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Sublethal Effects of Paraquat on Plasma Glucose Levels and Glycogen Reserves in Liver and Muscle Tissues of African Catfish (*Clarias gariepinus*) under Laboratory Condition

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Abstract: The effects of sublethal concentrations of paraquat (0.30, 0.15 and 0.00 mg L^{-1}) on the plasma glucose level and glycogen reserves in the liver and muscle tissues of *Clarias gariepinus* were investigated using static bioassays with continuous aeration for eight (8) weeks. The glycogen reserves in the liver and muscle tissues decreased significantly ($p<0.05$), the decrease being directly proportional to increase in paraquat concentrations and exposure period. On the other hand, the plasma glucose increases significantly ($p<0.05$), the increase was also directly proportional to the increase in concentrations of and exposure period.

Key words: Paraquat, plasma glucose, glycogen, muscle, liver, *Clarias gariepinus*, Nigeria

INTRODUCTION

In aquaculture, organophosphates are widely used to control a variety of agricultural pests as well as ectoparasites in fish. Fish accumulates these xenobiotic compounds through their gills (Das and Mukherjee, 2000). Many toxic substances in use today as control against pests have been known to have accumulative adverse effects on non-target organisms as reported by Omoregie *et al*. (1990). Pesticides in aquatic ecosystem may cause reduced productivity and biological activity (Awachie, 1980).

It has been reported that fish are extremely sensitive to aquatic pollution and shows severe physiological changes when exposed to sublethal concentrations of toxicants. According to Clarke *et al*. (1979) there is limited knowledge of the effects of daily exposure of fish to sublethal doses of pesticides, fertilizer and therapeutants. Both Chinabut *et al*. (1987) and Ufodike and Omoregie (1991) reported reduced growths in *Cyprinus Carpio* and *Oreochronis niloticus* when exposed to sublethal concentrations of Dipterex (Trichlorofon) and Gammalin 20 and Actelli 25EC.

The use of haematogical studies in fisheries is growing in importance for toxicological research, environmental monitoring and fish health conditions. According to Das and Mukherjee (2000) and Ghosh (1989) reported an increase in blood glucose of *Labeo rohita* and *Clarias batrachus* when exposed to sublethal concentrations of Quinalphos and Organophosphates. Cicik and Engin (2005) reported significant decrease in the levels of glycogen reserves in the liver and muscle tissues of *Cyprinus carpio* when exposed to sublethal concentrations of cadmium.

On the other hand, because glycogen reserves in the liver and muscle tissues of fish under stress are used as an emergency energy supply, changes in the glycogen levels in these tissues could indicate the health status of fish population. The sensitive characters of these parameters (plasma glucose and glycogen reserves of liver and muscle tissues) to detect the presence of toxicants is the reason why they are chosen as variables in this toxicological study, exposing the fish to sublethal concentrations of paraquat.

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Paraquat, a diquarternary nitrogen compound is an herbicide used to control weeds on farms in the tropic. Because of its common use on farms in the tropic, a good portion may be washed into streams and ponds with potential deleterious effects on aquatic biota. The literature on the effects of paraquat on catfish, biochemical, histopathological and haematological parameters are scanty. Thus, it is necessary to study the deleterious effects of paraquat on the histopathological as well as haematological parameters of this important species (*Clarias gariepinus*).

Life span, condition factor, reproduction and health are all functions of metabolic events in fish exposed to pesticides. This study aimed to determine the effects of sublethal concentrations of paraquat on the levels of glucose in the plasma and glycogen reserves in the liver and muscles of *Clarias gariepinus* in a static bioassay with continuous aeration.

