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Acute Haematological Effects of Sublethal Levels of Paraquat on the African Catfish, *Clarias gariepinus* (Osteichthyes: Clariidae)

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Abstract: Haematological variables related to oxygen transport (total erythrocyte counts TEC, haemoglobin levels Hgb and haematocrit [packed cell volume, PCV]), calculated haematological indices (mean erythrocyte haemoglobin concentration MEHC, mean erythrocyte haemoglobin MEH and mean erythrocyte volume MEV) and total leucocyte counts TLC varied ($p<0.05$) between control and paraquat treated *Clarias gariepinus* following 96 h exposure. There were significant increases ($p<0.05$) in the levels of TEC, Hgb, PCV, MEV, MEH and MEHC while TLC decreased significantly ($p<0.05$). The variations observed were directly proportional to the toxicant concentrations.

Key words: Paraquat, haematology, anaemia, *Clarias gariepinus*, Nigeria

INTRODUCTION

Herbicides make up about 40% of the production of pesticides in the world. They have been shown to cause deleterious effects on fish health (Banhawy *et al*., 1996). Synthetic herbicides are commonly used by farmers to control weeds and nuisance aquatic vegetations around rivers, lakes and reserviours. However, these pesticides ultimately find their way to these water bodies, inducing adverse impacts on fishes living therein (Tsuda *et al*., 1997). The widespread use of herbicides has resulted in a steady increase in water pollution, evoking considerable damage of phytoplankton and zooplankton, thus depleting essential sources of the food chain for fish (Montanes *et al*., 1995).

Paraquat (1, 1-dimethyl, 4,4-bi pysidinium) is an herbicide used in the tropics to eradicate weeds with potential toxicity on fish as a result of runoff into aquatic system.

The African catfish *Clarias gariepinus* is an ecological important and commercially valued fish for the Nigerian fishing industry (Ita, 1980). These mud fish are frequently and widely cultured in ponds and they also occur freely in Nigerian's Natural Freshwater. According to Musa and Omoregie, (1999) fish are intimately associated with the aqueous environment, physical and chemical changes in the environment are rapidly reflected as measurable physiological changes in fish.

The use of haematological technique in fish culture is growing in importance for toxicological research, environmental monitoring and fish health conditions. Many works has been conducted on haematological changes of pesticides in the fish such as Das and Mukherjee (2000) Adebayo *et al*. (2005) and Patnaik and Patra (2006). Sampath *et al*. (1993) noted that there is a possibility that studies on fish blood might reveal conditions within the body of the fish long before there is any outward manifestation of disease.

Literature on the effects of paraquat on *Clarias gariepinus* haematological parameter are scanty. Since paraquat is extensively applied in agriculture for weed eradication in Nigeria, it is pertinent to study its hazardous effect on the aquatic system, it is assumed, that the residue might affect the fish as a result of runoff into the water body. The study investigated the effects of paraquat on some selected blood parameters (TEC, TLC, HGB and PCV) and haematological indices (MEHC, MEH and MEV) of *Clarias gariepinus*.

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MATERIALS AND METHODS

Apparently healthy specimens of *Clarias gariepinus*, same broodstock, mean weight and length of 112.57±0.32 g and 24.45±0.18 cm were obtained from a private fish farm at Obiaruku town, Delta State, Nigeria and were transported in containers to the laboratory. In the laboratory fishes were kept in large plastic bowls containing 60 L of clean tap water and acclimatized for 14 days to the laboratory conditions, during which they were provided with artificial feed (grower's mash from Bendel Feed and Flour Mill, Ewu Nigeria) and ground shrimps obtained locally to avoid possible effects of starvation on any of the haematological parameters of the fish. Mortality of less than 1% was observed during acclimatization period. Fish of both sexes were used without discrimination.

Ten fish each were introduced into three aquaria with replicates. Each aquarium had the following concentrations 0.3, 0.15 and 0.0 mg L^{-1} of paraquat as paraquat chloride. The exposure period lasted for 96 h. The toxicant and test waters were renewed every 24 h to maintain a relatively constant concentration of paraquat. No mortalities occurred in any group during the experimental period.

However, during the exposure period the fish were not fed, while some water quality parameters were monitored according to APHA (1995). The water quality parameters monitored included temperature, dissolved oxygen, free carbon IV oxide, total alkalinity and pH.

The following blood parameters: packed cell volume/haematocrit (PCV/HCT), total leucocyte count (TLC), total erythrocyte count (TEC) and haemoglobin level (HGB) and haematological indices of mean erythrocytic volume (MEV), mean erythrocytic haemoglobin (MEH) and mean erythrocytic haemoglobin concentration (MEHC) were determined and calculated after the exposure period of 96 h.

Five fish from each test aquarium were sacrificed for the haematological assessment. Blood was collected by cardiac puncture according to Blaxhall and Daisley (1973). To determine PCV/HCT, 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 sample for TEC, TLC and haemoglobin 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. TEC was performed with microscope Nebauer count chambers diluting the blood (20 times) in Toisson's solution and TLC was performed with microscopic Nebauer count chambers diluting the blood (200 times) in Turk's solution. Haematological indices were calculated from the equations given by Anderson and Klontz (1965).

$$\text{MEHC (\%)} = \frac{\text{Haemoglobin (g dL}^{-1}\text{)}}{\text{PCV (\%)}} \times 100$$

$$\text{MEH } (\rho\text{g}) = \frac{\text{Haemoglobin (g dL}^{-1}\text{)}}{\text{TEC (106 L}^{-1}\text{)}} \times 10$$

$$\text{MEV } (\mu^3) = \frac{\text{PCV (\%)}}{\text{TEC (106 L}^{-1}\text{)}} \times 10$$

Students' t-test was employed to calculate the significant difference between control and experimental means. Probability values of 0.05 were considered statistically significant.

