HAEMATOLOGICAL AND SERUM BIOCHEMICAL CHANGES IN *Clarias gariepinus* EXPOSED TO SUB-LETHAL CONCENTRATIONS OF WATER SOLUBLE FRACTIONS OF CRUDE OIL.

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ABSTRACT

The exploitation of crude oil resource in Nigeria has resulted in the continuous exposure of fish to crude oil and its refined products. In this study, alterations in haematological and biochemical parameters were evaluated after exposure of *Clarias gariepinus* to water soluble fraction of crude oil. The 96 h-LC_{50} of crude oil determined by probit regression was 224.74 mg/l. Fish were exposed to 4 sub-lethal concentrations (30, 45, 60 and 75% of the LC_{50} corresponding to 67, 101, 135 and 169 mg/l respectively) of the oil and a control. After 90 days of exposure, blood was collected and used for haematological and biochemical analyses. Results showed a significant ($P<0.05$) reduction in the number of red blood cells, and values of packed cell volume and haemoglobin with no definite trend in the values of computed haematological indices. Glucose content (mg/dl) increased significantly ($P<0.05$) from 289.00 in control to 384.67 in the highest concentration (33% increase) indicating the occurrence of gluconeogenesis to supplement additional energy needed to meet the increased metabolic demands. A significant ($P<0.05$) inverse relationship between the concentration of crude oil and values of total protein, albumin and globulin was also observed. The mean plasma alanine aminotransferase, alkaline phosphatase, aspartate aminotranferase, creatinine and urea activities of all fish, which have been exposed to sub-lethal concentrations of crude oil were significantly higher ($P<0.05$) in comparison with their respective control. The upsurge in the activity of these enzymes is a direct reflection of alterations in the hepatic structural integrity and kidney disorder.

Key Words: Crude oil, Haematology, Biochemistry, *Clarias gariepinus*

INTRODUCTION

Nigeria is one of the leading oil producers in the world, with the Niger Delta region the epicenter of oil and gas production (Sam *et al*., 2017). However, one of the major environmental problems in this region since the inception of oil exploration, processing and transportation by multinational oil companies and pipeline vandalization has been that of oil spillage (Eriegha and Sam, 2020). Fish and aquatic invertebrates in the Niger Delta waters are continuously being exposed to crude oil and its refined products as a consequence of the petroleum spills occurring every year (Kadafa, 2012). Crude oil varies considerably in its toxicity according to its origin or the method of refining and the length of exposure to air that encourages the escape of highly volatile component. In order to minimize these sources of disparity, it is important to state the source of crude oil and standardize the method of preparation of test solutions. The use of the water soluble fraction (WSF) in toxicity testing represents what is actually occurring in nature after a spill (Anderson *et al*., 1974). Clinical pathological assays are relevant tools in evaluating the physiological and pathological statuses, that aid clinicians to arrive at proper diagnoses of diseases, guide prognosis and asses the toxicity of chemical substances (V´azquez Guerrero, 2007). Haematology and serum biochemical assessment, along with physical examination, help to determine the indicators of stress response and sensitive biomarkers crucial to physiological functions, and predict pathological processes in the vital internal organs (V´azquez Guerrero, 2007). They also help to establish the presence or absence of disease of an organ, and determines the nature and extent of a disease process by serial performance of laboratory tests for the internal organs where little or no clinical signs of disease are observed even when seriously ill within the aquatic environment (Kim *et al*., 2008).

The African catfish, *Clarias gariepinus* (Burchell, 1822) has exceptional qualities that have increased its importance in ecotoxicological studies. It is one of the most important fish species currently being cultured both inside and outside its natural range of tropical and subtropical environments. This species has been used previously in laboratory studies and have been shown to be a suitable organism for monitoring the effects of xenobiotics (Omitoyin *et al*., 2006). Several authors have investigated the effect crude oil on fish especially on survival and growth (Olaifa, 2012; Ikeogu *et al*., 2013), while a few others have evaluated its effects on the biochemical and haematological components (Awoyinka *et al*., 2011; Rostam & Soltani, 2016). However, there is little information on the composition of the crude oil causing such toxicity. The objective of this study was to evaluate the sub-lethal effects of water soluble fractions of crude oil in *C. gariepinus* using the hematological and biochemical parameters as biomarkers and also present information on the composition of the WSF of the oil causing such toxicity.

