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Macrocytic-normochromic anaemia in the African catfish *Clarias gariepinus* (Siluriform: Clariidae) exposed to *Paraquat* under laboratory conditions

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ABSTRACT. Introduction: Agricultural pesticides are among the main causes of pollution in aquatic ecosystems, and they can lead to physiological changes in fish. For example, blood alteration and damage to haemopoietic tissue can be associated with pathological conditions related to water. **Objective**: To describe the effects of certain levels of pesticide on a Nigerian fish species. **Methods:** Macrocytic-normochromic anaemia was induced in *C. gariepinus* at intervals of 1,7 and 14 days (sub-lethal concentrations of *Paraquat*: 0; 0,03; 0,05; 0,07 and 0,09mg/l). **Results:** Blood dyscrasias was observed with a significant (p<0,05) decrease in haemoglobin, haematocrit, red blood cells, white blood cells, lymphocytes and monocytes. Mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH), Neutrophils, Eosinophil and Basophil increased significantly (p<0,05) with increasing concentrations of the toxicant while Mean corpuscular haemoglobin concentration (MCHC) remained normal. **Conclusion**: Sublethal concentrations of paraquat induced macrocytic-normochromic anaemia in the catfish *C. gariepinus*.

Keywords: Toxicity, herbicides, stun, haematology, disorder, poisoning.

RESUMEN. "Anemia macrocítico-normocrómica en el bagre africano *Clarias gariepinus* (Siluriform: Clariidae) expuesto a Paraquat en laboratorio" **Introducción:** Los plaguicidas agrícolas se encuentran entre las principales causas de contaminación en los ecosistemas acuáticos y pueden provocar cambios fisiológicos en los peces. Por ejemplo, la alteración de la sangre y el daño al tejido hematopoyético pueden estar asociados con condiciones patológicas relacionadas con el agua. **Objetivo:** Describir los efectos de ciertos niveles de plaguicidas en una especie de pez de Nigeria. **Métodos:** Se indujo anemia macrocítico-normocrómica en *C. gariepinus* a intervalos de 1, 7 y 14 días (concentraciones subletales de Paraquat: 0; 0,03; 0,05; 0,07 and 0,09mg/l). **Resultados:** Hubo discrasias sanguíneas con una disminución significativa (p <0,05) de hemoglobina, hematocrito, glóbulos rojos, glóbulos blancos, linfocitos y monocitos, el volumen corpuscular medio (MCV), la hemoglobina corpuscular media (MCH), los neutrófilos, eosinófilos y basófilos aumentaron significativamente (p <0,05) con concentraciones crecientes del tóxico, mientras que la concentración de hemoglobina corpuscular media (MCHC) se mantuvo normal. Conclusión: Las concentraciones subletales de paraquat generaron anemia macrocítico-normocrómica en el bagre *C. gariepinus*.

Palabras clave: Toxicidad, herbicidas, aturdimiento, hematología, desorden, intoxicaciones.

One of the main causes of pollution of aquatic ecosystems is agricultural pesticides, which have adverse effects on the environment (Bahmani et al., 2001). The rate at which herbicides and pesticides are being use in agriculture (including commercial and household production of vegetables) for the control of pest and weeds are causing chemical pollution of aquatic environment (Aghoghovwia & Izah, 2018). Determination of the toxic compounds in aquatic environments and their effects on aquatic organisms is a fundamental issue in ecotoxicological studies (Bagheri, 2007). Pesticides are generally used by farmers to control weeds, remove aquatic plants in rivers, lakes and water reservoirs, which generally have harmful effects on aquatic animals' health (Abubakar et al.,

2019). Within a few weeks of using these pesticides in agricultural activities, they entered to aquatic ecosystems through surface runoff and subsurface drainage (Abubakar et al., 2019).

Fish is directly associated with the aquatic environment, hence, the incidence of physical and chemical changes in the aquatic environment could leads to measurable physiological changes in fish (Adihikari et al., 2004). In recent years, the incidence of mortality due to pesticides, industrial effluents and sewage contamination has been reported in Iran (Agha-Mohammadi et al., 2012). Use of hematology parameters is expanding in aquaculture activities, particularly in toxicology researches, environmental monitoring and in evaluation of aquatic animals' health (Verma & Agarwal, 2007). One of the most important and reliable indicators for the health assessment and fish physiology is measurement of blood parameters which are influenced by the nutritional and environmental factors (Abubakar, 2016). Fish blood parameters can be used as a bio-indicator (Abubakar, 2013).

