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Some Haematological Responses of *O. Niloticus* to Acute and Sublethal Concentrations of Paraquat

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Abstract
Haematological characteristics have been used widely in clinical diagnosis in man .The application of haematological techniques is valuable for fishery biologists in assessing fish health and monitoring stress. The results of the study showed that of acute toxicity test, *O. niloticus* exposed to (0.00,9.60,10.40,11.20,12.00,14.20,15.20 and 16.00 mgL-1) of paraquat showed that fish were put under stress as evidenced by a dose –dependent reduction in haematocrit value, haemoglobin content, mean corpuscular haemoglobin concentration, and % neutrophil as compared to its baseline values. The acute effect on the blood was further confirmed by the photomicrograph of the blood cells at 96-hour, which showed total destruction and senility of blood cells. In the sub-lethal toxicity tests of paraquat to *0reochromis.niloticus*, the study showed a significant decrease in the haemoglobin (Hb), haematocrit (Hct) and mean corpuscular haemoglobin concentration (MCHC) with increase in concentrations and in exposure periods of 8-weeks which could be due to haemodilution and an increase in erythrocyte destruction. This may indicate that the herbicide induced an effect similar to production of anaemia.

Keywords: Acute toxicity, Sublethal toxicity, bioassay, haematological response, paraquat, *O.niloticus.*

1. Introduction
McClay and Vars [1] first demonstrated that alteration in the blood and damage to haemopoietic tissue in fish can be associated with pathological conditions related to water –borne pollutant. Halsband and Halsband [2] while studying the Threshold effects of various contaminants, noted that the blood was affected prior to any other visible changes in fish. Haematocrit measurements reported by Allison *et al.* [3] showed no difference between exposed and control cut throat trout. Hunt and Gilderhus [4] found the same with the exposure of blue gills to sodium arsenite. Other workers, however, have noted a significant reduction in haematocrit values. Andrews *et al* [5] observed an increase in haematocrit values of blue gills exposed to 0.05 mg\L of heptachlor for four hours, but they returned to normal after 28 days. Blood is the most accessible elements of the teleost bodyflow, consequently its variables are commonly used as direct or inferential indicators of functional state [6, 7].
The objective of this study was to evaluate the effect of acute and sublethal concentrations of paraquat on *O. niloticus* blood. *O. niloticus* was chosen, because it is of local economic importance and common in African freshwaters.

2. Materials and Methods
2.1 Source and Maintenance of Experimental Animals
Fish used in this study were obtained from the Ahmadu Bello University dam. Fingerlings of *O. niloticus* of weight range of 6.97-7.72 g and mean weight of 7.35 g were conveyed in an ice box containing sufficient water from the dam, which is the natural habitat of the fish.
In the Laboratory, the dam water was gradually replaced with dechlorinated tap water over a period of 4hours during which the aquarium tanks were aerated. The fingerlings were acclimatized at a temperature range of 21.5-24 ^{0}C for two weeks prior to the commencement of the assay and Natural daylight photoperiod of 12/12 was maintained. The Fish were fed daily during acclimation but the fish were not fed 24hours prior to commencement of the acute toxicity assay and during the assay. The fish were fed twice daily during the long term assay.

2.2 General experimental procedure.
During the assay, eight 30.5 cm *30.5 cm *92.5 cm of glass aquaria with test solutions of 0.00 (control), 9.60, 0.04, 11.20, 12.00, 14.20, 15.00 and 16.00 mg/l of paraquat that were replicated but the treatment aquaria were used and replicated subsequently. The fish were exposed to the water tanks and control water tank too in the acute toxicity test. Renewal of water was by siphoning three quarters of the test solution and the facal materials using a rubber hose. The remaining test solution was then topped with a fresh test solution while the control was topped with dechlorinated water during acclimation.

2.3 Haematology
Determination of haemoglobin (Hb) haematocrit (Hct), mean corpuscular haemoglobin concentration (MCHC) and leucocyte differential counts were carried out on three (3) of the surviving fish in each test solution of 9.60 mgl^{-1}, 11.20 mgl^{-1}, 12.00 mgl^{-1} and 14.20 mgl^{-1} and control, but none could be carried out on fish in solution of concentration of 16.00 mgl^{-1} because they had all died by the end of 96-hour. The histogram was plotted to compare the effects the different concentration of paraquat had on the blood of test organisms. Statistical analysis was carried out to check the significance of the differences. Ventilation rates and tail fin movement rates were graphically represented using histograms.

