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Biochemical Changes In Plasma and Liver of *Clarias Gariepinus* Exposed To Paraquat

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Abstract: *Effect of sublethal concentrations of paraquat (2.00, 4.00, 6.00 and 8.00mg/L) and a control were examined in the African catfish Clarias gariepinus in the laboratory. The enzymes, aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) and electrolytes, sodium (Na^+), potassium (K^+) and chloride (Cl^-) were examined in the plasma and the liver of the fish. Results showed that AST and ALP increased significantly (P>0.05), while ALT either decreased or increased significantly (P>0.05) in the plasma. In the liver, AST activity either decreased or increased significantly (P>0.05) while that of ALP showed decreased activity only at 2.00mg/L concentration. ALT only showed a significant (P>0.05) decrease at 6.00mg/L concentration. Plasma Na^+ increased significantly (P>0.05) in levels in all the exposure concentrations, while K^+ only increased in the middle exposure concentrations (4.00 and 6.000mg/L). Cl^- decreased significantly (P.0.05) in all the exposure concentrations. In the liver, there were marked significant (P>0.05) decrease in all the ions (Na^+, K^+ and Cl^-) in all the test concentrations except K^+ ion at 2.00mg/L. The result showed that paraquat induced serious biochemical changes in the catfish Clarias gariepinus after sublethal exposure which could be deleterious to the fish.*
Key words: *Plasma, liver, Clarias gariepinus, environment*

I. Introduction

In the dry season, pesticides are known to cause serious environmental problems because during this period, the dilution capacity of the water system is low and thus increasing the risk of high concentrations of toxic chemicals (Adedeji, 2010). Increasing population, industrialization, urbanization and agricultural activities have become sources of water pollution and major concern to humanity (Yousafzai and Shakoori, 2011). Most of these chemicals through diffuse sources such as runoffs, leaching, spray drift and discharge of waste contaminate the water environment (Singh *et al.*, 2010). Pesticides include a wide variety of chemicals with great difference in their mode of action, uptake by the body, metabolism and elimination from the body and toxicity to target and non target organisms. Poisoning risk of pesticide depends largely on dose, toxicity, duration of exposure and sensitivity of the organism to the toxicant (Singh *et al*., 2010).

Many aquatic organisms are known to concentrate or accumulate toxic solutes from their habitat without any obvious damage to themselves and therefore act as toxicant amplifiers, making the toxicants available to predators at dangerously high levels. Several cases of the adverse effects of environmental pollution on fish and its consumers have been reported (Yuan *et al*., 2004; Dix, 1981; Karthikeyan *et al*., 2006; Iweala and Okeke, 2005). More commonly, aquatic organism are subjected to long- term stresses from exposure to sublethal concentrations. However, in the long run, these sublethal concentrations may also prove to have negative effect on animals as do lethal concentrations (Yuan *et al*., 2004). Sublethal concentrations alters the bahaviour (Gabriel and Edori, 2010), feeding habits, school group positions, reproductive rates (Murty, 1986), glucose and glycogen levels (Kori-Siakpere *et al* ., 2007) and reduction in body weight (Gabriel *et al*., 2009) of organisms in general.

Paraquat, a nitrogen based compound used as herbicide in the control weeds on farms was first synthesized in 1882. The pesticidal property was discovered much later in 1959 . It is in use the world over and is found to cause severe, acute and chronic poisoning when water borne (Harley, 1979). It readily dissolves and dissociates in water or aqueous solutions (Amdur *et al*., 1991). It is one of the most widely used herbicide globally and comes next to glyphosate. It is sold in over 130 countries for use on large and small farms, plantations and estates and in non weed control. It is a quick acting, non selective herbicide, which destroys green plant tissue on contact and by translocation within the plant (Zeneca,1996). It also causes liver damage and renal failure as the kidney tries to remove absorbed paraquat (Dial and Dial, 1995).

