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RESEARCH ARTICLE

Changes in behavior and hematological parameters of freshwater African catfish *Clarias gariepinus* (Burchell 1822) following sublethal exposure to chloramphenicol

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Abstract

The present study was aimed at evaluating the effects of different concentrations of the most commonly used fish antimicrobial drug, chloramphenicol (CAP), on the behavior and hematological parameters of *Clarias gariepinus*. Fish specimens were exposed to three (2.5, 5.0 and 10.0 mg L^{-1}) sublethal concentrations of CAP and a control. Abnormal behavioral changes were observed in fish exposed to higher concentration of CAP. Blood erythrocytes were sampled on days 1, 5, 10 and 15 postexposure to evaluate hematological parameters. Results showed concentration- and time-dependent significant increase in packed cell volume after day 5 of exposure ($p < 0.05$). Hemoglobin values also significantly decreased from day 5, whereas values of mean cellular volume significantly decreased throughout the experimental period ($p < 0.05$). A mixed trend was observed in the mean values of red blood cells, white blood cells, mean cellular hemoglobin and mean cellular hemoglobin concentration as well as neutrophils. Activities of lymphocytes were significantly increased in all CAP-treated fish during the exposure period, whereas no significant differences were observed in values of monocytes, eosinophils and basophils among the treatment groups and control. Consequently, precautions must be taken, especially when high concentrations of CAP are used in long-term treatments of *C. gariepinus* in aquaculture.

Keywords

Behavioral changes, chloramphenicol, *Clarias gariepinus*, hematology, sublethal toxicity

History

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Introduction

Chloramphenicol (CAP) is a phenicol compound and a broad-spectrum antibiotic effective against a wide variety of Gram-positive and -negative bacteria, including most anaerobic organisms (Kreutzmann, 1977). Because of resistance and safety concerns, it is no longer a first-line agent for any infection in developed nations, but in low-income countries, however, CAP is still widely used because it is inexpensive and readily available (Fergusoma et al., 2005; Ye et al., 2008). The most serious adverse effect associated with CAP treatment is bone marrow (BM) toxicity, which may occur in two distinct forms: BM suppression, which is a direct toxic effect of the drug and is usually reversible, and aplastic anemia (a form of anemia when the BM ceases to produce sufficient red and white blood cells [RBCs and WBCs]). CAP prevents protein chain elongation by inhibiting peptidyl transferase activity of the bacterial ribosome. It specifically binds to A2451 and A2452 residues in the 23 S ribosomal RNA of the 50 S ribosomal subunit, preventing peptide bond formation. Veterinary use of pharmaceuticals and other personal care products (PPCPs), such as nutraceuticals (bioactive food supplements) and other consumer chemicals, including fragrances and sun-screen agents, could result in diffuse dispersion or release into the aquatic environment comparable to, for instance, pesticides (Kümmerer, 2004). CAP is degraded by biological, chemical and photolytic means and undergoes oxidation, reduction and condensation reactions upon exposure to light in aqueous solution. Photochemical decomposition of CAP *in vitro* by ultraviolet-A light leads to the formation of *p*-nitrobenzaldehyde, *p*-nitrobenzoic acid and *p*-nitrosobenzoic acid (de Vries et al., 1994). The half-life of CAP in soil at 25 °C is 4.5 days; in pond water, the half-life is 10.3 days at 25 °C and pH 8 and 20.8 days at 37 °C and pH 6. The pharmaceutical drug, CAP, and other PPCPs have been detected from groundwater, river water, ocean water, sediments and soil, thus posing a risk to economically important nontarget species (Halling-Sørensen et al., 1998). Hirsh et al. (1999) reported on the detection of CAP in the effluent of one sewage treatment plant and in surface water in the United States at concentrations of 0.56 and 0.06 µg/L^{-1}, respectively. CAP was also detected in water (112.3 ng/L^{-1}) and sediment (0.196 mg/kg^{-1}) from a freshwater aquaculture pond in China (Lu et al., 2009). In Nigeria,

