



HAEMATOLOGICAL RESPONSES OF AFRICAN CATFISH (*Clarias gariepinus*, BURCHELL, 1822) JUVENILES EXPOSED TO ACUTE CONCENTRATIONS OF BUTACHLOR (HERBICIDE).

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ABSTRACT

The possible changes in the haematological parameters of *Clarias gariepinus* juveniles exposed to acute concentrations of butachlor was examined in this study. One hundred and twenty juveniles of *C. gariepinus* were used for the study. The acute toxicity bioassay was a completely randomized design consisting of six treatments, with varying concentrations of butachlor (0.0, 0.6, 0.7, 0.8, 0.9, and 1.0 mg/l) all in duplicates. The post-exposure haematological parameters results revealed that the exposed fish showed significant ($p < 0.05$) progressive reduction in packed cell volume (PCV), haemoglobin (Hgb), total protein (TP) and total red blood cells count (TRBC) as the concentration of the toxicant increases. However, the values of total white blood cells count (TWBC), tends to increase with an increase in concentration of the toxicant. The absolute blood indices, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), showed fluctuating values though no significant differences ($p > 0.05$) was observed among the treatments.

Keywords: Acute, Butachlor, *Clarias gariepinus* Haematology, Toxicity

INTRODUCTION

Water contamination due to the application of agrochemical pesticides (Herbicides in particular) is regarded as one of the major challenges to the conservation of aquatic ecosystems (Umar and Aisami, 2020; Umar and Shukor 2020). The increase in the usage of these chemicals has been necessitated by ever-increasing global population with the consequential effect of increase in demand for food production (Ani *et al.*, 2018). Currently, farmers rely so much on pesticides (herbicides) for greater harvest and most often apply them excessively beyond or against manufacturers' recommendation with no worries about possible short- and long-term ecological effects (Ani *et al.*, 2018). Herbicides are harmful chemicals introduced into the environment to eradicate weeds and they end up in the natural water bodies, and their contamination of aquatic system has attracted the attention of researchers worldwide (Dutta and Dalal 2008). Aquatic pollution is a global and major problem of this century owing to the addition of various pollutants in water bodies through many anthropogenic ways and the subsequent changes in the natural qualities of water (Voltz *et al.*, 2005). The changes in water qualities due to pollutants adversely affect non-target organisms especially fish and this leads to massive mortalities in acute and chronic exposure (Velcheva *et al.*, 2012, Sabae *et al.*, 2014)

Fishes are more frequently exposed to pollutants because it is believed that regardless of where the pollution occurs, it will eventually end up in the aquatic environment. They also represent one of the key

elements to evaluate the ecological status of the open water bodies (Scardi *et al* 2008, Hermoso *et al* 2010).

Though, toxicity testing in organisms involves a multi-marker approach such as body mass indices, food conversion ratio, specific growth rate, hepatosomatic index (HSI), gonado-somatic index (GSI) (Kesbic *et al.*, 2020; Parino *et al.*, 2020), however hematological parameters are considered the most promising (Parino *et al.*, 2018; Fazio *et al.*, 2019). The use of haematological parameters is gaining more attention for toxicological research, environmental monitoring and assessment of fish health conditions (Akinwole *et al.*, 2016, Shah and Altindag, 2004). This is because blood parameters change very fast with the happenings around the fish, they are considered pathophysiological indicators of the whole body and therefore are important in diagnosing the structural and functional status of fish exposed to toxicants (Maheswaran *et al.*, 2008).

Butachlor ($C_{17}H_{26}ClNO_2$) is a chloroacetanilide herbicide which was originally developed by Monsanto Co. (USA) in 1968 and commonly used as a pre-emergence herbicide in Asia and Africa (Liu *et al.*, 2011). It is reportedly produced by the reaction of chloroacetyl chloride with azomethine 2, 6-diethylaniline and formaldehyde, followed by treatment with n-butanol (Dwivedi *et al.*, 2012).

The aim of the present study is to investigate the possible changes in the blood parameters that the herbicide can cause to the to the exposed fish.

MATERIAL AND METHODS.