Alteration in the blood and damage to haemopoietic tissue in fish can be associated with pathological conditions related to water–borne pollutant (Babatunde et al., 2014). Blood haematological and biochemical characteristics of fish are important evidences of their physiological processes and reflect the relationship between aquatic ecosystem characteristics and their health status (Osman & Kloas, 2010). Changes in blood parameters in response to environmental conditions area response to environmental stress and can be considered as an important bio-indicator (Adihikari et al., 2004). Haematological parameters have been used as biomarkers for physiological and pathological alterations in fishery managementand disease investigation (Dogan & Can, 2011). These parameters show the number of red blood cells (RBC), haemoglobin (Hb), white blood cells (WBC) and packed cell volume (PCV) (Dogan & Can, 2011). In addition, blood indices have been used, including mean corpuscular volume (MCV), meancorpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) to evaluate toxic stress of environmental contaminants (Dogan & Can, 2011)

There are some publications on the effects of paraquat on *Clarias gariepinus* heamatological parameters (e.g. Kori-Siakpere et al., 2007), but, overall, there are few documented reports of effects of paraquat on the health status of fish in Nigeria (Abubakar et al., 2019). Paraquat (1, 1-dimethyl-1, 4, 4 Bipyridinium dichloride) is a non-selective contact herbicide (Gao et al., 2010) that acts in the presence of light which it absorbs at 260nm to desiccate the green parts of plants (Abubakar et al., 2019). Paraquat is an herbicide used in destroying weeds in tropical regions; it's active ingredients are destructive to biota (Bahmani et al., 2008). In Nigeria, local fisher men use paraquat to harvest fish in high proportion (Banaee et al., 2008). It is pertnemt to study its harzadious effects on aquatic system (Kori-Siakpere et al., 2007). In world wide application, It is second only to glyphosate (Banaee et al., 2013). It is highly toxic to aquatic organisms (Katagi, 2010).

Paraquat toxicity may be attributed to its redox potential, which involves cyclic reduction-oxidation reactions that produce ROS and depletion of nicotinamide adenine dinucleotidephosphate hydrogen (Tsai, 2013). Photodegradation of paraquat results in formation of N-methylisonicotinic acid which may further decomposes to yield methylamine-hydrochloride and carbon dioxide (Huang et al., 2013). Paraquat can affect the cardiac contraction and opercula ventilation in fish (Tortorelli et al., 1990).

Anemia is a reduction in the total number of circulating erythrocytes or a decrease in the quality or quantity of haemoglobin (Abubakar, 2016). Macrocytic-normochromic anemia is megaloblastic anemias which are characterized by defective deoxyribonucleic acid (DNA) synthesis that produces a pattern of ineffective erythropoiesis, resulting in unusually large stem cells in the marrow that mature into unusually large erythrocytes (macrocytes) in the circulation (Abubakar et al., 2019). In addition to an increase in size (diameter), the thickness and volume of the cell also increase (Abubakar, 2016). When the haemoglobin decreases significantly, the fish experiences the

classic symptoms of anemia: weakness, fatigue, paresthesias of fins and difficulty in swimming (Abubakar et al., 2019).

African catfish (*C. gariepinus*) is frequently and widely cultured in Nigerian waters including ponds (Abubakar et al., 2019) and is an ecologically important and commercially valued fish in Nigeria (Abubakar et al., 2019). There are few information on toxicity of paraquat (1, 1-dimethyl-1, 4, 4 Bipyridinium dichloride) on catfish by the local fishermen in Nigeria.

The objective of this work was to evaluate sublethal paraquat-induced macrocytic-normochromic anaemia in *C. gariepinus*.

MATERIALS AND METHODS

Juveniles of *C. gariepinus* (mean body weight 19,47 ± 1,05; mean standard length, 20,00 ± 0,45cm) were obtained from Tee jay Fish and Feeds Farm Ogidi, Ilorin, and transported in oxygenated polythene bags to the Central Laboratory, University of Ilorin where they were acclimatized for 2 weeks in a plastic glass aquarium (30x30x60cm) capacity. The fishes were kept in the glass aquaria to observe any visible pathological symptoms. Before introducing into the aquarium, fishes were treated with 0,1% potassium permanganate ($KmNo_4$) solution to obviate any dermal infection. The experimental protocol and procedures used in this study were approved by the University of Ilorin, Ilorin, Nigeria; Ethical Review Committee (Protocol Identification Code: UERC/AGR/199; UERC Approval Number: UERC/ASN/2021/2191) and conform with the "Guide to the care and use of Animals in Research and Teaching (Ethical Principles for Medical Research: Declaration of Helsinki).

