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RESEARCH ARTICLE

Hematological, biochemical, and biometric changes in *Clarias gariepinus* exposed to antipsychotic drug chlorpromazine

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Abstract

Chlorpromazine (CPZ) is a neuroleptic and antipsychotic medication for individuals suffering from schizophrenia and other medical conditions. This study investigated the effects of CPZ on the hematological, biochemical, and biometric characteristics in juvenile *Clarias gariepinus*. The fish were exposed to 0.53, 1.06, and 2.11 mgL^{-1} CPZ for 15 days after which they were withdrawn from the toxicant and allowed to recover for 5 days. Blood were sampled from the fish on days 1, 5, 10, 15, and during the 5-day recovery for hematological and biochemical analysis, and thereafter, the fish were sacrificed for the morphometric analysis. While the values of the white blood cells significantly increased in the exposed fish, the hemoglobin, red blood cells, and packed cell volume decreased. Compared with the control, there were no significant differences in the values of the blood derivatives in the exposed fish. The values of protein and glucose reduced, but those of aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase were significantly elevated. Though there was no significant difference in the condition factor, a significant increase in hepatosomatic index occurred on day 15 at 5.28 mg/L CPZ. After the 5-day withdrawal from the drug, most of the studied parameters returned to the control values. The present study indicated that CPZ is toxic to fish and should be used with utmost care to guard against toxicological effect on non-target organisms.

Keywords Chlorpromazine · Behavior · Biochemistry · Hematology · Morphological parameters · Fish

Introduction

Chlorpromazine (CPZ) is a drug popularly used for the treatment of psychotic challenges in humans. It is also used in treatment of children with behavioral and attention disorders. The mechanism of action of the drug is the blockage of the dopamine receptors in the central nervous system such as the brain (Suzuki et al. 2013). This in turn lowers the accumulation of excess dopamine in the brain, thus leading to various degrees of suppression of the psychotic symptoms (Roy et al. 1984). The high level of pharmaceuticals and personal care products in the aquatic environments reported recently calls for concern due to the eco-toxicological effects on non-target organisms (Ebele et al. 2020). Studies indicate that CPZ was harmful to aquatic micro-algal species (Porsbring et al. 2008), *Daphnia magna* (Oleveira et al. 2016), and *Carassius auratus* (Li et al. 2008). In a related report, CPZ elevated brain acetylcholinesterase values, lipid peroxidation, and caused variations in the values of antioxidant enzymes in African catfish *Clarias gariepinus* (Atama et al. 2020). Further, pharmaceuticals and personal care product residues in the aquatic system can cause irreversible changes in the hematological, biochemical, and processes in the organisms (Nwani et al. 2014a). Chlorpromazine residues of about 5 to 364 ng L^{-1} was detected in effluents from waste water treatment plant in Beijing, China (Yuan et al. 2013), while concentrations up to 0.9 to 2.6 ng L^{-1} had been detected in surface water in Tago

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River Spain (Fernandez et al. 2010). Though the presence of CPZ in various water bodies has been reported, information regarding the hematological, biochemical, and biometric profile of *C. gariepinus* after exposure to CPZ is scarce. The African Catfish *C. gariepinus* is one the commercial indigenous fish species preferred due to its fast growth, nutritional quality, easy domestication, high reproductive potential, and adaptability to extreme water quality condition. The present study was aimed at determining the 96-h lethal concentration (LC_{50}) of CPZ and investigate whether sublethal CPZ concentrations can induce changes in hematological, biochemical, and biometric parameters in indigenous African Catfish *C. gariepinus* juveniles.

Materials and methods

Procurement of fish and drug

African catfish *C. gariepinus* juveniles ($n = 300$), mean length 30.13 ± 0.25 cm and weight 152.38 ± 2.24 g, were procured from Freedom Fish Farm Limited Nsukka and transported to the Fisheries Wet laboratory of our institution where they were acclimatized for 2 weeks in three concrete ponds ($3 \times 1 \times 1$ m^3) using non-chlorinated tap water. They were given 35% crude protein diet three times daily. The behavioral responses of the fish such as opercula movement, erratic swimming, air gulping, mucus secretions, and skin coloration were observed and recorded. Commercial pharmaceutical preparations of CPZ with the trade name Largactil (100 mg CPZ) manufactured by Oubari Pharmaceutical Ltd, Aleppo-Syria, under license from Aventis Laboratory-France was used.