Preparation of Test Solution

A commercially available herbicide Butatex® with Butachlor as an active ingredient was used for the study. The herbicide was purchased from an agrochemical shop in Sabon-gari market, Zaria. Stock of the test chemical was prepared by dissolving 1 mg of Butachlor in 1 litre of test water in a conical flask in accordance with Dede and Kagbo (2001).

Range finding test was carried out to check for the concentration of the herbicide that was used for the definitive tests. This was done by placing five nominal concentrations (0.5, 1.0, 1.5, 2.0 and 2.5 mg/l) of the herbicide in separate tanks and ten fishes were stocked in each tank, mortality of the stocked fish was observed at 0, 12, 24, 48, 72, and 96 hours. The concentrations were graded using lower ranges until 80-90% mortality was recorded in the highest concentration and 20-30% for the lowest concentration as suggested by Martins *et al.* (2008).

Acute Toxicity Test

A four-day static toxicity bioassay was conducted in the Fisheries Laboratory of the Department of Biological Sciences Ahmadu Bello University, Zaria, using juveniles of *C. gariepinus*. The experiment was a completely randomized design with five treatments and a control in duplicates, (0.0, 0.6, 0.7, 0.8, 0.9 and 1.0 mg/l). Butachlor was added to each glass tank (30 cm x 30 cm x 45cm) containing 20l of de-chlorinated tap water. The addition was done for varying concentrations using a syringe and allowed to stand for 10 minutes for the toxicant to be evenly distributed. Ten fishes were allotted to each tank.

Water quality monitoring.

Selected water quality parameters were monitored every twenty-four hours in all the tanks. Temperature, electrical conductivity, total dissolved solids and pH were determined using a pen type Hanna instrument (Model- HI 98129, HI 98130) while dissolved oxygen was determined using modified Winkler's reagents (APHA, 2005).

Blood sampling

Blood was collected from the fish by severing the caudal peduncle following the method described by Blaxhall and Diasely (1973), and the following blood parameters were determined

Packed cell volume (PCV)

Packed Cell Volume (PCV) was determined using a micro-westegren method as described by Blaxhall and Daisely (1973). The reading was taken with the aid of a micro-haematocrit reader and expressed as the volume of the erythrocytes per 100cm³.

Total white blood cells Count (TWBC)

Total white blood cells count was done using Shaw's solution A (neutral red (25mg), sodium chloride (0.9g), distilled water (100mls) and B (crystal violet (12mg), sodium citrate (3.8g), distilled water (100ml). The blood was drawn up to the 0.5 mark on the stem of a white cell pipette. Solution A was drawn to shake the bulb of the pipette halfway and then filled to 101, mark with solution B. A few drops were then dispensed into the haemocytometer. The cells in the four large squares of the chamber (Hesser, 1960) were counted with 4mm objective and X40 eyepiece microscope. The number of cells was multiplied by 500 to obtain the total number of white blood cells per cubic millimetre (mm³) of blood (Hesser, 1960).

Total red blood cells count (TRBC)

Hendricks solution was used for the TRBC, blood was drawn just beyond 0.5 mark of the haemoglobin pipette wiped with cotton wool and adjusted the volume to exactly 0.5 mark. The pipette was filled to 101, mark with the diluting fluid and shaken for 30 minutes to ensure thorough mixing. The diluted suspension of cells was thereafter drawn into the Neubauer's haemocytometer chamber. The haemocytometer was placed under the microscope and the cells within the boundaries of five small squares of the haemocytometer (Hesser, 1960) were counted with 4mm objectives and X 40 eyepiece of the microscope. The number of cells was multiplied by X10 and this gave the total number of cells per cubic millimetre (mm³) of blood (Hesser, 1960).

Differential Leucocyte Count (DLC)

Two drops of blood were positioned on a slide, made into a thin smear with another slide and left to dry. The smear was fixed with absolute methanol, then stained with Giemsa's stain and 170 buffered distilled water. It was allowed to stand for about 20-30 minutes after which the slide was rinsed again with buffered distilled water and allowed to air-dried. Counting was made by the use of a microscope and the parameters counted include neutrophils, lymphocytes, basophils, eosinophils, monocytes and band.

