The in vitro effect of water soluble fraction of crude oil on the biochemical, hematological and enzymological parameters of clariid catfish juveniles and sub adults

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Abstract
Investigations on the effect of exposing catfish juveniles and sub adults to water soluble fraction (WSF) of crude oil at LC_{50} was carried out in vitro. The experiment was in duplicates labeled A_1, B_1, C_1, D_1, E_1, and A_2, B_2, C_2, D_2, E_2. WSF of crude oil was introduced into ten aquaria containers filled with 3L of borehole water with ten juveniles of ***Clarias gariepinus*** added into each container at different concentrations of 0, 12, 24, 36 and 48 mg/l. After exposing the fish for 3H to the toxicant, the first blood sample was collected from batches A (A_1, B_1, C_1, D_1, E_1), and B (A_2, B_2, C_2, D_2, E_2). At the close of the experiment (after 96 hours), the second blood sample was collected. Haematological parameters (PCV, RBC, MCV, MCH and MCHC) of *C. gariepinus* exposed to different concentrations of WSFs of crude oil were significant ($P < 0.05$) whereas WBC, Hb, and MCHC were not significant ($P > 0.05$). Biochemical parameters of WSFs of crude oil after 3 hours and 96 hours showed that Ast, cholesterol, triglyceride, albumin, glucose, total bilirubin, ALK and urea were significantly different ($P < 0.05$) while total protein, globulin, and Alkaline phosphate, were not significant ($P > 0.05$) signaling compromised kidney function. The very high AST and ALK with increase in concentrations depict much damage to the liver and muscle of the fish due to toxic effect of wsf. Though no mortality was recorded at exposure times, the WSFs of crude oil were seen to be toxic to *C. gariepinus* and contributed to the environmental stressors resulting to the abnormal conditions in heamatological, biochemical parameters and enzymatic changes observed in the fish.

Keywords: biochemical, haemotological, enzymological, toxicological, oil spill, catfish
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INTRODUCTION

The impact of oil spills on coastal and marine environment can be short and long term (Dicks 1999). The degree of damage caused by oil spill event depends primarily upon the quality of oil spill, the chemistry and properties/types of the oil and the sensitivity of the biological resources impacted, but the overall effect of crude oil on aquatic flora and fauna depend on a great number of factors acting separately or in concert (Adewoye, 2010). The effect of oil spill on aquatic lives are caused by either the physical nature of the oil (physical contamination and smothering) or by it chemical components (toxic effects) and accumulation leading to tainting. Aquatic lives may also be affected by cleanup operation or indirectly through physical damage to the habitats in which plants and animals live (Cooney *et al.*, 2001,).

Ekweozor (1989) reported that frequent spillage of crude oil and its products in Creeks and rivers of the Niger Delta have resulted in a marked reduction in the number of both freshwater and marine creatures. Earlier report has also shown that oil pollution impact negatively on fishery resources (Afolabi *et al.*, 1985). There has therefore, been a regular and constant increase of contamination of the natural environment and most especially the aquatic ecosystem. Azed (2005) observed that the eggs and young stages (fingerlings) of fish are especially vulnerable to the toxic effect of water soluble components of crude oil and its refined product. Nwabueze and Agbogidi (2010) found reductions in weight of *Heterobranchus bidorsalis* due to exposure to water soluble fraction of crude oil.

Oil pollution, one of the environmental consequences of crude oil exploration and exploitation activities produces aqua-toxicological effects, which are deleterious to aquatic life (Kori-Siakpere, 2000; Agbogidi *et al.*, 2005; Mona *et al* 2014; Zaki, *et al.*, 2014). A

variety of pollutants including crude oil and its product are known to induce stress condition, which impair the health of fish (FEPA, 1991). Most often the degree of toxicity of pesticides on aquatic organisms is determined via the haematological, biochemical and enzymological parameters (Saravanan *et al*., 2011).

The activities of enzyme have been widely used as sensitive biochemical indicators prior to the occurrence of hazardous effects in aquatic organisms (Suvetha *et al*., 2015). Phosphatase plays active role in the regulation of various metabolic processes and the attendant changes in acid and alkaline phosphatase activities in fish can be used as indices of growth, illness and spawning (Matusiewicz and Dabrowski, 1996) and also early warning of sensitive stress indicators (Suvetha *et al*., 2015). Cholinesterase acts on the nervous system of the fish and their measurement is extensively used as a indicator of exposure to many xenobiotics (Whitacre and Nunes, 2011).

