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Hematological Parameters and Serum Biochemistry of *Clarias gariepinus* Juveniles Exposed to Vestaline® (Pendimethalin) Herbicide

Odo Joel Inya[1, *], Amuta Elizabeth[2], Umeh Ebele[2], Azua Emmanuel[2], Odo Edna[1]

[1]Department of Fisheries and Aquaculture, University of Agriculture, Makurdi, Nigeria

[2]Department of Biological Sciences, University of Agriculture, Makurdi, Nigeria

Email address:
odojoel@gmail.com (O. J. Inya)

[*]Corresponding author

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Abstract: The study was carried out to determine the effects of sub lethal concentrations of vestaline® (pendimethalin) herbicide to haematological and serum biochemistry of juveniles of African catfish (*Clarias gariepinus*) using static bioassays. The toxicity was carried out using 180 healthy and active fish of mean weight of 27.97±0.03 g which were divided into six treatments with each treatment having ten fish and the setup was in triplicate. The fish were exposed to (1.89 mg/l, 3.79 mg/l, 5.68mg/l, 7.57mg/l. 9.46mg/l and 0.00 mg/l)) concentrations of Vestaline® (Pendimethalin) herbicide for a period of eight weeks during which the fish were fed at 5% of their body weight twice daily. Data obtained were analyzed for descriptive statistics (mean and standard error of means).The data were further subjected to analysis of variance (ANOVA) at $P \leq 0.05$ using Minitab. Results showed that Haematological parameters reduced significantly ($P<0.05$) in amount of HCT, Hb and RBC with increase in concentration an indication of Anaemia. While WBC increased significantly ($P<0.05$) with increase in concentration. All were dose dependant. The results of Serum Biochemistry showed significant difference ($P<0.05$) in all the parameters determined. There was significant ($P<0.05$) decrease in Total protein and Albumin while Cholesterol, Alanine aminoTransferase, Aspartate aminoTransferase and Creatine all increased significantly ($P<0.05$) with increase in concentration. Vestaline® (Pendimethalin) herbicide from this study is toxic on *Clarias gariepinus* juveniles so its use around aquatic environment should be done with care.

Keywords: *Clarias gariepinus*, Vestalin® (Pendimethalin), Hematology

1. Introduction

The use of pesticides through agricultural practice can find its way into aquatic ecosystem and affect non target organisms. This can be possible through indiscriminate use, careless handling, accidental spillage, or discharges of this herbicide from farm lands by rain into natural waterways which can have harmful effects on fish population and other forms of aquatic life and may contribute long term effects in the environment [8]. Agricultural chemicals use and their residual effects on non- target organism have not been largely considered in Nigeria [8].

Vestaline® is liquid herbicide of the group of of dinitroaniline, its active ingredient is Pendimethalin. It is a class of herbicides used in premergence and postemergence applications to control annual grasses and certain broadleaf weeds. Pendimethalin is approved in Europe, North America, South America, Africa, and Asia for different crops including cereals (wheat, barley etc), corn, soybeans, rice, potato, legumes, fruits, vegetables, and ornamental plants [12].

Haematological analysis can be used for monitoring the health status of fish from the information's it provides [9].

Hematological indices can vary depending on so many factors ranging from environmental factors to fish species, age, sexual maturity, the toxicant, length of exposure, water quality, and health condition [8].

Clarias species is a widely distributed fish which constitutes one of the major fisheries in Asia and Africa. Some records have shown that Clarias contributes about 14% of over 6,000 tonnes of annual fish production from all fisheries sectors [7]. The common species found in Nigeria are *Clarias gariepinus*, *Clarias angullaris* and *Clarias lazare*. The *Clarias gariepinus* is a prominent cultured species because of its hardiness and fast growth rate. Several investigations have been carried out on various toxicants with *Clarias* sp. [4]. The objective of this study was to evaluate the effects of Vestalin Herbicide on the heamatological parameters and blood serum biochemistry of Clarias *gariepinus* juveniles.