Design for sublethal exposure

The 96-h median lethal concentration (LC_{50}) of CPZ in the present study was calculated to be 10.56 mg L^{-1} for *C. gariepinus* juveniles. Three sub-lethal CPZ concentrations, viz. 2.112 mgL^{-1} (1/5th of 96-h LC_{50}), 1.056 mgL^{-1} (1/10th of 96 h), and 0.528 mgL^{-1} (1/20th of 96-h LC_{50}), were selected for the in vivo experiment. The fish were divided into four treatment groups (groups I, II, III, and IV). Group I was exposed to 0.528 mgL^{-1} CPZ, group II to 2.11 mgL^{-1}, group III to 1.06 mg L^{-1}, and group IV which served as control to dechlorinated water only. The groups were set in glass aquaria ($60 \times 30 \times 30$ cm) in replicates of three, each containing ten fish. The test solution was renewed with fresh preparations of the test drug every 48 h to ensure that the concentrations remain the same. Prior to the renewal of the test solution, the water in each test aquarium was siphoned out completely and the aquarium cleaned to avoid contamination. The level of CPZ in the aquaria was analyzed using liquid chromatography–mass spectrometry (LC–MS) (Ajima et al. 2017) and did not significantly differ from the nominal concentrations. During the sublethal experiment, no mortality was observed during the 20-day exposure. Thereafter, the fish were withdrawn and kept in drug-free aquarium for another 5 days for possible recovery. The fish were fed daily throughout the experiment with about 3% of total body weight an hour before renewal of the test solution to avoid mortality. The physico-chemical parameters of the test water (APHA, AWWA, WPCE 2005) were dissolved oxygen 7.14 mgL^{-1}, temperature 27.83 °C, pH 12.3, and conductivity 247.5 μScm^{-1}.

Assessment of behavioral changes

Assessment of the behavioral changes in *C. gariepinus* exposed to CPZ was determined according to the method of Ogueji et al. (2019). Behavioral changes such as swimming rate, air gulping, and opercula movement were observed over 5–10 min and recorded. Also, alteration in skin color and abnormal mucus secretions in exposed fish were monitored under the same period.

Blood sample collection

Prior to blood collection, the fish were immobilized by anesthetizing with MS-222 (tricane methanesulfonate). Blood sample collection was made on the first day of exposure to CPZ and subsequently, on days 5, 10, 15, and 20 (i.e., 5-day recovery period). About 3.0 mL of blood was obtained by cardiac puncture using a hypodermic heparinized syringe and transferred into small EDTA bottle. Two fish from each replicate experiment and control were sampled on each sampling day. Every sampled fish was removed from the experimental system to avoid multiple blood collection from the same fish. One-half of the blood was used for estimation of the hematological parameters. The remaining half was centrifuged at 10,000 g at 4 °C for 20 min to separate the plasma which was used for the estimation of biochemical parameters.

Hematological assays

Red blood cell count was determined by the method of Ochei and Kolhatkar (2008). The blood specimen was diluted 1:200 with RBC diluting fluid and cells were counted under high power (×40) objective of a microscope by using a counting chamber. The number of cells was calculated and reported as the number of red cells/cu.mm of whole blood. The white blood cell count, PCV, and hemoglobin concentration (Hb) were determined following the method described by Ochei and Kolhatkar (2008). Erythrocyte indices, such as mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH), and mean

corpuscular volume (MCV), were calculated from the results of RBC count, Hb, and PCV according to standard formulas (Dacie and Lewis, 1984):

$$\text{MCV (fl)} = \frac{\text{PCV}(\%) \times 10}{\text{RBC count in millions/mm3}}$$

$$\text{MCH (pg)} = \frac{\text{Hb} \times \left(\frac{g}{dl}\right) \times 10}{\text{RBC count in millions/mm3}}$$

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

Estimation of biochemical indices

Randox diagnostic kits were used for biochemical tests. The activity of aspartate aminotransferase (AST) was assayed by the method of Reitman and Frankel (1957) as outlined in the Randox kit. Activities of alanine amino transferase (ALT) and alkaline phosphatase (ALP) were assayed as outlined in the kit. The protein and glucose level were determined using Randox Kit as described by Reitman and Frankel (1957).

Morphometric variations

During each sampling period, the standard lengths of the exposed fish and the control were measured to the nearest centimeters, while the weights were determined in grams using electronic weighing balance. The liver was excised and weight also measured. Following the methods of White and Fletcher (1985), the condition factor (CF) and hepatosomatic index (HSI) were calculated as

$$\text{CF} = \text{body weight(g)/standard length (cm)}^3 \times 100$$

$$\text{HSI} = \text{liver weight (g)/body weight (g)} \times 100$$

Statistical analysis

The statistical packages for social sciences (SPSS) version 23.0 (IBM Corporation, Armonk, NY) was used to analyze the data obtained. Two-way analysis of variance with CPZ concentration and exposure duration as fixed factors and biochemical and hematological parameters as dependent factors were used. Post hoc comparison was by LSD and p-values adjusted by the Bonferroni method. Values were presented as mean ± standard deviation, and level of significance set at $p < 0.05$. Effect size reported was partial eta squared (η_p^2).

Results

Changes in behavior in C. gariepinus exposed to CPZ

There were changes in behavioral responses such as loss of balance, skin coloration, abnormal mucus secretion, swimming rate, opercula movement, and air gulping during sublethal exposure and recovery of *C. gariepinus* to CPZ (Table 1). The skin coloration, mucus secretion, and air gulping increased with increased concentrations of CPZ, while swimming rate and opercula movement decreased. After 5-day recovery, the skin coloration, mucus secretion, and air gulping were reduced, while the swimming rate and opercula movement became stable (Table 2).

