Academic Journal of Current Research
Vol.9, No.1, January-2022;
ISSN (2343 – 403X);
p –ISSN 3244 – 5621
Impact factor: 7.91

HAEMATOLOGY STATUS OF *CLARIAS GARIEPINUS* (BURCHELL, 1822) SUB-ADULT EXPOSED TO PARAQUAT

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Abstract: Herbicides usage in agricultural fields to control weeds can be toxic to non-target organisms like fish and can affect overall fish health. The haematological status of *Clarias gariepinus* sub-adult was investigated. A total of thirty six *C. gariepinus* (weight 365.51± 92.57 g and length 37.77± 2.78 cm) were randomly allocated into nine glass tanks, each tank contained four fishes (4/tank; 12/treatment). A static-renewal method and single sub-lethal dose of 552mg/l of paraquat was used in the continuous exposure and intermittent exposure, while in the control paraquat was not added. In the intermittent exposure the fishes were exposed to paraquat for two days and returned back to clean water for two days alternately while in the continuous exposure the fishes were exposed to paraquat daily. The result showed there were no significant differences (ANOVA, $p>0.05$) in the values of the Packed cell volume (PCV), Red blood cell (RBC), Haemoglobin (Hb), and Mixed cells (monocytes, Basophils and Eosinophils) but there were significant increases in the White blood cell (WBC), Lymphocytes, Neutrophils (NEU) and platelets (thrombocytes). There were fluctuations in the total blood protein and sugar concentrations. In conclusion, the result of this experiment showed that paraquat (trade name: Paracot) has no negative effect on the haematological status of *C. gariepinus* sub-adult after 14 days in intermittent or continuous exposure.

Keywords: Continuous, pulse exposure, blood sugar, *C. gariepinus*, static-renewal

1.0. Introduction

The growth of human population and increasing activities associated with agriculture have resulted in a staggering release of herbicides. Herbicides (e.g, paraquat) are widely used to control weeds and increase productivity in agriculture. Globally, paraquat (1, 1-dimethyl-4, 4-bipyridinium) is one of the most broadly used herbicidal product (Suntres 2002; He *et al*., 2012). Paraquat is used as a defoliant and desiccant to aid in the harvesting of cotton, beans, soybeans, potatoes, sunflowers and sugarcane (Gwathway, 2007). Due to the high solubility and repeated use of paraquat in agricultural and non-agricultural areas, large quantities of paraquat could penetrate surface water through runoff with damaging effects to the biota (Marin-Morales, 2013). Studies with paraquat has shown that paraquat negatively impacts on the blood plasma activities of *Clarias gariepinus* (Ogamba *et al*., 2014); induce respiratory stress, erratic swimming and instant death of fish (Doherty *et al*., 2011); impair the physiological processes in *Clarias gariepinus* by significantly increasing the level of white blood cells, glucose aspartate amino-transferase, and alanine amino transferase (Nwani *et al*., 2015); alter the activity of several

enzymes, affect the cardiac contraction, opercula ventilation, and embryonic development in *Plecostomus Commersoni* (Tortoreli *et al*., 1990).

Haematology is one of the key tools to assess the health status of fish (George *et al*., 2017; Satheeshkumar *et al*., 2012; Gabriel *et al*., 2007). The measurement of blood parameters can be used as a tool for monitoring the physiological condition of fish in response to changes related to nutrition, water quality, disease and other stress factors on fish health (Adams *et al*. 1996; Bell-Olusoji *et al*., 2006). Changes in haematology of fish response to stressing agents are indicators of the stress stage of fish, producing useful information to curb any unfavourable condition that may affect the fish health. Previous studies were focused on early life stages of fishes, information on sub-adults are lacking.