The primary aim of this research work is to investigate the biochemical, enzymological and hematology effects of crude oil on aquatic organism, particularly, *Clarias gariepinus* (Catfish) under Laboratory conditions.

MATERIALS AND METHODS

PREPARATION OF WATER SOLUBLE FRACTION

Crude petroleum oil was obtained from the Department of Petroleum Resources (DPR) Port Harcourt, River State and was transported to Oceanographic Laboratory of the Instituted of Oceanography, University of Calabar Cross River State.

Following Afolabi *et al* (1988) procedure in the preparation of water soluble fraction (WSFs), 250 ml of crude oil was gently mixed with 750 ml of borehole water in a wind Chester bottle, shaken vigorously for two (2) hours and later poured into a glass separating funnel. This was allowed for twenty four (24) hours to effect complete phase

separation, after which clear WSFs at the lower layer of the separating funnel was obtained in a flat bottom flask.

TEST ANIMAL

A total of one hundred (100) juveniles and sub adults *Clarias gariepinus* (catfish) were obtained from the University of Calabar Fish Farm and Hatchery Complex and transported to the Institute of Oceanography laboratory. Their length ranged from 9.3 – 20.5 cm and 6g to 18g in weight measured using a meter rule and an electronic weighing balance respectively. The fish were randomly grouped into ten (10) different aquaria of 26.4 x 12.4 x 17.6 filled with three (3) liters of borehole water and acclimatized for 72 hours in duplicates. Groups A_1 A_2 served as control, while B_1 B_2, C_1 C_2, D_1 D_2 and E_1 E_2 served as the experimental groups.

THE EXPERIMENT AND TREATMENT

WSFs of crude petroleum oil was introduced into ten plastic containers filled with 3 liters of borehole water with a mixture of ten (10) juveniles and sub adults of *Clarias gariepinus* fish each at concentration of 0, 12, 24, 36 and 48 ml/l respectively. After 3 hours of exposure of *C. gariepinus* to the toxicant at the above five different concentrations, the blood samples were collected using sterilized scalpels to cut the fish in the musculature behind the opercula regions in the dorsal surfaces. The collected blood samples were kept in EDTA and Lithium Heparin labeled bottles to prevent the blood from clotting.

Biochemical and Enzymological study

The collected blood sera were used for further analysis of urea, sodium, potassium, chloride, Bicarbonate, Total Billirubin (CB), Aspartate Amino Transferase (AAT/AST/AsAT/ASAT) aka serum glutamic oxaloacetic transaminase (SGOT) test for checking liver damage, Alkaline phosphatase (AP), Total protein (TP), Albumin, Glubulin, Cholesterol, Triglycerides, Uric Acid (UA), Creatimin and Glucose using Glucose

oxidized method. No mortality was recorded but Blood samples of the fish at different concentrations were collected using a sterilized scalpel to cut the fish in the musculature behind the opercula region in the dorsal surface and emptied into EDTA and Lithium Heparin bottle to prevent the blood from clotting. The blood serums collected were used for analysis of Urea, Sodium, Potassium, Chloride, Bicarbonate, Total Bilirubin (TB), Conjugated Bilirubin (CB), Aspartate Amino Transferase (AAF), Alkaline Phosphate (AP), Total Protein (TP), Albumin, Glubulin, Cholesterol, Triglycerides, Uric Acid (UA), Creatimine and Glucose using glucose oxidized method. The results were read spectrophotometrically at 505 nm using chemistry analyser Model 19C Cad Novel GMBH.

Haematological parameters

Haematological parameters examined were PCV (packed cell volume), Hb (Haemoglobin) content estimated by cyanmethaemoglobin method (Dacie and Lewis, 1968), RBC (red blood cells) and WBC counts were calculated using haemocytometer (Rusia and Sood 1992), and indices of erythrocytes- MCV (Corpuscular volume), MCH (mean corpuscular haemoglobin) and MCHC (mean corpuscular haemoglobin concentration) using standard formulas (Dacie and Lewis, 1968) where.

