EVALUATION OF THE AMELIORATIVE ROLES OF VITAMINS A, C AND E ON HAEMATOLOGICAL PARAMTERS OF *CLARIAS GARIEPINUS* (BURCHELL, 1822) FINGERLINGS EXPOSED TO LEAD NITRATE

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ABSTRACT

The ever-increasing anthropogenic activities all over the world that usually lead to release of plethora of pollutants such as lead calls for concern. In the present study the effects of lead nitrate on the haematology of *Clarias gariepinus* and how such effects can be mitigated through administration of vitamins were investigated. *C. gariepinus* fingerlings (initial weight, 3-11g; standard length,7.9-9.4cm and total length, 8.9-10.9cm) were exposed to sub-lethal concentrations of Pb **(**00, 26mg/L, 44mg/L, 61mg/L and 79mg/L) with replicate in each case. 26mg/L each of the vitamins was administered across all bud. Fresh concentrations of both toxicant and vitamins were administered every 72 hours for a period of 12 weeks every time the water medium was changed according to standard methods. The various treatments group include Pb (Pb only), PbVA (Pb+vitamin A), PbVC ((Pb+vitamin C) and PbVE (Pb+vitamin E) with T1-T4 and replicates in each case. Three samples of the fish were randomly selected and sacrificed from each aquarium tank every 4th week of the exposure period. The blood collected were analyzed for White Blood Cell count (WBC), Red Blood Cells (RBC), Haemoglobin Concentration (HGB), Pack Cell Volume (PCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin Concentration (MCHC) and Platelet Count (PLT). The data generated were subjected to one-way analysis of variance at $P \leq 0.05$. The results indicated that in the Pb only group the mean values of WBC in T1 and T4 were significantly higher than other treatments. RBC mean values in T3 were significantly higher than other treatments. Hb, PCV and MCHC mean values in the control were significantly higher than other treatments. MCV and MCH mean values in T2 were significantly higher than other treatments. All the mean values of PLT were significantly higher than other treatments except the control. After the 8th week, the mean values of WBC in T2 were significantly higher than other treatments. The RBC, Hb, PCV, MCHC and PLT mean values in the control were significantly higher than other treatments. At the end of the 12th week, the mean values of WBC in T4 were significantly higher than other treatments. The PbVA treatments after 4 weeks of exposure indicated increased production values of WBC and PLT in all treatments. After the 4th week of exposure, the mean values of WBC in T3 were significantly higher than other treatments. The MCV and MCH mean values in T3 and T2, respectively were significantly higher than other treatments. Mean values of PLT in T4 were significantly higher than other treatments. After the 8th week of exposure, mean values of WBC in T4 were significantly higher than other treatments. The PLT mean values in T2 were significantly higher than other treatments. After the 12th week, the RBC, Hb and PCV mean values in T4 were significantly higher than other treatments. The PLT mean values in T4 were also significantly higher than other treatments. In sample subjected to PbVC after 4 weeks of exposure, the WBC mean values in T1 were significantly higher than other treatments. The RBC mean values in T3 were significantly higher than other treatments. The mean values of MCV and MCH in T2 were significantly higher than other treatments. The MCHC mean values in T1 were significantly higher than other treatments. After the 8th week of exposure, the mean values of RBC in T1 were significantly higher than other treatments. At the end of the 12th week of exposure, only the T2 mean values in all the parameters and treatments were significantly lower than other treatments. In samples exposed to PbVE treatments after four weeks, displayed higher values of WBC. MCV, MCH and MCHC values recorded in T2 were higher than other treatments. After the 4th week of exposure, the MCV mean values in T2 were significantly higher than other treatments. The mean values of PLT in other treatments were significantly higher than T1. There were no significance differences in all parameters after the 8th week of exposure. At the end of the 12th week of exposure however, the mean values of WBC in T4 were significantly higher than other treatments. The MCHC mean values in T1 were significantly higher than other treatments. The vitamins supplemented treatments displayed varying levels of ameliorations far better than the Pb only group. Amongst these, the PbVE performed better than others. The out-come of this research demonstrated the impact of vitamins A, C and E in ameliorating the effects of the toxicant and can serve as remedy in heavy metal toxication when appropriate concentrations are administered.

Key words: Ameliorative roles, *Clarias gariepinus*, haematological parameters, Pb toxicant, vitamin supplements.
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INTRODUCTION

Fish is a rich source of animal protein throughout the world. Due to its nutritional value [1], the demand for fish food has been on the increase with increasing human population [2, 3]. African catfish, *Clarias gariepinus* is an important commercial fish due to its high growth rate, high consumer acceptability, and ability to withstand poor water quality, and oxygen depletion [4, 5]. Fishes serve as early warning indicators of pollution in the aquatic systems and can be considered to be the most standard choice as test organisms because they are the best understood organism in the aquatic environment and its importance to man (and other organisms) as a source of protein [6]. The presence of toxicants in the aquatic environment has myriads of effects on fish physiology. Some of the parameters that have shown sensitivity to contaminants are RBC and WBC levels, clotting times, pack cell volume (PCV) and haemoglobin content. In line with this, Priyadharshani *et al*. [7] reported how immunological parameters such as total white blood cell (WBC) counts, differential WBC counts, spleen weight/body weight ratio and neutrophil per lymphocyte ratio were affected by heavy metals.

The presence of pollutants in aquatic environment of organisms such as fish can lead to the production of reactive oxygen species and consequently, oxidative stress. Heavy metals could be essential or non-essential. Heavy metals such as Fe, Cu, Zn, Ni, Co, Cr, and Mn are vital to human only at lower concentrations, but they become more toxic when they are taken up more than the bio-recommended limits [8]. It is also known that even essential metals may be toxic on the biological activities of organisms above certain concentrations [9]. Heavy metals such as lead, mercury, cadmium, etc., naturally occur in the soils, rocks and sediments with high concentrations [10]. The ability of heavy metals to bioaccumulate and biomagnifying and difficult to be eliminated from the body by ordinary metabolic activities make them one of the most dangerous sources of chemical water pollution to fish, causing big losses to fish and effects on the fish consumers [11]. Heavy metals are known to elicit oxidative stress in organisms when the threshold is exceeded. Heavy metals are also known to promote oxidative damage by increasing the cellular concentration of reactive oxygen species (ROS) in fish, consequently, a response of antioxidative defences [12].

