BIOCHEMICAL CHANGES IN THE LIVER OF JUVENILE AFRICAN CATFISH (*Clarias gariepinus*) EXPOSED TO CRUDE OIL

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Abstract
This study was carried out to examine the Biochemical changes in juvenile African catfish (Clarias gariepinus) exposed to crude oil. The study was conducted using 180 juvenile catfish with mean weight of 7.993 ±0.98g and mean total length of 9.7 ± 0.72cm exposed to different concentrations (0.0%, 0.1%, 0.25%, 0.5%, 0.75% and 1.0%) of crude oil for 20 days (480 hours). The experiment was laid out as a completely randomized design and replicated thrice. Data obtained from biochemical changes, in the liver of fish were analyzed using Genstat package (version 12.1). Means were separated by the New Duncan Multiple Range Test. There were significant differences P<0.05 between enzymatic activities of the liver, Alkaline Aminotransferase (ALP) Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) in comparison with the control. There was significant elevation of ALP (36 iµ/l) and AST (88 iµ/l) in concentrations 0.10% compared to the control. Results showed no significant differences (P>0.05) between total bilirubin concentration (ranges of 0.6 mg/dl to 0.8 mg/dl) and conjugated bilirubin concentration (ranges of 0.3 mg/dl to 0.4 mg/dl). The accumulation of crude oil in the liver might cause serious pathological damage following exposure

Keywords: Crude Oil, Biochemical, Liver, *Clarias gariepinus,* Enzymatic Activities of the Liver

Introduction

The effects of oil spill on aquatic lives are caused either by the physical nature of the oil or by its chemical components. The water soluble components of crude oils and refined products may prove toxic to fish and amphibians, with their eggs and young stages being especially vulnerable. Toxic effects are difficult to predict because individual samples of crude oil and refined products consist of many varied compounds of differing toxicities. Sublethal effects may be recorded at the biochemical, physiological or behavioural levels (Mason, 1981).

It is believed that fish possess the same biochemical pathways to deal with the toxic effects of endogenous and exogenous agents as do mammalian species, (Al-Akel *et al.,* 2010; Alkahem-Al-Balawi *et al.,* 2011).

Alamine amino transferase (ALT) and Aspartate amino transferase (AST) are liver specific enzymes and they are more sensitive measure of hepatotoxicity and histopathologic changes which can be assessed within shorter time (Balint *et al.,* 1997). Oluah (1999) noted that increase or decrease in the values of ALT and AST indicate tissue damage in liver, kidney, muscle and gill. Alterations in alkaline phosphate (ALP) activities in tissues, organs and serum have been reported in fish exposed to toxicants of varying concentrations (Jyothi and Narayan, 2000).

Haemoglobin and Red blood cell break down can be accelerated by exposure to toxicants. Bilirubin is a bile pigment and the end-product of red blood cell breakdown. High levels indicate liver damage while low levels are not considered clinically relevant (Patient.co.uk, 2010). Enzyme analysis has been widely used for rapid detection to predict early warning of pesticide toxicity (Duta and Areids, 2003). In exposure of *C. gariepinus* to different concentrations of cypermethrin, generally lowered activities of AST, ALT, ALP and LDH, in the gill muscle, kidney and liver were observed. Li *et al.,*(2004) obtained similar results when they exposed rainbow trout to carbamazepine for 42hrs. They observed that carbamazepine inhibited AST and ALT activities in the muscle, liver, gills and kidney and reported that enzymes in the liver, followed by kidney and the gill seem to be mostly affected by carbamazepine poisoning. Furthermore, Goel *et al.,* (1992) reported that the results of reduced activities of AST, ALT, ACP, ALP and LDH in various organs of fish (*Pontius conchonius*) exposed to mercury implies destruction in the tissues of the animals. ALT activity of fishes exposed to paper mill effluent at the mouth of the effluent discharge also decreased when compared to fishes downstream as reported by Adams *et al.,*(1996). This he assumed might be due to hepatoxicity which results in the liberation of the intracellular enzymes. The lower values of AST, ALT and ALP enzyme activities when compared to the controls showed that, inactive transamination and oxidative deamination has taken place. In the other hand, studies carried out by Das *et al.*,(2004) showed that there was an elevation in activity level of AST, ALT and ATP of Indian major carps exposed to nitrite toxicity and suggested that the elevation of the transferases is as a result of the diversion of the alphamino acids in the TCA cycle as keto acids to augument energy production.

