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Haematological effect of chlorfenapyr-exposed freshwater African mud catfish, *Clarias gariepinus* (Burchell 1822)

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ABSTRACT

The effects of chlorfenapyr pesticide were investigated on juvenile of Clarias gariepinus. Sub-lethal test was carried out using 5, 7, 9, 11 and 15 mgL^{-1} of chlorfenapyr pesticide for 96 hours, and peripheral blood was collected through the caudal vein and examined for haematological changes. There was significant reduction ($P<0.05$) in WBC when compared to the control. RBC increase was directly proportional to increase in chlorfenapyr dose and significantly differed from control ($p<0.05$). HGB increased in a dose-dependent manner. MCV values of all treatment groups, except 15 mgL^{-1}, were higher when compared to control. MCH was not significantly different from control. PLT decreased with increase in concentration and was lower for all treatment when compared with control. Results obtained in this study suggest that exposure to sub-lethal concentrations of chlorfenapyr can alter the haematological indices of non-target organisms.

Introduction

Pesticides are used globally for the control of pests and weeds during agricultural practices, and in the establishment and maintenance of lawn and recreational area (Helfrich *et al.*, 2009). Not disputing their benefits both domestically and industrially, it poses deleterious environmental and public health concerns (Ada *et al.*, 2011; Ada *et al.*, 2012; Ada *et al.*, 2013). Insecticides, as one of the widely used pesticides, can stimulate immune response (Pawar and Bhilave, 2019) and can induce deleterious histological, physiological and genetic effects (Olateju, 2019). According to Adewumi *et al.* (2018), the unregulated use and discharge of insecticides into aquatic environment have caused ecological problems to virtually all classes of aquatic organisms. Over time, its bioaccumulation may create potential health threat in higher trophic level, decreasing acetylcholinesterase activities, negatively affecting respiration, nervous coordination (Oh *et al.*, 1991; Adedeji *et al.*, 2008) and showing loss of equilibrium in fish (Auta and Ogueji, 2008; Nwani and Echi, 2013).

Chlorfenapyr, a pyrrole, is a newly synthesized insecticide against disease vector (Raghavendra *et al.*, 2011). Pyrroles are broad-spectrum pro-insecticides,

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which when activated by mixed function oxidases, causes the disruption of the respiratory pathways and proton gradients by uncoupling oxidative phosphorylation in mitochondria of the targeted organisms (Black *et al.*, 1994). This unique mechanism of metabolic pathway disruption in the mitochondrion seems not to exhibit cross-resistance, unlike the standard neurotoxic insecticides, against insects such as *Anopheles gambiae, Anopheles funestus, Culex quinquefasciatus* and *Cimex* spp (Berner, 1994; Mosha *et al.*, 2008; Tawatsin *et al.*, 2011), or beet armyworm (*Spodoptera exigua*) (Che *et al.*, 2013). Although, a cross-resistance to high level of pyrethroid, enhanced by esterase-mediated-resistance mechanism, has been reported in *Helicoverpa armigera* larvae (Gunning and Moores, 2002). The authors similarly observed stomach toxicity in the exposed cotton insects (*H. armigera*).

Chlorfenapyr was suggested a preferred alternative to synthetic pyrethroids since it is less toxic to aquatic vertebrates including mammals (Ingham *et al.*, 2012). However, considering that the biological features of fish are rapidly affected on exposure to stressors (Sahan *et al.*, 2007), therefore, quantifying some features in fish exposed to chlorfenapyr may serve as indicators and biomarkers that allow for rapid assessment of the health status and provide toxicity indicator about environmental pollution. The assessment of alterations in haematological parameters during exposure to toxicants in aquatic vertebrates is considered a potent biomarker of exposure (Blaxhall and Daisley, 1973; Oost *et al.*, 2003; Alimba and Laide, 2019; Alimba *et al.*, 2019). Blood is an important medium for systemic circulation in fish, therefore alterations in heamatological indices in response to water contamination is a sensitive biomarker of fish health (Seriani *et al.*, 2015; Alimba *et al.*, 2019). Generally, alterations in haematological parameters of fish precedes the onset of any morphological and degenerative damage (Mazon *et al.*, 2002). Also, the blood is a vehicle necessary for rapid mobilization against foreign substances introduced into the body, defending against allergies, inflammation, trauma and diseases (Bala *et al.*, 2019).

