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RESEARCH ARTICLE

RECOVERY MECHANISM IN FISHES WITH REFERENCE TO RECUPERATION OF HAEMATHOLOGICAL PARAMETERS IN *CLARIAS GARIEPINUS* ABERRATED BY HERBICIDE *PARAQUAT DICHLORIDE*

IKPESU T.O.

Department of Biological Sciences, Federal University Otuoke, Nigeria, +2348032312141, tomohwofasa@yahoo.com

IKPESU T.O.

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ABSTARCT : The rate of reocvery of *Clarias gariepinus* exposed to *Parquat dichloride* was examined by assessing responses of haemathological parameters in treatment and post treatment tests. The test was conducted under OECD test guideline 407 .Seventy five juveniles of *C. gariepinus* were used for the toxicity study .The fish were exposed to the to the range of concentrations observed in the field .Three fishes per test concentration in three replicates were exposed to varying concentrations of *Paraquat dichloride* (0.00 ,2.00, 4.00, 6.00, 8.00)µg/L in water for 28 days. After the exposure period, fish were transferred to a free insecticide aquarium. The treated groups were sampled at the end of the 28 days and at 1, 8 and 12 days post exposure .Haematological examination revealed that the treated fish showed significant ($p < 0.05$) progressive reduction in erythrocyte (RBC) count, leucocyte count (WBC), lymphocyte, haemoglobin content, haematocrit, mean corpuscular haemoglobin concentration (MCHC) and erythrocyte sedimentation rate (ESR). However, there were significant ($p < 0.05$) increased in mean corpuscular volume and values of mean corpuscular haemoglobin. The fish recovered spontaneously and the aberrated parameters in all the treatments normalised after 12 days in free insecticide aquarium . If haemathology affinity can predict in vivo pattern of aberration, there is great potential to use this affinity to predicts pollution and environmental animal susceptibility. Since haemathological alteration can return to a normal level, if exposure to contaminant is discontinuous .This is a good novelty that can be implored by aquaculturist especially in the area that is prone to oil spillage, surface run off and other environmnetal hazards.

INTRODUCTION

Pesticides can contaminate unintended land and water when they are sprayed aerially or when they escape from production sites and storage tanks or are inappropriately discarded [1]. The amount of pesticide that migrates from the intended application area is influenced by the particular chemical's properties: its propensity for binding to soil, its vapor pressure, its water solubility, and its resistance to being broken down over time [2] .Factors in the soil, such as its texture, its ability to retain water, and the amount of organic matter contained in it, also affect the amount of pesticide that will leave the area .Some pesticides contribute to global warming and the depletion of the ozone layer [3]

The impact of pesticides is often greater than what is intended by those who use them .Surface runoff into rivers and streams can be highly lethal to aquatic life, sometimes killing all the fish in a particular stream [4] .Pesticides can accumulate in bodies of water to levels that kill off zooplankton, the main source of food for young fish and can also kill off the insects on which some fish feed, causing the fish to travel farther in search of food and exposing them to greater risk from predators [5, 6].

Over 95% of herbicides reach a destination other than their target species, air, water, bottom sediments, and food [7] .Herbicides have been shown to be especially toxic to certain aquatic microorganisms, disrupting the photosynthesis process. Microorganisms are very important in aquatic ecosystems, as they are primary

producers, they cycle nutrients, and aid in decomposition. By negatively affecting microorganisms, pesticides in aquatic systems may have detrimental effects on higher trophic levels and disrupt the balance of the ecosystem. They also cause fish kills when the dead plants rot and use up the water's oxygen, suffocating the fish [7]

