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Behavioural, haematological, biochemical and histopathological alterations in African Catfish, *Clarias gariepinus* exposed to dragon (paraquat dichloride)

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Abstract

Paraquat herbicide, commonly used to control weeds in farms, could reach the aquatic environment and imperil non-target organisms through run-offs. This study was conducted to assess the impact of paraquat on African catfish *Clarias gariepinus*. Juvenile *C. gariepinus* weighing 19.06±7.43g, acclimatised for two weeks under laboratory conditions, were randomly divided into four groups of ten fish each and exposed to 0.00 (control), 794.33, 882.59 and 992.91µg/l of paraquat, labelled A, B, C and D, respectively. The experiment was performed in triplicate for 96 hours. Thereafter, two fish from each tank were removed, and blood was collected for haematological and biochemical analyses, while the kidneys were excised for histopathological examination. The results showed that red blood cells and packed cell volume decreased while the white blood cells increased significantly ($p<0.05$) compared to the control. The activities of aspartate aminotransferase and alkaline phosphatase significantly increased ($p<0.05$) while glucose and protein decreased significantly ($p<0.05$). Histopathological examination revealed concentration-dependent damage to the kidney of the paraquat-exposed groups. This study shows that paraquat is toxic to juvenile fish, therefore, caution should be taken in its application. Policies should be enacted and enforced to protect the environment and non-target species.

Introduction

The use of herbicides to improve crop yield to meet the steady rise in demand for food crops has been accepted globally (Okogwu *et al* 2015; Rohani 2023; Elebe 2024). Unfortunately, poor handling and extensive use of these agrochemicals have led to contamination of the aquatic environment and harming of non-target organisms such as fish (Nwani *et al* 2013; Amaeze *et al* 2020; Okogwu *et al* 2020; Aribisala *et al* 2022). USEP (2017) stated that three billion kilograms of pesticides are used worldwide annually, but only 1% of the total pesticides are effectively used to control insect pests on target plants (Bernardes *et al* 2015). This may result in bioaccumulation in organisms and biomagnification of these pesticides along the food chain in aquatic ecosystems, which may eventually affect human health (Thakur and Pathania 2020; Aribisala *et al* 2022).

Paraquat dichloride (N, N- dimethyl- dimethyl-4, 4 -bipyridinium dichloride), often referred to as paraquat is a commonly used herbicide (weed killer) in the world, second to glyphosate (Ashraf 2005; Ogamba *et al* 2014; Ike-Obasi and Ukazu, 2018; Okey *et al* 2021; Kachi and Ugwumba 2024). Paraquat is a peculiar, rapid-acting, non-selective contact herbicide used to exterminate fibrous-rooted grasses and weeds (Jacob *et al* 2024). Residues of paraquat have been detected in surface water, where it could cause cytogenetic damage, alter physiological processes, impair growth and reproduction, and cause the death of non-target species (Babatunde *et al* 2014; Jacob *et al* 2024).

Many fish are very sensitive to aquatic pollution and show both pathological and physiological alterations when exposed to xenobiotics, consequently, they are useful as sentinel organisms for aquatic pollution surveillance (Ike-Obasi and Ukazu, 2018). Several authors have used fish as bioindicators of aquatic ecosystem pollution, early detection of environmental problems and the toxicity of pesticides (Lakra and Nagpure 2009; Nwani *et al* 2010; Okogwu *et al* 2015; Ikhuoriah *et al* 2023; Mandal *et al* 2024). The choice of *Clarias gariepinus* in the present study was based on its ability to adapt easily to laboratory conditions, non-invasiveness and its high commercial value (Elebe *et al* 2018). Abnormal changes in fish behaviour are the most common and simplest indicators that the aquatic environment has been contaminated (Okogwu *et al* 2022). Often, behavioural changes, haematological, biochemical and histopathological alterations are used to assess the effect of herbicides on fish (Nwani *et al* 2013; Okogwu *et al* 2015; Kachi and Ugwumba 2024). Alterations in the haematological indices occur long before pathological damages are revealed (Khalesi *et al*

2017; Akhtar *et al* 2021; Witeska *et al* 2023; Alarape *et al* 2024).

