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INDUCTION OF MICROCYTIC-HYPOCHROMIC ANAEMIA IN *Clarias gariepinus* (BURCHELL, 1822) EXPOSED TO SUB-LETHAL CONCENTRATIONS OF 1, 1-DIMETHYL 4, 4-BIPIRIDILLIUM DICHLORIDE (PARAQUAT) UNDER LABPRATORY CONDITIONS.

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ABSTRACT

Microcytic-hypochromic anaemia was induced in *Clarias gariepinus* (Burchell, 1822) at intervals of 1, 7 and 14 days. Experimental fish were exposed to test water separately diluted with sub-lethal concentrations of paraquat of 0, 0.06, 0.07, 0.09 and 0.12mg/L. 14 days' exposure to the sub-lethal concentrations of the toxicant resulted in mirocytic-hypochromic anaemia in the exposed fish. Blood indices attributable to mirocytic-hypochromic anaemia was observed with a significant ($p<0.05$) decrease in haemoglobin, haematocrit, red blood cells, Mean corpuscular volume (MVC), Mean corpuscular haemoglobin (MCH), Mean corpuscular haemoglobin concentration (MCHC), white blood cells, lymphocytes, monocytes, Neutrophils and eosinophil compared with the fish in the control test.

Keywords: Toxicity, paraquat, *Clarias gariepinus*, microcytic-hypochromic and anaemia

INTRODUCTION

Determination of the toxic compounds in aquatic environments and their effects on aquatic organisms is a fundamental issue in ecotoxicological studies (Bagheri, 2007). One of the main causes of pollution of aquatic ecosystems is agricultural pesticide, which is used to deal with pests, weeds and agricultural diseases and they have adverse effects on the environment (Bahmani *et al.,* 2001). Within a few weeks of using these pesticides in agricultural activities, they entered to aquatic ecosystems through surface runoff and subsurface drainage (Abubakar *et al.,* 2019). Herbicides are generally used by farmers to control weeds, remove aquatic plants in rivers, lakes and water reservoirs, which generally have harmful effects on aquatic animals' health (Abubakar et al., 2019) Fish is directly associated with the aquatic environment, hence, the incidence of physical and chemical changes in the aquatic environment quickly leads to measurable physiological changes in fish (Adihikari *et al.,* 2004). In recent years, the incidence of mortality due to pesticides, industrial effluents and sewage contamination has been reported in Iran (Agha *et al.,* 2012). One of the most important and reliable indicators for the health assessment and fish physiology is measurement of blood parameters which are influenced by the nutritional and environmental factors (Abubakar, 2016). Blood characteristics of fish are one of the most important evidences of their physiological processes and reflect the relationship between aquatic ecosystem characteristics and their health status (Osman and Kloas, 2010). Having a normal range of fish blood parameters can be used as a bio-indicator (Abubakar, 2013). Changes in blood parameters in response to environmental conditions area response to environmental stress and can be considered as an important bio-indicator (Verma and Agarwal, 2007). Use of hematology parameters is expanding in aquaculture activities, particularly in toxicology researches, environmental monitoring and evaluation of aquatic animal health (Verma and Agarwal, 2007). There are few documented reports of effects of paraquat on the health status of fish. Paraquat(1,1-dimethyl-1,4, 4 Bipyridinium dichloride) is a non-selective contact herbicide. It acts in the presene of light which it absorbs at 260nm, to desiccate the green parts of all plants with which it comes in contact (Abubakar *et al.,* 2019). Paraquat is an herbicide that is used to destroy the weeds in tropical regions and it enters to aquatic ecosystems (Bahmani *et al.,* 2008). Paraquat is used in different parts of Iran with different climatic conditions and different agricultural activities, and the proposed amount of the herbicide is three liters per hectare for sugarcane plantations (Banaee *et al.,* 2008).

The African catfish (*Clarias gariepinus*) is an ecologically important and commercially valued fish in Nigeria (Abubakar *et al.,* 2019). These mudfish are frequently and widely cultured in Nigeria waters including ponds (Abubakar *et al.,* 2019). There is paucity of information on toxicity of paraqua (1,1-dimethyl-1,4, 4 Bipyridinium dichloride) on catfish by the local fishermen.

