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INDUCTION OF NORMOCYTIC-NORMOCHROMIC ANAEMIA IN CLARIAS GARIEPINUS (BURCHELL, 1822) EXPOSED TO ACUTE CONCENTRATION OF 1, 1-DIMETHYL 4,4-BIPIRIDILLIUM (PARAQUAT) UNDER LABORATORY CONDITIONS

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ABSTRACT

Normocytic-normochromic anaemia was induced in *Clarias gariepinus* (Burchell, 1822) in 96hrs. Experimental fish were exposed to test water separately diluted with acute concentrations of paraquat of 0, 15, 20, 25 and 30mg/L. A 96Hrs exposure to acute concentrations of the toxicant resulted in normocytic-normochromic anaemia in the exposed fish. Blood indices attributable to normocytic-normochromic anaemia was observed with a significant ($p<0.05$) decrease in haemoglobin, haematocrit, red blood cells compared with fish in the control test: Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were not significantly different ($p>0.05$). White blood cells, lymphocytes, monocytes, Neutrophils, eosinophil and basophil were also not significantly different ($p>0.05$) with increasing concentrations of the toxicant.

Keywords: *Toxicity, paraquat, Clarias gariepinus, normocytic-normochromic, and anaemia.*

INTRODUCTION

Environmental contamination with pesticides is a problem of a worldwide importance; data on their accumulation and excretion by fish are therefore valuable both for the assessment of the safety of pesticides for man and the extent of contamination of the fish in the environment (Auta, 2001). Pesticides are recognized as serious pollutants in the aquatic environment with the potential to cause deleterious effects on the biota, especially fish (Verma, *et al*., 1982). Over 5.5 billion liters of pesticides are sold around the world each year which consist of herbicides, insecticides, fungicides and rodenticides, but herbicides are sold in largest quantities followed by insecticides, fungicides and rodenticides (Mac-Ewen and Stephenson, 1975). Organophosphorus pesticides (OPs) are the most commonly used pesticides in the world due to their quick degradation (Eto, 1974). Indiscriminate disposal of pesticides into water bodies leads to contamination of aquatic environment. They pose a severe threat to aquatic organisms, which form important members of food chain (Satyaparameshwar *et al*., 2005). Pollution brings undesirable changes in the environment, which affects the biotic composition of the ecosystem. Most of the pollutants are either emitted to the atmosphere through gases which serve as medium, or are discharges to water bodies, or directly through the introduction of the chemical to attack particular organisms such as in pest control programs (Holden, 1977). In developing countries, the human population is exposed to pesticide compounds through drinking water and via the food supply, including fish (Hayes, 1982). In recent years, extensive use of pesticides has caused many problems due to their adverse effects on the aquatic ecosystem and human health (Hanke *et al.*, 1983). According to West and Biney (1991), besides habitat loss and over exploitation, pollution ranked third as the main cause of fish species loss, and that there are three main sources of aquatic pollution in Africa, Urban development, industrial waste and the use of pesticides. Contamination of water by pesticides either directly or indirectly kills, reduced fish productivity or elevated concentrations of undesirable chemicals in edible fish tissue which are deleterious to humans eating these fishes (Adedeji *et al*., 2012). The effects of pesticides concentrations on haematological parameters on different fish species have been studied by many investigators (Abubakar and Abdulsalami, 2013; Idi-Ogede and Smuel, 2018). Fish blood is being studied increasingly in toxicological research and environmental monitoring as possible indicator of physiological and pathological changes in fishery management and disease investigations (Mulcahy, 1975) as the blood in the gill has direct contact with the water medium and any unfavourable change in water could be reflected in the circulatory system(Adhikari *et al*., 2002).

Paraquat (1, 1-dimethyl 4, 4-bipiridillium), is a non-selective contact herbicide. It acts in the presence of light which it absorbs at 260nm, to desiccate the green parts of all plants with which it comes in contact (Hassall, 1990).

