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SUBLETHAL EFFECTS OF GAMMALIN 20® (LINDANE) ON THE HAEMATOLOGY OF *CLARIAS GARIEPINUS* (BURCHELL, 1822) JUVENILES

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ABSTRACT: *Five juveniles of Clarias gariepinus were exposed to sublethal concentrations (0, 0.25, 0.50, 0.75 ppm) of Gammalin 20 (Lindane) for 8 days to determine the effects on some hematological variables. The values of PCV (Packed Cell Volume), Leucocyte (Leuc), Haemoglobin (Hb), Platelets (Plt), Red Blood Cells (RBC) and Lymphocytes were significantly reduced (P ≤ 0.05) in all the treatment concentrations. Similar results were obtained with Monocytes (Mon.), Erythrocyte Sedimentation Rate (ESR) and Platelets while White Blood Cells (WBC), Neutrophils (Neut.), Lymphocytes (Lymp.) and Eosinophils (EOS) increased in values but were significant (P ≤ 0.05) in some treatment concentrations. There was a significant reduction of Hb and RBC resulting in macrocytic anaemia of the fish. Time, Concentration and Interaction produced major differences only in the values of WBC while haematological indices such as Mean Cell Volume (MCV), Mean Corpuscular Haemoglobin Concentration (MCHC) and Mean Corpuscular Haemoglobin (MCH) had no definite pattern during the exposure period, however, exposure to sublethal concentrations of Gammalin 20 caused changes in the blood characteristics of C. gariepinus which was not directly related to time and concentration.*

KEYWORDS: Clarias Gariepinus, Juveniles, Gammalin 20 (Lindane), Haematology, Sub-Lethal,

INTRODUCTION

Lindane (Gammalin 20) belongs to the Organochlorine (OC) pesticides class and one of the oldest classes of pesticides and a few OCs are still in use today. OC pesticides are so named because they include Carbon, Hydrogen, and Chlorine. Lindane has been used to control a wide variety of insect pest in agricultural, public health, and medicinal applications, sometimes, it is used to control fish diseases and ectoparasites and at very low doses it can also be used to sterilize pond bottom soil during pond preparation (Adhikari et al., 2004). Besides direct application, pesticides drifting from aerial to ground applications may contaminate the aquatic environment, thus endangering aquatic life directly and human life indirectly, hence, it is important that we understand the effects of pesticides on the physiology of fishes.

Changes in the haematology of fishes play an important role in monitoring and evaluation of possible physiological and pathological changes in vital organs (Mulcahy, 1975), furthermore, changes in blood characteristics of *C. gariepinus* caused by stress due to exposure to environmental pollutants have been studied by a number of authors (Adedeji et. al., 2008, Gabriel et.al., 2009, Gbadebo et. al., 2009, Adedeji, 2010, Abalaka, 2013, Ramesh and Nagarajan, 2013, Okomoda et. al., 2013, and Akani and Gabriel, 2015).

Adverse effects of pesticides on the blood of fish is possible because the gill of fish has direct contact with the water medium and any unfavorable changes in the water could be used to monitor the health status of fish as well as water quality. The effects of Lindane are primarily neurotoxic and generally produce a more rapid response in insects by increasing respiration to lethal levels. Lindane and its metabolites can be detected and measured in the blood and body fluids by clinical laboratory tests. Therefore, the present study is to determine the sub-lethal effects of Gamallin 20 (Lindane) on hematological parameters (PCV, MCHC, WBC, ESR, Leuc. and Plt.) of *Clarias gariepinus* juveniles.

MATERIALS AND METHOD

Juveniles of *Clarias gariepinus* (mean length - 9.52±2.40cm; mean weight, 4.08±2.25g) were obtained from the fish pond of Applied and Environmental Biology acclimated for a week under laboratory conditions and fed with 0.2 kg of pelleted feed from Taifeng Extrusion Feed™, during this period, the tanks were regularly cleaned and the water replaced. Twelve rectangular glass aquaria of forty litre capacity were utilized for the experiment, each aquarium was stocked with five fish and each treatment had three replicates including control. A two factor Randomized Complete Block Design (RCBD) was used.

Gammalin 20, manufactured by Chemical Allied Product Plc ® was procured off-the- shelf and stock solution was prepared by diluting 0.5 ml of Gammalin 20 in 999.5 ml of distilled water to give a stock of 1000 ml. Three concentrations of 0.25, 0.50, 0.75 ppm and one control (0.00) were prepared. The physico-chemical parameters (Temperature, pH, Dissolved Oxygen, and Total alkalinity) were analyzed according to Standard Methods by APHA (1992).