The aim of the present study was to determined mirocytic-hypochromic anaemia in *Clarias gariepinus* under laboratory conditions.

MATERIALS AND METHODS

Fish collection and transportation

One hundred and eighty (180) juveniles of African catfish, (*C. gariepinus*), average weight (21.48±3.32) g and length of (11.37±1.23) cm, respectively) were purchased from Tee jay Fish and Feeds Farm Ogidi, Ilorin, and transported in oxygenated polythene bags to the Central Laboratory, University of Ilorin where they were acclimatized for 2 weeks in a plastic tank (capacity of 300 L). Water was changed daily to acclimatize the C. gariepinus to the new environment. The fishes were fed daily with skretting fish feed (35% crude protein) at 3% body weight (morning and evening) during the period of acclimation. The fishes were accepted as well as adapted to the laboratory conditions when 3 mortalities were recorded for the 14 days. The water in the plastic tank was replaced with tap water every two days and uneaten and faecal matter was siphoned out. Feeding was terminated 24hours before the commencement of the experiment.

PROCUREMENT OF PARAQUAT AND ITS EXPOSURE

Paraqua(1,1-dimethyl-1,4,4Bipyridinium dichloride). A commercial formulation of paraquat (276 g/L) with trade-name Royaquat (CAS number 468514-7), manufactured by Anhui Costar Biochemistry cooperation limited (Anhui, China) was obtained from Aromokeye Trade Store.Unity Road, Ilorin, Nigeria.

EXPERIMENTAL DESIGN

The experimental design was a complete randomized design (CRD). One hundred and fifty (150) juvenile of *Clarias gariepinus* were randomly distributed into the tanks at a stocking rate of 10 fish per tank. The fifteen (15) tanks were assigned to 5 treatments (control inclusive). In order to determine the LC50, Method of Abubakar (2013) was adopted. The C. gariepinus were exposed to four different concentrations of paraquat (0, 1.2, 1.8, 2.4, and 3.0mg/L).

LETHAL TOXICITY TEST

Prior to the commencement of the lethal toxicity tests for Royaquat, a screening test was first conducted to ascertain the range to be used for the bioassay test. Dilutions were made from the stock solutions and only one test fish was put at a time and observed to determine the time mortality would occur. The concentrations from which serial dilutions will commence were arrived at after the test fish survived beyond 2 hours

on exposure to the toxicant. Observations and record of number of fish alive overturned and dead in each of the toxicant bioassay in test vessels were made at intervals of 3,6,12,24,48,72 and 96hours. Dead fish were identified when opercula movement ceased and there was no response to touch. From these data, LC50 values were calculated by Probit analysis using the US EPA software (Probit program version 1.5) and 1/15, 1/20, 1/25 and 1/30 were taken as sub-lethal using the method of Abubakr (2013).

ACUTE BIOASSAY TEST

The acute bioassay test for the determination of lethal effect of Royaquat on the test fish species were conducted using static renewal method. The following concentration of Royaquat was used 1.2, 1.8, 2.4 and 3.0mg/l respectively with a control with no toxicant. Each concentration level was triplicated. The desired stock solution was measured with a syringe and introduced into the 10 litres of water in the plastic tank. The mixture was allowed to stand for 30 minutes for proper mixing before introducing the test fishes. A stocking density of 10 fish per tanks was used following the method of Abubakr (2013).

Each tank was covered with a nylon mesh screen to prevent the fish from jumping out of the tank. The cover of each tank was perforated to allow air into the tanks and each was used to hold down the nylon mesh screen to the tank. Feeding was stopped 24 hours prior to and during the 96hour bioassay test. This was done to prevent interference with the metabolism and absorption of the extract by wastes in reconstituted areas. The test fishes (treatment and control) were observed for signs of toxicity with prompt recordings at 24h, 48h, 72h and 96h of exposure.

SUB-LETHAL TEST

From the LC50 values calculated by Probit analysis, 1/15, 1/20, 1/25 and 1/30 were taken as sub-lethal using the method of Abubakr (2013) to produce 0, 0.12, 0.09, 0.07 and 0.06mg/l The test fish were exposed to sub-lethal doses of the herbicide to determine the hematological effect. The concentrations were triplicated. The experiment had controls which were devoid of Royaquat. The experiment lasted for 14days. During the exposure of test fishes, selective sampling of three fish each was taken from each treatment concentration for hematological analysis at intervals of 24hours, 7 and 14days. However, the sampling of hematological analysis was limited to pre-exposure at the beginning and post-exposure at the end of the experiment.

