IN VITRO EFFECT OF PHARMACEUTICAL EFFLUENT ON HAEMATOLOGY AND BIOCHEMICAL RESPONSES IN AFRICAN CATFISH (*CLARIAS GARIEPINUS*)

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ABSTRACT

Pharmaceutical effluents collected from Bompai industrial area, Kano State were assessed for their physiochemical parameters, heavy metals and effect on haematology and biochemical changes in *Clarias gariepinus*. Laboratory analyses were performed using standard methods in a Completely Randomized Design (CRD). Five test solutions of the effluents (0, 25, 50, 75, and 100% v/v) were prepared for LC_{50} 96hr acute toxicity test. The LC_{50} for 96hr was 48.7% concentration of effluents by volume. Experimental fish were exposed to sublethal concentrations of 0.00% 2.43% (5/100 LC_{50}), 12.17% (25/100 LC_{50}), 24.35% (50/100 LC_{50}) and 36.52% (75/100 LC_{50}) for 28days. Physicochemical parameters recorded were higher than WHO recommended Standard with the exception of water temperature. Heavy metals concentrations decreased in the order of $Cr > Cu > Pd > Cd$. Red blood Cells count, haemoglobin concentrations, packed cell volume, lymphocytes and monocytes of the experimental fish decline significantly ($p<0.05$) when exposed with 2.43, 12.17, 24.35, 36.52% effluents compared to the control. White blood cell count, mean corpuscular haemoglobin, mean corpuscular volume, mean corpuscular haemoglobin concentrations, neutrophils and eosinophils were significantly higher ($P<0.05$) than the control. However, a significant increase ($P<0.05$) in the activities of serum aspartate aminotransferase (AST), Alkaline Phosphatase (ALP) and alanine aminotransferase (ALT) were recorded as the exposure period continued when compared with control. It is concluded that the effluents induces haematological and biochemical alterations. It is therefore recommended that regulatory bodies should adopt holistic approach on the aquatic pollution abatement, bearing in mind the negative impact to non-target organisms.

Keywords: sub-lethal effect, *Clarias gariepinus*, haematology, biochemical responses, pharmaceutical effluent

INTRODUCTION

Continuous contamination of aquatic environment globally is attributed to the illegal discharge of industrial, pharmaceutical and agricultural waste (Hosnia *et al*., 2015 and Alimba *et al.,* 2017). There has been concern in many countries as a result of deleterious effects of pollutants on the aquatic and terrestrial ecosystem ecology due to their accumulation through food chain (Ohioma *et al.,* 2009). Due to the discharge of untreated effluent into the water bodies, many aquatic environments in Nigeria have been reported to be polluted with pharmaceutical effluents, heavy metals, pesticides among other toxicants which alter the natural integrity of water and the physiological functions of the biota (Kanu and Achi, 2011 and Ado *et al*., 2014). The rapid population growth, industrialization and economic development of Kano city necessitate people to use contaminated water for irrigation activities to cater for the ever increasing population of the area (Yusuff and Sonibare, 2004 and Galadima and Garba, 2012).

However, Haematological investigation however, provides an insight on the immunological status in fish during exposure to toxicants (George *et al.,* 2017). Haematological parameters indicate different sensitivity to many environmental factors such as discharge from industries, domestic and natural input (Gabriel *et al.,* 2011 and Akinrotimi *et al.,* 2013). Akinrotimi *et al.* (2013) reported that haematological indices in fish culture is rapidly use in recent times for fish health conditions, toxicological survey and environmental monitoring. Akinrotimi and Amachree (2016) opined that when water quality is affected by toxicants, any physiological alterations will be reflected in the haematological values of the aquatic biota.

