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Haematological Effects of Water Soluble Fraction (Wsf) of Bonny Light Crude Oil (Blco) to The African Freshwater Catfish *Clarias Gariepinus* (Burchell, 1822)

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Abstract

Three hundred and sixty (360) juveniles of *Clarias gariepinus* were collected from Luis Fish Farm Warri, Delta State and transported to the Departmental Laboratory of Fisheries and Aquaculture, Ebonyi State University Abakaliki (latitude 6^0, 20' 49"N, longitude 8^0 06' 11"E), Nigeria. The experimental fish were exposed to 6 treatment concentrations (20, 10, 5, 2.5, 1.25 and 0.00) ml/l of WSF of BLCO in triplicate replications of 60 fish per treatment for 10 weeks. Mean cell count of red blood cell (RBC) reduced significantly ($P<0.05$) from $2.09\pm0.06 \times 10^6/mm^3$ in the control group to $1.4971\pm0.06x10^6/mm^3$ in the highest concentration exposed group (20ml/L). Similarly, significant reduction in packed cell volume (PCV) and haemoglobin content of RBC resulted to anaemic conditions in exposed fish. PCV decreased from 24.33±0.26% to 16.50±0.89% while haemoglobin decreased to 7.73±0.29g/dl from 12.05± 0.11g/dl. Total white blood cell (WBC) count suffered significant reduction than control and in the amount of its lymphocytes but increased significantly than control in the number of neutrophils. WBC reduced from $51.44\pm0.47x10^3/mm^3$ to $43.48\pm1.09x10^3/mm^3$, and lymphocytes from 91.55±2.24% to 78.0±0.5% while neutrophil increased to 11.52±1.3% from 3.58±0.27%. Mean corpuscular volume (MCV), MCH and mean corpuscular haemoglobin concentration (MCHC) did not differ significantly ($P>0.05$) with control group.

Key words: Haematological; crude oil; *Clarias gariepinus*

INTRODUCTION

Changes in haematological parameters of fish are frequently used to measure effects of toxicants and environmental contaminants. Prasad et al. (2006) observed marked changes in the blood of *Heteropneutes fossilis* exposed to crude oil which corresponds with earlier observations in rats *Rattus rattus* (Dede and Kaglo, 2002) and sea birds (Khan and Nag, 1993). Decreased red blood cell count, haematocrit (PCV), haemoglobin and mean cell volume have been reported in crude oil exposed Nile tilapia *Oreochromis niloticus* (Omoregie, 1998) and *Clarias gariepinus* (Gabriel et al., 2001). The decrease of red blood components in the croaker fish *Heteropneutes fossilis* was noted to be due to haemolysis of red blood cells that led to anaemic conditions in the exposed fish (Prasad et al. 2006). Erythropaenia, a condition of lowered RBC was reported in kerosene exposed African catfish *Clarias gariepinus* (Gabriel et al., 2007). This has been suggested by various authors to be due to different effect of toxicants: haematopoietic tissue damage in the kidney of fish (Singh and Singh, 1982), mutagenic damage in the erythrocyte (Khan and Nag, 1993) and swelling of the erythrocyte (Annune and Ahuma, 1998). Petroleum exposed Nile tilapia *Oreochromis niloticus* suffered similar fate of erythrocyte reduction (Omoregie, 1995) and it was suggested to be due to anaemic response occasioned by haemodilution (Omoregie et al., 1990; Sampath et al., 1993) or as a result of the inhibition and destruction of erythrocytes (Rai and Quayyan, 1984).

Leucopaenia, a condition in which white corpuscles of the blood are greatly reduced, have been reported in crude oil exposed fish (Zbanyszek and Smith 1984; Van Vuren et al., 1994; Omoregie, 1998). Leucopaenia often results to increased susceptibility to infectious

diseases due to lowered immune response (Omoregie, 1995, Kennedy and Farrel 2008). However other authors have reported leucocytosis, a temporary condition in which white blood cells are increased in number, in the African catfish *Clarias gariepinus* exposed to copper and lead (Annune and Ahuma, 1998), malachite green (Musa and Omoregie, 1999) and infected with bacteria *Pseudomonas fluorescens* (Ezeri, 2001). Toxicants differ in potency and mode of action and fish respond differently, producing various amounts of white blood cells (Cazenave et al., 2005). Increased number of leucocytes could be a defense mechanism (Ellis, 1981), to help destroy invading bacteria (Ezeri, 2001). Neutrophilia and thrombocytosis were reported in the African catfish *Clarias gariepinus* exposed to kerosene (Gabriel et al., 2007) , and malachite green (Musa and Omoregie, 1999). The neutrophils were noted to be most sensitive of the white blood sub-populations (Alkindi et al., 1996). Mercury exposed salmon *Oncorhynchus kisutch,* however indicated an increased neutrophils which was attributed to tissue damages (Storozhuk and Guleva, 1983). Reported increase in lymphocytes have been observed in fish exposed to environmental pollutants (Sampath et al., 1993; Banerjee, 1998) and petroleum (Gabriel et al., 2007), which they attributed to stimulation of the immune mechanism of the fish to eliminate effects of pollutants.

MATERIALS AND METHODS

Collection and Acclimation of Experimental Fish

Juveniles of *Clarias gariepinus* from same brood stock and age were collected from Luis Farm, Warri, Nigeria and transported in plastic containers to the Departmental (Wet) Laboratory of the Department of Fisheries and Aquaculture, Ebonyi State University, Abakaliki (latitude 6^0, 20' 49"N, longitude 8^0 06' 11"E), Ebonyi State, Nigeria. Thereafter the fish were acclimated to laboratory conditions in 50litres capacity plastic vats for 14 days and fed at 3% body weight, twice daily using 38% crude protein experimental diet.