Paraquat is widely used in Nigeria to control weeds of maize, yam and cassava cultivated in both upland and river basins. Run-offs from these farms flow into nearby water bodies, which serve as habitats to economically and ecologically important fish such as *C. gariepinus* (Okogwu 2011). Thus, resulting in harmful exposure of these fish to sublethal concentrations of the herbicides in their habitats (Nwonumara and Okogwu 2020).

Several authors have worked on the toxicity of paraquat to *C. gariepinus*; Ayanda *et al* (2017) on behavioural and physiological assessment of glyphosate and paraquat toxicity to the juveniles, Ogaga *et al* (2019) on the behavioural and acute toxicity response of the fingerlings exposed to paraquat dichloride, Jacob *et al* (2024) on paraquat-induced acute toxicity response in the juveniles. None of these studies combined behavioural, haematological, biochemical and histopathological parameters in a single study, though such a study would be useful in determining the overall toxicity of this herbicide to fish, which is essential for policy advocacy.

This study was a holistic assessment of the impact of paraquat on the behaviour, haematology, biochemistry and histopathology of the African catfish *C. gariepinus.* It is intended to influence policy decisions on the application of the herbicide, safety of the environment and conservation of non-target organisms such *C. gariepinus*.

Materials and methods

Procurement of experimental fish and herbicide

Three hundred and forty-five juveniles of *C. gariepinus* with a mean weight of 19.06±7.43g were acclimatised for two weeks in the Department of Applied Biology Research Laboratory, Ebonyi State University, Abakaliki. The fish were fed commercial floating pellet diets at 3% of their body weight per day during the period of acclimation. Water in the tanks was changed every 24 hours to eliminate faecal matter and ammonia. Then, groups of ten acclimated fish were randomly exposed to varying concentrations of paraquat (0.00, 1500.00, 1800.00, 2100.00, 2400.00 and 2700.00µg/l in 10 litres of water) in static tanks to determine the median lethal concentration (LC_{50}) of the herbicide, that is, the concentration that kills 50% of the experimental fish population. The concentrations were labelled Groups A, B, C, D, E and F in ascending order with A (0.00µg/L) as the control. The experiments were set in triplicate for 96 hours. The test media were changed every 48hours.

The LC_{50} value of paraquat for *C. gariepinus* was determined following modified probit analysis as described by Finney (1971), OECD (2014) and Okogwu *et al* (2022). Thereafter, the fish were randomly divided into four groups of ten fish each and exposed to varying concentrations of paraquat: 0.00 (control), 794.33, 882.59, and 992.91µg/l, labelled A, B, C, and D, respectively. The experiments were set in triplicates and the exposure lasted for 96 hours. Behavioural responses of the fish such as swimming rate, convulsions, hyperactivity, equilibrium status, fin movement, somersaulting activity, and operculum movement were observed in exposed as well as the control groups at 24, 48, 76 and 96 hours of exposure and recorded as suggested by OECD (2014). At the end of the 96 hours, two fish from each tank (six fish per group) were removed; the blood collected by puncture of the caudal vein into tubes containing anticoagulant potassium salt of ethylene diamine tetraacetic acid (EDTA), sodium fluoride tubes and plain tubes. Each chamber depth tube was labelled accordingly. The fish were then dissected and the kidneys were excised and processed for histopathological studies following the procedure of Si-Tayeb *et al* (2010), Bastiaenen (2020) and Khan *et al* (2021).

Analysis of some haematological parameters of the fish

Red Blood Cell (RBC) count was estimated by adding twenty microliter (20µl) of whole blood to 3.98ml of diluting fluid (10% sodium citrate) and mixed thoroughly. The first few drops were discarded after 5 minutes by holding the pipette vertically. Thereafter, the diluted blood was then introduced into the counting chamber and counted after three minutes using haemocytometer with the aid of a compound microscope. The RBCs in the four corner squares and one central square were counted. Hence;

$$\text{Total RBC (cells/L)} = \frac{\text{Counted erythrocytes}}{\text{Counted surface } (mm^2) \text{ x chamber depth x dilution}}$$

To estimate the White Blood Cells (WBC) count, 20µl of whole blood was added to 380µl of diluting fluid (acetic acid, with gentian violet) and mixed. Then, the counting chamber was charged with the well mixed diluted blood (after discarding the first five drops) with the aid of a pipette. Cells were allowed to settle in a moist chamber for 3 minutes. The four corners of the chamber were observed under a low power (×10) objective microscope and the cells were counted in all the four marked corner squares.