It has been reported that Pb can cause neurological, haematological, gastrointestinal, reproductive, circulatory, immunological, histopathological and histochemical changes_ all of these are related to the dose and time of exposure to Pb [13]. Among all the heavy metals, Cd, arsenic, mercury and lead pose highest degree of toxicity and that is of great concern to plants and human health [14]. They can also be classified as carcinogens [15].

Vitamins C and E supplementations have been reported to play a positive role in detoxification of mercury toxicity especially at lower concentrations [16]. The main biological function of vitamin E is its direct influence on cellular responses to oxidative stress through modulation of signal transduction pathway [17]. Vitamins E and C supplementation can induce protective effects on certain conditions after free radical-mediated cellular damage or disruption [18]. The cellular respiration in vertebrates depends on the availability of iron associated with Hb. The change in hemoglobin and leukocytes indicate the impact of lead and ascorbic acid [19] on the fish. In addition, Vitamin C has potent antioxidant activity against cadmium and mercury sensitive haematological parameters [20].

Changes in the haematological and genotoxic components of cat fishes have been reported from field and laboratory researches [21, 22, 23] but there is paucity of information on the effects of specific toxicants such as Pb and, especially when supplemented with vitamins. This is why the study attempted to bridge the gap in knowledge on the haematological effects of sub-lethal concentrations of lead toxicant and how vitamins A, C and E supplements can ameliorate such effects on *C. gariepinus* fingerlings with the aim of reducing to barest minimum the deleterious effects of lead toxicant.

MATERIALS AND METHODS

Samples/materials collection and acclimatization

A total number of four hundred (400) fingerlings of *C. gariepinus* were purchased from a commercial fish farmer and transported in 50L containers filled with water to the Old Farm Research Unit of the Department of Water, Aquaculture and Fisheries Technology, Bosso Campus, Federal University of Technology, Minna, Nigeria. The fishes were placed in fish ponds with water for acclimatization. The fishes were fed twice daily (morning and evening) with vital feed (3mm) for 14 days (2 weeks) for the acclimatization. The holding water was changed every two day throughout the period. The vitamins A, C and E granules (pellets) were purchased from commercial chemical stores. Five (500g) units of the granules in each case were used as the supplements in percentages corresponding to the sub-lethal concentrations of the treatments. The toxicant, Pb (2 pieces of 500g) analar grade were purchased from commercial chemical stores and stored in a cool dry condition throughout the period of the experiment. These toxicants were administered according to the sub-lethal concentrations corresponding to the sub-lethal concentrations of the treatments during the chronic phase of the exposure.

Experimental set-up

Five (5) treatments including control with two replicates in each treatment were set-up for the Pb, Vitamins A, C and E; and the sub-lethal exposures were run for a period of twelve (12) weeks. The five sub-lethal treatments of lead nitrate concentrations were: 00mg/L as control (CR), 26mg/L as T1, 44mg/L as T2, 61mg/L as T3 and 79mg/L as T4, respectively. Each treatment was in two replicates containing 15 fish in 20L plastic aquarium for the Pb, Vitamins A, C and E supplemented exposures. The minimum concentration of the toxicant serves the same concentration of the vitamins. The water was changed and fresh toxicant and the vitamins with the same set of concentrations were added at every 72 hours according to Organization for Economic Co-operation and Development [24] standards. Sampling was made from each trough randomly by picking out 3 samples every four (4) weeks for the analyses of the haematological parameters.

Determination of haematological parameters of *C. gariepinus* exposed to sub-lethal concentrations of lead

Blood samples were collected three times every 4th week from each treatment and replicate. The blood samples were collected with 1ml heparinized sterile syringe into EDTA test tubes containing little quantity of anti-coagulant [25, 26]. The method of collection involved the insertion of the syringe in between the opercula end and the pectoral fin on the ventral surface of the fish. The syringe was held perpendicularly and blood drawn out with suction pressure. Larger quantity of blood was drawn with this method in comparison to the little quantity available from the caudal end of the fish. White Blood Cell count (WBC), Red Blood Cells (RBC), Haemoglobin Concentration (HGB), Pack Cell Volume (PCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin Concentration (MCHC) and Platelet Count (PLT) of the blood collected from the samples of each treatment and replicate were determined in the Medical Laboratory Services of Minna General Hospital, Niger State**.** These parameters were determined using Mindray (BC-5300) Auto Hematology Analyzer for full blood count. This works on the principle of laser scatter, flow cytometry and chemical dye to provide reliable and accurate 5-part differentiation on blood cells.

Data Analyses

The blood parameters of the samples exposed to sub-lethal concentrations of the toxicant as well as those treatments supplemented with vitamins were analysed using One Way Analysis of Variance (ANOVA). The means were seperated by Duncan Multiple Range Test. The differences were considered significant at $P \leq 0.05$ using SPSS Statistical Package (version 20.0 for Windows).

RESULTS

Haematological parameters of *C. gariepinus* exposed to various treatments of Pb, Vitamins A, C and E for a period of four, eight and twelve weeks

Fish exposed to Pb treatments for a period of four weeks, there were increased values of production of WBC in all treatments when compared to the control. Also, there were drastic decreases in the production values of PLT in all treatments compared to higher values obtained in the control samples. Similarly, after the eight weeks of exposure, there were increased values of WBC in all treatments compared to the control. There were also decreased values of RBC, Hb and PCV in all treatments compared to the control group. Apart from T1 and T2, there were drastic reductions in values of PLT in all treatments compared to the control. Furthermore, there were increased values of WBC after twelve weeks of exposure in all treatments. Decreased values of RBC, Hb, MCV and PCV were also obtained in all treatments. Decreased PLT values were also recorded in all treatments compared to the control. From the statistical analysis after the 4th week of exposure, the mean values of WBC in T1 and T4 are significantly higher than T2 and T3. RBC mean values in CR and T3 are significantly higher than T2, T4 and T1. Hb and PCV mean values in the control are significantly higher than T1-T4. MCV mean values in T2 are significantly higher than CR, T1, T3 and T4. Likewise, MCH mean values in T2 are significantly higher than T1, CR, T3 and T4. MCHC mean values in the control are significantly higher than T1-T4. All the mean values of PLT are significantly different with the control significantly higher. (Table 1). After the 8th week, the mean values of WBC in T2 are significantly higher than T3, T4 and T1. The RBC, Hb, PCV, MCHC and PLT mean values in the control are significantly higher than T1-T4. (Table 2). Furthermore, at the end of the 12th week, the mean values of WBC in T4 are significantly higher than T1-T3 and CR. The RBC, Hb, PCV, MCV, MCH, MCHC and PLT in the control are significantly higher than T1-T4.