Preparation of Water Soluble Fraction (WSF) of Crude Oil

The method of preparation of water soluble fraction of crude oil proposed by UNEP (1989) was employed in this research. Clean well water and Bonny light crude oil, obtained from the Nigerian National Petroleum Company (NNPC) Port Harcourt, Nigeria were measured out in ratio of 10:1 into an aspirator and mixed thoroughly with rotatory magnetic stirring rod for 20 hours. Mixture were allowed to rest for 12 hours to demarcate layers. Thereafter separating funnel was used to separate out the water soluble fraction, which was corked as stock solution in 50l capacity gallons.

Haematological Analyses

Collection of Blood samples

Blood sample was collected from the caudal penduncle of fish with the aid of 2.5ml capacity syringes and hypodermal needles treated with anti coagulant, EDTA.

Erythrocyte Count

The counting of erythrocytes was carried out in a standard haemacytometer (Model-JSQA) following the method of Svobodava *et al.* (2003). Blood flowing from the cut at the caudal penduncle was carefully sucked into the haemoglobin pipette till its 0.5 mark was reached. Without allowing the blood to flow out, the diluting fluid (Haymen's Fluid) was drawn to the 101 mark on the pipette. Even distribution of blood cells was initially focused with the lower power followed by higher power for detailed observation. Red blood cells were counted in 5 out of 25 small squares which contained 16 smaller squares of 1/400mm3 size as contained in improved Neubuer counting chamber.

$$RBC\ (10^6/mm^3) = \frac{C \times D \times 100 \times 400}{S \times 80}$$

C = Number of cells counted
D = Diluting factor
S = Number of mm squares counted

Haematocrit (PCV) Determination

Blood filled heparinized micro-haematocrit capillary tubes were centrifuged at

12000 rounds per minute for 5 minutes, using a micro haematocrit reader at 540nm. Percentage of the haematocrit mean was recorded.

Haemoglobin estimation

The haemoglobin concentration was measured by the cyan-methemoglobin method using the method of Wickham et al. (1990). About 0.02 ml of blood was mixed with 4ml of Drabkins reagent (20mg Potassium ferricynide; 50 mg Potasium cyanide and 11ml of distilled water). Few minutes was allowed for haemoglobin to be oxidized by Potassium ferricynide to methemoglobin and be fully converted to cyanmethaemoglobin by Potassium cyanide. Thereafter, the absorbance at 540 nm wavelenght of the cyanmethaemoglbin equivalent to the maximum haemoglobin was read off using a standard colorimeter.

Leucocyte count

About 0.005ml of blood was dissolved in 0.19ml of Tureks solution .Gentain violet was used to stain WBC for clarity. White blood cells were counted 4 out of 16 squares in the Neubuer chamber of a standard haemacytometer.

$$WBC\ (10^3/mm^3) = \frac{C \times D \times 100 \times 10}{S \times 4}$$

C = Number of cells counted
D = Diluting factor
S = Number of mm squares counted

Differential count of WBC sub population

The differential count of leucocyte sub-populations were estimated using the method of Svobodava *et al*. (2003). A drop of blood was smeared on a glass slide and allowed to dry. Blood cells were then fixed with methanol fixative and stained with xanthene red and thiazene dye (blue). Differential cells of leucocyte were counted with the low power of microscope.

Mean Corpuscular Volume (MCV)

The average red blood cell size was estimated by dividing haematocrit value by RBC counts

$$MCV\ (Femtoliter) = \frac{Hct \times 10}{RBC}$$

Mean Corpuscular Haemoglobin (MCH)

Haemoglobin amount per red blood cell was estimated by dividing heamoglobin value by RBC

$$MCH\ (Picogram) = \left[\frac{Hb}{RBC}\right] \times 10$$

Mean Corpuscular Haemoglobin Concentration (MCHC)

The amount of haemoglobin relative to the size of the cell was estimated by dividing MCH by MCV

$$MCHC\ (g/dl) = MCH/MCV$$

Haematological parameter was conducted at the Federal Teaching Centre, Abakaliki.

Statistical Method

Results were subjected to one way Analysis of variance (ANOVA) using the Statistical Package for the Social Sciences (SPSS Version17) to determine significant difference between various treatments and control. The Duncan Multiple Range Test was used to separate differences among means. Differences were considered significant at ($P < 0.05$).

RESULT

Result of Red Blood Cell Count (RBC) of *Clarias gariepinus* Juveniles Exposed to Sublethal Concentration of WSF of Crude Oil for 10 Weeks

Mean number of red blood Corpuscles (RBC) counts ($10^6/mm^3$) of juveniles of *Clarias gariepinus* exposed to subacute concentrations of water soluble fraction of crude oil for 10 weeks reduced significantly ($P < 0.05$) in treatments 2,3,4 and 5 exposed groups below control value of $2.09 \pm 0.12 \times 10^6/mm^3$. Mean value of T_2 reduced to 1.61 million cells per cubic millimeter of blood and further in T_5 to $1.4917 \pm 0.07 \times 10^6/mm^3$ (Fig.1). Significant reduction in RBC count did not occur ($P > 0.05$)

in treatment 1 but mean values of RBC in treatments 2, 3, 4 and 5 were statistically different from each other. Along the period of exposure, within group differences in RBC did not occur in the control and treatment 1 but occurred in treatments 2, 3, 4 and 5 where RBC values in week 2, 6 and 10 differed from start, 4 and 8.