BLOOD COLLECTION AND HEMATOLOGICAL TEST

Blood sample was collected by caudal venous puncture using the method of Abubakr (2016) using 21Gx 11/2(0.8x 40 mm) syringe. The syringe was inserted under the skin of the ventral midline of the caudal peduncle of freshly euthanized fish and then eased towards the vertebral column until the base of the column was reached. The needle was withdrawn a fraction of a millimeter and blood sample was obtained. Blood were collected at intervals of 1, 7 and 14 days from both the tested and control fishes. The blood was put into EDTA vials and taken to Central Research and diagnostic laboratory, Tipper garage, Tanke, Ilorin for hematological analysis. Hematological analysis was carried out using veterinary hematology analyzer model (PR-3125 Plus). The blood samples were put under the probe of the hematology analyzer to suction the blood into the machine. Four reagents were used for the analysis. The four reagents were put inside four bottles: one reagent per bottle each and were connected to the back of the machine. The four reagents were: Lyse reagent, Diluent reagent, Cleaner reagent and Waste reagent. The machine is automated, displays the results on its screen which were printed out.

STATISTICAL ANALYSIS

The median lethal concentration (LC50) value was determined using the US EPA Software Probit analysis program, version 1.5. Data were analyzed with One-way analysis of variance (ANOVA) procedure using Statistical Product for Service Solution (SPSS version 16.0) for window. Statistical significance of differences between means was compared using Turkey (HSD) test.

RESULTS AND DISCUSSION

The mean values of the water quality parameters of the different sub-lethal concentrations of Paraquat and control media to which the test fish (*Clarias gariepinus*) were exposed for 14 days are presented in Table 1. The values of parameters were found not to be significantly different ($p<0.05$) from control samples after 14 days exposure period which means that Paraquat has no effect on the water parameters. This is in line with the study of Omoniyi *et al.,* (2002) on the sub-lethal effects of tobacco leaf dust on the haematological parameters of *Clarias gariepinus*. The temperature (25.590C-27^{0}C) and pH (7.9-8.2) were within the range recommended for aquaculture practices. The water quality parameters studied were within the standard meant for aquaculture purposes. Researchers have reported pH of 6.5-9.0 to support fish life. Okomoda and Ataguba(2011) reported the pH of 6.5-8.5. Basically, in aquatic ecosystem, temperature is very essential for the survival of fishes through metabolism. As such, inability of fish to adapt to the environment could cause a change in their physiological response which could lead to mortality. The temperatures recorded in this study were within the ambient temperature of the area and values of surface water resources in Nigeria (Ben-Eledo *et al.,* 2017a). Hence, the fishes may not have died due to temperature. Dissolved solids contain inorganic and

some organic compounds (Boyde, 2015). From the result of this study, temperature increases as the TDS increases in each treatment, this is in line with the findings of Raz (2019) who reported that in bodies of water like rivers, higher levels of TDS changes the mineral content of the water which is important for the survival of aqua-biotas. Also, dissolved salts can dehydrate the skin of aquatic animals which can be fatal and also in line with the findings of Adewoye *et al.,*(2005) who observed changes in characteristic features might have resulted from the organic loads in the water. The mean values of the water quality parameters of the different sub-lethal concentrations of Paraquat and control media to which the test fish (*Clarias gariepinus*) were exposed for 14 days are presented in Table 1. The values of parameters were found not to be significantly different ($p<0.05$).

Table 1: Physico-chemical parameters of test solutions on Clarias gariepinus (mean ± SD) for chronic(sub-lethal) test.

Concentration	PH	Temperature	TDS
(mgL^{-1})		(^0C)	(mgL^{-1})
0	$8.05±0.21^a$	$25.59±0.82^{ab}$	$776.38±189^a$
0.06	$8.07±0.18^a$	$25.80±0.84^{ab}$	$828.08±179^a$
0.07	$8.06±0.21^a$	$25.85±0.82^{ab}$	$828.31±201^a$
0.09	$7.92±0.40^a$	$25.91±0.83^{ab}$	$836.92±193^a$
0.12	$7.92±0.40^a$	$26.39±0.71^a$	$853.31±208^a$

Values of parameters with the same superscript along the same column are not significantly different at ($p<0.05$).