2. Materials and Methods

2.1. Study Area

The study was carried out in the General purpose Laboratory, Department of Fisheries and Aquaculture, University of Agriculture Makurdi, Benue State Nigeria.

2.2. Sample Collection

A total number of 250 (Two hundred and fifty) *Clarias gariepinus* juveniles were used throughout the study. Healthy and active juveniles of African catfish *Clarias gariepinus* were collected from University of Agriculture Makurdi fish farm for the study. The fish were transported in well aerated plastic containers to the laboratory. The fish were acclimatized in the laboratory for two weeks during which they were fed with commercial floating feed (coppens) at 5% of their body weight. Unconsumed feeds were removed and water replenished twice a week as recommended by [13].

2.3. Experimental Procedure

A preliminary range finding test was carried out based on the concentration of the active ingredient in order to know which concentrations to be used. The concentration was done using a serial dilution formula $C_1V_1 = C_2V_2$. The result obtained from the range finding test provided a guide for the concentrations. The concentrations that were used are; 1.89 mg/l, 3.79 mg/l, 5.68 mg/l, 7.57 mg/l and 9.46 mg/l.

A total of eighteen (18) glass aquaria were used for the study. Ten juveniles of *Clarias gariepinus* were introduced into each aquarium containing 20 liters of water into which the concentrations of vestalin as mentioned above were introduced. The experiment was carried out for a period of 56 days. At the end of the experiment, blood samples were collected by randomly selecting fish from the various treatments using a 2 mm needle and syringe. The blood was placed in ethylene-diamine-tetra-acetic acid (EDTA) bottles to prevent coagulation. The blood samples were transported in ice packed to the University of Agriculture Makurdi Vertinary teaching Hospital, North bank laboratory where they were analyzed using an automated hematology analyzer mindrey vet model 2800 while serum biochemistry was determined using UV spectrophotometer. [8].

3. Data Analysis

Data collected from the study was analyzed for descriptive statistics (mean and standard error of means) using Minitab 17. The data was further subjected to analysis of variance (ANOVA) at 0.05% level.

4. Result

The mean HCT, Hb, RBC, WBC, Platelet count, derived erythrocyte indices (MCV, MCH and MCHC) and Leucocytes Differential Counts (Lymphocytes, Neutrophil, Basophil, Eosinophil, Monocyte and Heterophil) of the fishes exposed to Vestalin herbicide are presented in table 1. Significant variations ($P < 0.05$) were observed between the various blood parameters with different concentrations of the toxicant.

HCT, Hb and RBC of Clarias *gariepinus* juveniles exposed to different concentration of Vestalin® herbicide showed a dose-dependent relationship in which there was significant ($P<0.05$) decrease in their values with increase in concentration. Treatment 1(0.00 mg/l) had the highest HCT of 32.50±0.50% while treatment 6 (9.46 mg/l) had the lowest of 21.11±0.03%. Treatment 1(0.00 mg/l) had the highest Hb of 10.93±0.04 g/L while treatment 6 (9.46 mg/l) had 7.29±0.06 g/L. Also in RBC, Treatment 1(0.00 mg/l) had the highest value of 6.23±0.02($x10^{12}$/L) while treatment 6 (9.46 mg/l) had the lowest value of 3.67±0.04($x10^{12}$/L). WBC and platelets increased significantly ($P<0.05$) with increase in concentration with the control having the lowest WBC of 2.55±0.05, platelets of 38.25±0.05 and treatment 6 (9.46 mg/l) had the highest WBC value of 5.76±0.05 and platelets 63.26±0.06.

On the other hand, derived Erythrocyte indices MCH, MCV and MCHC although differ significantly ($P < 0.05$) but did not follow any trend. In MCH, 5.68 mg/l had the highest value followed by 3.79 and 9.46 while there was no significant difference in MCH for 0.00 mg/L, 1.89 mg/L and 7.57 mg/L because they have the same superscript. For MCV, 3.79 mg/l had the highest value followed by 5.68, 9.46, 1.89 and 7.57 mg/l respectively. There was no significant difference ($p>0.05$) in MCHC across the treatments.