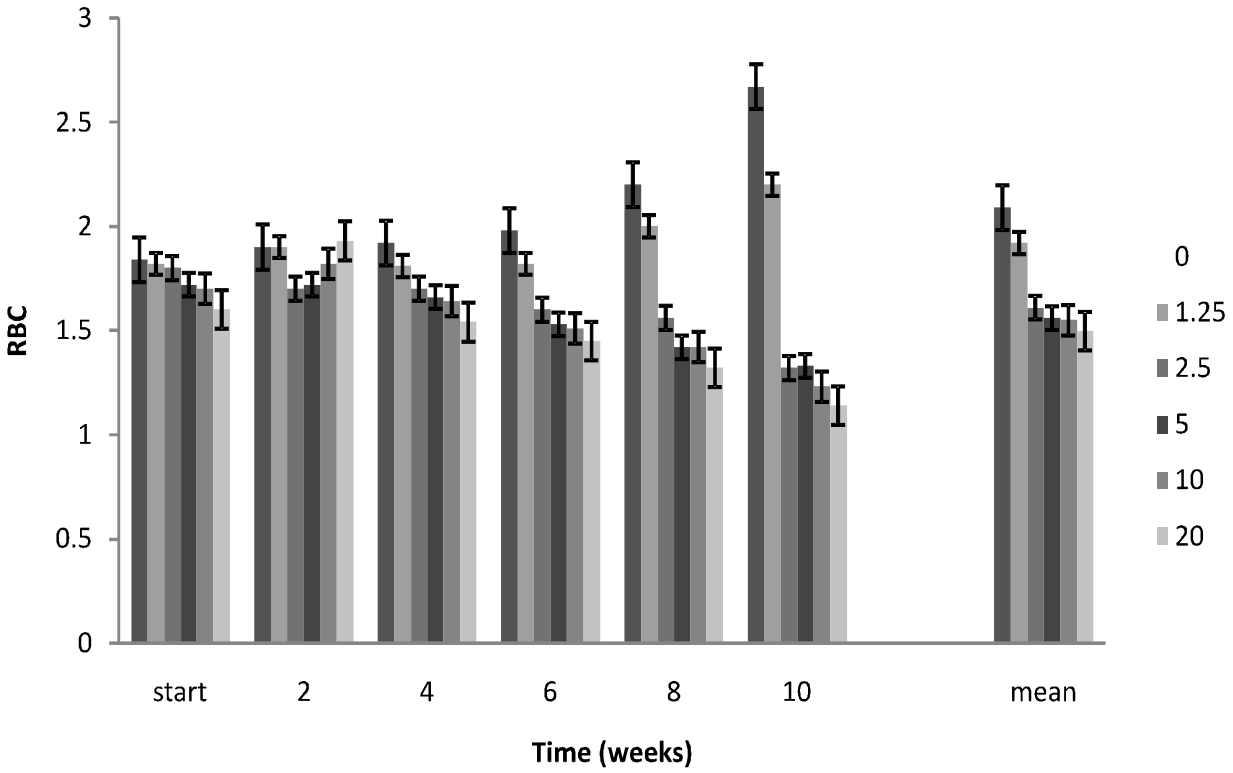

Fig.1: Mean Number of RBC±SE (10^6/mm^3) of *Clarias gariepinus* Exposed to Subacute Concentration of WSF of Crude oil for 10 weeks

Result of White Blood Cell Count (WBC) (x10^3/mm^3) of *Clarias gariepinus* Juveniles Exposed to Sublethal Concentrations of WSF of Crude Oil for 10 weeks

Mean number of White Blood Cell Count (WBC) +SE (x10^3/mm^3) of juveniles of *Clarias gariepinus* exposed to subacute concentrations of WSF of crude oil for 10 weeks was significantly reduced ($P < 0.05$) from mean value of 51.44±0.47 x 10^3/mm^3 in the control group to 50.20±0.14 x10^3/mm^3 in T_1 and further to 43.48±1.09x10^3/mm^3 in T_5 exposed group. White blood cell number in group of fish exposed to T_3, T_4 and T_5 did not vary significantly from each other ($P > 0.05$) but differed significantly ($P < 0.05$) from those exposed to treatments 1 and 2. Along the exposure period, WBC values in weeks 2, 6 and 10 was different from weeks 8, 4 and start within treatments 2, 3. 4, and 5 but did not occur in group exposed to treatment 1. Leucocyte reduction may have led to leucopaenia. Increased number of cell was generally recorded in week 2, a condition known as leucocytosis but gradually reduced from week 4 along the exposure period (Fig 2).

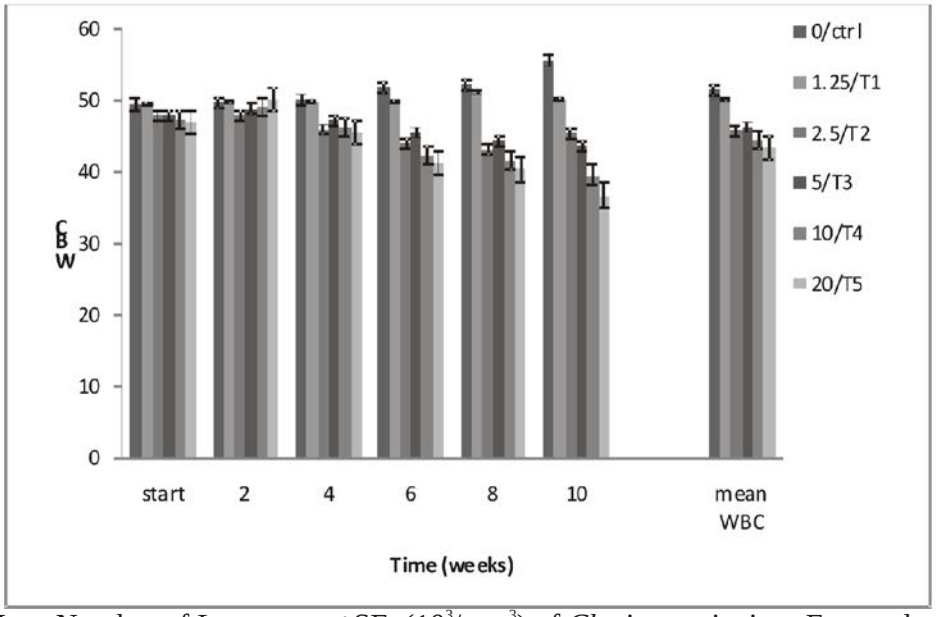

Fig.2: Mean Number of Leucocytes±SE ($10^3/mm^3$) of *Clarias gariepinus* Exposed to Subacute Concentrations of WSF of Crude oil for 10 weeks.

