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Haematology and Erythropoietic Response in the Catfish, *Clarias albopunctatus* (Lamonte and Nichole 1927), Exposed to Sublethal Concentrations of Gammalin 20 (Lindane)

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Abstract
Haematological changes in the fish *Clarias albopunctatus* (Lamonte and Nichole, 1927) subjected to sublethal concentrations (0, 0.25, 0.5, 0.75, and 1.0 µg/L) of Gammalin 20 for 21 days were studied in a static bioassay renewal system. Compared with the control, the erythocytic count haematocrit values and haemoglobin concentrations were significantly reduced ($P > 0.05$) in the treatment groups. These parameters also differed in the treatment groups. There was significant leucocytosis ($P > 0.05$) in the fish exposed to sublethal concentrations of Gammalin 20. The Gammalin-20-exposed fish suffered macrocytic anaemia.

Key Words: Haematology, Erythropoietic, Catfish, Gammalin

Introduction

Gammalin 20 is an organochloride insecticide which is commercially available as 20% aqueous lindane preparation. Despite the restricted use of organochloride pesticides, on account of their persistence in the environment, Gammalin 20 is currently widely used by farmers in Nigeria. This and other indiscriminately applied terrestrial pesticides are washed into water bodies as run-offs, Gammalin 20 is also illegally used by local fishermen. This would result in changes in the water

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quality with concomitant alterations in the internal physiology of the fish and also massive fish mortality (Ware, 1983).

The assay of some physiological and biochemical parameters in the fish blood, have been used to assess the subtle perturbations in the internal milieu of fish due to input of anthropogenic xenobiotics. Changes in the blood chemistry have been reported in fish exposed to endoshlphan (Gimeno et al., 1994), paraquet (Simon et al., 1983; Oluah and Njoku, 2001). Fernando and Andrew (1992) reported that lindane causes hyperglycaemia by promoting glycogenolysis in *Anguilla anguilla*. Similar observation was made on *Oreochromis* exposed to Gamalin 20 (Omoregie et al., 1990). Haematological parameters are also good indicators of chemical and heavy metal pollution (Van Vuren, 1986; Nussey et al., 1995; Oluah, 2001) as well as stress and hypoxia (Cassillas and Smith, 1977). Haematological changes are thus, regarded as "early warning" signal of aquatic contamination (Nussey et al., 1995).

The objective of this study was to investigate the effect of sublethal concentrations of Gammalin 20 on the haematological parameters of the catfish, *Clarias albopunctatus* (Lamonte and Nichole 1927).

MATERIALS AND METHODS

Fish Collection

The fish used in the study were collected from the Anambra River at Ogurugu. The fish were transported to the laboratory in a plastic fish transport container and were acclimatized to the laboratory condition for three weeks before the commencement of the study. In the laboratory, the fish were disinfected with 1% potassium permanganate solution for two minutes. Thereafter, they were rinsed in a clean tap water.

Experimental Design

A total of one hundred and fifty fish (mean weight 28.26 ± 1.67 g) were used for the study. The fish were divided into five groups of thirty (30) fish per group. Each group was further subdivided into three replicates experiments of 10 fish per replicate. The groups were treated as follows: groups 1, 2 and 3 were exposed to 0.25 µg/l 0.5, and 0.75 µg/l of Gammalin 20, respectively. The fourth group was treated with 1.0 µg/l Gammalin 20 while the fifth group was exposed only to tap water (as the control). During the experimental period, the fish were fed 3.% body weight 35% crude protein diet daily at 8.00 h.

The static bioassay method was adopted for the study. The water and Gammalin 20 (0, 0.25, 0.5, 0.75, and 1.0 µg/L) were changed every morning. This served to maintain the toxicant concentration and avoid the accumulation of waste products throughout the study, period. The experiment lasted for three weeks during which fish in each treatment group were sampled at seven days interval. Two fish from each replicate were used for haematological assessment. Blood from the fish was collected by the cardiac puncture method using disposable hypodermic syringe and by caudal severance.

Haematological Studies

The haematocrit was determined by filling one capillary tube with blood from each fish and centrifuging in a microhaematocrit (Hawksley England) at 300 rpm for 5 minutes at room temperature. Soon after, the haematocrit was read using a haematocrit reader and reported as percentage of the whole blood.

The haemoglobin was determined using the cyanmethaemoglobin method (Blaxhal and Dasiley, 1973). The erythrocyte count was determined using microscope neubauer counting chamber after diluting the blood with Toisson's solution. Similarly, the leukocyte count was performed with microneubauer count chamber following dilution with Turk's solution. The haematological indices were calculated following Tort and Torres (1988) method.

Statistical analysis was performed using the one-way analysis of variance (ANOVA) at 5% level of significance. Any significant differences were partitioned with the least significant difference (LSD).

Table 1: Changes in the haematological parameters (mean ± SE) of *Clarias albopunctatus* exposed to Gammalin 20 for 7days.