Hematological changes in C. gariepinus exposed to CPZ

Chlorpromazine exposure was associated with reduction in PCV, RBC, Hb, and increased WBC (Fig. 1). PCV ($F_{3,40} = 56.505$, $p < 0.0001$, $\eta_p^2 = 0.809$), RBC ($F_{3,40} = 76.935$, $p < 0.0001$, $\eta_p^2 = 0.852$), and Hb ($F_{3,40} = 129.704$, $p < 0.0001$, $\eta_p^2 = 0.907$) decreased significantly from the drug exposure. The decreases in all three parameters were duration dependent ($F_{4,40} = 19.188$, 52.279, 69.256, $p < 0.0001$, respectively), and most pronounced on day 15. Leucocytosis induced by the drug was significant ($F_{3,40} = 185.543$, $p < 0.0001$, $\eta_p^2 = 0.933$), and similarly duration dependent ($F_{3,40} = 124.674$, $p < 0.0001$, $\eta_p^2 = 0.926$), effect noticed from day 5 and continued until 5-day post withdrawal. The values of the neutrophil, basophil, eosinophil, lymphocytes, and monocytes were not affected by CPZ (Supplementary Table S1).

The hematological derivatives MCV, MCH, and MCHC did not show any obvious change due to exposure of *C. gariepinus* to CPZ (Supplementary Table S2). Change in MCV was significantly different overall between the groups ($F_{3,40} = 4.261$, $p = 0.011$, $\eta_p^2 = 0.242$), but the effect size was ~ 25.0% and cannot be said to be due to the drug. The source of the significant difference was not obvious from LSD

Table 1 Changes in behavior of *Clarias gariepinus* after 15-day exposure to sublethal concentrations of chlorpromazine and 5-day recovery

Exposure duration	Parameter	Chlorpromazine concentrations (mg/L)			
		Control	0.53	1.06	2.11
15 days	Skin coloration	–	++	++	+++
	Mucus secretion	–	++	++	+++
5-day recovery	Skin coloration	–	+	++	++
	Mucus secretion	–	+	+	++

Normal behavior (–, 0%), mild (+, < 10%), moderate (++, 10 to 50%), and high (+++, > 50)

Table 2 Effect of chlorpromazine on tail beat, opercula beat, and air gulping activities in *Clarias gariepinus*

Parameter (no/min)	Concentration (mg/L)	Duration of exposure (days)				
		1	5	10	15	5-day withdrawal
Tail beat	Control	12.05 ± 0.44^{a1}	12.46 ± 022^{a1}	13.11 ± 0.44^{a1}	12.71 ± 0.54^{a1}	12.55 ± 0.68^{a1}
	0.53	15.54 ± 0.34^{a1}	18.67 ± 0.51^{a2}	14.55 ± 0.60^{b1}	13.18 ± 0.80^{b1}	12.08 ± 0.45^{b1}
	1.06	18.22 ± 0.34^{a2}	20.44 ± 0.48^{a2}	13.64 ± 0.63^{b1}	12.27 ± 0.82^{b1}	11.35 ± 0.56^{b1}
	2.11	20.35 ± 0.40^{a2}	23.46 ± 0.45^{a2}	12.44 ± 0.33^{b1}	11.68 ± 0.72^{b1}	11.06 ± 0.66^{b1}
Opercula beat	Control	24.45 ± 1.10^{a1}	25.60 ± 1.12^{a1}	25.45 ± 0.20^{a1}	24.61 ± 0.40^{a1}	24.52 ± 0.24^{a1}
	0.53	35.46 ± 1.20^{a2}	36.09 ± 1.40^{a2}	34.29 ± 0.18^{a2}	33.23 ± 0.45^{a2}	28.35 ± 0.54^{a2}
	1.06	36.47 ± 1.23^{a2}	36.76 ± 1.30^{a2}	34.43 ± 0.39^{a2}	33.92 ± 0.69^{a2}	26.45 ± 0.55^{b1}
	2.11	37.15 ± 1.30^{a2}	38.25 ± 1.40^{a2}	34.17 ± 0.40^{b2}	33.56 ± 0.80^{b2}	26.82 ± 0.46^{b1}
Air gulping	Control	4.12 ± 0.12^{a1}	4.22 ± 0.33^{a1}	3.72 ± 0.18^{a1}	3.58 ± 0.08^{a1}	3.86 ± 0.07^{a1}
	0.53	6.42 ± 0.14^{a1}	7.86 ± 0.13^{a1}	6.43 ± 0.12^{a2}	5.08 ± 0.07^{a1}	3.54 ± 0.05^{a1}
	1.06	7.44 ± 0.24^{a1}	8.22 ± 0.14^{a2}	6.33 ± 0.15^{a2}	4.36 ± 0.11^{a1}	3.34 ± 0.10^{a1}
	2.11	7.55 ± 0.48^{a1}	9.14 ± 0.09^{a2}	6.41 ± 0.22^{a2}	3.25 ± 0.10^{a1}	3.46 ± 0.05^{a1}