The increase in the use of pesticides has increased both the hazards to human health and the pollution of the environment, and modern technology and industrialization is among the foremost factors for environmental pollution .Pesticides have the ability to bioaccumulate and biomagnify, and can bioconcentrate (i.e. become more concentrated) up to 70,000 times their original concentrations along the trophic level,where man is always at the top of trophic level [8] .Pesticides are sprayed onto food, especially fruits and vegetables, they percolate into soils and groundwater which can end up in drinking water .Pesticides can also enter the human body through inhalation of aerosols, dust and vapor that contain pesticides; through oral exposure by consuming food and water; and through dermal exposure by direct contact of pesticides with skin [9] .The effects of pesticides on human health are more harmful based on the toxicity of the chemical and the length and magnitude of exposure [10] .Farm workers and their families experience the greatest exposure to agricultural pesticides through direct contact with the chemicals [10] .Children may be exposed due to their closer proximity to the floor and natural tendency to put contaminated objects in their mouth .Pesticides tracked into the home from family members increase the risk of toxic pesticide exposure which is normally area specific. Also, toxic residue in food may contribute to a child's exposure to a certain pesticide and the chemicals can bioaccumulate in the body over time [11]

Exposure to pesticides can range from mild skin irritation to birth defects, tumors, genetic changes, blood and nerve disorders, endocrine disruption, and even coma or death [8] . pesticides targeted to disrupt insects can have harmful effects on the nervous systems of mammals, due to basic similarities in system structure. Both chronic and acute alterations have been observed in those who are exposed [10] .Exposure to pesticides may occur in postnatal early stages of development, in uterus, and even if either parent was exposed before conception took place. Reproductive disruption has the potential to occur by chemical reactivity and through structural changes to a system [12] . Though, there are benefits using pesticides, inappropriate use can counterproductive, many users are inadequately informed about potential short and long-term risks, and the necessary precautions in the correct application of such toxic chemicals are not always made [13]

Paraquat dichloride is very immobile in soil, does not hydrolyze, does not photodegrade in aqueous solutions, and is resistant to microbial degradation under aerobic and anaerobic conditions. The primary route of environmental dissipation of *paraquat* is adsorption to biological materials and soil clay particles. Due to the apparent adsorption strength of the herbicide for soil clays, the bound residues do not appear to be environmentally available. Nevertheless, since *paraquat* is persistent, it could potentially be found in surface water systems associated with soil particles carried by erosion. However, detections would not be considered to be representative of normal *paraquat* use (since it binds so strongly to soil clay particles and becomes environmentally inactive). When paraquat was sprayed onto bird eggs, it causes growth abnormalities in embryos and reduces the number of chicks that hatch successfully [14] .The herbicide is a non-selective contact herbicide, used in controlling pests of cultivated farmlands of rice, cotton, fruit, tea, potatoes, sugar cane and vegetable. It quickly kills a wide range of annual grasses, broad leafs, weeds and some perennial grasses when sprayed directly onto leaves. More so, the active ingredient is rapidly absorbed by clay and silt particles in the soil and does not leave any effective soil residue [15]. Repeated applications of this herbicide is practised for weed control of weed in agricultural field and thereby, large quantities find their way into the water bodies.

Fish serve as bioindicators of environmental pollution and therefore can be used for the assessment of the quality of aquatic environment [16] since they are directly exposed to chemicals resulting from agricultural production via surface runoff of water or indirectly through the food chain within the ecosystem [17] .*Clarias gariepinus* is widely distributed around the world with economic importance for fisheries and aquaculture. It is a good bioindicators for toxicological studies, because they are resistance to diseases, and good tolerance to a wide variety of environmental conditions [18].

The toxicity of a chemical is totally dependent on the concentration of the chemical in the organism, thus the use of aquatic bioassays are necessary in water pollution control to determine whether a potential toxicant is dangerous to aquatic life and if so, to find the relationship between the toxicant concentration and its effect on aquatic animals [19] and the tendency to recover if

condition improved. The application of environmental toxicology studies on non-mammalian vertebrates is rapidly expanding, and for the evaluation of the effects of noxious compounds [20] and the study of the hematological parameters of the fish responsive to pesticide stress is gaining recognition as we search for useful tools for monitoring the level of pollutants in the environment. The present study therefore, is to to assess the possibility of recovery from toxic in a natural setting using fish to determine in vivo required time for recovery of haemathological parameters in *C.gariepinus* induced with *Paraquat dichloride*

Materials and Methods

The juvenile stage of *C.gariepinus*, mean weight (140.00 ± 3.2g) and length (39.00 ± 1.03cm) were collected from the Department of Fisheries, Faculty of Agriculture, University of Benin, Benin City, Nigeria. They were acclimatized to laboratory conditions in holding glass tanks containing deionized water for two weeks before they were used for the experiments. The holding tanks were aerated with the help of air pump, cleaned and water renewed daily. Fish were fed on 30% protein pellets, unconsumed feed and faecal wastes were removed and water replenished regularly as recommended by [21]

Toxicity Test

The physicochemical parameters of the test media were kept within the one obtain from the natural environment in the tropical rainforest with the help of an air pump. Deionized water was used for acclimation as well as preparing test solutions.