2.4 Long-term Sub-Lethal Bio-Assay Exposure
In each case a fraction of the 96-hour LC_{50} was used to determine sub-lethal concentration rate as recommended by Amminikutty and Rege [8], Oladimeji and Ologunmeta [9] and Mohammed [6].
In the test with *O. niloticus* 1/6, 1/9, and 1/12 of the 96-h LC_{50} (12.25 mgl^{-1}) were used. These gave nominal concentrations of 2.04mgl^{-1}, 1.36 mgl^{-1} and 1.02 mgl^{-1} of paraquat. The values were rounded up to 2 mgl^{-1}, 1.4 mgl^{-1}, and 1.02 mgl^{-1} respectively.
Eight, 30.5 x 30.5 x 92.5 cm aquarium tanks were utilized in each set of the assay. Four tanks were used for the test, while the remaining four were duplicates. The changing was as described for the acute toxicity assay.
Appropriate volumes of stock solution were discharged into 50 litres of dechlorinated water in each of the test tanks except the control which contained only the diluted water.
The tanks were randomly arranged on the working table and left for 30 minutes to allow toxicant to be uniformly distributed in the water. Fish of weight range of 6.77–7.29 g and mean weight of 7.35±0.53 g were used. The fish were randomly assigned to give a load of sixteen (16) fish per test tank. They were fed to satiation once daily on compounded pelletized fish food. The feed was crushed into smaller units before being fed to the fish. The natural photoperiod of 12–h light and 12-h darkness that prevailed during the period of assay was used since the room was well lit with sunlight ray streaming into the laboratory.
Opercular ventilation rate and tail fin movement rate per minute were observed on four fish per tank marked by the little cut on tail fin once fortnightly.

2.5 Haematological Procedures
In the sublethal test, four fish were removed fortnightly from the sixteen in each tank, and stunned by the sharp blow at the juncture of cranium and the spine till they became immobile. [10].
Blood was withdrawn by cardiac puncture from the stunned fish using a 2 ml sterile plastic syringe and needle. Plastic instead of glass syringe was used to prevent quick coagulation (Smith *et al*., 1993) [11].
Blood was obtained from the test fish by cardiac puncture. The blood drawn was decanted into a vial containing 100 g/l EDTA. Five milligram EDTA per cm^3 blood was used (Owolabi, 1995) [12].
Determination of hematological parameters was carried out in 3 of the surviving fish in each test solution on 9.60,11,20,12.00 and 14.20 mg/L and control in acute toxicity test, but none could be carried out for test concentration of 16.00 mg/L, because they had all died by the end of the 96-hours.

The blood was allowed to air dry. It was then immersed in absolute methanol (5 g/L) (Owolabi, 1995) [12] for at least 5minutes.It was air dried again (Klontz, 1972) [13]. The Smear was then stained with Giemsa stain with 10% pH 6.4 phosphate buffer. The heamatocrit differential count was then taken from the slide. The microphotographs of the blood cells were taken. Blood samples for haematocrit were collected in heparinized capillary tubes, the end of the tubes was sealed with plasticine and the tubes spun for 7 minutes in a micro hematocrit centrifuge at a speed of 12,900 pm. The haematocrits were measured with a micro capillary reader.

2.6 Haemoglobin
The cyanomethaemoglobin technique was used (Wintrobe, 1962(14); Babatunde *et. al*, 2001) [15].

After thoroughly mixing the blood, 0.02 cm^3 of blood was placed into 4 cm^3 of Drabkin's reagent. The solution was gently mixed by inversion and allowed to stand for at least 10 min for full conversion of haemoglobin to cyanomethaemoglobin. The small coagulum formed are removed with a wooden stick. The Transmittance was read on an EEL spectrophotometer at a wavelength of 540 mm and the reading was converted to haemoglobin concentration in grams per 100 cm^3 by reference to a graph constructed using commercially available cyanomethaemoglobin standards.

2.7 Leucocyte Differential Count
The count was read on the slide with the aid of a microscope and a counter. Blood chart for blood components was prepared and used for identification of the different cell types. For the chart, aid was obtained from work done by KIontz (1972) [13] Blaxhall and Daisely (1973) (16) and Murad *et al.* (1990) [17]. The physicochemical parameters of test solutions such as temperature, dissolved oxygen, electrical conductivity, water hardness etc. were constantly determined at weekly intervals throughout the test periods.

2.8 Statistical Analysis
All data for paraquat treated fish were compared with the control using analysis of variance test (ANOVA) and Duncan Multiple Range Test (DMRT) at the 95% confidence level.