MATERIALS AND METHODS

The fish (*Clarias gariepinus*) mean weight and length 102.42±0.12 g and 28.83±0.32 cm were purchased from a fish farm at Sapele, Delta State of Nigeria. They were transferred in plastic container to the Zoology Research Laboratory; Delta State University, Abraka Nigeria. They were then acclimatized to laboratory condition for 14 days prior to exposure. Mortalities were less than 1% during acclimation period. Eight fish were stocked in each test aquarium with dechlorinated municipal aerated tapwater. Feeding was done twice daily (0800 and 1600 h) with pelleted fish feed (40% protein, Copens 3 mm). The exposure period was eight weeks during which some water parameters (temperature, total alkalinity, pH, dissolved oxygen, free carbon (IV) oxide and unionized ammonium) were monitored according to APHA /AWWA/WPCF (1980).

Paraeforce(e), a solution of paraquat dichloride containing 200 g paraquat per litre was purchased from an Agrochemical store in the market at Warri, Delta State of Nigeria. The following sublethal concentrations (0.30, 0.15 and 0.00 mg L^{-1}) were obtained after 96 h toxicity test. These concentrations were delivered into three aquaria in triplicates.

Two fish from each aquarium were sacrificed forthnightly and analysed for plasma glucose and glycogen reserves in liver and muscle tissues. Blood for plasma glucose analysis 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 2 h for analyses. The blood plasma obtained was stored under refrigeration at -4°C. Plasma glucose level (GOD/PAP method with Randox Laboratories UK).The mean values of the two fish taken from each test aquarium were recorded. The tissue glycogen levels in the muscles and liver were analysed using anthrone technique (Wedemeyer and Yasutake, 1977; Plummer, 1971).

To determine the glycogen reserve in the liver and muscle, the tissues were first wet weighted and placed into centrifuged tubes containing 3 mL of KOH solution (30%). The centrifuge tubes were kept in a hot water bath for 20 min. Then 0.5 mL of saturated Na_2SO_4 and 3 mL of ethyl alcohol (95%) were added, followed by boiling for a further 15 min. After being cooled all samples were centrifuged at 3500 rpm and the supernatants were discarded. The precipitation in the tube were dissolved in 2 mL of distilled water followed by addition of 2.5 mL of ethyl alcohol (95%). The tubes were then centrifuged at 3500 rpm for a further 10 min and the supernatants were discarded. The final precipitations in the tubes free of lipid and protein were then dissolved in 2 mL of HCL (5 m) and neutralizd with 0.5 m followed by dilution to 50 mL with distilled water before analysis (Wedemeyer and Yasutake, 1977). The glycogen levels in the samples were determined by the anthrone method (Plummer, 1971).

All data were treated with two-way Analysis of Variance (ANOVA) at 5% probability level to test for significant difference between treatment means.

RESULTS AND DISCUSSION

There were no significant differences ($p>0.05$) in water quality parameters monitored as shown in Table 1. However, there exist significant increase ($p<0.05$) in plasma glucose of fish (*Clarias gariepinus*) exposed to sublethal concentrations of paraquat (Fig. 1a), the increase being proportional

Table 1: Mean water quality values obtained during exposure of *Clarias gariepinus* to sublethal concentrations of Paraquat for eight weeks

	Paraquat concentration (mg)		
Parameters	0.30	0.15	0.00 (control)
Temperature (°C)	25.12 (0.03)	25.12 (0.03)	25.12 (0.03)
Dissolved oxygen (mg L^{-1})	6.84 (0.01)	6.84 (0.02)	6.84 (0.02)
Free carbon (IV) oxide (mg L^{-1})	4.32 (0.01)	4.32 (0.02)	4.32 (0.02)
Total alkalinity (mg L^{-1})	14.22 (0.01)	14.21 (0.01)	14.21 (0.02)
pH	6.86 (0.01)	6.90 (0.01)	6.91 (0.01)
NH_3 (Unionized)	0.03 (0.02)	0.03 (0.01)	0.02 (0.01)

