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Original Article

Effect of Sub-Lethal Doses of Lambda-Cyhalothrin on Leukocyte Sub-Population (Differential Count) of African Catfish *Clarias gariepinus* (Burchell, 1822).

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ABSTRACT

Static bioassay experiments were conducted to ascertain the sublethal effects of waterborne lambda-cyhalothrin (ranging from 0.0004mg/L-0.0016mg/L) on the leukocyte sub-population (differential count) of *C. gariepinus* at two weeks intervals for 8 weeks duration. The exposure resulted in alterations in the blood parameters. Fish exposed to the toxicant elicited significant neutrophilia (% rise) and highly significant ($p<0.001$) lymphopaenia at the high concentrations of 0.0008mg/Land 0.0016mg/L when compared with control. Equally, highly significant ($p<0.001$) neutropaenia and lymphocytosis, at the lowest concentrations of 0.0004mg/L compared with Control was obtained. Highly significant ($p\leq0.001$) duration dependent neutropaenia and lymphocytosis was recorded. Monocytes, basophils and eosinophils were scarcely observed. The result of this investigation showed that the entire physiology of the fish was disturbed due to exposure to the toxicants. The toxicants caused haematological disturbances which could lead to impairment of the fish ability to combat diseases, reduce its chances of survival and potentials for growth and reproduction.

Keywords: Toxicology, Lambda-cyhalothrin, Leukocyte differential count, *Clarias gariepinus.*

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INTRODUCTION

The application of pyrethroids as insecticides and antiparasitary preparations has increased markedly. Thus pyrethroids are successively replacing organophosphate pesticides. The main advantages of pyrethroids are their photo-stability, high effectiveness in low concentrations, easy disintegration and low toxicity to birds and mammals (Maud *et al*, 1998). However, fish are extremely susceptible to these substances. The 96-hLC_{50} value, determined in laboratory tests generally lies below 10μg/L (Bradbury and Coast, 1989). In fish cultures, pyrethroids are applied to control some parasitic diseases caused by e.g. *Lepeophtherius salmonis* in farm cultures of salmonids (Toovey and Lydon, 2000). Direct and indirect contamination of aquatic environment by pesticides may cause fish kills, reduce fish productivity and elevate concentrations of undesirable chemicals in edible fish tissues. Pesticides can enter fish through their skin and gills during respiration and orally when feeding and due to the lipophilicity of pyrethroids, they have

a high rate of gill absorption, which in turn would be a contributing factor in the sensitivity of fish to aqueous pyrethroid exposures (Rukiye *et al*., 2003). Fish seem to be deficient in the enzyme system that hydrolyzes pyrethroids. The main reaction involved in the metabolism of pyrethroids including lambda-cyhalothrin in mice and rats is ester cleavage mainly due to the action of carboxyesterase. Metabolism in fish is largely oxidative (Demoute, 1989). The role of blood as a supplier of essential nutrients, ions, gases and endocrine factors, coupled with its function as a reservoir for excretory products of metabolism means that alterations in blood parameters are often reflective of the overall toxic impacts of environmental contaminants (Dietrich *et al*., 2006).

Physicomorphological changes in blood indicate the changes in the quality of the environment and therefore blood parameters are important in diagnosing the functional status of the fish exposed to toxicants.

The purpose of this study is to investigate the hematological perturbations in the leukocyte sub-populations (differential count) of the freshwater catfish *Clarias gariepinus* at sublethal concentrations of lambda-cyhalothrin.

MATERIALS AND METHODS

Experimental Fish

Juveniles of *Clarias gariepinus* were purchased from Maigana fish farm in Zaria, Kaduna State Nigeria. The *Clarias* species averaging 14.33 $\pm$ 0.50cm standard length and body weight of 20.38 $\pm$ 1.25g were used for the study. The fish were conveyed to fisheries laboratory in a portable well-aerated white polythene bag containing water from the fish farm. They were held in large water baths of 160L capacity at 24.5 - 25.5^{0}C and acclimatized for two weeks in dechlorinated municipal water. During this period, the fishes were fed with pelleted diet containing 35% crude protein twice per day at 3% body weight. Also, the water in the glass aquaria was changed once every two days. The fishes were accepted as well adapted to laboratory conditions when less than 5% death was recorded for 14 day period (Reish and Oshida, 1987).

