

Effect of Paraquat Dichloride on Some Metabolic and Enzyme Parameters of *Clarias gariepinus*

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Abstract: The effects of paraquat dichloride on metabolic parameters (creatinine, albumin, total bilirubin, total urea and total protein) in the tissue (muscle and gill) of *Clarias gariepinus* were determined in a toxicity test. Juvenile *C. gariepinus* were exposed to varying sub-lethal concentrations of paraquat dichloride (0.05, 0.10, 0.20, 0.30 and 0.40 ppm) for four days. The results indicated a significant ($p < 0.05$) decrease in total protein and urea with increased concentration of the toxicant. The lowest value for total protein was recorded in the muscle (52.7 ± 0.02 SD) at 0.05 ppm compared to the control (55.0 ± 0.00 SD). The gill recorded the lowest value of urea (2.33 ± 0.05 SD) at 0.40 ppm compared to the control (2.37 ± 0.10 SD). The fluctuations were not significant ($p > 0.05$). Plasma AST values decreased as the concentration of the toxicant increased, (1.92 ± 5.20 μL SD being control value against 0.93 ± 3.55 μL SD at 0.40 ppm of toxicant). However, exposure to the herbicide did not alter the total Bilirubin. This tends to suggest that the liver may not have been affected by the toxicant. In all, the results indicate that the decrease in metabolite values in the muscle and gill of *Clarias gariepinus* could be due to stress caused by the herbicide.

Key words: *Clarias gariepinus*, gill, metabolic parameters, muscle, paraquat dichloride

INTRODUCTION

Paraquat dichloride, a herbicidal pesticide, is one of the most widely used herbicides in the world. Paraquat is quick-acting and non selective, killing green plant tissue on contact. It is also toxic to human beings when swallowed.

Paraquat applied to water for aquatic weed control purposes quickly disappears due to uptake by weeds and absorption by soil, silt and particulate suspended matter. The rate of disappearance is very variable, depending on the movement of water and the presence of mud or suspended matter. However, treatments within the range of 1 mg/L to 4 mg/L in water have resulted in only 0.1 mg/L or less of paraquat being detectable in water from 6 to 14 days after application. Decomposition of the killed weed is rapid, any remaining residue of paraquat thus is liberated and subsequently deposited on the bottom mud (Calderbank, 1968). Herbicides can be toxic to a wide variety of organisms. Studies of acute toxicity shows that birds exposed to herbicide experienced diminished reproductive ability in the form of cracked eggs. Also, herbicides in the soil inhibit the growth of mycorrhizal fungi, which grows with the root of many plants and aid in nutrient uptake, by reducing the number of fungal spores produced (Mackinnon and Freedman, 1993). The heavy treatment of soil with pesticides causes populations

of beneficial soil micro-organisms to decline, causing the soil to degrade. These pesticides are subsequently washed into the aquatic environment through run-offs and leaching. On entering the aquatic environment, they negatively affect the aquatic flora and fauna. According to the united states Environmental protection Agency (USEPA, 2007), herbicides contain an active ingredient which is highly toxic to both cold and warm water fishes and shown in a series of tests to cause vertebral deformities in fishes, and sub-lethal effects such as erratic swimming and bad breathing which increase the fishes changes of being eaten by predators (Liong *et al.*, 1988; McBride *et al.*, 1989). Kori-siakpere *et al.* (2007) reported that 96 h of exposure of *Clarias gariepinus* to paraquat dichloride revealed low values of plasma glucose, triglyceride and plasma protein levels compared to the control. Toxicant leads to a considerable loss of blood protein by renal excretion further augmenting its depletion in the blood (Sastry and Sharma, 1981). The genus *Clarias* are unique in their ability to survive a wide variety of environmental extremes (Akiri, 1987). They have highly efficient air-breathing organs which allow them to survive in oxygen depleted water (Jordan, 1978), and migrate for long distances of up to 1.2 km (Covertenary and Bruton, 1979). *Clarias species* are able to survive salinity increase of up to 25‰ (Chervinski, 1982), excessive crowding and rigours of high transport stress

(Inyang, 2008). *Clarias* species have high adaptability, fast growth rate in the natural and cultured environments and has proved to be successful aquaculture species (Reed and Bardach, 1979). It is difficult to assess the level of toxicity of pesticides on organisms in the aquatic environment because of the complex nature of aquatic ecosystems. Thus, toxicological methods have been seen as an adequate evaluation technique, of the effects of toxicants on aquatic populations (Rand and Petrocelli, 1985). The aim of this research is to evaluate the sub-lethal effects of paraquat dichloride on *Clarias gariepinus* by examining the alterations of metabolic parameters such as creatinine, total protein, albumin and urea in serum and tissues of organs (gills and muscles).