MATERIALS AND METHODS

Fish: Three hundred healthy juveniles of *C. gariepinus*, average weight 5.1 ± 0.3 g, procured from Aquatech College of Agriculture Fish Farm, Ibadan were used for the study. The fish were transported to the Experimental Unit of the Department of Fisheries and Aquaculture, University of Ibadan. Health status of the fish were ascertained based on the absence of lesions and other physical injuries on the body such as eroded mouth, hemorrhage, ulceration and other morphological diagnostic symptoms. After the examination, fish were kept in the laboratory for three weeks to allow for acclimation. During the period of acclimatization, they were fed twice a day with a commercial diet. Feeding of fish was terminated 24-hours before the experiment.

Preparation of Water Soluble Fraction of crude oil: Crude oil was obtained from the Afiesere oil field, near Ughelli in Delta State of Nigeria and was transported to the Department of Chemistry, Faculty of Science, University of Ibadan where water soluble fraction (WSF) of the oil was prepared using Anderson *et al.,* (1974). The Prepared WSF was also analysed using standard methods. Different concentrations of the WSF were prepared by diluting the stock WSF with water.

Experimental Procedure: Preliminary short-term (96 h) static toxicity test was performed in order to define suitable lethal concentration of WSF to *C. gariepinus* using Reish & Oshida (1987) and Odiete (2003). Nominal concentrations for definitive test were 56, 100, 180, 320, 576 mg/l and a control. All treatments and controls were conducted in triplicate, where 30 fish were randomly distributed for each treatment (n=10 per tank). Fish mortality was observed every 24 h. Mortalities recorded were expressed as percentages of the test populations and the median lethal concentration (LC_{50}) values were calculated by using regression equation method of probit analysis. The 96 hrs LC_{50} determined through probit analysis was 224.74mg/l. To evaluate the effects of the crude oil on Haematological and Biochemical parameters, fish were randomly exposed to 4 sub-lethal concentrations (30, 45, 60 and 75% of the LC_{50} corresponding to 67, 101, 135 and 169mg/l respectively) and a control group containing clean water in 30 L experimental tanks containing 20 fish each in triplicate. The tanks were filled with 20 L of the test solution and covered with a lid made of fine polyethylene gauze screen of 1mm mesh size to prevent the fish from jumping out of the containers. Experimental fish were fed *ad libitum* twice daily with a commercial feed containing 42% Crude Protein for 90 days.

Haematology and Biochemical analyses: Blood samples were withdrawn by caudal puncture, with the help of a needle and syringe from 3 randomly selected fish from each concentration (one fish from every replicate) into heparin tubes, and transported to the Department of Veterinary Pathology, University of Ibadan. Number of red blood cells (RBC) and white blood cells (WBC) were counted in a Neubauer chamber; packed cell volume (PCV) by the microhematocrit technique; and hemoglobin level (Hb) by the cyanomethemoglobin method. Haematological indices were computed using standard formulae. Total protein, albumin and alanine aminotransferase (ALT) were determined using haemocytometric. Alkaline phosphatase (ALP) level was determined by colourimetry while Urea and Creatinine by diacetyl reaction methods. The RANDOX® kit was used for the determination of the aspartate aminotranferase (AST), while Glucose was measured in the laboratory using an electronic blood glucose meter. Globulin was determined by subtracting albumin from total protein.

Statistical analysis: Data were expressed as mean ± SD (standard deviation). Mean values for the different experimental conditions were compared using one-way analysis of variance (ANOVA) followed by the Tukey's test. The significance level adopted was 95% ($P < 0.05$). Statistical analyses were performed using the software SPSS Version 20.