Table 1: Haematological parameters of *C. gariepinus* exposed to sub-lethal concentrations of Pb for a period of four weeks

Parameters	Treatments				
	CR	T1	T2	T3	T4
WBC (10^9cells/L)	9.80±0.12^{a}	19.50±0.87^{c}	16.00±0.58^{b}	19.50±0.87^{b}	19.00±1.15^{c}
RBC (Mil/mm^3)	3.60±0.06^{c}	2.15±0.20^{a}	1.95±0.09^{a}	2.60±0.12^{c}	2.80±0.00^{b}
Hb (g/dl)	10.05±0.14^{b}	6.05±0.20^{a}	7.00±0.35^{a}	6.65±0.55^{a}	6.25±0.32^{a}
PCV (%)	29.50±0.29^{b}	18.00±0.58^{a}	20.05±0.87^{a}	20.00±1.73^{a}	18.50±0.87^{a}
MCV (Fl)	81.50±2.02^{a}	86.50±10.68^{a}	104.50±0.29^{b}	76.00±3.46^{a}	75.50±2.60^{a}
MCH (Pg)	27.00±0.58^{ab}	29.00±3.46^{b}	35.50±0.29^{c}	25.00±1.15^{ab}	22.00±1.15^{a}
MCHC (g/dl)	33.50±0.29^{b}	31.50±1.44^{ab}	31.00±1.73^{ab}	29.50±0.29^{a}	29.00±0.00^{a}
PLT (Cmm)	206.00±1.15^{d}	103.00±1.15^{a}	107.00±0.00^{ab}	121.50±2.60^{c}	110.50±2.02^{b}

Values are presented as mean±SEM (Standard error of mean), Values with different alphabets as superscripts in a row are significantly different at $P \leq 0.05$. CR stands for the control at 00mg/L; T_1, treatment 1 at 26mg/L; T_2, treatment 2 at 44mg/L; T_3, treatment 3 at 61mg/L and T_4, treatment 4 at 79mg/L.

Table 2: Haematological parameters of *C. gariepinus* exposed to sub-lethal concentrations of Pb for a period of eight weeks

Parameters	Treatments				
	CR	T1	T2	T3	T4
WBC (10^9cells/L)	9.40±0.23^{a}	16.50±0.29^{b}	18.50±0.29^{c}	17.50±0.29^{bc}	17.00±0.58^{b}
RBC (Mil/mm^3)	3.60±0.12^{d}	2.90±0.06^{c}	2.20±0.06^{a}	2.60±0.17^{bc}	2.45±0.14^{a}
Hb (g/dl)	10.45±0.03^{d}	8.05±0.14^{c}	6.05±0.20^{a}	6.25±0.38^{ab}	6.80±0.06^{b}
PCV (%)	31.00±0.00^{d}	23.50±0.29^{c}	18.00±0.58^{a}	18.50±0.87^{ab}	20.00±0.00^{b}
MCV (Fl)	86.50±2.60^{a}	81.00±2.31^{a}	82.00±4.62^{a}	78.50±12.41^{a}	82.00±4.62^{a}
MCH (Pg)	28.50±1.44^{a}	27.50±0.87^{a}	28.50±2.02^{a}	24.50±3.18^{a}	27.50±2.02^{a}
MCHC (g/dl)	32.00±1.73^{b}	29.00±0.00^{ab}	29.00±0.00^{a}	29.50±0.29^{ab}	29.50±0.29^{ab}
PLT (Cmm)	227.50±7.79^{b}	179.00±41.57^{ab}	169.50±28.00^{ab}	120.00±6.35^{a}	114.00±4.04^{a}

Values are presented as mean±SEM (Standard error of mean) .Values with different alphabets as superscripts in a row are significantly different at $P \leq 0.05$. CR stands for the control at 00mg/L; T_1, treatment 1 at 26mg/L; T_2, treatment 2 at 44mg/L; T_3, treatment 3 at 61mg/L and T_4, treatment 4 at 79mg/L.

Table 3: Haematological parameters of *C. gariepinus* exposed to sub-lethal concentration of Pb for a period of twelve weeks

Parameters	Treatments				
	CR	T1	T2	T3	T4
WBC (10^9cells/L)	8.00±0.00[a]	17.00±5.78[b]	18.00±0.00[b]	18.50±2.89[b]	20.50±0.87[c]
RBC (Mil/mm^3)	3.45±0.14[b]	2.55±0.03[a]	2.55±0.09[a]	2.50±0.00[a]	2.60±0.06[a]
Hb (g/dl)	11.50±0.29[b]	7.00±0.17[a]	6.40±0.35[a]	7.00±0.17[a]	6.60±0.12[a]
PCV (%)	34.50±0.87[b]	20.50±0.29[a]	19.00±1.15[a]	20.50±0.29[a]	19.00±0.58[a]
MCV (Fl)	100.50±6.64[b]	81.00±1.73[a]	74.00±2.31[a]	82.00±1.15[a]	73.00±0.58[a]
MCH (Pg)	34.50±3.18[b]	27.50±0.87[a]	25.50±0.58[a]	28.00±0.58[a]	25.00±0.00[a]
MCHC (g/dl)	30.00±0.00[c]	27.00±0.58[a]	29.50±0.29[bc]	29.50±0.29[bc]	29.50±0.29[b]
PLT (Cmm)	247.00±5.20[c]	110.50±0.87[a]	163.00±34.06[b]	115.50±0.87[ab]	129.00±1.15[ab]

Values are presented as mean±SEM (Standard error of mean), Values with different alphabets as superscripts in a row are significantly different at $P \leq 0.05$. CR stands for the control at 00mg/L; T_1, treatment 1 at 26mg/L; T_2, treatment 2 at 44mg/L; T_3, treatment 3 at 61mg/L and T_4, treatment 4 at 79mg/L.