MCV (fl) = Hct (%) /RBC count in millions/ mm^3 x 10

MCH (pg) = Hb (g/dl) x RBC count in millions/ mm^3 x 10

MCHC (g/dl) = Hb (g/dl) /Hct (%) x 100

RESULTS

Mean Hematological Parameters of *Clarias gariepinus* after 3 hours and 96 hours of exposure to Water Soluble Fraction (WSF) of Crude Oil are illustrated in table 1.

Table 1: Mean Hematological Parameters of *Clarias gariepinus* after 3 hours and 96 hours of exposure to Water Soluble Fraction (WSF) OF CRUDE OIL

	AFTER 3 HOURS OF EXPOSURE					AFTER 96 HOURS OF EXPOSURE				
PARAMETERS	**0ML/L**	**12ML/L**	**24ML/L**	**36ML/L**	**48ML/L**	**0ML/L**	**12ML/L**	**24ML/L**	**36ML/L**	**48ML/L**
PCV (%)	36.00 ± 0.00	32.50 ± 0.50	34.00 ± 1.00	33.00 ± 1.00	34.00 ± 4.00	36.50 ± 0.50	31.50 ± 0.50	32.50 ± 0.50	31.00 ± 1.00	29.00 ± 1.00
WBC (10 9L^{-1})	6.47 ± 0.34	6.96 ± 0.76	5.93 ± 0.09	6.21 ± 0.90	6.97 ± 0.41	6.75 ± 0.08	6.55 ± 0.11	5.85 ± 0.15	6.01 ± 1.00	6.47 ± 1.46
Hb (g/dl)	12.20 ± 0.10	11.00 ± 0.20	11.75 ± 0.05	10.95 ± 0.65	11.55 ±1.35	12.50 ± 0.30	10.35 ± 0.05	10.90 ± 0.40	10.50 ± 0.20	9.65 ± 0.35
RBC (10 12L^{-1})	5.50 ± 0.10	5.25 ± 0.25	5.35 ± 0.05	5.65 ± 0.35	5.85 ± 0.15	5.65 ± 0.05	4.90 ± 0.10	5.10 ± 0.10	5.30 ± 0.20	5.45 ± 0.15
MCV (Femtolitre)	65.50 ± 1.20	62.00 ± 2.00	63.55 ± 1.25	58.55 ± 1.85	57.95 ± 5.35	64.60 ± 0.30	64.30 ± 0.30	63.75 ± 0.25	58.50 ± 0.30	53.20 ± 0.40
MCH (Pg)	22.20 ± 0.60	21.00 ± 0.60	21.05 ± 0.25	19.35 ± 0.50	19.70 ± 1.80	22.15 ± 0.35	21.15 ± 0.35	21.35 ±0.35	19.85 ± 0.35	17.70 ± 0.20
MCHC (g/dl)	33.90 ± 0.30	33.85 ± 0.05	33.10 ± 0.20	33.15 ± 0.95	33.95 ± 0.50	34.25 ± 0.35	32.85 ± 0.35	33.50 ± 0.70	33.85 ± 0.45	33.25 ± 0.05

Table 2: Mean Biochemical Parameters of *Clarias gariepinus* after 3 hours and 96 hours of exposure to Water Soluble Fraction (WSF) of Crude Oil

