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**Sublethal Effects of Paraquat on Some Plasma Organic Constituents
(Metabolic Parameters) of African Catfish: *Clarias gariepinus*
(Osteichthys-Clariidae)**

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Abstract: The plasma levels of glucose, protein, cholesterol and triglyceride were determined in *Clarias gariepinus* exposed to sublethal concentrations of paraquat (0.30 and 0.15 mg L⁻¹). There were significant decrease (p<0.05) in plasma glucose (showing hypoglycemia situation), plasma protein and triglyceride with a significant increase (p<0.050) in plasma cholesterol when compared with the control. These changes were directly proportional to the toxicant concentration and the exposure periods thus it is dose dependent and time-dependent.

Key words: Paraquat, plasma glucose, protein, cholesterol, triglyceride, *Clarias gariepinus*, Nigeria

INTRODUCTION

In aquaculture, organophosphates are widely used to control a variety of agricultural pests as well as ectoparasites in fish. Various chemicals entering the aquatic ecosystem through human activities, either accidentally or by design may cause adverse effects on the aquatic biota, including deleterious changes which disrupt metabolic activity at the biochemical level (Hirth, 1964; Das, 1998).

Paraquat was first synthesized in 1882, but its pesticide properties were not discovered until 1959 (Haley, 1979). It is in general use world wide and can cause severe, acute and chronic poisoning when it is waterborne (Haley, 1979), because it is very readily dissolves and dissociates in aqueous solution (Amdur *et al.*, 1991). Paraquat is a quick acting, non-selective compound that destroys green plant tissues on contact and by translocation with the plants (Ecobichon, 1999). Several authors have investigated the toxicity, uptake and tissue distribution and biochemical changes of pesticides on the fish (Das and Mukherjee, 2000; Das, 1998; Yildiz and Pulsatsii, 1999; Darwish *et al.*, 2002).

Since paraquat is extensively applied in agriculture for weed control in Nigeria, it is pertinent to study its hazardous effect on the aquatic system as it is assumed that the residue might affect the fish. Due to its food value, *Clarias gariepinus* is in high demand in Nigeria. It is also a candidate in fish culture system. Thus necessary to study the deleterious effects of paraquat on the metabolic parameters of this important species. The study investigated the effect of paraquat on the plasma glucose, protein, cholesterol and triglyceride of *Clarias gariepinus*.

MATERIALS AND METHODS

Apparently healthy juvenile life specimens of *Clarias gariepinus*, mean weight 43.20±2.35 g with a mean body length of 16.23±2.60 cm were collected from a commercial fish farm in Sapele, Delta State, Nigeria. The fish were transported to the Zoology Research laboratory at the Department of Zoology, Delta State University, Abraka; in an oxygenated bag and acclimatized for 2 weeks

prior to exposure. Borehole water was used throughout the acclimation and exposure periods. The physio-chemical parameters of water used were: temperature 24.50±2.0°C; pH 7.4, hardness 82 mg L⁻¹ (as CaCO₃), total alkalinity, 38 mg L⁻¹ (as CaCO) and dissolved oxygen concentration 8.20 mg L⁻¹.

Paraforce (e) a paraquat dichloride solution containing 200 g of paraquat per litre was obtained from a local agricultural store in Warri, Delta State Nigeria. For each experiment the required concentrations were prepared from fresh stock solutions.

Two sublethal treatments (0.15 and 0.30 mg L⁻¹) of paraquat and untreated (negative) control were tested for 96 h in a static system (140 L polythene [plastic] drum). Ten *Clarias gariepinus* were introduced into each aquarium in triplicates. The herbicide exposed fish and the control were fed twice (0800 and 1600) daily with commercial pelleted feed. Water in the enclosures was renewed every 24 h to maintain an approximately constant concentration of paraquat. During the period of exposure aeration was provided to each aquarium. No mortalities was recorded in any group during the exposure period.

Two fish each were sacrificed from each aquarium for blood analysis. Blood was collected from the vertebral column of the fish (Kori-Siakpere, 1998) with the aid of heparinized 2 mL disposable syringe and 21 gauge disposable hypodermic needle. The blood samples were centrifuged at 3,500 rpm for 10 min and the blood plasma obtained was stored under refrigeration at -4°C. The samples of a given batch were assayed simultaneously. The following assays were run on blood plasma samples. Triglycerides level (GPO-PAP method with Randox laboratories, U.K); cholesterol (Enzymatic End Point Method Randox Laboratories UK); glucose level (GOD/PAP method with Randox laboratories UK) and protein level (Biuret method with Randox Laboratories UK).

The data obtained were tested with one-way Analysis of variance test and Microsoft Excel 2000.

RESULTS

The mean plasma glucose in the control group of fishes ranged from 121.70±10.14 to 124.12±0.03 mg dL⁻¹ during the exposure period. The values of plasma glucose in the treated and control fish is represented in Table 1. The treated fish revealed low values of plasma glucose compared to the control values. The changes in the plasma glucose level are statistically significant (p<0.05) in both concentrations after 96 h of exposure.

The values of the plasma triglyceride in the treated and control fish are represented in Table 2. The plasma triglyceride in the control group of fishes ranging from 158.40±0.71-160.50±1.21 mg dL⁻¹.