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CAP residue of 0.837 ± 0.165 ppm has been detected from fish in a farm at Ibadan, Southern Nigeria (Adetunji et al., 2012). Because fish are particularly sensitive to the contamination of their environment, the presence of PPCPs in the water may significantly alter their physiological, biochemical and hematological processes. The increase in aquaculture production, especially in intensive systems, has encouraged farmers to use antibiotics to prevent bacterial diseases in farmed fish. These antibacterial drugs have been reported to have several hazards and side effects, such as immune suppression, nephrotoxicity, decreased growth and increased incidence of resistant bacterial strains as well as residue problems (Tafalla et al., 1999). Oxytetracycline (OTC), for example, was reported to suppress the immune functions in *Cyprinus carpio* and *Oncorhynchus mykiss* (Grondel et al., 1987; Siwicki et al., 1989). OTC and oxonilic acid (AO) suppress total WBC in *Oncorhynchus mykiss* (Lundén et al., 1998). Similarly, flunequine had a mitogenic effect in eel, *Anguilla anguilla* (Van der Heijden et al., 1996). Kreutzmann (1977) observed that CAP and OTC administration resulted in erythropoietic perturbation in eel *A. anguilla*, through decreased number of erythroblasts and dysfunctional erythrocyte metabolism. Florfenicol, a fluorinated analog of CAP, was reported to suppress lymphocyte proliferation and antibody responses in rainbow trout (Lundén & Bylund 2000, 2002). Caipang et al. (2009) also reported that florfenicol modulated the immune response and antioxidant defense system in Atlantic cod and may, in turn, affect their ability to resist bacterial pathogens. Sulfamerazine depressed some hematological and immunological parameters in rainbow trout (Saglam & Yonar, 2008). Barros-Becker et al. (2012) reported that exposure of Zebra fish larvae to OTC elicited widespread inflammation process though the larvae and, in turn, showed improved regeneration capacity.

There is a paucity of scientific documentation on CAP toxic effects on the behavior and hematological parameters in most indigenous fish species of Africa. *Clarias gariepinus* was selected for the present study because it is indigenous to Africa and can be found in other tropical countries of the world. It is of commercial importance and an aquaculture candidate that can narrow the gap between demand and supply of animal protein in developing countries. The species is also an attractive model for ecotoxicological research because of its availability throughout the season, wide distribution in the environment and easy acclimatization to laboratory conditions. The present study was thus designed to investigate the effects of sublethal concentrations of CAP on behavior and hematological parameters, including RBCs, WBCs, hemoglobin (Hb), packed cell volume (PCV), mean cellular volume (MCV), mean cellular hemoglobin (MCH), mean cellular hemoglobin concentration (MCHC) and differential WBC counts, in freshwater fish *C. gariepinus* under sublethal exposure.

Methods

Experimental fish and chemicals

A total of 300 freshwater African catfish *C. gariepinus* (Teugels; family: Clariidae; order: Siluriformes) were collected from nearby ponds and lakes with the help of local fishermen. They were transported to our laboratory at the University of Nigeria Nsukka (Nsukka, Nigeria) and thereafter given prophylactic treatment by bathing twice for 2 minutes in 0.05% $KMnO_4$ to prevent any dermal infection. The fish had mean (±standard deviation) wet weight and length of 16.42 ± 1.74 g and 29.43 ± 3.42 cm, respectively. They were acclimatized in three (300-L) plastic tank capacitites for 3 weeks using nonchlorinated tap water distributed in the University of Nigeria Nsukka. The fish were fed on 35% crude protein diet at 3% body weight daily at 7:00 a.m. Tanks were aerated continuously using an air pump to prevent dissolved oxygen (DO) depletion. The experiment was carried out in an indoor experimental system under the natural photoperiod of a 14:10 light-dark cycle prevalent at Nsukka (Nigeria) in the months of June to July when the experiment was conducted. The fish were treated in accord with the rules conforming to principles of laboratory animal care as set by the institutional animal care committee of the University of Nigeria Nsukka. The behavioral responses of fish were observed daily, and the control group showed normal behavior during the experimental period. The time at which the fish loses its sense of balance and displayed upside-down movement, faster opercular activity, surfacing and gulping of air, erratic swimming, hyperactivity and staying motionless on the bottom of the aquarium was noted. The physicochemical properties of the test water were analyzed daily using standard methods (APHA, AWWA, WPCE, 2005) and maintained within a normal range (water temperature, 25.60–26.40 °C; pH, 7.20–7.70; dissolved oxygen, 6.64–7.65 mg/L^{-1}, conductivity 250–305 μScm^{-1} and hardness in terms of $CaCO_3$ was 198–240 mg/L^{-1}, respectively). For the present study, commercial pharmaceutical preparations of CAP sodium succinate (99.9%; Imperial Drug Industries, Mumbai, India) containing 187.58 mg of CAP as the active ingredient were used as the stock solution.