	After 3 hours of exposure					After 96 hours of exposure				
Parameters	**0ml/L**	**12ml/L**	**24ml/L**	**36ml/L**	**48ml/L**	**0ml/L**	**12ml/L**	**24ml/L**	**36ml/L**	**48ml/L**
Cholesterol (mmol/L)	3.40 ± 0.10	3.35 ± 0.15	3.45 ± 0.05	3.60 ± 0.10	3.45 ± 0.05	3.40 ± 0.00	2.90 ± 0.10	2.60 ± 0.20	2.60 ± 0.40	2.25 ± 0.05
Triglyceride (mmol/L)	1.80 ± 0.10	1.70 ± 0.00	2.05 ± 0.15	1.75 ± 0.05	1.80 ± 0.20	1.85 ± 0.05	0.90 ± 0.20	0.75 ± 0.05	0.80 ± 0.00	0.80 ± 0.10
Tot Protein (g/L)	57.50 ± 0.50	58.00 ± 0.00	58.50 ± 1.50	60.50 ± 0.50	57.50 ± 1.50	59.00 ± 1.00	58.50 ± 0.50	60.00 ± 0.00	61.00 ± 1.00	61.50 ± 1.50
Albumin (g/L)	29.50 ± 0.50	32.50 ± 2.50	36.50 ± 0.50	32.00 ± 1.00	35.50 ± 1.50	31.00 ± 1.00	35.00 ±	35.00 ± 0.00	33.00 ± 1.00	41.50 ± 5.50
Globulin (g/L)	28.00 ± 1.00	25.50 ± 2.50	22.00 ± 2.00	28.50 ± 0.50	28.00 ± 3.00	28.00 ± 0.00	23.50 ± 2.50	25.00 ± 0.00	28.00 ± 0.00	20.00 ± 4.00
Urea (mmol/L)	2.70 ± 0.10	7.20 ± 1.70	7.55 ± 1.15	6.05 ± 0.25	5.55 ± 2.75	1.65 ± 0.05	6.35 ± 1.35	7.00 ± 0.90	6.80 ± 1.00	9.45 ± 0.55
Glucose (mmol/L)	5.65 ± 0.05	5.80 ± 0.10	5.75 ± 0.05	5.90 ± 0.10	5.90 ± 0.20	5.65 ± 0.05	7.05 ± 0.15	7.00 ± 0.00	7.20 ± 0.10	7.35 ± 0.15
Tot Bilirubin (umol/L)	4.95 ± 0.05	6.40 ± 0.70	6.35 ± 0.25	6.00 ± 0.80	5.75 ± 0.35	7.55 ± 0.15	7.30 ± 0.20	7.50 ± 0.40	6.90 ± 0.00	7.60 ± 0.30
Con Bilirubin Umol/L)	7.85 ± 0.15	10.80 ± 1.50	10.65 ± 0.45	10.85 ± 2.15	10.10 ± 0.20	13.65 ± 0.05	13.40 ± 0.60	13.85 ± 0.55	13.55 ± 0.25	14.35 ± 0.45
Ast (iu/L)	29.75 ± 0.35	28.85 ± 0.95	30.50 ± 0.60	32.10 ± 0.40	32.50 ±0.80	29.95 ± 1.35	28.70 ± 1.40	31.60 ± 1.70	32.20 ± 0.60	34.3 ± 0.30
Alk (iu/L)	15.55 ± 0.25	16.50 ± 0.10	15.95 ± 0.50	19.60 ± 1.10	25.05 ± 1.35	15.25 ± 0.25	32.25 ± 3.40	62.00 ± 6.40	64.00 ± 2.70	68.90 ± 1.20
Alkal. Phosphate (iu/L)	47.70 ± 0.40	46.95 ± 3.35	51.65 ± 1.75	50.15 ± 0.85	50.70 ± 1.40	51.80 ± 2.00	48.85 ± 1.95	49.10 ± 2.00	48.95 ± 0.15	49.70 ± 1.30

- Alk (alanine aminotransferase), Ast/SGOT= aspartate aminotransferase/serum glutamic oxaloacetic transaminase, ALP=Alkaline phosphatase

Table 3: Analysis of Variance of mean hematological and biochemical parameters of *Clarias gariepinus* exposed to different concentrations of water soluble fraction after 3 hours and 96 hours.

Indices	Significant Value	Inference
PCV (%)	0.091	Significant at P = 0.05
WBC (10 9L^{-1})	0.938	Not Significant at P = 0.05
Hb (g/dl)	0.484	Not Significant at P = 0.05
RBC (10 12L^{-1})	0.076	Significant at P = 0.05
MCV (Femtolitre)	0.021	Significant at P = 0.05
MCH (Pg)	0.017	Significant at P = 0.05
MCHC (g/dl)	0.458	Not Significant at P = 0.05
Cholesterol (mmol/L)	0.001	Significant at P = 0.05
Triglyceride (mmol/L)	0.002	Significant at P = 0.05
Tot Protein (g/L)	0.113	Not Significant at P = 0.05
Albumin (g/L)	0.058	Significant at P = 0.05
Globulin (g/L)	0.133	Not Significant at P = 0.05
Urea (mmol/L)	0.037	Significant at P = 0.05
Glucose (mmol/L)	0.001	Significant at P = 0.05
Tot Bilirubin (umol/L)	0.010	Significant at P = 0.05
Conj Bilirubin (Umol/L)	0.006	Significant at P = 0.05
Ast (iu/L)	0.033	Significant at P = 0.05
Alk (iu/L)	0.002	Significant at P = 0.05
Alkaline Phosphate (iu/L)	0.617	Not Significant at P = 0.05

