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Induction of Microcytic-Hypochromic Anaemia in *Clarias gariepinus* (Burchell, 1822) Exposed to Sublethal Toxicity of 2, 3-Dichlorovinyl Dimethyl Phosphate (Sniper 1000EC) Under Laboratory Conditions

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ABSTRACT

Microcytic-hypochromic anaemia was induced in *Clarias gariepinus* (Burchell, 1822) at intervals of 1, 14 and 28 days. Experimental fish were exposed to test water separately diluted with sub lethal concentrations of sniper 1000EC of 0, 0.27, 0.31, 0.41 and 0.55mg/L. A 28 days exposure to sublethal concentrations of the toxicant resulted in changes in heamatological parameters of the fish on the exposure days (1, 14 and 28). Blood dyscrasias attributable to microcytic- hypochromic anaemia was observed with a significant ($p<0.05$) decrease in haemoglobin, haematocrit, red blood cells, white blood cells, lymphocytes, monocytes, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC). Neutrophils, eosinophil and basophil increased significantly ($p<0.05$) with increasing concentrations of the toxicant.

Keywords: Toxicity, Sniper 1000EC, *Clarias gariepinus* and Haematological Parameters

INTRODUCTION

Indiscriminate disposal of pesticides into water bodies leads to contamination of aquatic environment. They pose a severe threat to aquatic organisms, which form important members of food chain (Satyaparameshwar, *et al*., 2005). Pesticides are recognized as serious pollutants in the aquatic environment with the potential to cause deleterious effects on the biota, especially fish (Verma, *et al*., 1982). Aquatic environment is the ultimate sink for all pollutants where they are going to affects the zoans more than their counterparts in the two environs of land and water. The agri and aquaventures necessitates the use of chemicals and these chemicals as contaminants were ever studied (Rand and Petrocelli, 1985).

In developing countries, the human population is exposed to pesticide compounds through drinking water and via the food supply, including fish (Hayes, 1982). In recent years, extensive use of pesticides has caused many problems due to their adverse effects on the aquatic ecosystem and human health (Hanke, *et al*., 1983). Contamination of water by pesticides either directly or indirectly kills, reduced fish productivity or elevated concentrations of undesirable chemicals in edible fish tissue which are deleterious to humans eating these fishes (Adedeji, *et al*., 2012). The effects of pesticides concentrations on haematological parameters of different fish species have been studied by many investigators (Abo-hegab, *et al*., 1991; El-Elaimy, *et al*., 1994; Mekkawy, *et al*., 1996). Fish blood is being studied increasingly in toxicological research and environmental monitoring as possible indicator of physiological and pathological changes in fishery management and disease investigations(Mulcahy, 1975) as the blood in the gill has direct contact with the water medium and any unfavourable change in water could be reflected in the circulatory system(Adhikari, *et al*., 2002).

Sniper 1000EC (2, 3-dichlorovinyl dimethyl phosphate), a brand of dichlorvos, is contact acting and fumigant insecticide (Abubakar, 2013). Like all organophosphates, it kills insects and other target organisms because of its toxicity to the nervous system. This is achieved by inhibition of enzyme acetylcholinesterase (AchE) that breaks down acetycholine at the receptor site for partial uptake into the nerve terminal. Without functioning AchE, accumulation of acetylcholine results in depolarizing block of muscle membrane, producing rapid twitching of involuntary muscles, convulsions, paralysis and early death. Indiscriminate use of Sniper 1000EC is common among local fishermen from Northern parts of Niger state.

The African catfish (*Clarias gariepinus*) is an important food fish in Nigeria, which is also good for research work (Abubakar, 2012). *Clarias gariepinus* is not only the most predominant fish species raised in aquaculture in Nigeria, but has also served as an experimental model of aquatic vertebrate for two decades (Cavaco, *et al*., 2001). Despite the indiscriminate use of Sniper 1000EC by local fishermen, there is a paucity of information on its toxicity.

The aim of the present study was to evaluate the effect of sublethal concentrations of sniper 1000EC on

haematological parameters of *Clarias gariepinus* (Burchell, 1822) under laboratory conditions.