The chronic test was conducted under OECD test guideline 407 [22] Fifteen glass aquaria were used with 3 replicates per treatment. Seventy five juveniles *C. gariepinus* were used for the toxicity study .The fish were fed with crude protein pellets (30%) twice daily .Freshly prepared test solutions were added on daily basis to maintain the concentration level after the waste have been siphoned out. The tanks cleaned daily .The water changed thrice weekly and aerated with the help of air pump. Fish and water quality parameters (pH, Temperature, dissolved Oxygen, turbidity and hardness) of the test solution were monitored throughout the duration of the experiment .Three fishes per test concentration in three replicates were exposed to the range of concentrations observed in the field (0.00 ,2.00, 4.00, 6.00, 8.00)µg/L in water for 28 days. After the exposure period, fish were transferred to insecticide free aquaria for 12 days. The treated groups and the control were sampled at the end of the 28 days . After the exposure period, fish were transferred to a insecticide free aquarium. The treated groups and control were also sampled at 1, 8 and 12 days post exposure. Slime and water present on the body surface of the fish were removed by using blotting paper. The blood samples was collected from the caudal vein, located beneath the backbone in a heparinized blood collecting duct using insulin syringe and needle.

Blood cells Determination

The whole blood was used for the estimation of the blood count. Erythrocytes and leucocytes were counted by method of [23] as modified by [24] using haemocytometer. Haemoglobin content was estimated by Cyanmethaemoglobin method [25] as modified by [24] haematocrit was estimated using microhematocrit method [26] while ESR was determined using wintrobe stand [27]

Erythrocyte Count (RBC): The glacial acetic acid lyses the red cells .The blood specimen was diluted 1:200 in R.B.C. pipette using Toission's solution as diluting fluid and cells were counted under high power (40 X objective) using a counting chamber. The red blood cells in the four corner squares and in the center square (marked in the diagrams as 'R') were counted as the number of red cells per cu mm (µl) of whole blood.

The following erythrocyte – related indices were calculated: mean corpuscular volume (MCV: μm^3/cell), mean corpuscular hemoglobin (MCH: pg/cell), and mean corpuscular hemoglobin concentration (MCHC: g l^{-1}) were calculated from RBC, Ht, and Hb

Erythrocyte Sedimentation Rate (ESR): The blood was taken from heparinized tube containing anticoagulatant into a tube and drawn to the zero mark. The tube was then placed on the wintrobe stand vertically for one hour. The fall of the red blood cell (RBC), was measure in mm / hr as the ESR.

Leukocyte count (WBC): The gentian violet slightly stains the nuclei of the leukocytes. The blood specimen is diluted 1:20 in a WBC pipette with the Turk's solution as diluting fluid and the cells are counted under low power of the microscope using a Burker chamber. Cells in all four W marked corners were counted and recorded. The numbers of cells in the blood were reported per cu mm (µl) of whole blood

Differential counts :Differential counts was done by making a thin film on a clean free slide with a spreader placed at an angle of 45^0C over a drop of blood on the slide. The spreader was allowed to touch the blood while spreading

along the edge of the spreader and this was moved along the slide to make a thin film. The thin film on the slide was allowed to dry. This was then stained with Leishmann stain for 10 minutes after which the Leishman stain was washed off. The film was air dried and examined under x 100 magnification (oil immersion) objective.