Analysis of Variance of hematological and biochemical parameters (Table 3) of *C. gariepinus* exposed to different concentrations of water soluble fraction of crude oil for 3 hours and 96 hours showed that PCV, RBC, MCV, MCH, cholesterol, triglyceride, urea, albumin, glucose, total bilirubin, conjugate bilirubin, Ast and Alk were significant ($P<0.05$) at probability level of 0.05 whereas WBC, hemoglobin, MCHC, total protein, globulin and Alkaline phosphate were not significant ($P>0.05$). RBC at 3H exposure time decreased at LC_{50} of 12 and 24 ml/L with a general decrease from the normal after 96H exposure period. PVC values were below the control at all levels of exposures. Though no mortality was recorded at

various exposure times, the biochemical, haematological and enzymological parameters deferred from the control and varied with increase in concentration (LC_{50}) and exposure times.

DISCUSSION

In aquatic science, measurement of haematological and biochemical indices is commonly used as a diagnostic tool in aquatic toxicology and biomonitoring (Soimasue *et al.,* 1995; Jee and Kang 2005). Exposure of aquatic organisms to crude oil and its derivatives has been proven to induce a variety of toxic effects in exposed aquatic animals. Also, petroleum hydrocarbons can act as a mediator in generating free radical in fish that are capable of causing various side effects in their internal and external tissues (Davison *et al.,* 1993; Al-kindi *et al,* 1996; Khan, 1998; Achuba and Osakwe, 2003; Khan, 2003; Zang *et al.,* 2004; Pachelo and Santos, 2016).

Hematological parameters of fish can be used as very sensitive indicators of changes in ecophysiological condition (Vinodhni and Norayamn, 2009). The general reduction of the blood parameters indicate anaemic condition caused by exposure of *Clarias gariepinus* to toxicant over the given period. Changes in haematological parameters of fish such as *C. gariepinus* reported in this study which is due to stress induced by environmental pollutant (water soluble fraction of crude oil) have been reported by several researchers (Onusiriuka and Ufodike, 2000; Ezeri, 2001, Gabriel, *et al,* 2001). These indices have been used in the effective monitoring of the responses of fish to the stressors including its health status under adverse conditions.

The variation in fish hematological parameters observed in this study may be due to physiological stress in the exposed fish. This finding is in agreement with findings of Rostem, and Soltani (2016) who observed variation in hematological parameters of

juvenile Beluga (*Huso linnacus*) exposed to water soluble fraction of crude oil. The variations in haematological indices observed in this study when exposed to different concentrations of WSF of crude oil are a defensive mechanism against crude oil toxicity through stimulation of erythropoiesis which corresponds with studies on *Sarotherodon melanotheron* (Oriakpono, *et al*, 2012), *Oreochromis mossambicus* (Hwang, *et al*, 1989) and eels (Kirsch and Mayer, 1973). Haemoglobin is known to be a sophisticated oxygen delivery system which provides the amount of oxygen required by the tissues (Voet and Voet, 1990). According to Blaxhall and Daisley (1973; 2006), haemoglobin estimation is a reliable or good indicator of anaemic conditions in fish. Generally, the hemoglobin value of fish depends on the oxygen carrying capacity of the blood (Larsson, *et al*, 1985).

In this study, the observed significant variation ($P<0.05$) in hemoglobin value of the exposed fish may be attributed to less oxygen content in the blood of the exposed fish. Moreover, lower Hemoglobin values are indication of shrinkage of cell due to toxicant stress on the erythropoietic tissue (Saravanan *et al*., 2011). Also, Rostem, and Soltani, (2016) attributed the significant variation in hemoglobin concentration of the fish exposed to crude oil to either an increase in the rate of hemoglobin destruction or a decrease in the rate of hemoglobin synthesis. The decreases in haemoglobin concentrations indicate considerable restrictions in the fish's ability to provide oxygen sufficiently to their tissues resulting to a decreases of physical activities of the fish (Nussey, 1994).