In another development, PbVA treatments in the first four weeks of exposure indicated increased production values of WBC in all treatments. Similarly, increased values of PLT were obtained in all treatments. Reduced values of PCV in all treatments were also recorded. On the other hand, samples exposed for eight weeks indicated that there were increased production values of WBC. However, more or less the same values of MCV, MCH and MCHC and PLT (nearly equal to PLT values of the control) were obtained. Moreover, samples exposed for a period of twelve weeks also displayed increased values of WBC. From the statistical analysis, after the 4th week of exposure, the mean values of WBC in T3 are significantly higher than T1, T4, T2 and CR. The MCV mean values in T3 are significantly higher than T2, Cr, T4 and T1. More so, MCH mean values in T2 are significantly higher than T3, T1, CR and T4. Likewise, mean values of PLT in T4 are significantly higher than T3, T2, T1 and CR. (Table 4). After the 8th week of exposure, mean values of WBC in T4 are significantly higher than T2, T3, T1. T3 mean values are significantly higher than T1, T2 and T4. The PLT mean values in T2 are significantly higher than T1, T3 and T4. (Table 5). After the 12th week on the other hand, the mean values of WBC in T4 and T1 are significantly higher than T2 and T3. The RBC, Hb and PCV mean values in T4 are significantly higher than T1-T3. The PLT mean values in T4 are significantly higher than T1-T3. (Table 6).

Table 4: Haematological parameters of *C. gariepinus* exposed to sub-lethal concentration of Pb supplemented with vitamin A for a period of four weeks

Parameters	Treatments				
	CR	T1	T2	T3	T4
WBC (10^9cells/L)	9.80±0.12^{a}	15.50±0.29^{bc}	13.50±0.29^{b}	16.00±0.58^{c}	15.00±1.15^{bc}
RBC (Mil/mm^3)	3.60±0.06^{b}	3.10±0.17^{a}	2.80±0.17^{a}	2.65±0.14^{a}	3.00±0.17^{a}
Hb (g/dl)	10.50±0.14^{b}	7.85±0.55^{a}	8.10±0.17^{a}	7.45±0.32^{a}	7.20±0.23^{a}
PCV (%)	29.50±0.29^{b}	23.00±1.73^{a}	24.00±0.58^{a}	22.00±1.15^{a}	21.50±0.87^{a}
MCV (Fl)	81.50±2.02^{ab}	73.50±1.44^{a}	86.00±3.46^{bc}	94.50±3.18^{c}	81.50±4.33^{ab}
MCH (Pg)	27.00±0.58^{abc}	24.50±0.29^{ab}	29.00±1.15^{c}	28.50±2.60^{bc}	24.00±0.58^{a}
MCHC (g/dl)	33.50±0.29^{c}	31.50±1.44^{ab}	29.00±0.00^{a}	29.50±0.29^{ab}	29.50±0.29^{ab}
PLT (Cmm)	206.00±1.15^{a}	211.50±0.29^{ab}	211.50±6.06^{ab}	212.50±0.87^{ab}	217.00±2.31^{b}

Values are presented as mean±SEM (Standard error of mean). Values with different alphabets as superscripts in a row are significantly different at P≤ 0.05. CR stands for the control at 00mg/L; T_1, treatment 1 at 26mg/L; T_2, treatment 2 at 44mg/L; T_3, treatment 3 at 61mg/L and T_4, treatment 4 at 79mg/L.

Table 5: Haematological parameters of *C. gariepinus* exposed to sub-lethal concentration of Pb supplemented with vitamin A for a period of eight weeks

Parameters	Treatments				
	CR	T1	T2	T3	T4
WBC (10^9cells/L)	9.40±0.23^{a}	13.50±0.87^{bc}	12.50±0.29^{b}	14.00±0.00^{bc}	15.00±1.15^{c}
RBC (Mil/mm^3)	3.60±0.12^{b}	3.30±0.17^{ab}	2.90±0.06^{a}	3.15±0.20^{ab}	3.05±0.09^{a}
Hb (g/dl)	10.45±0.03^{c}	9.50±0.17^{b}	9.00±0.17^{a}	9.65±0.03^{b}	9.30±0.00^{ab}
PCV (%)	31.00±0.00^{d}	28.00±0.58^{b}	26.50±0.29^{a}	29.00±0.00^{c}	27.00±0.00^{a}
MCV (Fl)	86.50±2.60^{a}	85.50±6.06^{a}	91.00±2.89^{a}	93.00±5.78^{a}	88.50±2.60^{a}
MCH (Pg)	29.50±1.44^{a}	28.50±2.02^{a}	31.00±1.15^{a}	30.50±2.02^{a}	31.50±0.29^{a}
MCHC (g/dl)	32.00±1.73^{b}	29.00±0.00^{a}	29.00±0.00^{a}	29.50±0.29^{ab}	29.50±0.29^{ab}
PLT (Cmm)	227.50±7.79^{a}	221.50±2.02^{a}	242.00±4.62^{b}	219.00±1.15^{a}	219.50±0.87^{a}

Values are presented as mean±SEM (Standard error of mean), Values with different alphabets as superscripts in a row are significantly different at $P \leq 0.05$. CR stands for the control at 00mg/L; T_1, treatment 1 at 26mg/L; T_2, treatment 2 at 44mg/L; T_3, treatment 3 at 61mg/L and T_4, treatment 4 at 79mg/L.