2.0. MATERIALS AND METHOD

2.1. Procurement and Acclimation of Experimental Fish

One hundred (100) *C. gariepinus* juveniles (weight 250 to 300g) were obtained from Agro-protein synergy, off oil mill market, Port Harcourt, Rivers State, and transported in plastics troughs to the aquaculture centre, Department of Fisheries and Aquatic Environment, Rivers State University, (RSU) Port Harcourt. In the centre, the fish were acclimated for three weeks in concrete tank. During this period, the fish were fed, tanks were cleaned and water renewed daily. The fish were fed twice daily (morning and evening) 9:00 and 18:00 hours. Feeding was suspended 24 hours before the commencement of the experiment. The fish were transferred immediately to the glass tanks after taking initial length and weight measurements and the top of aquaria were covered to prevent fish escape.

2.2. Range Finding Test

After two weeks of acclimation, a 96hrs range finding test was carried out. Paraquat-dichloride 276.g/l (Trade name: Paracot, manufactured by Hubei Xianlong Chemical Industry. Co., Ltd., China) was prepared in several concentrations representing different percentages (0, 1, 2, 2.5, 5, 10, 20, 40 and 80%) of the herbicides. Five fish were introduced in each glass trough containing 20 liter of borehole water. The required amount of paraquat (mg/l) for each percentage was added to give nominal concentrations of 0. 276, 552, 690, 1380, 2760, 5520, 11040 and 22080 mg/l. A static-renewal system where the test water was renewed and dosed every 24 hours for 96 hours was employed. Glass tanks were checked daily for behavioral changes and mortality of fish, if any mortality is noticed, fish was removed, disposed immediately and recorded. At the end of the 96 hours, there were mortalities (50-100%) in all concentrations apart from the 0, 276 and 552 mg/l concentrations were there were 100% survival, hence 552 mg/l was chosen as the sub-lethal concentration for the experiment.

2.3. Experimental Design

A complete randomized Design was used. Thirty-six *C. gariepinus* sub-adults (Total length 37.65±2.85cm and whole wet weight 362.64±95.88g mean±SD:) were allocated randomly to nine rectangular glass tanks representing three treatments, control, intermittent and continuous exposure (ie., 4 fish/tank; 12 fish/treatment). Each tank contained 30L of clean borehole water. The control tanks were not exposed (no added paraquat) while the intermittent and continuous exposure tanks were dosed to give a nominal concentration of 552mg/l paraquat. Fish were exposed according to Amachree et al., (2013), fish in the continuous exposure were exposed daily to paraquat immediately after daily water change, while the fish in intermittent exposure were exposed to paraquat for two days and returned to fresh borehole water every two days alternately for 14 days.

Prior to the experiment, the glass aquaria were soaked with 2% Nitric acid for two days, double rinsed with borehole water and dried before use. For day 0 (before exposing the treatment groups to paraquat) control group, three fish were collected and kept in a separate glass tank for one day

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ISSN (2343 – 403X);
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to adjust to experimental condition. Thereafter, fish were collected, measured (total length, cm and weight, g) and blood taken for haematology and biochemistry.

2.4. Estimation of Water Quality Parameters

During the study, the water quality parameters (temperature ℃, pH and dissolved oxygen (DO), mg/l) were measured before daily water change. Temperature and pH measurement was with a pentype meter model: 20/pay-20, while Dissolved oxygen levels were determine with a DO meter (DO 700, EXTECH instruments, Aflir, company).

2.5. Blood Sample Collection

Blood was collected by kidney puncture through the genital opening. Blood samples were collected with 5ml disposable syringe attached to a 21 guage hypodermic needle (Agary pharmaceutical, Ltd, China) for full blood count, blood protein and blood sugar

2.5.1. Full Blood Count, Plasma Protein and Blood Sugar measurements

Blood for full blood count was collected and preserved in a pre-labelled green bottle containing ethylenediaminetetraacetic acid, EDTA (Blaxhall and Daisley, 1973) and thereafter analyzed according to manufacturer's instruction using the Mindray model: BC-2800 Auto Haematology analyzer.

Blood for total protein was collected, preserved in a pre-labelled blue bottle containing Lithium and analysed according to manufacturer's instruction with the evolution 3000-semi auto biochemistry analyzer.