MATERIALS AND METHODS

Thirty eight juvenile African catfish *Clarias gariepinus* (mean weight 149.207 ± 1.437 g SD; mean length 26.25 ± 2.1 cm SD) were obtained from a fish farm at Aquaculture Research Center, Aluu, Port Harcourt, Rivers State. They were transported in 50 L trough whose mouth was covered with a net, by car to the Department of Biological sciences laboratory, Niger Delta University, Amassoma Bayelsa State, where the assays were conducted between August and September, 2009. On arrival the fish were acclimated individually in circular aquaria for seven days. The top of the aquaria were covered with fishing nets to prevent escape of the fish. The fishing net had a slit at the middle for clearing the aquaria. The water was changed and the aquarium washed with a piece of foam at 10-11 h daily, and also the fish were fed at the same time with their feed, once a day. A trial test was carried out using the toxicant paraquat dichloride. Five concentrations of paraquat were prepared from the original solution (350 g/L) using the formula described by Grinshaw (1978). The concentrations of the trial test were prepared by pipetting 0.01, 0.02, 0.03, 0.03, 0.04, 0.05, 0.05, 0.06 mL of the original concentration of paraquat into 30 L of water in the aquaria to make 0.05, 0.1, 0.2, 0.3 and 0.4 ppm solution. The test solution was renewed daily immediately after washing and the fishes were fed once daily as in the acclimation period of seven days. The purpose of the trial test was to determine the range of concentration to be used for the definitive test.

Sub-ethal concentrations for the definitive test were prepared based on the range finding test. The concentration was the same as the trial test. The test solution was renewed after washing the aquaria.

The physicochemical characterization of the water used for fish bioassay was carried out using standard methods (APHA, 1998) and the following values were obtained: Temperature 26°C, pH 6.20-6.37, Dissolved oxygen 5.38-7.21 mg/L, alkalinity 15.25-17.09 mg/L, conductivity 99.50-136.12 $\mu\text{s}/\text{cm}$ and turbidity 0.42-0.50

NTU (Inyang *et al.*, 2010). Three fishes were exposed to each concentration of paraquat dichloride. The water was filled to 25 L point before the introduction of the toxicant, after which the water was made up to the 30 L mark.

At the end of the experiment, the fishes were dissected and the gills and muscles separated. Then 0.5 g each of the organs were macerated with pestle and mortar. To each of the macerated samples, 5 mL of percloric acid was added and centrifuged at the rate of 3000 rpm for 10 min. The supernatants were removed and stored in plain bottles at -20°C for analysis.

The samples were analysed for the metabolites: Creatinine, Albumin, Total Bilirubin, Total urea and Total protein in conformity with the standard methods as described by APHA (1998). Alkaline and aspartate aminotransferase (ALT&AST) and acid phosphatase (ACP) measurements were done according to Zimmerman (1978). Various statistical tools such as analysis of variance and f-test were used where appropriate for data analysis (Wahua, 1999).

RESULTS

Generally, there were decreases in all values of the metabolites in the muscles and gills except total bilirubin (Table 1, 2). For total protein, the lowest value was recorded in the muscle (54.7 ± 0.20 SD) compared to the gill (62.9 ± 0.20 SD) at the highest concentration of the toxicant (0.4 ppm) each. The muscle recorded the lowest value of albumin (27.3 ± 0.00 SD) compared to the gill (30.0 ± 0.20 SD) at 0.3 ppm of the toxicant each. The lowest value of urea (2.33 ± 0.05 SD) was recorded in the gill at 0.4 ppm concentration of paraquat. For creatinine, the lowest value was recorded in the muscle (0.4 ± 0.60 SD) at 0.4 ppm concentration of paraquat, while the highest value of creatinine for both muscle and gill tissues (0.6 ± 0.00 SD) and (0.97 ± 0.05 SD), respectively, were recorded at 0.1 ppm concentration of paraquat.

Again, all the metabolites (total protein, albumin, urea, creatinine and total bilirubin), recorded higher values in the gill than the muscle at the various concentrations of paraquat (0.00, 0.05, 0.10, 0.20, 0.30, 0.40 ppm). However, total bilirubin did not record any observable change in both organs (muscle and gill).