The African catfish, *Clarias gariepinus* is an ecologically important and commercially valued fish for the Nigerian fishing industry (Ita, 1980). These mudfish are frequently and widely cultured in ponds and they also occur freely in Nigerian natural fresh waters. The demand for this fish species by almost 75% of Nigerian population has necessitated the cropping of it in large number using poisons (herbicides). There is paucity of information on toxicity of paraquat on catfish despite its indiscriminate use by the fish farmers.

The aim of the present study was to evaluate normocytic-normochromic anaemia in *Clarias gariepinus* under laboratory conditions.

MATERIALS AND METHODS

Procurement of test fish

Clarias gariepinus juveniles (total length: between 15.5 and 18.2 cm) were obtained from a reputable fish farm in Ilorin and transported in oxygenated polythene bags to the laboratory. 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% $KmNO_4$ solution to obviate any dermal infection.

Acclimation of test fishes

Fishes were acclimatized to laboratory conditions for a period of two weeks. No mortality was recorded during the acclimation period. The fishes were fed with pelleted feed containing 35 % crude protein at 3% body weight per day. Daily ration was divided into three portions and fed thrice per day. After acclimatization, fishes were kept in different concentrations of paraquat in different aquaria. The test solutions were renewed fortnightly.

SOURCES OF PARAQUAT AND ITS EXPOSURE

Paraquat (1, 1-dimethyl 4, 4-bipiridillium) was purchased from Ilorin central market. Non-renewal toxic test method (APHA, 1992) was used. Fishes were exposed to acute concentrations for 96hours. Control fish were also maintained under identical conditions without the toxicant.

The experimental design was a randomized complete block design. A total of one hundred and fifty (150) juvenile of *Clarias gariepinus* were randomly distributed into the tanks at a stocking rate of 10 fish per tank. The fifteen (15) tanks were assigned to 5 treatments (control inclusive). In order to determine the LC_{50}, the *C. gariepinus* were exposed to four different concentrations of paraquat (0, 15, 20, 25 and 30mg/L. LC_{50} value obtained using EPA Probit Analysis programme version 1.5 was 20.02 mg/L (Abubakar, 2013).

COLLECTION OF BLOOD.

Blood samples were collected from both the control and experimental fish at intervals of 1, 14 and 28days. 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 32 mm) syringe. The blood was put into EDTA vials and taken to Medical diagnostic laboratory in Ilorin for analysis using methods described by Blaxhall and Daisley (1973) 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:

Hemoglobin determination is the quickest method for detecting anemia. 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 10.0 milliliters 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: $X\ \% = \frac{XC14\ gm\ \text{haemoglobin per 100 ml of blood.}}{100}$

Determination of pack cell volume:

Pack Cell Volume (PCV) was carried out by micro-westegreen method as described by Blaxhall and Diasely (1973). 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 techniques of red blood counts of fish blood are similar in most respects to those used in mammalian counts. However, the diluting fluids normally used for mammalian counts were not applicable to fish bloods. Gorder's and Hayme's diluting solutions were distorted after a few minutes. 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 (counting chamber) was a Neubauer, the pipette was held to the edge between the cover slip and the chamber, and capillary action drew the diluted suspension of cells into the chamber. 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 (mm^3) of blood (Mallum *et al.*,2015).

ERYTHROCYTE INDICES

Content of the erythrocyte from values obtained for the erythrocyte count, the haemoglobin concentration and haematocrit.

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

$$MCV = \frac{\text{Haematocrit}}{\text{Erythrocyte count (millions/Cu.mm)}} \times 10$$

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:

$$MCH = \frac{\text{Haemoglobin(gm/100ml) x 10}}{\text{Erythrocyte count (millions/Cu.mm)}}$$

It is expressed in pictogram (pg).

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

$$MCHC\ (g\ 100ml^{-}) = \frac{\text{Haemoglobin (gm/ 100ml) x 100}}{\text{Haematocrit}}$$

It is expressed in gram deciliter (gdL^{-1}).