Sub-lethal exposure test lasted for eight days and was carried out with the aid of an aquaria which was filled with 10 litres of dechlorinated water before the various concentrations of the stock solutions were added and made up to 20 liters, stirred vigorously for one minute before introducing the fish, the solutions were renewed daily and at the end of the exposure period blood samples were collected from the kidneys of a minimally restrained fish, with the aid of a 2cm^3 disposable syringes and stored in labeled Ethylene Diamine Tetra-acetic Acid (EDTA) bottles. The fish was then kept in pesticide free water.

BLOOD ANALYSIS

Packed Cell Volume (PCV), Haemoglobin (HB), and Red Blood Cells (RBC)

The above parameters were determined using the methods in Blaxhall and Daisley (1973)

Platelets (PLT)

Platelet counts were done based on the Rees and Ecker's method (Brown, 1976).

Haematological Indices

The haematological indices were calculated according to Cheesbrough (1992), using the formula below:

MCV (fl*) = $PCV\ (1/l))/RBC\ x\ 10^{-12})/l\ )$

- *A femtolitre (fl) is 10^{-15} of a litre*

MCH (pg*) = $\frac{\text{HB in g/l}}{\text{RBC X } 10^{-12}/\text{l}}$

- *A pictogram (pg) is 10^{-12} of a gram*

MCHC (g/dl) = $\frac{\text{HB in g/l}}{\text{PVC } (1|\text{l})}$

Erythrocyte Sedimentation Rate

This was analyzed according to Wintrobe, 1978.

Leucocrit

Leucocrit was calculated in accordance with McLeay and Gordon, 1977

Data Analysis

The data were subjected to a two-way ANOVA and mean separation was done with the Duncan Multiple Range Test (DMRT) at 0.05 probability level unless otherwise indicated (Ogbeibu, 2005).

RESULTS/FINDINGS

There were no obvious signs of disease or abnormality in the physical conditions of the fish and the behavior of the experimental fish during the period of acclimation, although, a 10% mortality was recorded. Table 1 shows the mean haematological variables of *Clarias gariepinus* exposed to different concentrations of Gamalin 20 for 8 days. In most of the exposure concentration, the values of Hb (Haemoglobin), RBC (Red Blood Cell), PVC (Packed Cell Volume) fell below that of control and the values were significantly different at $P < 0.05$ level with the various concentrations, time of exposure and their interactions.

Table 1: Mean (± SD, DMRT) Haematological Variables of *Clarias Gariepinus* Exposed to Different Concentrations of Gamalin 20 for 8 days

S/no.	Haematological variables	Concentrations (%)			
		0.00	0.25	0.50	0.75
1.	Haemoglobin (g/l)	8.05 ± 0.76[d]	7.75 ± 0.76[c]	7.36 ± 0.76[b]	6.51 ± 0.76[a]
2.	RBC ($x10^9$/l)	4.01 ± 0.45[d]	3.67 ± 0.45[c]	3.32 ± 0.45[b]	3.01 ± 0.45[a]
3.	PCV (1/l)	30.26 ± 0.57[d]	28.55 ± 0.57[c]	25.31 ± 0.57[b]	21.16 ± 0.57[a]
4.	Leucocrit ($x10^9$/l)	3.95 ± 0.47[d]	3.81 ± 0.47[c]	3.53 ± 0.47[b]	3.38 ± 0.47[a]
5.	W.B.C. ($x10^3$/µL)	18.37 ± 0.75[a]	21.02 ± 0.75[a]	22.13 ± 0.75[b]	22.10 ± 0.75[b]
6.	Neutrophils ($x10^9$/l)	23.30 ± 0.72[a]	24.84 ± 0.72[a]	25.43 ± 0.72[a]	28.20 ± 0.72[b]
7.	Lymphocytes ($x10^9$/l)	57.55 ± 1.18[c]	51.09 ± 1.18[b]	51.10 ± 1.18[b]	45.76 ±1.18[a]
8.	Eosinophils (10^9/l)	3.20 ± 0.29[a]	4.18 ± 0.29[b]	4.45 ± 0.29[b]	5.63 ± 0.29[c]
9.	Monocytes (10^9/l)	2.78 ± 0.15[a]	3.30 ± 0.15[b]	3.92 ± 0.15[c]	4.38 ± 0.15[d]
10.	ESR (mm/hr)	4.38 ± 0.15[a]	5.34 ± 0.15[b]	5.95 ± 0.15[c]	6.38 ± 0.15[d]
11.	Platelets (10^9 g/l)	122.55±1.97[d]	109.06±1.97[c]	100.89±1.97[b]	90.96±1.97[a]

