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Paraquat-Induced Acute Toxicity Response in Juvenile African Catfish *Clarias gariepinus* (Burchell, 1822)

***[1]JACOB, US; [1]OBOT, OI; [1]UMOH, EM; [2]AKANGBE, OA**

**[1]Department of Fisheries and Aquatic Environmental Management, Faculty of Agriculture, University of Uyo, Uyo, Akwa Ibom State, Nigeria*
[2]Department of Zoology, University of Ibadan, Oyo State, Ibadan, Nigeria

*Corresponding Author Email: udujacob@yahoo.com
**ORCID ID: https://orcid.org/0009-0000-4730-2995*

Co-Authors Email: ofonmbukobot@uniuyo.edu.ng; udujacob@yahoo.com; akangbeolusola@gmail.com

ABSTRACT: The extensive application of Paraquat in agricultural and non-agricultural sectors has led to severe physiological and environmental consequences. Hence, this paper was aimed at investigating the Paraquat-induced acute toxicity response for 98 hours in juvenile African catfish *Clarias gariepinus* using standard methods. The mean weight and mean total length of the fish were 60.67 ± 0.2 g, and 23.06 ± 0.2 cm. The fish were randomly distributed into a transparent plastic aquarium (22.6 x 18.5 cm). Paraquat was introduced at concentrations of 0.00, 0.4, 0.8, 1.2, 1.6, and 2.0 mg/l into the aquaria with the corresponding percentage mortality for 0, 30, 40, 55, 75, and 100% respectively. The observed behavioral changes and mortality were time and concentration-dependent. Symptoms of toxicity exhibited by the fish include loss of equilibrium, startle responses, hyperactivity, abnormal swimming, hemorrhage, and general restlessness. The median lethal concentration (LC_{50}) estimated by probit analysis was 1.017 mg^{-1}. The physicochemical parameters of the aquaria were in the following range DO, 3.00 - 5.80 mgL^{-1}, temperature, 19.75 - 21.90 ^{o}C, TDS 10.10 - 20.20 mgL^{-1}, alkalinity 40.80 - 91.00 mgL^{-1}, and pH, 6.7 - 8.04 respectively. The measured haematological parameters showed a concentration-depending pattern of alterations due to paraquat exposure. The highest haematological parameters such as TWBC, 124.50 $\times 10^{9}L^{-1}$, TRBC, 2.01 $\times 10^{12}L^{-1}$, Tplt, 85.00 $\times 10^{9}L^{-1}$, and LYM, 84 % were recorded in 2.0 mgL^{-1} paraquat concentration. This result revealed the toxic effects of Paraquat on juvenile *Clarias gariepinus.* Hence, the need for its regulation in both agricultural and non-agricultural sectors.

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Herbicides constitute about 40% of environmental chemicals globally with significant negative impacts on organisms and the environment (Doherty, *et al.*, 2011). The use of herbicides to control weeds is a global practice in the agricultural sector (FAO, 2008; Doherty, *et al.*, 2011). Unfortunately, these practices have resulted in the release of toxic chemical compounds into the environment with severe negative impacts in non-target organisms (Annune *et al*., 1994; Doherty *et al.*, 2011). Synaptic herbicides used to control weeds and unwanted aquatic plants in farmlands, rivers, lakes, and reservoirs enters into the terrestrial and aquatic environment with severe adverse effects on the biota (Tsuda *et al.*, 1997; Bamidele, *et al.*, 2018). Approximately three million people are poisoned and about 200,000 die each year globally from pesticide poisoning. Regrettably, majority of these individuals are from developing countries (FAO, 2000). The use of Paraquat in developed countries is restricted, and in some case

*Corresponding Author Email: udujacob@yahoo.com
**ORCID ID: https://orcid.org/0009-0000-4730-2995*

banned. However, in developing country like Nigeria, herbicides are used extensively in both agricultural and non-agricultural sectors. Herbicide and other agro-chemicals enter the environment through different pathways which include the use of chemicals to control weeds, pests, and preservation (UNEP, 2003; Arivu, *et al.,* 2016). The combine interaction effects between agricultural chemicals and the environment usually produce more toxic compounds with negative physiological effects on organisms and the environment (UNEP, 2003). Herbicides at high concentrations may affect the survival, growth, development, and reproduction of organisms (Rahman *et al*., 2002). From United States Environmental protection Agency (USEPA, 2007), herbicides contain an active ingredient with severe toxicity that can cause vertebral deformities in fishes, erratic swimming and impaired breathing which make fish vulnerable to predators (Liong *et al*., 1988; McBride *et al*., 1989). Therefore, effective risk management for agricultural chemicals requires understanding the pathways of entry into the environment and their mode of interaction in the environment (Oliveira *et al.,* 2009).