Values with different numeric superscripts along a column for each parameter were significantly different, while values with different alphabetic superscripts across a row were significantly different ($p < 0.05$)

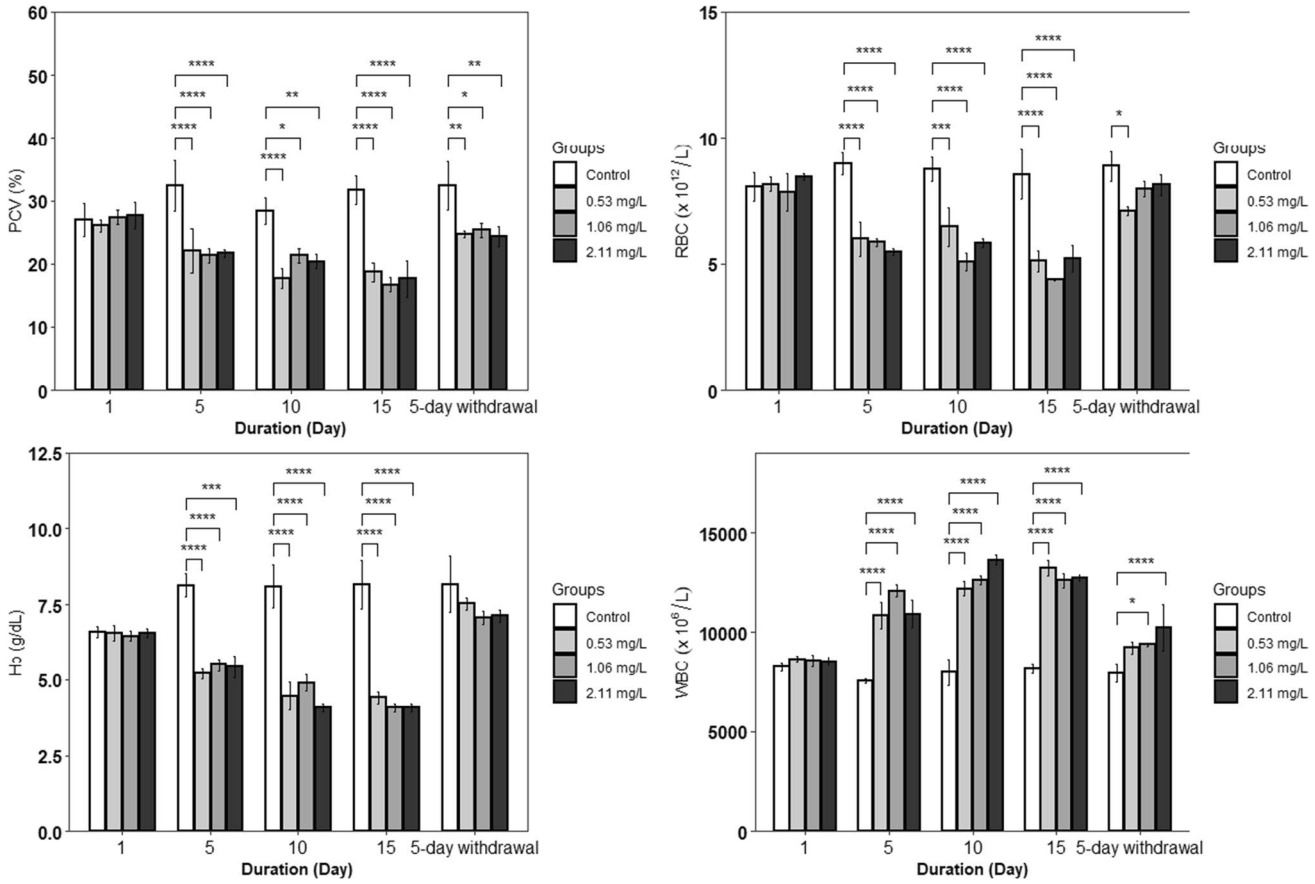

Fig. 1 Changes in packed cell volume (PCV), red blood cell count (RBC), hemoglobin concentration (Hb), and white blood cell count (WBC) on 15-day exposure of juvenile *Clarias gariepinus* to CBZ. Significantly different at $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$, $^{****}p < 0.0001$