Means with the same superscript in the row are not significantly different ($p \geq 0.05$)

Values of PCV (Packed Cell Volume), Leucocyte (Leuc), Haemoglobin (Hb), Platelets (Plt), Red Blood Cells (RBC) and Lymphocytes were significantly reduced ($P \leq 0.05$) in all the treatment concentrations. Similar results were obtained with Monocytes (Mon.), Erythrocyte Sedimentation Rate (ESR) and Platelets while White Blood Cells (WBC), Neutrophils (Neut.), Lymphocytes (Lymp.) and Eosinophils (EOS) increased in values but were significant ($P \leq 0.05$) in some treatment concentrations.

Table 2 shows the mean haematological indices of Clarias gariepinus exposed to different concentrations of Gamalin 20. The Mean Cell Volume (MCV), Mean Corpuscular Haemoglobin Concentration (MCHC) and Mean Corpuscular Haemoglobin (MCH) had no definite pattern during the exposure period and were not significant in some exposure concentrations, however, exposure to sublethal concentrations of Gammalin 20 caused changes in the blood characteristics of *C. gariepinus* which was not directly related to time and concentration.

Table 2: Mean (± SD, DMRT) Haematological Indices of *Clarias Gariepinus* Exposed to Different Concentrations of Gamalin 20 for 8 days

S/no.	Haematological indices	Concentration (%)			
		0.00	0.25	0.50	0.75
1.	MCV (fl)	76.75 ± 1.72[b]	82.09±1.72[c]	81.59±1.72[c]	73.40 ± 1.72[a]
2.	MCHC (g/dl)	20.57±0.45[a]	21.45±0.45[a]	22.69±0.45[a]	22.80±0.45[c]
3.	MCH (pg)	27.41±0.88[a]	26.99±0.88[a]	27.86±0.88[c]	30.53±0.88[b]

Means with the same superscript in the row are not significantly different ($p \geq 0.05$)

Table 3 shows the results of physicochemical analysis, which indicated that there were no significant differences between the values of the various variables and were within the recommended tolerance range for cultured fish (Mackereth, 1963), consequently, water quality was not thought to be the cause of physiological changes in the fish.

Table 3: Mean (±SD) Physico-Chemical Variables of Different Concentrations of Gamalin 20.

S/no.	Physico-chemical variables	Concentrations (%)			
		0.00	0.25	0.50	0.75
1.	Temperature (^oC)	26.00 ± 0.02^a	26.15 ± 0.01^a	26.29 ± 0.01^a	26.03 ± 0.01^a
2.	D.O (Mg/L)	7.31 ± 0.01^a	7.29 ± 0.03^a	7.20 ± 0.03^a	7.13 ± 0.02^a
3.	pH	5.59 ± 0.01^a	6.00 ± 0.01^a	5.05 ± 0.01^a	6.03 ± 0.02^a
4.	Alkalinity (Mg/L)	25.57 ± 0.02^a	26.53 ± 0.01^a	25.60 ± 0.03^a	25.90 ± 0.01^a

Means with the same superscript in the row are not significantly different ($p \geq 0.05$)

DISCUSSION

Blood is a path physiological reflector of the whole body and therefore, blood parameters are important in diagnosing the structural and functional states of fish exposed to toxicants. (Lindane) Gammalin 20 is relatively toxic to birds and mammals but extremely toxic to aquatic organisms, including fish, amphibians and invertebrates (Jolley et al., 2000).

Physicochemical variables recorded in this study were observed not to differ significantly and were within tolerance range suggested for cultured fish by Mackereth (1963), similar observations were also reported by Adigun, (2005), Kolo et al., (2008), Kolo et al., (2009) ,Okomoda and Ataguba (2011) and Okomada et al., 2013 for other toxicants , thus , observations of water quality variations were not thought to affect the fish , rather the toxicant may have had an effect on the physiological processes of the fish which were observed through haematological variables.