4.1. Leucocytes Differential Counts

There was significant difference ($P<0.05$) in Leucocytes Differential Counts. Lymphocytes and Neutrophil shows decrease with increase in concentration with Treatment 1(0.00 mg/l) having the highest Lymphocytes of 56.50±0.50% and Neutrophil value of 50.20±0.10% where as treatment 6 (9.46 mg/l) had the lowest Lymphocytes of 45.40±0.50% and Neutrophil of 45.20±0.50. Basophil, Eosinophil, Monocyte and Heterophil all increased significantly ($P<0.05$) with increase in concentration with the control, Treatment 1(0.00 mg/l) having the lowest values while treatment 6 (9.46 mg/l) had the highest values for all the parameters mentioned.

4.2. Serum Biochemistry

After the exposure, there were found significant (P<0.05) alterations in the concentrations of the determined Biochemistry parameters compared to the control group. The results showed that there was significant (P<0.05) decrease in Total protein and Albumin where as there was an increase in Cholesterol, Alanine amino Transferase, Aspertate amino Transferase and Creatine with increase in concentration. Treatment 1 had the highest total protein of 4.20±0.10 while treatment 6 have, 2.86±0.03 this trend was also found in Albumin. Cholesterol, Alanine amino Transferase, Aspertate amino Transferase and Creatine all increased significantly (P <0.05) with increase in concentration with the control, Treatment 1(0.00 mg/l) having the lowest values while treatment 6 (9.46 mg/l) had the highest values for all the parameters.

Table 1. *Mean Hematological Parameters of Clarias gariepinus juveniles exposed to concentrations of Vestaline® (Pendimethalin) Herbicide for eight weeks.*

Parameters	Concentration (mg/L)						P-Value
	0.00	1.89	3.79	5.68	7.57	9.46	
HCT (%)	32.50±0.50^{a}	32.30±0.20^{a}	28.35±0.15^{b}	26.17±0.06^{c}	24.78±0.03^{d}	21.11±0.03^{e}	<0.01
Hb (g/L)	10.93±0.04^{a}	10.25±0.05^{b}	9.39±0.02^{c}	9.05±0.01^{d}	8.37±0.03^{e}	7.29±0.06^{f}	<0.01
RBC (x10^{12}/L)	6.23±0.02^{a}	5.91±0.02^{b}	4.70±0.02^{c}	4.55±0.05^{d}	4.77±0.05^{c}	3.67±0.04^{e}	<0.01
WBC (x10^{9}/L)	2.55±0.05^{e}	2.72±0.00^{e}	3.80±0.10^{d}	4.77±0.04^{c}	5.36±0.04^{b}	5.76±0.05^{a}	<0.01
PLT (x10^{9}/L)	38.25±0.05^{f}	41.15±0.03^{e}	45.66±0.06^{d}	51.75±0.05^{c}	57.85±0.05^{b}	63.26±0.06^{a}	<0.01
MCH (g/dL)	17.66±0.02^{c}	17.31±0.01^{c}	19.89±0.04^{b}	20.47±0.38^{a}	17.70±0.03^{c}	19.87±0.05^{b}	<0.01
MCV (g/dL)	53.12±0.02^{d}	54.55±0.05^{c}	59.68±0.06^{a}	58.11±0.09^{b}	52.48±0.04^{e}	58.08±0.01^{b}	<0.01
MCHC (g/dL)	33.28±0.01	31.80±0.02	33.36±0.14	34.60±0.01	33.74±0.04	34.35±0.05	0.06^{ns}
Lymphocyte (%)	56.50±0.50^{a}	52.30±0.29^{b}	50.45±0.15^{c}	47.85±0.05^{d}	45.40±0.20^{e}	45.50±0.50^{e}	<0.01
Basophil (%)	0.35±0.05^{e}	0.61±0.01^{d}	0.80±0.01^{d}	1.08±0.06^{c}	1.45±0.05^{b}	1.80±0.10^{a}	<0.01
Eosinophil (%)	5.60±0.10^{c}	5.90±0.10^{bc}	6.20±0.10^{ab}	6.20±0.10^{ab}	6.35±0.15^{ab}	6.60±0.20^{a}	0.01
Monocyte (%)	4.80±0.10^{c}	6.85±0.35^{b}	8.15±0.15^{ab}	9.80±0.10^{a}	8.20±0.10^{ab}	9.80±0.00^{a}	<0.01
Heterophil (%)	32.30±0.20^{d}	33.20±0.00^{c}	35.80±0.10^{b}	36.50±0.50^{b}	37.70±0.10^{a}	38.25±0.05^{a}	<0.01
Neutrophil (%)	50.20±0.10^{a}	48.45±0.15^{b}	47.30±0.20^{c}	46.40±0.10^{d}	45.20±0.00^{e}	45.50±0.50^{e}	<0.01
Basophil (%)	0.35±0.05^{e}	0.61±0.01^{d}	0.80±0.01^{d}	1.08±0.06^{c}	1.45±0.05^{b}	1.80±0.10^{a}	<0.01