	Concentration (µg/l)				
Parameters*	Control	0.25	0.50	0.75	1.0
Erythrocyte (10^6/mm^3)	3.35 ± 0.72	3.05 ± 0.61	2.80 ± 0.48	2.49 ± 0.32	2.29 ± 0.18
Haemoglobin (g/dl)	15.1 ± 1.64	12.0 ± 1.06	7.2 ± 1.12	7.5 ± 1.08	6.6 ± 1.26
Haematocrit (%)	29.0 ± 1.50	28.1 ± 1.40	25.3 ± 1.50	24.9 ± 1.60	22.9 ± 1.20
MCV (µm^3)	86.5 ± 6.21	92.1 ± 5.02	90.3 ± 2.20	100.0 ± 3.68	100.0 ± 4.6
MCHC (%)	51.6 ± 1.84	42.8 ± 3.10	28.6 ± 2.1	30.1 ± 2.09	28.7 ± 1.88
MCH (pg)	38.3 ± 2.62	56.2 ± 2.08	26.8 ± 1.82	30.1 ± 2.09	28.7 ± 1.88
Leucocyte (10^4/mm^3)	4.65 ± 1.88	11.5 ± 4.06	14.85 ± 3.1	20.0 ± 4.64	31.05 ± 2.6

*MCV = Mean Corpuscular Volume, MCH = Mean Corpuscular haemoglobin, MCHC = Mean Corpuscular Haemoglobin Concentration.

Result and Discussion

The result of the study showed that there were alterations in the blood parameters of *C. albopunctatus* exposed to sub-lethal concentrations of Gammalin 20 for 21 day (Tables 1, 2 and 3). There were no significant changes in the haematological parameter of the control fish, throughout the study period.

Table 2: Changes in the haematological parameters (mean ± SE) of *Clarias albopunctatus* exposed to Gammalin 20 for 14 days

	Concentration (µg/l)				
Parameters*	Control	0.25	0.50	0.75	1.0
Erythrocyte (10^6/mm^3)	3.52 ± 0.61	2.89 ± 0.84	2.56 ± 0.48	2.27 ± 0.56	2.15 ± 0.68
Haemoglobin (g/dl)	15.0 ± 1.60	11.4 ± 1.02	10.2 ± 1.10	6.0 ± 1.91	4.0 ± 1.02
Haematocrit (%)	34.5 ± 1.47	29.7 ± 1.81	26.0 ± 1.30	24.0 ± 1.30	24.0 ± 1.86
MCV (µm^3)	96.59 ± 3.27	102.76 ± 4.68	115.56 ± 5.80	105.22 ± 10	111.52 ± 5.40
MCHC	42.6 ± 1.24	39.4 ± 1.29	40.0 ± 1.54	26.43 ± 1.14	18.9 ± 1.08
MCH	42.6 ± 1.24	39.4 ±1.29	40.0 ± 1.54	26.43 ± 1.14	18.9 ± 1.08
Leucocyte (10^4/mm^3)	17.4 ± 1.10	19.6 ± 1.19	24.35 ± 1.30	39.7 ± 1.61	44.1 ± 1.48

*MCV = Mean Corpuscular Volume, MCH = Mean Corpuscular haemoglobin, MCHC = Mean Corpuscular Haemoglobin Concentration.

Table 3: Changes in the haematological parameters (mean ± SE) of C. *albopunctatus* Exposed to Gammalin 20 for 21 days.

	Concentration (µg/l)				
Parameters*	Control	0.25	0.50	0.75	1.0
Erythrocyte (10^6/mm^3)	3.37 ± 0.61	2.60 ± 0.79	2.32 ± 0.51	2.14 ± 0.64	1.84 ± 0.6
Haemoglobin (g/dl)	12.5 ± 1.44	9.0 ± 0.68	10.2 ± .08	5.4 ± 0.83	3.0 ± 0.6
Haematocrit (%)	34.7 ± 1.47	28.7 ± 1.78	26.6 ± 1.37	23.4 ± 1.28	22.0 ± 1.8
MCV (µm^3)	98.1 ± 4.5	110.3 ± 5.20	114.5 ± 4.80	109.4 ± 5.10	114.7 ± 6.1
MCHC (%)	36.1 ± 1.20	31.5 ± 1.05	38.4 ± 1.52	23.8 ± 1.28	13.5 ± 1.09
MCH (pg)	35.2 ± 1.61	34.7 ± 1.34	43.8 ± 1.27	25.1 ± 1.80	15.4 ± 1.06
Leucocyte (10^4/mm^3)	12.2 ± 1.04	17.2 ± 1.18	29.6 ± 1.88	40.9 ± 1.80	39.4 ± 106

*MCV = Mean Corpuscular Volume, MCH = Mean Corpuscular Haemoglobin, MCHC = Mean Corpuscular Haemoglobin Concentration.