Exposure of *C. gariepinus* to sublethal levels of Gammalin 20 resulted in a significant time and concentration dependent decrease in the values of PVC, Hb and RBC which may be an indication of the deleterious effects of the chemical pollutant on the body cells fluid. Anderson and Klontz (1965) reported that haemoglobin determination , red blood cell counts and haematocrit are recommended as check on the health of fish stocks , however , the observed decrease in erythrocyte count, Red blood Cells (RBC); Haemoglobin (HB) and Pack Cell Volume (PCV) of *C gariepinus* during exposure period agreed with the trend noticed in fish species exposed to chemical irritants (Sancho et al., 2000; Seth and Saxena, 2003; Adhikari et al., 2004,Okomoda et al., 2013 , Akani and Gabriel , 2015 and Edori et al., 2013).

Packed cell volume (haematocrit) is a vital tool for assessing the amount of plasma and blood corpuscles in the blood and also used in determining the oxygen carrying capacity of the blood (Larsson et al., 1985), nevertheless, Wedemeyer et al., 1983 reported that the haematocrit value is not easily altered as other parameters and should be used in conjunction with erythrocyte and leucocytes counts, haemoglobin content, osmotic fragility and differential leucocytes count , while , Ragnar , 1992 reported that hematocrit values of fish blood range from almost 0 to more than 50% for surface feeding species and in most Teleost the value was between 20 and 40% . A decrease in the PCV values after exposure to Gamalin 20 as observed in this study may be indicative of anaemia and haemodilution , similar observations were noted by Ahmad et al., 1995 , furthermore ,variations in the PCV and Hb levels in the blood of the a freshwater Teleost (*Labeo rohita*) were reported to indicate changes in membrane permeability, a complement to the histological findings on hematopoietic tissues (Adhikari et al., 2004).

The increase in MCHC and MCH suggested that the anaemia was macrocytic. Svoboda *et al.*, (2001) noted that increase in the rate of erythrocyte may lead to anaemia destruction in hemopoietic organ. The macrocytic anaemia condition in *C. gariepinus* may possibly be due to reduction of RBC and PCV which was also reported in a number of fish species exposed to sublethal concentrations of Lindane (Anandkumar et al., 2001).

Reduction in oxygen carrying capacity of the blood will result in a toxic internal environment due to build up of metabolic wastes in the organs or tissue resulting in respiratory stress. Similar observations were made in *Sarotherodon. mossambicus* exposed to an organophosphate (Ramaswamy et al., 1996). Under this condition the fish will attempt to escape from the toxic environment once the threshold is exceeded. Grillistch et al., (1999) reported that *Tilapia guineensis* exhibited behavioural responses to acute and sublethal chemical stress.

The time-dependent increment in the number of WBC (White blood cell) in *C. gariepinus* exposed to Gammalin 20 may be due to haemoconcentration (Adhikari et al., 2004) and stimulated lymphopoiesis and/or enhanced release of lymphocytes from lymphopoeloid tissues (Meenakala, 1978). This pattern of response of leucocyte (WBC) could be associated with the presence of pollutant induced tissue damage and severed disturbances of the non specific immune system leading to increased production of WBC (Das and Mukherjee, 2003), similarly, increases in WBC have been reported in fish species exposed to toxicants (Das and Mukherjee, 2003; Adhikari et al., 2004).

Changes in the number of platelets in fish have been reported in fish species exposed to environmental chemicals (Mahajan and Dheer, 1983), however, it must be noted that platelets are cell fragments which circulate within the blood with a life span of about 10 days. The middle of the night and morning hours are time of platelet activation (Farell, 2011). The decrease in the number of Platelets with time of exposure in this study suggests that the chemical interfered with thrombocytopoiesis in the bone marrow and this agrees with the observation of Hall and Roger (1984). There was a positive time dependent increase in the values of *C. gariepinus* exposed to Gammalin 20 which was contrary to previous studies on other fish species (Mclealy and Gordon, 1977, Das and Mukherjee, 2003) , this may be due to the differences in the sources of stress.

CONCLUSION

Results from this study indicate that sub-lethal concentrations of Gammallin 20 could cause changes in the blood characteristics of *Clarias gariepinus*. However, the pattern of the changes during exposure and withdrawal were not directly related to the time and exposure concentrations. Remarkable changes in WBC, Neutrophils, Monocytes and Lymphocytes were observed during acclimation, as such, the fishes were subjected to environmental stress, which resulted in the increase of these parameters to enhance the natural defense mechanisms of *Clarias gariepinus*.

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