Table 6: Haematological parameters of *C. gariepinus* exposed to sub-lethal concentration of Pb supplemented with vitamin A for a period of twelve weeks

Parameters	Treatments				
	CR	T1	T2	T3	T4
WBC (10^9cells/L)	8.00±0.00^{ab}	13.50±0.87^{b}	7.33±3.67^{ab}	4.00±4.00^{a}	14.50±0.29^{b}
RBC (Mil/mm^3)	3.45±0.14^{b}	2.90±0.06^{ab}	1.87±0.93^{ab}	0.93±0.93^{a}	3.25±0.14^{b}
Hb (g/dl)	11.50±0.29^{b}	8.45±0.09^{ab}	6.53±3.27^{ab}	2.93±2.93^{a}	9.65±0.09^{b}
PCV (%)	34.50±0.87^{b}	24.50±0.29^{ab}	18.67±9.33^{ab}	8.67±8.67^{a}	28.50±0.29^{b}
MCV (Fl)	100.50±6.64^{a}	84.50±2.60^{a}	66.67±33.33^{a}	30.67±30.67^{a}	88.00±2.89^{a}
MCH (Pg)	34.50±3.18^{a}	29.00±1.15^{a}	23.33±11.67^{a}	10.33±10.33^{a}	30.00±1.15^{a}
MCHC (g/dl)	30.00±0.00^{a}	28.00±0.58^{a}	19.33±9.67^{a}	9.67±9.67^{a}	29.50±0.29^{a}
PLT (Cmm)	247.00±5.20^{ab}	219.50±0.87^{ab}	162.00±81.00^{ab}	78.00±78.00^{a}	259.50±13.00^{b}

Values are presented as mean±SEM (Standard error of mean), Values with different alphabets as superscripts in a row are significantly different at $P \leq 0.05$. CR stands for the control at 00mg/L; T_1, treatment 1 at 26mg/L; T_2, treatment 2 at 44mg/L; T_3, treatment 3 at 61mg/L and T_4, treatment 4 at 79mg/L.

In the case of samples exposed to PbVC for a period of four weeks, there were increases in WBC and PLT values. After eight weeks of exposure, there were increased productions in values of WBC with higher T1 values. There were increased values of Hb and MCV as from T2 to T4. Furthermore, after twelve weeks of exposure, there were increased values of WBC. From the statistical analysis, after 4 weeks of exposure, the WBC mean values in T1 are significantly higher than T3, T4 and T2. The RBC mean values in T3 are significantly higher than T1, T2 and T4. The mean values of MCV in T2 are significantly higher than T1, T3 and T4. Likewise, the MCH mean values in T2 are significantly higher than T1, T4 and T3. The MCHC mean values in T1 significantly higher than T2-T4. (Table 7). After the 8th week of exposure, the mean values of RBC in T1 are significantly higher than T2-T4. (Table 8). Furthermore, at the end of the 12th week of exposure, only the T2 mean values in all the parameters and treatments are significantly lower. (Table 9).

Table 7: Haematological parameters of *C. gariepinus* exposed to sub-lethal concentration of Pb supplemented with vitamin C for a period of four weeks

Parameters	Treatments				
	CR	T1	T2	T3	T4
WBC (10^9cells/L)	9.80±0.12^{a}	12.00±0.00^{c}	10.50±0.29^{ab}	11.00±0.58^{b}	11.00±0.00^{b}
RBC (Mil/mm^3)	3.60±0.06^{b}	3.35±0.03^{a}	3.25±0.03^{a}	3.75±0.03^{c}	3.30±0.06^{a}
Hb (g/dl)	10.05±0.14^{c}	8.95±0.03^{ab}	9.45±0.26^{bc}	9.05±0.20^{ab}	8.60±0.35^{a}
PCV (%)	29.50±0.29^{c}	27.00±0.00^{ab}	28.00±0.58^{bc}	27.00±0.58^{ab}	25.50±0.87^{a}
MCV (Fl)	81.50±2.02^{ab}	80.00±0.58^{ab}	86.00±2.31^{b}	82.00±3.46^{ab}	77.00±1.15^{a}
MCH (Pg)	27.00±0.58^{b}	26.50±0.29^{b}	31.00±0.58^{c}	23.50±0.87^{a}	26.00±0.58^{b}
MCHC (g/dl)	33.50±0.29^{c}	31.50±0.87^{b}	29.50±0.29^{a}	29.00±0.00^{a}	30.00±0.00^{a}
PLT (Cmm)	206.00±1.15^{a}	226.50±4.33^{b}	225.00±4.61^{b}	234.00±5.20^{b}	226.00±2.31^{b}

Values are presented as mean±SEM (Standard error of mean), Values with different alphabets as superscripts in a row are significantly different at P≤ 0.05. CR stands for the control at 00mg/L; T_1, treatment 1 at 26mg/L; T_2, treatment 2 at 44mg/L; T_3, treatment 3 at 61mg/L and T_4, treatment 4 at 79mg/L.

Table 8: Haematological parameters of *C. gariepinus* exposed to sub-lethal concentration of Pb supplemented with vitamin C for a period of eight weeks

Parameters	Treatments				
	CR	T1	T2	T3	T4
WBC (10^9cells/L)	9.40±0.23^{a}	11.00±0.58^{a}	10.00±0.00^{a}	10.00±0.00^{a}	6.67±3.33^{a}
RBC (Mil/mm^3)	3.60±0.12^{b}	3.85±0.09^{b}	3.15±0.03^{ab}	3.15±0.14^{ab}	2.00±1.00^{a}
Hb (g/dl)	10.45±0.03^{a}	10.10±0.17^{a}	10.80±0.17^{a}	10.80±0.75^{a}	7.13±3.57^{a}
PCV (%)	31.00±0.00^{a}	30.00±0.58^{a}	32.00±0.58^{a}	32.00±2.31^{a}	20.67±10.33^{a}
MCV (Fl)	86.50±2.60^{a}	78.00±0.00^{a}	103.00±0.00^{a}	100.50±2.60^{a}	68.67±34.33^{a}
MCH (Pg)	28.05±1.44^{a}	26.00±0.00^{a}	35.50±0.29^{a}	36.00±2.31^{a}	23.33±11.67^{a}
MCHC (g/dl)	32.00±1.73^{a}	30.50±0.29^{a}	32.50±1.44^{a}	29.50±0.29^{a}	19.33±9.27^{a}
PLT (Cmm)	227.50±7.79^{a}	214.50±1.44^{a}	223.50±7.80^{a}	227.50±7.22^{a}	144.60±72.33^{a}

Values are presented as mean±SEM (Standard error of mean). Values with different alphabets as superscripts in a row are significantly different at $P \leq 0.05$. CR stands for the control at 00mg/L; T_1, treatment 1 at 26mg/L; T_2, treatment 2 at 44mg/L; T_3, treatment 3 at 61mg/L and T_4, treatment 4 at 79mg/L.