Acclimation of test fishes: No mortality was recorded during acclimation period. The fishes were fed with pelleted feed containing 35% crude protein at 3% body weight twice per day. Two third of the water in the tank were changed every forty-eight hours to avoid the accumulation of food residue and waste metabolite. Feeding was terminated 24 hours before the commencement of the experiment. After acclimatization, fish were kept in different concentrations of Paraquat (1, 1-dimethyl- 1, 4, 4 Bipyridinium dichloride) for the experiment.

Sources of paraquat and its exposure: Paraquat (1, 1-dimethyl- 1, 4, 4 Bipyridinium dichloride) with trade mark name Gramoxone manufactured by Syngenta, USA was purchased from Muritala Muhammed Road in Ilorin. Kwara Stata, Nigeria. Fishes were exposed to acute concentrations of paraquat for 96 hours. Control fish were also maintained under identical conditions without the toxicant.

Experimental design: The experimental design was completely randomized design (CRD) with three replicates. One hundred and fifty (150) juvenile of *C. gariepinus* were randomly distributed into the glass aquarium at a stocking rate of 10 fish glass aquarium. Fifteen 30cm X 30cm X 60cm glass aquarium was assigned to 5 treatments (control inclusive). In order to determine the LC_{50}, Method of Abubakar (2013) was adopted. The *C. gariepinus* were exposed to four different concentrations of paraquat (0; 1,3; 1,6; 1,8 and 2,0mg/L).

Lethal toxicity test: Prior to the commencement of the lethal toxicity tests for the paraquat, a screening test was first conducted to ascertain the range to be used for the bioassay test. Dilutions were made from the stock solutions and only one test fish was put at a time and observed to determine the time mortality would occur. The concentrations from which serial dilutions will commence were arrived at after the test fish survived beyond 2 hours on exposure to the toxicant.

Observations and record of number of fish alive overturned and dead in each of the toxicant bioassay in test vessels were made at intervals of 3, 6, 12, 24, 48, 72 and 96 hours. Dead fish were identified when opercula movement ceased and there was no response to touch. From these data, LC_{50} values were calculated by Probit analysis using the US EPA software (Probit program version 1.5) and 1/15, 1/20, 1/25 and 1/30 were taken as sub-lethal using the method of Abubakar (2013).

Acute bioassay test: The acute bioassay test for the determination of lethal effect of Paraquat on the test fish species were conducted using static renewal method. The following concentration of Paraquat was used: 1,3; 1,6; 1,8 and 2,0mg/L respectively with a control with no toxicant. Each concentration level was triplicated. The desired stock solution was measured with a syringe and introduced into the 10 Liters of water in the plastic tank. The mixture was allowed to stand for 30 minutes for proper mixing before introducing the test fishes. A stocking density of 10 fish per tanks was used following the method of Abubakar (2013). Each tank was covered with a nylon mesh screen to prevent the fish from jumping out of the tank. The cover of each tank was perforated to allow air into the tanks and each was used to hold down the nylon mesh screen to the tank. Feeding was stopped 24 hours prior to and during the 96hour bioassay test. This was done to prevent interference with the metabolism and absorption of the extract by wastes in reconstituted areas. The test fishes (treatment and control) were observed for signs of toxicity with prompt recordings at 24h, 48h, 72h and 96h of exposure.

Sub-lethal test: From the LC_{50} values calculated by Probit analysis, 1/15, 1/20, 1/25 and 1/30 were taken as sub-lethal using the method of Abubakar (2013) to produce 0; 0,09; 0,07; 0,05 and 0,03mg/L. The test fish were exposed to sub-lethal doses of the herbicide to determine the hematological effect. The concentrations were triplicated. The experiment had controls which were devoid of Paraquat. The experiment lasted for 14days. During the exposure of test fishes, selective sampling of three fish each was taken from each treatment concentration for hematological analysis at intervals of 24hours, 7 and 14days. However, the sampling of hematological analysis was limited to pre-exposure at the beginning and post-exposure at the end of the experiment.

Collection of blood: Blood samples were collected from both the control and experimental fish at the end of fourteen (14) days. The fish were stunned with a gentle knock on the head. The stunned fish was placed in a trough and blood was taken by caudal venous puncture using 23GX 11/4 (0,6 x 32mm) syringe. The blood was put into EDTA vials and taken to Central Research Laboratory, University of Ilorin for analysis using methods described by Abubakar (2013) at a wavelength of 540µm. The haematological parameters analyzed were Haemoglobin (Hb), packed cell volume (PCV), red blood cell (RBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), white blood cells (WBC) and its differential counts.