Moreover, activity of biochemical indices in fish such as levels of glucose, plasma proteins, Urea and Creatinine and enzymes, like alkaline phosphates (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase have been used widely in assessing oxidative stress of environmental contaminants (Nte and Akinrotimi, 2011 and Elias *et al.,* 2020). Biochemical indicators and metabolic enzymes mainly indicates stress animals tissues induced by many environmental influence (George *et al.,* 2017).Therefore biochemical examination of blood serum is a vital tool in assessing the physiological status of fish health in the aquatic ecosystem (Doherty *et al.,* 2010 and Elias *et al.,* 2020). Oxidative stress occurs in fish when there is an imbalance between the generation of ROS and production of antioxidants (Nafiu and Ibrahim, 2019). In view of the forgoing this research aimed at evaluating an *In vitro* effect of pharmaceutical effluent on haematology and biochemical responses in African catfish (*Clarias gariepinus*).

MATERIALS AND METHODS

Collection and Analysis of Pharmaceutical Effluents

The pharmaceutical effluents was collected in triplicate in a 30 litre dark plastic container from the outlet of a pharmaceutical industry, located at Bompai Industrial Area, Kano where a significant proportion of pharmaceutical and other industries operate. Prior to the analysis, the effluents were filtered through 70nm mesh size plankton net to eliminate algal growth. The sample was immediately analyzed for the following physicochemical parameters using the protocol adopted by Reddy (2018). These include: Colour, pH, Total Dissolved Solid (TDS), Dissolved Oxygen (DO), Biochemical Oxygen Demand (BOD) and Electrical Conductivity. Cadmium, Chromium, Copper and Lead were analyzed using double acid digestion with Atomic Absorption Spectrophotometer (Buck Scientific VGP 210) at Soil Science Department, Bayero University Kano using the method described by Gregg (1989), as adopted by Ibrahim and Nafiu (2017).

Source of Experimental Fishes

A total of 250 fish samples with mean weight range of 22.5 ± 0.22 -24.5 ± 0.20g, mean length 10.5 ± 0.27 -11.5 ± 0.11cm were used for the study. They were procured and maintained in aquaria at Rumbun Kifi Fish Farm, along Shiekh Jaafar Road, Kano State, Nigeria. They were treated by bathing in 0.05% potassium permanganate (KMnO$_4$) solution for two (2minutes) to minimize any dermal infections during the treatment. The health status of selected fish was determined based on the presence or absence of physical injuries and other morphological deformations as adopted by Adekunle (2015). The specimens were acclimatized for two weeks (14 days) under laboratory conditions in a semi-static system of 28°C Temperature and 40% Relative Humidity prior to the commencement of the experiment as described by Adeboyejo *et al.* (2011).They were fed with fixed feeding regime using commercial trout pellets daily at 2% body weight. The faecal were siphoned off daily to reduce ammonia content in water.

Experimental Design

The experimental and control juveniles fishes *C. gariepinus* was subjected to Completely Randomized Design (CRD) with 3-levels of exposure to the varying concentrations of the pharmaceutical effluents in a 40 x 80 x 40 cm dark plastic tank containing 50L of dechlorinated and aerated water.

Range Finding Test

Definitive concentration for range-finding test was carried out to determine the definitive concentration for testing the pharmaceutical effluents using the procedure described by OECD (2011) No.236 adopted by Bamidele *et al.* (2018). The concentration of the pharmaceutical effluents collected from the point source in which some fishes survived after 96 hours to the lowest concentration that no organism survived was used for the actual acute toxicity test.

Acute Toxicity Test

The acute toxicity test to determine the 96h LC50 of the pharmaceutical effluents was conducted according to the method described by Reddy (2018). Five test solutions (20%, 40%, 60%, 80% and 100% v/v) were prepared by diluting the effluents with distilled water while the sixth solution 0% served as a control, which contains tap water only. The test solution was changed on every alternate day to counter-balance the decreasing the effluents concentrations. The experiment was carried in triplicate for 12, 24, 48, 72 and 96 hours. Fish mortality due to the exposure to the pharmaceutical effluents was recorded up to 96 h at 24 h interval to obtain LC50 values of the effluents. The LC50 of the effluents was conducted using the probit analysis described by Finney (1971). Probit analysis was determined by fitting a regression equation through graphical interpolation. Logarithms of the effluents concentration was considered on the x-axis while percentage mortality on the y-axis. The point of intersection with the 50% survival line is regarded as LC$_{50}$ and was found to be 48.7%.