Paraquat is a unique, fast-acting, non-sensitive, contact herbicide used to eradicate fibrous-rooted grasses and weeds. Because of its high solubility and repeated application in agricultural and non-agricultural sectors, large quantity and residue of paraquat have been detected in surface water with damaging effects (Babatunde *et al*., 2014). Though Paraquat is a moderately toxic chemical with relatively low bio-accumulation factor, at low concentrations, paraquat can cause cytogenetic damage, alter physiological processes, and impair the growth, reproduction, and death, of non-target species (Omitoyin *et al*., 2006; Babatunde *et al*., 2014). Several authors have reported the toxic effects of paraquat on tissues and cellular components of organisms including fish (Ogamba *et al*., 2011; Deivasigamani, 2015).

Kori-siakpere *et al*. (2007) reported that 96 hours exposure of *Clarias gariepinus* to paraquat dichloride resulted in altered levels of plasma glucose, triglyceride and plasma protein. This ultimately led to considerable loss of blood protein by renal excretion (Sastry and Sharma, 1981). Oruç *et al.* (2004) reported that pesticides may result in an immense disruption of the ecological balance triggering severe damage to non-target organisms, including fish of commercial importance. However, exposure to pesticide can resulted in the synthesis of reactive oxygen species (ROS) and altered the antioxidant defenses in organisms. The effect of ROS include damage of important biological molecule such as lipids, proteins, carbohydrates, and nucleic acids (Monserrat *et al.,* 2007). Peroxidation of unsaturated fatty acids and the corresponding increase in tissue malondialdehyde levels is the major symptoms of oxidative damage. It is fundamental to investigate haematological parameters of fish in response to different concentration of Paraquat. This will provide critical information on public health implication of herbicide and other related environmental chemical. Hence, this study was aimed at investigating paraquat-induced acute toxicity responses in juvenile *Clarias gariepinus* using haematological indices.

MATERIALS AND METHODS

Experimental Setup, Fish, and Management: This study was conducted in the Laboratory of the Department of Fisheries and Aquatic Environmental Management, University of Uyo, Nigeria. The University of Uyo guideline for the care and use of animals for scientific research was strictly adhered to. Juvenile African catfish *Clarias gariepinus* mean weight of 60.67 ± 0.2 g and mean length of 21.90 ± 0.2 cm were purchased from a commercial fish farm in Uyo, Akwa Ibom State. The fish were transported in a plastic aerated container to the Laboratory. The fish were randomly distributed into six different aquaria (22.6 x 18.5 cm), filled with dechlorinated tap water. They were fed with commercial fish feed (pelleted) containing 40% crude protein at 3% of their body weight. Unconsumed feed and fecal waste were removed and the water was changed twice a week. The paraquat solution used for this study was purchased from Ekponwa Chemical Store in Ukana Offot Street, Uyo, Nigeria.

Before conducting the range finding test, the juveniles were starved for 24 hours. This was to reduced pollution that may arise from the decomposing fecal droppings. Additionally, it was also to ensure that the fish were relaxed and not exposed to external stress that could arise from feeding (Akinsorotan *et al*., 2019). The range finding test was conducted using specific factor 10 to determine the concentration of paraquat as described by (Solbe, 1995; Rahman *et al.*, 2002). From the range finding test, six graded concentrations of 0.00, 0.4, 0.8, 1.2, 1.6, and 2.0 mg/l of paraquat were used for the definitive test. The experiment was set in duplicates to obtain the 96 h LC_{50} value of paraquat. During the test, the mortality and survival rates were checked at intervals. Deceased fish were removed to avoid pollution of the water. Fish behaviours such as a loss of equilibrium, startle responses, hyperactivity, abnormal swimming, hemorrhage, and general restlessness were observed. The lethal concentration values of paraquat were calculated for 96 h using the probit analysis method

described by (Finney, 1978). The physico-chemical parameters of the aquaria were monitored every 24 h as described by (Eaton, 2005).

Blood collection: Before blood collection, each fish was anesthetized with tricaine methanesulfonate MS 222. Blood was collected by puncturing the caudal vein with a heparinized syringe needle and stored in small ethylene diamine tetraacetic acid-treated vials. Whole blood samples were used for the estimation of hematological parameters such as White Blood Cell Count (WBC), Red Blood Cell Count (RBC), Haemoglobin (HGB), Hematocrit (HCT), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), and Mean Corpuscular Haemoglobin Concentration (MCHC), total Platelet (Tplt) and lymphocyte (LYM). Erythrocyte indices, such as MCHC, MCH, and MCV, were calculated using the standard formula:

$$\text{MCHC (\%)} = \frac{Hb\left(\frac{g}{100}\,mL\right)}{\frac{PCV}{100}\;mL}\;x\;100 \quad (1)$$

$$\text{MCH}\left(\text{pg}^{-\text{cell}}\right) = \frac{Hb\left(\frac{g}{dL}\right)}{RBC\ count\ in\ \frac{millions}{mm^3}}\;x\;10 \quad (2)$$

$$MCV(fl)^{-cell} = \frac{PCV}{RBC\ count\ in\ millions/mm^3}\;x\;10 \quad (3)$$

Statistical Analysis: The mean lethal concentration LC_{50} for the selected periods of exposure was subjected to probit analysis using SPSS (Version 23). The result was considered significant at ($P < 0.05$).