RESULTS

The results of the physical and chemical properties of the water soluble fraction (WSF) of the crude oil used for the bioassay is presented in Table 1. Total petroleum hydrocarbon (TPH) was 1820mg/l. Changes in haematological parameters of *C. gariepinus* after 90 days of exposure to sub-lethal concentrations of crude oil are presented in Table 2. The result showed a significant ($P<0.05$) reduction in the values of red blood cells (RBC), packed cell volume (PCV) and haemoglobin. RBC reduced from 3.32×10^{6}µl

in control to 1.87×10^{6}µl in highest concentration (75% of LC_{50} i.e. 168.56mg/l) while PCV decreased from 32.67% in control to 22.20% in highest concentration. Haemoglobin reduced drastically from 11.37 in control to 6.63g/l in highest concentration. Although no absolute pattern of changes in the values of all the computed haematological indices (mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration and mean corpuscular volume) were observed, analysis of variance (ANOVA), however detected significant difference ($P<0.05$) between them.

Mean values and standard deviation of biochemical parameters of *C. gariepinus* after exposure to sub-lethal concentrations of water soluble fractions of crude oil is presented in table 3. The Glucose content (mg/dl) increased significantly ($P<0.05$) from 289.00 in control to 384.67 in the highest concentration (168.46mg/l). The mean plasma ALP, ALT, AST, Urea activities of all the affected set of fish, which have been exposed to sub-lethal concentrations of crude oil were significantly higher ($P<0.05$) in comparison to their respective control. Exposure of *C. gariepinus* to WSF of crude oil also led to significant increase in urea compared to control. The urea level was 8.63 mg/dl in the control group and increased to 10.97 mg/dl in the highest concentration (168.56 mg/l). No definite trend was observed in the values of creatinine. Although, the lowest value was observed in control (0.53mg/dl) compared to 0.87 mg/dl in highest concentration.

Table 1: Properties of water soluble fraction used for the bioassay

Parameter	Value
Apparent Colour (K_2PtCl_6)	5
Appearance	Clear
Temperature (^oC)	25.5
pH (at stated temperature above)	6.94
Alkalinity ($mgCaCO_3/L$)	173
Chloride (mg/L)	16.3
Total Hardness ($mgCaCO_3/L$)	169
Calcium (mg/L)	60
Magnesium (mg/L)	9.73
Total Dissolved Solids (mg/L)	321
Total Solids (mg/L)	377
Biological Oxygen Demand (mg/L)	6.97
Chemical Oxygen Demand (mg/L)	764
Turbidity (FTU)	38.5
Nitrate (mg/L)	2.18
Sulphate (mg/L)	27.4
Phosphate (mg/L)	3.18
Total Petroleum Hydrocarbon (mg/L)	1820
Lead (mg/L)	0.92
Copper (mg/L)	0.50
Cadmium (mg/L)	0.53
Chromium (mg/L)	0.44
Nickel (mg/L)	0.36
Iron (mg/L)	1.02

ND- Non Detectable, FTU- Furan Transform Unit

Table 2: Haematological parameters of *C. gariepinus* following exposure to sub-lethal concentrations of crude oil. Data are means ± S.D (n=3).

Parameters	**% Concentration LC_{50}**				
	0%	30%	45%	60%	75%
PCV (%)	32.67 ± 1.53^{c}	30.33 ± 1.15^{bc}	28.67 ± 0.58^{b}	26.67 ± 2.08^{b}	22.20 ± 3.73^{a}
Hb (g/L)	11.37 ± 0.32^{d}	9.83 ± 0.28^{c}	8.73 ± 0.32^{b}	8.83 ± 0.29^{b}	6.63 ± 0.11^{a}
RBC ($\times 10^6$µl)	3.32 ± 0.06^{c}	3.15 ± 0.08^{c}	3.08 ± 0.09^{c}	2.46 ± 0.06^{b}	1.87 ± 0.29^{a}
WBC ($\times 10^3$µl)	12.22 ± 0.26^{a}	14.33 ± 1.04^{bc}	12.67 ± 0.29^{ab}	15.22 ± 0.37^{c}	13.52 ± 0.88^{abc}
MCV (fL)	98.24 ± 5.66^{a}	96.19 ± 2.63^{a}	93.10 ± 1.11^{a}	108.29 ± 8.67^{ab}	122.27 ± 14.45^{b}
MCH (pg)	34.19 ± 1.58^{ab}	31.19 ± 1.04^{ab}	28.40 ± 1.94^{a}	35.85 ± 0.44^{b}	35.91 ± 4.99^{b}
MCHC (g/dL)	34.82 ± 0.88^{b}	32.46 ± 1.93^{ab}	30.49 ± 1.75^{ab}	33.24 ± 2.53^{ab}	29.33 ± 1.53^{a}

Different letters indicate significant difference mean values among treatments ($P < 0.05$).