MCV, MCH and MCHC

The values of MCV, MCH and MCHC were determined using the formulae below (Ghai,1986)

$$\text{MCV} = \frac{\text{PCV} \times 10}{\text{TRBC}}$$
$$\text{MCH} = \frac{\text{Hb} \times 10}{\text{TRBC}}$$
$$\text{MCHC} = \frac{\text{Hb} \times 100}{\text{PCV}}$$

Data Analyses

The data obtained from hematological was subjected to one way analysis of variance (ANOVA) at $p < 0.05$ alpha level using SAS version 9.0. Where significant differences was observed, Duncan's Multiple Range Test (DMRT) was employed to separate the means.

RESULTS

Fish Mortality was recorded in all the exposed groups, whereas no mortality was recorded in the control (table 1), the recorded mortality was found to be dose – dependent. The highest mortality rate 16 (80%) was recorded at the highest concentration (1mg/L) and the lowest concentration (0.6mg/L) has the lowest mortality rate 5 (25%).

The acute effects of butachlor on some haematological parameters of *C. gariepinus* juveniles are presented in Table 2, Packed cell volume, (PCV) haemoglobin, (HGB) and total red blood cells count (TRBC) of the control group were significantly higher ($P < 0.05$) than the exposed groups. The values of PCV, HGB, and TRBC were found to decrease with an increase in the concentration of the toxicant. The values of total white

blood cells count (TWBC) of the exposed groups were significantly higher ($P < 0.05$) than the control group. Similarly, it was also observed that as the concentration of the toxicant increased, the values of TWBC also increased. Total protein was found to be higher in the control group than the exposed groups, though there was no significant difference ($p > 0.05$) in the values. Neutrophils and lymphocytes also show fluctuating values though not significant with the control group having the highest values. Eosinophils, monocytes, basophils were all not detected, whereas mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration also showed mixed trend values though these values were not significantly ($P > 0.05$) different. The highest values of MCV and MCH (62.00 ± 1.98 and 20.50 ± 0.68) were recorded in the concentrations 0.9 mg/L and 0.7mg/L respectively. The least value of MCV 55.00 ± 1.9797 was recorded at the highest concentration (1.00 mg/L), MCH was lowest in the exposed groups (0.6 and 0.7mg/L). Similarly, the least value of MCHC was at the control group though there was no significant difference ($p > 0.05$) between the control and the exposed groups (Table 2).

Table 1: Mortality rate, percentage mortality and probit kill values of *C. gariepinus* juveniles exposed to acute concentrations of butachlor

Concentrations (mg/l)	Log of Conc.	No. Exposed	Mortality	% mortality	Probit kill
0.00	0.000	20	0	0	0.00
0.60	-0.221	20	5	25	4.33
0.70	-0.155	20	7	35	4.61
0.80	-0.096	20	9	45	4.87
0.90	-0.045	20	13	65	5.40
1.00	0.000	20	16	80	5.84

Table 2: Acute effects of butachlor on some haematological parameters of *C. gariepinus* juveniles

Conc. (mg/l)	PCV (%)	HGB (g/dl)	TWBC ($\times 10^9/l$)	TRBC ($\times 10^{12}/l$)	TP (g/dl)	NEUTR. (%)	LYMPH. (%)
Control	39.00 ^a	12.80 ^a	12.15 ^c	6.45 ^a	4.20 ^a	12.50 ^a	82.50 ^a
0.60	31.50 ^b	10.45 ^b	12.45 ^c	5.35 ^b	3.55 ^a	11.00 ^a	85.00 ^a
0.70	30.00 ^{bc}	10.00 ^{bc}	14.05 ^{bc}	5.15 ^b	3.10 ^a	9.50 ^a	84.50 ^a
0.80	28.50 ^{bcd}	9.50 ^{bc}	15.15 ^b	4.90 ^{bc}	2.80 ^a	11.00 ^a	88.00 ^a
0.90	28.00 ^{cd}	9.30 ^c	15.15 ^b	4.50 ^c	2.90 ^a	11.00 ^a	86.50 ^a
1.00	26.50 ^d	8.80 ^c	19.10 ^a	4.55 ^c	3.60 ^a	16.50 ^a	89.50 ^a
SEM	0.90	0.33	0.60	0.13	0.51	2.15	2.71

Means with the same superscript along the columns were not significantly different ($P > 0.05$)

Key: PCV= packed cell volume, Hgb = haemoglobin, TWBC= total white blood cells, TRBC= total red blood cells, TP= total protein, NEUTR= neutrophils, LYMP= lymphocytes,

Table 2 cont'd: Acute Effects of Butachlor on Red blood cells indices of *C. gariepinus* Juveniles.