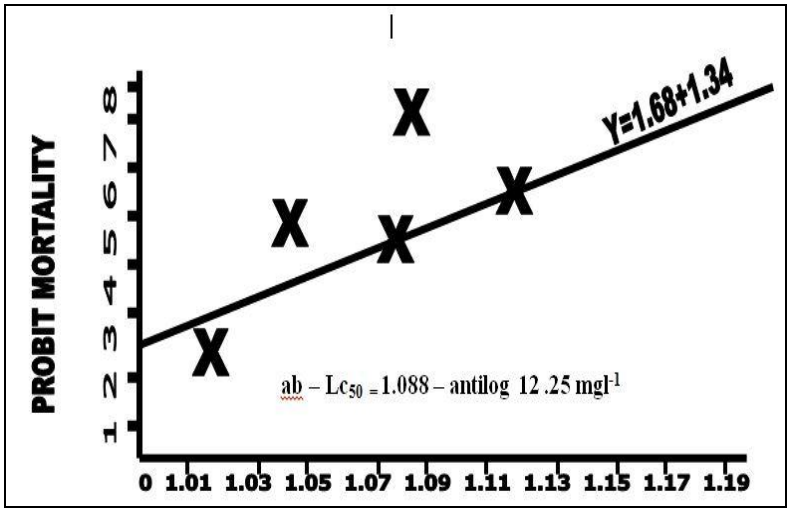
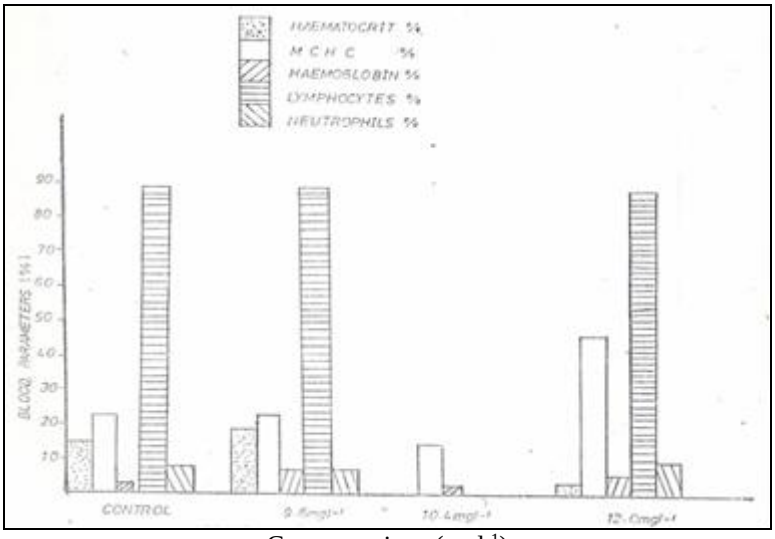


Fig 1: Linear relationship between probit mortality (%) and $\log_{10}$ concentration (mgL^{-1}) of *O.niloticus* exposed to various acute concentrations of paraquat.



Concentrations (mgl^{-1})

Fig 2: Blood parameters of *o. niloticus* exposed to paraquat for 96hr

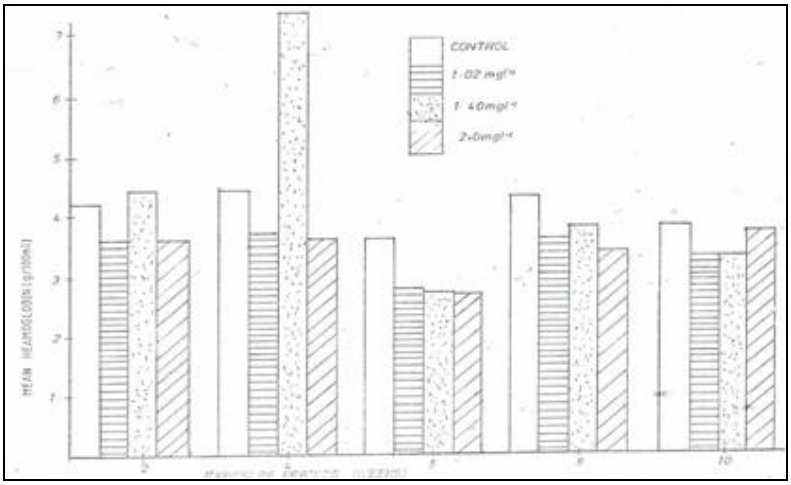


Fig 3: Mean haemoglobin (gl100ml blood) of *O. niloticus* exposed to paraquat for 10 weeks at 23 ^{0}C ± 0 samples are presented

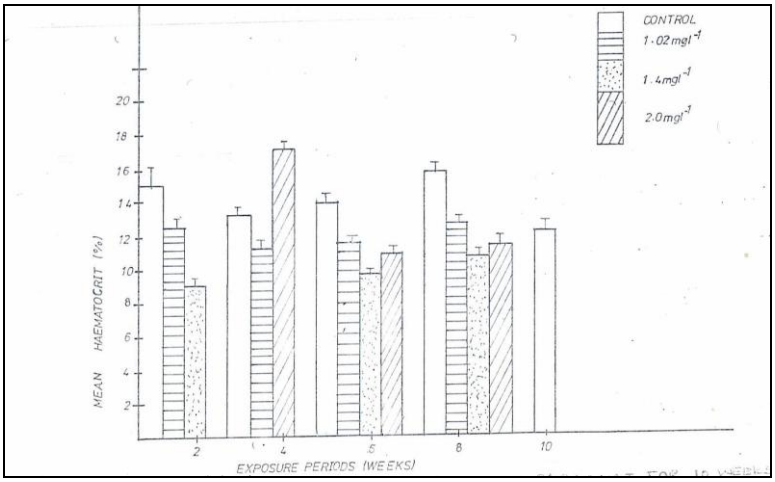


Fig 4: Haematocrit of *O. niloticus* exposed to various concentrations of paraquat