Standard error in parentheses

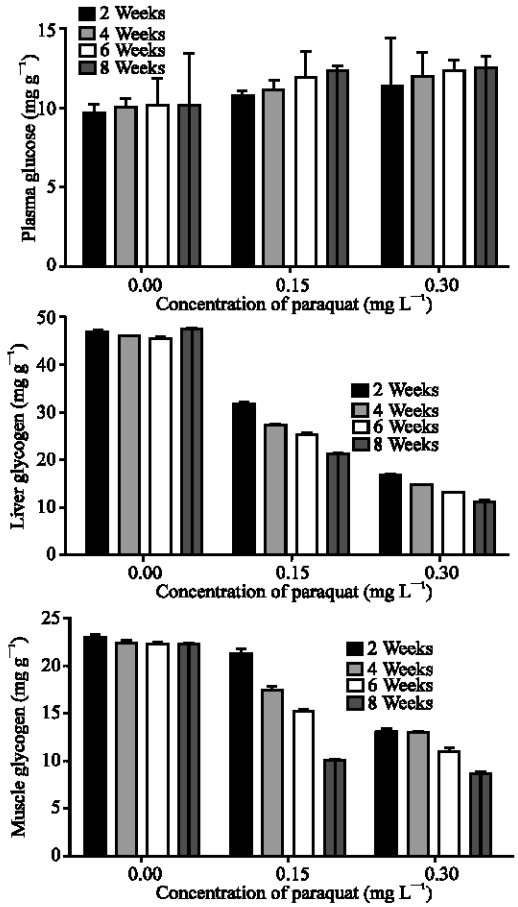

Fig. 1: Changes in the mean values of plasma glucose (a) liver glycogen (b); muscle glycogen and (c) *Clarias gariepinus* exposed to various sublethal concentrations of paraquat over a period of 8 weeks

to the toxicant concentrations and exposure period. The slight but not significant ($p>0.05$) changes observed in the plasma glucose of the control is attributed to stress during blood sampling which is reported by Barnhart (1969) can lead to slight variation in blood chemistry. The glycogen reserves in the liver and muscle tissues decreased significantly ($p<0.05$) when compared with those of the control fish (Fig. 1b and 1c), the decrease was also observed to be directly proportional to increase in paraquat concentrations and the exposure period.

The increase in plasma glucose noticed in the present study could be attributed to difference in respiration and activity as pointed out by Das and Mukherjee (2000). The progressive accumulation of Plasma Glucose reported in this investigation revealed that *Clarias gariepinus* exposed to sublethal concentrations of paraquat became hyperglycaemic. Omoregie *et al*. (1990) for stressed that tilapia showed marked hyperglycaemic response to stressed environment conditions as a result of incomplete metabolism of the blood sugar due to impaired osmoregulation. Increased blood glucose concentration results from an imbalance between the hepatic output of glucose and the peripheral uptake of the sugar (Coles, 1980). In the present study, exposure to different concentrations of paraquat caused an increase in the plasma Glucose level leading to lethargy. The decrease levels of liver and muscle glycogen reserves of *Clarias gariepinus* in this study may be as a result of paraquat stimulating the activities of enzymes that work in glycogenolysis as supported by Cicik and Engin (2005).

The dose-department liver and muscle glycogen reserve depletion observed in this investigation may be due to inefficient absorption of soluble glucose from the intestine as a result of the swallowing paraquat in the test medium. It has been demonstrated that liver glycogen levels decreased in *Oncorhynchus mylass* as a result of the activation of glycolytic enzymes via catecholamines under lack of food and hypoxic conditions (Vijayan and Moon, 1992). Heath (1987) reported that catecholamines may deplete glycogen reserves in stressed fish by stimulating glycogenolysis and gluconeogenesis.

Paraquat, a commonly used herbicide in the agricultural sector at sublethal concentrations results in an increase of plasma glucose and decrease in glycogen reserves of the liver and muscle tissues of *Clarias gariepinus*. Nevertheless, it is obvious that the herbicide has deleterious effects on *Clarias gariepinus* as observed through the present study.

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