Key: TDS (Total dissolved solids)

EFFECTS OF SUB-LETHAL CONCENTRATIONS OF PARAQUAT ON BLOOD PARAMETERS

The results of blood parameters of C.gariepinus exposed to sub-lethal concentration of paraquat are discussed below. The hematological responses and values obtained in the exposed and control groups at various sub-lethal concentrations are presented in Tables, 2, 3, 4 and 5. The blood indices in each treatment varied significantly ($p<0.05$) and were dose-dependent.

HEMATOLOGICAL PARAMETERS OF *Clarias gariepinus*

The results of hematological parameters on exposed C.gariepinus indicated a consistent reduction in the values of Hb, PCV, RBC, MCV, MCH, MCHC, WBC, Neutrophils, lymphocytes and monocytes respectively (microcytic-hypochromic anaemia) at ($p<0.05$) on various days of exposure.

DAY 1

Table 2:Heamatological parameters of ***Clarias gariepinus*** exposed to sub-lethal concentrations of paraquat(Mean ± SD) on Day 1.

	Concentration(mg/L)				
Parameters	**Control**	**0.06**	**0.07**	**0.09**	**0.12**
Hb(gdL^{-1})	$6.99±1.762^a$	$6.97±2.970^a$	$6.80±2.022^a$	$6.73±1.411^a$	$5.57±0.103^b$
PCV (%)	$18.93±4.509^c$	18.90±6.385c	$18.03±7.239^c$	$14.47±8.145^d$	$9.27±7.251^a$
RBC($x10^{12}$ L^{-1})	$1.67±0.727^a$	$1.64±0.509^b$	$1.45±0.639^c$	$1.17±0.118^d$	$1.10±0.732^e$
MCV(FI)	$144.43±6.082^a$	$135.03±3.078^b$	$127.90±3.576^c$	$119.70±2.626^d$	$108.83±5.982^e$
MCH(pg)	$55.78±5.740^a$	$49.60±0.721^b$	$49.00±3.897^b$	$47.40±2.227^c$	$42.50±2.180^d$
MCHC(gdL^{-1})	$55.82±4.112^a$	$44.57±9.181^b$	$40.00±4.246^b$	$39.90±1.758^g$	$38.20±3.151^f$
WBC($x10^9L^{-1}$)	$7093±8.235^a$	$7083±5.378^b$	$7053±7.276^c$	$6067±3.001^d$	$5633±1.150^e$
Neutrophils (%)	$63.0±3.699^d$	51.0±0.700a	$48.7±3.955^b$	$46.0±0.529^f$	$24.0±0.200^g$
Lymphocytes (%)	$97.59±2.261^a$	$94.13±0.666^b$	$94.03±4.759^b$	$93.70±0.964^c$	$92.47±3.700^d$
Monocytes (%)	$1.25±0.518^c$	$1.20±0.400^c$	$1.10±0.854^c$	$0.97±0.058^a$	$0.70±0.100^a$

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

Hb-Haemoglobin; PCV-Packed cell volume; RBC-Red Blood cell count; MCV-Mean Corpuscular Volume; MCH-Mean Corpuscular Haemoglobin; MCHC-Mean Corpuscular Haemoglobin Concentration; WBC-White Blood cell count

Data: Day 7

Table 3: Heamatological parameters of Clarias gariepinus exposed to Sub-lethal concentrations of paraquat(Mean ± SD) on Day 7