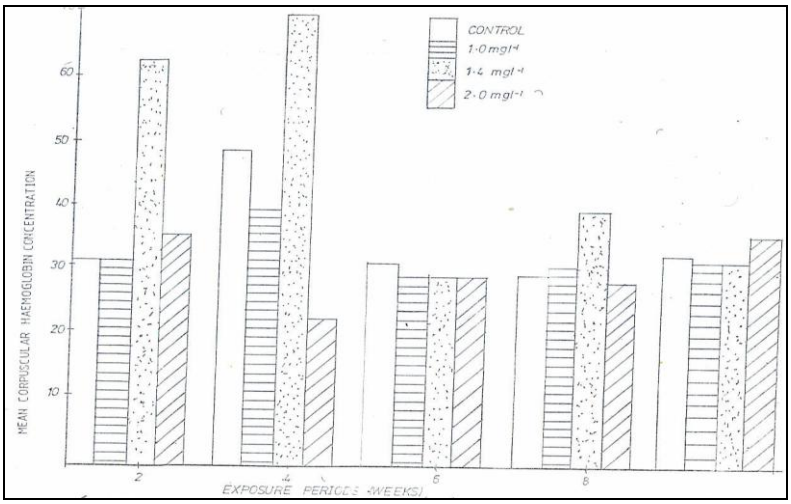


Fig 5: Mean corpuscular haemoglobin content of o. niloticus exposed to paraquat for

3. Results

The LC_{50} was 12.25mgl $^{-1}$ and 95% confidence limit was 1.06 mg/l. The result showed that in the acute toxicity the concentrations below 9.60 mg/l were not lethal to the fish. Increase in concentration to 10.40 mgl^{-1} resulted in 20% mortality. The highest mortally (100%) was recorded at a concentration of 16.00 mgl^{-1} at 48 hours.

Table 1: Water quality values during toxicity assays with *O. niloticus* exposed to paraquat.

Parameter	Range	MEAN + SD (N=4)
Temperature	21.90-24.00	27.7±0.80
Dissolved oxygen (mg/L)	5.20-6.10	5.7±0.70
Conductivity (Umhos)	3.50-5.5 *10^2	4.5*10^2 ± 0.10
Hardness (mg/L of $gcaco_3$)	20.0-35.00	28.0±0.80
Alkalinity (mg/L)	14.00-19.60	15.0±0.10
Ph	6.80-7.80	7.3±0.50

The physico-chemical parameters of the test solutions did not differ significantly ($P<0.05$) from those for the control during the exposure period (Table 1). Signs of toxicosis observed in the exposed fish included loss of balance, air gulping and hemorrhaging of the gills and fins in the acute toxicity test and signs were more pronounced in the fish exposed to aquaria with higher test –water concentrations. The above behavioural patterns were not observed in the sublethal assay. Only increased sluggish movement and reduction in food consumption were observed (Babatunde *et al.* 2001) [15].

Table 2: Means haemoglobin (g/100 ml blood), haematocrit and mean corpuscular haemoglobin concentration (MCHC) of *O. niloticus* exposed to sub-lethal concentration of paraquat for 8 weeks at 22.7 ^{0}C ± 0.8 means ±S.E of 4 fish are presented.

Conc	**Weeks Of Exposure**				
Mg1^{-1}	2	4	6	8	10
HAEMATOCRIT%					
0.00	14.5±0.71	13.0±1.41	11.5±0.71	14.5±0.71	11±0
1.02	12.0±1.41	9.5±0.70	9.5±0.7	14.0±1.41	-
1.40	7.5±0.71	0±0.0	9.0±0.0	9.5±0.07	-
2.00	0. ±0.0	17.0±1.40	9.5±0.70	12.0±2.80	-
Neutrophils (Granulocytes)%					
0.00	4.67±0.01	4.45±.07	3.6±.4	4.3±0.42	3.72±0.19
1.02	3.59±.01	3.70±.14	2.75±.7	3.6±0.57	3.26±0.07
1.40	4.35±.07	7.29±.01	2.7±.07	3.8±0.28	3.30±0.14
2.00	3.67±.07	3.58±.04	2.7±.07	3.4±0.28	3.65±0.07
	MCHC (HB/HCT)				
0.00	32.7±0.07	48.8±0.71	32.7±.01	29.65±1.5	32.70±0.07
1.02	32.7±0.07	40.0±0.35	30.0±.35	26.15±2.19	32.00±0.41
1.40	62.9±0.41	73.0±0.35	30.0±.41	40. ±0.0	32.00±0.00
2.00	36.0±0	22.5±0.00	30.0±.07	28.85±450	30.00±3.50

Base line values determined before fish were allotted to the exposure groups were 5.55±0.35, 17.5±0.71, 31.70±0. 74, 82±5.66, 14±5.65, for haemoglobin (HB) hematocrit (Hct) MCHC, lymphocyte (L) and Neutrophil (N) respectively. Means ±S.E of 4 fish are represented.