Biochemicals are the assessable body contents for checking the toxicity of any chemicals (Singh *et al*, 2010) and the results of such biochemical parameters results in serious outcome in the form of various diseases in fishes/ animals and also reveals underlying physiological conditions of the organs/ tissues of organisms (Obomanu et al, 2009).

The fish *Clarias gariepinus* is selected for this study because of its availability and its commercial importance. The aim of this study is to investigate the effect of paraquat on plasma and liver biochemistry with particular reference to enzymes and electrolytes in *Clarias gariepinus*.

II. Materials And Methods

Clarias gariepinus (220.50 ± 15.33g mean weight and 30.43 ± 1.22cm mean total length) were bought from a private farm in Port Harcourt close to the University of Port Harcourt in Rivers State Nigeria. They were transported to the Chemistry Department Laboratory of the Ignatius Ajuru University of Education, Port Harcourt. The fishes were acclimated to laboratory conditions in individual tanks for seven days with bore hole water with 10 litre effective volume in a 30 litre plastic aquaria. The mouths of the aquaria were covered with 1mm nylon mesh to prevent escape of fish from the aquaria. The fish were fed with 31- 35% crude protein diet at 2% biomass during the acclimation and the experimental period.

Completely randomized design was used in the experiment with five treatment levels and four replicates. The test solutions were prepared from a stock of 20g/l solution and diluted to the desired concentrations (2.00, 4.00, 6.00 and 8.00mg/L) and a control. The choice of these concentrations were based on range finding test (trial test). Fishes were fed during the experimental period as was in the acclimation. The solutions for the assay were renewed daily to maintain the concentrations. At the end of the experimental period (21 days), blood was collected from the kidney by inserting 21G size needle perpendicularly behind the anal fin and blood flows freely into the needle immediately it touches the kidney. The blood samples were then transferred into 5ml heparin bottles for biochemical analysis. The fishes were killed by striking a blow on the head and dissected to remove the liver. 0.5g of the samples (liver) were macerated and mixed with 5ml of physiological saline for enzyme assay. Another 0.5g of the homogenate sample was mixed with de-ionized water and centrifuged at 3000 rpm for ten minutes. The supernatant was transferred into plain bottles and stored frozen ($-2^{O}C$).

The samples were taken to the laboratory for electrolytes and enzymatic analysis. Sodium ion (Na^{+}), potassium ion (K^{+}) and chloride ion (Cl^{-}) concentrations were determined using the automatic analyzer and optimal test by means of flame photometry as was described by Schales and Schales, (1941). Aspartate transaminase (AST) and alanine transaminase (ALT) were assayed by the method of Reitman and Frankel, (1957), while alkaline phosphatase (ALP) was assayed by the method of Babson et al., (1966). The concentrations of the various electrolytes were expressed as Meq/L, while that of the enzymes were expressed as IU/L. The data obtained were subjected to analysis of variance (ANOVA) to determine if significant differences existed between the means in the parameters at different levels of contamination. Where differences existed, Duncan's multiple range test (DMRT) was used to compare the difference between the means (Zar, 1984).