In vivo exposure experiment

One hundred and eighty fish from the acclimatized batch were used for the *in vivo* experiment. The aquaria were aerated continuously to avoid DO depletion. The fish were not fed 24 hours before commencement of the exposure to CAP and throughout the duration of the experiment, as recommended by Ward & Parrish (1982) and Reish & Oshida (1987). The fish were randomly divided into four groups of 60 fish each without regard to sex. Each group was further randomized into three replicate experiments of 20 fish per replicate in 40-L glass aquaria ($60 \times 30 \times 30$ cm size). Fish in the first and second treatment groups were exposed to tap water only (control) and 2.5 mg/L^{-1} of CAP, respectively. The third and fourth groups were treated with 5.0 and 10.0 mg/L^{-1} CAP, respectively. The experiment was conducted in a static renewal bioassay system in which the water and CAP were changed daily to maintain constant CAP concentration. The test concentrations (2.5, 5.0 and 10.0 mg/L^{-1}) were prepared by dilution from the stock. The dose schedule selected was based on preliminary investigation involving a range of doses and from a previous report of CAP in fishes (Kasagala & Pathiratne, 2008).

Blood collection and hematological analysis

Blood was collected on the first day (1 d) of exposure to CAP. Thereafter, blood was collected on the 5th (5 d), 10th (10 d) and 15th (15 d) days postexposure. On each sampling day, 5 fish from each replicate experiment was anesthetized with MS 222 to minimize stress. Blood was obtained by cardiac puncture using a hypodermic heparinized syringe. The collected blood sample was transferred into small vials, which were also previously rinsed with heparin. Every sampled fish was removed from the experimental system to avoid getting blood a second time from the same fish.

RBC counts were estimated using a Neubauer hemocytometer, as described by Rusia and Sood (1992). Briefly, 0.02 mL of blood was pipetted from the blood sample and added to 4 mL of the RBC diluting fluid (Toisson's solution) in a clean test tube to make a 1:200 dilution of the blood sample. The diluted blood sample was loaded onto a Neubauer counting chamber, and all RBCs in the five groups of 16 small squares in the central area of the Neubauer chamber was counted using a light microscope at 40× objective. The number of cells counted for each sample was multiplied by 10 000 to obtain the RBC count per microliter of blood. Hematocrit (PCV) was determined using the microhematocrit method of Nelson and Morris (1989), in which the capillary tubes were filled with blood and centrifuged for 5 minutes at $14\,000 \times g$ using a microhematocrit centrifuge (Hawkesley & Sons, Ltd, Lancing, UK) at room temperature. Soon after centrifuging, the hematocrit was read using the microhematocrit reader. The result was expressed as the percentage of whole blood. Hb determination was done using the cyanmethemoglobin method (Blaxhall & Daisley, 1973). Briefly, 0.02 mL of blood was mixed with 4 mL of Drabkin's solution. This was allowed to stand for 10 minutes for full color development to occur. Absorbance was read at 540 nm with a Unican spectrophotometer against the blank. For determination of leucocytes, 0.02 mL of blood was pipetted into a small test tube containing 0.38 mL of WBC diluting fluid (Turk's solution).to make a 1:20 dilution of the blood sample. The diluted sample was loaded on to the Neubauer counting chamber, and all cells on the four corner squares were counted using a light microscope at 10× objective. The total number of WBCs was calculated in $mm^3 \times 10^4$. While counting, the numbers of different types of leucocytes (neutrophils, monocytes, lymphocytes, eosinophils and basophils) in the blood smears were identified as described by Hibiya (1982) and Chinabut et al. (1991). The number of each type of leukocytes was calculated as a percentage. Erythrocyte indices, such as MCHC, MCH and MCV, were calculated from the results of RBC count, WBC count, Hb and PCV (Dacie & Lewis, 1984) according to standard formulae:

$$\text{MCHC (g/dl)} = \frac{\text{Hb (g/dl)}}{\text{PCV (\%)}} \times 100.$$

$$\text{MCH (pg/cell)} = \frac{\text{Hb (g/dl)} \times 10}{\text{RBC count in millions/mm}^3}.$$

$$\text{MCV (fl/cell)} = \frac{\text{PCV (\%)} \times 10}{\text{RBC count in millions/mm}^3}.$$

Statistical analysis

The data obtained were statistically analyzed by using SPSS 16.0 software (SPSS, Inc., Chicago, IL). Data were subjected to one-way analysis of variance and Duncan's multiple range tests to determine the significant difference at the 5% probability level. Results were expressed as means ± standard error.

Results

Behavioral changes in fish

Behavioral responses of fish were observed daily. The control and the group exposed to 2.5 mg/L^{-1} of CAP showed normal swimming behavior and natural coloration of skin during the experimental period. Abnormal behavioral changes, such as swimming near the water surface, loss of equilibrium, erratic swimming, circling movement, hyperactivity and staying motionless on the bottom of aquarium tank, were however observed in fish exposed to 5.0 mg/L^{-1} of CAP on day 15 and in fish exposed to 10.00 mg/L^{-1} of CAP 10 days after the start of the experiment. The observed clinical signs of the toxic effects of CAP on the fish were lightening in skin color of the body surface, erosion of fins and tails and increased mucus secretions from the whole body. No fish mortality was recorded in both the experiment and control groups throughout the experimental period.

Hematological parameters

Changes in hematological profiles, such as PCV, RBC, WBC, Hb, MCV, MCH and MCHC, are presented in Table 1. PCV was not significantly affected after CAP exposure on day 1, but it however induced both concentration- and time-dependent significant decrease ($p < 0.05$) in PCV from day 5 onward. No significant differences were observed in the RBC count among the treatment groups at different sampling intervals, except on day 15, where exposure to 10.00 mg/L^{-1} of CAP significantly reduced RBC counts ($p < 0.05$), when compared to the control. No significant difference in WBC counts was observed in fish specimen exposed to CAP on days 1 and 5. However, continued exposure of the fish to the drug resulted in significant increase in WBC counts ($p < 0.05$) in all tested concentrations on the 10th and 15th days. No significant change was observed in Hb values on day 1 postexposure, but there was a significant decrease in all fish groups exposed to CAP from day 5 onward, compared to the control ($p < 0.05$). There was both a concentration- and time-dependent significant decrease ($p < 0.05$) in MCV values in all treated groups, compared to the control, during the whole experimental period. MCH values were significantly reduced in 10.00 mg/L^{-1} of CAP on the 1st day and in 5.00 and 10.00 mg/L^{-1} on the 5th and 10th days of exposure, respectively, compared to the control ($p < 0.05$). Exposure to CAP on day 15 resulted in a significant decrease of MCH values at all tested concentrations ($p < 0.05$), compared to the control. No significant differences in MCHC were evidenced after exposure to CAP on days 1 and 5. Exposure to the drug, however, induced a significant increase in MCHC at 5.00- and 10.00-mg/L^{-1} concentrations on day 10 and at all concentrations on day 15. The effects of CAP to differential WBC counts are shown in Table 2. Neutrophil values were

Table 1. Effects of exposure to sublethal CAP concentrations on RBC parameters in *C. gariepinus*.