Observation of Behavioural Responses

During daily behavioural response examination, thirty (30) minutes observation was used out after each exposure. The behaviour assessment described by Nwani *et al.* (2013) was adopted. These includes: erratic swimming, skin discoloration, loss of equilibrium, mortality, mucous secretion and haemorrhage.

Sub-Acute Toxicity Test

The experimental fishes were challenged to four sublethal concentrations of 0.00%, 2.43% (5/100 LC50), 12.17% (25/100 LC50), 24.35% (50/100 LC50) and 36.52% (75/100 LC50) for 28days. During the exposure period, fresh effluent was added in every 48 hours to maintain the concentration level before the wastes are siphoned as adopted by Ezenwaji *et al.* (2013).

Haematological Studies

At the end of the exposure period of 28 days, haematological examination was carried out according to the procedure

described by Dahunsi and Oranusi (2013). Blood samples was collected from experimental and control fish with heparinized plastic syringe, fitted with 21gauge hypodermic needle from the liver, behind the anal fins and stored in labelled ethylene diamine tetra-acetic acid (EDTA) bottles. The blood samples collected was analysed for Packed Cell Volume (PCV), haemoglobin (Hb), Red Blood Cells (RBCs), White Blood Cells (WBCs), WBCs differential count for lymphocytes, monocytes, eosinophils and neutrophils. Red blood indices such as Mean Corpuscular Haemoglobin Concentration (MCHC), Mean Cell Volume (MCV) and Mean Corpuscular Haemoglobin (MCH) were calculated as follows as adopted by Nafiu and Ibrahim (2019).

$$MCHC\ (g/dl) = \frac{Hb\,(g/dl)\times 100}{Pcv\,(\%)}$$

$$MCH\ (pg/cell) = \frac{Hb\,(G/dl)\times 10}{RBC\ count\ in\ million\ mm-6}$$

$$MCV\ (fl/cell) = \frac{PCV\,(\%)\times 10}{RBC\ count\ in\ million\ mm-6}$$

Biochemical Analyses
At the end of the exposure period of 28 days, Blood sample for biochemical investigation was collected from the experimental and control fish groups by piercing caudal vein using Ethylenediamine tetra acetic acid (EDTA) as an anticoagulant. Plasma was obtained from the whole blood by the centrifugation at 5000 rpm for 15 min. The aspartate aminotransferase (AST), Alkaline phosphatase (ALP) and alanine aminotransferase (ALT) activities were determined at Biochemistry Laboratory, Bayero University Kano, using Randox kits as adopted by Ezenwaji *et al.* (2013).

Statistical Analysis
Statistical analysis was conducted using SPSS (Version 23.0). Data from the 3 replicates of the experiment was subjected to one-way Analysis of Variances of (ANOVA). Treatment means was separated using DMRT at 5% probability level. Probability level of less than 5% ($p< 0.05$) was considered significant.

RESULTS
Physicochemical and Heavy metals parameters
The mean values of physicochemical parameters of the of pharmaceutical effluents revealed that pH had 9.6, Electrical conductivity (1879μS/cm), Dissolved Oxygen (3.4mg/L), Biochemical Oxygen Demand (4.7mg/L), Total Dissolved Solids (675.3mg/L), water temperature (28.4°C) and turbidity (27.4). The values recorded above with exception of pH and water temperature were within the FEPA standard limits for irrigation waters (Table 1). The results of heavy metals concentrations revealed that Cadmium had 0.001mg/L, followed by Chromium with 2.76mg/L, Pb (1.88mg/L) and Cu (1.68 mg/L).The values recorded above with the exception of Chromium and Lead was within the maximum limit set by FEPA for surface fresh waters (Table 2). The heavy metal concentrations in of pharmaceutical effluents decreased in the order of Cr > Cu >Pb > Cd.