Heamatological tests. Haemoglobin: The sahil's-Hellige haemoglobin determination was performed as follows: The sallied pipette was filled slightly above the $20mm^3$ mark, the pipette was wiped with a soft absorbent tissue to remove excess blood and the volume was adjusted to exactly $20mm^3$ by blotting the tip. The blood was expelled into a calibrated (transmission) test tube containing 10,0mm of 0,1N hydrochloric acid, and the pipette was raised several times in the acid solution. The sample was allowed to stand for not less than 3 minutes before reading the values in the colorimeter. The intensity of color was measured at a wavelength of 540µm and was recorded as percent transmission.

Calculation: X % = XC14 gm haemoglobin per 100 ml of blood.
100

Determination of pack cell volume: Pack Cell Volume (PCV) was carried out by micro-westegreen method as described by Abubakar et al. (2019). The blood sampled from the severed caudal peduncle was drawn into micro-haematocrit tube. The tubes were sealed with wax and centrifuged for 5 - 6 minutes. The PCV was measured with the aid of a microhaematocrit reader and expressed as the volume of the erythrocytes per 100cm^3.

Red blood cell count: The standard RBCC diluting pipette and a 1:200 dilution were used for the red blood cell count. Blood was drawn just beyond the 0,5 mark on the pipette. The tip of the pipette was wiped with a soft absorbent tissue to adjust the volume to exactly the 0,5 mark. The pipette was immediately filled to the 101 mark with Hendricks diluting fluid. Partial rotation of the pipette while being filled assured the complete mixing of the blood and diluting fluid, and prevented clotting. With its ends griped between the thumb and second finger, the pipette was then shaken for 30 to 60 seconds. After the pipette had been shaken, a few drops of the diluted blood were expelled from it. Control of over flow of fluid was maintained by replacing the index finger over the bulb end of the pipette. The haemocytometer was then placed under the light microscope, and the cells were counted. The haemocytometer is divided into ruled areas 1mm^2, with the centre square millimeter divided into 25 groups of 16 small squares. The cells within the boundaries of five of these small squares were counted. Each corner plus the center group were counted when the red blood cell count was computed, the number of cell counted in all five squares was multiplied by 106, this gave the total number of cells per cubic millimeter (mm3) of blood (Mallum & Sogbesan, 2015).

Erythrocyte indices: Calculated mean values that reflect the size, weight and haemoglobin content of individual erythrocytes.

Means Corpuscular Volume (MCV) express the average volume of the individual erythrocyte and is calculated from the formula.

MCV= Haematocrit x 10 Abubakar (2016)
Erythrocyte count (millions/Cu.mm)
It is expressed in femtoliter (Fl)

Means Corpuscular Haemoglobin (MCH) is the amount of haemoglobin by weight, in the average erythrocyte and is calculated thus:

MCH= Haemoglobin (gm/100ml) x 10 Abubakar (2016)
Erythrocyte count (millions/Cu.mm)
It is expressed in pictogram (pg)

Means Corpuscular Hemoglobin Concentrations (MCHC) is the concentration of haemoglobin in the average erythrocyte and is calculated thus:

MCHC (g $100ml^{-}$) = Haemoglobin (gm/100ml x 100 Abubakar (2016)
Haematocrit
It is expressed in gram deciliter (gdL^{-1})

Total leucocytes count: Leucocytes were counted using Shaw's solution A and B. The blood was drawn up to the 0,5mark, solution A was added to fill the bulb of the pipette approximately half filled, and mixed. Then, the pipette was removed from solution A and filled to the mark 101 with solution B. The pipette was then shaken for 30 to 60 seconds. After the pipette had been shaken, a few drops of the diluted blood were expelled from it. A few drops were expelled and the haemocytometer was filled in the manner described previously. For comparison of the total number of leucocytes, the cells in the four large squares noted by the large cycle were counted. The total number of cells counted multiplied by 500, determined the total number of leucocytes per cubic millimeter (mm^3) of blood (Abubakar, 2016).

Statistical analysis: Data were analyzed with One-Way analysis of variance (ANOVA) procedure using Statistical Package for Social Sciences (SPSS version 16.0) for window. Statistical significance of difference among means was compared using Turkey test ($P < 0,05$).