$$\text{Total WBC (cells/l)} = \frac{\text{Counted white blood cells}}{\text{Counted surface } (mm^2) \text{ x chamber x dilution}}$$

The Packed Cell Volume (PCV) was determined by centrifuging 1ml blood sample in the microhaematocrit centrifuge at 10,000 rpm for 5 minutes. Spinned tubes were placed into a specially designed scale and the PCV was read as a percentage of the whole blood.

$$\text{PCV\%} = \left(\frac{\text{Packed RBC column height}}{\text{Total blood volume height}}\right) \times 100$$

The Haemoglobin (Hb) concentration was determined using the cyanomethaglobin technique as outlined by Ochei and Kolhatkar (2008). Whole blood (20µl) was added to 4ml of Drabkin's solution in a test tube in a 1:250 dilution, then well mixed, allowed to stand for 10 minutes at room temperature and the absorbance read colorimetrically at 540nm with Drabkin's solution as a blank.

$$\text{Haemoglobin (g/L)} = \frac{\text{Reading of test x conc standard}}{\text{Reading of standard}}$$

The erythrocyte sedimentation rate indices; Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin Concentration (MCHC) and Mean Cell Haemoglobin content (MCH) were estimated from the primary indices, RBC, WBC, PVC and Hb. The MCV was also estimated using PCV % values and the erythrocyte counts and expressed in ($\times 10^6 mm^3$) (Anderson and Klontz 1965).

Biochemical analysis

The activities of aspartate aminotransferase (AST) were assayed by the method of Reitman and Frankel (1965) as outlined in the Randox kit. The samples of the serum (0.1ml) were pipetted into the sample test tubes, and 0.1ml of distilled water was pipetted into the blank test tube. Then, 0.5ml phosphate buffer, L-aspartate, and beta-oxoglutarate were pipetted into both the blank and serum sample test tubes, respectively. The entire reaction medium was well mixed and incubated for 30 minutes in a water bath at 37°C. Immediately after incubation, 0.5ml of 2,4-dinitrophenylhydrazine was added to the blank and the serum sample test tubes and allowed to stand for exactly 20 minutes at 25°C. Finally, 5.0ml of 0.4 sodium hydroxide solution was added to both the blank and serum sample test tubes, respectively and mixed thoroughly. The absorbance was read at a wavelength of 546 nm after 5 minutes.

$$\text{AST} = \frac{\text{Abs of sample} - \text{Abs of blk}}{\text{Slope}}$$

Abs of sample = absorbance of sample, Abs of blank = absorbance of blank

Thereafter, the activities of ALT were assayed by the same method of Reitman and Frankel (1957) as outlined in Randox Kit.

$$\text{ALT} = \frac{\text{Abs of sample} - \text{Abs of blank}}{\text{Slope}}$$

Abs of sample = absorbance of sample, Abs of blank = absorbance of blank

To determine the activities of alkaline phosphatase (ALP), 50µl of serum, 50µl of standard and 50µl distilled water were added into three test tubes labelled serum, standard and blank.

Then, 0.05ml of ALP substrate was added into the labelled test tubes, mixed gently for 3 minutes and incubated for exactly 10 minutes at 37°C. After that, ALP colour developer (2.5ml) was added at timed intervals of 1 minute and mixed well. The absorbance was measured at 630nm.

$$\text{ALP} = \frac{\text{Absorbance of sample x value of sample}}{\text{Absorbance of standard}}$$

The total protein content was determined using the method of Tietz (1995). Into three test tubes labelled serum, standard and blank were added 20µl of serum, 10µl standard and 10µl distilled water, respectively. Thereafter, 0.5ml of Biuret reagent composed of NaOH, Na-K- tartrate, potassium iodide and cupric sulphate was added to all the test tubes and incubated for 3 minutes at 25°C. The absorbance was measured at 500nm and protein concentration calculated as follows:

$$\text{Protein concentration (g/dl)} = \frac{\text{absorbance of sample x standard conc.}}{\text{absorbance of standard}}$$

A commercial enzyme kit (Glu L 1000, PLIVA-Lachema, Czech Republic) was used to determine the glucose concentration. Samples were added to a solution of glucose oxidase, peroxidase and 4-aminoantipyrine; 10 minutes later in incubation, samples were moved to 96-well microtiter plate (200µl) and their absorbance (500nm) was measured by the plate reader (Tecan Sunrise, USA). The absorbance of glucose standard (10mmol/l) was used to determine the glucose concentration using the quantification limit of 0.02mmol/l; working range of 0.065–45mmol/l, repeatability 1.05%, and the working volume of 10µl.