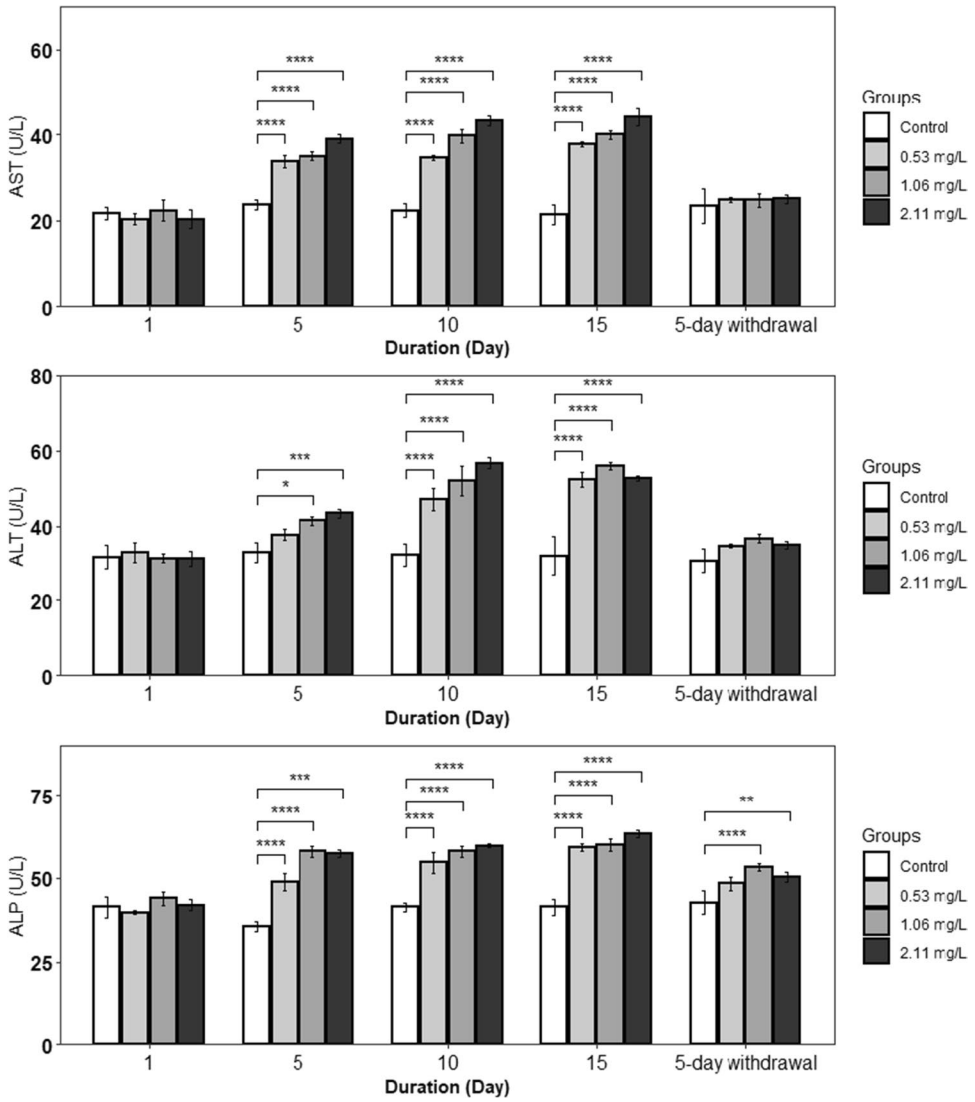

Fig. 2 Changes in blood aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) on 15-day exposure of juvenile *Clarias gariepinus* to CBZ. Significantly different at $*p<0.05$, $**p<0.01$, $***p<0.001$, and $****p<0.0001$

before and after Bonferroni adjustment. Similarly, the source of a weak significant difference between the MCH of the groups ($F_{3,40}=5.127$, $p=0.004$, $\eta_p^2=0.278$) was not obvious from multiple comparisons. The values of MCHC also did not differ in the control and the exposed groups.

Biochemical changes in C. gariepinus exposed to CPZ

The activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) were significantly affected by the 15-day exposure of *C. gariepinus* to CPZ (Fig. 2). AST (concentration: $F_{3,40}=141.934$, $p<0.0001$, $\eta_p^2=0.914$; duration: $F_{4,40}=184.371$, $p<0.0001$, $\eta_p^2=0.949$), ALT (concentration: $F_{3,40}=78.081$, $p<0.0001$, $\eta_p^2=0.854$; duration: $F_{3,40}=110.624$, $p<0.0001$, $\eta_p^2=0.917$), and ALP (concentration: $F_{3,40}=176.100$, $p<0.0001$, $\eta_p^2=0.930$; duration: $F_{4,40}=93.178$, $p<0.0001$, $\eta_p^2=0.903$) activities were increased significantly with increasing CPZ concentration and exposure duration. The effect of CPZ on all three parameters was significantly enhanced by prolongation of exposure ($p<0.0001$, $\eta_p^2\sim0.800$). The effect of CPZ on AST, ALT, and ALP activity waned on 5-day withdrawal of the fish from the drug.