Result of Packed Cell Volume (PVC) of *Clarias gariepinus* Juveniles Exposed to Sublethal Concentrations of WSF of Crude Oil for 10 Weeks

Mean packed Cell Volume (PCV) of juveniles of *Clarias gariepinus* exposed to subacute concentrations of WSF of crude oil for 10 weeks reduced significantly ($P < 0.05$) among treatment means below those of the control group with mean value 24.3±0.26% in the control to 19.67±0.49% in T_2 and further below control to 16.5±1.5±0.89% in those exposed to T_5. Reduction of PCV between group of fish exposed to treatment 4 and 5 did not differ statistically but differences occurred between mean values of group of fish exposed to treatment 1 and 2. Along exposure period, PCV values varied within groups exposed to 5ml/l, 10ml/l and 20ml/l (Fig.3).

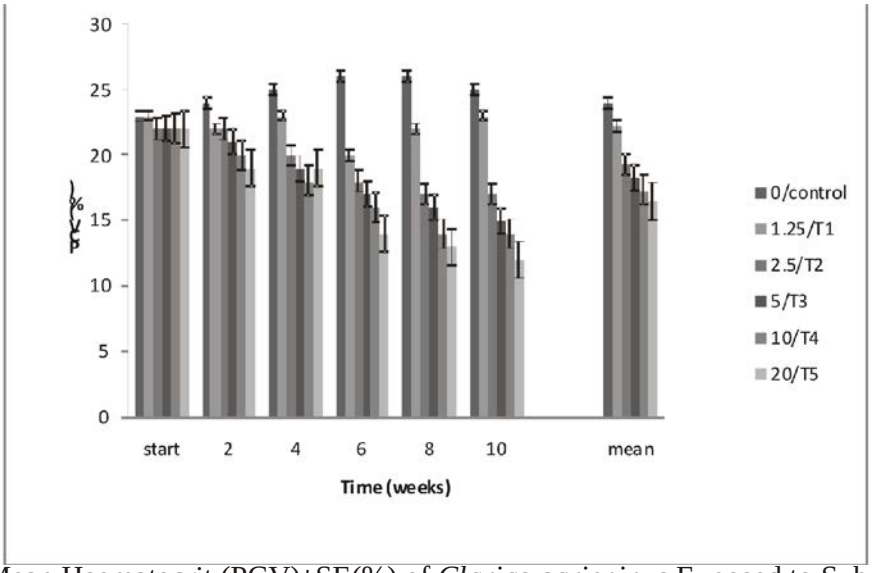

Fig.3: Mean Haematocrit (PCV)±SE(%) of *Clarias gariepinus* Exposed to Subacute Concentrations of WSF of Crude oil for 10 weeks

Result of Heamoglobin Content of *Clarias gariepinus* Juveniles Exposed to Sublethal Concentration of WSF of Crude Oil for 10 Weeks

Mean Heamoglobin content of juveniles of *Clarias gariepinus* exposed to subacute levels of WSF of crude oil for 10 weeks reduced significantly ($P<0.05$) below those in control group in treatments 2, 3 and 5 below mean control value of 12.05±0.48 g/dl of haemoglobin reduced to 9.45±0.28 , 8.89±0.24 and 7.73±0.30 g/dl) in T_2, T_3 and T_5 respectively. Significant difference exited in treatments 2,3, and 5 along the period of exposure. Lowered heamoglobin content indicated anaemic condition in exposed groups (Fig. 4).