Conc. (mg/L)	MCV (fL)	MCH (pg)	MCHC (%)
0.00	60.30	19.75	32.50
0.60	58.75	19.30	33.00
0.70	58.25	19.30	33.00
0.80	58.25	19.40	33.00
0.90	62.00	20.50	33.00
1.00	55.00	19.40	33.00
SE	1.10	0.70	0.21

Means with the same letter superscript along the columns are not significantly different ($P>0.05$)

MCV= mean corpuscular volume, MCH= mean corpuscular haemoglobin MCHC= mean corpuscular haemoglobin concentration.

DISCUSSION

Mortality rates of the exposed groups during the first 24 hours were highest at all concentration levels but declined as exposure times increased. This trend may be as a result of the fact that the fish tend to have developed immunity against the toxicant as exposure time progressed mainly due to the increase in the production of white blood cells. This was also reported by Alagoa *et al.* (2015) when they assess the acute toxicity of the herbicide uproot® (Isopropylamine salt) on the survival of juveniles of *Clarias gariepinus*

Exposure of *Clarias gariepinus* to acute concentrations of butachlor caused a significant decrease in packed cell volume (PCV), Haemoglobin (Hgb), and total red blood cells count of the fish. The significant reduction in these blood parameters is an indication of severe anaemia that might have been caused by the destruction of red blood cells (Kori-Siakpere *et al.*, 2009), and haemodilution resulting from impaired osmoregulation across the gill epithelium (Adeyemo, 2005; Aderolu *et al.*, 2010; Okomoda *et al.*, 2010). Gaafar *et al.* (2010) reported that prolonged reduction in haemoglobin content might be deleterious to oxygen transport and degeneration of the erythrocytes could be due to toxicological conditions in fish exposed to Butachlor. The anaemic condition caused by the reduction in total red blood cells count could also be attributed to erythropoiesis and osmoregulatory dysfunction (Jenkins and Smith, 2003). The decreased values of haemoglobin concentration, packed cell volume and erythrocyte in the present study could also be attributed to the inability of the fish to deliver sufficient oxygen to hematopoietic tissues suggesting poor physical activity (Hussain *et al.*, 2014).

The reduction in haemoglobin in Butachlor exposed fish could be due to the destruction of red blood cells as red blood cells are carriers of haemoglobin (Kori-Siakpere *et al.*, 2009). However, white blood cells (WBC) count was found to increase with increasing level of the toxicant, this increase is likely due to increased immune mechanism of the exposed fish species stimulated to fight against the toxicant (Ayuba and Ofojekwu, 2002; Solomon and Okomoda, 2012). In the present study, the decreased values of serum total proteins in the exposed groups might be due to the direct effect of the utilization of body's available protein to meet the required energy demand in trying to overcome the stress caused by the toxicant (El-Sayeed *et al.*, 1996; Bayero, 2017).

The red blood cells indices MCV, MCHC and MCH are important biomarkers in the diagnosis of anaemia in animals (Sharaf *et al.*, 2010; Ghaffar *et al.*, 2014). However, fluctuations in these haematological (MCH, MCV and MCHC) values found in the present study though, not significant could be due to toxic effects of butachlor as also supported by lower values of erythrocyte, haemoglobin and packed cell volume in the present study (Kori-Siakpere, 2011).

CONCLUSION

The acute exposure of butachlor to juveniles of *Clarias gariepinus* revealed a significant reduction in packed cell volume (32%) total red blood cells, (29.45%) and haemoglobin (31.25%) which are clear signs of anaemia, whereas total white blood cells increased with (36.40%) which implies that the exposed groups (0.6, 0.7, 0.8, 0.9, and 1.0 mg/l) were affected by the toxicant and hence the reduction in the values of PCV, TRBC and HGB as well as increase in the values of TWBCs.

RECOMMENDATION

Since Butachlor as a herbicide causes serious reduction in the major blood parameters which can lead to death of the fish; it's usage by farmers especially near waterbodies should be monitored so as to prevent mass mortality to non-target organisms especially fish.

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