Bilirubin level was determined using the diazo method as detailed in (Jendrassik and Grof 1938) and Elebe (2022).

Histopathological assessment

The kidney tissues were fixed in 10% normal-saline and then dehydrated using different grades of alcohol ranging from 50% to absolute alcohol for 30 minutes each. The dehydrated tissues were cleared of the alcohol by immersing them through three (3) changes of xylene for 10 minutes each, and were then impregnated and infiltrated in molten paraffin in a hot air oven for 30 minutes each and paraffin blocks of the tissues were made. The blocks of the tissues were sectioned at 5µm thickness using a rotary microtome. The sectioned tissues were dewaxed in xylene for 10 minutes, rehydrated and counterstained with hematoxylin and eosin (H&E), then dried after 2 minutes and the micrograph taken under the light microscope (Si-Tayeb *et al* 2010; Virovic-Jukic *et al* 2022).

Data analysis

The dose-dependent mortality rate and the median lethal concentration (LC_{50}) were determined using probit analysis. Descriptive statistics were used to calculate the mean and standard errors of the variables measured. Mean values were presented in tables. Two-way

Analysis of Variance (ANOVA) was used to test the difference in the variables measured between the treatments. Values were considered significant at $p<0.05$. All statistical analyses were carried out using Statistical Package for Social Sciences (SPSS) version 21.1.

Ethical approval

All fish-related procedures were carried out in accordance with the approval (EBSU/DRIC/UREC/VOL.04/062) of the Ethical Committee of Ebonyi State University, Abakaliki.

Results

Impact of paraquat on behavioural parameters of *C. gariepinus*

The fish in the control exhibited normal behaviour throughout the bioassay while the fish subjected to different concentrations of paraquat displayed uncoordinated behaviours at different times of exposure as shown in Table 1.

Median lethal concentration (LC_{50}) of paraquat exposed to *C. gariepinus*

The LC_{50}, which is the concentration of the herbicide that will kill 50% of experimental fish, was 7,943.29µg/l (Figure 1).

Haematological effects of sublethal concentrations of the paraquat on *C. gariepinus*

The RBC value decreased from 9.42±0.87 to 8.66±0.66 million/mm^3. The Hb varied from 6.50±0.34 to 7.30±0.60g/dl and PCV decreased from 32.04±0.98 to 30.03±0.87 (%). However, WBC increased significantly ($p<0.05$) in all the paraquat treatments when compared to the control. There were significant ($p<0.05$) changes in the values of MCH, MCV and MCHC in all the paraquat treatments compared to the control (Table 2).

Biochemical effects of paraquat on *C. gariepinus*

A significant ($p<0.05$) increase in the value of AST was only recorded in the group exposed to 992.91µg/l of paraquat. The ALP showed significant ($p<0.05$) increase in all exposures while changes in ALT values were not significant in all paraquat exposures compared to the control ($p>0.05$). Glucose and protein showed significant ($p<0.05$) decrease while bilirubin increases were not significant when compared to the control (Table 4).

Histopathological changes in kidney of *C. gariepinus*

The kidney from the control 0.00µg/l (Group A) showed normal kidney architecture with normal glomeruli (NG) and normal renal tubules (NRT). However, histopathological examination in the exposed groups revealed concentration-dependent damage to the organ. Fish exposed to 794.33µg/l (Group B) showed shrinking of the glomeruli (SG) and mild tubular necrosis (MTN). The kidney of the fish from Group C (882.59µg/l) showed complete loss of glomeruli and moderate tubular necrosis (mtn) while those exposed to 992.91µg/l (Group D) revealed severe tubular necrosis (STN) and focal distortion of renal tissue (FDRT) (Plate 1).

Discussion

Studies on the impact of toxicants on fish have become widely accepted, as environmental monitoring protocols and behavioural alterations in fish are the simplest and most sensitive indicators of the presence of toxicants in aquatic environments (Yanchev 2020). The erratic swimming, gulping of air, convulsion, strong fin and opercular movement observed in the present study agree with the observations made in several toxicity studies (Nwani *et al* 2013; Okogwu *et al* 2015; Ike-Obasi and Ukazu 2018; Okogwu *et al* 2022; Ikhuoriah *et al* 2023 and Elebe 2024).