Table 9: Haematological parameters of *C. gariepinus* exposed to sub-lethal concentrations of Pb supplemented with vitamin C for a period of twelve weeks

Parameters	Treatments				
	CR	T1	T2	T3	T4
WBC (10^9cells/L)	8.00±0.00^b	9.50±0.29^b	3.00±3.00^a	9.50±2.89^b	10.00±0.00^b
RBC (Mil/mm^3)	3.45±0.14^b	3.40±0.17^b	1.23±1.23^a	3.75±0.03^b	3.60±0.12^b
Hb (g/dl)	11.50±0.29^b	10.05±0.14^b	3.40±3.40^a	10.85±0.20^b	11.60±0.23^b
PCV (%)	34.50±0.87^b	29.50±0.29^b	10.00±10.00^a	32.00±0.58^b	34.50±0.87^b
MCV (Fl)	100.50±6.64^b	89.50±6.35^b	27.00±27.00^a	86.00±1.78^b	95.50±0.87^b
MCH (Pg)	34.50±3.17^b	30.00±2.31^b	9.00±9.00^a	28.50±0.29^b	32.00±0.00^b
MCHC (g/dl)	30.00±0.00^b	33.00±1.73^b	10.00±10.00^a	29.50±0.29^b	30.00±0.00^b
PLT (Cmm)	247.00±5.20^b	224.00±10.97^b	85.33±85.33^a	239.00±1.15^b	226.50±8.37^b

Values are presented as mean±SEM (Standard error of mean). Values with different alphabets as superscripts in a row are significantly different at $P \leq 0.05$. CR stands for the control at 00mg/L; T_1, treatment 1 at 26mg/L; T_2, treatment 2 at 44mg/L; T_3, treatment 3 at 61mg/L and T_4, treatment 4 at 79mg/L.

In addition, samples exposed to PbVE treatments after four weeks, displayed higher values of WBC. MCV, MCH and MCHC recorded in T2 with higher values than other treatments. There were increases in the production values of blood PLT in all treatment. After eight weeks of exposure, there were increased values of WBC. The increased values of MCV were recorded in T2 to T4. Moreover, after the twelve weeks, there were also increased values of WBC, slightly decreased values of RBC, Hb, PCV and MCV in all treatments. From the statistical analysis, after the 4th week of exposure, the mean values of WBC in T1 and T3 are significantly higher than T2 and T4. The MCV mean values in T2 are significantly higher than T1, T3 and T4. Also, the mean values of PLT in T2-T4 are significantly higher than T1. (Table 10). There were no significance differences after the 8th week of exposure. (Table 11). At the end of the 12th week of exposure however, the mean values of WBC in T4 are significantly higher than T3, T2 and T1. The MCHC mean values in T1 are significantly higher than T2-T4. (Table 12).

Table 10: Haematological parameters of *C. gariepinus* exposed to sub-lethal concentration of Pb supplemented with vitamin E for a period of four weeks

Parameters	Treatments				
	CR	T1	T2	T3	T4
WBC (10^9cells/L)	9.80±0.12^a	15.00±0.58^b	12.50±0.87^{ab}	14.50±0.87^b	12.50±1.44^{ab}
RBC (Mil/mm^3)	3.60±0.06^b	3.00±0.00^a	2.95±0.09^a	3.15±0.03^a	3.15±0.14^a
Hb (g/dl)	10.05±0.14^c	7.85±0.26^a	9.00±0.06^b	7.50±0.17a	8.50±0.23^b
PCV (%)	29.95±0.29^c	23.00±0.58^a	26.50±0.29^b	22.50±0.58^a	25.00±0.58^b
MCV (Fl)	81.50±2.02^{ab}	76.50±2.02^a	89.50±3.75^b	79.50±4.33^a	79.00±1.73^a
MCH (Pg)	27.00±0.58^b	25.50±0.87^{ab}	30.00±1.15^c	23.50±0.29^a	26.50±0.29^b
MCHC (g/dl)	33.50±0.29^b	32.00±1.73^{ab}	29.50±0.29^a	29.50±0.29^a	29.50±0.29^a
PLT (Cmm)	206.00±1.15^a	208.00±2.31^a	223.50±4.33^b	225.00±3.18^b	219.50±1.44^b

Values are presented as mean±SEM (Standard error of mean). Values with different alphabets as superscripts in a row are significantly different at $p \leq 0.05$. CR stands for the control at 00mg/L; T_1, treatment 1 at 26mg/L; T_2, treatment 2 at 44mg/L; T_3, treatment 3 at 61mg/L and T_4, treatment 4 at 79mg/L.

Table 11: Haematological parameters of *C. gariepinus* exposed to sub-lethal concentration of Pb supplemented with vitamin E for a period of eight weeks

Parameters	Treatments				
	CR	T1	T2	T3	T4
WBC (10^9cells/L)	9.40±0.23[a]	7.33±3.67[a]	7.33±3.67[a]	7.33±3.67[a]	9.33±4.67[a]
RBC (Mil/mm^3)	3.60±0.12[a]	2.13±1.07[a]	1.87±0.93[a]	2.00±1.00[a]	2.00±1.00[a]
Hb (g/dl)	10.45±0.29[a]	5.80±2.90[a]	6.53±3.27[a]	6.27±3.13[a]	6.53±3.27[a]
PCV (%)	31.00±0.00[a]	17.33±8.67[a]	19.33±9.67[a]	18.00±9.00[a]	19.3±19.66[a]
MCV (Fl)	86.50±2.60[a]	54.00±27.00[a]	68.67±34.33[a]	60.00±30.00[a]	64.00±32.00[a]
MCH (Pg)	28.50±1.44[a]	18.00±9.00[a]	23.33±11.67[a]	20.67±10.33[a]	21.33±10.67[a]
MCHC (g/dl)	32.00±1.73[a]	19.33±9.67[a]	19.33±9.67[a]	20.00±10.00[a]	20.00±10.00[a]
PLT (Cmm)	227.50±7.79[a]	148.00±74.00[a]	144.67±72.33[a]	147.33±73.67[a]	142.67±71.33[a]

Values are presented as mean±SEM (Standard error of mean), Values with different alphabets as superscripts in a row are significantly different at $P \leq 0.05$. CR stands for the control at 00mg/L; T_1, treatment 1 at 26mg/L; T_2, treatment 2 at 44mg/L; T_3, treatment 3 at 61mg/L and T_4, treatment 4 at 79mg/L.