RESULTS

Haematological parameters of *C. gariepinus* after 96 hrs exposure to paraquat Blood dyscrasias attributable to paraquat poisoning were observed in *C.gariepinus* at various exposure days (1, 7 and 14). Haemoglobin, haematocrit and red blood cell decreased (pancytopenia) with increase in sublethal concentrations of Paraqua (1, 1-dimethyl- 1, 4, 4 Bipyridinium dichloride) at all levels compared with their control. Analysis of Erythrocyte indices revealed Macrocytic-normochromic anaemia. MCV and MCH increased with increase in concentrations along different levels compared with their controls while MCHC remained normal. Analysis of White blood cell (WBC) revealed immuno-deficiency disorder as evidenced in leucopenia (loss of immunity/ decreased WBC). Analysis of differential White blood cells (neutrophils and lymphocyte) indicated joint disorder as evidenced in decrease in neutrophil, lymphocyte, Eosinophil and basophils at ($P<0,05$) in the exposed fish species (Table 1).

DAY 1

Table 1

Heamatological parameters of *C. gariepinus* exposed to sub-lethal concentrations of paraquat (Mean ± SD) on Day 1

Parameters	Concentration(mg/L)				
	Control	0,03	0,05	0,07	0,09
Hb (gdL^{-1})	5,99 ± 1,762^a	5,97 ± 2,970^a	5,80 ± 2,022^a	5,73 ± 1,411^a	4,57 ± 0,103^b
PCV (%)	19,93 ± 4,509^c	18,90 ± 6,385^c	17,03 ± 7,239^c	13,47 ± 8,145^d	8,27 ± 7,251^a
RBC ($x10^{12}\ L^{-1}$)	1,67 ± 0,727^a	1,54 ± 0,509^b	1,35 ± 0,639^c	1,17 ± 0,118^d	1,10 ± 0,732^e
MCV (Fl)	106,83 ± 5,982^e	120,70 ± 2,626^d	127,90 ± 3,576^c	135,03 ± 3,078^b	148,43 ± 6,082^a
MCH (pg)	41,50 ± 2,180^d	45,40 ± 2,227^c	47 00 ± 3,897^b	49,60 ± 0,721^b	54,78 ± 5,740^a
MCHC (gdL^{-1})	57,82 ± 4,112^a	57,57 ± 9,181^b	5,00 ± 4,246^b	57,90 ± 1,758^g	57,20 ± 3,151^f
WBC ($x10^9L^{-1}$)	7 093 ± 8,235^a	7 083 ± 5.378^b	7 053 ± 7,276^c	6 067 ± 3,001^d	5 633±1.150^e
Neutrophils (%)	59,0 ± 3,699^d	53,0 ± 0,700^a	48,7 ± 3,955^b	46,0 ± 0,529^f	34,0 ± 0,200^g
Lymphocytes (%)	97,59 ± 2,261^a	95,13 ± 0,666^b	93,03 ± 4,759^b	91,70 ± 0,964^c	87,47 ± 3,700^d
Monocytes (%)	1,36 ± 0,518^c	1,24 ± 0,400^c	1,15 ± 0,854^c	0,92 ± 0,058^a	0,73 ± 0,100^a

Mean of parameters with the same superscripts along the rows are not significantly different at $p>0,05$.

Hb-Haemoglobin; PCV-Packed cell volume; RBC-Red Blood cell count; MCV-Mean Corpuscular Volume; MCH-Mean Corpuscular Haemoglobin; MCHC-Mean Corpuscular Haemoglobin Concentration; WBC-White Blood cell count

Day 7

Table 2

Heamatological parameters of *C. gariepinus* exposed to Sub-lethal concentrations of paraquat (Mean ± SD) on Day 7

Parameters	Concentration(mg/L)				
	Control	0,03	0,05	0,07	0,09
Hb (gdL^{-1})	5,99 ± 1,762^{a}	5,90 ± 2,950^{a}	5,80 ± 2,022^{a}	4,47 ± 1,41^{b}	3,47 ± 0,153^{c}
PCV (%)	18,93 ± 4,509^{a}	17,13 ± 4,939^{b}	15,57 ± 4,969^{c}	14,23 ± 3,027^{d}	12,13 ± 0,666^{e}
RBC ($x10^{12}\ L^{-1}$)	1,90 ± 0,727^{a}	1,74 ± 0,669^{b}	1,43 ± 0,289^{c}	1,24 ± 0,208^{d}	0,97 ± 0,112^{g}
MCV (FI)	108,97 ± 3,021^{f}	122,6 ± 3,947^{d}	130,9 ± 7,877^{c}	138,6 ± 5,515^{b}	154,43 ± 6,082^{a}
MCH (pg)	42,03 ± 5,401^{d}	46,17 ± 5,405^{c}	48,60 ± 2,722^{c}	51,37 ± 4,927^{b}	57,78 ± 5,740^{a}
MCHC (gdL^{-1})	57,82 ± 4,112^{b}	57,73 ± 6,612^{b}	75,13 ± 4,531^{b}	57,03 ± 3,587^{b}	57,20 ± 5,025^{b}
WBC ($x10^{9}L^{-1}$)	7 093 ± 8,235^{g}	6 730 ± 7,956^{f}	5 390 ± 3,528^{d}	4 693 ± 3,672^{a}	3 607 ± 7,705^{b}
Neutrophils (%)	63,0 ± 3,699^{a}	59,7 ± 1,550^{b}	48,7 ± 1,429^{c}	21,3 ± 2,055^{d}	11,0 ± 0,458^{e}
Lymphocytes (%)	97,59 ± 2,261^{a}	95,30 ± 2,088^{b}	93,23 ± 1,986^{b}	91,87 ± 4,654^{c}	87,13 ± 4,678^{d}
Monocytes (%)	1,25 ± 0,518^{a}	0,73 ± 0,551^{b}	0,63 ± 0,551^{b}	0,60 ± 0,529^{b}	0,27 ± 0,115^{b}