III. Results

Activities of AST in the plasma were all raised above the control value. The increase in activity of AST was not concentration dependent, with the highest value obtained at 4.00mg/L (143.34 ± 6.99IU/L) as against the control value (43.13 ± 4.43IU/L). The activities of ALT were either lower or higher than the control value. The same value was observed at 2.00 and 6.00mg/L, which was 10.00 ± 0.00IU/L, while the values observed at 4.00 and 8.00mg/L were 25.00 ± 3.31 and 20.63 ± 1.63IU/L respectively as against the control value of 12.50 ± 1.25IU/L. The activity of ALP also showed a non concentration dependent increase with the highest value obtained at 4.00mg/L (71.67 ± 3.61IU/L), followed by the value at 6.00mg/L (45.00 ± 4.02 IU/L) as against the control value of 19.63 ± 3.71 IU/L (Table 1). In the liver, AST increased only at 2.00mg/L (222.50 ± 0.00IU/L). Lower values were observed at 4.00mg/L (200.84 ± 4.69IU/L) and 6.00mg/L (164.38 ± 4.60IU/L) as against the control value and that observed at 8.00mg/L (206.25 ± 4.69IU/L). The activities of ALT in the control and 8.00mg/L was 15.00 ± 1.45IU/L. Slight increase in activity was observed at 2.00mg/L (18.13 ± 2.25IU/L) and 4.00mg/L (16.67 ±1.45IU/L). However, there was an observed decrease at 6.00mg/L (12.50 ± 1.25IU/L). The activities of ALP the liver decreased at 2.00mg/L (51.63 ± 2.64IU/L) as against the control value of 64.50 ± 4.63IU/L. The activities of ALP in all other concentrations were higher than the control value, with the highest at 8.00mg/L (262.50 ± 16.22IU/L), followed by that observed at 4.00mg/L and 6.00mg/L which were 94.17 ± 2.55 and 78.32 ± 3.60IU/L respectively (Table 2).

Plasma Na^{+} ion increased in value in all the concentrations of the test solution as against the control value (477.50 ± 10.15Meq/L). The highest value was observed at 6.00mg/L (593.13 ± 13.65Meq/L), followed by the value at 8.00mg/L (525.63 ± 15.37Meq/L). The levels of K^{+} ion were higher than the control value (22.44 ± 2.61Meq/L) at 4.00mg/L and 6.00mg/L which were 25.42 ± 2.06 and 27.88 ± 2.02Meq/L respectively. The values obtained at 2.00mg/L (16.00 ± 1.54Meq/L) and 8.00mg/L (21.00 ± 3.10Meq/L) were lower than the control value. The levels of Cl^{+} ions were lower than the control value in all the test concentrations. The least value was observed at 4.00mg/L (195.00 ± 5.37 Meq/L), which was followed by that observed at 8.00mg/L

which was 237.00 ± 9.45Meq/L (Table 3). Liver Na^+ ion decreased in all the exposure concentrations. The decrease was highest at 2.00mg/L (372.50 ± 8.88Meq/L) and least at 4.00mg/L (400.84 ± 9.34 Meq/L) as against the control value of 413.13 ± 6.51Meq/L. The values of K^+ ion in the liver increased slightly only at 2.00mg/L (18.00 ± 2.37 Meq/L), while decreased levels were observed in all other concentrations with the highest decrease at 6.00mg/L (14.25 ± 1.25Meq/L) as against the control value of 16.32 ± 1.17Meq/L. The value of Cl^- ions in the liver were all lower than the control value (175.00 ± 10.75 Meq/L). The least value of 60.84 ± 5.03Meq/L was observed at 4.00mg/L (Table 4).

Table 1. Plasma AST, ALT and ALP in Clarias gariepinus exposed to paraquat for 21days

Concentration of paraquat (mg/L)	AST(IU/L)	% of control	ALT(IU/L)	% of control	ALP(IU/L)	% of control
0.00	43.13±4.43^{cd}	100	12.50±1.25^{c}	100	19.63± 3.71^{e}	100
2.00	51.25 ±3.73^{c}	118.84	10.00±0.00^{d}	80.00	41.19± 4.34c	209.89
4.00	143.34±6.99^{a}	332.37	25.00±3.31^{a}	200.00	71.67 ± 3.61^{a}	365.17
6.00	56.28 ± 5.58^{c}	130.50	10.00±1.00^{cd}	80.00	45.00± 4.02^{b}	229.30
8.00	94.38 ± 5.37^{b}	218.84	20.63± 1.63^{b}	165.00	35.25 ±6.60^{d}	176.62

Means with the same alphabet in the same column are not significantly different (P>0.05)

Table 2. Liver AST, ALT and ALP in Clarias gariepinus exposed to paraquat for 21days .