Concentration(mg/L)					
Parameters	**Control**	**0.06**	**0.07**	**0.09**	**0.12**
Hb(gdL^{-1})	$6.99±1.762^{a}$	$6.47±2.950^{a}$	$6.20±2.022^{a}$	$5.80±1.41^{b}$	$4.47±0.153^{c}$
PCV (%)	$18.93±4.509^{a}$	$16.13±4.939^{b}$	$15.57±4.969^{c}$	$14.23±3.027^{d}$	$11.13±0.666^{e}$
RBC($x101^{2}$ L^{-1})	$1.67±0.727^{a}$	$1.54±0.669^{b}$	$1.43±0.289^{c}$	$1.34±0.208^{d}$	$0.97±0.112^{g}$
MCV(FI)	$144.43±6.082^{a}$	$118.6±5.515^{b}$	$114.9±7.877^{c}$	$111.6±3.947^{d}$	$103.97±3.021^{f}$
MCH(pg)	$55.78±5.740^{a}$	$53.37±4.927^{b}$	$46.60±2.722^{c}$	$46.17±5.405^{c}$	$45.03±5.401^{d}$
MCHC(gdL^{-1})	55.82±4.112a	$42.73±6.612^{b}$	$40.13±4.531^{c}$	$40.03±3.587^{c}$	$38.20±5.025^{g}$
WBC($x10^{9}L^{-1}$)	$7093±8.235^{g}$	$6730±7.956^{f}$	$5390±3.528^{d}$	$4693±3.672^{a}$	$3607±7.705^{b}$
Neutrophils (%)	$63.0±3.699^{a}$	$59.7±1.550^{b}$	$48.7±1.429^{c}$	$21.3±2.055^{d}$	$11.0±0.458^{e}$
Lymphocytes (%)	$97.59±2.261^{a}$	$96.30±2.088^{b}$	$96.23±1.986^{b}$	94.87±4.654c	$75.17±4.678^{d}$
Monocytes (%)	$1.25±0.518^{a}$	$0.73±0.551^{b}$	$0.63±0.551^{b}$	$0.60±0.529^{b}$	$0.27±0.115^{b}$

Mean of parameters with the same superscripts along the rows are not significantly different at p>0.05.

Hb-Haemoglobin; PCV-Packed cell volume; RBC-Red Blood cell count; MCV-Mean Corpuscular Volume; MCH-Mean Corpuscular Haemoglobin; MCHC-Mean Corpuscular Haemoglobin Concentration; WBC-White Blood cell count

Data: Day 14

Table 4:Haematological parameters of Clarias gariepinus 14 exposed to sub-lethal concentrations of paraquat(Mean ± SD) on Day 14

Concentration(mg/L)					
Parameters	Control	0.06	0.07	0.09	0.12
Hb(gdL^{-1})	$6.99±1.762^{a}$	$6.07±3.308^{a}$	$5.67±2.610^{b}$	$4.60±1.300^{b}$	$2.35±1.850^{c}$
PCV (%)	$18.93±4.509^{a}$	$14.90±4.357^{b}$	$13.60±5.339^{c}$	$12.63±7.543^{d}$	$11.67±2.248^{e}$
RBC($x10^{12}$ L^{-1})	$1.67±0.727^{a}$	$1.19±1.187^{b}$	$1.12±1.120^{c}$	$0.97±0.520^{d}$	$0.69±0.047^{e}$
MCV(FI)	$144.43±6.082^{a}$	$126.7±1.268^{b}$	$123.8±1.825^{c}$	$120.4±3.934^{g}$	$119.03±2.348^{f}$
MCH(pg)	$55.78±5.740^{f}$	$49.00±3.160^{a}$	$48.00±4.423^{b}$	$44.30±3.974^{n}$	$42.07±5.672^{c}$
MCHC(gdL^{-1})	$55.82±4.112^{a}$	$38.20±4.490^{b}$	$35.80±3.987^{c}$	$35.03±5.977^{c}$	$29.00±6.245^{d}$
WBC($x10^{9}L^{-1}$)	$7093±8.235^{a}$	$5167±5.636^{b}$	$3997±6.623^{d}$	$3307±3.014^{g}$	$2620±1.416^{ag}$
Neutrophils(%)	$63.0±3.699^{m}$	$48.7±2.589^{n}$	$23.7±1.767^{a}$	$21.7±1.531^{b}$	$19.0±0.854^{d}$
Lymphocytes (%)	$97.59±2.261^{a}$	$97.53±1.002^{a}$	$97.10±2.254^{a}$	$94.47±2.706^{b}$	$91.17±3.095^{d}$
Monocytes (%)	$1.25±0.518^{a}$	0.67±0.351b	0.57±0.153b	$0.53±0.493^{b}$	$0.27±0.058^{b}$

Mean of parameters with the same superscripts along the rows are not significantly different at p>0.05.