The objective of this study therefore was to assess the biochemical changes in the liver of the African catfish juveniles exposed to different concentrations of crude oil

Materials and Methods

The exposure test was carried out at Faculty of Agriculture Laboratory, University of Benin and lasted for period 480 hours. Crude oil for the study was collected from the Lease Automatic Custody Transfer (LACT) unit of the Nigerian Petroleum Development Company (NPDC) at Pan Ocean, Oredo Flow Station. A sample was diluted with borehole water to obtain concentrations of 0.00% (Control), 0.10%, 0.25%, 0.50%, 0.75% and 1.00% by volume (Sunmonu and Oloyede, 2006).

Healthy live juveniles of the African catfish (*Clarias gariepinus*) were collected from Samdoc Fish Farm, Sapele Road, Benin City. They were acclimated for seven days to laboratory conditions with borehole water and fed with 2mm Coppens Feed at 4% body weight daily, at 8.00 am and 4.00 pm respectively during the study period.

Biochemical Analysis

Biochemical procedures and examination were carried out at the Haematology and Chemical Pathology Laboratory of the University of Benin Teaching Hospital, Benin City Nigeria.

These are specifically the liver function test. Liver function test measures the concentration of various proteins and enzymes in the blood that are produced by the liver cells or released when liver cells are damaged (Virtual Medical Centre.com, 2010). They include: Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Alkaline Phosphatase (ALP), Bilirubin and Conjugated bilirubin.

Blood samples collected were placed in capped plastic tubes for analysis, centrifuged at 120g for 5 minutes at 2°C in order to remove the blood cells and debris. The resulting supernatants were used to analyze the above parameters. These were measured spectrophotometrically (Photometer 5010®-Boehringer Mannheim) following the procedures defined in the commercial test kits (Biolabo, France).

Statistical Analysis

Data obtained were subjected to one-way Analysis of Variance (ANOVA), using Genstat (12 Edition). Significant treatment means were separated using the New Duncan Multiple Range Test

Results

Biochemical Parameters (Liver Function Tests)

In Figure 1, the specific activities of ALP in the liver of catfish exposed to various concentrations of crude oil for 20 days were significantly different ($P<0.05$). The highest level was however, obtained in catfish exposed to 0.10% crude oil (36I µ/L).

Figure 2 shows the level of ALT in the serum of catfish exposed to various concentrations of crude oil compared with the control, a significant reduction ($P<0.05$) was obtained in the concentrations 0.10% (25I µ/L) and 1.0% (26I µ/L).

There were no significant differences ($P>0.05$) between the concentrations 0.5% and 0.75% and 0.10% and 1.0%

Significant differences ($P<0.05$) existed in the AST activities in the liver of catfish exposed to various concentrations of crude oil for 20 days. The highest level was observed in 0.10% (88I µ/L). While the lowest level was observed in 0.50% (82I µ/L).

There were no significant differences ($P>0.05$) between control (0.00%) and 0.75 % and 1.0 % (Figure 3).

Figures 4 and 5 show the level of total bilirubin (TB) and conjugated bilirubin (CB) in the serum of catfish exposed to various concentrations of crude oil. There were no significant differences (P>0-05) between the various concentrations of crude oil after 20 days of exposure. However, lowest level of total bilirubin (0.6 mg/dL) and conjugated bilirubin (0.3 mg/dL) were observed in 0.25 %.