MATERIALS AND METHODS

Procurement of Test Fish

Juveniles of *Clarias gariepinus* (mean body weight 19.47± 1.05; mean standard length, 20.00± 0.45cm) were obtained from a reputable fish farm in Minna and brought to the laboratory. The fishes were kept in the glass aquaria to observe any visible pathological symptoms. Before introducing into the aquarium, fishes were treated with 0.1% $KmNO_4$ solution to obviate any dermal infection.

Acclimation of Test Fishes

Fishes were acclimatized to laboratory conditions for a period of two weeks. No mortality was recorded during acclimation period. The fishes were fed with pelleted feed containing 35 % crude protein at 3% body weight per day. Daily ration was divided into three portions and fed thrice per day. After acclimatization, fishes were kept in different concentrations of sniper 1000EC in different aquaria. The test solutions were renewed fortnightly.

Sources of Sniper 1000EC and Its Exposure

Sniper 1000EC (2, 3-dichlorovinyl dimethyl phosphate) was purchased from Minna central market. Renewal toxic test method (APHA, 1992) was used. Fishes were exposed to sub-lethal concentrations for 28 days. Control fish were also maintained under identical conditions without the toxicant.

Experimental Design

The experimental design was a complete randomized design. A total of one hundred and fifty (150) juvenile of *Clarias gariepinus* were randomly distributed into the tanks at a stocking rate of 10 fish per tank. The fifteen (15) tanks were assigned to 5 treatments (control inclusive). In order to determine the LC_{50}, the *C. gariepinus* were exposed to four different concentrations of sniper 1000EC for 96hrs. LC_{50} value obtained using EPA Probit Analysis programme version 1.5 was 8.21mg/l and one fifteen (1/15), one twenty (1/20), one twenty fifth (1/25) and one thirty (1/30) were taken as sublethal using the method of Abubakar (2013) to produce 0, 0.27, 0.31, 0.41and 0.55mg/L respectively.

Collection of Blood and Heamatological Tests

Blood samples were collected from both the control and experimental

fish at intervals of 1, 14 and 28days. The fish were stunned with a gentle knock on the head. The stunned fish was placed in a trough and blood was taken by caudal venous puncture using 23G x 11/4 (0.6 x 32 mm) syringe. The blood was put into EDTA vials and taken to Medical diagnostic laboratory in Minna for analysis using methods described by Blaxhall and Daisley (1973) at a wavelength of 540nm. The haematological parameters analyzed were Haemoglobin (Hb), packed cell volume (PCV), red blood cell (RBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), white blood cells (WBC) and its differential counts.

STATISTICAL ANALYSIS

All the data generated were managed with Microsoft Office Excel 2003. The data were analyzed with One-Way analysis of variance (ANOVA) procedure using Statistical Package for Social Sciences (SPSS version 16.0) for window. Statistical significance of difference among means was compared using Turkey (HSD) test.

RESULTS

Alteration in blood which is attributable to sublethal concentrations of sniper 1000EC were observed **28** days after exposure**.**

Haematological Parameters of the Exposure Concentrations and Duration in *Clarias gariepinus*

Exposure of *Clarias gariepinus* to sublethal concentrations of sniper 1000EC for 28 days resulted in Pancytopenia anaemia (consistent decrease in the values of Hb, PCV and RBC). The anaemia was the same along different sublethal concentration levels. Analysis of Erythrocyte indices (MCV, MCH and MCHC) revealed Microcytic-hypochromic anaemia. The values of Erythrocyte indices of MCV, MCH and MCHC for the exposed groups were significantly ($p<0.05$) lower than their respective control values. There were insignificant increase ($p>0.05$) in the values of WBC as evidence in immune response. Differential white blood cells analysis revealed no pronounced joint disorder with insignificance different between neutrophil and lymphocyte of the exposed groups and their controls ($p>0.05$). The values for the exposed groups of monocyte, eosinophil and basophil fluctuated relative to their control groups (Table 1).