Means on the same row with different superscript are statistically significant (P<0.05), ns = not significant.
HCT=Haematocrit, PLT=Platelets.
Hb=Haemoglobin, MCV=Mean Corpuscular Volume.
RBC=Red blood cell, MCH=Mean Corpuscular Haemoglobin.
WBC=White blood cell, MCHC=Mean Corpuscular Haemoglobin Concentration.
(g/dL)=grams per deciliter.

Table 2. *Mean of some Serum Biochemistry of Clarias gariepinus juveniles exposed to concentrations of Vestaline® (Pendimethalin) herbicide.*

Parameters	Concentration (mg/L)						P-Value
	0.00	1.89	3.79	5.68	7.57	9.46	
TP	4.20±0.10^{a}	3.90±0.10^{b}	3.87±0.05^{b}	3.93±0.02^{b}	3.72±0.05^{b}	2.86±0.03^{c}	<0.01
ALB	2.85±0.05^{a}	2.55±0.05^{b}	2.45±0.05^{bc}	2.25±0.05^{cd}	2.21±0.09^{d}	1.35±0.03^{e}	<0.01
CHOL	93.70±0.20^{f}	97.45±0.05^{e}	102.40±0.20^{d}	109.40±0.10^{c}	112.50±0.10^{b}	113.20±0.10^{a}	<0.01
ALT	24.2±0.10^{f}	28.80±0.10^{e}	31.45±0.15^{d}	39.75±0.05^{c}	42.40±0.10^{b}	45.50±0.10^{a}	<0.01
AST	69.65±0.15^{e}	73.25±0.05^{d}	76.00±1.00^{c}	78.35±0.15^{b}	90.45±0.15^{a}	91.50±0.50^{a}	<0.01
Creat	0.08±0.00^{e}	0.91±0.01^{a}	0.16±0.01^{cd}	0.13±0.01^{d}	0.17±0.00^{bc}	0.19±0.00^{b}	<0.01

Means on the same row with different superscript are statistically significant (P<0.05).
TP=Total Protein, ALB=Albumin;
CHOL=Cholesterol ALT=Alanine amino transferase;
AST=Aspertate amino transferase;
CREAT=Creatine.

5. Discussion

Haematological parameters observed during the sub-lethal studies includes, Red Blood counts (RBC), White Blood Counts (WBC), Haemoglobin (Hb), Haematocrit (HCT), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), Neutrophils, Lymphocytes, Eosinophils, Basophils and Monocytes and Heterophil. this were done because Haematological and profiles of blood can provide important information about the internal environment of organisms [18].