Table 3: WBC (Leucocyte) differential blood count (%) for blood of *O. niloticus* exposed to sublethal concentration of paraquat for 10 weeks. Means ± S.E. of 4 fish are presented.

Conc	**Weeks of Exposure**				
Mg1^{-1}	**2**	**4**	**6**	**8**	**10**
Lymphocytes					
0.00	**83**±0.38	**90**±0.07	**90**±0.41	**77**±1.4	**88**±0
1.02	**88**±0.71	**86**±0.41	**89**±0.07	**89**±0.71	**-**
1.40	**84**±0.71	**0.0**	**91**±0.07	**96**±0.0	**-**
2.00	**0**	**89**±0.71	**88**±.35	**99**±4.0	**-**
Neutrophils (Granulocytes)%					
0.00	**16**±.71	**10**±.41	**10**±0.10	**22**±2.8	**-**
1.02	**12**±.07	**10**±.1.4	**9**±1.4	**10**±0.7	**-**
1.40	**16**±.35	**0.0**	**9**±.35	**4**±0.0	**-**
2.00	**0.0**	**11**±0	**12**±.07	**14**±1.4	**-**

(-) or (0) refers to senile or completely destroyed blood thus no values for the blood parameters

3.1 Effects of Paraquat on Haematological Parameters in Sub-Lethal Test

Haemoglobin and haematocrit of the control fish did not deviate much from the baseline values throughout the test period (Table 2). The hematocrit of the control decreased slightly with time from an initial 15% to 12% at the sixth week of exposure, after which value increased to 14.5%. Haematocrit of *O. niloticus* exposed to different concentration of paraquat showed a dose-dependent relationship in which there was decrease in haematocrit value to increase in concentration. However, at week 4, blood of fish exposed to paraquat concentration of 1.40 mgl^{-1} showed total denaturation of blood cells. The same phenomenon was seen in the high toxicant concentration (2.0 mgl^{-1}) at week 2. Statistical analysis showed a significant difference in both the within and between treatment. Thus a dose and period dependent effects are shown.

3.2 Haemoglobin

The haemoglobin concentration of O. niloticus exposed to various sublethal concentrations of paraquat are represented in Table 2. The results show that after 2 weeks of exposure there was a decrease in haemoglobin value to with increase in concentration of the toxicant. Haemoglobin concentration in blood of fish exposed to toxicant concentration 0f 1.40 mgl^{-1} was close to that of control. The same pattern was observed after 6 weeks of exposure, indicating a dose-dependent decrease in haemoglobin concentration. Analysis of variance shows a significant difference in various haemoglobin values of the fish exposed to different sublethal concentrations (ANOVA and DMRT < .05). The haemoglobin content of the fish blood decreased with increase in exposure duration till 6 weeks, followed by an increase in hemoglobin values by the 8th week. There is a significant difference between control.

The mean corpuscular haemoglobin concentration (MCHC) values of *O. niloticus* exposed to various paraquat concentration, are presented in Table 2. The baseline mean value is 31.70%, which shows no significance difference from that of control throughout the duration of the experiment except at two weeks when the value increased only to return to baseline value thereafter. Values of MCHC of fish exposed to toxicant concentration of 2.0 mgl^{-1} showed a decrease right from the beginning but latter towards the end an increase in value. Fish exposed to 1.40 mgl^{-1} paraquat showed the greatest variation in values. Statistical analysis shows a significant difference ($P<0.05$).

The lymphocytes (%) of *o. niloticus* exposed to various concentrations of paraquat are presented in Table 3. The results show that at the start of the experiment, the lymphocytes of the control fish was 83%. The value increased with increase in concentration as well as increased in exposure for 1.40 mgl^{-1} and 2.0 mgl^{-1}. Anova and DMRT showed significant difference between the treatment groups. The percentage (%) neutrophils of o. niloticus exposed to various concentrations of paraquat are presented in Table 3. The mean neutrophilic value of control does not deviate from that of basal value. There is a decrease in percentage neutrophils with the highest toxicant concentration of 2.00 mgl^{-1}, showed an increase at 8^{th} and 10weeks. Anova showed that there is a significant difference between the various concentrations (ANOVA & DMRT $P<0.05$) There was denaturing of the blood at concentration 2.00 mgl^{-1} at 2 weeks. The same effects was observed at week 4 in toxicant concentration of 1.40mgl^{-1}. At 10 weeks fish in various toxicant concentrations showed the same condition with no neutrophil and lymphocyte values recorded. These test was extended to 10 weeks because of the observation of high incidence of death at 10 weeks with all environment and water parameters being constant as before to see if death was due to environmental factors or effects of toxicant on fish blood.