Exposure of *C. gariepinus* to CPZ resulted in reduction of protein in a duration-dependent manner (Fig. 3). Significant reduction in serum protein occurred on days 5, 10, and 15 of exposure in all drug concentrations ($F_{3,40}=33.779$, $p<0.0001$, $\eta_p^2=0.717$). The magnitude of hyperproteinemia associated with the drug exposure was similar from days 5 to 15, and normalcy returned on withdrawal of the fish from the drug. CPZ exposure was associated with significant reduction in serum glucose concentration ($F_{3,40}=109.525$, $p<0.0001$, $\eta_p^2=0.891$). Though the glucose concentration was significantly elevated during the exposure period ($F_{4,40}=58.776$, $p<0.0001$, $\eta_p^2=0.855$), the concentration in exposed fish was not different

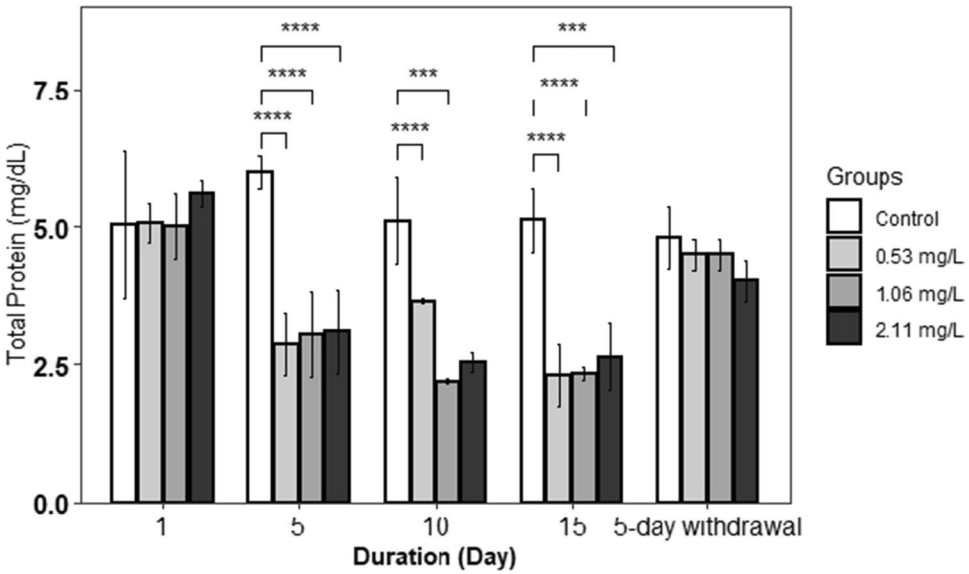

Fig. 3 Changes in serum total protein levels in *Clarias gariepinus* after 15-day exposure to chlorpromazine and 5-day recovery. Bars with different alphabet labels were significantly different for a given day, while bars with different numeric labels were significantly different between durations for a given chlorpromazine concentration. Significantly different at ***$p < 0.001$ and ****$p < 0.0001$

between day 5 and day 15. Glucose concentration returned to same level as control on 5-day withdrawal of the fish (Fig. 4).

Changes in morphological parameters

There were changes in CF and HSI in the fish exposed to CPZ. Although there were concentration- and duration-dependent variations in the CF, the LSD test showed no significant differences ($p > 0.05$). HSI of the fish were different between the groups ($F_{3,40} = 4.956$, $p = 0.005$, $\eta_p^2 = 0.271$), though the effect size was 27.1%. A significant increase in HSI occurred on day 15 in fish at 5.28 mg/L CPZ and explained the significant interaction effect ($F_{12,40} = 3.813$, $p = 0.001$, $\eta_p^2 = 0.534$ (Fig. 5).

Discussion

Pharmaceuticals are among the emerging contaminants used in human and veterinary medicine that are currently detected in the environment (Ebele et al. 2020). There is palpable concern over their presence in the environment due to their effects on non-target organisms like fish. Though our tested CPZ concentrations exceeded the maximum residue limit of 2.6 ngL^{-1} detected in fresh water system in Tajo Spain (Fernandez et al. 2010) but in view of its increasing use and indiscriminate disposal of hospital and other related pharmaceutical wastes in Nigeria and many developing countries, the concentrations of CPZ in the aquatic bodies may be higher,

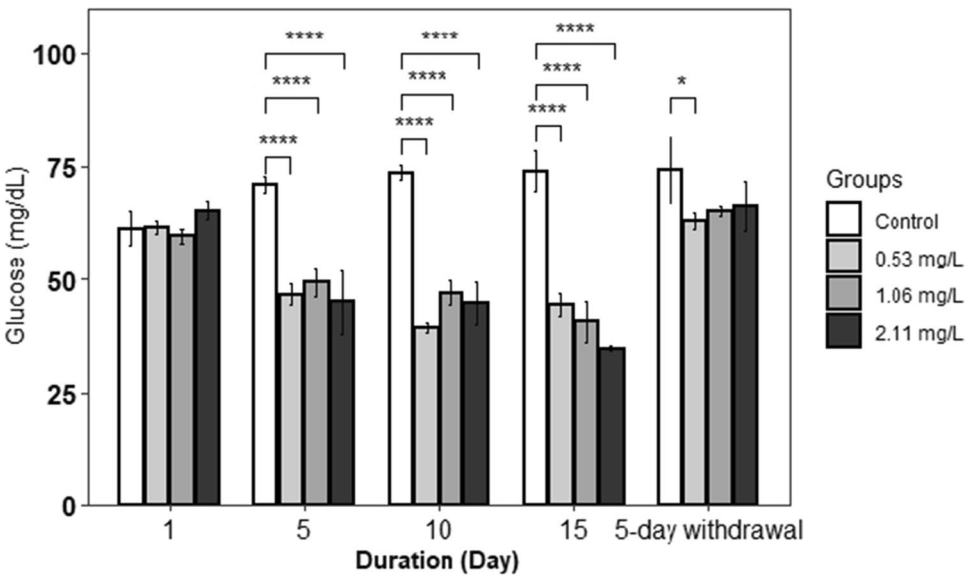

Fig. 4 Changes in serum glucose levels in *Clarias gariepinus* after 15-day exposure to chlorpromazine and 5-day recovery. Bars with different alphabet labels were significantly different for a given day, while bars with different numeric labels were significantly different between durations for a given CPZ concentration. Significantly different at *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, and ****$p < 0.0001$