Determination of Sublethal Concentrations of Pesticides and Feeding Test

From the result of acute bioassay, a fraction of 1/5, 1/10 and 1/20 of the 96hLC_{50} was used to determine sublethal concentrations for the pesticides as recommended by Oladimeji and Ogunmeta (1987) and Mohammed (1995).

Four aquaria tanks of the same size were used for three replicates each for the toxicants.

Appropriate volumes of the stock solution were dispensed using micro-syringe into 25 litres of dechlorinated municipal water in each of the test tanks, except the Control. The juveniles were exposed to sublethal concentrations of toxicants for 8 weeks. The concentrations used for chronic study of the toxicant were 0.0004 mg/L, 0.0008 mg/L, 0.0016 mg/L and control (0.00 mg/L). Each treatment was in triplicate. The fishes were randomly assigned to give a loading of 10 fish per tank. Fishes were fed to 3% body weight and with 35% crude protein level pelleted diet. The natural photoperiod of 12 hours light / 12h dark was maintained. To avoid variation in toxicant concentration, test solutions were changed every 12hrs. Biodegradation occurring in this time frame is less than 10% of the initial concentration (Flores *et al,* 1980).This helps to maintain toxicant strength and the level of dissolved oxygen, as well as minimizing the level of ammonia during the experiment.

Differential Count Analysis

EDTA-treated sample bottles was used to collect blood bi-weekly after severance of the caudal vein. Two drops of blood was placed on a clean slide and made into a thin

smear with another slide and left to dry. The smear was fixed with absolute methanol and then stained with Giemsa's stain and 1% buffered distilled water. It was allowed to stand for about 20 - 30 minutes after which the slide was washed again with buffered distilled water and allowed to air-dry. The slide was examined under the oil immersion objective of the microscope, and the cells are counted using a leukocyte differential counting machine.

Data Analysis

Probit analysis (Finney, 1971) was used to calculate 96hLC_{50} values for the pesticide. The data collected from growth and nutrient utilization parameters were subjected to analysis of variance (ANOVA) using the GenStat® 4.2 (2006) statistical analysis software and Duncan multiple range test (DMRT) was used to test for differences between different levels of treatment and to separate means respectively, where applicable (Duncan, 1955). Test of significance was at the 5% level.

96-h LC_{50} / No observed effects concentration (NOEC)

Laboratory 96-h LC_{50} was estimated to be 0.008 mg/L (Figure 1). Based on this, NOEC for the exposure of *C. gariepinus* to lambda-cyhalothrin was calculated to be 8x 10^{-3}mg/L and 9x 10^{-3}mg/L for 96-h LC_{50}. NOECs range of 8.0x10^{-4} - 8.0x10^{-5}mg/L for 96-h LC_{50}.

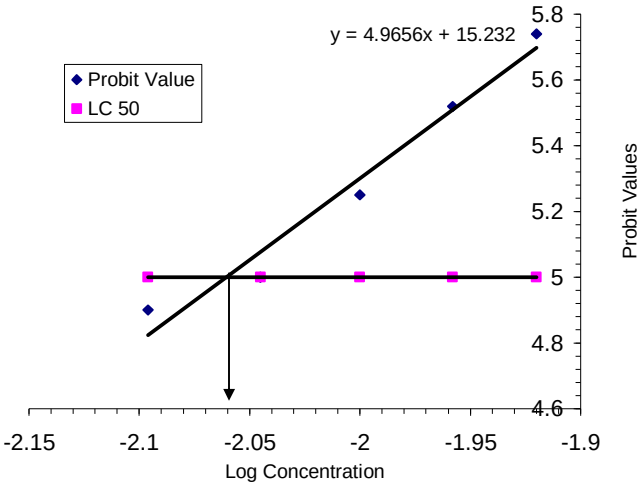

Fig 1: 96h LC_{50} of Lambda-cyhalothrin for juveniles of *C. gariepinus*

Leukocyte differential count due sub lethal exposure of fish to lambda-cyhlothrin

Results of Leukocyte differential count due to sub-lethal exposure of fish to lambda-cyhalothrin are presented in Tables 1 and 2. The percentage neutrophils of the control fish were significantly higher ($p \leq 0.001$) than those of fish exposed to 0.0004 mg/L, but significantly lower ($p \leq 0.001$) than those of fish exposed to 0.0008 mg/L and 0.0016mg/L sublethal concentrations of lambda-cyhalothrin. Percentage lymphocytes of the control group were significantly lower ($p \leq 0.001$) than those of fish exposed to 0.0004 mg/L of the toxicant, but significantly higher ($p \leq 0.001$) than those of fish exposed to 0.0008 mg/L and 0.0016 mg/L concentrations of the same toxicant.