RESULTS AND DISCUSSION

Fish mortality: The result of the mortality rate is shown in Table 1. There was no mortality in the control experiment throughout the 96 h. The lowest mortality was recorded in 0.4 mg/l, while the highest mortality was recorded in 2.0 mg/l. This result revealed that mortality was dose-dependent.

Changes in physicochemical parameters: Changes in physicochemical parameters are presented in Table 2. There was a significant ($p < 0.05$) difference in mean values for some physicochemical parameters. pH and Dissolved oxygen in the control group were significantly different from the other test solution at ($P < 0.05$).

Behavioural changes: The exhibit normal behaviour in the control group was normal while the ones introduced into different concentrations of the herbicide showed different abnormal behaviors such as loss of equilibrium, startle responses, hyperactivity, abnormal swimming, hemorrhage, and general restlessness. However, abnormal behavior was observed to increase with increased concentration.

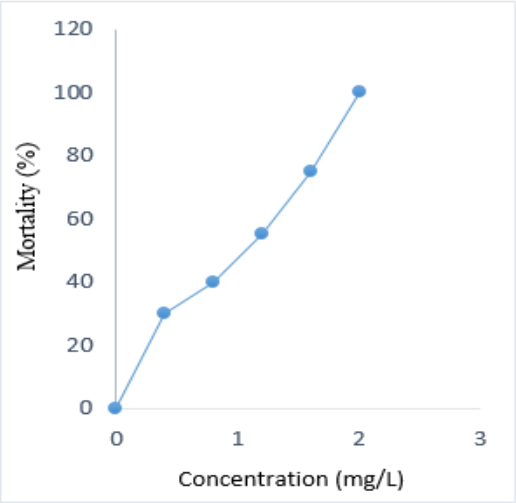

Fig 1. Graph of probit analysis

Haematological parameters: Table 3 shows the results of the hematological parameters. The RBC, PCV, and Hb values in the exposed fish significantly decreased ($P<0.05$) compared to the control group. There was an increase in the WBC count in the exposed fish throughout the duration of the experiment. Variations in the hematological indices indicated that MCV, MCH, and MCHC were significantly lower ($P<0.05$) during the experiment in the treated groups when compared to the control.

In this study, the 96 h LC_{50} value of paraquat was 1.017 mg/l (Fig. 1). This indicates that the toxicant had a serious negative effects on the fish. The behavioral changes in exposed fishes were time and concentration-dependent. These abnormal behaviours may be due to neurotoxic effects and irritation to the perceptive system of the fish due to the accumulation of acetylcholine at synaptic junctions through the inactivation of acetylcholinesterase and stimulation of the peripheral nervous system resulting in a higher metabolic rate (Rao *et al*., 2005). The results of this study agree with the reports of (Ayoola, 2008; Ojikutu *et al*. 2013). This also agreed with the report that the behavioural responses of fish to most toxicants are the most sensitive indicators of potential toxic effects (EIFAC, 1973). It was also similar to the report by (Pathak and Kumar, 2020) who reported acute toxic effect of mercuric chloride on *H. fossilis.* Haematological parameter is important in assessing fish health (Blaxhall, 1972) and monitoring stress response (Solvio and Olkari, 1976).

Table 1. Cumulative mortality of *C. gariepinus* exposed to various concentrations of paraquat.

		Numbers of mortality (hrs)					
Concentration mg/L	Number of fish exposed	24	48	72	96	Mortality (%)	Survival (%)
0.0	20	0	0	0	0	0	100
0.4	20	0	1	2	3	30	70
0.8	20	0	1	2	5	40	60
1.2	20	1	2	2	6	55	45
1.6	20	2	3	3	7	75	25
2.0	20	4	4	4	7	100	0

Table 2: Variation in Physicochemical parameters recorded during the experiments

Concentrations	pH (g/l)	Alkalinity(mg/L)	TDS (mg/L)	Temperature °C
0.0	6.31±0.29^{a}	87.85±2.50^{a}	12.060±0.05^{a}	23.80± 0.00^{a}
0.4	4.91±0.34^{b}	54.64±3.09^{a}	11.710± 0.20^{a}	24.80±0.01^{a}
0.8	4.86±0.30^{b}	59.79±2.57^{a}	10.550±0.56^{bc}	21.79± 0.01^{a}
1.2	4.72±0.12^{b}	54.05±1.36^{a}	10.319± 0.23^{c}	22.83± 0.02^{a}
1.6	4.94±0.38^{b}	43.97±2.05^{a}	11.600±0.20^{a}	25.80± 0.02^{a}
2.0	4.68±0.17^{b}	45.90±1.49^{a}	11.465± 0.47^{ab}	23.83± 0.01^{a}

Data are presented as mean and standard deviation and different superscripts represent levels of significance ($p < 0.05$).