Blood for sugar level was collected and preserved in a pre-labelled yellow bottle (fluoride oxalate) for easy identification and kept in a cooler containing ice. Before the blood for sugar level was analyzed, the blood was brought to room temperature. A drop of blood was placed on the stripe and read on the ACCU check machine (Mannheim Germany, model: GB).

3.0. RESULTS AND DISCUSSION

3.1. Physico-Chemical Parameters of the Experimental Water

The physico-chemical parameters of the experimental water were taken weekly during the experimental period (Table 1). The results indicated that there were no significant differences ($p>0.05$) for pH for all the treatments including the control. However, there were time effects. For example, the control and continuous exposure on day 14 showed significant time effect compared to day 0 ($p<0.05$).

Temperature did not show treatment effect for all days apart of day 14 where the intermittent exposure showed a significant increase compared to the control and continuous exposure ($p<0.05$). Temperature showed a significant time effect for all days, for example all treatments on day 7 and 14 were significantly different from those of day 0.

There was a transient significant increase in the control compared to the treatments for DO. However, at the end of the experiment there was no significant difference in all treatments for DO concentration. The values were 5.01 ± 0.33, 4.26 ± 0.13 and 4.78 ± 0.59) for the control, intermittent and continuous exposure respectively.

Table 1: Water quality measurement during 14 days exposure to paraquat dichloride

Parameters	Treatments	Exposure days		
		0	7	14
pH	Control	5.53 ± 0.42**a**	6.77 ± 0.06**a#**	6.80 ± 0.20**a+**
	Intermittent	5.17 ± 0.15**a**	6.40 ± 0.26**a#**	6.53 ± 0.47**a**
	Continuous	5.47 ± 0.29**a**	6.50 ± 0.26**a#**	6.77 ± 0.15**a+**
Temperature (° C)	Control	27.87 ± 0.15**a**	31.47 ±0.55**ab#**	30.93 ± 0.55**b+**
	Intermittent	27.73 ± 0.35**a**	31.97 ± 0.15**a#**	32.77 ± 1.02**a+**
	Continuous	27.93 ± 0.23**a**	30.90 ± 0.20**b#**	31.13 ± 0.32**b+**
Dissolved oxygen (mg/l)	Control	5.02 ± 0.15**a**	6.24 ± 0.52**a#**	5.01 ± 0.33**a#**
	Intermittent	5.20 ± 0.66**a**	4.35 ± 0.46**b**	4.26 ± 0.13**b**
	Continuous	5.85 ± 0.12**a**	4.53 ± 0.05**b#**	4.78 ± 0.59**ab+**

Different letters within the exposure day indicates a significant treatment effect (ANOVA, $p<0.05$). # indicates

a significant time effect within treatment compared to the previous exposure day (ANOVA, $p<0.05$). + represents a significant time effect compared to day zero (day 0, initial fish stock) (ANOVA, $p<0.05$).

3.2. Heamatological Status of Clarias gariepinus sub-adults during sub-lethal exposure to paraquat

3.2.1. Full Blood Count

The results of the full blood count is shown on Table 2. There were no significant difference among the treatments for PCV, RBC, Hb, Mixed cells (i.e., monocytes, basophils and eosinophils) but there were some time-dependent effects.

At the end of the experiment, WBC and neutrophils showed a significant increase in the intermittent exposure compared to the control and the continuous exposure. There were time-dependent effect, for example, on day 14 all treatments were significantly increased compared to day 0 ($p<0.05$).

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Table 2. Heamatology of Clarias gariepinus sub-adult during 14 days exposure to paraquat

