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Chronic Effects of Paraquat on Haematology of African Catfish *(Clarias gariepinus)*

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ABSTRACT

The toxic effect of paraquat dichloride on the juveniles of *Clarias gariepinus* was investigated. Juvenile *C. gariepinus* were exposed to varying lethal concentrations (0.5, 1.0, 1.5, 2.0 and 2.5 mg/l) of paraquat dichloride for 4 days and sub-lethal (0.02, 0.04, 0.06, 0.08 and 1.0mg/l) concentrations for 30 days. The control group was free of the toxicant. The haematological properties (pack cell volume PCV, red blood cell counts RBC, platelets and haemoglobin HB, platelets, monocytes, lymphocytes, esinophils, neutrophils and basophils) of the exposed and control groups were examined using standard methods. Results showed that PCV, RBC, Platelets and HB values decreased significantly while the WBC increased with increasing concentrations of paraquat dichloride. It is concluded that the use of paraquat as a herbicide adversely affects the wellbeing of non-target organisms like *C. gariepinus*. We therefore recommend the discontinued use of this herbicide as it toxic to non-target organisms including fish and man.

Keywords: Paraquat, toxicity, haematology, *Clarias gariepinus.*

INTRODUCTION

Paraquat is a commonly used herbicide with trade name gramoxone and an active ingredient called N, N′-dimethyl-4,4′-bipyridinium dichloride (Gao *et al*., 2010). In usage, it is second only to glyphosate in world wide application (Banaee *et al*., 2013). However, it is highly toxic to man (Huang *et al.*, 2013) and aquatic organisms (Katagi, 2010).

The increasing use of herbicides and pesticides in agriculture (including commercial and household production of vegetables) for the control of pest and weeds causes chemical pollution of aquatic environment (Aghoghovwia and Izah, 2018). Fish and other aquatic animals are very sensitive to aquatic pollution and show both pathological and physiological alterations when exposed to these xenobiotics (Gabriel and Edori, 2010). The quality of fish food is inexorably

linked to the health of fish which itself is dependent on the level of pollutants in the aquatic environment (Verma *et al*., 2004). In fish, paraquat has been reported to alter the activity of several enzymes. Thus, paraquat can affect cardiac contraction, opercula ventilation, and embryonic development. Toxins cause an increase in energy requirements for maintaining homeostasis (Ensibi *et al*., 2013).

Fish are among the organisms used in eco-toxicological studies probably due to their sensitive to the aquatic pollutants. To this effect cat fish especially *Clarias gariepinus* (Aghoghovwia and Izah, 2018) is the most common group of fish use in toxicological studies in Nigeria. It was, therefore, used as the test organism in this research to investigate the impact of paraquat on the haematological properties of fish.

Materials and Method

Source of Experimental Fish

One hundred and eighty (180) juveniles of *Clarias gariepinus* were purchased from African Regional Aquaculture Centre (ARAC), Aluu, Rivers State, Nigeria. The *Clarias* species averaging 12.14 ± 1.76cm standard length and body weight of 4.45 ± 1.37g were used for the study. The fish were conveyed in a well aerated container half-filled with water from the rearing ponds in the farm premises to the holding units at the toxicology laboratory of ARAC where the experiment was conducted.

Preparation of Test Solution

The herbicide paraquat was purchased from a commercial outlet in Port Harcourt. Out of 276g/L paraquat, stock solution of 55.2g was prepared by diluting measured volumes (i.e 199.824mls of paraquat in 20L de-chlorinated tap water for 10mg/l concentration. The de-chlorinated tap water

used had the same physical and chemical properties with the one used in acclimatizing the fish. The control solutions were made up of only de-chlorinated tap water.

Test for Range Finding and Definitive Test (Pilot Study)

For each test concentration of the herbicide, smaller holding containers of 20L each were used. The test solutions and the controls were run in triplicates. For each triplicate, the test solutions and controls were prepared and labeled, thoroughly mixed and allowed to stand for about 5 minutes before use. Range finding test described by Inyang *et al.* (2008) was adopted in this study:

mls x stock solution = aquarium water (ml) x desired concentration (mg/l) …… eqn. 1

or

$N_1V_1 = N_2V_2$ …… eqn 2

(Seiyaboh *et al*., 2013).

Where N_1= Manufacturer's concentration (276g)

N_2= concentration of test solution desired

V_1= Volume of the original solution added

V_2= volume of the test solution (20litres).