Table 1: Mean Values of Physicochemical Parameters of Pharmaceutical Effluents obtained from Bompai Industrial Area, Kano State, Nigeria

Sampling Sites	Effluent	Control	Standard limits
Water temperature (°C)	28.4±0.23	26.7±1.56	<40°Cmg/L*
DO (mg/L)	3.4±0.02	6.4±0.04	5.0-9.0mg/L**
BOD (mg/L)	4.7±1.01	3.1±0.12	3.0-6.0mg/L**
TDS (mg/L)	675.3±2.66	197.9±1.56	<500mg/L***
Electrical Conductivity (μS/cm)	1879±1.09	210.4±0.67	<1000 μ/Scm**
Turbidity (NTU)	27.4±0.01	27.6±1.12	<25 NTU***
pH	9.6±0.78	7.7±0.16	6.0-9.0*

*FEPA (1991), **FME (2001), ***WHO (2011)

Table 2: Mean Values of Heavy metals of Pharmaceutical Effluents obtained from Bompai Industrial Area, Kano State, Nigeria

Parameters	Effluent	control	WHO (2011)
Cu (mg/L)	1.68±0.16[a]	ND	0.05
Pb (mg/L)	1.88±0.01[a]	ND	0.10
Cd (mg/L)	0.001±0.10[a]	ND	0.01

Cr (mg/L)	2.76±0.00[a] ND 0.05		

ND: not detected

Behavioural Responses in the Experimental fish

With regards to the physical and behavioural changes at the beginning of the treatment, the fish exposed to the pharmaceutical effluents revealed erratic swimming, loss of reflex, hyperventilation, in consistent jumping and discolouration with increase in the concentrations of the effluent (Table 3).

Table 3: Behavioral Responses Examined on *Clarias gariepinus* during 96h Exposure to Pharmaceutical Effluent

Exposure time in Hours	Concentration (%)	behaviour erratic swimming	Loss of reflex	Hyperventilation	Inconsistent jumping	discolouration
	0.00	-	-	-	-	-
	2.43	-	-	-	-	-
24	12.17	-	-	+	-	-
	24.35	-	-	+	-	-
	36.52	+	+	+	+	-
	0.00	-	-	-	-	-
	2.43	-	-	-	-	-
48	12.17	-	-	-	-	-
	24.35	-	-	+	+	-
	36.52	+	+	+	+	-
	0.00	-	-	-	-	-
	2.43	-	-	-	-	-
72	12.17	-	-	-	-	-
	24.35	-	+	+	+	-
	36.52	+	+	+	+	-
	0.00	-	-	-	-	-
	2.43	-	-	-	+	-
96	12.17	-	-	+	+	-
	24.35	+	+	+	+	-
	36.52	+	+	+	+	-

Key: - = absent   + = Present

Haematological indices

Sub-lethal Effect of pharmaceutical effluents on haematological indices is presented in Table 4. Packed cell volume (PCV) and haemoglobin (Hb) revealed no significant difference (p>0.05) between the all treatments and control, with exception of the highest concentrations of 36.52% in which significant difference (p< 0.05) was recorded. Control sample had significant higher mean PCV value of 33.01% while the lowest mean value of 18.41% was obtained from fish challenged with 36.52% of the effluents. The haemoglobin (Hb) showed no significant difference (p> 0.05) between the control and treated fishes at all concentrations with the exception of 36.52% treatment which differed significantly (p<0.05) with the control. The control had the highest mean value of 12.70g/dl while at 36.52% the haemoglobin value declined to 7.42g/dl. Furthermore, the RBCs count of the experimental fish reduced significantly (p<0.05) from 38.01 ± 0.60^6/mm^3 in control samples compared to the highest concentration of 36.52% pharmaceutical effluents in which $12.45\pm0.91 0^6$ /mm^3 was recorded. Findings of toxic effect of pharmaceutical effluents on WBCs count revealed no significant difference (p> 0.05) among all the challenged and control group. Control group had highest mean count of $32.73\pm0.51 \times10^3$ mm^3, while the experimental fish challenged with 36.52% had the highest mean count of $57.20\pm0.72\times10^3$ mm^3.