Clarias gariepinus (freshwater mud catfish), the most consumed fish in Nigeria and many other African countries (Conceicao *et al.*, 1998; Aderolu *et al.*, 2017; Marimuthu *et al.*, 2019; Adeniyi *et al.*, 2021), has been widely used as suitable fish model for assessing the hematological alterations induced by individual and mixture of metals (Adeyemo, 2007), insecticides (Adedeji *et al.*, 2008), and chemicals in effluent (Alimba *et al.*, 2019). This study investigated alterations in haematological parameters of *C. gariepinus* exposed to sub-lethal concentrations of chorfenapyr.

Materials and Methods

Fish collection and acclimatization

Juveniles of *C. gariepinus* (Burchell, 1822) (Family: Clariidae, Order: Siluriformes) were procured from the Tilapia Resort in Abak Local Government Area and were transported in fresh water to the Department of Animal and Environmental Biology Laboratory, University of Uyo. The fishes were acclimatized for 48 hours and fed *ad libitum* with commercial feed (Coppens commercial feed, Coppens International Helmond, Netherlands) containing 35% crude protein. To maintain hygiene, the water was renewed every 48 h to prevent the accumulation of metabolic wastes and unconsumed food particles.

Experimental procedure and preparation of chlorfenapyr stock solution

Chlorfenapyr was obtained from the University of Uyo Insectary Laboratory and used to prepare the stock solutions. 500 mg of chlorfenapyr was measured and dissolved in 10 L of distilled water to give a stock solution of 50 mgL^{-1}. Five different concentrations; 5, 7, 9, 11 and 15 mgL^{-1}, prepared in accordance with previous study (Esenowo *et al.*, 2021), wherein the genotoxicity of chlorfenapyr was determined in the same size *C. gariepinus*, were used for the sub-lethal exposure. Ten (10) fish per group, randomly selected from the acclimatized stock, were used for the acute toxicity study which lasted for 96 h. Dechlorinated tap water was used as the negative (-ve) control. The length and weight of fish were measured in all the treatment and control groups.

Blood sampling and analysis

Blood samples were collected randomly from five fishes per group using heparinized syringe and hypodermic needle, into ethylenediamine tetraacetic acid (EDTA) solution precoated sample bottles. The haematological indices were measured in accordance with standard methods (Blaxhall and Daisley, 1973; Wedemeyer *et al.*, 1977). Briefly, the improved Neubauer haemocytometer (Dacie and Lewis, 1991) was used to measure the Red blood cell (RBC), Leucocytes (LCT) and thrombocyte count. The improved Neubauer counter was used to determine the white blood cells (WBC), while blood film stained with May-Grunwald-Giemsa stain (Mirale, 1982) was used to determine the differential counts such as neutrophils, lymphocytes and monocytes. Packed cell

volume (PCV) was determined by the microhaematocrit method, while the cyano-haemoglobin method was used to determine haemoglobin (Hb) concentration using diagnostic kits from Sigma diagnostics, USA. The blood parameters evaluated were blood haemoglobin (HGB), red blood cell (RBC), white blood cell (WBC), platelet (thrombocyte) (PLT) and pack cell volume (PCV). The mean cell haemoglobin (MCH) and mean cell volume (MCV) values of the red blood indices were calculated according to Brown (1980) using the formula:

$$MCH = Hb \times 10/RBC$$
$$MCV = PCV \times 10/RBC$$

Statistical analysis

Data obtained from the experiments were collated and subjected to ANOVA using MS Office Excel Package, version 10 and the differences among means tested at $p<0.05$. Results were expressed as means ± standard deviation.

Results

Length and Weight Measurement

The length (cm) and weight (g) of *C. gariepinus* were measured before and after treatment respectively and are presented in Table 1, respectively. Except in 5 mgL^{-1}, all other groups showed average increase in length of *C. gariepinus* against the slight increase recorded in the control. Similarly, there was an average weight gain by fish in all treatment groups except 7 mgL^{-1}. Increase in average length of the fish was directly proportional to increase in the average weight. Fishes exposed to 11 mgL^{-1} (10.66%) and 9 mgL^{-1} (22.08%) recorded highest increase in length and weight, respectively. Notably, increase in the average length and weight of fish was not concentration dependent.