Table 3: Biochemical alterations in *C. gariepinus* following exposure to sub-lethal concentrations of crude oil. Data are means ± S.D (n=3).

Parameter	**% Concentration of LC_{50}**				
	0%	**30%**	**45%**	**60%**	**75%**
Glucose (mg/dl)	289.00 ± 7.00^{a}	312 ± 2.08^{b}	343.00 ± 16.09^{c}	360.33 ± 2.08^{c}	384.67 ± 3.21^{d}
Total Protein mg/dl)	8.87 ± 0.15^{d}	8.07 ± 0.11^{cd}	7.33 ± 0.58^{bc}	6.83 ± 0.76^{b}	5.53 ± 0.15^{a}
Albumin (mg/dl)	2.77 ± 0.06^{d}	2.53 ± 0.06^{cd}	2.30 ± 0.10^{bc}	2.10 ± 0.10^{b}	0.97 ± 0.25^{a}
Globulin (mg/dl)	6.11 ± 0.20^{b}	5.53 ± 0.15^{ab}	5.03 ± 0.49^{ab}	4.73 ± 0.86^{a}	4.57 ± 0.21^{a}
ALP	192.67 ± 9.45^{a}	202.67 ± 6.66^{ab}	208.00 ± 8.89^{ab}	218.67 ± 4.16^{b}	223.00 ± 9.00^{b}
Urea (mg/dl)	8.63 ± 0.06^{a}	9.73 ± 0.06^{b}	9.97 ± 0.25^{b}	10.63 ± 0.15^{c}	10.97 ± 0.15^{c}
ALT	26.00 ± 1.00^{a}	29.00 ± 1.00^{b}	30.00 ± 1.00^{b}	30.67 ± 0.58^{bc}	32.67 ± 0.57^{c}
AST	175.67 ± 4.04^{a}	179.67 ± 2.08^{ab}	190.00 ± 3.00^{cd}	186.67 ± 3.05^{bc}	195.00 ± 2.00^{d}
Creatinine (mg/dl)	0.53 ± 0.28^{a}	0.67 ± 0.06^{ab}	0.63 ± 0.06^{ab}	0.77 ± 0.06^{bc}	0.87 ± 0.06^{c}

Different letters indicate significant difference mean values among treatments ($P < 0.05$).

DISCUSSIONS

The value of TPH recorded in the water soluble fractions of the oil was considerably higher than the report by Ogbeibu (2011) who observed values of 35.79 and 827.3mg/l for water and sediment analysis respectively in Ethiope-Benin River after a spill. Presence of some metals has also been linked with pollution. Values of metals in WSF of crude oil utilized for the assay were, however, slightly lower in comparison to the values obtained (Ogbeibu, 2011) in the Ethiope-Benin River. Differences in these values could be attributed to weathering which include spreading, evaporation, dissolution, dispersion into the water column, water-in-oil emulsification, photochemical oxidation, microbial degradation, adsorption to suspended particulate matter and stranding on the shore or sedimentation to bottom sediments of oil after spillage (Wang & Stout, 2007). Alterations in haematological parameters of *C. gariepinus* induced by environmental pollutants, diseases or attack by pathogens have been reported by a number of workers (Ezeri, 2001; Eriegha *et al*., 2019). A reduction of Hb and RBC in Persian sturgeon (*Acipenser persicus*) was also reported after acute exposure to crude oil (Rostam & Soltani (2016). Smith *et al.* (1979) ascribed the decrease in RBC to haemolysis resulting in haemodilution (a mechanism for diluting the concentration of the pollutant in the circulatory system). The PCV value of all fish exposed to the crude oil were lower than reference value for healthy fish provided by Etim *et al.* (2009). The diminution in these values could be attributed to some form of anaemia. Sunmonu & Oloyede (2008) have reported a significant reduction in red blood count, haemoglobin and PCV in *C. gariepinus* exposed to WSF of crude oil and attributed same to the suppression of erythropoiesis by the toxic components of the crude oil. The value of PCV also depends on the oxygen carrying capacity of the blood. The observed decrease in PCV value after exposure to the crude oil may be due to the lower oxygen content in the blood of *C. gariepinus*. Moreover, lower PCV values also indicate shrinkage of cell due to toxicant stress on erythropoirtic tissue (Saravanan *et al*., 2011). The reduction in haemoglobin content signifies that the fish's ability to provide sufficient oxygen to the tissues is restricted considerably and will result in decrease of physical activity (Kori-Isiakpere *et al*., 2009). The significant decrease in the haemoglobin