Table 3: Changes in fish hematological parameters

Concentration (mg/l)	TWBC ($\times10^{9}L^{1}$)	TRBC ($\times10^{12}L^{1}$)	HGB (gdL^{-1})	HCT (%)	MCV (fl)	MCH (pg)	MCHC ($g\ dL^{-1}$)	Tplt ($\times10^{9}L^{-1}$)	LYM (%)
0.0	107.60	2.06	7.60	26.70	143.10	35.30	28.50	64.00	65.00
0.4	110.10	2.01	7.30	27.20	140.60	33.70	27.30	66.00	67.00
0.8	115.80	1.19	7.50	27.30	139.10	26.10	27.50	68.00	68.00
1.2	117.40	1.14	7.40	26.90	134.30	20.30	40.00	78.00	78.00
1.6	120.10	1.01	7.20	28.60	132.80	19.20	25.20	80.00	80.00
2.0	124.50	1.18	6.90	27.40	130.90	16.30	36.50	85.00	84.00

Here, hematological analysis revealed that paraquat has a depressive effect on the hematopoietic tissues of the fish. These were observed due to the decreased in haematological parameters of juvenile African catfish exposed to acute concentration. This was already reported by (Ayoola, 2008). However, this may suggest anemic condition because deficiency in oxygen transport usually occurs from anemia (Robert, 1978; Pandey *et al.,* 2003; Nwani *et al.,* 2013). The increase in TWBC and lymphocytes may be due to the activity of paraquat which causes the immune system of the fish to respond by producing more WBC to provide strong defense system against the toxic effect of the paraquat. The increase in platelet count was concentration-dependent. Increased lymphocytes in the exposed group also suggested immunological response of the fish to paraquat which agreed with (Olatayo, 2005; Adewoye, 2010; Kayode and Shamusideen, 2010; Emmanuel *et al*. 2012). A decreased erythrocyte count has been reported in *Cirrhinus mrigala* exposed to ibuprofen (Prusty *et al.,* 2011) which also agreed with the reports of the findings from this study. The cytotoxic effect of pesticides on fish generally resulted in synthesis of ROS, which leads to oxidative stress (Pereira *et al.,* 2013, Nwani *et al.,* 2013). Decreased TRBC reported in this study may be due to erythrocyte membrane lipid peroxidation which subjects the fish to hemolysis caused by paraquat exposure which is in line with (Pereira *et al.,* 2013).

The reduction in MCV of exposed fish revealed erythrocytic shrinkage leading to microcytic anemia which may be due to osmoregulatory imbalances. The reduction in the HGB concentration, MCH, and MCHC suggesting that paraquat could have inhibitory effects on HGB biosynthetic pathway by modulating the utilization of delta-aminolevulinic acid (Nussey *et al.,* 1992). However, this may impaired the oxygen-carrying capacity of the blood. Leucocytes regulate immunological functions in animals and fish. In the present study, leucocytosis parameters revealed abnormal immune protective response.

Environmental variables such as temperature, pH, alkalinity and TDS are important physico-chemical which can be used to assess the status of pollution of the experimental media and to determine the well-being of organism. (Adeogun *et al.,* 2011; Jacob *et al.,* 2023). In this study, the highest pH was 6.31±0.29 g/l while the lowest was 4.68±0.17 g/l. Alkalinity ranged from 43.97±2.05 to 87.85±2.50 mg/L. The TDS of the experimental media ranged from 10.319±0.20 to 12.060±0.05 mg/L. The temperature of the media

ranged from 21.79±0.01 to 25.80±0.02 ℃. This primary abiotic variable plays a critical role in controlling organisms' body physiology, life-history processes, development (Jacob *et al.,* 2023).

Conclusion: Acute concentration of paraquat is toxic to juvenile *Clarias gariepinus.* This was observed due to their behavioural changes such as loss of equilibrium, startle responses, hyperactivity, abnormal swimming, hemorrhage, and general restlessness. Changes in hematological parameters indicate damage to physiological processes in the fish. Therefore, it is imperative to adopt precautionary measures before and after the use of paraquat in agricultural and non-agricultural sectors to prevent unintended toxic effect in the environment and organisms.

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