During the study period, the mean values of MCV, MCH and MCHC of the experimental fish increased significantly (P<0.05) when exposed to different concentrations of the effluents. Lowest value of MCH (16.70±1.15 pg), MCV (33.61±1.10 fl) and MCHC (24.50±0.30 g/dl) was recorded in

the control samples while the highest mean value was recorded at 36.52% with 29.59±1.56 pg, 67.81±0.47 fl and 39.01±1.09 g/dl respectively. The lymphocytes and monocytes revealed no significant difference (P> 0.05) between the exposed and control fish, although their values decrease with increase in the effluent concentrations (Table 4). The control had the highest mean value of 54.70±0.33% and 13.56% for the lymphocytes

and monocytes while at the highest effluent concentrations of 36.52%, 34.34% and 8.43% were recorded respectively.

No significant differences (p>0.05) examined in all the challenged and control fish in the neutrophils and eosinophil indices. They increase with increase in the effluent concentrations (Table 4).

Table 4. Effect of Pharmaceutical Effluents on Haematological Indices in *C. gariepinus* after Twenty eight days of Exposure

Parameters	0.00(control)	2.43%	12.17%	24.35%	36.52%
PCV (%)	33.01±0.15[a]	22.11±0.86[a]	21.09±0.11[a]	19.10±1.91[a]	18.42±0.22[c]
RBC (10^6/mm^3)	38.01±1.10[a]	34.01±0.21[a]	23.21±1.11[a]	16.10±1.44[a]	12.45±1.01[a]
WBC (10^3/mm^3)	32.73±0.51[a]	60.37±1.78[b]	61.88±3.28[b]	63.72±3.19[b]	57.20±0.72[b]
Hb (g/dl)	12.70±0.10[a]	7.10±0.35[a]	6.77±0.41[a]	6.09±0.67[a]	7.42±0.21[a]
Lymphocytes (%)	54.70±0.51[c]	42.67±0.21[a]	40.90±1.28[a]	35.19±0.01[a]	34.34±0.81[a]
Neutrophil (%)	18.33±0.33[a]	20.77±1.11[b]	23.34±0.78[b]	24.05±0.56[b]	20.77±1.11[b]
Monocytes (%)	13.56±1.81[a]	10.45±0.10[a]	9.89±1.71[a]	9.71±0.10[a]	8.43±0.10[a]
Eosinophil (%)	4.09±0.18[a]	4.39±0.10[a]	5.01±1.00[a]	6.45±0.72[a]	9.71±0.11[a]
MCV (fl)	30.05±0.32[a]	33.61±1.10[a]	38.10±0.10[a]	45.71±0.34[a]	67.81±0.47[a]
MCH (pg)	11.03±1.00[a]	16.70±1.15[a]	19.01±0.11[a]	24.17±1.01[a]	29.59±1.56[b]
MCHC (g/dl)	26.21±0.06[a]	24.50±0.30[a]	37.19±1.18[b]	39.00±0.33[a]	39.01±1.09[a]

Means with the different superscript in a row differ significantly (P<0.05)

Biochemical Analysis

Results of toxic effects of pharmaceutical effluents on serum biochemical parameters on experimental fish revealed significant differences (p<0.05) (Table 5). During the study period, the mean serum AST values of the experimental fish increased at 28 days when challenged to pharmaceutical effluents. Lowest activity was obtained in the control group with 34.20±1.01U/L and the highest of 46.72 ± 0.83U/L was obtained at 36.52%. The AST activity between control and treatment did not differ significantly (P>0.05).

The activity of ALT was highest (55.27±0.42U/L) in experimental fish challenged with 36.52% concentration (Table 2). The lowest values were observed at Group 2.43%, 12.17% and 24.35% which were 45.10 ± 1.01U/L, 45.57 ± 1.86U/L and 51.13 ± 1.42U/L respectively as against the control value of 41.18 ±0.67U/L. Statistically there was no significant difference between the groups (P>0.05).

The alkaline phosphatase (ALP) results on experimental fish revealed significant differences (p<0.05) between the treatments 2.43%, 12.17%, 24.35%, 36.52% when compared with the control. No significant differences (p>0.05) recorded between the control and the pharmaceutical effluents treatment. Although, 36.52% treated fish recorded the highest mean value of 30.89±0.57 u/L while 2.43% pharmaceutical effluents recorded the lowest of 23.91 ± 0.67u/L.