Parameter (s)	Concentration (mg/L)	Duration (days) 1	5	10	15
PCV (%)	Control	30.66 ± 2.08^{a1}	30.33 ± 1.52^{a1}	29.00 ± 2.00^{a1}	30.00 ± 2.00^{a1}
	2.5	30.00 ± 4.35^{a1}	21.00 ± 1.00^{b2}	16.67 ± 6.65^{b3}	15.12 ± 8.54^{b3}
	5	28.67 ± 1.15^{a1}	17.67 ± 3.21^{b2}	16.33 ± 5.85^{b2}	12.17 ± 5.92^{b3}
	10	20.61 ± 3.21^{b1}	16.67 ± 2.52^{b2}	12.67 ± 5.03^{b3}	10.00 ± 1.00^{c3}
RBC ($\times 10^6$ cells/mm^3)	Control	1.33 ± 1.33^{a1}	1.43 ± 1.73^{b1}	1.42 ± 3.22^{a1}	1.54 ± 2.84^{a1}
	2.5	1.16 ± 0.15^{a1}	1.12 ± 0.25^{a1}	1.11 ± 0.40^{a1}	1.08 ± 0.45^{a1}
	5	1.16 ± 0.57^{a2}	1.06 ± 0.11^{a1}	1.05 ± 0.20^{a1}	1.06 ± 0.20^{a1}
	10	1.13 ± 0.12^{a1}	1.04 ± 0.30^{a1}	1.03 ± 0.53^{a1}	0.91 ± 0.56^{b1}
WBC ($\times 10^4$ cells/mm^3)	Control	3.50 ± 1.59^{a1}	3.50 ± 1.50^{a1}	3.76 ± 3.76^{a1}	5.90 ± 5.90^{a1}
	2.5	3.52 ± 0.39^{a1}	3.56 ± 2.53^{a1}	6.70 ± 1.02^{b2}	7.88 ± 2.9^{b2}
	5	3.55 ± 0.25^{a1}	3.69 ± 2.76^{a1}	8.00 ± 0.90^{b2}	9.34 ± 0.45^{b2}
	10	3.59 ± 0.70^{a1}	3.72 ± 0.69^{a1}	8.34 ± 0.74^{b2}	11.93 ± 1.97^{c3}
Hb (g/dL)	Control	10.20 ± 0.72^{a1}	10.16 ± 0.35^{a1}	10.08 ± 1.00^{a1}	10.06 ± 1.85^{a1}
	2.5	10.14 ± 1.45^{a1}	6.96 ± 0.35^{b2}	6.30 ± 1.15^{b2}	5.58 ± 4.27^{b2}
	5	10.12 ± 1.01^{a1}	5.52 ± 1.08^{b2}	5.16 ± 1.69^{b2}	4.08 ± 1.70^{b2}
	10	10.10 ± 1.07^{a1}	5.07 ± 0.85^{b2}	4.33 ± 2.51^{b2}	2.00 ± 0.50^{c3}
MCV (fl/cell)	Control	185.60 ± 10.88^{a1}	183.46 ± 10.3^{a1}	182.09 ± 6.07^{a1}	180.81 ± 6.23^{a1}
	2.5	174.18 ± 10.92^{b1}	148.69 ± 8.60^{b2}	133.73 ± 6.41^{b3}	118.15 ± 2.06^{b4}
	5	163.05 ± 6.98^{c1}	135.17 ± 9.59^{c2}	130.92 ± 9.43^{b2}	114.25 ± 3.82^{b3}
	10	137.30 ± 4.95^{d1}	107.71 ± 4.19^{d2}	103.24 ± 1.03^{c2}	102.24 ± 2.24^{c3}
MCH (pg)	Control	49.95 ± 13.8^{a1}	49.90 ± 2.39^{a1}	45.04 ± 11.54^{a1}	47.41 ± 10.13^{a1}
	2.5	48.33 ± 2.40^{a1}	47.31 ± 6.05^{a1}	44.63 ± 9.06^{a1}	39.07 ± 11.03^{b2}
	5	48.18 ± 13.9^{a1}	38.24 ± 9.90^{b2}	35.46 ± 14.72^{b2}	28.67 ± 41.94^{c3}
	10	38.29 ± 1.63^{b1}	36.11 ± 35.0^{b1}	31.64 ± 33.29^{b2}	26.12 ± 15.6^{c3}
MCHC (g/dL)	Control	33.25 ± 0.03^{a1}	34.21 ± 1.14^{a1}	40.01 ± 3.89^{a1}	40.02 ± 10.11^{a1}
	2.5	33.33 ± 0.19^{a1}	35.17 ± 0.09^{a1}	43.63 ± 0.15^{a1}	50.10 ± 2.54^{b1}
	5	33.52 ± 2.9^{a1}	35.19 ± 1.95^{a1}	50.01 ± 2.03^{b1}	51.30 ± 2.11^{b1}
	10	34.26 ± 0.96^{a1}	35.88 ± 0.98^{a1}	50.02 ± 4.07^{b1}	53.40 ± 3.00^{b1}