The results of this study revealed decrease in Haematocrit (HCT), Haemoglobin (Hb), Red blood cell (RBC), Platelets (PLT) but an increase in white blood cells (WBC). This does not agree with findings of [6] who reported a significant increase in RBC count of *C. gariepinus* when subjected to Zn treatment, The attributed the red blood cell elevation to blood cell reserve combined with cell shrinkage as a result of osmotic alterations of blood by the action of the metal.

[11] observed that higher concentration of pendimethalin herbicide cause significant drop in the number of erythrocytes and leukocytes, hemoglobin concentration, haematocrit level, mean corpuscular hemoglobin, mean

corpuscular volume and mean corpuscular hemoglobin concentration level of *Cyprinus carpio*. [1] reported that diazinon exposed African catfish (*Clarias gariepinus*) showed significantly lower values of erythrocyte count, leukocyte count, hemoglobincontent, and hematocrit compared to the control group after 96 h of exposure. [3], reported similar trend of result. Their experiment showed that exposure of *Labeo rohita* to sub-lethal levels of cypermethrin and carbofuran resulted in significant decrease in blood erythrocyte count. [16] Found significant decrease in erythrocyte in fish *Channa punctatus* after exposure to sub lethal dose of mercury toxicant. [17] Also reported that total erythrocyte and leukocyte counts were significantly lower in the blood of *Rhamdia quelen* exposed to glyphosate herbicide relative to the same type of cells counted in the blood of non-exposed fish.

Haemoglobin is the red pigment contained in the erythrocytes. it functions physiologically in the transport of dissolved gases, principally, dissolved oxygen and carbon dioxide within the body of the fish. The decrease in the Haematocrit (HCT) and Hemoglobin (Hb) in this study could be attributed to gill damage and or impaired osmoregulation causing anaemia and haemo dilution or can be attributed to the lysing of erythrocytes. Similar reductions have been reported by [21] when they exposed fish to polluted environment under laboratory conditions.

Inhibition of erythropoiesis and increase in the rate of erythrocyte destruction in hematopoietic organs is a major cause of decrease in RBC count [15]. In the present study, the significant decrease in RBC and hemoglobin content might have resulted from the lowering of the oxygen content of the water due to the presence of the toxicant in the test media.

Leucocytes are involved in immunological system function and their numbers increase significantly with increase in exposure period. This may be attributed to immunological responses of the fish to pesticides. Such an increase in WBC count occurs by the increase in lymphoperisis or enhanced release of lymphocytes from lymphoid tissues [22]. The increase in WBC count in the present study indicates the stress condition of the fish resulting to stimulation of immune system to produce antibodies to cope up with stress due to tissue damage or may be related to compensatory response of lymphoid tissues to circulating lymphocytes.

Platelets count for the fish exposed increased significantly with increase in concentration. The Mean Corpuscular Haemoglobin Concentration is a good indicator of red blood cell swelling [26]. MCHC although varied but shows no significant difference ($p>0.05$) across the treatments. The fluctuation in the mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) in the present study, indicates that concentration of haemoglobin in red blood cells were much lower in the exposed fish, resulting in aneamic condition. [10] Observed similar fluctuations.

There was sharp increase in the leucocytes differential count of *Clarias gariepinus* juveniles exposed to sub-lethal concentration of vestaline ie percentage of Lymphocyte, heterophils, monophils, Heterophil, basophils and eosinophils. In this study, lymphocytes had the highest percentage compared to the rest leucocytes differential count and it is known that lymphocytes constitute the majority of white blood cells present in the blood of fish. The significant increase in lymphocyte value with increase in concentration obtained compared to the control in this study reflected the quick intervention needed in the stress situation. Lymphocytes are natural killer cells and at these concentrations, more T-cells and B-cells were needed to destroy antigens and produce more antibodies.

Monocytes are phagocytic in action. It increases during sub lethal toxicity. They are a type of white blood cell involved in the immune response to foreign substances.