These were prepared by transferring 199.8mls, 399.6mls, 599.4mls, 799.8mls and 999.6mls of the original concentration of paraquat and making it up to 20L de-chlorinated tap water. The concentrations of toxicants in the five holding tanks were prepared by adding 0.00mg/l, 144.8mls, 289.6mls, 434.4mls, 579.6mls and 724.4mls gotten from a stock solution of 55.2g into 20L of de-chlorinated tap water and converted into 0.0 (control), 0.5, 1.0, 1.5, 2.0 and 2.5mg/l. which is the final concentration for the acute toxicity test.

Experimental Setup

Ten fish samples were held in containers of 20L capacity and acclimatized for two weeks in de-chlorinated water which was changed daily, the top of the containers was covered with a net to control escape of fish. Eighteen 20L capacity containers were used in all. During this period, the fishes were fed with commercial feed containing 35% crude protein twice per day at 4% body weight.

The following blood parameters, packed cell volume (PCV), and haemoglobin level, red blood cells, white blood cells, platelets, monocytes, lymphocytes, esinophils, neutrophils and basophils were determined and calculated after the exposure period of 30days.

Five fish from each test aquarium were sacrificed for the haematological assessment. Blood was collected by cardiac puncture. To determine PCV, fresh blood samples were collected into heparinized microhaematocrit tubes sealed with plasticine at one end and centrifuged for 5 min at 3,000 rpm. The mean values of PCV were measured with a microhaematocrit reader. Blood samples were collected in a tube containing EDTA to prevent blood coagulation. Haemoglobin levels were obtained by means of Boehringer-Mannheim commercial kits, based on colorimetric determinations.

Determination of Sub-lethal Concentrations of Herbicide

Six 20L containers of same size were used in three replicates for the toxicants and a control. Appropriate volumes (0.2mls, 0.4mls, 0.6mls, 0.8mls and 1.0mls of stock solution were dispensed using a 5ml syringe and measuring cylinder into 20L of dechlorinated tap water in each of the tanks except the control. The juveniles were exposed to nominal concentrations of paraquat for 30 days. The concentrations used for the chronic study are 0.0 (control) 0.02, 0.04, 0.06, 0.08 and 0.1mg/l of toxicants for 30 days.

Experimental Design

The experimental design was a completely randomized design (CRD) with five treatments levels of paraquat and a control with each level having three replicates.

Results

The RBC, PCV, Hb and platelets values in the exposed fish significantly decreased ($P<0.05$) compared to the control group. However, parameters such as WBC, neutrophils, esinophils, and monocytes increased progressively as the toxicant concentrations increased when compared to the control The variations observed were proportional to the herbicide concentration. PCV reduced from 30.19mg/l in the control to 21.29mg/l in the 1.0mg/l paraquat concentration. Haemoglobin HB counts also reduced from 9.72mg/l in the control to 7.44mg/l in the 1.0mg/l herbicide exposure. Platelets reduced significantly and progressively from 279.51mg/l in the control to 155.71mg/l in the 1.0mg/l paraquat concentration. These reductions were statistically significant at $p=0.05$.

The highest increase of 26.4mg/l (control) to 38.67mg/l in neutrophils, was observed in the 1.0mg/l concentration as compared to the control, while the least increase of 1.07mg/l in the control to 4.99mg/l in the 1.0mg/l concentration was observed in eusinophils,. No traces of basophils were found in the fish juveniles during the experiment (Table 1).

Table 1: Haematology in C. *gariepinus* Exposed to Paraquat Dichloride for 30 Days (Mean ± S.D)