Hb-Haemoglobin; PCV-Packed cell volume; RBC-Red Blood cell count; MCV-Mean Corpuscular Volume; MCH-Mean Corpuscular Haemoglobin; MCHC-Mean Corpuscular Haemoglobin Concentration; WBC-White Blood cell count

Table 5: Summary of heamatological parameters of Clarias gariepinus at the various days of exposure to sub-lethal concentrations of (Mean± SD)

Duration of exposure (Days)				
Parameters	Control	1	7	14
Hb(gdL^{-1})	$6.99±1.762^{a}$	$6.51±1.828^{a}$	$5.74±1.644^{b}$	$4.67±2.267^{c}$
PCV (%)	$18.93±4.509^{d}$	$15.16±7.255^{b}$	$14.29±3.900^{c}$	$13.20±4.871^{ad}$
RBC($x10^{12}$ L^{-1})	$1.67±0.727^{g}$	$1.44±0.0499^{a}$	$1.32±0.319^{b}$	$0.94±0.718^{d}$
MCV(FI)	$144.43±6.082^{c}$	$122.87±3.815^{a}$	$112.27±5.090^{b}$	$122.48±2.343^{a}$
MCH(pg)	$55.78±5.740^{a}$	$47.13±2.246^{b}$	$47.79±4.614^{b}$	$45.84±4.307^{c}$
MCHC(gdL^{-1})	$55.82±4.112^{d}$	$40.66±4.584^{a}$	$40.27±4.938^{a}$	$34.51±5.174^{b}$
WBC($x10^{9}L^{-1}$)	$7093±8.235^{a}$	$6459±4.201^{b}$	$5105±4.715^{c}$	$3773±4.172^{d}$
Neutrophils(%)	$63.0±3.699^{a}$	$42.4±1.346^{b}$	$35.2±1.363^{c}$	$28.3±1.635^{d}$
Lymphocytes(%)	$97.59±2.261^{a}$	$93.58±2.022^{b}$	$90.64±3.352^{c}$	$95.07±2.264^{d}$
Monocytes (%)	$1.25±0.518^{a}$	$0.99±0.353^{b}$	$0.56±0.437^{b}$	$0.51±0.264^{b}$

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

Hb-Haemoglobin; PCV-Packed cell volume; RBC-Red Blood cell count; MCV-Mean Corpuscular Volume; MCH-Mean Corpuscular Haemoglobin; MCHC-Mean Corpuscular Haemoglobin Concentration; WBC-White Blood cell count.

This research work revealed microcytic-hypochromic anaemia in C.gariepinus exposed to sublethal concentrations of paraquat (Tables 2, 3, 4 and 5). The reductions observed in the hematological parameters (HB, PCV, RBC, MCV, MCH, MCHC, WBC, Neutrophils, Basinophils and lymphocytes) of ***C. gariepinus*** exposed to paraquat demonstrates the fact that pesticides cause a significant reduction in the haematological parameters of fish (Ada *et al.,* (2012). These reductions could also be the product of impaired erythropoiesis and rapid haemolysis of the RBC. Furthermore, the reduction in MCV in the present study reveals erythrocytic shrinkage leading to microcytic anaemia. This is in agreement with the work of Idi-Ogede and Samuel (2018) who also observed microcytic-hypochromic anaemia in *Oreochromis niloticus* exposed to sublethal toxicity of 2,3-dichlorovinly dimethyl phosphate (sniper 1000EC). This reduction in MCV in the paraquat exposed fish could also arise due to osmoregulatory imbalances. Li *et al.,* (2011) reported that verapamil reduced the erythrocyte count, Hb and PCV in O. mykiss. Reduction in the values of these parameters was also reported in Prochilodus lineatus exposed to clomazone Pereira *et al.,* (2013) and in Labeo rohita exposed to fenvalerate (Prusty *et al.,* 2011). Reduction in the erythrocyte count was reported in C. mrigala exposed to ibuprofen (Saravanan *et al.,* 2012) and C. albopunctatus exposed to acetellic (Mgbenka *et al.,* 2005). The results from this study are also consistent with the results of Abubakar *et al.,* (2019) who reported Normocytic-Normochromic anaemia in C.gariepinus exposed to paraquat. It also agreed with the results in Heteropneustes fossilis, exposed to adrin and fenvalerate (Thakur and Bais, 2000), and in Salmo gardneri and Mystus vittatus exposed to pesticides (John, 2007). Studies have shown that pesticides have cytotoxic effects in fish which impose severe oxidative stress on the fish (Mikula *et al.,* 2009; Nwani *et al.,* 2013. The anaemic exposure could be as a destruction of RBC (Abubakar, 2013). Generally, leucocytes modulate immunological functions in animals, including fish. The observed leucocytosis in the present study indicated abnormal immune protective response to paraquat intoxication. It also suggested that paraquat stimulated the immune system with a concomitant release of lymphocytes from the lymphomyeloid tissue as a defense response. It resulted in the leucocytosis and or lymphocytosis, which altered body physiology. Leucocytosis was also reported in *C. carpeo* exposed to lindane (Saravanan *et al.,* 2011), and in O. niloticus exposed to deltamethrin (El-Sayed *et al.,* 2007). White blood cells are the in smaller number compared with red blood cells and they have the defensive role in the body of organisms (Ezeri, 2001). Changes in the levels of white blood cells following exposure to paraquat may be due to disturbances in the process of hematopoiesis and subsequent reduction or non-specific immune weakening in fish (Kumar *et al.,* 2011).White blood cells include lymphocytes, neutrophils, esinophils, monocytes and basophils, each of which plays a different role in the body of fish. Lymphocytes are seen in the blood circulation, lymphoid organs and other tissues, especially during inflammatory reactions. Lymphocytes are involved in the immune