Haemoglobin: 0.05ml of blood sample was taken from heparinized tube containing anticoagulatant into a colorimeter tube (an instrument for measuring and specifying colour by comparison with established set of standard colour). Contents were mixed and allowed to stand for 10 minutes before reading. Photoelectric colorimeter was read at 540nm

Hematocrit: (Ht v/v ratio or %) Blood sample was taken from heparinized tube containing anticoagulatant into a capillary and centrifuge for five (5) minutes and allowed to stand for 5 minutes and measure with Haematocrit reader express in percentage.

Statistical Analysis

Students't-test and one-way analysis of variance SPSS (14.0 version), SPSS Inc, Chicago, USA, was employed to calculate the significance of the differences between control and experimental means and within various treatments and post treatment .P values of 0.05 or less were considered statistically significant [28]

RESULTS AND DISCUSSION

The water quality parameters in the control and various treatments were within the range obtain in the natural habitat; the temperature remained between 27.00 $\pm$ 0.58 ◦C and 26.67 $\pm$ 1.15◦C; pH between 7.27 $\pm$ 0.25 and 7.16 $\pm$ 0.25, rate of dissolved oxygen was kept between 8.73 $\pm$ 0.12 and 8.12 $\pm$ 0.10, turbidity between 3.25 $\pm$ 1.03 and 3.86 $\pm$2.01 and hardness between 180 $\pm$ 1.90 and 210 $\pm$ 0.06 .

Paraquat dichloride is one of the widely used herbicides that could be persistent and mobile in soil and water, and it is known to be one of the most common terrestrial and aquatic contaminants [29] .The use of haemathological parameters to predict in vivo response and recovery from contaminants has not been seriously considered in toxicology. Until now little is known about recovery time for complete recovery of haemathological parameters after exposure to pesticides especially herbicide such as *P. dichloride.*

If haemathology affinity can predict in vivo pattern of aberration, there is great potential to use this affinity to predicts pollution and environmental animal susceptibility [30]. It should be noted that haemathological alteration can return to a normal level, if exposure to contaminant is discontinuous

Red Blood Indices: In this investigation, during the 28 days exposure period and post treatment duration, no mortality occurred, but the fishes were sluggish and remain at the bottom toward the end of 28 days. Response of the fish exposed to different concentrations and control varies significantly ($p < 0.05$) and the level of variation increased with increase in the concentrations of the toxicant. The red blood indices of *C.gariepinus* exposed to different treatments of *paraquat dichloride* at 28 days are shown in Table 1. The fish exposed had significant ($p < 0.05$) lower erythrocyte count, haemoglobin content, haematocrit, mean corpuscular haemoglobin concentration (MCHC) and erythrocyte sedimentation rate (ESR) with increased in concentrations of the toxicant. In contrast, there was sharp increased in mean corpuscular volume (MCV) and fluctuation in values of mean corpuscular haemoglobin (MCH). The reduction in total erythrocytes noticed in the present study showed that the induced fish became anaemic, which may be due to erythropoiesis and osmoregulatory dysfunction [31, 32]. Similar reduction in the erythrocytes of *O. niloticus* exposed to Gammalin 20 and Actellic 25 EC was reported by [33] and malathion in freshwater catfish [34] .The reduction in haemoglobin in experimental animals might be destruction of red blood cell as reported by [35] , where he stated that exposure to heavy metals or pesticides leads to reduced haemoglobin content and haematocrit via disorders in haemopoietic and accelerated disintegration of erythrocyte cell membrane . The decrease in blood haemoglobin and red blood cells may also be due to the presence of stressor which causes haemodilution to occur as a result of impaired osmoregulation [36] .The decrease in packed cell volume indicated that the fish is suffering from anaemia, .This corroborated the finding of [37] where fishes exposed to atrazine experienced decreases in the packed cell volume levels and fall in the number of red blood cells. The MCV value was significantly ($p < 0.05$) increased in the fish treated with *Paraquat dichloride* than the control .The significant increased of mean corpuscular volume (MVC) may be associated with decreased in synthesis of haemoglobin and red blood cell numbers in bone marrow . Similar observation was reported by [38] in common carp exposed to atrazine. MCV measures the average volume of red blood cell by dividing the hematocrit by RBC. Under a microscope, stained red blood cells with a high MCV appear larger than cells with a normal or low MCV. When