RESULTS

The water quality parameters monitored showed no significant difference ($p<0.05$) as shown in Table 1.

Table 1: Mean values of water quality parameters[+] resulting from 96 h exposure of *Clarias gariepinus* to the various sublethal concentrations of paraquat

	Water quality parameters					
Concentration ($mg\ L^{-1}$)	pH	Temperature (°C)	Dissolved oxygen ($mg\ L^{-1}$)	Free carbon (IV) oxide ($mg\ L^{-1}$)	Total Alkalinity ($mg\ L^{-1}$)	NH3 (Unionized) ($mg\ L^{-1}$)
0.30	6.85 (0.12)	27.00 (0.01)	7.41 (0.02)	3.98 (0.21)	28.40 (0.30)	0.03 (0.02)
0.15	6.84 (0.11)	27.50 (0.02)	7.42 (0.10)	3.80 (0.12)	28.40 (0.48)	0.03 (0.01)
0.00 (control)	6.85 (0.13)	27.00 (0.01)	7.41 (0.02)	3.85 (0.06)	28.40 (0.35)	0.02 (0.01)

[+]The values are expressed as the mean (SE)

Table 2: Mean values haematological parameters[#] resulting from 96 h exposure of *Clarias gariepinus* to the various sublethal concentrations of paraquat

	Haematological parameters/indices						
Concentrations ($mg\ L^{-1}$)	PCV (%)	HGB ($g\ dL^{-1}$)	TEC ($10^6\ L^{-1}$)	TLC ($10^3\ L^{-1}$)	MEHC (%)	MEH (ρg)	MEV (μ^3)
0.30	26.50 (0.68)	9.55 (0.22)	4.80 (0.18)	4.78 (0.09)	36.04 (0.68)	19.95 (4.59)	55.21 (0.22)
0.15	34.20 (0.32)	12.56 (0.12)	5.10 (0.06)	2.75 (0.09)	36.70 (0.98)	12.28 (3.89)	67.05 (0.32)
0.00 (control)	36.60 (0.45)	14.60 (0.08)	6.45 (0.08)	1.50 (0.06)	39.89 (0.51)	22.64 (3.41)	56.74 (0.55)

[#]The values are expressed as the mean (SE) and for each parameter, mean values were calculated from 5 fishes from each exposure and control; each result was duplicated so that the mean (SE) given here were calculated from 10 observations

The haematological variables related to oxygen transport (total erythrocyte counts TEC, haemoglobin levels HGB and haematocrit [packed cell volume, PCV]), calculated haematological indices (mean erythrocyte haemoglobin concentration MEHC, mean erythrocyte haemoglobin MEH and mean erythrocyte volume MEV) and total leucocyte counts TLC varied ($p<0.05$) between control and paraquat treated *Clarias gariepinus* following 96 h exposure as shown in Table 2.

There were significant increases ($p<0.05$) in the levels of TEC, Hgb, PCV, MEHC and MEH while TLC decreased significantly ($p<0.05$). The variations observed were directly proportional to the toxicant concentrations.

DISCUSSION

The data obtained from this investigation show that Paraquat causes a stress inducing effect on freshwater teleosts during exposure period as indicated by the alternations in blood parameters. In such cases the fish became more susceptible to infections and may be more vulnerable to predators.

The values of PCV, HGB, TEC, MEV, MEH and MEHC decreased significantly ($p<0.05$) while TLC increased significantly ($p<0.05$) which was directly proportional to the paraquat concentrations. The trend obtained in this study is in accord with the findings of Kori-Siakpere (1998), Das and Mukherjee (2000), Kori-Siakpere *et al*. (2005) and Patnaik and Patra (2006).

The significant reduction in the values of HGB and TEC as the concentration of extracts increases in an indication of severe anaemia caused by paraquat on the exposed fish. The anaemic response could be as a result of destruction/the inhibition of erythrocyte production, haemodilution and it could as well as a result of the destruction of intestinal cells by the toxicant (Samprath *et al*., 1993; Saheny and Johal, 2000; Patnaik and Patra, 2006).

Reduced TEC may be due to swelling erythrocyte or haemolysis which may be due to increased protein-carbon IV oxide in the blood. It may also be as a result of anaemia which is possibly due to haemodilution resulting from impaired osmoregulation across the gill epithelium as reported by Wedemeyer *et al*. (1984) and Svoboda *et al*. (2001). However, the decrease in the erythropoietic activity of the kidney (Santhakumar *et al*., 1999) or the haemodilution resulting from impaired osmoregulation across the epithelium (Wedemeyer *et al*., 1984) may be cause of decrease in TEC level of *Clarias gariepinus* exposed to paraquat concentrations.

Increase in TLC has been attributed to several factors such as increase in thrombocytes, lymphocyte or squeezing of leucocytes in peripheral blood (Agrawal and Srivastava, 1980). Leucocytes are involved in the regulation of immunological function of body (Santhakumar *et al*., 1999). An increase in TLCs thus occurs as a protective response to stress (Das, 1998).

Conclusively, the 96 h bioassay test performed on the toxic effects of paraquat on *Clarias gariepinus* showed that the toxicant has significant effects ($p<0.05$) on the haematological parameters examined.

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