Haematological Parameters

Table 2 shows the blood parameters of juveniles of *C. gariepinus* exposed to sublethal concentrations of chlorfenapyr. In this study, there were significant variations in WBC, RBC, HGB, HCT, MCV, MCH and PLT observed. The control group had the highest WBC mean value of 193.23±1.18 while the exposed group 5 mgL^{-1} had the least mean value 166.76±5.05. Chlorfenapyr induced decrease in WBC of exposed fish, however, this was not concentration dependent. Highest mean RBC value of 2.68±0.10 was recorded from 15 mgL^{-1} while group 7 mgL^{-1} recorded the least mean value of 0.82 ±1.02. HGB had the highest value of 11.60±0.31 recorded in 15 mgL^{-1} concentration whereas 5 mgL^{-1} had the least value of 2.70±5.0. 5 mgL^{-1} group recorded the least HCT mean value of 7.87±17.43 while 15 mgL^{-1} had the highest value of 36.07±0.12. Mean values of 126.97±0.17 and 166.2 ±0.17 were recorded as the least and highest MCV for 15 mgL^{-1} and 9 mgL^{-1}, respectively. Mean values of 40.10 ±0.70 and 47.40 ±0.21 were recorded as the least and highest MCH for 7 mgL^{-1} and 9 mgL^{-1}, respectively. As shown in Figure 1, there was a trend of decrease in PLT level with increase in concentration of chlorfenapyr. This observation is contrary to the observation recorded for RBC, which indicated an increase with increase in chlorfenapyr concentration. With increase in the concentration of chlorfenapyr, MCV peaked at 9 mgL^{-1} and declined.

Table 1. Mean and percentage increase in the length and weight of *C. gariepinus*.

Concentration (mgL^{-1})	Initial length (cm)	Final length (cm)	Mean Length (cm)	%	Initial weight (g)	Final weight (g)	Mean weight (g)	%
control	29.70	30.20	29.95±0.25	1.66	172.6	174.0	173.3±0.70	0.80
5	30.90	30.25	30.58±0.33	-2.15	169.0	190.5	179.75±10.75	11.29
7	31.75	34.50	33.13±1.38	7.97	188.0	178.0	183.00±5.00	-5.62
9	27.50	30.40	28.95±1.45	9.54	150.0	192.5	171.25±21.25	22.08
11	27.25	30.50	28.88±1.63	10.66	128.5	153.0	140.75±12.25	16.01
15	32.50	35.50	34.00±1.50	8.45	228.5	282.0	255.25±26.75	18.97

Table 2. Hematological parameters (Mean ± SD) of *C. gariepinus* exposed to sublethal concentration of chlorfenapyr.

Toxicant Conc.	WBC ($x10^3/mm^3$)	RBC ($x10^6/mm^3$)	HGB	HCT (%)	MCV (μ^3)	MCH (%)	PLT
Control	193.23±1.18^b	2.1167 ±0.03^a	8.83 ±0.22^{ab}	28.47±0.64^b	135. ±1.53^{ab}	41.27 ±0.62^{ab}	15.3 ±12.3^a
5 mgL^{-1}	166.76±5.05^a	1.85 ±0.06^a	2.70 ±5.0^a	7.87±17.43^a	140.07 ±5.0^b	40.90 ±0.30^a	13.33 ±2.33^a

Toxicant Conc.	WBC ($x10^3/mm^3$)	RBC ($x10^6/mm^3$)	HGB	HCT (%)	MCV (μ^3)	MCH (%)	PLT
7 mgL^{-1}	172.20±8.65^a	0.82 ±1.02^a	6.57±0.94^{ab}	10.47±14.4^a	141.17 ±7.7^b	40.10 ±0.70^a	8.67 ±2.73^a
9 mgL^{-1}	177.03 ±0.09^a	1.52 ±0.01^b	7.10±0.06^{ab}	25.10 ±0.12^b	166.2 ±0.17^c	47.40 ±0.21^c	5.00 ±0.00^a
11 mgL^{-1}	181.20±0.52^{ab}	2.59 ±0.09^b	10.03±0.56^b	27.10 ±0.61^b	165.97±0.09^c	41.00 ±0.44^{ab}	7.70 ±1.60^a
15 mgL^{-1}	174.87 ±3.12^a	2.68 ±0.10^b	11.60±0.31^b	36.07 ±0.12^b	126.97±0.17^a	41.70 ±0.17^{ab}	0.00 ±0.0^a
(P < 0.05)	0.016	0.000	0.121	0.013	0.000	0.000	0.45

Means in the same column with different superscripts differ significantly (P<0.05)

Discussion

In this study, there were no behavioural changes observable with fishes in the control group as they remained active, rapidly responding to stimuli at all times and their skin colour remained normal. On the contrary, behavioural responses were observed in the chlorfenapyr treatment groups, which included restlessness, frequent vertical movements towards the water surface to gulp in air, torpidity, docility, erratic and uncoordinated movements.