Table 12: Haematological parameters of *C. gariepinus* exposed to sub-lethal concentration of Pb supplemented with vitamin E for a period of twelve weeks

Parameters	Treatments				
	CR	T1	T2	T3	T4
WBC (10^9cells/L)	8.00±0.00[a]	10.50±0.29[b]	13.50±0.29[c]	14.00±0.58[c]	16.50±0.29[d]
RBC (Mil/mm^3)	3.45±0.15[b]	2.90±0.06[a]	3.00±0.00[a]	3.15±0.58[a]	2.90±0.58[a]
Hb (g/dl)	11.50±0.29[b]	9.45±0.03[a]	8.80±0.35[a]	9.25±0.29[a]	8.80±0.06[a]
PCV (%)	34.50±0.87[c]	27.50±0.29[b]	25.50±0.87[a]	27.00±0.00[ab]	26.00±0.00[ab]
MCV (Fl)	100.50±6.64[b]	95.00±2.89[ab]	85.00±2.89[a]	86.00±2.31[a]	89.50±1.45[ab]
MCH (Pg)	34.50±3.18[a]	32.50±0.87[a]	29.00±0.58[a]	29.50±0.29[a]	29.50±0.29[a]
MCHC (g/dl)	30.00±0.00[a]	32.00±1.16[b]	29.00±0.58[a]	29.50±0.29[a]	29.50±0.29[a]
PLT (Cmm)	247.00±5.20[b]	219.00±4.62[a]	229.00±4.61[a]	216.50±2.80[a]	231.00±5.78[a]

Values are presented as mean±SEM (Standard error of mean). Values with different alphabets as superscripts in a row are significantly different at P≤0.05. CR stands for the control at 00mg/L; T_1, treatment 1 at 26mg/L; T_2, treatment 2 at 44mg/L; T_3, treatment 3 at 61mg/L and T_4, treatment 4 at 79mg/L.

DISCUSSION

Haematological indices are of different sensitivity to various environment factors and chemicals [27]. In this regard, samples of *C. gariepinus* exposed to sub-lethal concentrations of Pb environment exhibited increased WBC production in all treatments after 4 weeks of exposure. There were drastic decreases in blood platelets in all treatments which are significantly different when compared with the control. This probably buttresses the need for the body to react and elicit defence against the xenobiotic in its environment. The same drastic decrease in the blood platelets occurred in the 8th week except in T1 and T2 (lower concentrations), increased level of WBC, reduced RBC, Hb and PCV. This scenario also continued in the 12th week. The Hb and PCV mean values in the control are significantly higher than T1-T4. As duration and concentration increase the need for engagement of the body's defence mechanisms also probably increased. The ability of the body's defence mechanisms to utilize platelets in combating the effects of the toxicant may have been overwhelmed especially in higher concentrations, hence, the drastic reduction. In line with this, Mohamed *et al*. [28] reported how polluted main basin of Lake Mariout environment clearly indicate a significant decrease in RBCs, Hb, Hct, and platelet counts while a significant increase in MCH, MCHC, and WBCs in the *C. gariepinus* samples obtained from them. The findings from this research are also in conformity with Ikeogu *et al*. [29] when they found

that RBC counts, Hb, and Ht of *C. gariepinus* decreased following exposure to 20 and 35 mg/L of $Pb(NO_3)_2$; and that, hematological parameters, such as red blood cells (RBCs), hemoglobin (Hb), and hematocrit (Hct) showed significant ($p < 0.05$) concentration-dependent decreases in fish exposed to $Pb(NO_3)_2$ during both periods [30]. At week 8, the WBC mean values in T2 are significantly higher than T1, T3 and T4. Likewise, WBC mean values in T4 are significantly higher than T1-T3 and CR at week 12. The control values in RBC, Hb, PCV, MCHC and PLT are significantly higher than T1-T4. This probably indicates the utilization of these parameters in combating the effects of the toxicant. There also seemed to be struggle for survival of the fish especially at the 12th week of exposure culminating in increased generation of the white blood cells to counteract the effects of the toxicant. In line with the findings of this research, Abdel-Warith *et al*. [30] also reported how RBCs, haemoglobin (Hb) and Haematocrit (Hct) showed significant ($P<0.05$) concentration dependent decreases in fish exposed to $Pb(N0_3)_2$. Likewise, Zaki *et al*. [31] reported that long term exposure of *Clarias lazera* to Pb and Hg caused a gradual increase in WBCs count. Furthermore**,** Verma *et al*. [32] reported how PCV decreased drastically relative to control after 28 days of exposure of *Heteropneustes fossilis* to the highest concentration of Pb and that, there were fluctuations in the values of MCV in all treated groups with the highest value of 195.12µm^3 recorded at the initial stage (7 days) of exposure; and a maximum decline in MCH (7.09%) observed in 2.65ppm concentration after 21 and 28 days of exposure.

In samples exposed to sub-lethal concentrations of Pb supplemented with vitamin A, there were increased WBC values, increased values of PLT (which were nearly constant in the 8th and 12th weeks), reduced values of RBC, Hb and PCV after the 4th and 8th weeks of exposure. The blood platelets in T4 were also significantly higher than T1-T3. The increased WBC and the significance of PLT in T4 probably points to the fact that there has to be up-regulation of the body's defence mechanisms to deal with the effects of the toxicant. These findings are in conformity with Olatunji *et al*. [33] who reported an increase in blood cell count and platelet while there was a decrease in Haematocrit, MCV, MCH, LYMH, HMB, and RBC in higher concentration of 750mg/l throughout the test in juvenile catfish, *C. gariepinus.* In like manner also, Ugwuja *et al*. [34] reported how PCV, Hbc were significantly reduced whie the total WBC, MCH, MCHC and platelets were significantly elevated in treatments with Pbc group. The presence of vitamin A in the environment of the fish perhaps, created the avenue for the utilization of the body's defence systems especially the blood platelets in combating the effects of the toxicant unlike in the Pb only treatments.