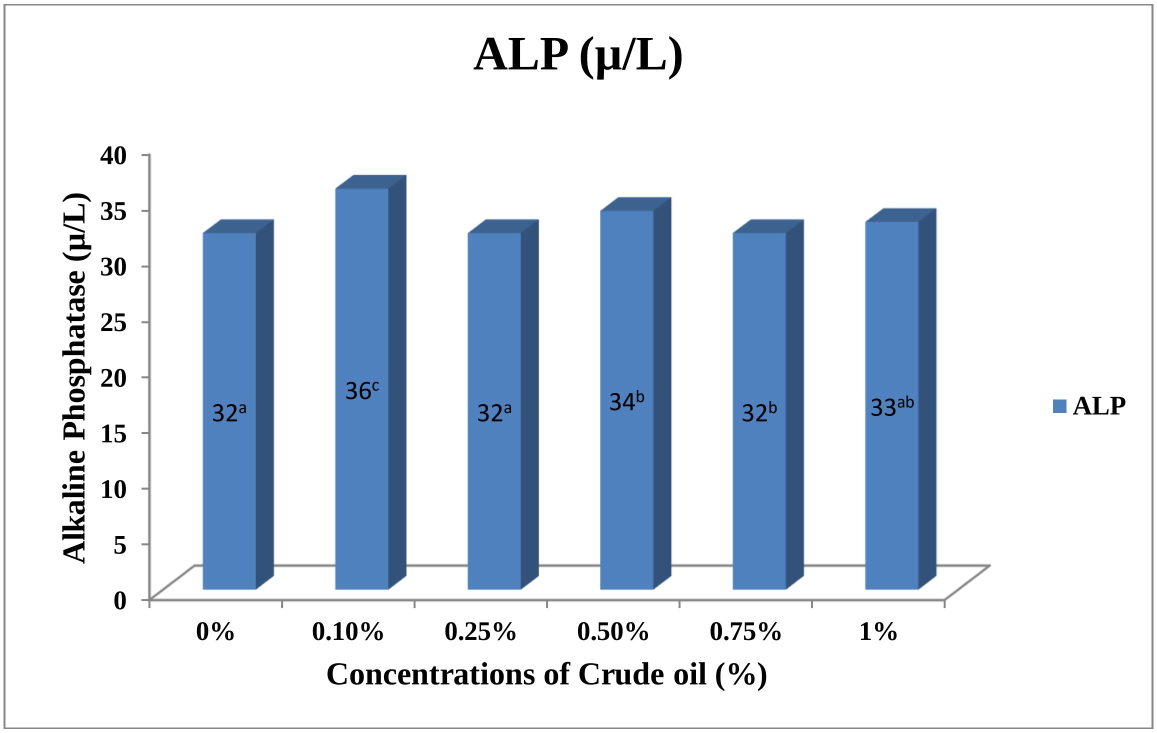

Figure 1: Alkaline Phosphatase Concentration in the blood of *Clarias gariepinus* with different concentration of crude oil.
abc Means with different superscripts in the bars differ significantly

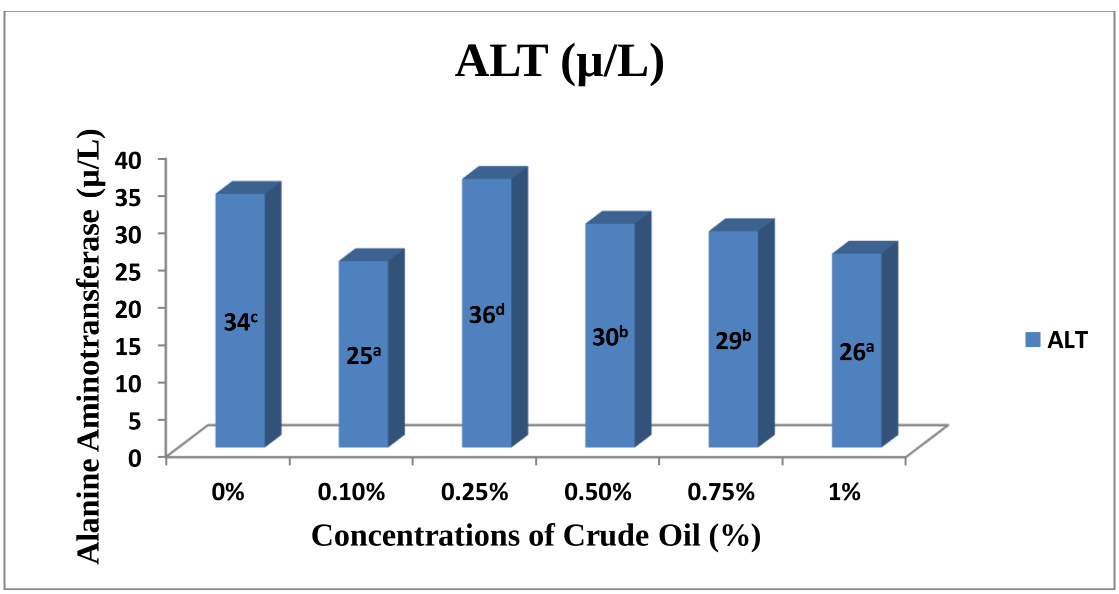

Figure 2: Alanine Aminotranferase in the blood of *Clarias gariepinus* with different concentrations of crude oil.
abc Means with different superscripts in the bars differ significantly