When the effects of duration were considered (Table 2) both neutrophils and lymphocytes were observed to exhibit time

dependent responses. Neutrophils in the exposed group of fish decreased steadily and significantly ($p \leq 0.001$) from week 2 to week 8, while significant increases in percentage lymphocytes with increasing periods of exposure were recorded (Table 2). No traces of basophils, eosinophils and monocytes were found in the fish blood (Tables 1and 2).

Sublethal effect of lambda-cyhalothrin on leukocyte sub-population (Differential Count) of *C. gariepinus* in 8 weeks

Toxicant elicited significant neutrophilia (% rise) and highly significant ($p \leq 0.001$) lymphopaenia (% fall) at the high concentrations of 0.0008 mg/Land 0.0016 mg/L when compared with control. Equally highly significant ($p \leq 0.001$) neutropaenia and lymphocytosis, at the lowest concentrations of 0.0004mg/L compared with control was obtained. When the interaction effects were considered, highly significant ($p \leq 0.001$) duration dependent neutropaenia and lymphocytosis was recorded. The lowest neutrophil count (7.5%) was recorded with the highest concentration and longest duration of exposure (Table 3). Lymphocyte counts increased with increasing duration of exposure. The highest lymphocyte count (92.5%) was obtained with the highest (0.0016mg/L) sublethal concentration and longest duration of exposure (Table 4). Several cells were observed undergoing mitotic division and no basophils, eosinophils and monocytes were observed.

Table 1: The effect of sublethal doses of lambda-cyhalothrin on leukocyte sub-population (Differential count) of *C. gariepinus*

Concnetration (mg/L)	Neutrophils (%)	Lymphocytes (%)	Basophils (%)	Eosinophils (%)	Monocytes (%)
Control	16.75 ± 0.36^{c}	83.25 ± 0.36^{b}	0.00	0.00	0.00
0.0004	14.00 ± 1.46^{d}	86.00 ± 1.46^{a}	0.00	0.00	0.00
0.0008	20.00 ± 0.88^{a}	80.00 ± 0.89^{d}	0.00	0.00	0.00
0.0016	18.75 ± 3.09^{b}	81.25 ± 3.09^{c}	0.00	0.00	0.00

Means with the same superscript along columns are not significantly different ($p \leq 0.05$)
(Mean values $\pm$SD) n=8

Table 2: The effect of duration of sublethal doses of Lambda-cyhalothrin on leukocyte sub-population (Differential Count) of *C. gariepinus*

Time (Weeks)	Neutrophils (%)	Lymphocytes (%)	Basophils (%)	Eosinophils (%)	Monocytes (%)
2	22.00 ± 1.46^{a}	78.00 ± 1.46^{d}	0.00	0.00	0.00
4	19.50 ± 1.50^{b}	80.50 ± 1.50^{c}	0.00	0.00	0.00
6	15.00 ± 0.96^{c}	85.00 ± 0.95^{b}	0.00	0.00	0.00
8	13.00 ± 1.73^{d}	87.00 ± 1.74^{a}	0.00	0.00	0.00

Means with the same superscript along columns are not significantly different ($p \leq 0.05$)
(Mean values $\pm$SE) n=8

Table 3: Duration Interaction Effect of Sub-lethal Doses of Lambda-cyhalothrin on the Leukocyte Differencial Count (Neutrophils) of *C. gariepinus*

Concentration	Biweekly Leucocyte differential count (neutrophils)			
Mg/L	2	4	6	8
0.000	17.50 ± 0.50^{fg}	16.50 ± 0.50^{gh}	15.50 ± 0.50^{hi}	17.50 ± 0.50^{g}
0.0004	19.50 ± 0.50^{de}	15.50 ± 0.50^{hi}	11.50 ± 0.50^{j}	9.50 ± 0.50^{k}
0.0008	23.50 ± 0.50^{c}	20.50 ± 0.50^{d}	18.50 ± 0.50^{ef}	17.50 ± 0.50^{g}
0.0016	27.50 ± 0.50^{a}	25.50 ± 0.50^{b}	14.50 ± 0.50^{i}	7.50 ± 0.50^{l}