Table 1: Haematological Parameters of *Clarias gariepinus* Exposed to Sublethal Concentrations of Sniper 1000EC (Mean ± SD)

	Concentration(mg/L)				
Parameters	**Control**	**0.27**	**0.33**	**0.41**	**0.55**
Hb(gdL^{-1})	7.4 ±0.21[a]	6.3±0.13[b]	5.8±0.30[c]	5.5±0.30[c]	5.2±0.28[c]
PCV (%)	29.3±1.63[a]	26.7±1.51[b]	25.9±1.62[b]	25.1±1.63[b]	24.0±1.60[b]
RBC($x10^{12}L^{-1}$)	2.43±0.03[a]	2.39±0.03[a]	2.35±0.05[a]	2.32±0.07[a]	2.24±0.10[a]
MCV(Fl)	119.88±5.84[a]	117.78±5.24[a]	109.60±5.30[b]	107.94±5.24[b]	105.77±4.84[b]
MCH(pg)	30.95±1.22[a]	26.13±0.66[b]	24.95±1.28[c]	23.66±0.84[c]	22.83±0.99[d]
MCHC (gdL^{-1})	26.20±0.94[a]	23.401.55[b]	22.42±1.48[b]	21.89±1.32[b]	21.57±1.27[b]
WBC($x10^{9}L^{-1}$)	4725±3.43[a]	4730±2.22[a]	4733±2.04[a]	47361.51[a]	4739±2.22[a]
Neutrophils %)	64.2(±0.39)[a]	64.4(±0.40)[a]	64.4(±0.22)[a]	64.7(±0.94)[a]	64.4(±0.57)[a]
Lymphocytes (%)	27.7±0.63[a]	28.7±0.44[a]	22.4±0.42[b]	28.4±0.19[a]	29.9±0.52[a]
Monocytes (%)	5.6±0.23[a]	4.6±0.18[b]	4.7±0.24[b]	4.1±0.24[b]	4.7±0.49[b]
Eosinophils (%)	1.9±0.20[a]	1.8±0.26[a]	1.9±0.14[a]	2.1±0.53[a]	2.3±0.49[a]
Basophils (%)	0.6±0.16[a]	0.5±0.14[a]	0.7±0.22[a]	0.6±0.22[a]	0.7±0.06[a]

Means of parameters with the same superscript along the rows are not significantly different at $p>0.05$.

Hb – Haemoglobin;
PCV – Packed Cell Volume;
RBC – Red Blood Cell Count;
MCV – Mean Corpuscular Volume;
MCH – Mean Corpuscular Haemoglobin;
MCHC – Mean Corpuscular Haemoglobin Concentration;
WBC – White Blood Cell Count

Haematological Parameters in *Clarias gariepinus* at Various Exposure Duration

Haematological parameters of *Clarias gariepinus* on tested days (1, 14 and 28) compared with their control reflected Microcytic-hypochromic anaemia in which Hb, PCV, RBC, MCV, MCH and MCHC decreased significantly ($p<0.05$) within exposure days. There were insignificant different in the values of WBC (immune response) at exposure days compared with their controls ($p>0.05$). Differential White blood cells analysis (Neutrophi, Lymphocyte, monocyte, eosinophil and basophils) showed no pronounced joint disorder at exposure days (1, 14 and 28) with insignificant different in the values of exposed and control groups at $p>0.05$ (Table 2).

Table 2: Haematological Parameters of Clarias *gariepinus* at the Various Duration of Exposure to Sniper 1000EC (Mean ± SD)

	Duration of Exposure (Days)		
Parameters	**1**	**14**	**28**
Hb(gdL^{-1})	5.7 ± 0.26^a	6.0 ± 0.33^b	6.5 ± 0.13^b
PCV (%)	24.9 ± 1.81^a	25.8 ± 1.71^b	27.8 ± 1.27^c
RBC($x10^{12}L^{-1}$)	2.31 ± 0.07^a	2.33 ± 0.06^a	2.44 ± 0.03^a
MCV(Fl)	107.20 ± 5.25^a	109.92 ± 5.97^b	115.98 ± 4.15^c
MCH(pg)	24.52 ± 0.72^a	25.58 ± 1.28^b	27.01 ± 1.10^c
MCHC (gdL^{-1})	22.57 ± 1.05^a	23.03 ± 1.59^a	23.50 ± 1.28^a
WBC ($x10^9L^{-1}$)	4732 ± 2.13^a	4734 ± 2.04^a	4731 ± 2.68^a
Neutrophils (%)	64.6 ± 0.47^a	64.1 ± 0.57^a	64.6 ± 0.46^a
Lymphocytes (%)	28.3 ± 0.34^a	28.3 ± 0.41^a	28.2 ± 0.53^a
Monocytes (%)	4.4 ± 0.19^a	5.1 ± 0.33^b	4.7 ± 0.47^a
Eosinophils (%)	2.1 ± 0.30^a	1.9 ± 0.35^a	2.0 ± 0.32^a
Basophils (%)	0.6 ± 0.19^a	0.6 ± 0.16^a	0.6 ± 0.13^a

Means of parameters with the same superscripts along the rows are not significantly different at $P>0.05$.