Concentration of paraquat (mg/L)	AST(IU/L)	% of control	ALT(IU/L)	% of control	ALP(IU/L)	% of control
0.00	206.25 ± 4.69^{a}	100	15.00± 1.45^{ab}	100	64.50 ± 4.63^{d}	100
2.00	222.50 ± 0.00^{a}	107.88	18.13± 2.25^{a}	120.83	51.63 ± 2.64^{e}	80.04
4 .00	200.84± 4.69^{ab}	97.37	16.67 ± 1.45^{ab}	111.10	94.17 ± 2.55^{b}	145.99
6.00	164.38 ± 4.60^{b}	79.70	12.50 ± 1.25^{c}	83.33	78.32 ± 3.60^{c}	121.42
8.00	206.25 ± 4.69^{a}	100	15.00 ± 1.45^{ab}	100	262.50 ±16.22^{a}	406.98

Means with the same alphabet in the same column are not significantly different (P>0.05)

Table 3. Plasma Na^+, K^+ and Cl^- in Clarias gariepinus exposed to paraquat for 21days .

Concentration of paraquat (mg/L)	Na^+ (Meq/L)	% of control	K^+(Meq/L)	% of control	Cl^- (Meq/L)	% of control
0.00	477.50 ± 10.15c	100	22.44 ± 2.61b	100	329.38 ± 4.68^{a}	100
2.00	523.13 ± 20.00b	109.55	16.00 ± 1.54c	71.30	274.33 ± 6.14^{b}	83.30
4.00	495.84 ± 19.94c	103.84	25.42 ± 2.06ab	113.26	195.00 ± 5.37^{d}	59.20
6.00	593.13 ± 13.65a	124.21	27.88 ± 2.02a	124.22	275.00 ± 8.20^{b}	83.49
8.00	525.63 ± 15.37b	110.08	21.00 ± 3.10b	93.58	237.00 ± 9.45^{c}	72.11

Means with the same alphabet in the same column are not significantly different (P>0.05)

Table 4. Liver Na^+, K^+ and Cl^- in Clarias gariepinus exposed to paraquat for 21days .

Concentration of paraquat (mg/L)	Na^+ (Meq/L)	% of control	K^+ (Meq/L)	% of control	Cl^- (Meq/L)	% of control
0.00	413.13 ± 16.51^{a}	100	16.32 ± 1.17^{ab}	100	175.00± 10.75^{a}	100
2.00	372.50 ± 8.88^{b}	90.17	18.00 ± 2.37^{a}	110.33	79.38 ± 4.68^{c}	45.36
4.00	400.84± 9.34^{a}	97.03	16.09 ± 2.74^{ab}	98.59	60.84 ± 5.03^{c}	34.76
6.00	378.13 ± 13.97^{b}	91.53	14.25 ± 1.05^{b}	87.34	170.63 ± 6.67^{a}	97.50
8.00	388.75 ± 16.66^{b}	94.10	15.94 ± 3.75^{ab}	97.70	133.13 ± 9.80^{b}	76.07

Means with the same alphabet in the same column are not significantly different (P>0.05)

IV. Discussion

The major function of the liver is the detoxification of the onslaught of chemical substances that come in contact with organisms by metabolizing xenobiotics to non harmful substances. Therefore in the event of contact with xenobiotics or toxicants, the chemistry of the liver is altered so as to counter the effect of pollutants (Abdullaev *et al*., 1985; Obomanu *et al*., 2009; Yousafzai and Shakoori, 2011). Enzymes and electrolytes are part of liver function test which are reliable indicators of liver metabolism and wellness of organism under test (Oksama and Kristoffersson, 1980; Sreekala and Zutshi, 2010; Iweala and Okeke, 2005; Uboh *et al*., 2011).