Mean of parameters with the same superscripts along the rows are not significantly different at p>0,05.
Hb-Haemoglobin; PCV-Packed cell volume; RBC-Red Blood cell count; MCV-Mean Corpuscular Volume; MCH-Mean Corpuscular Haemoglobin; MCHC-Mean Corpuscular Haemoglobin Concentration; WBC-White Blood cell count

Day 14

Table 3

Haematological parameters of *C. gariepinus* exposed to sub-lethal concentrations of paraquat (Mean ± SD) on Day 14

Parameters	Concentration(mg/L)				
	Control	0,03	0,05	0,07	0,09
Hb (gdL^{-1})	5,99 ± 1,762^{a}	5,67 ± 3,308^{a}	5,60 ± 2,610^{b}	4,17 ± 1,300^{b}	2,35 ± 1,850^{c}
PCV (%)	18,93 ± 4,509^{a}	14,90 ± 4,357^{b}	13,60 ± 5,339^{c}	12,63 ± 7,543^{d}	11,67 ± 2,248^{e}
RBC ($x10^{12}\ L^{-1}$)	1,67 ± 0,727^{a}	1,19 ± 1,187^{b}	1,12 ± 1,120^{c}	0,97 ± 0,520^{d}	0,69 ± 0,047^{e}
MCV (FI)	119,03 ± 2,348^{f}	120,4 ± 3,934^{g}	123,8 ± 1,825^{c}	126,7 ± 1,268^{b}	144,43 ± 6,082^{a}
MCH (pg)	44,07 ± 5,672^{c}	47,30 ± 3,974^{n}	49,00 ± 94,423^{b}	52,00 ± 3,160^{a}	59,78 ± 5,740^{f}
MCHC (gdL^{-1})	57,82 ± 4,112^{a}	57,20 ± 4,490^{a}	57,80 ± 3,987^{a}	57,03 ± 5,977^{a}	57,00 ± 6,245^{a}
WBC ($x10^{9}L^{-1}$)	7 093 ± 8,235^{a}	5 167 ± 5,636^{b}	3 997±6,623^{d}	3 307 ± 3,014^{g}	2 620±1,416^{ag}
Neutrophils (%)	63,0 ± 3,699^{m}	48,7 ± 2,589^{n}	23,7 ± 1,767^{a}	21,7 ± 1,531^{b}	19,0 ± 0,854^{d}
Lymphocytes (%)	97,59 ± 2,261^{a}	95,53 ± 1,002^{a}	93,10 ± 2,254^{a}	91,47 ± 2,706^{b}	87,17 ± 3,095^{d}
Monocytes (%)	1,25 ± 0,518^{a}	0,67 ± 0,351^{b}	0,57 ± 0,153^{b}	0,53 ± 0,493^{b}	0,27 ± 0,058^{b}

Mean of parameters with the same superscripts along the rows are not significantly different at p>0,05.
Hb-Haemoglobin; PCV-Packed cell volume; RBC-Red Blood cell count; MCV-Mean Corpuscular Volume; MCH-Mean Corpuscular Haemoglobin; MCHC-Mean Corpuscular Haemoglobin Concentration; WBC-White Blood cell count

Table 4

Summary of heamatological parameters of *C. gariepinus* at the various days of exposure to sub-lethal concentrations of (Mean ± SD)