Values with different alphabetic (lowercase) superscripts differ significantly ($p < 0.05$) between different concentrations within the same exposure duration. Values with different numeric superscripts differ significantly ($p < 0.05$) between different exposure periods within the same concentration. Results are expressed as mean ± standard error of the mean.

Table 2. Effects of exposure to sublethal CAP concentrations on differential WBC counts (percentage) in *C. gariepinus*.

Parameter (s)	Concentration (mg/L)	Duration (days) 1	5	10	15
Neutrophils	Control	18.66 ± 3.21^{a1}	18.68 ± 5.02^{a1}	19.33 ± 2.08^{a1}	20.33 ± 2.51^{a1}
	2.5	19.33 ± 1.52^{a1}	20.66 ± 12.2^{a1}	20.73 ± 2.08^{a1}	23.00 ± 2.64^{a1}
	5	19.37 ± 3.00^{a1}	22.66 ± 10.06^{a1}	25.33 ± 2.08^{a2}	26.33 ± 2.30^{b2}
	10	21.25 ± 4.16^{a1}	24.66 ± 11.37^{b1}	25.66 ± 2.51^{b2}	26.01 ± 2.00^{c2}
Lymphocytes	Control	52.33 ± 4.93^{a1}	53.11 ± 2.00^{a1}	55.33 ± 2.51^{a1}	54.00 ± 2.64^{a1}
	2.5	60.67 ± 2.30^{b1}	60.67 ± 13.3^{b1}	62.43 ± 2.08^{b1}	63.33 ± 2.08^{b1}
	5	61.00 ± 2.00^{b1}	65.33 ± 10.06^{b1}	67.66 ± 0.57^{b2}	67.01 ± 1.00^{c3}
	10	62.00 ± 3.46^{b1}	66.33 ± 10.06^{b2}	68.31 ± 5.77^{c3}	68.13 ± 4.16^{d4}
Monocytes	Control	0.30 ± 0.01^{a1}	0.30 ± 0.01^{a1}	0.33 ± 0.02^{a1}	0.34 ± 0.01^{a1}
	2.5	0.30 ± 0.01^{a1}	0.34 ± 0.02^{a1}	0.36 ± 0.08^{a1}	0.35 ± 0.05^{a1}
	5	0.41 ± 0.03^{a1}	0.54 ± 0.01^{a1}	0.53 ± 1.52^{a1}	0.50 ± 1.54^{a1}
	10	0.43 ± 0.33^{a1}	0.40 ± 0.00^{a1}	0.52 ± 0.57^{a1}	0.50 ± 0.00^{a1}
Eosinophils	Control	1.46 ± 1.73^{a1}	1.50 ± 0.04^{a1}	1.60 ± 0.06^{a1}	1.61 ± 0.57^{a1}
	2.5	2.00 ± 1.00^{a1}	2.16 ± 0.57^{a1}	2.50 ± 0.57^{a1}	2.54 ± 0.00^{a1}
	5	2.10 ± 1.54^{a1}	2.30 ± 0.00^{a1}	2.34 ± 0.00^{a1}	2.30 ± 0.00^{a1}
	10	2.30 ± 1.73^{a1}	2.40 ± 0.00^{a1}	1.10 ± 0.00^{a1}	1.80 ± 0.00^{a1}
Basophils	Control	1.30 ± 0.00^{a1}	1.33 ± 0.57^{a1}	1.33 ± 0.57^{a1}	1.35 ± 0.00^{a1}
	2.5	1.32 ± 0.00^{a1}	1.66 ± 1.54^{a1}	1.71 ± 0.00^{a1}	2.00 ± 0.00^{a1}
	5	1.34 ± 0.00^{a1}	1.60 ± 0.00^{a1}	1.90 ± 0.00^{a1}	1.90 ± 0.00^{a1}
	10	1.50 ± 0.00^{a1}	1.70 ± 0.00^{a1}	1.80 ± 0.00^{a1}	1.88 ± 0.00^{a1}