Table 1: Mean plasma glucose (mg dL⁻¹) of *Clarias gariepinus* exposed to sublethal concentrations of paraquat after 96 h

Concentration (mg L ⁻¹)	Exposure period (h)			
	12	24	48	96
0.30	110.69 (0.01)	106.69 (0.01)	99.33 (0.03)	95.18 (0.02)
0.15	122.39 (0.02)	118.92 (0.05)	110.66 (0.02)	106.86 (0.04)
0.00 (control)	124.12 (0.03)	123.42 (0.02)	122.92 (0.06)	121.70 (0.14)

Standard error in parenthesis

Table 2: Mean plasma triglyceride (mg dL⁻¹) of *Clarias gariepinus* exposed to sublethal concentrations of paraquat after 96 h

Concentration (mg L ⁻¹)	Exposure period (h)			
	12	24	48	96
0.30	38.51 (0.14)	30.29 (0.01)	37.91 (0.02)	22.01 (1.03)
0.15	130.25 (0.07)	105.52 (0.02)	99.52 (0.02)	86.57 (0.08)
0.00 (control)	160.50 (1.21)	159.16 (0.70)	158.51 (0.91)	158.40 (0.71)

Standard error in parentheses

Table 3: Mean plasma cholesterol (mg dL⁻¹) of *Clarias gariepinus* exposed to sublethal concentrations of paraquat after 96 h

Concentration (mg L ⁻¹)	Exposure period (h)			
	12	24	48	96
0.30	40.01 (0.14)	80.61 (0.21)	89.01 (0.01)	130.01 (0.61)
0.15	38.14 (0.02)	68.91 (0.10)	85.00 (0.08)	89.01 (0.01)
0.00 (control)	37.85 (0.13)	38.02 (0.21)	38.12 (0.11)	38.61 (0.01)

Standard error in parentheses

Table 4: Mean Plasma Protein (mg dL⁻¹) of *Clarias gariepinus* exposed to sublethal concentrations of paraquat after 96 h

Concentration (mg L ⁻¹)	Exposure period (h)			
	12	24	48	96
0.30	46.91 (0.01)	44.97 (0.01)	40.71 (0.01)	36.64 (0.04)
0.15	49.22 (0.03)	48.03 (0.04)	44.72 (0.03)	37.85 (0.03)
0.00 (control)	49.39 (0.01)	49.28 (0.03)	48.56 (0.03)	48.39 (0.01)

This variation is insignificantly different at the level tested ($p > 0.05$). However, the variation may be attributable to stress during handling. Treated fish showed low values of triglyceride levels when compared to the control. Changes in the triglyceride levels are statistically significant ($p < 0.05$) in both concentrations after 96 h of exposure.

The plasma cholesterol in the control group of fishes ranged from 37.85 ± 0.13 to 80.61 ± 0.21 mg dL⁻¹. The changes in the cholesterol levels is statistically not significant ($p > 0.05$) which may be as a result of stress during handling. The values of the plasma cholesterol in the treated and control fish are represented in Table 3. Treated fish showed high values of cholesterol levels than the control. Change in the cholesterol levels are statistically significant ($p < 0.05$) in both concentrations after 96 h of exposure.

The plasma protein in the control group of fishes ranged from 48.39 ± 0.01 to 49.39 ± 0.01 mg dL⁻¹. The changes in the plasma protein obtained is statistically not significant ($p > 0.05$), which may also be as a result of handling stress. The values of the plasma protein in the treated and control fish are represented in Table 4. Treated fish showed low values of plasma protein levels than the control. Changes in the protein levels are statistically significant ($p < 0.05$) in both concentrations after 96 h of exposure.

DISCUSSION

The content of glucose in the blood of cultural fishes depends chiefly on the kind of ingested carbohydrate feed (Chavin and Young, 1970). Hypoglycaemia may accompany intensive physical effort (Driedac and Kiceniuk, 1976). The present study revealed, that in the cause of the exposure of the experimental fish to paraquat, there was a significantly decreased plasma glucose levels compared to the controls. This decrease in plasma glucose (hypoglycaemia) situation was supported by Das *et al.* (2004) when *Catla* fingerlings were exposed to sublethal concentration of nitrite and Kori-Siakpere (1998) in *Clarias gariepinus* exposed to sublethal concentration of petroleum.

Triglycerides are known to be lipolytically broken down to glyceride and fatty acid. During exposure periods, the role of glyceride as glucose precursors becomes more important. The muscle metabolism changes as well. The muscles stop using glucose and restrict their ketone utilization to necessary energy being supplied via oxidation of fatty acid. The decrease in plasma triglyceride levels observed allows to pressure that the lipolysis proceeding during exposure period was the major source of energy (Simeno *et al.*, 1981; Herming and Palezny, 1987; Friedrich and Stepanowska, 2001).

Cholesterol content in the blood is linked to lipid metabolism and depends on the calorific value of the food. As a consequence of action of negative environmental factors (stress and pollution) the same fish species exhibited hypercholesterolemia (Perrier *et al.*, 1972). The present study demonstrates that the exposure of fish to paraquat after 96 h caused statistically significant increase in the level of cholesterol; which is in agreement with the results of Klyszejko and Lyczywek (1999).

Plasma protein level in the blood is linked to the kind of feed, experimental exposure (Szeroro *et al.*, 1996) and also to the health status of the fish (Wedemeyer and Mc Leay, 1981). It is obvious that exposure of fish for a long time to most toxicants including paraquat interferes with protein metabolism. The present study supports the observations by Das *et al.* (2004) and Das and Mukheyee (2000) in this regard, who opined that such an interference results in the depletion of total protein in the plasma of fish when exposed to paraquat. This toxicant lead to a considerable loss of blood protein by renal excretion further augmenting its depletion in the blood (Sastry and Sharma, 1981).

CONCLUSION

Paraquat a commonly used herbicide by the agricultural sector at sublethal concentrations can reduce the plasma protein, glucose and triglyceride with elevated levels of cholesterol of fish exposed to it for a long period of time. However, it is important to evaluate the residual effects of this herbicide in different body tissues of fish as they are ultimately consumed by man.

Nevertheless, it is obvious that the herbicide has deleterious effects on fish as observed through the present study.

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