According to Rostem, and Soltani, (2016), crude oil can affect RBC, causing a hemolysis by a disruptive effect on the erythropoietic tissues of spleen and kidney. In this study, RBC values were significantly different ($P<0.05$) in the exposed fish and this findings agrees with those of Rostem, and Soltani, (2016) for juvenile Beluga (*Husohuso*).

This indicates that chronic exposure of *Clarias gariepinus* to water soluble fraction of crude oil can result in or stimulate erythropoiesis in *C. gariepinus*. According to Health (1995), erythrocytes are produced in the haematopoietic tissue, situated in the spleen and head kidney of fish. It has been established that a reduction in the quantity and quality of erythrocytes as observed in this study will lead to a deteriorated oxygen supply (Kori-Siakpere, Martin and Ikomi 2009). This implies that fish exposed to crude oil toxicity will suffer from deteriorated oxygen supply which is very dangerous to fish health. Nasir and Hemtoush (2010) observed a linear reduction in hemoglobin which suggests an anemic condition in the crude oil treated fishes. Sudakov (1992) reported that the toxic components especially those in crude oil are capable of changing blood chemistry thereby inducing anaemia by causing bone marrow hypoplasia and interference with platelet production in the exposed animals, hence resulting in the reduced values.

The reduction in the RBC may be attributed to the presence of environmental stressors which manifest in form of a change in the environment resulting to haemagglutination due to impaired osmoregulation (Rottman, Francis-Floyd and Durborow 1992) or erythropoiesis in the organs responsible for the production of RBC. According to Oriakpono *et al.,* (2012), packed cell volume (PCV) is an important haematological parameter that changes with fish activity and environmental stress. In this study, PCV values of the exposed fish for 96 hours varied ($P<0.05$) significantly with increasing concentration of water soluble fraction of crude oil which corroborates findings of Oriakpono *et al.,* (2012) for *Sarotherodon melanotheron* exposed to crude oil. Oriakpono *et al.,* (2012) attributed these findings to changes in water balance, which could lead in a decrease in blood volume and an increase in the white blood cells resulting in

reduced PCV. However, white blood cells (WBC) and mean corpuscular haemoglobin concentration (MCHC) of the exposed fish did not increase significantly (P>0.05) even after 3 hours and 96 hours comparison. This may indicate that water soluble fraction of crude oil did not influence WBC values of *Clarias gariepinus* significantly. This result disagrees with the finding of David *et al.*, (2002) who reported an increase in size and monocytes of *Tilapia guineensis* and *S. melanotheron* after exposure to industrial effluents. Ajani, *et al*, (2007) attributed the increase in WBC to recruitment of more cells to combat the stressor. Anyanwu *et al*., (2007) also attributed the increase in WBC to non specific immune response to stress as a result of interaction of prolactin and cortisol hormones to restore ion balance in isosmotic salinity (and a stimulation of the immune system in response to toxicity of crude oil). The values of calculated haematological indices including MCHC, MCH, and MCV are crucial in the diagnosis of anaemia in most animals (Coles, 1986). The variations obtained in these haematological indices (MCV, MCH and MCHC) in the present study could be due to a defense against the toxic effect of crude oil through the stimulation of erythropoiesis (Mouse, 1999). According to Kori-Siakpere, *et al*., (2009), the decrease in MCV and low hemoglobin level indicate that the red blood cells have shrunk, either due to hypoxia or microcytic anaemia; microcytosis been due to the decrease in the haematocrit values. The fluctuation in the MCH values clearly indicates that the concentration of haemoglobin in the red blood cells were much lower in the exposed fish than in the control over the exposure period, thus indicating an anaemic condition. The MCHC is a good indicator of red blood cell swelling (Wepener, *et al.* 1992). The significant decreases in the MCHC values in the exposed fish are thus probably an indication of swelling of the red

blood cells and/or a decrease in hemoglobin synthesis. Pimpao *et al.*, (2007) posited that the alteration of these parameters can be used as health indicators of the aquatic environment and also provide early warning tools in monitoring environmental quality. A vital tool in the field of risk assessment is an understanding on the adaptation and recovery (Du *et al*., 2009; Wu *et al*., 2005).