Values with different alphabetic (lowercase) superscripts differ significantly ($p < 0.05$) between different concentrations within the same exposure duration. Values with different numeric superscripts differ significantly ($p < 0.05$) between different exposure periods within the same concentration. Results are expressed as mean ± standard error of the mean.

significantly higher on the 5th and 10th days of exposure in the highest CAP treatment (10.00 mg/L^{-1}) group, when compared to the control ($p < 0.05$). The neutrophil count was, however, significantly higher in the entire CAP-tested concentrations on day 15, compared to the control ($p < 0.05$). Lymphocytes were the most dominant leucocyte species in the peripheral blood of *C. gariepinus* exposed to CAP. When compared to the control group, there was a significant increase in lymphocytes in the CAP-exposed fish ($p < 0.05$). There was no difference in the percentage number of

monocytes and basophils in the treatment group, when compared to the control. Also, eosinophils did not vary in the CAP-treated fish throughout the study period.

Discussion

The observed behavioral alterations in the CAP-exposed fish indicate internal disturbances of the body system and may be attributed to neurotoxic effects of the drug by acetylcholinesterase (AChE) inhibition, which removes the neurotransmitter, acetylcholine, and the subsequent interference with normal transmission in cholinergic synapses and neuromuscular junctions of the nervous system, thus affecting the normal functioning of nerves (Miron et al., 2005). Wang et al. (2009) reported that exposure of Tra catfish (*Pangasianodon hypophthalmus*) to enrofloxacin antibiotic severely depressed AChE activities in various organs of the fish. In fish, it has been observed that the inhibition of AChE was associated with disorders of respiration, swimming and feeding behavior (Payne et al., 1996).