Haematological analysis is one of the basic tools used to assess the impact of organic and inorganic substances on fish. The decrease in the RBC, PCV and Hb count in the present study agrees with the report of Kachi and Ugwumba (2017), who reported significant ($p<0.05$) decrease in these parameters during their study on the sublethal effect of paraquat on *C. gariepinus*. Chris *et al* (2022), also observed decrease in RBCs, Hb and hematocrit, in *C. gariepinus* exposed to different doses of xylene. Okey *et al* (2021), suggested that the continuous decrease in the values of RBC of *C. gariepinus* exposed to paraquat indicates an impairment of the erythropoietic process. The decline in the RBCs could also be due to paraquat-induced anaemia, which might be attributed to erythropenia and damage to haematopoietic organs (Okogwu *et al* 2022). The reduction in Hb and PCV could be due to the adverse effect of paraquat inhibiting the haemapoietic activity and increased breakdown of RBC membranes (Kuhn *et al* 2017). On the other hand, the significant ($p<0.05$) increase in the WBC count disagrees with the initial decrease observed in the values of WBC reported by Kachi and Ugwumba (2017) but agrees with the toxicity reports of many researchers (Okogwu *et al* 2015; Okey *et al* 2021; Shahjahan *et al* 2022 and Jacob *et al* 2024). The increase in WBC could be attributed to defence mechanisms employed by the fish to mitigate paraquat assault.

Alterations in biochemical parameters are also key indicators of the health of fish exposed to toxicants (Younis *et al* 2012 and Rohani 2023). The significant ($p<0.05$) increase in the value of AST and ALP in the present study agrees with the report of Ayanda *et al* (2015) in their studies on the acute toxicity of glyphosate and paraquat-based herbicide on the African catfish (*C. gariepinus*) using some biochemical indicators. Significant increase in the activities of liver enzymes recorded in this study indicates hepatic damage due to paraquat exposure, which causes the liver to release these enzymes. However, the release of AST into the bloodstream can be triggered by damage in many other organs such as the brain, pancreas, heart, kidneys, lungs, and skeletal muscles (Nwonumara and Okogwu 2020).

Table 1: Behavioural parameters of *C. gariepinus* exposed to paraquat

	Pesticide Concentration (µg/l)															
	Control				794.33				882.59				992.91			
Time of exposure (hours)	24	48	72	96	24	48	72	96	24	48	72	96	24	48	72	96
Behaviour																
Hypersensitivity	-	-	-	-	++	++	+	+	++	++	+	+	++	++	+	+
Equilibrium Status	+++	+++	+++	+++	++	++	+	+	+	+	+	+	+	+	+	+
Swimming Rate	++	+	++	++	++	++	++	++	++	++	++	++	++	++	++	++
Gulping of Air	-	-	-	-	-	+	+	+	+	+	++	++	++	++	++	++
Convulsion	-	-	-	-	-	-	-	-	+	+	-	-	++	++	++	-
Somersaulting	-	-	-	-	-	-	-	-	+	+	-	-	++	++	+	_
Fin movement	++	++	++	++	++	++	++	++	++	++	++	++	+++	++	+	+
Opercular movement	++	++	++	++	++	++	++	++	++	++	++	++	++	++	+	+

Key: - = None, + = Mild; ++ = Moderate; +++ = Strong

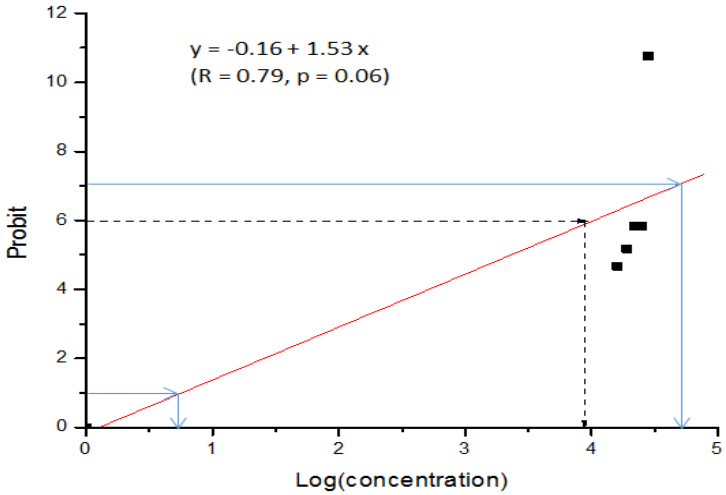

Figure 1. Median Lethal Concentration (LC_{50}) of paraquat of *C. gariepinus* using probit method

