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EFFECT OF ACTELLIC 25 EC ON THE DIFFERENTIAL LEUCOCYTE COUNTS OF THE CATFISH *Clarias albopunctatus* (NICHOLE & LAMONTE, 1953)

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ABSTRACT

The changes in the total and differential leucocyte count in the fish Clarias albopunctatus exposed to sublethal concentrations of actellic 25 EC (0, 0.3, 0.5, 0.8 and 1.0 μg/l) were studied for 18 days in a static renewal bioassay system. Compared with the control, there was significant leucocytosis (P<0.05) in the actellic -exposed fish. The total leucocyte counts also differed significantly (P <0.05) in the treatment groups. The lymphocytes were the dominant leucocyte subgroup in the blood of the fish. There was significant lymphocytosis in the actellic 25 EC-exposed fish. Decreased eosinophils, monocytopenia and neutropenia were evident in the treatment groups. These observations are indications of the mobilization of the body's defense system due to Actellic25 challenge leading to leucopoiesis.

Key words: Actellic, *Clarias*, Stress, Leucocyte, Differential count

INTRODUCTION

The application of pesticides either to boost food production or in the control of pests, results in environmental contamination especially in the developing countries. These chemicals eventually get to the aquatic habitats either as run-off or directly during aerial spray or while washing of the containers. This would result in changes in the physico-chemical qualities of the water body with attendant effects on the physiological and indeed the health of faunal populations living therein.

The response of the leucocytes to the changes in water quality and chemicals is variable (Nussay et al., 1995; Srivastava and Narain, 1982; Ezzat, *et al.,* 1974). Lecucocytosis was reported in some fish species exposed to pesticides (Mathiessien, 1981; Srivastava and Narain, 1982; Santhakumar *et al.,* 1999; Mgbenka *et al.,* 2003). Oluah and Nwosu (2003) also reported increased leucocyte count in *C. albopunctatus* chronically exposed to Brewery effluent. Leucocytopenia however, was reported in Coho salmon exposed to Kraft pulp mill effluent (Mcleay, 1975). Lymphocytosis accompanied by neutropenia was reported in *Anguilla anguilla* (Krutzmann, 1979) treated with drugs. Neutropenia was also reported in channel catfish during hypoxia (Scott and Rogers, 1981). On the contrary, neurophilia was observed in fish during bacterial infection (Hines and Spira, 1973) and stress (Slicher, 1961).

In recent times, there is increasing interest on the effect of heavy metals and chemicals and or / harmful substances on the haematology of *Clarias* species on the account of their socio-economic importance in the fish food supply in Nigeria.

This study represents part of our continuing contribution to the physio-ecology of the Clariid fishes. The purpose of the study was to investigate the effect of sublethal concentrations of actellic 25 on the leucocyte and differential white blood cell count of the fish *C. albopunctatus.*

MATERIALS AND METHOD

The fish samples were caught from Anambra River Nigeria, using local traps. The fish were transported to the laboratory in a plastic container and thereafter acclimatized for 14 days at water temperature of 28 ^{o}C. The 150 fish (66.3 ± 2.48 g mean weight) used in the study were randomly divided into five groups of 30 fish each. Each group was further randomized into three replicates experiments of 10 fish per replicate. The fish in group 1 and 2 were exposed to 0.3 and 0.5 μg/l actellic, respectively. The fish in groups 3 and 4 were exposed to 0.8 and 1.0 μg/l actellic, respectively. The fish in the fifth group which served as the control was exposed to tap water only. The experiment lasted for 18 days in a static bioassay system. The water and the pesticide were changed every 24 hour to maintain constant concentration and avoid the accumulation of waste metabolites and food remains. The feeding regime and blood collection methods were as described by Oluah and Nwosu (2003).The leucocyte count was made using improved Neubauer haemocytometer after diluting the blood 1:100 with Shaw's solution (Shaw, 1930).

The results were analyzed using one way analysis of variance (ANOVA) followed by F-LSD post hoc test. The significance level was taken as $P < 0.05$.

RESULT

The effects of Actellic 25 EC on the total leucocyte and differential white blood cell counts of the fish *C. albopunctatus* are shown in Tables 1, 2, 3 and 4. The result showed that the total leucocyte in the treatment groups were significantly higher ($P < 0.05$) than the control. Also the leucocyte counts in the treatment groups were significantly different ($P < 0.05$).