A statistically significant difference in neutrophil count was observed between 2 and 4 weeks. There was an insignificant increase in the mean values after 6 and 8 weeks of exposure. Toxicant concentration of 1.02 mgl^{-1} and 1.40 mgl^{-1} cause a decreased in percentage neutrophil with increase in exposure period while toxicant concentration 2.00 mgl induced an

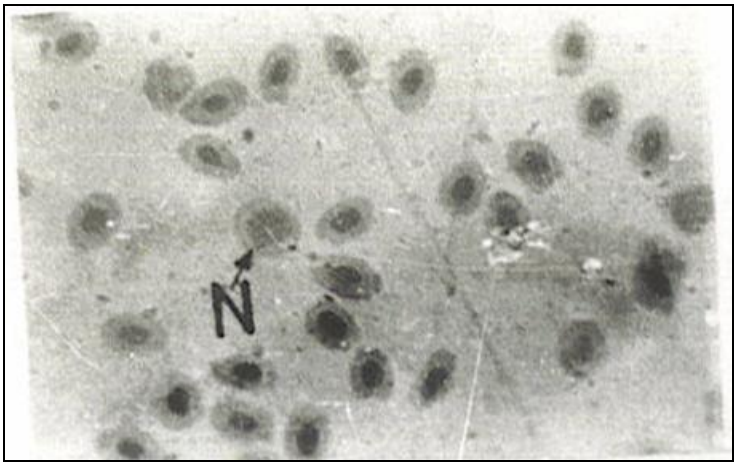


Plate I: Blood of control fish shows well demarcate cytoplasm and nucleus. x100 Arrow N shows a neutrophil.

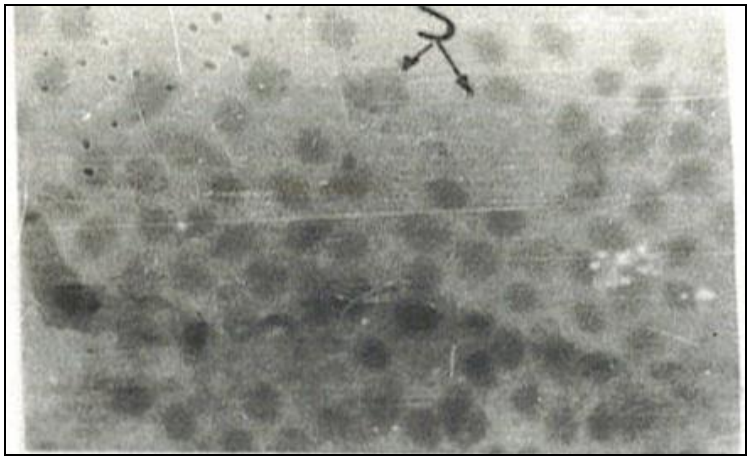


Plate II: Blood of *O. niloticus* exposed to 12 $mg1^{-1}$ of paraquat at 60-h acute toxicity test (Leishan stain) x100 shows completely denatured cells.

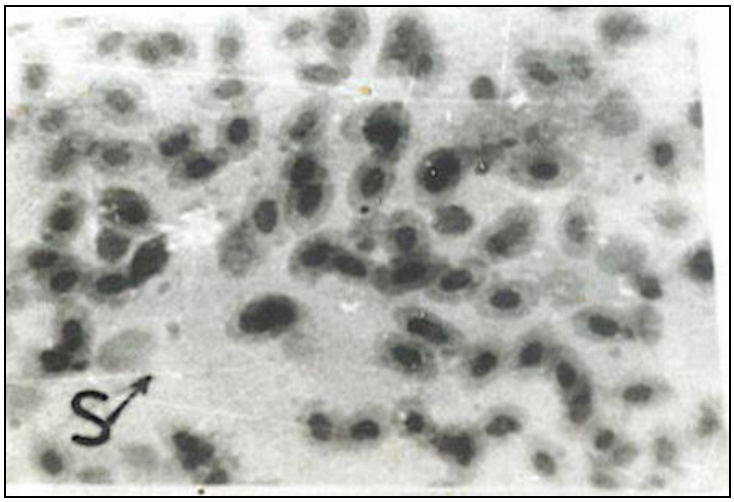


Plate III: Blood of *O. niloticus* exposed to 1.02 mg 1^{-1} of paraquat at 6 weeks. Shows few senile (destroyed) erythrocytes (Arrow S) x100.