Table 2: Haematological parameters (mean±SE) of juvenile *C. gariepinus* exposed to paraquat

	Pesticide concentrations (µg/l)			
Blood Parameters	control	4.50	5.44	6.10
RBC ($\times10^6$/µl)	9.67 ± 0.86^a	9.42 ± 0.87^a	8.66 ± 0.66^a	8.94 ± 0.56^a
WBC ($\times10^3$/ µl)	8750 ± 6.80^a	9400 ± 7.20^b	9800 ± 8.50^b	9600 ± 8.20^b
Hb (g/dl)	6.90 ±0.56^a	7.30 ±0.60^a	6.50 ± 0.45^a	6.50 ± 0.34^a
PCV (%)	32.00 ± 1.03^a	32.04 ± 0.98^a	0.03 ± 0.87^a	331.02 ± 1.05^a
MCH (pg)	7.12 ± 0.90^a	7.75 ± 0.84^a	7.51 ± 0.70^a	31.02 ± 1.05^a
MCV (fl)	33.09 ± 1.00^a	33.97 ± 1.54^a	34.64 ± 1.02^a	34.68 ± 1.12^a
MCHC (g/l)	21.56 ± 0.44^a	22.81 ± 0.38^a	34.64 ± 1.02^a	20.97 ± 0.42^a

RBC - red blood cell, WBC - white blood cell, Hb - haemoglobin, PCV - pack cell volume, MCH - mean corpuscular haemoglobin, MCV - mean corpuscular volume, MCHC - mean corpuscular haemoglobin concentration.
Values with different alphabetic superscripts differ significantly ($p< 0.05$) between concentrations within the same row

Table 4: Biochemical parameters of the peripheral blood of *C. gariepinus*

	Pesticide concentrations (µg/l)			
Biochemical Parameters	control	4.50	5.44	6.10
AST (IU/L)	40.01 ± 2.45^a	42.02 ± 2.67^a	40.03 ± 2.11^a	48.01 ± 1.97^b
ALP (IU/L)	68.02 ± 3.87^a	80.03 ± 3.67^b	84.12 ± 2.11^b	78.03 ± 4.56^b
ALT (IU/L)	64.04 ± 2.11^a	60.05 ± 2.01^a	68.10 ± 2.89^a	66.11 ± 3.01^a
Glucose(mg/dL)	80.20 ± 4.02^a	71.10 ± 2.73^b	68.20 ± 2.91^b	66.23 ± 1.97^b
Protein (mg/dL)	8.40 ± 0.56^a	4.21 ± 0.34^b	3.62 ±0.14^b	4.93 ± 0.38^b
Bilirubin (mg/dL)	4.30 ± 0.15^a	5.64 ± 0.18^a	5.40 ± 0.21^a	5.66 ± 0.32^a

AST - Aspartate aminotransferase, ALP - Alkaline phosphatase, ALT - Alanine aminotransferase
Values with different superscripts differ significantly ($p< 0.05$) between concentrations within the same row.