Parameters	Treatments	Exposure days		
		0	**7**	**14**
Packed cell volume (%)	Control	38.33 ± 2.08	32.00 ± 5.66**a**	36.00 ± 5.66**a**
	Intermittent		29.00 ± 7.07**a**	30.00 ± 1.41**a**+
	Continuous		30.00 ± 1.41**a**#	41.00 ± 2.83**a**+
White blood cells (10^{12} cells l^{-1})	Control	229.33 ± 4772.14	80.60±19516.15**a**#	63.15. ± 5586.14**a**+
	Intermittent		49.75 ± 33021.89**a**	88.75 ± 10535.89**b**+
	Continuous		73.85 ± 22132.44**a**#	64.10 ± 13152.19**a**+
Red blood cells (10^{12} cells l^{-1})	Control	2.40 ± 0.49	1.66 ± 0.88**a**	2.81 ± 0.11**a**+
	Intermittent		1.56 ± 1.30**a**	3.12 ± 0.28**a**
	Continuous		1.90 ± 0.71**a**	2.78 ± 0.08**a**+
Haemoglobin (%)	Control	12.78 ± 0.69	10.67 ± 1.89**a**	12.00 ± 1.89**a**
	Intermittent		9.67 ± 2.36**a**	10.00 ± 0.47**a**+
	Continuous		10.00 ± 0.47**a**#	13.67 ± 0.94**a**
Lymphocytes (%)	Control	45.13 ± 8.91	74.25 ± 15.63**a**#	60.35 ± 8.41**a**
	Intermittent		32.45 ± 24.11**b**	13.85 ± 3.46**b**+
	Continuous		30.60 ± 26.16**b**	52.75 ± 7.00**a**
Neutrophils (%)	Control	42.23 ± 5.72	20.60 ± 11.60**a**#	29.30 ± 12.59**a**
	Intermittent		53.60 ± 32.53**a**	79.50 ± 2.40**b**+
	Continuous		54.55 ± 33.59**a**	37.40 ± 6.93**a**
Platelets (10^{9} cells l^{-1})	Control	15.00 ± 7.00	24.00 ± 0.00**a**	24.50 ± 0.71**a**+
	Intermittent		27.00 ± 21.21**a**	8.00 ± 5.66**b**
	Continuous		49.00 ± 42.43**a**	29.00 ± 0.00**c**+
Mixed cells i.e., Monocytes, Basophils and Eosinophils (%)	Control	12.60 ± 3.28	5.15 ± 4.03**a**#	13.30 ± 4.24**a**
	Intermittent		13.95 ± 8.41**a**	6.65 ± 1.06**a**+
	Continuous		14.85 ± 7.42**a**	12.85 ± 4.17**a**

Different letters within the exposure day indicates a significant treatment effect (ANOVA, $p<0.05$). # indicates a significant time effect within treatment compared to the previous exposure day (ANOVA, $p<0.05$). + represents a significant time effect compared to day zero (day 0, initial fish stock) (ANOVA, $p<0.05$).

There were both treatment and time-dependent significant difference for lymphocytes. On the day, the control showed a significant increase compared to the intermittent and continuous exposures. However, at the end of the experiment (day 14) the intermittent exposure showed a significant decreased (13.85 ± 3.46 %) compared to the

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control (60.35 ± 8.41 %), continuous (52.75 ± 7.00 %) and day 0 (45.13 ± 8.91 %).

There were no significant difference among treatments for platelets on day 7. However, at the end of the experiment, the treatments including control were significantly different from each other (Table 2).

3.2.2. Plasma Protein

The result of the plasma total protein is showed in Figure 1. There was no treatment dependent effect, at the end of the experiment the values were 19.05 ± 8.13, 18.05 ± 4.45 and 52.90 ± 45.40 for control, intermittent and continuous exposure respectively. There were some time-dependent difference, at the end of the experiment the control and the intermittent exposure were significantly decreased compared to the day 0 (37.70 ± 7.78 mg ml^{-1}).

4.2.3. Blood sugar

Unlike the total protein, the blood sugar showed a transient significant decreased in the treatments compared to the control. However, at the end of the experiment, there were no significant time dependent differences in all treatments including the control, values were 3.36 ± 1.18, 4.30 ± 1.84 and 3.77 ± 0.31 mmol l^{-1} for control, intermittent and continuous exposure respectively (Figure 2).