Plasma AST values decreased as the concentration of the toxicant increased, Table 3. 0.93 ± 3.55 μL SD was recorded at 0.40 ppm compared to control that had 1.92 ± 5.20 μL SD. The lowest inhibition was recorded at 0.05 ppm (1.26 ± 0.50 μL SD). Liver ALT recorded slight elevation in values except at 0.50 ppm (0.85 ± 0.96 μL S.D) (Table 4). Slight increase in values was recorded in liver AST except at 0.49 ppm. Liver ALP values showed slight decrease as the concentration of the toxicant except at 0.10 ppm.

Table 1: Metabolites in gill

Conc. of Paraquat (PPM)	Total (g/L) Protein	Albumin (g/L)	Urea ($\mu\text{mol/L}$)	Creatinine ($\mu\text{mol/L}$)	Total Bilirubin ($\mu\text{mol/L}$)
0.00	68.00 \pm 0.00 ^a	380.00 \pm 0.60 ^a	2.73 \pm 0.10 ^c	0.73 \pm 0.10 ^c	0.001 \pm 0.03 ^a
0.05	66.00 \pm 0.00 ^a	37.30 \pm 0.20 ^a	3.10 \pm 0.00 ^a	0.60 \pm 0.00 ^d	0.001 \pm 0.03 ^a
0.10	68.00 \pm 0.60 ^a	37.30 \pm 0.20 ^a	3.13 \pm 0.05 ^a	0.97 \pm 0.05 ^a	0.001 \pm 0.03 ^a
0.20	63.30 \pm 0.50 ^b	34.00 \pm 0.00 ^b	2.93 \pm 0.05 ^b	0.35 \pm 0.69 ^e	0.001 \pm 0.03 ^a
0.30	59.40 \pm 0.30 ^{bc}	30.70 \pm 0.20 ^c	2.68 \pm 0.05 ^c	0.77 \pm 0.05 ^{bc}	0.001 \pm 0.03 ^a
0.40	62.90 \pm 0.20 ^b	32.00 \pm 0.00 ^c	2.33 \pm 0.00 ^d	0.80 \pm 0.00 ^b	0.001 \pm 0.03 ^a

Within column, mean \pm SD with different superscripts are significantly different at ($p < 0.05$)

Table 2: Metabolites in muscle

Conc. of Paraquat (PPM)	Total (g/L) Protein	Albumin (g/L)	Urea ($\mu\text{mol/L}$)	Creatinine ($\mu\text{mol/L}$)	Total Bilirubin ($\mu\text{mol/L}$)
0.00	55.00 \pm 0.00 ^b	27.00 \pm 0.00 ^c	2.80 \pm 0.00 ^b	0.57 \pm 0.05 ^b	0.001 \pm 0.03 ^a
0.05	52.70 \pm 0.20 ^c	30.30 \pm 0.20 ^a	2.70 \pm 0.00 ^b	0.30 \pm 0.26 ^d	0.001 \pm 0.03 ^a
0.10	59.0 \pm 0.00 ^a	31.00 \pm 0.60 ^a	3.23 \pm 0.05 ^a	0.60 \pm 0.00 ^a	0.001 \pm 0.03 ^a
0.20	61.00 \pm 0.00 ^a	27.70 \pm 0.20 ^b	2.60 \pm 0.24 ^{bc}	0.63 \pm 0.00 ^a	0.001 \pm 0.03 ^a
0.30	57.00 \pm 0.00 ^{ab}	27.00 \pm 0.00 ^c	2.50 \pm 0.00 ^c	0.47 \pm 0.05 ^c	0.001 \pm 0.03 ^a
0.40	54.70 \pm 0.20 ^b	28.3 \pm 0.20 ^b	2.70 \pm 0.20 ^b	0.04 \pm 0.60 ^e	0.001 \pm 0.03 ^a

Within column, mean \pm SD with different superscripts are significantly different at ($p < 0.05$)

Table 3: ALT, AST, ACP in plasma of *Clarias gariepinus* exposed to paraquat dichloride for 96 h (Mean \pm SD)

Conc. of Paraquat (ppm)	ALT (μL)	AST (μL)	ACP (μL)
0.00	0.73 \pm 9.80 ^b	1.92 \pm 5.20 ^a	8.00 \pm 5.00 ^a
0.10	1.05 \pm 2.50 ^a	1.26 \pm 0.50 ^{bc}	3.53 \pm 4.44 ^d
0.20	0.79 \pm 1.26 ^b	1.30 \pm 0.91 ^b	5.37 \pm 5.76 ^b
0.30	0.69 \pm 2.01 ^{bc}	1.59 \pm 2.05 ^b	3.20 \pm 6.69 ^d
0.40	0.62 \pm 6.50 ^c	1.70 \pm 0.02 ^a	4.65 \pm 0.02 ^c
0.50	0.71 \pm 5.50 ^c	0.93 \pm 3.55 ^c	4.20 \pm 0.63 ^c