The increase in WBC counts recorded in this research Suggest that the antigens (herbicide) stimulated the production of more WBC to improve the health status of the fishes which however, agreed with the reports of [2]

Serum biochemistry analysis provides information that can be used for monitoring the health condition of fishes. Biochemical changes depend on the fish species, age, sexual maturity and health condition. Analyses of serum biochemical constituents' levels have shown useful information in detection and diagnosis of metabolic disturbances and diseases in fishes [2].

All biological activities are regulated by enzymes and hormones which are also proteins and proteins plays useful role in the metabolism and regulation of water balance, thus, Proteins play a vital role in physiology of living organisms. Albumins in fish are involved in metabolism and perform the functions of transporting substances necessary for life activities [14].

The present results showed that Total protein (TP) and Albumin decreased as the concentration of the toxicant increases. This finding is similar to the report of other study on Pendimethalin herbicide exposed to *Oreochromis niloticus* that reported a decrease in total protein and Albumins [19]. The decrease was attributed to the inhibitory effect of dinitroanalin on the protein synthesis. Also [2] reported a decrease in total protein and Albumin with increase in concentration of *Oreochromis mossambicus* exposed to Pyrazosulfuron –ethyl toxicant.

These results deviated from the findings of [20] that reported increase in total protein and Albumins with increase in concentration. The variation may be attributed to difference in the toxicant. The reduction of protein (hypoproteinaemia) in this study suggests an increase in proteolytic activities and possible utilization of their products for metabolic purpose to overcome stress. While the reduction in Serum albumin (hypoalbuminaemia) can be attributed to the liver damage.

Alanine amino transferase (ALT) and Aspertate amino transferase (AST) are normally found in the liver but in disease conditions or injuries, when the cells are destroyed, these enzymes are released into blood and their high concentration in blood are considered as indicator of abnormality.

There was an increase observed in AST and ALT concentrations in vestalin exposed fish. Similar increased activities of AST and ALT were reported by other authors. [25] reported Increase in transaminase activities in *Clarias gariepinus* exposed to aqueous extract of Nigeria crude oil. also [19] Reported increased AST and ALT concentrations when *Oreochromis niloticus* were exposed to stomp Pendimethalin herbicide The increase in these parameters indicated stressed based tissue impairment. According to [24], change in activities of transaminase indicates amplified transamination processes and an increase in transamination occurs with amino acid input into the TCA cycle to cope with the energy crisis during pesticide stress.

Creatinine is derived from catabolism of creatine found in muscle, tissue and its catabolism to creatinine occurs at a steady rate. Severe kidney damage will lead to increased creatinine levels. Result showed that cholesterol and Creatinine increased as the concentration of the toxicant increases. The increase cholesterol could be attributed to necrosis of the liver by the toxicant which leads to the impairment in the metabolism of this parameter thus resulting in significant increase in the concentration of the parameter. Similar result was recorded by [27]. While the alteration in the levels of serum creatinine may, therefore, be due to an alteration in glomeruli filtration rate. Becouse excretion occurs through a combination of glomerular filtration and tubular secretion. [5].

6. Conclusion

In conclusion, the results have revealed that sub-lethal concentrations of Vestaline® (Pendimethalin) herbicide are harmful directly or indirectly to *Clarias gariepinus* juveniles because Changes were observed in hematological parameters such as haemoglobin, erythrocyte count, MCV, MCH, MCHC, Hct and Leucocytes Differential Counts of *Clarias gariepinus* such as Lymphocytes, Neutrophil, Basophil, Eosinophil, Monocyte and Heterophil to be dose dependent.

Serum Biochemistry of exposed *Clarias gariepinus* juveniles such as Total protein, Albumin, Cholesterol, Alanine amino Transferase, Aspertate amino Transferase and Creatine were also dose dependent. The results of Serum Biochemistry and haematological parameter values indicate that the exposed fish were faced with serious metabolic crisis.

The results of the study suggest that exposure of *C. garipinus* to Vestaline® could lead to alterations in hematological parameters; therefore it should be used with caution and in a sustainable manner, as it could be hazardous to aquatic biota.

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