compared with the control, no significant ($p > 0.05$) alteration in the values of MCH and MCHC registered during exposure to the herbicide .Mean corpuscular haemoglobin (MCH) measures the average amount of haemoglobin within a red cell. A similar measurement, mean corpuscular haemoglobin concentration (MCHC), expresses the average concentration of haemoglobin in the red blood cells. Contrary to this finding, [39] reported that MCHC levels increased in tilapia *Oreochromis mosambicus* when it was exposed to cadmium .Erythrocytes sedimentation rate was significant ($p < 0.05$) increased compared with control .The similar responses was registered in common carp after acute exposure of phenitrothion, and dichloros [40]

Table 1: Red blood cell indices in *O.niloticus* at 28 days sub lethal exposure to different concentrations (μg/L) of *Paraquat dichloride*

Conc. (ug/L)	0.0000	2.0000	4.0000	6.0000	8.0000
	Mean ± SD	Mean± SD	Mean ± SD	Mean ± SD	Mean ± SD
ESR (mm / hr)	24.00± 4.0^{abcd}	12.67±2.08^{a}	11.00± 0.00^{b}	8.67± 1.15^{c}	8.00± 0.00^{d}
RBC(mill/cmm)	2956.67±35^{abcd}	1129±77.16^{a}	1110±97.39^{b}	1006± 8.72^{c}	932.67±115.76^{d}
Haemoglobin(g/L)	126.5± 1.9^{abcd}	91.63±1.24^{b}	73.35±17.02^{c}	66.75±11.11^{d}	70.10± 9.38^{a}
PCV (%)	102.67± 6.4^{abcd}	62.00±7.84^{a}	62.00± 8.00^{b}	57.33± 4.16^{c}	50.33± 2.89^{d}
MCV (μm^3/cell)	34.74± 2.58^{a}	47.89±5.35^{b}	59.72± 2.36^{c}	67.01± 4.45^{d}	74.71± 9.32^{e}
MCH(pg/cell)	0.07± 0.00^{a}	0.08± 0.01^{b}	0.07± 0.01^{c}	0.07± 0.01^{d}	0.07± 0.01^{e}
MCHC (gl^{-1})	12.38± 0.91^{a}	15.2± 1.89^{b}	11.84± 2.44^{c}	11.71± 2.42^{d}	13.97± 2.06^{e}

Mean with the same superscript in the row are significantly different * ($p < 0.05$)

White Blood Indices: White blood cell indices of *C.gariepinus* exposed to different treatment of *paraquat dichloride* at 28 days are shown in Table 2. The WBC contents in the fish exposed to *P. dichloride* increment was dose dependent and varies significantly ($P < 0.05$) when compared with the control .Higher leucocyte count, lymphocyte, granulocytes, neutrophile and basophils were recorded in both experimental groups when compared with the reference fish. Eosinophils and monocyte count concentrations were comparable with the control. The increased in WBCs content recorded in this work could be due to the attempt of the fish to fight against the antigens (pollutant) and this augmented the production of more WBC to improve the health status of the fish .Comparable findings was reported by [31] when *Channa punctatus* was exposed to fenvalerate. Significant increament ($p < 0.05$) of neutrophils in smear of the fishes were observed during exposure to different concentrations of this herbicide .The most common and important cause of neutrophilia is infection and the degree of elevation often indicates the severity of the infection. Poisonings, and severe disease, like kidney failure cause neutrophilia [41]. This finding is similar to observation made by [42] where neutrophils and eosinophils elevation was reported in *heteropneustes fossilis*, induced with dimethoate in 96h LC_{50} concentration.

Recuperation: The fish recovered spontaneously and the aberrated parameters in all the treatments normalised after 12 days in free insecticide aquarium. The changes in the erythrocyte parameters during recovery at 1, 8 and 12 days in paraquat free water are shown in table 3,4 and 5, while the white blood indices during recovery period at 1, 8 and 12 days in a pesticide free water are shown in table 6 , 7 and 8.

CONCLUSION

Until now little is known about recovery pattern or length of time required for complete recovery of haemathological parameters in pisces especially the benthics under natural circumstances. The results of this investigation revealed that the recovery of the haemathological parameters is dose dependent as the parameters gradually normalized and were completely recovered after 12 days in *paraquat* free water.

