



Oxidative stress, biochemical, lipid peroxidation, and antioxidant responses in *Clarias gariepinus* exposed to acute concentrations of ivermectin

Emmanuel Ogueji¹ · Christopher Nwani² · Christian Mbah³ · Stanley Iheanacho¹ · Friday Nweke⁴

Received: 14 January 2019 / Accepted: 11 November 2019 / Published online: 5 March 2020
© Springer-Verlag GmbH Germany, part of Springer Nature 2020

Abstract

The short-term effects of ivermectin (IVMT) on the oxidative stress and biochemical parameters of *Clarias gariepinus* juvenile was assessed under semi-static conditions at concentrations of 9 to 25 $\mu\text{g L}^{-1}$ for up to 4 days. Juveniles were highly sensitive to ivermectin, with an LC_{50} of 15 $\mu\text{g L}^{-1}$. The antioxidant enzyme profile assessed included glutathione reductase (GR), superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT). General stress biomarkers such as serum glucose, protein, alkaline phosphatase (ALP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were also determined at 24-h, 48-h, 72-h, and 96-h exposure durations. Lipid peroxidation showed significant ($p < 0.05$) decreases in higher concentrations (21 $\mu\text{g L}^{-1}$ and 25 $\mu\text{g L}^{-1}$) and durations of exposure (72 h and 96 h). Significant concentration-dependent increases ($p < 0.05$) were recorded in the liver function enzymes, superoxide dismutase (SOD), catalase (CAT), and glutathione reductase (GR) when compared to the control. GPx decreased significantly ($p < 0.05$) in higher concentrations (21 $\mu\text{g L}^{-1}$ and 25 $\mu\text{g L}^{-1}$) and durations of exposure (48–96 h). Protein showed significant concentration-dependent decreases, while glucose recorded a mixed trend. The changes in the hepatic antioxidant enzyme activities and serum metabolites were indicative of oxidative stress induced by IVMT. This showed that IVMT is toxic to fish and should be used with utmost caution.

Keywords Freshwater fish · Fish health · Oxidative stress · Pharmaceuticals · Pollution

Introduction

Global fish production peaked at about 171 million tons in 2016, with aquaculture representing 47% (FAO 2018). Aquaculture has been reported to be the fastest growing animal production sector worldwide (Iheanacho et al. 2018). Increased demands for fish as a source of animal protein are

the main reason for the industry's growth. Because of expansion of the industry, the culture methods have become more intensive for producing higher yields perhaps resulting in the outbreak of diseases. The use of veterinary drugs in aquaculture for prophylaxis or chemoprophylaxis purposes became imperative in order to avoid economic disasters (Cabello 2006). Ivermectin has been reported to be a broad-spectrum antiparasitic drug and efficient in controlling nematodes and parasitic arthropods in aquaculture (Campbell 1989). Over 5 billion doses of this drug are sold worldwide (Shoop and Soll 2002). The drug is a type of abamectin, which was ab initio, extracted from the actinomycete *Streptomyces avermitilis*. The drug has been reported to be effective for treating parasitic diseases in swine, equines, bovine, and canines (Campbell et al. 1983; Forbes 1993). Ivermectin is also used for the treatment of diseases in humans like onchocerciasis, strongyloidiasis, ascariasis, trichuriasis, and enterobiasis (Varo et al. 2010; Thiripurasundari et al. 2014). The use of IVMT for the treatment of *Lepeophtheirus salmonis* infestation in salmon and *Salmincola californiensis* infection in rainbow trout have been reported (Johnson and Margolis 1993;

Responsible editor: Thomas Braunbeck

✉ Emmanuel Ogueji
oguejikey@yahoo.com

- ¹ Department of Fisheries and Aquaculture, Alex Ekwueme Federal University, Ndufu Alike, Ikwo, Abakaliki, Ebonyi State, Nigeria
- ² Department of Zoology and Environmental Biology, University of Nigeria Nsukka, Nsukka, Nigeria
- ³ Department of Zoology, Ahmadu Bello University, Zaria, Nigeria
- ⁴ Department of Biology/Microbiology/Biotechnology, Alex Ekwueme Federal University, Ndufu Alike, Ikwo, Abakaliki, Ebonyi State, Nigeria

