determined according to phenolphthalein monophosphate method (1954) using Randox kits. The results were statistically analyzed using one-way analysis of variance (ANOVA) model of 1996 and Pearson’s correlation.

RESULTS

Liver and plasma enzymes

The liver enzymes profile of the *C. gariepinus* after 28 days of exposing to different concentrations of diesel is presented in Table 1, while the plasma enzymes after 28 days of exposure is presented in Table 2. The mean liver AST activity of all the treated fish groups (B to D) were significantly lower ($p< 0.05$) than that of the control (untreated Group A) after 14, 21 and 28 days of exposure (Table 1).

The mean ALT activity of the control (untreated Group A) was significantly lower ($P < 0.05$) than that of treated Groups B, C and D after 14 days of exposure (Table 1). However the mean liver ALT activity of all the other fish groups (B to D) exposed to diesel were significantly lower ($p< 0.05$) than that of the control (Group A) after 21 days.

After 28 days of exposure, the mean liver ALT of Groups B and C were significantly lower ($p<0.05$) than that of the control. The mean liver ALP activity of all the fish groups (B to D) exposed to diesel were significantly lower ($p<0.05$) than that of the control (Group A) after 14, 21, and 28 days of exposure (Table 1).

Table 1. Effect of different concentrations of diesel on the liver enzymes of *C. gariepinus* after 14, 21 and 28 days of exposure.

Concentration (ppm) of diesel	AST (I/U)	ALT (I/U)	ALP (I/U)
14 days of exposure			
Group A 0 (control)	20.33 ± 1.33^{c}	16.00 ± 0.00^{a}	85.00 ± 2.03^{d}
Group B (0.3)	17.00 ±1.00^{b}	19.67 ± 1.33^{b}	58.00 ± 0.58^{a}
Group C (0.6)	13.00 ± 0.00^{a}	17.00 ± 0.00^{ab}	65.67 ± 0.88^{b}
Group D (0.9)	17.00 ± 1.00^{b}	19.67 ± 1.33^{b}	73.33 ± 2.73^{c}
21 days of exposure			
Group A 0 (Control)	23.00 ± 0.00^{b}	26.67 ± 3.84^{a}	84.33 ± 0.67^{a}
Group B (0.3)	16.00 ± 0.00^{a}	21.00 ± 0.00^{a}	78.00 ± 10.50^{a}
Group C (0.6)	17.00 ± 1.00^{a}	25.00 ± 2.31^{a}	66.67 ± 0.88^{a}
Group D (0.9)	21.67 ± 1.33^{b}	25.00 ± 0.00^{a}	69.33 ± 1.20^{a}
28 days of exposure			
Group A 0 (Control)	34.33 ± 1.67^{c}	39.00 ± 0.00^{b}	88.33 ± 0.33^{c}
Group B (0.3)	27.00 ± 0.00^{a}	26.33 ± 1.33^{a}	84.33 ± 1.86^{bc}
Group C (0.6)	28.33 ±1.33^{ab}	29.00 ± 0.00^{a}	73.00 ± 2.52^{a}
Group D (0.9)	31.00 ± 0.00^{bc}	37.00 ± 3.00^{b}	80.33 ± 1.86^{b}

Result expressed as mean ± S.D. Values with same super script on the colunm are not significantly different from each other (p<0.05).

Table 2. Effect of different concentrations of diesel on the plasma enzymes of *C. gariepinus* after 14, 21 and 28 days of exposure.

Concentration (ppm) of diesel	AST (I/U)	ALT (I/U)	ALP (I/U)
14 days of exposure			
Group A (control)	10.00 ± 0.00^{a}	5.33 ± 2.31^{a}	75.33 ± 4.73^{a}
Group B (0.4 ml)	7.33 ± 0.58^{b}	8.00 ± 0.00^{ab}	86.00 ± 3.46^{b}
Group C (0.6 ml)	10.00 ± 0.00^{b}	9.33 ±.2.31^{b}	84.67 ± 3.21^{b}
Group D (0.8 ml)	16.00 ± 0.00^{c}	17.00 ± 0.00^{c}	85.33 ± 2.05^{b}
21 days of exposure			
Group A (control)	9.00 ± 1.73^{a}	5.33 ± 2.31^{a}	76.00 ± 2.65^{a}
Group B (0.4 ml)	10.00 ± 3.00^{a}	13.67 ± 1.67^{b}	84.7 ± 4.16^{b}
Group C (0.6 ml)	11.00 ± 1.73^{a}	18.33 ± 2.31^{c}	86.67 ±1.53^{b}
Group D (0.8 ml)	21.67 ± 4.62^{c}	22.33 ± 2.31^{c}	87.33 ± 2.08^{b}
28 days of exposure			
Group A (control)	16.00 ± 0.00	8.00 ± 0.00^{a}	80.67 ± 2.89^{a}
Group B (0.4 ml)	17.00 ± 1.73^{a}	22.33 ± 4.62^{b}	84.67 ± 3.51^{ab}
Group C (0.6 ml)	23.00 ± 4.00^{b}	29.00 ± 0.00^{c}	83.00 ± 2.00^{ab}
Group D (0.8 ml)	27.00 ± 0.00^{b}	34.00 ± 5.00^{c}	87.33 ± 1.15^{b}

Result expressed as mean ± S.D. Values with same super script on the colunm are not significantly different from each other (p<0.05).