Within column, mean \pm SD with different superscripts are significantly different at ($p < 0.05$)

Table 4: ALT, AST and ALP in liner of *Clarias gariepinus* exposed to paraquat dichloride for 20 h (Means \pm S.D)

Conc. of Paraquat (ppm)	ALT (μL)	AST (μL)	ACP (μL)
0.00	0.85 \pm 0.96 ^d	0.88 \pm 0.38 ^{bc}	5.50 \pm 0.14 ^a
0.10	1.30 \pm 0.25 ^b	0.92 \pm 0.06 ^b	6.00 \pm 1.99 ^a
0.20	2.75 \pm 5.05 ^a	1.01 \pm 0.12 ^a	5.03 \pm 1.72 ^b
0.30	1.20 \pm 0.16 ^b	1.04 \pm 6.51 ^a	4.90 \pm 1.93 ^c
0.40	1.02 \pm 1.58 ^c	0.80 \pm 0.82 ^c	5.17 \pm 1.31 ^b
0.50	0.58 \pm 0.35 ^e	1.01 \pm 0.46 ^a	5.01 \pm 0.14 ^b

Within column, mean \pm SD with different superscripts are significantly different at ($p < 0.05$)

DISCUSSION

There were no significant ($p > 0.05$) variations in the mean values of the water quality parameters throughout the study period. The values recorded were comparable with the control values, except a slight fall in pH and dissolved oxygen at 0.30 ppm concentration of paraquat. This result is in consonance with the work of Onwusiruka and Ufodike (1994) that exposed *Clarias gariepinus* to Akee apple and sausage extracts and reported minimal variation in the values of the water quality parameters. The values recorded in this study have been reported to be within the tolerance ranges of warm water fish species. Therefore, the water quality parameters may not have caused the observed changes in the metabolites of *Clarias gariepinus* in this study. Total protein, urea, and albumin in the gills decreased with increased concentration of paraquat dichloride. However, creatinine in gills did not vary significantly ($p > 0.05$). Inyang (2008) reported similar results in plasma total protein, albumin, glucose

and organ's total urea and creatinine of *Clarias gariepinus* exposed to diazinon. Similarly, Sastry *et al.* (1982) and Das and Mukharjee (2000), observed that exposure of fish for a long time to most toxicants including pesticides interferes with protein metabolism, depletion of total protein in the plasma and serum of fish. The decrease in total protein and albumin levels may be due to impaired synthesis of protein or enhanced loss of protein via excretion and is also suggestive of some problem in the kidney (Jee *et al.*, 2005). Similarly, the decrease in creatinine levels in fish exposed to paraquat dichloride compared to the control suggest that creatinine was completely used up by the muscle as a result of the stress induced by the toxicant. However, the very low levels of total bilirubin recorded in both organs in this study suggest that the liver may not have been affected by the toxicant. On the other hand, the general decrease in the levels of the metabolic parameters of *Clarias gariepinus* in the cause of acute exposure to paraquat dichloride was in conformity with

the reports by Kori-Siakpere (1998) when *Clarias gariepinus* was exposed to sub-lethal concentrations of petroleum, and Das *et al.* (2004) when catta fingerlings were exposed to sub-lethal concentration of nitrite.

It is important to note, however that the low concentration of paraquat dichloride that induced the observed abnormal changes in *Clarias gariepinus* metabolism and enzymes obviously showed the level of toxicity of paraquat dichloride on organism.

The liver contains numerous enzymes, some of which are also present in the plasma in very low concentration. These enzymes have no known function in plasma other than to provide information about hepatic state and disorders. Liver AST, ALT and ACP receded in some concentration, evidence that there might be problem in the livers. Similar result was also obtained by Zimmerman (1978) when he exposed *C. carpio* to carbon tetrachloride. The author reasoned that these could be used as a measure of the extent of hepatic damage. ALT and AST slightly decreased in some treatment levels in plasma, hence the inhibition is not dose dependent.

In conclusion, the levels of the metabolites, protein and creatinine, as well the activities of the enzymes AST, ALT and ACP in the muscle and plasma of *Clarias gariepinus* respectively, could be good bio-indicators of the effect of paraquat dichloride and possibly also, of other stressors in the aquatic environment.

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