	Duration of exposure (Days)			
Parameters	**Control**	**1**	**7**	**14**
Hb (gdL^{-1})	5,99 ± 1,762[a]	5,51 ± 1,828[a]	5,74 ± 1,644[b]	4,67 ± 2,267[c]
PCV (%)	18,93 ± 4,509[d]	15,16 ± 7,255[b]	14,29 ± 3,900[c]	13,20 ± 4,871[ad]
RBC ($x10^{12}$ L^{-1})	1,67 ± 0,727[g]	1,44 ± 0,0499[a]	1,32 ± 0,319[b]	0,94 ± 0,718[d]
MCV (FI)	112,27 ± 5,090[b]	117,48 ± 2,343[a]	122,27 ± 5,090[c]	144,43 ± 6,082[d]
MCH (pg)	45,84 ± 4,307[c]	47,13 ± 2,246[b]	49,79 ± 4,614[g]	55,78 ± 5,740[a]
MCHC (gdL^{-1})	57,82 ± 4,112[d]	57,66 ± 4,584[d]	57,27 ± 4,938[d]	57,51 ± 5,174[d]
WBC ($x10^{9}L^{-1}$)	7 093 ± 8,235[a]	6 459 ± 4,201[b]	5 105 ± 4,715[c]	3 773±4.172[d]
Neutrophils (%)	63,0 ± 3,699[a]	42,4 ± 1,346[b]	35,2 ± 1,363[c]	28,3 ± 1,635[d]
Lymphocytes (%)	97,59 ± 2,261[a]	93,58 ± 2,022[b]	90,64 ± 3,352[c]	95,07 ± 2,264[d]
Monocytes (%)	1,25 ± 0,518[a]	0,99 ± 0,353[b]	0,56 ± 0,437[b]	0,51 ± 0,264[b]

Mean of parameters with the same superscripts along the rows are not significantly different at p>0.05.
Hb-Haemoglobin; PCV-Packed cell volume; RBC-Red Blood cell count; MCV-Mean Corpuscular Volume; MCH-Mean Corpuscular Haemoglobin; MCHC-Mean Corpuscular Haemoglobin Concentration; WBC-White Blood cell count.

DISCUSSION

These findings showed that *C. gariepinus* is sensitive to sublethal concentrations of Paraquat (1, 1-dimethyl- 1, 4, 4 Bipyridinium dichloride). The induction of macrocytic-normochromic anaemia in the exposed *C. gariepinus* by paraquat in this study is in agreement with an earlier report by Abubakar and Abdulsalami (2013) who observed microcytic-hypochromic anaemia in *C. gariepinus* exposed to sublethal toxicity of Sniper 1000EC. Reduction in the erythrocyte count was reported in *C. mrigala* exposed to ibuprofen (Saravanan et al., 2012) and *C. albopunctatus* exposed to acetellic (Mgbenka et al., 2005). Reduction in erythrocyte count, haematocrit value and haemoglobin content of *C.gariepinus* in this study might be due to blood haemorrhage owning to un equilibrium of osmotic pressure inside and outside blood cells (Abubakar, 2016), and can also be attributed to haemodilution of blood due to the bleeding of fish organs and cells (Abubakar, 2013). This is in agreement with Kori-Siakpere et al. (2007) who observed reduction in PCV, HGB, TEC, MEV, MEH and MEHC in *C. gariepinus* exposed to paraquat. This is also in agreement with Ada et al. (2012) who reported significant reductions in hematological parameters C*. gariepinus* exposed to paraquat. The reductions could be attributed to the product of impaired erythropoiesis and rapid haemolysis of the RBC.

The reduction in blood parameters was an indication of anaemia caused by increase in the concentration of the toxicant (Kori-Siakpere et al., 2007). A decrease in the concentration of haemoglobin in blood is usually caused by the effect of pollutant in gills as well as decrease in oxygen carrying capacity which also suggests anaemia in catfish (Abubakar, 2013). This is in line with Kori-Siakpere et al. (2007) who reported significant reduction in the values of HGB in *C. gariepinus* exposed to paraquat. A possible cause is the ceasing of erythrocyte production (Patnaik & Patra, 2006). Li et al. (2011) observed that verapamil reduced the erythrocyte count, Hb and PCV in *O. mykiss*. Reduction in the values of these parameters was also observed in *Prochilodus lineatus* exposed to Clomazone (Pereira et al., 2013) and in *Labeo rohita* exposed to fenvalerate (Prusty et al., 2011). Reduction in the erythrocyte count was reported in *C. mrigala* exposed to ibuprofen (Saravanan et al., 2012) and in *C. albopunctatus* exposed to acetellic (Mgbenka et al., 2005). Haematological indices such as RBC, haemoglobin and haematocrit indicate secondary responses of an organism to pollutants (O'Neal & Weirich, 2001).