The mean plasma ALP activity of all the treated fish groups were significantly higher (p<0.05) than that of the untreated fish group after 14, 21 and 28 days respectively (Table 2). The mean plasma of ALT activity of all the treated fish groups were significantly higher (p<0.05) than that of the untreated fish group (control) after 14, 21 and 28 days of exposure to diesel. After 14 days of exposure to diesel, the mean serum AST of the Group D fishes were significantly lower (p<0.05) than that of the control while that of the Group D was significantly higher (p<0.05) than that of the control (Table 2). After 21 days post- exposure, the mean serum AST of the Group D was significantly higher (p<0.05) than that of the control and other treated groups, but after 28 days of exposure, fishes in Groups C and D had a significantly higher (p<0.05) plasma AST than the control and Group B. No

sex-related significant differences (P > 0.05) was observed. In plasma enzymes, a low significant relationship was observed between enzyme ALP and AST (r=0.338, P<0.05), low significant relationship was observed between enzyme ALT and ALP (r=0.485, P<0.05) also a low significant relationship was observed between enzyme ALT and AST (r=0.868, P<0.01).

DISCUSSION

Diesel produces many hazardous compounds which are potent immunotoxicants and carcinogenic for living being. Diesel induced variable degenerative changes in the structural integrity of hepatic cells and its enzymes. These toxic effects of diesel are equally exerted on a variety of tissues and organs of fish (Azad, 2005; Dimichele and Taylor, 1978). The result obtained in this study in liver enzymes suggests that ingestion of diesel increased serum mean ALT level which indicated myocardial infarction, cirrhosis and stress; similiar increases were observed in serum mean ALP, AST and ALT in plasma enzymes. This is similar to the study of a Wedemeyer and Yasutake (1977) which reported that exposure of various stressor usually elicits changes in liver enzymes with the lowest concentration having the highest effect. Although liver pathology, hepatocellular lipid vacuolization was suggested by McCan et al. (1978) to be non-specific liver lesions induced by exposure to a variety of hydrocarbon at toxic levels. The significant increase in plasma enzyme activity indicated that diesel stimulated aspartate aminotransferase which is a mitochondria enzyme. The increase in the plasma could be due to toxic injury caused by diesel which stimulated tissue repair through protein turnover and increased respiration. Nivedita et al. (2002) reported a similar work although fresh water fish was exposed to diethylphttalate. Assessment of plasma and liver enzyme activities can be considered as diagnostic tool to determine the physiological status of cells or tissue (Manoj, 1999; Tyson and Sawhney, 1985). Alteration in plasma enzyme activities of fish resulting from toxicants or contaminant affecting various cells, immune system, tissues and organs of fish have been reported (Sastry and Subhadra, 1985; Gill et al., 1991; Begun, 2004; Gabriel and George, 2005). The mean serum ALP and ALT activities were similar to the work, Fatma and Nahed (2000) in which they reported significant increase in ALP and ALT which may be indicative of generalized pathological changes and damage to specific organs of *C. gariepinus* . The increase of plasma ALP, ALT and AST in the fishes contradicted that of Inyang et al. (2010) although, *C. gariepinus* was exposed to sublethal concentration of Diazinons. A decrease in transaminases in liver enzyme suggested that there was no tissue damage (Ayalogu et al., 2001; Luskova et al., 2002; Oluah and Amalu, 1998). The decreases in ALP may be due to the fall in rate of synthesis of glycogen resulting from low mtabolim demand (Shaffi, 1979) and decrease in metabolic transport (Begum, 2004; Edquisit et al., 1992). The minimal change in fish condition may be due to the fact that the various concentration used by Gabriel et al. (2009) and Inyang et al. (2010) were below threshold value or before sampling, the fish was able to detoxify and excrete the waste thereby maintaining its physiological condition without negative effect.

Conclusion

This research has shown that plasma enzymes activities was increased due to the effect of diesel while liver enzyme showed a decrease in activities, therefore, even the minute concentration of diesel in the environment can cause a lot of physiological changes. Since toxicity occurred with low concentrations of diesel with a subsequent rise in enzymes activities of *C. gariepinus,* Crosby (1991) noted that fish affected by these toxicant would like-wise affect other biological systems. Exposure to diesel pollution is a recognized risk fresh water for cardiorespiratory mortality and morbidity in fish.

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