Means with the same superscript along columns are not significantly different ($p< 0.05$) (Mean values $\pm$SE) n=2

Table 4: Duration Interaction Effect of Sub-lethal Doses of Lambda-cyhalothrin on the Leukocyte Differencial Count (Lymphocytes) of *C. gariepinus*

Concentration	TIME (Weeks)			
Mg/L	2	4	6	8
0.000	82.50 ± 0.50^{f}	83.50 ± 0.50^{ef}	84.50 ± 0.50^{dc}	82.50 ± 0.50^{f}
0.0004	80.50 ± 0.50^{g}	84.50 ± 0.50^{dc}	88.50 ± 0.50^{c}	90.50 ± 0.50^{b}
0.0008	76.50 ± 0.50^{h}	79.50 ± 0.50^{g}	81.50 ± 0.50^{g}	82.50 ± 0.50^{f}
0.0016	72.50 ± 0.50^{j}	74.50 ± 0.50^{i}	85.50 ± 0.50^{c}	92.50 ± 0.50^{a}

Means with the same superscript along columns are not significantly different ($p< 0.05$) (Mean values $\pm$SE) n=2

DISCUSSION

Lambda-cyhalothrin induced significant duration-dependent neutropaenia lymphocytosis count. The duration dependent decreases and increases in the percentage subpopulation of lymphocytes and neutrophils may be associated with the nature of immunological challenge to which the fish was exposed at a particular period of time and in the various sublethal concentrations. Neutrophils being phagocytotic in function, their increase may have been associated with invasion of the system by pathogenic micro-organisms, viruses and debris, which may have been occasioned by tissue and organ damage due to toxic insult. Evidence of phagocytotic activity was seen in the histopathology result of liver tissue exposed to 0.045mg/L chlorpyrifos for four weeks (Ogueji *et al*, 2007). Ogueji *et al* (2007) reported that aggregated macrophages with haemosiderosis activity were numerous in the liver of *C.gariepinus* exposed to sublethal concentration of chlorpyrifos-ethyl. When the percentage subpopulation of neutrophils decreases, it means that the phagocytotic capacity of the fish blood and therefore its ability to resist infection has been compromised. Suppression of neutrophil response has also been shown in studies with fish exposed to metals (Faisal and Huggett, 1993). And according to Lohner *et al.* (2001) reduced neutrophil numbers are possibly indicative of reduced or disrupted phagocytotic capacity and reduced disease resistance.

The duration-dependent increase in lymphocytes counts may be associated with enhanced release of lymphocytes from lymphomyeloid tissues. This could be an adaptive mechanism to burst the immune system of the fish and give it the positive survival value needed in the sublethal toxic environment or possibility of leukaemia due to prolonged toxic insult. Ogueji (2008) reported dose and duration dependent degenerations in the gill and liver of *C.gariepinus* exposed to sublethal concentrations of lambda-cyhalothrin. In the current investigation, monocytes, basophils and eosinophils were scarcely observed. This indicated an extremely low concentration of these cell types in the fish blood. According to Ranzani-Paiva (1995), it was difficult to preserve basophils, and this was the main reason why basophils were difficult to

identify in fish blood. Modra *et al.* (1998) observed low concentration of eosinophils and the absence of basophils in several fish species, such as *Cyprinus carpio*, *Tinca tinca*, *Siluris glanis* and *Oncorhinchus mykiss.* Romao *et al.* (2006) also reported absence of eosinophil, basophil and granulocyte in *Hoplias malabaricus* and *Geophagus brasiliensis* collected from the wild.

CONCLUSION

The present findings showed hypersensitivity of leukocyte sub populations to Lambda-cyhalothrin and these changes may be due to immunological reactions leading to antibody production to help fish cope with stress induced by the chemical. It is therefore recommended that:
Administrative environmental regulations and legal instruments in Nigeria should be strengthened and adequately enforced with respect to importation and discharge of toxic substances into the aquatic environment. We must use our biomonitoring technology to predict the effects of new chemical substances likely to reach aquatic ecosystems. In addition, we must use our biomonitoring tools to predict the future ecological effects of chemical substances and utilize this knowledge to prevent these substances from reaching hazardous concentrations in the ecosystem.

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