This study had shown that haemathology is a reliable bioindicator to monitor pesticide and other environmental contaminants. It is hereby recommended that aquaculturist in an area that is prone to contamination, should expose the fish to water in a pond sited in a placid environment that is holistic free from contaminants before they are sold to the public.

Table 2: White blood cell and differential counts in the plasma of *C.gariepinus* exposed to sublethal concentrations (µg/L) of *paraquat dichloride* for a period of 28 days

Conc. (ug/L)	0.0000	2.0000	4.0000	6.0000	8.0000
Indices($G.1^{-1}$)	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
WBC	921.33± 35.23 ^{a}	936.38±2.02 ^{b}	981.67±19.50 ^{c}	1102.67±3.06 ^{d}	1587.33±11.02 ^{g}
Lymphocytes	57.33± 1.15 ^{abc}	68± 4.00 ^{de}	74± 3.46 ^{a}	89.33± 2.31 ^{bd}	125.33± 2.31 ^{ce}
Neutrophils	3.67± 0.58 ^{ab}	14.67±1.15 ^{a}	16.67± 1.15 ^{b}	18.00± 0.00 ^{c}	26.67 ± 2.08 ^{abc}
Basophils	4.00± 0.00 ^{acd}	3.00± 0.00 ^{b}	2.00± 1.00 ^{a}	2.00± 1.00 ^{c}	1.33± 0.58 ^{d}
Monocytes	3.67± 0.58 ^{abc}	2.33± 0.58 ^{b}	1.33± 0.58 ^{a}	1.00± 0.00 ^{b}	1.00± 0.00 ^{c}
Eosinophils	2.33 ± 0.58 ^{a}	2.00± 0.00 ^{b}	2.00 ±0.00 ^{c}	2.00 ± 0.00 ^{d}	2.00± 1.00 ^{e}

Mean with thesame superscript in the row are significantly different * ($p < 0.05$)

Table 3: Red blood cell indices in *C.gariepinus* at 24hrs post treatment in insecticide free de-ionized water

Conc. (ug/L)	0.0000	2.0000	4.0000	6.0000	8.0000
Indices	Mean ± SD	Mean± SD	Mean ± SD	Mean ± SD	Mean ± SD
ESR (mm / hr)	24.00± 4.0 ^{abcd}	15.00±1.12 ^{b}	13.65± 0.30 ^{b}	14.60± 1.11 ^{c}	14.40± 2.10 ^{d}
RBC(mill/cmm)	2956.67±35 ^{abcd}	1178±3.86 ^{b}	1119±10.21 ^{c}	1012± 3.43 ^{d}	1120±45.20 ^{b}
Haemoglobin(g/L)	126.5± 1.9 ^{abcd}	90.20±1.44 ^{b}	72.21±07.00 ^{c}	69.15±8.05 ^{a}	74.19± 3.25 ^{b}
PCV (%)	102.67± 6.4 ^{abcd}	61.54±1.14 ^{a}	62.00± 8.00 ^{b}	55.30± 6.12 ^{c}	53.13± 1.02 ^{d}
MCV (μm^{3}/cell)	34.74± 2.58 ^{a}	45.80± 3.30 ^{b}	58.42± 2.36 ^{a}	57.01± 2.40 ^{c}	54.22± 5.20 ^{d}
MCH(pg/cell)	0.07± 0.01 ^{a}	0.06± 0.01 ^{b}	0.03± 0.01 ^{c}	0.07± 0.01 ^{d}	0.07± 0.01 ^{e}
MCHC (gl^{-1})	12.38± 0.91 ^{a}	16.2± 0.02 ^{b}	10.84± 2.44 ^{c}	11.71± 2.42 ^{d}	12.33± 1.23 ^{e}

Mean with the same superscript in the row are significantly different * ($p < 0.05$)

Table 4: Red blood cell indices in *C.gariepinus* at 8 days post treatments in insecticide free de-ionized water