Total leucocytes count:

Shaw's solutions A and B allowed differentiation between leucocytes, erythrocytes and thrombocytes. Both solutions were filtered just prior to use. Solution A was made fresh each day, solution B was stable for several days. Leucocytes were counted using Shaw's solution A and B. The blood was drawn up to the 0.5

mark, 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 as in the erythrocyte count. 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 (Hesser, 1960).

All the data generated were managed with Microsoft Office Excel 2003. The data were analyzed with One-Way analysis of variance (ANOVA) procedure using Statistical Product for Service Solution (SPSS) version 16.0 for window. Statistical significance of difference among means was compared using Turkey (HSD) test.

RESULTS AND DISCUSSION

Alterasion in blood attributable to acute concentrations of paraquat were observed at end of 96hours bioassay.

Haematological parameters of acute concentrations of paraquat on *C. gariepinus* .

Exposure of *C. gariepinus* to acute concentrations of paraquat for 96hrs resulted in normocytic-normochromic anaemia (consistent decrease in the values of Hb, PCV and RBC). The anaemia was the same along different sublethal concentration levels. Analysis of Erythrocyte indices (MCV, MCH and MCHC) revealed normocytic-normochromic anaemia. The values of Erythrocyte indices of MCV, MCH and MCHC for the exposed groups were not significantly ($p>0.05$) different with that of control values. White blood cells, lymphocytes, monocytes, Neutrophils, eosinophil and basophil were also not significantly different ($p>0.05$).

There were insignificant increase ($p>0.05$) in the values of WBC as evidence in immune response. Differential white blood cells analysis revealed no pronounced joint disorder with insignificance different between neutrophil and lymphocyte of the exposed groups and their controls ($p>0.05$). The values for the exposed groups of monocyte, eosinophil and basophil fluctuated relatively to their control groups (Table 1).

Table 1: Haematological parameters of *Clarias gariepinus* exposed to acute concentrations of paraquat (mean ± SD)

		Concentration(mg/L)		
Parameters	Control	15.00	20.00	25.00
Hb(gdL-1)	14.5± 0.67a	12.7± 0.62b	13.5± 0.70c	11.39± 0.50e
PCV (%)	45.2±2.06a	44.7±1.85e	42.0±1.88d	38.7± 0.12 b
RBC(x 1012L-1)	5.7±0.22a	5.4.±0.14d	5.2±0.14f	4.1± 0.01 b
MCV(Fl)	81.3±2.94b	81.0±2.20b	81.0±1.41b	81.0± 0.31b
MCH (pg)	27.1±0.99a	27.6±0.73a	27.3±0.91a	27.9± 0.61a
MCHC (gdL-1)	33.61±0.02ab	33.31±0.03ab	33.34±0.03ab	33.29± 0.00 ab
WBC (x109L-1)	5408±10.20ac	5612±15.00ad	5710±6.68ae	5806± 5.00af
Neutrophil (%)	68.0±1.41a	68.6±1.28b	68.3±2.16b	68.1±1.13 b
Lymphocytes (%)	26.0±1.37a	26.5±1.35a	26.4±0.82a	2680± 0.43a
Monocytes (%)	5.0±1.25a	5.0±1.42a	5.0±0.79a	5.0± 1.34 a
Eosinophils (%)	0.0±0.00b	0.0±0.01b	0.0±2.16b	0.0± 0.00 b
Basophils (%)	0.0±0.00a	0.0±0.00a	0.0±0.01a	1.0± 0.00b

Means 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.