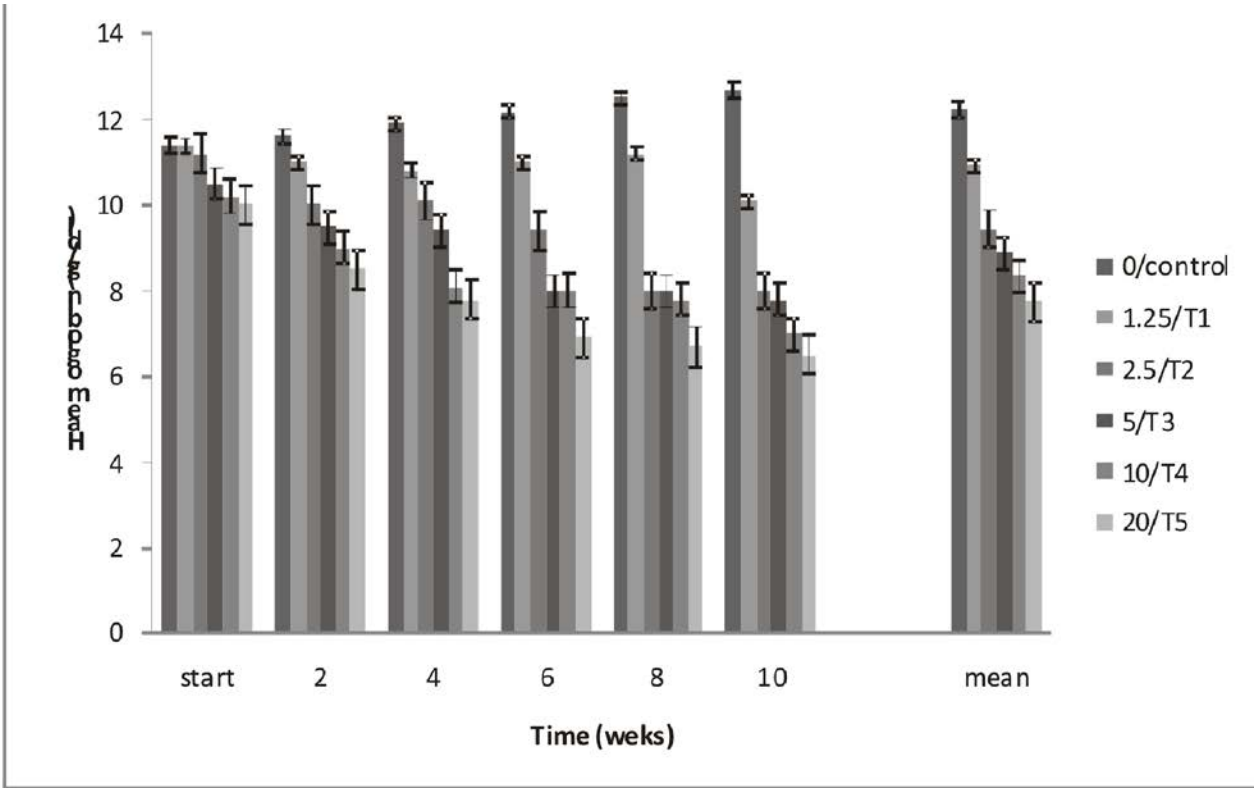

Fig.4: Mean Haemoglobin±SE (g/dl) of *Clarias gariepinus* Exposed to Subacute Concentration of WSF of Crude oil for 10 weeks.

Result of Neutrophilic Leucocyte of *Clarias gariepinus* Juveniles Exposed to Sublethal Concentration of WSF of Crude Oil for 10 Weeks.

Mean number of neutrophilic leucocyte for juveniles of *Clarias gariepinus* exposed to subacute concentrations of WSF of crude oil indicated significant increase ($P < 0.05$) above control value of 3.58±0.27% among groups exposed to T1 (5.31±0.49%), T_2 7.31±0.76%, T_3 8.58±0.82%, $T_4$10.47±1.04%, and T_5 11.53±1.3%,. with the greatest increase among those exposed to T_5 (Fig 5). Mean difference did not exist between treatments 2 and 3 but differences occurred between group of fish exposed to treatments 1, 4 and 5. Along the period of exposure, neutrophil level during weeks 2, 6 and 10 was different from its amount during start, week4 and week8 respectively. Significant increase in neutrophils above control fish may have resulted in neutrocytosis in those exposed to WSF of crude oil.

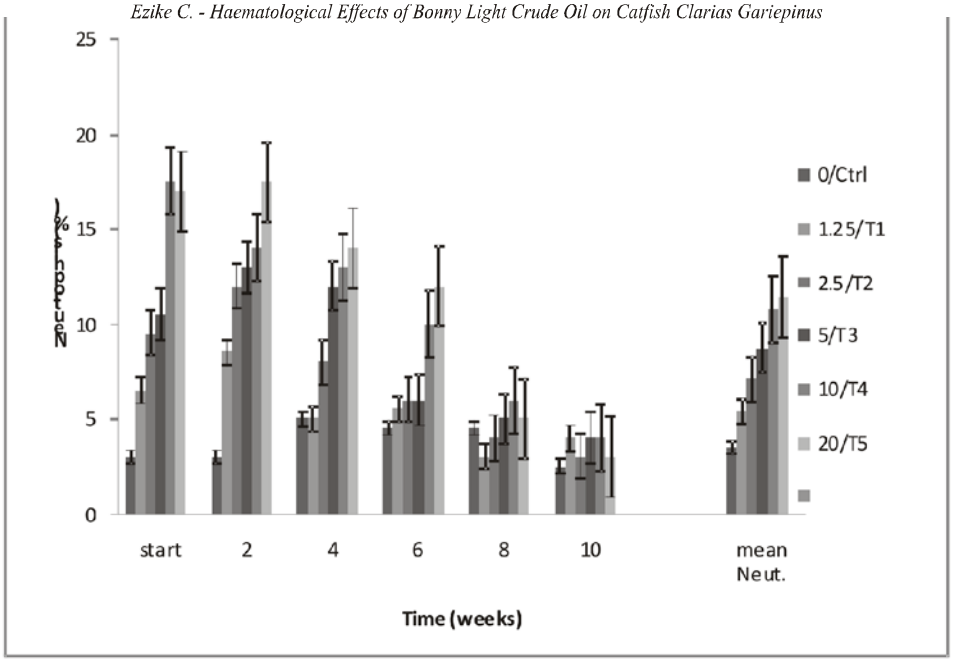

Fig.5: Mean Number of Neutrophils±SE (%) of *Clarias gariepinus* Exposed to Subacute concentrations of WSF of Crude oil for 10 weeks

Result of Lymphocytes of *Clarias gariepinus* Exposed to Sublethal Concentrations of WSF of Crude Oil for 10 Weeks

Mean number of Lymphocytes sub populations in exposed catfish *Clarias gariepinus* to crude oil indicated significant dose dependent decrease ($P < 0.05$) below mean control value of 91.55±0.53% to 80.72±0.45% in T_4 and where the only significant difference was shown to have taken place. A consideration along exposure period also indicated level of lymphocyte difference in weeks2, 6 and 10 when compared with lymphocyte levels at start, week4 and week8 (,Fig. 6). Decrease in exposed group of fish to 10ml/l may have resulted to lymphopeania. No statistical difference took place in group of fish exposed to treatments 1, 2, 3, and 5.