Table 5: Effect of Pharmaceutical Effluents on biochemical Indices in *C. gariepinus* after Twenty eight days of Exposure

pharmaceutical effluents (%)	AST (IU/L)	ALP (IU/L)	ALT (IU/L)
0.00	34.20±1.01 [a]	19.30 ± 1.48[a]	41.18 ±0.67[a]
2.43	35.12 ±1.10[a]	23.91 ± 0.67[a]	45.10 ± 1.01[b]
12.17	37.20 ± 1.61[b]	25.60 ± 0.91[a]	45.57 ± 1.86[b]
24.35	39.51 ± 1.01[a]	27.17 ± 0.82[b]	51.13 ± 1.42[a]
36.52	46.72 ± 0.83[a]	30.89±0.57 [a]	55.27±0.49[a]

DISCUSSION

Physicochemical analysis of the pharmaceutical effluents revealed that the pH value recorded of 8.6 is within the limit set by APHA (2005) for surface fresh water which can support productivity of aquatic organisms such as fish (Ado *et al*., 2014). The electrical conductivity recorded during the study period is beyond the maximum discharge limit of $1000\mu S/cm$ set by FME (2001). Dissolved oxygen recorded in the pharmaceutical effluents sample is below the standard limit of 5mg/l which cannot satisfactorily support aquatic life as reported by Ademola *et al*. (2017). The turbidity of the pharmaceutical effluent exceeded WHO (2002) permissible limit of 25NTU for surface fresh water bodies. The high turbidity recorded indicates that the effluent contains some dissolved substances that can pose problems to aquatic biota (Ibrahim, 2009). The high TDS recorded could be due to the dissolved salts in the effluent as reported by (Ibrahim, 2009). From the concentrations of the heavy metals concentrations recorded Chromium had the highest value of 2.76mg/L while Cd had the lowest value of 0.001mg/L. Heavy metals concentrations in the effluents decreased in the order of Cr> Cu> Pb> Cd. The high mean concentration of Cu and Cr obtained in this finding is in consistent with the work of Samson (2015). Ishaq *et al*. (2011) reported that the harmful effect of Copper is mainly due to its cupric Cu^{2+} form which is commonly found in the water sediment. Pb has been reported to cause toxicological implication in aquatic biota (Samson, 2015). In other vertebrates for instance, Galadima and Garba (2012) reported lead poisoning that resulted in learning disabilities, behavioural problem, kidney damage and poisoning of several vital enzyme in the central nervous system in children exposed to lead oxide in Zamfara State. The concentrations of Cr and Cu recorded were higher than the permissible limits and the peculiar problems attributed to heavy metals in an aquatic ecosystem is their accumulation via food chain with long term effect (Ibrahim and Nafiu, 2017).

During the 96h of exposure, the experimental fish displayed various stressful anomalies such as inconsistent jumping, hyperventilation, general body weakness, loss of reflex, erratic swimming which were noticeable particularly among fishes exposed to the highest concentrations of the effluents. These behavioral changes are in response to toxicant present in the effluents as opined by Rakesh and Kumar (2019). Hyperventilation examined indicates respiratory impairment, resulted from the effect of the pharmaceutical effluents on the gills of the experimental fish as observed by Roopadevi and Somashekar (2012). General body weakness, loss of reflex and eventual 100% mortality at 96hr occur at higher effluents concentrations. This might be attributed to the depletion of oxygen in the body of the experimental fish which led to the disruption of carbohydrate metabolisms and fishes that could not tolerate the effluents become unconscious and subsequent

death as observed by Barboza *et al*. (2018). The 96h LC_{50} value of the pharmaceutical effluents recorded in the present finding was 48.7% which depicted that the effluents are toxic to fish as reported by Agunbiace and Moodley (2016). Mortality recorded was dose and concentration-dependent as obtained in previous findings by Akan *et al*. (2009) in tannery and textile effluents, Alimba *et al.* (2015) in abattoir effluents (96 h LC50 = 6.28 %). Sublethal concentrations of the effluents may not cause immediate death of the aquatic biota, but the bioaccumulation of these pollutants for long period of time could have potential health impact not only to the aquatic biota such as fish but to organisms at higher trophic level including man as reported by Safahieh *et al.* (2012).