This research work revealed normocytic-normochromic anaemia in *Clarias gariepinus* exposed to acute concentrations of paraquat for 96hours under laboratory conditions (Table 1). According to the findings, the test chemical could be ranked toxic (Wagner, *et al*., 1995). Findings demonstrated that *C.gariepinus* is sensitive to paraquat. The pesticide stress caused the normocytic-normochromic anaemic condition by destroying mature erythrocytes, resulting in a reduced RBC, and disrupting- synthesizing mechanisms (Adhikari, *et al.*, 2002). Normocytic may be due to decrease in haematocrit during exposure. Similar pattern has been detected in *Labeo*

umbratus after exposure to various pollutants (Van Vuren, 1986). The anaemia observed in the test species could have been due to destruction of erythrocytes or inhibition of erythropoiesis and haemosynthesis (Gabriel *et al.*, 2007). Abubakar and Abdulsalami (2013) observed microcytic-hypochromic anaemia in *Clarias gariepinus* exposed to sublethal toxicity of Sniper 1000EC. Idi-Ogede and Samuel (2018) also recorded microcytic-hypochromic anaemia in *Oreochromis niloticus* exposed to sublethal toxicity of Sniper 1000EC. Benarji and Rajendranath (1990) reported reduced hematopoiesis followed by anaemia due to exposure to acute concentration of dichlorvos. Reduction in erythrocyte count, haematocrit value and haemoglobin content of *C.gariepinus* can be attributed to such factors as (1) blood haemorrhage due to un equilibrium of osmotic pressure inside and outside blood cells (Heath, 1987), and (2) haemodilution of blood due to damage and bleeding of fish organs (Movotny and Beeman, 1990). The reduction in blood parameters was an indication of anaemia caused by this toxicant as the concentration increased. 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 tilapia. Haematological indices (RBC count, concentration of haemoglobin and haematocrit) have been reported to indicate secondary responses of an organism to pollutants (O'Neal and Weirich, 2001). MCV, MCH and MCHC values were calculated using the PCV, Hb and RBC. Insignificant difference in MCV, MCH and MCHC were indication of normocytic-normochromic (Idi-Ogede, 2016). The anaemic exposure could be as a result of destruction of RBC (Abubakar, 2013) or haemodilution as reported by Sampath *et al*., (1993). Joshi *et al*., (2002) made a similar observation on blood parameters of C. batrachus exposed to Lindane and Malathion which are pesticides. Changes in WBC and differential counts have been reported to play important roles in the state of health of *C. gariepinus* (Ezeri, 2001; Omoregie and Oyebanji, 2002). Changes in leucocyte number and appearance have been correlated to alterations in immune system performance (Hine and Wain, 1988). Changes in neutrophils, eosinophils and basophils indicated stress condition in the fish and similar reports have been made by several authors including Johansson-Sjobeck *et. al*. (1978) and Anyanwu *et al.*, (2007).In vertebrates, white blood cells (leucocytes) are a primary line of immunological defence. The leucocytes are involved in the regulation of immunological function of the body (Santhakumar *et al.*, 1999). Accordingly, one of the most elementary ways to assess the immune system is to explore changes in the number or appearance of the four main types of circulating leucocytes, that is, lymphocytes, thrombocytes, granulocytes and monocytes (Tierney *et al.*, 2004). Changes in leucocytes also occur when fish are stressed and environmental quality is altered (Casillas and Smith, 1977). Wedemeyer *et al.*, (1984) reported that changes in leucocytes and erythrocytes of fish were strong indicator of stress due to the presence of toxicants in the aquatic environment.

CONCLUSION

Our observations revealed that the abnormalities in haematological parameters of *C.gariepinus* as result of exposure to acute concentrations of paraquat under laboratory conditions resulted in normocytic-normochromic anaemia attributable to stress due to the presence of the toxicant. Based on our observation, we therefore conclude that paraquat exposure has stressor effects on heamatological parameters of the exposed fish species. The study recommends that proper education of farmers on the danger of paraquat to the environment should be done by extension field officers. Attempts should also be made to monitor the use of paraquat by local fishermen. The Manufacturing industries should look into ways of reducing the potency of paraquat to non-target organisms such as fish, and they should also be compelled to state categorically the effect of paraquat to non-targeted aquatic organisms.

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