response of aquatic animals by antibody production (immunoglobulin). White blood cell differential count in this study showed that the percentages of lymphocytes in all the exposed concentrations after 14 days were measured less than the control group. Decrease in the percentage of lymphocytes exposed to pesticides was reported by different researchers. Nussey *et al.,* (2015) investigated the effects of sub-lethal concentrations of diazinon on grass carp(Ctenopharyngodon idella) and reported significant increase ($P<0.05$) in percentage of neutrophils and significant reduction ($P>0.05$) in the percent of WBC and monocytes compared with the control group incontrast to the results of this study. According to the results of this study, the blood indices including MCV, MCH and MCHC of *C. gariepinus* exposed to paraquat were lower than the control group ($P < 0.05$). Sarikaya and Yilmaz (2003) studied the toxicity effects of pesticide Lindane on blood indices (MCV, MCH and MCHC) of common carp, Cyprinus carpio with significant reduction ($P < 0.05$) in blood indices (MCV, MCH and MCHC) compared with the control group. Satarri (2003) reported reduction in hematological indices (MCV, MCH and MCHC) of Cichlasoma dimerus' exposed to acute toxicity of Endosulfan. The reduction in size and quantity of hemoglobin of red blood cells is measured by the indices MCV, MCH, MCHC which can be a sign of anemia in fish (Feiz, 2010). The anemia may be as a result of stress due to bacterial infections (Silveira-Coffigny *et al.,* 2004) and exposure to agricultural pesticides (Mohammadian *et al.,* 2010). The presence of a large percentage of immature red blood cells in the bloodstream may be a reason for reduction of MCV and MCH. On the other side, reduction of MCHC in this study may be due to decreased in production of hemoglobin after exposure to the toxicant (Kazemi *et al.,* 2011).

CONCLUSION

This study revealed microcytic-hypochromic anaemia in *C.gariepinus* exposed to sublethal concentrations of 1, 1-dimethyl- 1, 4, 4 Bipyridinium dichloride brought about as a result of abnormalities in MCV, MCH and MCHC-haematological parameters of the exposed *C.gariepinus* under laboratory conditions attributable to stress. Thus, it was concluded that Paraquat exposure has stressor effects on hematological parameters of the exposed fish species.

RECOMMENDATION

In line with the conclusion, it is recommended that:

1- The manufacturing industries should look into ways of reducing the potency of Paraquat to non-target organisms such as fish, and they should be compelled to state categorically the effect of Paraquat to non-target organisms.

2- Agricultural extension officers should enlighten farmers on the dangers of Paraquat to the environment and water body at large.

3- Attempt should be made to monitor the use of Paraquat by local fishermen.

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