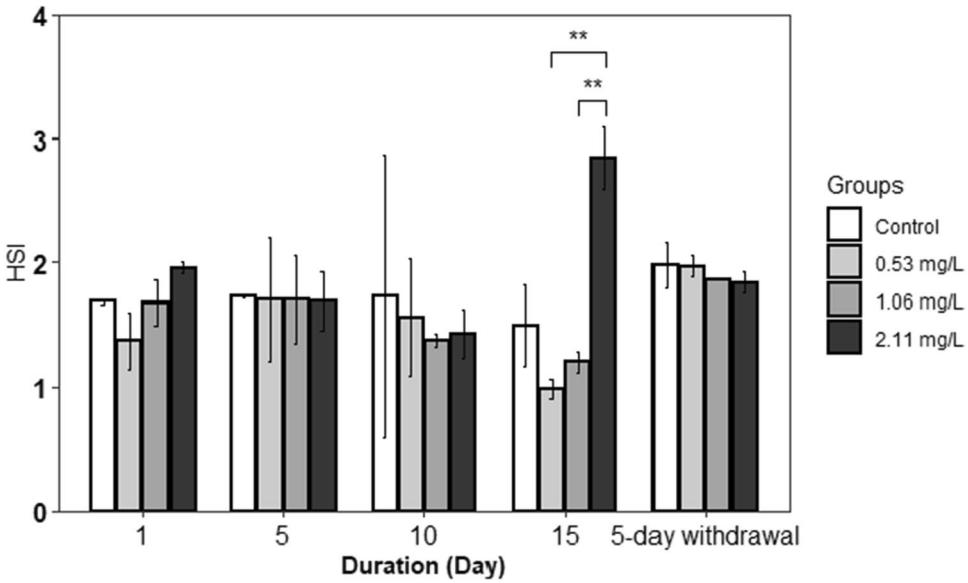

Fig. 5 Changes in hepatosomatic indices (HSI) of juvenile *Clarias gariepinus* on 15-day exposure to CBZ. Significantly different at $*p < 0.05$ and $**p < 0.01$

thus, suggesting the environmental relevance of the test concentrations.

Studies on acute toxicity have been used widely in assessing water quality parameters by various agencies in toxicology. In the present study, the LC_{50} of 10.56 mgL^{-1} obtained for *C. gariepinus* exposed to CPZ is higher than the 0.32 mgL^{-1} obtained by Li et al. (2008) for *Carassius auratus* exposed to the same drug. Reports indicate that differences in LC_{50} is dependent on exposure durations, water parameters, method of estimation, pH, size, age, species, hardiness, and biotransformation in the test organism used (Ogueji et al. 2019; Melefa et al. 2020). Our test organism *Clarias gariepinus* is hardy and can withstand harsh environmental conditions and thus less sensitive to CPZ than *C. auratus*, hence the higher LC_{50} observed.

Exposure of juvenile *C. gariepinus* to CPZ elicited behavioral changes such as abnormal mucus secretion, skin coloration, and air gulping, which were more pronounced at higher CPZ concentrations. The increased mucus secretions in the fish exposed to higher concentrations of CPZ may be a physiological adaptation to protect the fish from the toxic effect of the drug. The color change observed may also be due to the dispersion of pigment cells in the chromatophores due to the presence of the drug (Ogueji et al. 2019). Other responses such as opercula movement and swimming were less pronounced with increasing CPZ concentrations. Similar behavioral responses were reported in *Carassius auratus* exposed to CPZ (Li et al. 2008); *C. gariepinus* exposed to chloramphenicol, CPZ, and tramadol (Nwani et al., 2014a; Ogili et al. 2021); and *Danio rerio* exposed to tramadol (Bachour et al. 2020). Zhang (2003) also reported behavioral abnormalities in animals after administration of small doses of CPZ.