Roberts et al. 2004). Ivermectin binds with high affinity to glutamate-gated chloride channels which occur in invertebrate nerve and muscle cells, causing an increase in the permeability of the cell membrane to chloride ions. This influx causes hyper polarization of the nerve or muscle cell, consequently resulting in paralysis or death of affected invertebrates (Oliveira et al. 2016). Besides the glutamate channel, IVMT also regulates other inhibitory ligand-gated channels, e.g., γ -aminobutyric acid (GABA)-gated chloride channels occurring in the peripheral nervous system of invertebrates and in the central nervous system of vertebrates (Duce and Scott 1985). Since these channels are inhibitory, the effect of IVMT is to enhance the inhibitory neurotransmission produced by GABA and glutamate in invertebrates which results in paralysis of muscle cells (Geary and Moreno 2011). Ivermectin, while paralyzing body wall and pharyngeal muscles in nematodes, has no such impact in mammals, as it cannot cross the blood-brain barrier into the mammalian central nervous system, where GABA receptors are located. Evidence has shown that fish has porous blood-brain barrier to toxicants like IVMT due to low efficacy of its P-glycoprotein.

Alarm has been raised over increased use of IVMT as the larger percentage of this drug is excreted in an unmetabolized form through the feces of treated animals (Athanasopoulou et al. 2002). Ivermectin and its metabolites usually find their ways into aquatic ecosystem through discharges from effluents and surface runoff (Thiripurasundari et al. 2014). The drug has been reported to adversely affect the fish and certain aquatic organisms like crustaceans and benthic polychaetes (Black et al. 1997). Ivermectin exist in the range of ng/L on surface waters (Iglesias et al. 2012) and has been identified as one of the pharmaceuticals that persist in the sediment for months or years (Boxall 2010).

Investigation of the fate of IVMT and its metabolites was carried out in aerobic sediment/water systems using radiochemical analysis (Prasse et al. 2009). Authors observed a rapid sorption of IVMT to sediments and claim that this was due to IVMT high octanol/water partitioning coefficient ($K_{ow} = 1651$, $\log K_{ow} = 3.2$). Halley et al. (1993) argued that since IVMT undergo rapid degradation in light and soil and bind tightly to soil and sediment, they will not accumulate and will not undergo translocation in the environment, thereby minimizing any environmental impact on nontarget organisms. Boonstra et al. (2011) studied the effect of IVMT in the plankton-dominated indoor microcosms and reported that the half-life (dissipation time 50%; DT_{50}) of IVMT in water phase ranged from 1.1 to 8.3 days. Study on the aerobic transformation of IVMT in soil mixed with feces gave DT_{50} values of 93 days and 240 days, depending on soil type and mode of application (Hally et al. 1989). However, Carlsson et al. (2013) reported that IVMT can pose a threat to nontarget organisms even at low

concentrations (ng/L). *Daphnia magna* is one of the fresh water species found to be the most sensitive to IVMT that has an LC_{50} value of 0.025 ppb (Halley et al. 1993).

Reactive oxygen species (ROS) may be generated from xenobiotics during biotransformation processes (Ogueji et al. 2017b). There have been reports that ROS when produced in excess reacts with macromolecules to elevate the level of protein denaturation, lipid peroxidation, and changes in antioxidant enzyme activities (Blahova et al. 2013; Pereira et al. 2013). Oxidative stress occurs when there is imbalance in the population of ROS and the antioxidant enzymes to depopulate them (Halliwell and Gutteridge 1999). The intracellular enzymatic antioxidants that scavenge ROS include catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), and glutathione reductase (GR). Excess ROS leads to lipid peroxidation and subsequent leakage of liver function enzymes such as alkaline phosphatase (ALP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) into the blood (Arise and Malomo 2009). Ivermectin have been used in agriculture and horticulture for the protection of fruits, cotton, vegetables, and ornamentals (Dybas 1989). Extensive works have been done on acute toxicity of ivermectin to invertebrates and fish embryos particularly zebrafish and few other species (Carlsson et al. 2013; Blahova et al. 2013). In 2003, approximately 56 million Africans were taking a single annual dose of ivermectin (BIO 2013). Regrettably, there has been scanty scientific information on ivermectin-induced toxic effects on the antioxidant and liver function enzyme profiles in most native fish species in Africa. *C. gariepinus* juveniles was selected as a model for the study because it is the most popularly cultured fish species in Africa, hardy, adaptable to laboratory conditions, and widely distributed in tropical freshwater ecosystems. The aim of the study was to (1) determine the 96-h LC_{50} of IVMT for *C. gariepinus* juveniles (2) Determine if IVMT have effect on the biomarkers of oxidative stress and other biochemical endpoints that reflect on the health of the fish. The endpoints obtained from this study would be useful to monitor the acute and sublethal effects of IVMT in freshwater fish.