The result also showed that the lymphocytes, neutrophils, monocytes, eosinophils and the basophils were the recognizable types of white blood cells found in the peripheral blood of *C. albopunctatus*. These were classified as granulocytes or agranulocytes, depending on the presence or absence of granules in their cytoplasm. The lymphocytes are the most dominant leucocyte type in the blood of *C. albopunctatus*. In the control, the leucocytes were significantly higher ($P<0.05$) when compared with the treated groups. Lymphocytosis occurred with increased duration of exposure. The monocytes, which are round cells with oval nuclei with clumped chromatin, are the second agranulocytes in the blood. When compared with the control, there was significant decrease ($P<0.05$) in the monocytes in the Actellic-exposed fish. Monocytopenia occurred with increasing duration of exposure.

The neutrophil, eosinophils and basophils were the granulocytes in the blood of *C. albopunctatus*. The neutrophils were significantly reduced in the treated groups ($P < 0.05$) when compared with the control. The neutrophil was the dominant granulocyte in the fish *C. albopunctatus.* Neutropenia was most pronounced in the fish exposed to 1.0μg/l actellic 25 EC. The second most numerous granulocytes was the eosinophils which decreased significantly ($P < 0.05$) in the treatment group when compared with the control. The eosinophils also decreased with exposure time to Actellic. The least abundant granulocyte in the peripheral blood of *C. albopunctatus* was the basophils.

DISCUSSION

The result of the study showed that Actellic 25 EC had significant effect on the leucocyte and differential white blood cell count in *C. albopunctatus.* According to Nussey *et al.,* (1995), the sustained leucocytosis in the actellic 25 exposed fish represented a physiological response to infection. Earlier studies had demonstrated that leucocytosis was observed in fish subjected to pollution by insecticides (Mathiessien, 1981); Van Vuren, 1986; Santhakumar *et al.,* 1999; Mgbenka et al., 2003), heavy metals (Misha and Srivastava, 1980; Dick and Dixon 1985, Nussey *et al.,* 1995; Oluah, 2001) and Brewery effluent (Oluah and Nwosu, 2003). The increase in the proportion of lymphocytes in the *C. albopunctatus* exposed to actellic agreed with the earlier work of Mgbenka *et al.,* (2003) in the same species exposed to gammalin 20. Srivastava and Narain (1982) also reported increased lymphocyte number in H*eteropneustes fossillis* treated with endrin and nuvacron. Similarly, lymphocytosis was reported in *Ictalurus punctatus* subjected to hypoxia (Grizzle and Rogers, 1976; Scoot and Rogers, 1981). On the other hand, leucocytopenia was reported in Coho salmon treated with kraft pulp mill effluent (McLeay, 1975).

The decreased number of monocytes and neutrpophils in *C. albopunctatus* with exposure to actellic 25 EC and duration agreed with the report of Nussey et al., (1995) on *Oreochromis mossambicus* treated with Copper. Monocytopenia and neutropenia have been reported in *C. albopunctatus* exposed to gammalin 20 (Mgbenka *et al.,* 2003) and brewery effluents

Table1: The mean total and differential leucocyte count *C. albopunctatus* exposed to 0.3 μg/l actellic

Types of leucocytes (%)	*Exposure Period (days)*			
	Control	**6**	**12**	**18**
Agranulocytes(%)				
Lymphocytes	60.50 ± 1.69	64.50 ± 1.38	72.50 ± 1.04	71.00 ± 1.64
Monocytes	13.50 ± 0.82	12.50 ± 0.62	11.50 ± 0.39	9.50 ± 1.03
Granulocytes				
Neutrophils	22.0 ± 1.74	17.5 ± 1.09	16.00 ± 1.17	18.50 ± 1.42
Eosinophils	4.0 ± 0.08	4.0 ± 0.10	_	1.00 ± 0.01
Basophils	_	1.50 ± 0.52	_	_
Total leucocyte	**4.70 ± 1.80**	**11.70 ± 1.75**	**20.5 ± 1.46**	**54.84 ± 1.51**