Decrease in red blood cells, Haemoglobin concentrations and PCV value recorded in the exposed fish (*C. gariepinus*) is an indicator of anaemic condition in the exposed fish. The decrease in RBCs count could be attributed to the deleterious effects of the effluents component on the fish's hematopoietic system by inhibiting erythropoiesis through transferrin dysfunction as reported by Ishaq *et al.* (2011). The decrease in RBCs count could be attributed to the iron synthesis disruption or due to the inhibitory effect of some toxic chemicals in the pharmaceutical effluents on the enzyme system responsible for haemoglobin synthesis (George *et al.,* 2017). The significant decrease in PCV and Hb with increasing concentration and exposure time of experimental fish indicates haemolysis of the erythrocytes due to impaired osmoregulation as a result of reduction in dissolved oxygen concentrations within the fish's respiratory system. This agrees with findings of Akhila *et al.* (2007). A decrease in RBCs count, Hb and PCV has been examined in previous findings by Al-asgah *et al.* (2015) and Alimba *et al.* (2016) in *O. niloticus* and *C. gariepinus* respectively. George *et al.* (2017) opined that the significant decrease in PCV may be associated to disruption in osmoregulation thereby causing anaemia and haemodilution**.** In most vertebrates, including fishes, erythropoietic activity is controlled by erythropoietin produced in the kidney which further helps erythropoiesis by inducing haemopoietic stem cells to distinguish into erythroblasts which form RBCs (John *et al.,* 2014).

White blood cells (WBC) confer defense against infectious agent caused by toxicants and other microbial agents (Reddy, 2018). In the present finding increase in WBC count with increase in pharmaceutical effluents concentrations recorded may be attributed to the activation of the experimental fish's defense mechanism and the immune system as reported by Alimba *et al.* (2019). WBC count contribute largely in the control of immunological function in an organisms, therefore, alterations in the WBC counts after exposure to the effluents might cause reduction in non-specific immunity of the experimental fish or protective response in animal under environmental stress as reported by Adewumi *et al.* (2018).

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Increased WBC count in experimental fish challenged with sub-lethal concentrations revealed leukocytosis that is associated with leucocytic response in organisms under stress as reported by Dahunsi and Oranusi (2013). Frequent release of lymphocytes under toxicant stress could lead to elevation in WBC count as observed Akinrotimi *et al.* (2012) on exposure of *Clarias gariepinus* to Cypermethrin. Increase in WBC count obtained in the present research is attributed to excitation of the defense mechanism by the exposed fish to withstand the impact of the effluents. This is in tandem with work of Shaheen and Akhtar (2012) and Reddy (2013).

Increase in MCV, MCH and MCHC observed in the effluents exposed fish, although not statistically significant is an indicator of macrocytic hyperchromic anemic condition induced by the effluents as reported by (Reddy, 2018). Macrocytic hyperchromic anemia has been associated with defects in DNA synthesis during erythropoiesis (Alimba *et al.,* 2017). The erythrocyte indices such as MCV, MCH and MCHC has been reported to alter in the homeostatic system of fish physiology (Adeogun and Chukwuka, 2012). Fluctuations of these indices directly correspond with the values of RBC count, Hb concentration, and PCV. However, slight fluctuations were recorded in the MCH and MCV as compared to the control. It was observed from the present finding that the WBCs differential count, lymphocytes and monocytes reduced significantly with an increase in the pharmaceutical effluents. There was also an increase in the neutrophils and eosinophils count compared to the control value. The decrease in lymphocytes and monocytes could be due to interactive effect of the pharmaceutical effluents components on the metabolic and hemotopoietic activities in the fish tissues or due to the kind of the challenging immune system in which the experimental fish was exposed to as reported by Adewumi *et al.*. (2018). The decrease in lymphocytes and monocytes might be attributed to toxicants present in the effluents that facilitate the detoxification pathways by increasing the activity of some antioxidant enzymes such as CAT, GSH and SGT which could trigger cellular oxidative damage and may lead to decrease in lymphocytes and monocytes as observed by Alimba *et al.* (2019) during exposure of *C. gariepinus* to effluents from pharmaceutical industry. Cellular oxidative disruption occur when antioxidant and detoxifying processes is insufficient in an organisms (Adewumi *et al.,* 2018). An increase in the neutrophils and eosinophils count examined may be associated with detoxifying mechanism against the effluents induced toxicity in the blood stream (George *et al.*, 2017). Decrease in lymphocytes and increase in neutrophils has been reported by Akinrotimi *et al.* (2012) during exposure of *C. gariepinus* juveniles to cypermethrin.