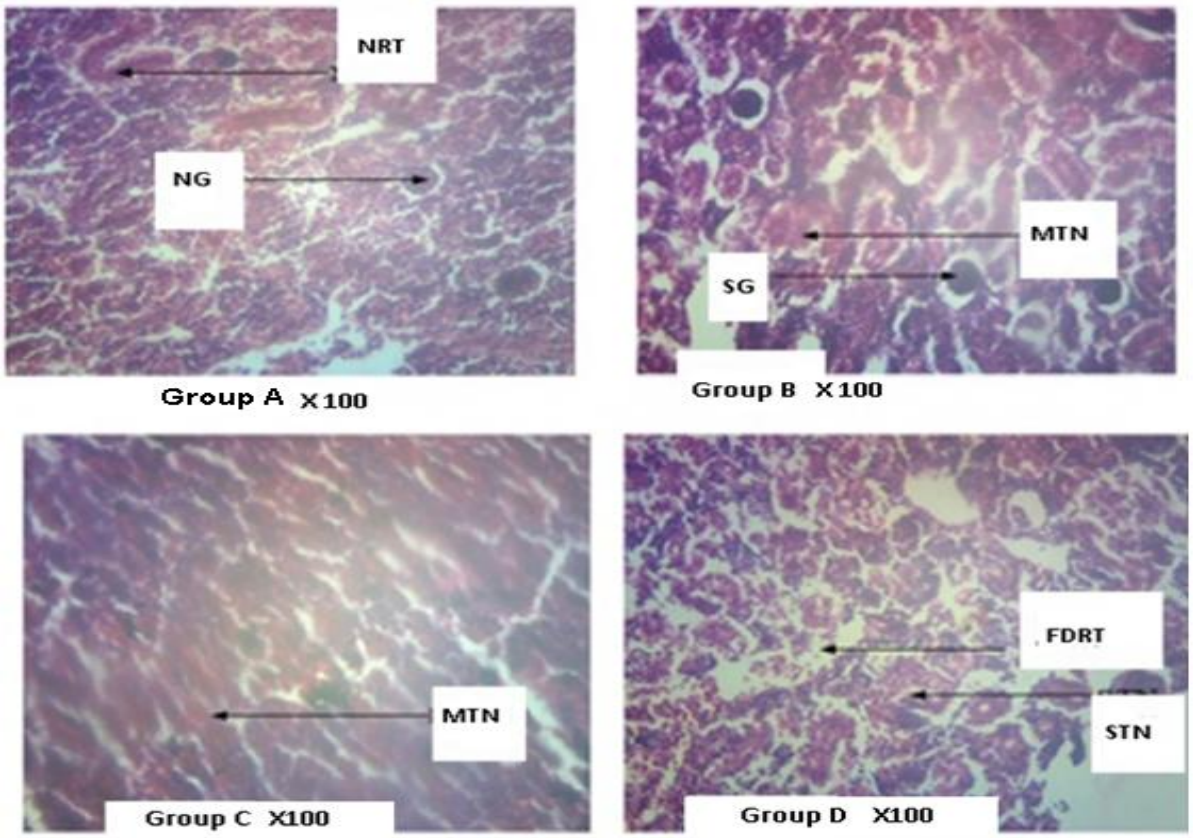

Plate 1. Histopathological impact of paraquat on the kidney of *C. gariepinus* at 96-hour exposure
Control (Group A, 0.00µg/l) showing normal glomeruli (NG) and Normal Renal Tubules (NRT); **Group B** (4.50µg/l) showing Shrinking of the Glomeruli (SG) and Mild Tubular Necrosis (MTN); **Group C (5.44µg/l)** showing Moderate Tubular Necrosis (MTN); **Group D (6.10 µg/l)** showing severe tubular Necrosis (STN) and Focal Distortion of Renal Tissue (FDRT).

Shrinking of the glomeruli, mild tubular necrosis, complete loss of glomeruli and moderate tubular necrosis, severe tubular necrosis including focal distortion of renal tissue shown in the kidney of exposed fish in this study align with the report of Sayed *et al* (2023) who reported renal dysfunctions in the form of glomerular swelling with melanomacrophages aggregation, diffuse interstitial inflammatory cell infiltration, necrosis, vacuolar degeneration in epithelial cells lining the renal tubules and interstitial hemorrhages between tubules in *Oreochromis niloticus* exposed to xenobiotics. Okogwu *et al* (2015) reported extensive damage of the kidney as renal and intracardiac haemorrhages, which depict loss of blood due to cellular damage and inferred that this could lead to organ failure. Ogamba *et al* (2014) reported similar results from fish exposed to a variety of toxicants. These pathological expressions suggest that paraquat could potentially induce oxidative stress. Oxidative stress has been reported to cause cell, tissue and organ damage (Okogwu *et al* 2014; Okogwu 2016; Nwonumara and Okogwu 2020).

Conclusion

Paraquat is toxic to fish due to adverse effect on the behaviour, alteration of some haematological and biochemical parameters and damage to kidney of *Clarias gariepinus*. Therefore, caution should be taken in the application of this herbicide, especially near aquatic ecosystem and protective policies should be enacted and enforced to ensure the protection of the environment and conservation of non-target organisms like fish.

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Conflict of interest

The authors declare no conflict of interest.

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