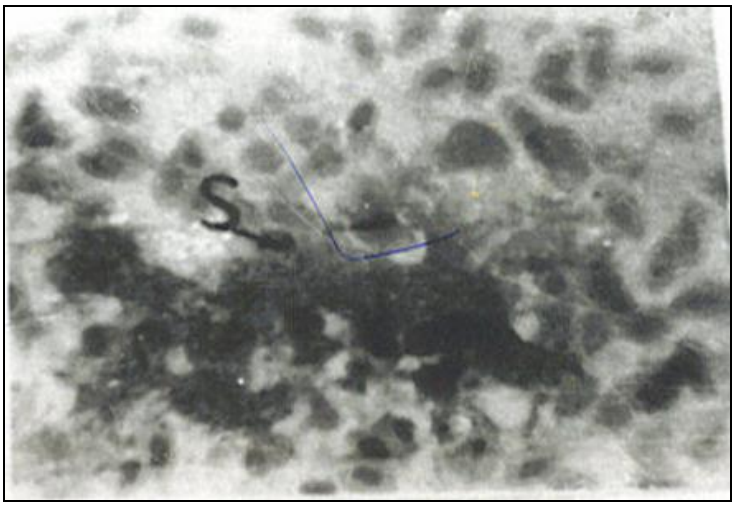


Plate IV: Blood of *O. niloticus* exposed to 1.4 mg1^{-1} of pareaquat at 6 weeks. Shows highly haemolysed blood cells. (Arrow S) x100.

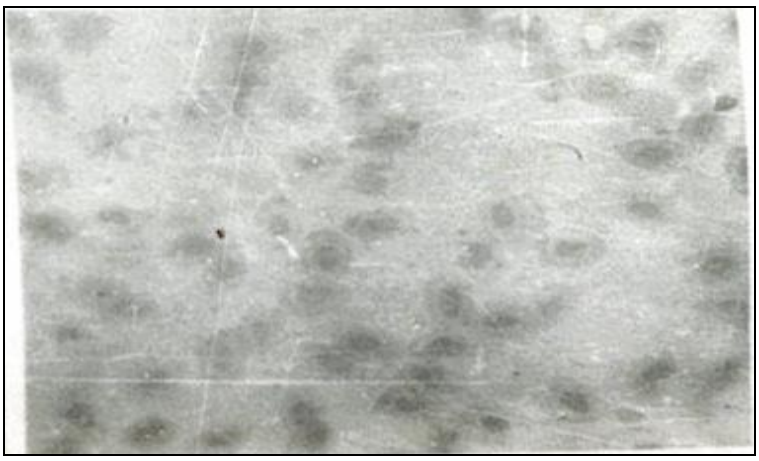


Plate V: Blood of *O. niloticus* exposed to 2.0 mg1^{-1} of paraquat at 6weeks. Shows few destroyed cells (Arrow S) x100.

4. Discussion

4.1 Haematological effects

Haematological characteristics have been widely used in clinical diagnosis in man. The application of haematological techniques are valuable for fisheries biologist in assessing fish health [18] and monitoring stress [19]. Eisler [20] demonstrated by studies on the blood of northern puffers that changes in blood prior to onset of more striking physiological and morphological changes can be indicative of unfavorable aquatic environment. Anees [21] note from his work on haematological abnormalities in *C. punctatus* exposed to sublethal and chronic levels of organophosphorus insecticides that the blood was affected prior any other visible changes in fish. Haemoglobin concentration, red blood cells count and haematocrit are particularly recommended as tests which could be carried out on routine basis in fish hatcheries as a check on health of fish [22]. The results of this study show that *o. niloticus* exposed various acute concentrations of paraquat were put under stress, as evidence by a dose-dependent reduction in haematocrit value, haemoglobin content, MCHC, And percentage neutrophil as compared to its baseline or control. The acute effects on the blood was further confirmed by the photomicrograph of the blood cells at 96 hr. the blood cells were completely rendered senile with reduced size and indistinguishable cytoplasmic content. This could be due to increased fragility or rate of destruction of circulating erythrocytes [23]; [24]. Leucocyte count showed no significant changes. In the sublethal toxicity test of paraquat to *O. niloticus*, the significant decrease in the haemoglobin, haematocrit and MCHC with increase in concentrations and in exposure periods observed could be due to an inhibition of erythrocyte production, an increase in the erythrocyte destruction and haemodilution [23, 25]. This may indicate that the herbicide induced an effect similar to the production of anaemia. reported [20, 25] for fishes exposed to different toxicants.