Ignoring the impact of pesticide pollution in water bodies has resulted in several consequences. Blood variables respond swiftly to low doses of pollutants (Osman *et al*., 2018), hence haematology is among the most quantitative method in evaluating the hazardous effects of pollutants on aquatic organisms (Hedayati *et al*., 2019). Reports have shown that exposing fish to pesticides has led to reduction in haematological value due to haemo-synthesis, osmoregulatory dysfunction and even increase in the rate of erythrocyte destruction in haematopoietic organs and erythropoiesis (Jenkins *et al*., 2003; Seth and Saxena, 2003). As an established fact, blood, being the oxygen and nutrient transport vessel of the body, is imperative for homeostasis. A decrease in haemoglobin (Hb) level is tantamount to a reduction in oxygen-carrying capacity of blood to tissues. This can contribute to stress, hypoxia and anaemic conditions further triggering metabolic and respiratory misfit (Ada *et al*., 2012). In this study, the changes in the behaviours (restlessness, gasping for air, etc.) may be attributed to nervous reactions of the organism to the irritating effects of the toxicants. Though responses to chemical compound may vary depending on different factors such as species of fish, duration of exposure, type, concentration and quality of chemical, the alterations in haematological parameters are either reversible or irreversible (Cameron, 1970; Soivio and Oikari, 2006). The study showed a marked decrease in WBC in all treatment groups when compared with the control. WBCs, which respond immediately after exposure to foreign substances, act as defense mechanism and regulate the non-specific immunological function in the fish. It is also noted that WBC production is necessary to fight against invading foreign bodies (Joshi *et al*., 2003). Chlorfenapyr can induce high level total WBC when compared with other insecticides (Ghayyur *et al*., 2021). However, chlorfenapyr, in our study, did not stimulate an increase in WBC and may be due to the concentrations level of toxicants investigated. When compared with the control, RBC increased in all chlorfenapyr treatment and this increment was directly proportional to increase in concentration of chlorfenapyr. High RBC count may be caused by exposure to chemicals, dehydrated plasma or other problems. Studies have shown that fish experiencing this had significant higher RBC (Affonso *et al*., 2002; Pakanit and Kinchareon, 2011) and this increase in circulating RBC can result from spleen contraction (Moura *et al*., 1997).

Meanwhile, 5, 7 and 9 mgL^{-1} of chlorfenapyr elicited a decreased in HGB level when compared to control. Increase in HGB may be attributed to increase in RBC. Variables, such as MCV and MCH, demonstrate the current physiological state of the animal and are used to evaluate stress. MCV values of all treatment groups, except 15 mgL^{-1} concentration, were higher when compared with control. This is as a result of the increase in RBC. MCH, in this study, shows that the average quantity of haemoglobin present in a single RBC was fairly normal. Studies have postulated that increase in MCV and MCH suggests the occurrence of macrocytic normochromic type of anaemia (Gabriel and Ugbomeh, 2016). Also, increase in MCV may be caused due to endomitosis which results to the haemodilution as suggested by Anand-kumar (1994).

PLT, in the study, decreased with increase in concentration and was lower for all treatments when compared with the control. PLT is responsible for blood clotting and releases a substance called thromboplastin that initiates blood clotting reaction (Pandey and Shukla, 2005). The increase in the concentration of chlorfenapyr in the study decreased

the rate of PLT production, hence reducing the tendency for blood to clot.

Conclusions

This study showed that sub-lethal concentrations of chlorfenapyr on juveniles of *C. gariepinus* caused alterations in haematological parameters. These alterations were also evident in the behavioural responses dependent on the concentration of the toxicant. Consequently, we recommend an interdisciplinary intervention that encourages safe handling and use of chlorfenapyr and its derivatives as a matter of urgency to avert chlorfenapyr-induced poisoning of non-targeted organisms.

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