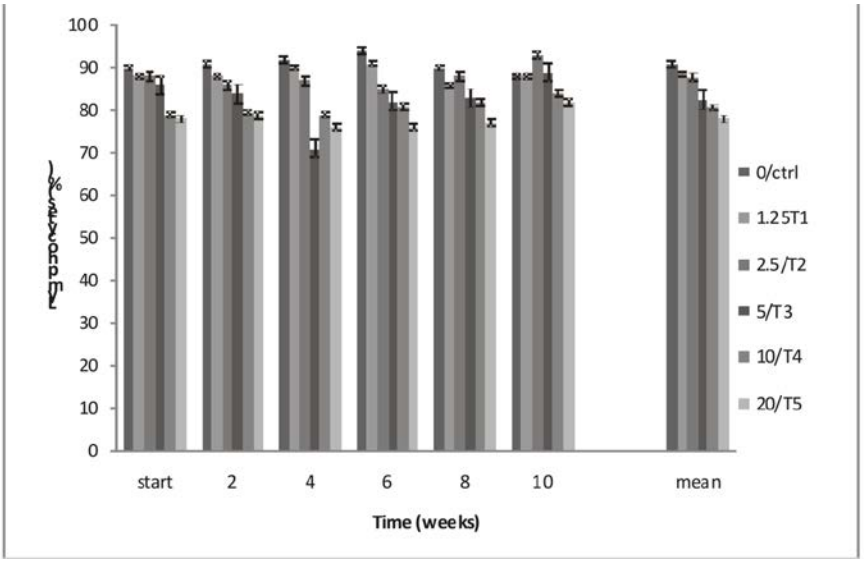

Fig.6: Mean Number of Lymphocytes±SE (%) of *Clarias gariepinus* Exposed to Subacute concentrations of Crude oil for 10 weeks

Result of Mean Corpuscular Volume (MCV) of *Clarias gariepinus* Juveniles Exposed to WSF of Crude oil for 10 Weeks

Mean Corpuscular Volume of Juveniles of *Clarias gariepinus* exposed to subacute concentrations of WSF of crude oil did not vary significantly (P > 0.05) among treatment means and control. (Fig 7). A range of 118.52±2.83 to 130.31±3.52 Femtoliters observed in control and treatment 2 exposed group did not show that differences took place between the various treatment concentrations and period of exposure.

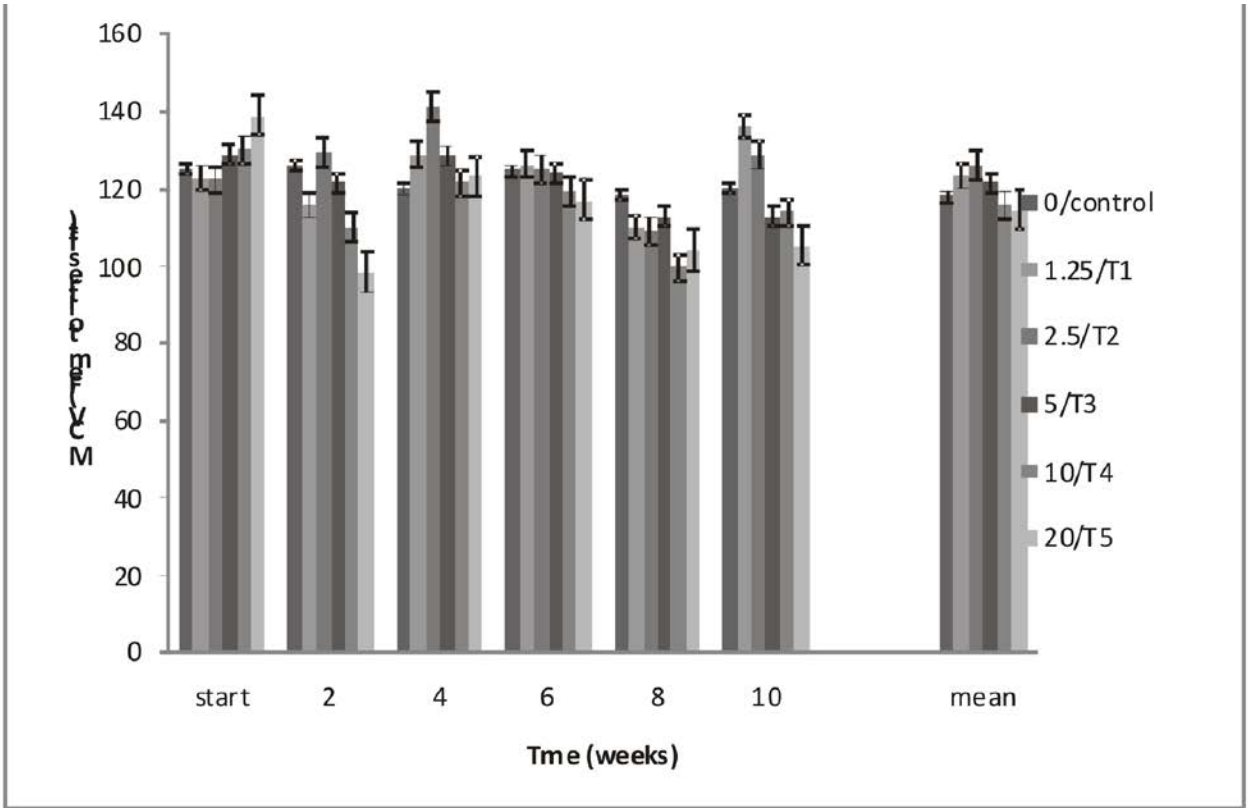

Fig.7: Mean Corpuscular Volume MCV±SE (ft) of *Clarias gariepinus* juveniles Exposed to WSF of Crude oil for 10 weeks

Result of Mean Corpuscular Haemoglobin Concentration (MCHC) of *Clarias gariepinus* Juveniles Exposed to Sublethal Concentration of WSF of Crude Oil for 10 Weeks

There was no significant difference in MCHC among treatment means of *Clarias gariepinus* juveniles exposed to subacute concentrations of crude oil. Mean corpuscular haemoglobin concentration (MCHC) between exposed group of fish and control fish did not differ significantly (P > 0.05). There was also no observable difference in MCHC within the various groups of fish along the period (Fig. 8).