Eisler and Edmunds [26] reported that erythropenia (deficiency in number of red blood cells) was reflected by the reduced haemoglobin content and haematocrit as well as in ESR (Erythrocyte Sedimentation Rate). He further reported that Anaemia generally causes an increase in gamma-globulins, as reported in northern puffers following endrin toxicosis. Srivastava, [27] showed that hypercoagulability of whole blood in fish exposed to heptachlor was due to thrombocytopaenia; that is, blood coagulation was retarded in the fish as thrombocyte counts decreased. However, in the present study, higher coagulability (Hyper coagulability) as well as high haemolytic action in blood samples of fish exposed to high toxicant concentrations were recorded. McLeay. [23] on his work on fish exposed to pulp mill effluent observed a significant decrease in the RBC count and haematocrit which was attributed to possibility of inhibition of erythrocyte production and increase in the rate of erythrocyte destruction or haemodilution. Wedemeyer and Yasatake [28], suggested that decrease in these parameters could be due to haemodilution because of impaired osmoregulation. Mount and Putnicki [29] and Zambariborsch and Biu [30] were of the opinion that marked decrease in total number of erythrocytes, leucocytes and thrombocytesafter exposure of *heteropneustes fossilis* to heptachlor was unlikely to be due to haemodilution because of hyperchloraemia occurring at 48 hr and 96 hr of test period. These changes, according to them, clearly indicated blood dyscrasia (a development disorder of the blood) induced by the heptachlor in the fish which interfered with the haemopoiesis and/or alteration of cell membranes by hydrolysis of acetylcholine in the body fluid. Musa [31] reported a decrease in haematocrit, haemoglobin, content and RBC counts in *C. gariepinus* exposed to malachite green (oxalate). The decrease was suggested to be possibly due to the effect of the chemical on the membrane at pase, the glycolytic enzymes in the erythrocytes and glutathione. In this study, with the histopatological results on the gills in which there was marked destruction especially at high toxicant concentrations, decrease in haemoglobin, haematocrit and MCHC could be attributed to haemodilution due to impaired osmoregulation as consequences of gill damage.

It is known that lymphocytes constitute the majority of white blood cells present in the peripheral blood of apparently normal salmonids [32]. In this study, lymphocytes had the highest percentage compared to neutrophils and monocyte. However, there was slight increase with increase in concentration of toxicant to which they were exposed and periods, which possibly indicate chronic effects. Neutrophils increased with increased concentrations but decreased with periods.

Grizzle) [33] observed neutrophils in *Ictalurus punctatus* 1-3 days after exposure to 0.1 mgl^{-1} malachite green. The large increase in neutrophils was the most drastic change. Weineb and Yokoyama [34, 35] explained that neutrophils respond to inflammatory conditions following turpentine injections. This neurophil is an indicator of acute effect. There was no indication of inflammatory response in *o. niloticus* exposed to paraquat in this study. It was observed that fish exposed to toxicant concentration of 1.4 mgl^{-1} showed marked effect of toxicant on the fish right from the beginning. At week 6 there was an increase in number of destroyed red blood cells by haemolysis as seen in the photomicrograph (plate iv) but gradual acclamation could be observed in the blood cells at toxicant concentration of 2.0 mgl^{-1} with less hemolyzed cells. By the 10^{th} week haemolysis had taken place in all treatment groups except the control. This could be due to stress response on the blood cells of the fish.

The results of hematology test 0f O. niloticus exposed to acute level of paraquat after 96 hr showed that the values for hemoglobin (g/100 ml), hematocrit (%) and mean corpuscular showed a dose dependent decrease in (MCHC) all these could be due to haemodilution. Similar findings were reported after exposure of *Clarias albopunctatus* to sublethal actellic concentrations [24]. Anaemia associated with erythropenia has been reported by several fresh water fish species [25, 36, 37].

Insignificant variation in MCH and MCHC values could be due to lesser effects of the toxicant on these parameters. Giron-peres *et al.* [38] reported that chlorpyrifos had no effects on MCH and MCHC of Nile Tilapia.

Yayi *et al.* [39] reported reduction in the values of RBC, Hb and Pvc on the *O. niloticus* exposed to cypermethrin at sublethal level. This agrees with this work in which a dose-dependent decrease in haemoglobin, hematocrit and MCHC values in the blood of *O. niloticus* exposed to paraquat was observed in sub-lethal test as well as acute toxicity test. However, total denaturing of the blood cells at high concentrations of 14.20 and 15.20 mgl^{-1} during the acute toxicity test was recorded. Romeo *et al.* [7] reported absence of eosinophils, basophils and granulocytes in *Hoplias malabaricus* and Geophagus brasiliensis *brasiliensis*. In *O. niloticus* used for this work Neutrophils and lymphocytes were observed but Basophils,

Eosinophils were absent.

In acute toxicity test, lymphocytes (%) decreased slightly with decrease in toxicant concentrations while neutrophils (%) showed no appreciable difference in value.

In sublethal test, there was dose-dependent increase in Lymphocyte value and close-dependent decrease in neutrophils value possibly due to haemodilution [15, 39].

Hematological increase are considered to be pathophysiological indicators of toxicant effects. Decrease in Hb content, Pvc/Hct of test fish, as compared to control may impair oxygen supply to various tissues, thus resulting in a slow metabolic rate and low energy production and indicates the worsening of an organism state and developing anaemia, whereas the total white blood cells increased significantly, with increase in exposure periods, which may be attributed to immunological responses of the fish to heavy metal/pesticides [40, 41, 42, 43].

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