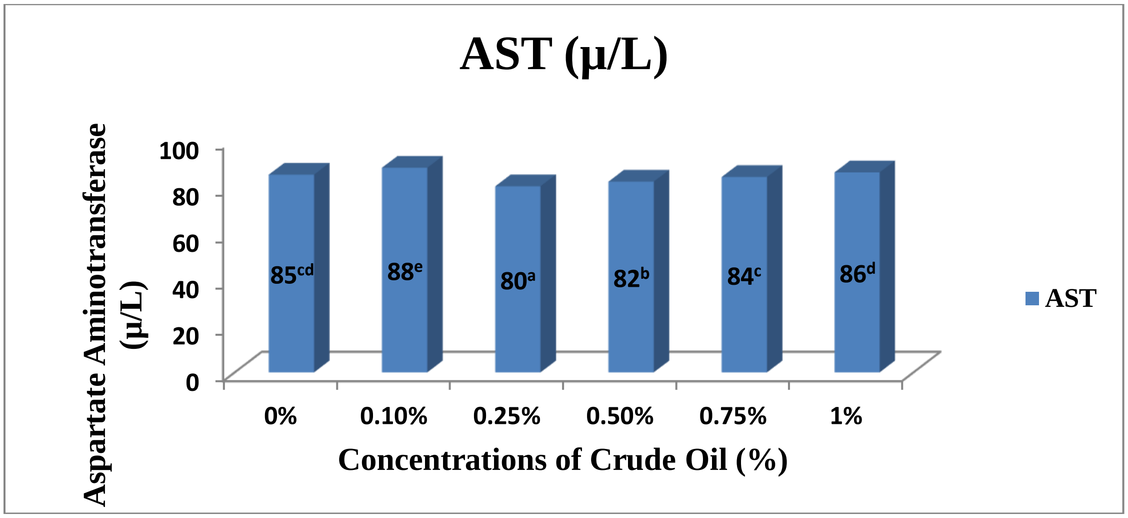

Figure 3: Aspartate Aminotranferase in the blood of *Clarias gariepinus* with different concentrations of crude oil.

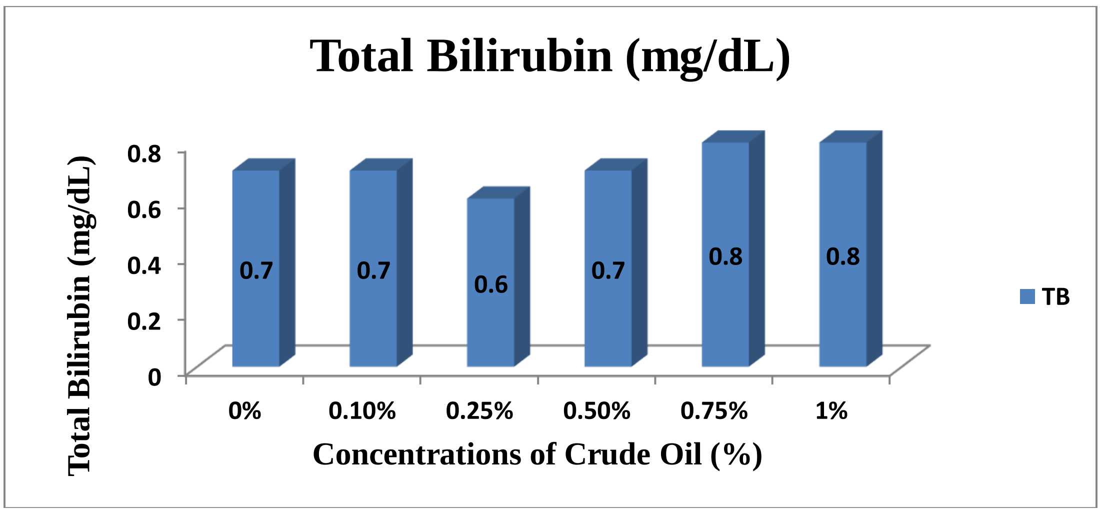

Figure 4: Total Bilirubin (TB) in the blood of *Clarias gariepinus* with different concentrations of crude oil.