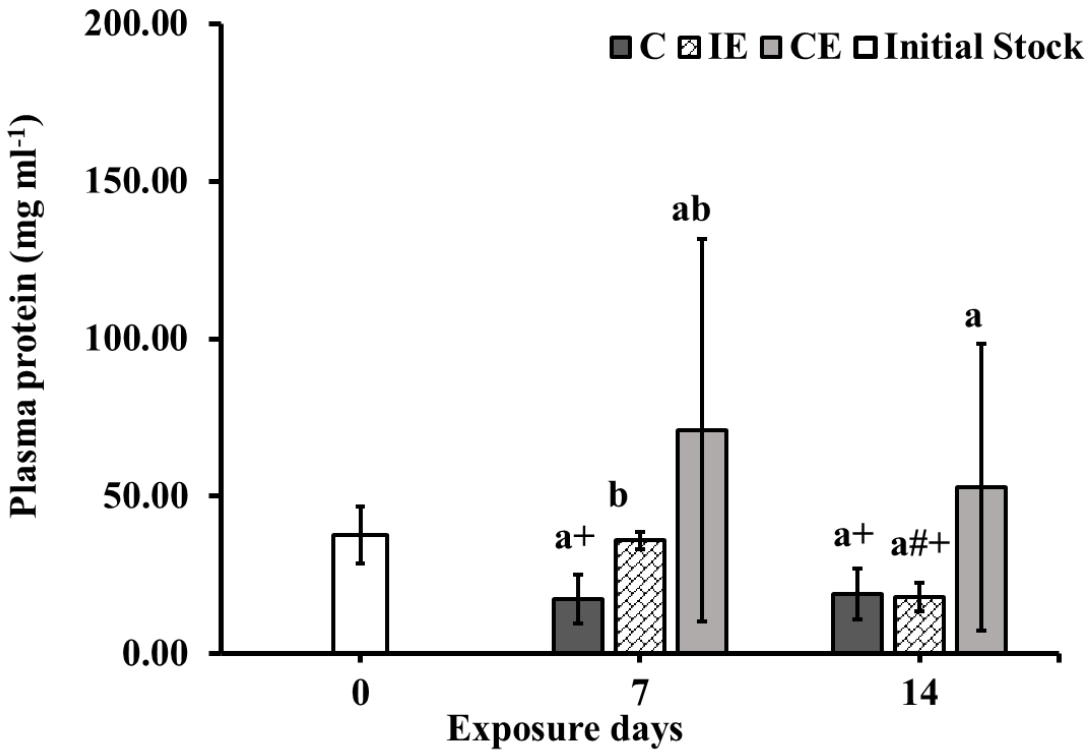

Figure 1. Plasma total protein concentration of *Clarias gariepinus* sub-adult during 14 days exposure to paraquat. Data are means ± SD, n=3. Different letters within the exposure day indicates a significant treatment effect (ANOVA, $p<0.05$). # indicates a significant time effect within treatment compared to the previous exposure day (ANOVA, $p<0.05$). + represents a significant time effect compared to day zero (day 0, initial fish stock) (ANOVA, $p<0.05$).

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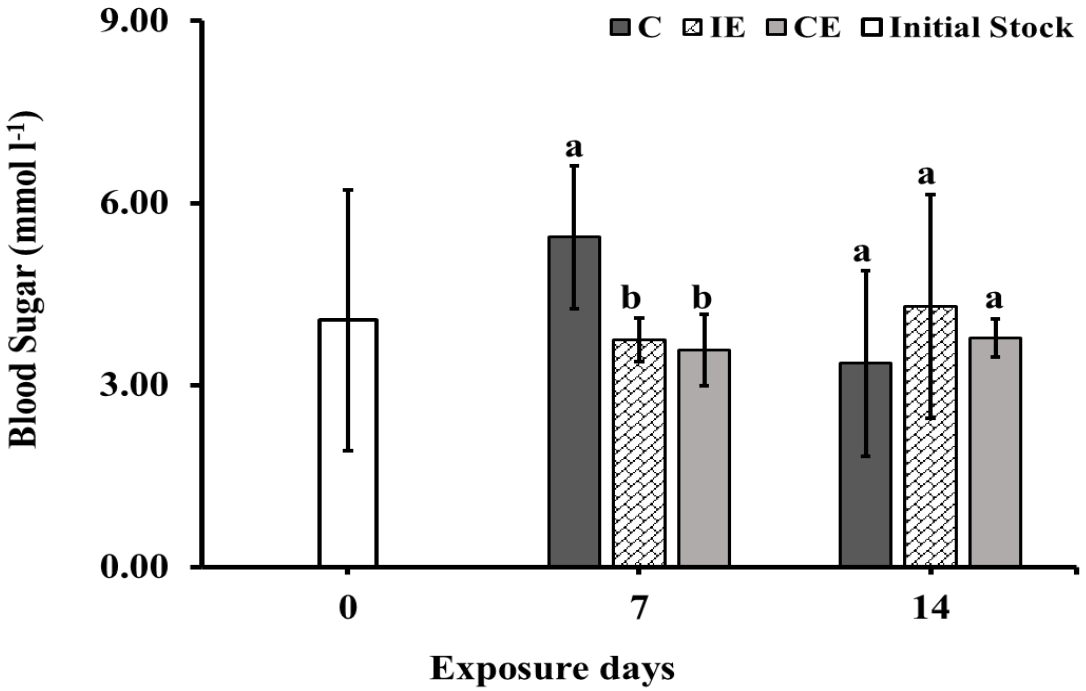