Conc. (ug/L)	0.0000	2.0000	4.0000	6.0000	8.0000
Indices	Mean ± SD	Mean± SD	Mean ± SD	Mean ± SD	Mean ± SD
ESR (mm / hr)	24.00± 4.0 ^{a}	20.96±0.10 ^{b}	21.30± 1.10 ^{c}	20.11.20± 0.12 ^{d}	18.20± 1.18 ^{e}
RBC(mill/cmm)	2956.67±35 ^{abcd}	2504±30.86 ^{a}	2679±12.09 ^{a}	2650± 12.90 ^{c}	2590 ±36.10 ^{d}
Haemoglobin(g/L)	126.5± 1.9 ^{a}	120.40±3.40 ^{b}	119± 10.02 ^{c}	113±13.19 ^{d}	118.20± 8.32 ^{e}
PCV (%)	102.67± 6.4 ^{a}	98.50±1.04 ^{b}	90.08± 14.20 ^{c}	94.10± 18.30 ^{d}	89.30± 12.34 ^{e}
MCV (μm^{3}/cell)	34.74± 2.58 ^{a}	30.20± 1.30 ^{b}	31.30± 4.10 ^{c}	32.11± 1.20 ^{d}	28.10± 2.10 ^{e}
MCH(pg/cell)	0.07± 0.01 ^{a}	0.07± 0.01 ^{b}	0.06± 0.21 ^{c}	0.07± 0.01 ^{d}	0.07± 0.01 ^{e}
MCHC (gl^{-1})	12.38± 0.91 ^{a}	11.68± 0.02 ^{b}	11.82± 2.44 ^{c}	11.98± 1.02 ^{d}	10.90± 2.20 ^{e}

Mean with the same superscript in the row are significantly different * ($p < 0.05$)

Table 5: Red blood cell indices in *C.gariepinus* at 12 days post treatments in insecticide free de-ionized water

Conc. (ug/L)	0.0000	2.0000	4.0000	6.0000	8.0000
Indices	Mean ± SD	Mean± SD	Mean ± SD	Mean ± SD	Mean ± SD
ESR (mm / hr)	24.00± 4.0 ^{a}	24.96±0.10 ^{b}	24.30± 1.10 ^{c}	23.90± 0.12 ^{d}	24.10± 2.10 ^{e}
RBC(mill/cmm)	2956.67±35 ^{a}	2953±12.00 ^{b}	2959±8.09 ^{c}	2955± 12.20 ^{d}	2953 ±12.12 ^{e}
Haemoglobin(g/L)	126.5± 1.9 ^{a}	126.40±3.20 ^{b}	126.9± 6.00 ^{c}	125.80±6.19 ^{d}	126.00± 1.12 ^{e}
PCV (%)	102.67± 6.4 ^{a}	103.00±1.04 ^{b}	102.08± 8.20 ^{c}	102.10± 11.30 ^{d}	121.70± 9.34 ^{e}
MCV (μm^{3}/cell)	34.74± 2.58 ^{a}	34.20± 1.60 ^{b}	34.30± 3.10 ^{c}	34.13± 1.70 ^{d}	34.10± 3.60 ^{e}
MCH(pg/cell)	0.07± 0.00 ^{a}	0.07± 0.01 ^{b}	0.07± 0.21 ^{c}	0.07± 0.01 ^{d}	0.07± 0.01 ^{e}
MCHC (gl^{-1})	12.38± 0.91 ^{a}	12.08± 0.02 ^{b}	12.82± 1.40 ^{c}	12.29± 1.00 ^{d}	12.25± 1.10 ^{e}

Mean with the same superscript in the row are significantly different * (p < 0.05)

Table 6: White blood cell differential counts in *C.gariepinus* at 24 hrs post treatments in insecticide free de-ionized water