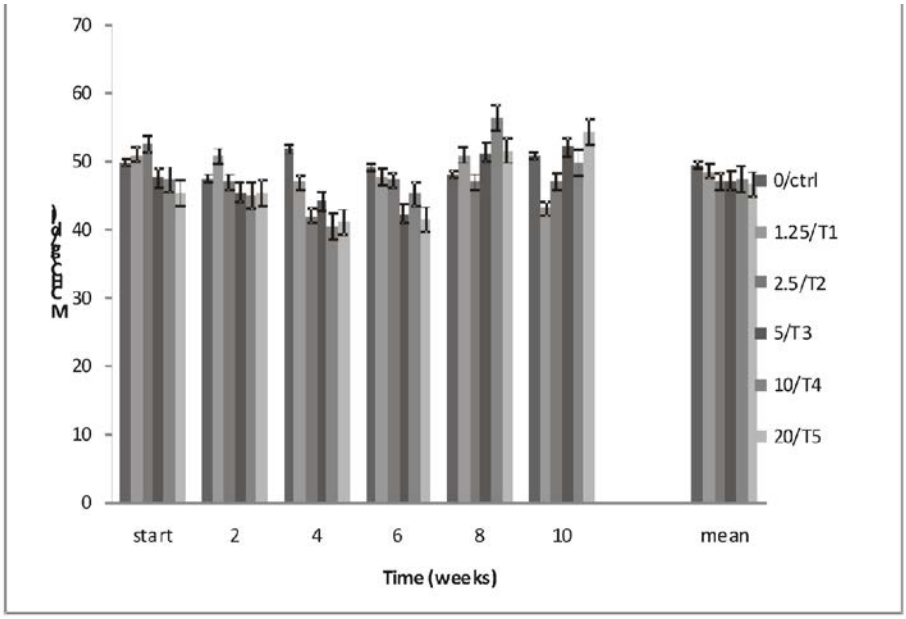

Fig.8: Mean Corpuscular Haemoglobin Concentration MCHC ±SE(g/dl) of *Clarias gariepinus* juveniles Exposed to WSF of Crude oil for 10weeks.

Result of Mean Corpuscular Haemoglobin (MCH) of *Clarias gariepinus* Juveniles Exposed to Sublethal Concentration of WSF of Crude Oil for 10 Weeks

There was no significant difference (P >0.05) in mean corpuscular haemoglobin MCH in the various group of fish exposed to subacute concentrations of water soluble fraction of Bonny light crude oil for 10 weeks. A mean range of 58.19±1.23Pg to 52.63±1.49Pg of MCH observed in control fish and treatment 5 exposed groups of fish did not indicate statistical changes in values of MCH of fish. (Fig.9). Significant difference along the period was not demonstrated in the various group of fish exposed to WSF of Bonny light crude oil for 10 weeks.

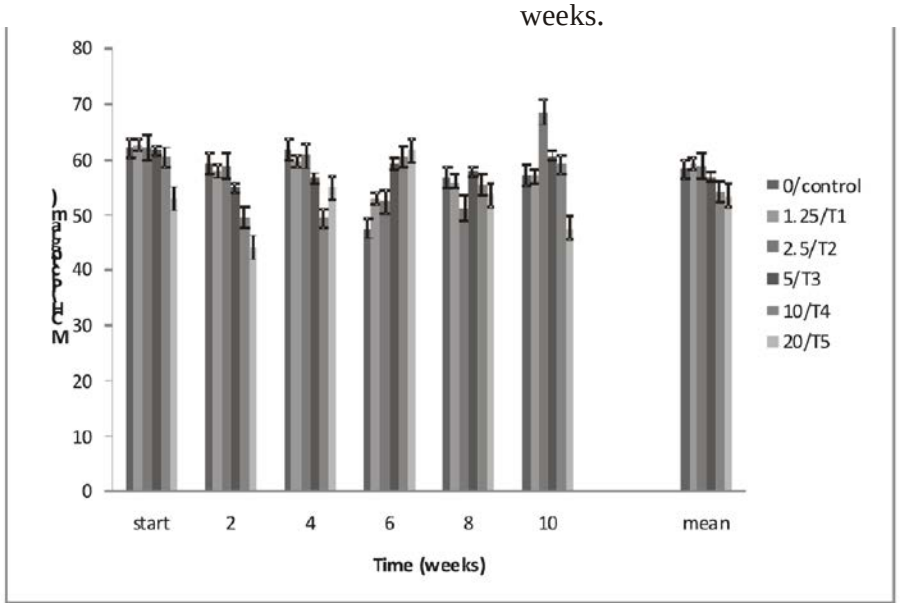

Fig.9: Mean Corpuscular Haemoglobin MCH ±SE(pg) of *Clarias gariepinus* juveniles exposed to WSF of crude oil for 10weeks