Decrease in WBC and differential counts affect the health of *C.gariepinus* (Ezeri, 2001; Omoregie & Oyebanji, 2002). Joshi et al. (2002) made a similar observation on blood parameters of *C. batrachus* exposed to Lindane and Malathion which are pesticides. Kumar et al. (2011) observed changes in white blood cells of *C. gariepinus* exposed to paraquat which he attributed to disturbances caused by hematopoiesis and subsequent reduction or non-specific immune weakening in fish. White blood cells are small in number compared to red blood cells. Changes in the levels of white blood cells as a result of exposure to paraquat may be due to disturbances in the process of hematopoiesis and non-specific immune weakening in fish (Kumar et al., 2011). White blood cells include lymphocytes, neutrophils, esinophils, monocytes and basophils, each of which plays a different role in the body of fish. Lymphocytes are involved in the immune response of aquatic animals by antibody production (immunoglobulin). White blood cell differential count in this study showed that the percentages of lymphocytes and platelets in all the exposed concentrations after 30 days were measured less than the control group (Kumar et al., 2011).

Changes in white blood cell- differential count after exposure to paraquat may be due to disruption in the process of the hematopoietic and subsequent reduction or suppression of non-specific immune in Benni fish and then body strength of fish exposed to Paraquat reduces and they are easily susceptible to pathogens Kumar et al. (2015). Decrease in the percentage of lymphocytes exposed to pesticides was reported by different researchers. Nussey et al. (2015) investigated the effects of sub-lethal concentrations of diazinon on grass carp, *Ctenopharyngodon idella* and reported significant increase ($P<0,05$) in percentage of neutrophils and significant reduction ($P>0,05$) in the percent of WBC and monocytes compared with the control group which are in agreement with the results of this study.

Leucocytosis was reported in *C. carpeo* exposed to lindane (Saravanan et al., 2011) and in *O. niloticus* exposed to deltamethrin (El-Sayed et al., 2007). Increase in MCV and MCH with normal MCHC were indication of Macrocytic-normochromic anemia as observed in this study, similar results was reported by Abubakar (2013) who worked on effect of acute and sublethal concentrations of sniper 1000EC on *C. gariepinus*. The increase in MCV and MCH might be due to swelling of the erythrocytes resulting in a macrocytic anaemia (Abubakar, 2013). Abubakar & Abdulsalami (2013) attributed the increase in MCV to the swelling of the RBC as a mechanism that reduces the concentration of an irritating factor in the circulatory system. Macrocytic-normochromic anemia is associated with increase rate of secondary malignancies of cancer of the stomach, pancrease and myeloid leukemia (Goldberges et al., 2001). The results from this study is in agreement with Abubakar et al. (2019) who reported Normocytic-Normochromic anaemia in *C.gariepinus* exposed to paraquat under laboratory conditions. It also agreed with the work of Thakur & Bais (2000) who exposed *Heteropneustes fossilis* to adrin and fenvalerate. The results of this work are also in agreement with the report of John (2007) on exposure of pesticide to *Salmo gardneri* and *Mystus vittatus* respectively. Generally, leucocytes modulate immunological functions in animals, including fish. Anemia associated with erythropenia has been reported in several freshwater fish species (Auta et al., 2002). The observed leucocytosis in the present study indicated abnormal immune protective response to paraquat intoxication (Abubakar et al., 2019). It also suggested that paraquat stimulated the immune system with a concomitant release of lymphocytes from the lymphomyeloid tissue as a defense response (Abubakar et al., 2019).

In conclusion, sublethal concentrations of Paraqua (1, 1-dimethyl- 1, 4, 4 Bipyridinium dichloride) induced macrocytic-normochromic anaemia in the exposed *C. gariepinus* under laboratory conditions. Proper research on the danger of 1, 1-dimethyl- 1, 4, 4 Bipyridinium dichloride to aquatic environment is required while manufacturers should be compelled to state categorically the effect of paraquat to non-targeted aquatic organisms on their labels.

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ETHICAL, CONFLICT OF INTEREST AND FINANCIAL STATEMENTS

The authors declare that they have fully complied with all pertinent ethical and legal requirements, both during the study and in the production of the manuscript; that there are no conflicts of interest of any kind; that all financial sources are fully and clearly stated in the acknowledgements section; and that they fully agreed with the final edited version of the article.

The declaration of the contributions of each author to the manuscript is as follows: M.I.A: Conceptualization of research work and designing of experiment: M.I.A and A.I: Execution of field/lab experiment. M.I.A and A.I: Data collection. A.I: Analysis of data. M.I.A and A.I: Interpretation of data. M.I.A and A: Preparation of manuscript.

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