Figure 2. Blood Sugar concentration of *Clarias gariepinus* sub-adult during 14 days exposure to paraquat. Data are means ± SD, n=3. Different letters within the exposure day indicates a significant treatment effect (ANOVA, $p<0.05$). # indicates a significant time effect within treatment compared to the previous exposure day (ANOVA, $p<0.05$). + represents a significant time effect compared to day zero (day 0, initial fish stock) (ANOVA, $p<0.05$).

4.0. DISCUSSION

4.1. Physico-Chemical Parameters of the Experimental Water

The results of the physico-chemical parameter of the experimental water indicates that paraquat did not have adverse negative effect of the water quality. The results are within the acceptable values in the study area.

4.2. Heamatological Status of C. gariepinus sub-adults during sub-lethal exposure to Paraquat

4.2.1. Full blood count

Haematological parameters are routinely used for the evaluation of physiological, environmental and husbandry stressors in fishes. In recent years good management practices have been advocated as effective ways of reducing stress in aquaculture (Gabriel, *et al*., 2007). Several factors have been reported to affect haematological responses in fish. These include sex, age, size, environmental and physiological conditions. Exposing fish to pollutant can cause stress in fish, which can be evaluated from their blood analysis (Patti and Kulkarni, 1993). At the end of the experiment in the present study, the result did not show any significant difference in all treatments including the control in all the haematological parameters measured apart from the WBC, lymphocytes, neutrophils and platelets. The WBC, lymphocytes, Neutrophils and

platelets represent an important function in the cell immunity of fish (Thrall *et al*., 2007). The differences as compared to the control could be as a result of innate responses of the fish to the presence of the paraquat. According to Svobodova *et al.* (2006) lymphocytes were reduced in fish exposed to pesticides.

4.2.2. Plasma Protein

There were no differences in plasma protein for all treatments. However, Osman *et al*. (2009) and Nkpondion *et al*. (2016) reported reduction in protein content of the blood of *Clarias gariepinus* exposed to different pollutants. According to Alkahem *et al*. (1998) total protein in blood reduces as a result of the fish been under stress as it mobilizes protein from body to meet up energy loss as a result of biotransformation and excretion of strange chemicals.

4.2.3. Blood Sugar

In this present study, there was a significant transient decrease in the blood sugar level on day 7. However at the end of the experiment, blood glucose levels were not different in all treatment. This is contrary to the report of Kori-Siakpere *et al.* (2007) who reported increase in plasma glucose of fish with time of exposure and concentration of paraquat.

4.3. Conclusion

In conclusion, the result of this experiment showed that paraquat dichloride 276mg/l (trade name: Paracot) did not impact negatively on the haematological status of *C. gariepinus* sub-adult after 14 days exposure. This might be as a result of the size of fish used as earlier works reported have used early life stages of *Clarias gariepinus*.

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