Table 1 : Mean Blood Parameters ±SE of *C.gariepinus* juveniles exposed to subacute concentrations of WSF of crude oil for 10weeks

Parameters	20	10	5	2. 5	1.25	0
RBC 10^6/mm^3	1.49±0.06^e	1.55±0.03^d	1.56±0.06^c	1.61±0.03^b	1.87±0.02^a	2.09±0.07^a
WBC 10^3/mm^3	43.48±1.1^d	44.41±0.84^d	46.26±0.46^d	45.87±0.43^c	50.20±0.14^b	51.44±0.47^a
PCV (%)	16.5±0.89^d	17.66±0.76^d	19.05±0.65^c	19.66±0.49^b	22.66±0.11^a	24.33±0.27^a
Haemoglobin(g/dl)	7.73±0.29^a	8.34±0.24^a	8.89±0.99^b	9.45±0.27^c	10.81±0.16^a	12.05±0.11^c
Neutrophils(%)	11.53±1.35^d	10.47±1.03^e	8.58±0.83^c	7.31±0.75^c	5.30±0.49^b	3.58±0.27^a
Lymphocytes (%)	78.0±0.50^a	80.72±0.44^b	84.67±2.1^a	87.83±0.61^a	88.50±0.38^a	91.55±0.53^a
MCV (Ft)	114.54±3.3^a	115.73±2.33^a	121.43±1.62^a	130.31±3.5^a	123.33±2.07^a	118.51±2.83^a
MCHC (Mg/dl)	49.21±3.9^a	47.44±1.1^a	47.24±0.87^a	47.19±0.73^a	48.59±0.64^a	49.51±0.35^a
MCH(Pg)	52.6±1.4^a	54.1±0.90^a	56.9±1.3^a	58.8±0.9^a	59.3±0.5^a	58.2±1.2^a

Means on a row with the same superscript did not differ significantly but Means with different superscript di ffered significantly. Mean separation by Duncan 's Multiple Range Test at 5%level of significanc e (P<0.05)

DISCUSSION

Discussion on the Effects of WSF of Crude Oil on the Haematology of *Clarias gariepinus* Exposed to WSF of Crude Oil for 10 Weeks

The value of 2.09 ± 0.2 x 10^6/mm^3 as RBC mean value for control group of fish was within the normal RBC range reported in Adams (2004), who reported normal RBC value for *Clarias gariepinus* to be 2.17 ± 0.03 x 10^6/mm^3 . The value of 12.22 ± 0.018g/dl and 24.3 ± 2.6% reported as control mean values of Haemoglobin and PCV were within normal values of fish reported by Adams (2004) and Omitoyin (2006). The recorded significant reduction in RBC in exposed group than control in this research is somewhat in disagreement with Prasad et al. (2006) who recorded increased values of these parameters when they exposed catfish H. *fossillis* to crude oil water soluble fraction but it aggress with Omoregie (1995) on Nile tilapia fish *Oreochromis niloticus* exposed to crude oil water soluble fractions and Gabriel et al. (2007) 0n on *Clarias gariepinus* exposed to kerosene and to gasoline (Ezike and Ufodike, 2009). Decrease in these values may be due to haemolysis caused by haemodilution effects of water soluble fraction of crude oil into the circulatory system of the exposed group of fish. Sigh and Sigh (1982) noted that damages of haematopoietic tissues in the kidneys and spleen and aggregation of cells at the gills contributed to the above decrease of circulating RBC cells among exposed group of fish. Annune and Ahuma (1998) noted that swelling of the erythrocytes lead to erythropaenia in *Clarias gariepinus* exposed

heavy metal components of petroleum. Erythrocyte reduction in fingerlngs of *Clarias gariepinus* subjected to tobacco exposition was destroyed by toxicant as reported by Musa et al. (2013)

Dose dependent reduction in White Blood Cell below control group in this investigation is similar to reports of Prasad et al. (2006) when they exposed *Heteropheustes fossilis* to crude oil; in crude oil exposed *Clarias gariepinus* (Van Vuven et al., 1994); in crude oil exposed *Clarias gariepinus* juveniles and rats (Sonmonu and Oloyede 2008). Significant dose dependent increase in White Blood Cell was however shown to have occurred in juveniles of *Clarias gariepinus* exposed to copper and lead (Annue and Ahuma, 1998) and same observation was made by Musa and Omoregie (1999) when they exposed *Clarias gariepinus* to malachite green and *Clarias gariepinus* exposed to tobacco (Musa et al., 2013). Kochhann et al. (2013) however did not notice WBC changes in *Colossoma macropomum* linked to crude oil. These authors seem to have agreed that toxicant differ in potency and mode of action hence fish tend to respond differently with varying potency of toxicants.

Pollutants and other stressors as recorded in this research and several studies elicit changes in White Blood Cell subpopulations (Ellis, 1981). In this investigation, crude oil Water Soluble Fraction resulted in dose dependent increase in neutrophils and decrease of lymphocytes. Gabriel et al (2007) reported similar effects in *Clarias gariepinus* exposed to kerosene as well as in *Tilapia guineensis* exposed to industrial effluents (Akinrotimi et al., 2013) and assigned neutrophils as the most sensitive subpopulation of the White Blood Cell. The reported no significant difference in MCV and MCHC with control as well as non significant decrease in MCH than control may suggest that anaemic condition in exposed fish did not result to hypochromic anaemia (Hoffbrand et al., 2006). Our findings suggest that water soluble fraction of Bony Light crude oil caused a depletion of blood cells which affected other blood parameters in which RBC reduction led to lowering of PCV and haemoglobin. However, leucocyte and lymphocyte reduction may have triggered increased neutrophil number to address anaemic conditions elicited by hydrocarbon metabolites to macromolecules of blood cells.

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