Table 2: The mean total and differential white blood cell count in *C. albopunctatus* exposed to 0.5 μg/l actellic

Typesofleucocyte(%)	*Duration of Exposure (days)*			
	Control	**6**	**12**	**18**
Agranmcocytes				
Lymphocytes	60.50 ± 1.69	65.0 ± 1.48	81.0 ± 1.92	80.5 ± 1.86
Monocyte	13.50 ± 0.82	16.0 ± 0.66	3.5 ± 0.11	3.5 ± 0.02
Granulocyte				
Neutrophil	22.0 ±1.74	18.0 ± 1.20	15.5 ± 1.09	16.0 ± 1.40
Eosinophils	4.0 ± 0.08	1.0 ± 0.02	-	-
Basophil	-	1.0 ± 0.01	-	-
Total WBC	**4.70 ±1.80**	**15.05 ± 1.03**	**27.60 ± 1.26**	**25.00 ± 1.58**

(*Values are means of 5 determinations*)

Table 3: The mean total and differential white blood cell counts in *C. albopunctatus* exposed to 0.8 μg/l actellic

Leucocyte type	*Duration of Exposed (days)*			
	Control	**6**	**12**	**18**
Agrannulocyte				
Lymphocytes	60.5 ± 1.69	67.0 ± 1.88	82.5 ± 1.56	83.0 ± 1.09
Monocytes	13.50 ± 0.82	15.0 ± 1.06	4.0 ± 0.12	_
Grannulocytes				
Neutrophil	22.0 ± 1.74	17.0 ± 1.09	13.0 ± 1.11	16.0 ± 1.20
Eosinophil	4.0 ± 0.08	_	0.5 ± 0.04	_
Basophil	_	1.0 ± 0.01	_	0.5 ± 0.03
Total leucocyte	**4.10 ± 1.80**	**20.65 ± 1.30**	**32.6 ± 1.74**	**39.9 ± 1.83**

(*Values are means of 5 determinations*)

Table 4: The mean total and differential white blood cell counts in *C. albopunctatus* exposed to 1.0 μg/l

Leucocyte types (%)	*Duration of Exposed (days)*			
	Control	**6**	**12**	**18**
Agrannulocytes				
Lymphocytes	65.5 ± 1.69	72.0 ± 1.98	94.5 ± 1.36	85.00 ± 1.55
Monocytes	13.5 ± 0.82	10.5 ± 0.76	3.0 ± 0.08	_
Grannulocytes				
Neutrophil	22.0 ± 1.74	17.0 ± 1.18	12.0 ± 0.84	10.0 ± 0.66
Eosinophil	4.0 ± 0.08	_	_	0.50 ± 01
Basophil	_	_	_	_
Total leucocyte	**470 ± 1.80**	**31.45 ± 1.19**	**39.70 ± 1.24**	**39.8 ± 1.60**

(*Values are means of 5 determinations*)

(Oluah and Nwosu, 2003). Neutropenia was also reported in *Barbus conchonius* exposed to mercury (Gill and Pant, 1985) as well as in *Pleuronectes flascens* treated with cadmium (Johansen-Sjobeck and Larsson, 1978). Kreutzmann (1977) also observed neutropenia in *Anguilla anguilla* treated with drugs.

On the other hand, neutrophilia was reported in rainbow trout after the administration of adjuvant (Finn and Nielson, 1971) and in *Cyripinus carpio* due to *Ichthyophthirius multifilis* infection (Hines and Spira, 1973). Slicher (1961) also reported neutrophilia in stressed fish.

In this study, the basophil was the least abundant (1 %) leucocytes in *C. albopunctatus*. This agreed with report of Ward (1969) that the basophils constitute about 1% of the leucocytes in the lungfish *Neoceratodus forsteri*. Also, the review by Ellis (1977) showed that the basophils are very few or even absent in some fishes. In conclusion, the observed leucocytosis, hymphocytosis, monocytopenia, and neutropenia are indications of stress and concomitant infection in *C. albopunctatus* exposed to sublethal actellic 25 EC concentrations. With standardization, information on the changes in the white blood cell types in fish could be applied as a diagnostic tool in Ichytho-toxicological monitoring and assessment of aquatic pollution.

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