The erythrocyte count decreased progressively with increasing concentrations and exposure period to Gammalin 20. Compared with the control, there was significant reduction in the erythrocyte count in the treatment groups ($P < 0.05$).

The haematocrit also decreased with increasing sub-lethal concentrations of Gammalin 20. Compared with the control, the haematocrit values were significantly reduced in the treatment groups ($P < 0.05$). There was significant difference in the haematocrit values in the treatment group ($P < 0.05$). However, within each treatment group, this parameter did not vary significantly with duration of exposure ($P < 0.05$). The haemoglobin concentration differed in the treatment group ($P < 0.05$). The erythrocyte morphological indices exhibited noticeable alterations during the chronic exposure to sub-lethal concentrations of Gammalin 20. The mean corpuscular volume (MCV)

increased with both concentration and duration of exposure ($P > 0.05$) (Tables 1, 2, and 3). Both the mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) decreased with increasing Gammalin 20 concentrations and exposure period.

The observed erythrocyte count *of C. albopunctatus* with increasing Gammalin 20 concentration and exposure time is consistent with the earlier report of Omoregie et al. (1990) on the effect of monocrotophos, an organophosphate which caused a significant reduction in the erythrocyte count in *Labeo umbratus*. Also, diuron (Reddy et al., 1992) and malathion (Dutta et al., 1992) had similar adverse effects on the fish erythrocyte.

The observed reduction in both the haematocrit and haemoglobin concentrations in C. *albopunctatus* exposed to Gammalin 20 is in accord with the result of Santhakumar et al. (1999) on *Anabas testudineus* chronically exposed to monocrotophos which had decreased haematocrit and haemoglobin concentrations. Similarly, Demael et al. (1980) reported that tench treated with potassium nitrate had reduced haematocrit values. The reduction in the haematocrit values *C. albopunctatus* exposed to sub-lethal Gammalin 20, may be attributed to the decrease in erythrocyte number in the peripheral blood. Other polychlorinated biphenyls (PCBs) like Aroclor are known to cause decrease in erythrocyte count, haematocrit and haemoglobin concentrations in vertebrates (Flick et al., 1965; Iturri et al., 1974). Larson et al. (1985) observed that MCV provides clue as to the status or size of the erythrocyte as well as showing abnormal or normal cell division during erythropoiesis. The observed significant increase in the MCV as well as the reduction in both the haemoglobin and erythrocyte count are indicative that *C. albopunctatus* had swollen erythrocyte and had suffered macrocytic anaemia during the exposure to Gammalin 20. Evidence from the data suggests that the decreased haematocrit and indeed anaemia may not be due to haemodilution but could have resulted from the reduction in erythrocyte counts as a result of impaired erythropoiesis due to Gammalin 20.

In fish, like in other vertebrates, erythropoiesis is controlled by erythropoetin that promotes the differentiation of erythroblasts from the erythropoietic stem cells (Gordon et al., 1967; Santhakumar et al., 1999). It has been shown that testosterone had effect on erythropoiesis through the enhancement of erythropoietin reduction (Gordon et al., 1967; Malgor and Fisher, 1970; Santhakumar et al., 1999). Nowicki and Norman (1972) reported that PCBs cause decreased erythrocyte formation by facilitating testosterone breakdown in the liver. Also, Iturri et al. (1974) reported that estrogen inhibits the biosynthesis of erythropoietin precursor. In this study, the observed significant reduction in the erythrocyte count could probably result from the inhibition of erythropoietin production and the promotion of hepatic catabolism of testosterone by Gammalin 20. Reddy et al. (1992) reported that haemoglobin biosynthesis is promoted by erythropoietin through the activation of pyridoxal phosphate in developing erythrocyte. Thus, the significant reduction in haemoglobin concentration in *C. albopunctaus* may be due to the inhibition of pyridoxal phosphate formation in the fish treated with Gammalin 20. Therefore, the macrocytic anaemic condition observed in this study could be due to impaired erythropoietin as a compensatory adaptive response to Gammalin 20 intoxication.

Compared with the control, the white blood cell count (WBC) increased significantly with increasing Gammalin 20 concentration and exposure period. The leukocyte number in the treatment groups was significantly higher than the control ($P < 0.05$). Mortality was observed only in the

treatment groups treated with 1.0 µg/L Gammalin 20 on 18th and 20th day. Similar leucocytosis was observed in *A. testudineus* exposed to monocrotophos (Santhakumar, 1999), in *Labeo* exposed to agrochemicals (Van Vuren, 1986; Srivastava and Narain,1982) but not in *Ictalurus punctatus* subjected to hypoxia (Scott and Rogers, 1981)

Leucocytes are inextricably involved in immunological function of the body. Thus the significantly elevated leucocyte number in the Gammalin 20- exposed fish represented part of the immunological defence mechanism to protect the fish against concomitant infection due to Gammalin 20 exposure. The study showed that Gammalin 20 had significant effect on the erythropoietic activity and other haematological parameters of *C. albopuctatus*.

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