Blood parameters have been used in assessing the health condition of fish as they are the first target for xenobiotic action in the body (Melefa et al. 2020). Exposure of *C. gariepinus* to CPZ sublethal concentrations induced reduction in PCV, RBC, and Hb values. The reduction in the values of these parameters in the fish may be due to hemolysis and impairment of their synthesis by CPZ. Similar results have been reported in *C. gariepinus* exposed to drugs such as praziquantel (Nwani et al. 2016), ivermectin (Ogueji et al. 2019), and clotrimazole (Melefa et al. 2020). Our results indicate that CPZ provoked significant elevation of WBC in the peripheral blood of the exposed fish. The increased WBC levels may indicate immuno-protective activity elicited against the drug. The elevated WBC in the blood may also be due to the stimulation of the T-lymphocytes by CPZ (Campbell 1996). Similar increase in WBC was obtained in *C. gariepinus* exposed to chloramphenicol (Nwani et al. 2014b), diazepam (Ogueji et al. 2018), and clotrimazole (Melefa et al. 2020). Ajima et al. (2017) also reported the elevation of WBC in verapamil-exposed *Oreochromis niloticus*. Hematological indices are useful in determining the type of anemia in organisms. In the present study, there were no significant difference in the values of MCV, MCH, and MCHC in exposed fish compared to the control. Similar to our finding, Nwani et al. (2014a) reported no significant difference in MCV, MCH, and MCHC values in *C. gariepinus* exposed to praziquantel. Li et al. (2011) also reported no significant change of MCHC in *Onchorrynchus mykiss* exposed to carbamazepine. Contrary to our report however, increased MCV, MCH, and MCHC values were reported in *C. gariepinus* exposed to clotrimazole (Melefa et al. 2020) and acetylsalicylic acid (Siddeswaran et al. 2020). The leucocyte differentials in *C. gariepinus* exposed to CPZ were comparable to the control throughout the duration of

the experiment. Similar observations have been recorded in other fish exposed to different toxicants (Velisek et al. 2009; Mohammad et al. 2012; Nwani et al. 2016).

CPZ-associated concentration and duration-dependent elevation in activities of AST, ALT, and ALP occurred in *C. gariepinus*. The significant increase of these parameters in the exposed fish indicates stress-induced liver damage and may be attributed to the hepatotoxic effects of CPZ. Our result is in agreement with the reports of other authors that recorded elevation of biochemical parameters in pharmaceutical-exposed fish (Owoade et al. 2019; Odo et al. 2020; Siddeswaran et al. 2020). Protein and glucose are important in living organisms and may be used as indicators of environmental stress (Saravanan et al. 2012). There was significant decrease in the protein and glucose levels in *C. gariepinus* exposed to CPZ. The decrease in protein may be due to its increased use to make up for the high metabolic activities that may have induced stress and hepatic damage (Dogan and Can 2011). Decrease in food intake and tissue damage due to the drug may also account for the decreased protein levels in the exposed fish (Neff 1985). The decrease in glucose level as observed in the present study may be due to possible impairment of the kidney which may have inhibited glucose biosynthesis as a result of damage to the liver cells (Ogueji et al. 2017). Further investigations are, however, needed to conclusively validate our report. The return of the protein and glucose to the control levels after the 5-day withdrawal from CPZ may indicate that the impairments were due to the drug.

Condition factor gives an indication of wellbeing of the fish in the environment. In the present study, CF showed no obvious response to the CPZ exposure. Similar to our report, Melefa et al. (2020) reported that CF was not altered in *C. gariepinus* exposed to clotrimazole. Further, there was no significant difference in CF in *Oncorhynchus mykiss* exposed to clotrimazole and carbamazepine (Li et al. 2011). No significant change in CF was also reported in *C. gariepinus* exposed to praziquantel (Nwani et al. 2014a) and ivermectin (Odo et al. 2020). Our result thus implies that CPZ may have no major influence on the ratio of length to weight of the fish. Further research may explain the underlying mechanisms involved and whether same results will be obtained in other test models. Exposure of *C. gariepinus* to CPZ resulted in elevation of HSI which was significantly different from the control on day 15 at 5.28 mg/L CPZ. Changes in the HSI have also been reported in fish species exposed to pharmaceuticals and other substances (Sogbanmu et al. 2018; Odo et al. 2020).

Conclusion

The study indicates that CPZ elevates the values of WBC, PCV, RBC, decreases that of Hb but the MCV, MCH, MCHC values, and white blood differentials were comparable to the control. The values of aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase significantly increased but protein and glucose decreased. Condition factor and hepatosomatic indices in the exposed fish showed mixed pattern. CPZ is toxic to *C. gariepinus* and its potential ecological risks to aquatic biota on a long term should be further investigated.

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Author contribution This work was carried out in collaboration among all authors. Martins Nnamdi Okpe and Ifeanyi Oscar Aguzie designed the study and performed the laboratory analysis. Chukwuebuka Christabel Eze and Martin Abdubala Okpanachi wrote the protocol and the first draft of the manuscript. Hope Chinwe Ezinwa and Christopher Didigwu Nwani managed the statistical analyses of the study. Uduak Aletan, Henrietta Ijeoma Kelle, and Maureen N Chukwu managed the literature searches and edited the final manuscript. All authors read and approved the final manuscript.

Data availability Not applicable.

Declarations

Ethical approval The fish were treated in accordance with the rules conforming to principles of laboratory animal care as obtained from the ethical committee on the use of experimental animals Faculty of Biological Science, University of Nigeria Nsukka (UNN-ECFBS-00234).

Consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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