concentrations may also be due to either an increase in the rate at which the haemoglobin is destroyed or to a decrease in the rate of haemoglobin synthesis (Reddy & Bashamohideen, 1989). Fluctuation in WBC after exposure to the crude oil is an indication of stress. Increase in WBC may be due to recruitment of more cells to combat the stressor (Ajani *et al*., 2007). The undulating changes in values of computed haematological indices observed in this study similar to the values reported by Eriegha *et al*. (2017). The slight decrease observed at some concentrations is an indication that crude oil induces a decrease in haematopoiesis and anaemia induction in the fish (Seth & Saxena, 2003).

The high levels of glucose observed in the exposed fish suggest that the fish were under severe stress during the period of exposure. Petroleum hydrocarbons have been reported to increase plasma glucose in various fish species. Al-Kindi *et al.* (1996) have also reported a significant elevation in plasma glucose concentrations after 3h exposure to water soluble fraction of crude oil and an increase of over 50% after 48h in *Pleuronectes flesus*. Elevation of glucose which is part of stress response in fish as well as occurrence of gluconeogenesis to supplement additional energy needed to meet the increased metabolic demands have also been reported by Zutshi *et al*. (2010) in *Labeo rohita* retrieved from a polluted water body. There was a significant ($P<0.05$) inverse relationship between the concentration of crude oil and values of total protein, albumin and globulin. Depletion of total protein content may be due to breakdown of protein into free amino acids under the effect of crude oil exposure. Our observations are in accordance with the report of Rostam & Soltani (2016) who noted a steady decline of total protein in *Acipenser persicus* after exposure to crude oil. Also, the significant decrease in globulins levels from 6.11mg/dl in control to 4.57mg/dl in fish with highest concentration crude oil could be due to a disruption in protein biosynthesis (Banaee & Ahmadi, 2011).

The significant ($P<0.05$) increase in the level of plasma ALP, ALT, AST, Urea activities of all the affected set of fish in comparison to their respective control is a direct reflection of alterations in the hepatic structural integrity. These enzymes are located in the cell cytoplasm and are emptied into the circulation once the cellular membrane is damaged (Lin *et al*., 2002). Elevated level of different hepatic biotransformation of enzymes with different sensitivity level after the severe exposure of the fish *Scophthalmus maximus* (juvenile turbot) to Prestige fuel oil has been reported by Martin-Skiltona *et al.* (2008). The reduced activity of AST and ALT, which are marker enzymes in the liver, indicates that crude oil damages the hepatocytes. Similar observations have been reported by Ezenwaji *et al.,* (2013). Elevation of the serum urea and creatinine may be attributed to kidney disorder (Zaki *et al*., 2009). Kidney damage may result in reduced renal blood flow with reduction in glomerular filtration rate, resulting in azotemia characterized by increase in blood urea nitrogen, uric acid and creatinine. Increased blood urea could also occur at times of impaired kidney function, liver diseases and cardiac arrest (Abdelmoneim *et al*., 2008).

CONCLUSION

The study revealed that exposure to water soluble fraction of crude oil (which approximates what is actually occurring in nature after a spill) has profound influence on the haematological and biochemical characteristics in juveniles of *C. gariepinus*. This is a typical indication to stress response upon introduction of the xenobiotic. Therefore, the health status of aquatic organisms can be compromised if aquatic organisms are exposed to sub-lethal concentrations of crude oil. Consequently, there is the need for all stakeholders in the oil industry such as government, oil companies and communities to take urgent measures in order to prevent the discharge of crude oil into the environment. Also, the authors suggest that other biomarkers utilized in pollution studies such as genetic markers should be investigated to substantiate these claims and used in environmental monitoring.

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