The release of very high AST and Alk into the blood of the fish at all the concentrations is an indication of injury to the liver or muscle due to wsf. Ast and Alk values were higher than the control and increased with increase in concentration and duration of experiment depicting damage to the liver and probably other organs such as the heart or kidney. Alkaline phosphate occurred in moderate amount showing less damage to liver or bone disorders. It also helps to break down protein in the body.

According to Nasir and Hemtoush (2010) the degree of ecosystem contamination by toxicants can be evaluated by use of biochemical indices. Biochemical parameters of *Clarias gariepinus* exposed to different concentrations of water soluble fraction of crude oil after 3 hours and 96 hours showed that Ast, urea, albumin, cholesterol, triglyceride, glucose, total bilirubin, and conjugate bilirubin, were significantly different ($P<0.05$) total protein, globulin, and Alkaline phosphate were not significant ($P>0.05$). The very high urea content in the fish juveniles with increase in concentration depicts very high compromise of the kidney function as a result of the toxic effect of wsf Glucose level increased steadily above the normal at all levels of concentrations as a result of the toxic effect of wsf. The higher levels of total bilirubins formed with increased concentration is an indication of the breakdown of rbc in the fish due to the toxic effect of wsf.

Proteins are naturally composed of fibrinogens, globulins and albumins which are responsible for the distribution of important materials from one part of the body to another during circulation. In living Organisms, protein is also known to possess or exhibit transporting, nutritive, protective, buffering and energetic properties or functions (Inyang, *et al*., 2010). In animal system, protein assessment is often used as part of laboratory diagnosis to ascertain the extent of wellness of an organism (Edori, *et al.*, 2013). In this study, changes in total protein content of *C. gariepinus* after exposure to WSF of crude oil agrees with findings of Inyang, *et al*., (2010) and Edori, *et al*., (2013) who reported changes in protein content of *C. gariepinus* exposed to toxicants.

General alterations were observed in the biochemical parameters of the exposed fish which was not time and concentration dependent and this observation is similar to findings of Edori, *et al*., (2013), Edori and Konne (2015) on *Tympanotonus fuscatos* and *Placopecten magellanicus*. According to Sambasiva Rao (1999), decrease in total protein content is caused by the degradation and utilization of degraded products for metabolic processes which results in increase in free amino acids due to the decreased incorporation of amino acids in protein synthesis. The variation observed in enzyme activities could be attributed to the crude oil toxicity and is similar to observation of Mahmoud, *et al*, (2011), Ramesh *et al.*, (2015) and Edori and Ekpete (2014) who reported varying degrees or changes in enzyme activities induced by crude oil respectively on rat, *Cyprinus carpio* and periwinkles (*Tympanotonus fascatus)*. According to Gabriel *et al*., (2009), these enzymes (AST, ALP and ALP) are clinically important in the diagnosis of hepatic (hepatocellular) damage or disease. In the event of damage in the organs, especially those of hepatic importance such as the liver, spleen or the kidney, these enzymes leak from the organs to

the blood and this alters the permeability of the cell membrane to an appreciable degree (Gad, 2007). The alteration of enzyme (AST, ALT and ALP) in any organism indicates a disturbance in the general physiological structure of important organs or tissues and the membrane transport (Roy, 2002). Also, the direct interference of crude oil with the tissues of *Clarias gariepinus* could be responsible for the change in the activities of these enzymes (AST, ALT and ALP) which resulted in the changes observed in the fish biochemistry. Hepatic, gill and kidney glutathione reductase as well as glutathione S-transferase, and catalase activities were markedly elevated after two or four weeks of exposure (Jee and Kang 2005). The result of our analysis equally conforms to that of Jee and Kang (2005) that enzymatic activities increased with concentrations at all levels of exposure.

Conclusion

From the results obtained in this study, it is suggested that water soluble fraction of crude oil is an environmental stressor which leads to abnormal alteration in hematological and biochemical parameters in fishes. The alteration ~~of~~ in some hematological and biochemical parameters is an indication that crude oil is toxic to *Clarias gariepinus* and its environment. This underscores the need for adequate and timely environmental check by government and other relevant agencies to protect the aquatic environment from the effect of crude oil toxicity since this fish is one of the most consumed and cheap protein source for the coastal inhabitants ~~of areas~~.

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