Conc. (ug/L)	0.0000	2.0000	4.0000	6.0000	8.0000
Indices($G.1^{-1}$)	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
WBC	921.33 ± 35.23^{a}	921.00 ± 1.02^{b}	900.27 ± 12.50^{c}	901.20 ± 5.00^{ab}	890.21 ± 6.02^{abc}
Lymphocytes	57.33 ± 1.15^{g}	52 ± 2.80^{de}	50 ± 2.70^{a}	50.20 ± 1.28^{bd}	50.00 ± 3.50^{ce}
Neutrophils	3.67 ± 0.58^{g}	3.60 ± 1.05^{a}	3.60 ± 1.25^{b}	4.20 ± 0.00^{c}	4.36 ± 1.30^{abc}
Basophils	4.00 ± 0.00^{g}	3.00 ± 0.00^{b}	3.00 ± 1.00^{a}	3.00 ± 1.00^{c}	3.33 ± 0.58^{d}
Monocytes	3.67 ± 0.58^{g}	3.30 ± 0.58^{b}	3.34 ± 0.58^{a}	3.60 ± 2.20^{b}	3.20 ± 1.00^{c}
Eosinophils	2.33 ± 0.58^{g}	2.30 ± 1.00^{b}	2.20 ± 0.00^{c}	2.30 ± 1.00^{d}	2.30 ± 2.00^{e}

Mean with the same superscript in the row are significantly different * (p < 0.05)

Table 7: White blood cell and differential counts in *C.gariepinus* at 8 days post treatments in insecticide free de-ionized water

Conc. (ug/L)	0.0000	2.0000	4.0000	6.0000	8.0000
Indices($G.1^{-1}$)	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
WBC	921.33 ± 35.23^{a}	921.20 ± 1.02^{b}	917.20 ± 12.50^{c}	917.00 ± 5.00^{d}	920.20 ± 4.02^{e}
Lymphocytes	57.33 ± 1.15^{a}	57.00 ± 2.80^{b}	57.00 ± 2.70^{c}	57.20 ± 2.30^{d}	57.30 ± 2.10^{e}
Neutrophils	3.67 ± 0.58^{a}	3.63 ± 1.05^{b}	3.63 ± 1.25^{c}	3.60 ± 0.01^{d}	3.61 ± 2.10^{e}
Basophils	4.00 ± 0.00^{a}	4.00 ± 0.00^{b}	4.00 ± 2.10^{c}	4.00 ± 1.00^{d}	4.20 ± 0.42^{e}
Monocytes	3.67 ± 0.58^{a}	3.60 ± 0.18^{b}	3.64 ± 0.28^{c}	3.66 ± 2.00^{d}	3.70 ± 1.30^{e}
Eosinophils	2.33 ± 0.58^{a}	2.30 ± 1.00^{b}	2.29 ± 0.00^{c}	2.30 ± 1.00^{d}	2.29 ± 2.00^{e}

Mean with the same superscript in the row are significantly different * (p < 0.05)

Table 8: White blood cell differential counts in and *C.gariepinus* at 12 days post treatments in insecticide free de-ionized water

Conc. (ug/L)	0.0000	2.0000	4.0000	6.0000	8.0000
Indices($G.1^{-1}$)	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
WBC	921.33 ± 35.23^{a}	921.32 ± 1.02^{b}	921.20 ± 8.50^{c}	921.20 ± 2.10^{d}	921.10 ± 2.13^{e}
Lymphocytes	57.33 ± 1.15^{a}	57.70 ± 1.80^{b}	57.60 ± 5.70^{c}	57.32 ± 2.70^{d}	57.35 ± 1.10^{e}
Neutrophils	3.67 ± 0.58^{a}	3.65 ± 1.15^{b}	3.65 ± 2.15^{c}	3.65 ± 0.05^{d}	3.69 ± 3.12^{e}
Basophils	4.00 ± 0.00^{a}	4.00 ± 0.00^{b}	4.00 ± 2.10^{c}	4.00 ± 1.00^{c}	4.20 ± 0.42^{d}
Monocytes	3.67 ± 0.58^{abc}	3.64 ± 3.18^{b}	3.64 ± 2.28^{a}	3.67 ± 1.00^{c}	3.66 ± 2.30^{d}
Eosinophils	2.33 ± 0.58^{a}	2.31 ± 1.00^{b}	2.30 ± 0.00^{c}	2.34 ± 1.00^{d}	2.30 ± 1.00^{e}

Mean with the same superscript in the row are significantly different * (p < 0.05)

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