The observed general increase in the plasma enzymes and the corresponding increase in the liver enzymes in this study is an indication of underlying liver injury in the fish. Elevation of plasma AST, ALT and ALP shows liver damage which may be hepatitis or necrosis of cells (Dame, 1980; Gabriel and George, 2005; Yousafzai and Shakoori, 2011). The cellular component of the liver which seems to be affected most is the cellular membrane. Toxicants have been observed to either have increased the cellular membrane permeability thereby enhancing enzyme leaching or leaking out of the liver to the blood or may have reduced the permeability, forcing the enzymes to accumulate in the cells (Gabriel *et al*., 2009; Yousafzai *et al*., 2011).
Alteration of enzymes is a direct measure of histopathological effects on the liver. In this study, since there was elevation of the enzymes both in the plasma and the liver, it implies that the liver of the fish has the ability to regenerate these enzymes in the face of paraquat assault. Increase in AST and ALT indicates active transamination, so as to maintain energy cycle (Adams *et al*., 1996). ALP in the cellular external membrane plays the major role in phosphate metabolism and it prevents the external membrane from being damaged (Dirrieu and Tran-Minh, 2002). Its increase may be due to phosphate ingestion by the fish. The increase in this enzyme implies overproduction of phosphate bound esters, which helps in the maintenance of cell integrity (Gabriel et al, 2009; Gabriel and George, 2005).

The regulation of ions (Na^+, K^+ and Cl^-) are essential in the maintenance of the rate of water influx and ion efflux in aquatic organism. Due to the inflow and outflow of ions, organisms in freshwater maintain an internal homeostatis. These ions whether in free form or bound play major roles roles in cellular metabolism (Karthikeyan et al., 2006; Oksama and Kristoffersson, 1980). The exposure of Clarias gariepinus altered the ions in both plasma and liver of the fish. Na^+ ion increased in the plasma and decreased in the liver, K^+ ion fluctuated in the plasma, but decreased in the liver, while Cl^- ions decreased both in the plasma and the liver.

The increase in plasma Na^+ and its decrease in the liver is an indication of injury in the liver, which may have leaked into the blood. The decrease in Na^+ ion concentration suggests a change in the permeability properties of the liver. The fish tried to maintain iso-osmotic condition in the intercellular organ by pumping out Na^+ into the blood (Karthikeyan et al., 2006; Gabriel et al, 2009). The decrease in Cl^- ions indicated a fall in osmolality of the liver and the plasma.

Na^+ Cl^- ions are principally responsible for the maintenance of osmotic pressure in the fish (Potts and Parry, 1964) and their decrease directly infers a decrease in the osmolality of the blood/ plasma. These ions (Na^+ and Cl^-) has been used often as index of osmoregulation and are found to react similarly in adverse environmental situation and therefore are useful indicators of stress in animals (Karthikeyan et al., 2006). According to Foskeft (1983), blood chlorine is known to be an important extracellular anion for the regulation of cation-anion balance and osmotic pressure and this conforms to the concomitant decrease in Cl^- ion along with Na^+ ion in this study. Decrease in Cl^- and Na^+ ion concentration may be due to inhibition of active ion transport (Oksama and Kristoffersson, 1980) and the uncoupling of phosphorylation due to paraquat influence.

K^+ ion is majorly found in the intercellular fluid and also found in the intercellular fluid. K^+ ion functions in carbohydrate metabolism in nerve fibres of animals. Decrease in K^+ ion concentration or its increase affects the heart function and may cause a neurotoxic damage to the central nervous system (CNS) of the fish (Adedeji, 2010). The balance between K^+ and Na^+ ion is essential in the stability of the fish and are involved in the transport of high energy compounds such as Na^+, K^+ ATPase (Gabriel et al., 2009).

The result of this study reveals underlying injury (necrosis) in the liver of the fish, *Clarias gariepinus* and this leads to the depletion of ATP, which finally leads to death of the cells (Shimizu, *et al*., 1996). The responses of the biomarkers indicated a good response to paraquat toxicity and therefore could be used as indices of paraquat toxicity in the environment.

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