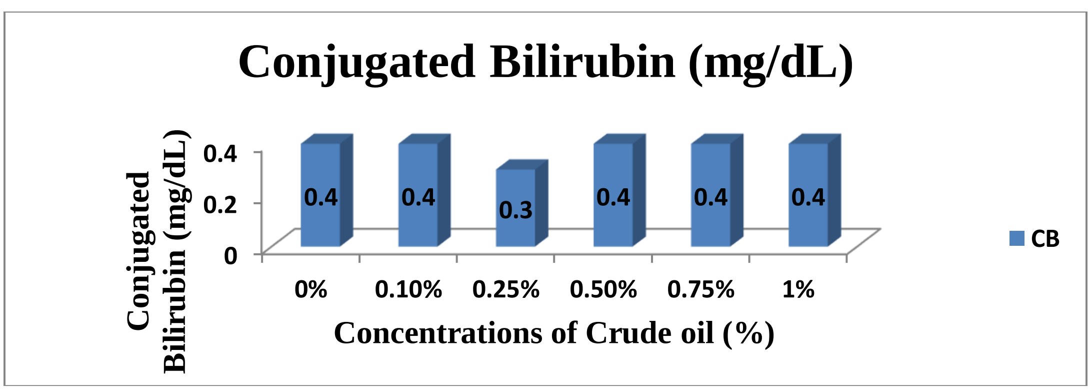

Figure 5: Conjugated Bilirubin (TB) in the blood of *Clarias gariepinus* with different oncentrations of crude oil. [abc] Means with different superscripts in the bars differ significantly

Discussion

The values of ALT obtained in this study did not follow a definite pattern. There was a decrease in 0.10 % concentration (25 IU/L) relative to the control (34 IU/L) followed by an increase in 0.25 % concentration (36 IU/L) and then a decrease in 0.50 % (30 IU/L), 0.75 % (29 IU/L) and 1.0 % (26 IU/L).

ALT generally showed a decline in activity from that of the control (34 IU/L) except 0.25 % (36 IU/L), which corroborated the findings of Gabriel, *et al.*, (2009) and Sunmonu and Oloyede (2006). Abdel-Tamneab, *et al.*, (2001) also observed a similar result in the liver ALT of the Nile tilapia after exposure to mercury. These workers ascribed the reduction in the enzyme activity to liver necrosis caused by the toxicants and a possible damage to the hepatocytes. ALP and AST recorded elevations at 0.10 % and 1.0 % when compared to the control (0.0 %). Sepici-Dincel *et al*., (2009) observed similar increase in activities of AST and ALT in muscle and liver of the common Carp (*Cyprius carpio*) exposed to 10 µg/L of cyfluthrin. Yildirim *et al.,* (2006) also observed similar changes in *Oreochromis niloticus* exposed to deltamithrin for four days and observed increase in enzyme activities in the gill, liver and kidney and that the observed enzyme elevation is intended to increase the role of proteins in the energy production during toxicant stress.

The accumulation of PAHS in the liver, stomach and other organ might cause serious pathological damage following exposure Vutukuru *et al.,* 2007). Vutukuru *et al.* (2007) reported that when liver cell is damaged, tissue specific enzymes are released into the blood stream, thus making the enzymes level in the blood to go up. Values obtained in this study are contrary to the values of Myburgh *et al*., (2008) who recorded reference ranges of 4.4 to 16.4 mg/ dL for sharp tooth catfish (*Clarias gariepinus*) and 0.0 to 6.8 mg/dL for channel catfish. There were no significant differences (P>0.05) between the bilirubin and conjugated bilirubin levels in the treatments relative to the control. Low levels of bilirubin and conjugated bilirubin may indicate that the livers of catfish in all the treatments have not been severely damaged and this may explain why mortality of fish was not recorded throughout th e period of exposure (Orowe, 2013).

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