Parameters	Concentration (mg/l)					
	0.00	**0.02**	**0.04**	**0.06**	**0.08**	**0.1**
PCV	30.19 ± 7.48[c]	29.25 ± 1.74[b]	27.00 ± 1.54[b]	24.85 ± 0.95[a]	23.11 ± 1.47[a]	21.29 ± 1.38[a]
HB	9.72 ± 5.82[c]	9.03 ± 0.48[c]	8.96 ± 0.42[b]	8.50 ± 0.35[b]	8.04 ± 0.18[b]	7.44 ± 0.20[a]
RBC	5.31 ± 2.87[c]	4.60 ± 0.13[b]	4.40 ± 0.15[b]	4.21 ± 0.13[b]	4.08 ± 0.09[b]	3.78 ± 0.18[a]
WBC	3.78 ± 3.28[a]	6.46 ± 0.62[b]	7.34 ± 0.41[c]	8.19 ± 0.56[c]	9.02 ± 0.31[d]	9.47 ± 0.13[d]
PLAT	279.51 ± 15.46[c]	260.73 ± 6.77[c]	239.37 ± 14.17[b]	209.80 ± 12.54[b]	177.44 ± 15.01[a]	155.71 ± 10.08[a]
NEU	24.67 ± 2.03[a]	30.0 ± 5.00[b]	32.00 ± 3.00[b]	33.33 ± 2.89[b]	35.00 ± 6.56[c]	38.67 ± 5.77[c]
LYM	71.67 ±6.86[d]	63.33 ± 4.16[c]	60.67 ± 2.15[c]	57.33 ± 3.05[b]	53.00 ± 5.29[b]	47.33 ± 4.04[a]
MON	2.67 ± 2.31[a]	4.00 ± 1.00[b]	5.33 ± 0.57[b]	6.67 ± 2.08[c]	7.67 ± 0.57[c]	9.00 ± 0.00[d]
EOS	1.07 ± 2.08[a]	2.67 ±1.15[a]	3.00 ± 1.00[b]	3.67 ± 1.15[b]	4.33 ± 1.15[c]	4.99 ± 1.73[c]
BAS	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]

Discussion

The significant reductions observed in some of the hematological parameters (PCV, HB, RBC, Platelets, neutrophils and lymphocytes) of C. *gariepinus* exposed to paraquat demonstrates the fact that pesticides cause a significant reduction in the haematological parameters of fish Ada *et al,* (2012). These reductions could also be the product of impaired erythropoiesis and rapid haemolysis of the RBC.

Furthermore, the reduction in MCV in the present study reveals erythrocytic shrinkage leading to microcytic anaemia. This reduction in MCV in the paraquat exposed fish could also arise due to osmoregulatory imbalances. Li *et al*. (2011) reported that verapamil reduced the erythrocyte count, Hb and PCV in *O. mykiss*. Reduction in the values of these parameters was also reported 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 *C. albopunctatus* exposed to acetellic (Mgbenka *et al.*, 2005). The results from this study are also consistent with the trend in *Heteropneustes fossilis*, exposed to adrin and fenvalerate (Thakur *et al.*, 2000), and in *Salmo gardneri* and *Mystus vittatus* exposed to pesticides (John, 2007).

Studies have shown that pesticides have cytotoxic effects in fish which impose severe oxidative stress on the fish (Mikula *et al*., 2009; Nwani *et al.*, 2013). The anaemia reported in this study may be attributed to erythrocyte membrane lipid peroxidation that predisposed it to rapid sequestration or haemolysis brought on by paraquat exposure. Samthakumar *et al*. (1999) noted that monocrotophos exposure resulted in the destruction of interstitial cells in Benni fish and inhibited erythropoeitic processes through the inhibition of erythropoietin, which may result in anaemia.

The reduction in the Hb concentration could also suggest that paraquat could have inhibited the Hb biosynthetic pathway by interfering or inhibiting the utilization of delta-aminolevulinic acid. This may have an adverse impact on the oxygen carrying capacity of the blood. Generally, leucocytes modulate immunological functions in animals, including fish. The observed leucocytosis in the present study indicated abnormal immune protective response to paraquat intoxication. It also suggested that paraquat stimulated the immune system with a concomitant release of lymphocytes from the lymphomyeloid tissue as a defense response. It resulted in the leucocytosis and or lymphocytosis, which altered body physiology. Leucocytosis was also reported in *C. carpio* exposed to lindane (Saravanan *et al.,* 2011), and in *O. niloticus* exposed to deltamethrin (El-Sayed *et al.,* 2007).

White blood cells are the smaller number compared with red blood cells, and they have the defensive role in the body of organisms. Changes in the levels of white blood cells following exposure to paraquat may be due to disturbances in the process of hematopoiesis and subsequent reduction or 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 seen in the blood circulation, lymphoid organs and other tissues, especially during inflammatory reactions. 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.

According to results of this study and other researches, 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 the 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, similar results were also observed in this study.

Conclusion and Recommendations

The results of the present study has indicated that the commercial formulation of paraquat is toxic and has the potential to impair the haematological properties of the African catfish. Further studies are required to investigate the immune-toxicological mechanisms of action of these herbicides in *C. gariepinus* and other fish species.

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