Vitamin C is an important chain-breaking antioxidant and enzyme co-factor against heavy metals. In samples exposed to sub-lethal concentration of Pb supplemented with vitamin C, there were increased values of production of WBC and PLT, and higher values of MCV and MCH after the 4th week of exposure. Also, at the 8th week there were increased WBC and reduced RBC. There was probably the need for the repair of the damaged tissue due to haemolysis and up-regulation of the defence system of the fish to deal with the deleterious effects of the toxicant. Abdulkareem *et al*. [25] also attributed the reduction in the level of RBC, Hb and HCT in the fish to the destruction of RBC and haemolysis caused by the presence of dichorvos; while increased level of WBC could be due to defence mechanism exhibited by the fish. Increased values of MCV and MCH may have been occasioned by the presence of vitamin C. The PLT values in other treatments were nearly at par with those of the control most likely because of the presence of the vitamin; since vitamin C supplementation in animals exposed to heavy metals not only affects the reduction of oxidative stress, but also significantly reduces the levels of these metals in different tissues such as blood, liver, kidney, muscle, gills, brain [35, 36, 37]. Also, vitamin C treatment was reported to be successful in increasing the haemogobin and haematocrit levels to normal levels [38]. Furthermore, Saraswati *et al*. [39] reported how vitamin C ameliorated the effects of dimethoate on *C. batrachus*. The production mean values of RBC in T1 are significantly higher than T2-T4 at week 8. This is probably because the effects became more over bearing in higher concentrations. Mahmoud *et al*. [40] reported that *C. gariepinus* exposed to 7 mg/l of lead exhibited a significant decrease in their RBCs, Hb, and Hct. Also, similar findings were reported by Abdulkareem *et al*. [25] when they showed that the values of RBC, Hb and HCT of *C. gariepinus* exposed to different concentrations of dichlorvos for 96hrs decreased as the concentration of the toxicant increased in comparison to the control and that the, WBC and platelets increased in the fish as the concentration of the toxicant increases. Kim and Kang [41] also reported significant decrease in the haematocrit and haemaglobin level of *Sebastes schlegelli* exposed to chromium.

Haematological and biochemical profiles of blood can provide important information about the internal environment of the organism [42]. From the results of the samples exposed to sub-lethal concentrations of Pb and supplemented with vitamin E, there were increased WBC and PLT values, reduced RBC, Hb and PCV after the 4th week of exposure. The

same scenario played out in the parameters indicated except PLT in the 8 and 12th week of exposure. The body defence system probably fought with these parameters to combat the deleterious effects of the toxicant. In like manner, Satish *et al*. [43] reported significant decrease in the RBC counts, WBC counts, Hb and PCV levels after exposure of the fish, *Channa punctata* to acephate and the MCV, MCH and MCHC levels of the blood on the other hand, were significantly increased. Also, Gaafar *et al*. [44] reported that prolonged reduction in haemoglobin content is deleterious to oxygen transport and degeneration of the erythrocytes could be due to pathological condition in fish exposed to toxicant. The WBC mean values in T4 are significantly higher than T1-T3 at the end of the 12th week of exposure probably because the need for up-regulation of body's defence system to ensure survival as the concentration and duration increased has become more important than ever. Similar findings by Neelima *et al*. [45] indicated that WBC, MCV and MCHC showed increasing trend at sub-lethal exposure to cypermethrin. The MCHC mean values in T1 are significantly higher than T2-T4. This is probably due to the presence of vitamin E in the environment of the fish samples. The physical status of the samples in this treatment may also have manifested physiologically. Given that, this is the lowest concentration of the exposure the effects of the toxicant may have been diminished to the point of out-performing the other higher concentration. Vitamins E and C have been shown to have chelating abilities, affecting the reduction in the amount of accumulated metals in different tissues to various organisms, including fish [36]. Likewise, the total antioxidant capacity in erythrocytes also returned to values between 58.9% and 67.7% in workers exposed to Pb after treatment with vitamins E and C supplements for a year, a level similar to those in exposed non-Pb workers [46]. In addition to the foregoing, Suleiman *et al*. [47] reported that fish pretreated with vitamin C and E significantly suppressed the adverse haematological effects of the toxicant. In the same vein, Ebuehi *et al*. [48] indicate that oral administration of vitamins C and E significantly reduced the blood lead concentration, ameliorates the hepatic damage and significantly reduced the oxidative stress in the brain of rats.

CONCLUSION AND RECOMMENDATION

The results from the samples exposed to Pb toxicant and then supplemented subsequently with vitamins A, C and E displayed varying levels of ameliorations and improvements in mitigating the effects of the toxicant. In the Pb only group at the end of the 12th week, the mean values of WBC in T4 were significantly higher than other treatments. All other parameters in the control were significantly higher than other treatments.

C. gariepinus samples subjected to PbVA treatments after 4 weeks of exposure indicated increased production values of WBC and PLT in all treatments. After the 8th week of exposure, mean values of WBC in T4 were significantly higher than other treatments. After the 12th week, the PLT, RBC, Hb and PCV mean values in T4 were significantly higher than other treatments. In the higher concentrations the blood parameters are mostly elevated where there were needs to up-regulate the defence systems to counter the effects of the toxicant. In sample subjected to PbVC after 4 weeks of exposure, the WBC and MCHC mean values in T1 were significantly higher than other treatments. The mean values of MCV and MCH in T2 were significantly higher than other treatments. After the 8th week of exposure, the mean values of RBC in T1 were significantly higher than other treatments. At the end of the 12th week of exposure, only the T2 mean values in all the parameters and treatments were significantly lower than other treatments. In this treatment group the buffering capacity of the vitamin was evident in lower concentrations.

Samples exposed to PbVE treatments after four weeks, displayed higher values of WBC. MCV, MCH and MCHC recorded in T2 with higher values than other treatments. The mean values of PLT in other treatments were significantly higher than T1. There were no significance differences in all parameters after the 8th week of exposure. At the end of the 12th week of exposure however, the mean values of WBC in T4 were significantly higher than other treatments. The MCHC mean values in T1 were significantly higher than other treatments.

The vitamins supplemented treatments displayed varying levels of ameliorations far better than the Pb only group. Amongst these, the PbVE performed better than others. The out-come of this research demonstrated the impact of vitamins A, C and E in ameliorating the effects of the toxicant and can serve as remedy in heavy metal toxicities when appropriate concentrations are administered.

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