Hb – Haemoglobin;
PCV – Packed Cell Volume;
RBC – Red Blood Cell Count;
MCV – Mean Corpuscular Volume;
MCH – Mean Corpuscular Haemoglobin;
MCHC – Mean Corpuscular Haemoglobin Concentration;
WBC – White Blood Cell Count

DISCUSSION

This study clearly demonstrated that Sniper 1000EC induced sublethal effects on *C. gariepinus* exposed under laboratory conditions. According to the findings, the test chemical could be ranked toxic (Wagner, et *al.,* 1995). Findings demonstrated that *C. gariepinus* is sensitive to Sniper 1000EC. Reduction in erythrocyte count, haematocrit value and haemoglobin content of *C. gariepinus* can be attributed to such factors as (1) blood haemorrhage due to un equilibrium of osmotic pressure inside and outside blood cells (Heath, 1987), and (2) haemodilution of blood due to damage and bleeding of fish organs (Movotny and Beeman, 1990). The reduction in blood parameters was an indication of anaemia caused by this toxicant as the concentration increased. A decrease in the concentration of haemoglobin in blood is usually caused by the effect of pollutant in gills as well as decrease in oxygen carrying capacity; which also suggests anaemia in Catfish.

Haematological indices (RBC count, concentration of haemoglobin and haematocrit) have been reported to indicate secondary responses of an organism to pollutants (O'Neal and Weirich, 2001). MCV, MCH and MCHC values were calculated using the PCV, Hb and RBC. Decrease in MCV, MCH and MCHC were indication of Microcytic-hypochromic anaemia (Abubakar, 2013). Microcytic-hypochromic anaemias are characterized by abnormally small red cells with insufficient haemoglobin content (Lee, 1993). Microcytic-hypochromic anaemia can result from a variety of conditions that are caused by (1) disorders of iron metabolism, (2) disorders of porphyrin and heme synthesis, or (3) disorders of globin synthesis (Lee, 1993). The anaemic exposure could be as a result of destruction of RBC which is an indication of lack of iron (Abubakar, 2013) or haemodilution as reported by Sampath *et al.,* (1993). Iron plays a principal role in erythropoiesis, as it is necessary for proliferation and maturation of red blood cells and for haemoglobin synthesis (Abubakar, 2013). Iron deficiency anaemia develops slowly through three stages. (1) The body's iron stores for erythropoiesis are depleted. (2) Low iron transported to the bone marrow and iron-deficient erythropoiesis begins. Stage 3 begins when the small haemoglobin-deficient cells enter the circulation in sufficient numbers, replacing normal erythrocytes that have grown old and have been removed from the circulation (Lee, 1993). Joshi *et al.,* (2002) made a similar observation on blood parameters of *C. batrachus* exposed to Lindane and Malathion which are pesticides. The pesticide stress caused the microcytic anaemic condition by destroying mature erythrocytes, resulting in a reduced RBC, and disrupting iron-synthesizing mechanisms (Adhikari, *et al*., 2002). Microcytosis may be due to decrease in haematocrit during exposure. Similar pattern has been detected in *Labeo umbratus* after exposure to various pollutants (Van Vuren, 1986). Changes in WBC and differential counts have been reported to play important roles in the state of health of *C. gariepinus* (Ezeri, 2001; Omoregie and Oyebanji, 2002). Changes in neutrophils, eosinophils and basophils indicated stress condition in the fish and similar reports have been made by several authors including Johansson-Sjobeck *et al.,* (1978) and Anyanwu *et al.,* (2007).

CONCLUSION

The abnormalities in haematological parameters of *C. gariepinus* as a result of exposure to sublethal

concentrations of sniper 1000EC under laboratory conditions resulted in microcytic-hypochromic anaemia attributable to abnormally small red blood cell, haemodilution and impaired haemoglobin synthesis which make the study to recommend that the use of Sniper 1000EC by local fishermen be banned to save aquatic lives in Nigerian inland water bodies. This pesticide is highly poisonous to fish and its public awareness should be enhanced in the country.

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