Analysis of the hematological parameters in fish can provide important information about the physiological status of the fish and can be used as health indicators in the aquatic environment (El-Sayed et al., 2007). The progressive decrease in RBCs and Hb in *C. gariepinus* treated with CAP is an indication of an impaired erythropoietic process and even erythroblastopenia, which, in turn, disrupts the maturation of erythrocytes. This appears discernible in the significant reduction of the MCV in the CAP-treated fish, when compared to the control. Also, the reduction of Hb value in the treatment groups, when compared to the control, is an indication that the Hb biosynthetic process was adversely affected. This could limit the oxygen-carrying capacity of the fish blood. Earlier studies (Kasagala & Pathiratne, 2008; Kreutzmann, 1977) had shown that antibiotics cause damage to hemopoietic tissues. Thus the anemia observed in the present study, when compared to the control group, represents one of several adverse effects of CAP on hemopoietic processes and thus calls for close monitoring of peripheral blood cells of farm animals treated with antibiotics. Similar findings were reported in European eel (*A. anguilla*) exposed to CAP and OTC (Kreutzmann, 1977), *C. carpio* exposed to CAP (Kasagala & Pathiratne, 2008), *Oreochromis niloticus* exposed to praziquantel (El-Banna et al., 2008) and in *O. mykiss* exposed to verapamil (Li et al., 2011). There was significant increase in WBCs after day 5 and 10 exposure of the fish to CAP, respectively. Similar to the present finding, Saravanan et al. (2011) reported a significant increase in WBCs in *C. carpio* exposed to pharmaceutical drugs clofibric acid and diclofenac. Significant increase in WBCs has also been reported in *Cirrhinus mrigala* (Saravanan et al., 2012) after exposure to different concentrations of pharmaceutical drug ibuprofen. WBCs are involved in the regulation of immunological function in many organisms and the observed increase in WBC count in CAP-treated fish after some days of exposure indicates immune and protective response (Saravanan et al., 2011). The significant decrease in PCV after day 5 exposure to CAP may be the result of an appreciable decline in hematopoiesis. The hematological indices of the CAP-treated fish were adversely affected. Blaxhall & Daisley (1973) reported that mature erythrocytes have larger volume than immature ones. Decreased MCV shows that erythrocytes shrunk as a result of water imbalance, stress, microcytic anemia or the production of immature erythrocytes. The significant reduction in MCV in CAP-treated fish, when compared to the control, is an evidence of the production and subsequent release of immature erythrocytes into the general circulation to compensate for the adverse effects of CAP on the hemopoietic tissues of the fish. Exposure of *C. gariepinus* to CAP also resulted in a significant decrease in MCHC, except on day 15, and MCH only on day 15. The significant reduction in MCH and MCHC is a good indication of defective Hb biosynthesis in the fish. Similar decreases in MCV, MCH and MCHC have been reported in fish exposed to different concentrations of pharmaceutical drugs (Kasagala & Pathiratne, 2008; Li et al., 2010; Velisek et al., 2009). Changes in differential leukocyte count are recognized as a sensitive indicator of environmental stress and provide an overview of the integrity of the immune system (Cole et al., 2001). CAP treatment has a stimulatory effect on the lymphocyte whose number increased significantly in the peripheral blood of *C. gariepinus*. A similar increase was found in *O. mykiss* treated with sulphadiazine and trimethoprin mixture (Lundén & Bylund, 2002), with a concomitant rise in antibody titer. Lymphocytosis was also reported in *A. anguilla* treated with CAP (Kreutzmann, 1977). Antibiotics-induced lymphocytosis was found in rainbow trout, *O. mykiss* given oral administration of florfenicol (Lundén et al., 1999) and in *A. anguilla* treated with flumequine (Van der Heijden et al., 1996). On the contrary, lymphocytopenia was observed in *O. mykiss* and in *C. carpio* treated with OTC and AO (Lundén et al., 1998; Rijkers et al., 1980). Pharmacological studies (Grondel et al., 1987; Horsberg et al., 1994) have shown that metabolic products of CAP, florfenicol and OTC accumulate, bind to and affect kidney tissues in *Salmo salar* and *C. carpio*. In consequence thereof, the balance of immunocompetent cells and RBCs in the bloodstream is affected. The observed neutrophilia in *C. gariepinus* resulting from CAP treatment is in agreement with the increased neutrophil in zebra fish (*Danio rerio*) larvae treated with OTC (Barros-Becker et al., 2012). Apart from the significant increase in lymphocytes throughout the duration of the experiment and neutrophils on day 15, CAP had no significant effect on monocytes, eosinophils and basophils. Similar to the present finding, no significant difference in these leukocyte differentials have likewise been reported in fish exposed to various concentrations of toxicants (Mohammad et al., 2012; Nwani et al., 2012; Roy & Nath, 2012; Velisek et al., 2009).

In Nigeria, there is no published information on the level of CAP or any other pharmaceutical products in the aquatic ecosystem. This lack of monitoring data calls for systematic environmental monitoring programs for such products in our water bodies in view of the hazards and side effects associated with their excessive use.

Conclusion

In conclusion, the results of the present study indicate that sublethal exposure to the pharmaceutical drug, CAP, alters

behavioral and hematological parameters in *C. gariepinus*. The studied parameters could provide useful information for evaluating the physiological effects of CAP on *C. gariepinus*, but more-detailed laboratory study are needed before they can be established as special biomarkers for monitoring pharmaceuticals in the aquatic environment.

Acknowledgements

All experiments were carried out in accord with the guidelines for the care of experimental animals as approved by the ethical committee of the University of Nigeria (Nsukka, Nigeria).

Declaration of interest

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