With regards to biochemical analysis, serum ALT, ALP and ALT enzyme activities in organism have been used as biomakers of toxicants contaminating the aquatic ecosystem

(Ezenwaji *et al.,* 2013). The present finding showed that experimental fish treated with varying sub lethal concentrations of the pharmaceutical effluents were significantly elevated compared with control group. The increased in the ALT, ALP and AST activity could be attributed to the disruption of cellular permeability and hepatocytes as reported by Kavya *et al.* (2016).The large quantity of AST and ALT released in the blood plasma of *C. gariepinus* could serve as a counter mechanism against the effluents induced toxicity. Similar observation was reported by Reddy (2018) in his study on the toxic effects of waste water in cat fish *Heteropneutus fossilis*. The high level of ALT, ALP and AST might indicates disrupted liver metabolism or cellular injuries in various tissues of the exposed fish. This observation is consistent with findings of Ajaz (2015) in *Clarias batrachus* exposed to parathion. The present finding revealed that some toxic substances present in the pharmaceutical effluents might have facilitate the detoxification processes by elavating the activity of ALT and AST enzymes which could results to the of lipid peroxidation in the exposed cells / or tissues (Akinwande *et al.*, 2016). Elevation activity of ALT, AST and ALP might contribute to the cellular oxidative damage which occur when antioxidant and detoxifying processes is insufficient in the body as reported by Srivastava and Reddy (2017) .The elevated level of AST activity recorded in present finding based on dose – dependent manner might be due to myocardial infarction or liver disorder as reported by Ezenwaji *et al.* (2013). It might also be due to the presence of some substances that possibly interfere with catalytic interconversion of amino acids and α-ketoacids by amino group in ALT. Al-Asgah *et al.* (2015) recorded increased AST activity in *Oreochromis niloticus* exposed to various concentrations of cadmium chloride. The elevated activities of ALP, AST and ALT in the present study demonstrates disruption activities of liver or enhanced transamination as observed by Kavy *et al.* (2016). Increased ALP activity in the treatment group compared with control may be attributed with biliary obstruction of the experimental fish liver by effluents or due to primary hyperparathyroidism as reported by Shaheen and Akhtar (2012). Sreelekshmy *et al.* (2016) reported an increased in AST in fish tissue due to metabolic activity distortion caused by industrial effluents.

CONCLUSION AND RECOMMENDATIONS

It can be concluded that water quality parameters (physicochemical and heavy metals) of the effluents were higher than the recommended Standard for Surface Freshwaters. The effluents altered haematological and biochemical indices as it induces fluctuations from the control group of the fish under investigation (*C. gariepinus).* The adverse effects recorded are based on the dose-dependent manner. This indicates the constituents of pharmaceutical

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effluents as potential toxicant that are capable of altering blood cell indices and causing varying pathological effect in the fish's blood stream. The indiscriminate discharge of these effluents in the water body could be a threat to the immediate aquatic ecosystem and its biota. It is therefore recommended that appropriate regulatory bodies should come up with holistic approach on the aquatic pollution abatement. Continuous monitoring and promulgation of stringent pollution mitigation laws against illegal discharge of effluents into water bodies should be enforced, bearing in mind the negative impact to many non-target organisms.

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