Materials and methods

Fish collection and maintenance

The juveniles of *C. gariepinus* were obtained from the Department of Fisheries and Aquaculture farm, Federal University Ndufu Alike, Ikwo, Ebonyi State. Fish were transported in 50 L plastic container to the Fish wet Laboratory. Fish were subjected to a 2-min bath treatment with 0.05% potassium permanganate (KMnO₄) to prevent skin infections (Ogueji et al. 2017b). The fish was acclimated for 14 days in a tarpaulin tank (10 × 8 × 3 m) and

fed twice (7:00 a.m.–6:00 p.m.) daily with commercial feed (Coppens International, Helmond, Netherlands) containing 45% crude protein. Feces and other devastate materials were siphoned off everyday from the water in experimental tanks to avoid fouling.

Test chemical

In the current study, ivermectin (CAS No: 70288-86-7, Empirical Formula: $C_{15}H_{16}C_{13}N_3O_2$, 97% purity) was purchased from Sigma-Aldrich. Dimethyl sulfoxide (DMSO) was purchased from Mark Pharmacy Ltd., Abakaliki, and used ($100 \mu\text{L L}^{-1}$ and purity 99%) to make the stock solution due to their low water solubility. Test solutions were subsequently prepared by successive dilution of the stock solution in water.

Acute toxicity test

Acute toxicity test was performed according to methods of APHA (2005). Ivermectin was dissolved in water with the addition of dimethyl sulfoxide as a solvent at 0.1% DMSO (Carlsson et al. 2013). Five treatments and two controls were used: The fish in group 1 contained only dechlorinated tap water without the drug and served as first control. Fish in group 2 contained 0.1% DMSO in water and served as second control. The fish in groups 3 to 7 were exposed to $9 \mu\text{g L}^{-1}$, $13 \mu\text{g L}^{-1}$, $17 \mu\text{g L}^{-1}$, $21 \mu\text{g L}^{-1}$, and $25 \mu\text{g L}^{-1}$ ivermectin, respectively, and were prepared from the stock. A total of 210 fish ($197.39 \pm 2.34 \text{ g}$ and $27.36 \pm 0.23 \text{ cm}$ total length) were randomly assigned to 21 experimental glass tanks ($60 \times 30 \times 30 \text{ cm}$) holding ten fish per tank. Tanks were calibrated and filled with 40 L of dechlorinated tap water. Test was performed using a semi-static method with solutions renewed every 24 h. The feeding was discontinued 24 h before the experimental run as previously described by Reish and Oshida (1987). Survival and mortality were recorded at 24, 48, 72, and 96 h. Fishes were considered dead when the opercula movement ceased and when there was no response to gentle prodding. Dead fish were checked and removed every 24 h. The LC_{50} values of IVMT at different durations of exposure were estimated using probit analysis as recommended by Finney (1971). The safe level of IVMT was obtained based on the methods of (Hart et al. 1948; Sprague 1977; CWQC 1972; NAS/NAE 1973; CCREM 1991; IJC 1977). Water quality parameters were monitored daily throughout the duration of the experiment using a water quality test kit (Pro-Lab Diagnostics, Toronto, Canada), and the respective mean values were as follows: temperature $28.6 \pm 0.2 \text{ }^\circ\text{C}$, pH 6.1 ± 0.42 , dissolved oxygen $4.96 \pm 0.15 \text{ mg/L}$, conductivity $145.3 \pm 2.7 \mu\text{Scm}^{-1}$, hardness $43.2 \pm 7.8 \text{ mgL}^{-1}\text{CaCO}_3$, and alkalinity $28.8 \pm 2.0 \text{ mL}^{-1}$.

Biochemical and antioxidant enzymes assay

One fish from each replicate (in both the treatment groups and control) was sacrificed after anesthetizing with tricaine methanesulfonate (MS-222) to minimize stress. This was carried out at 24- to 96-h exposure durations. The following biochemical parameters ALP, AST, and ALT were measured as previously described by Lawrence and Burk (1976). The fish were dissected, and liver and gill tissues were removed and washed in 0.9% sodium chloride (NaCl) solution, and blended in pre-chilled potassium phosphate buffer (1:10 w/v, 0.1 M, pH 7.0). One part of the homogenate was used for the estimation of lipid peroxidation (LPO), while the other part was centrifuged for 20 min at 10,500 rpm under $4 \text{ }^\circ\text{C}$ to obtain the supernatant which was stored at $4 \text{ }^\circ\text{C}$ for enzyme assay. For each of the parameters, five technical replicates were made, and the average was recorded as means \pm SE. The rate of NADPH oxidation at 340 nm in combined reaction with GR was used in the determination GPx activity. Lawrence and Burk (1976) method was used for the estimation of specific activity using 6.22 mMcm^{-1} extinction coefficient. The activity was expressed in unit/min/mg protein. Spectrophotometer was employed to determine tissue CAT activities by estimating the rate of H_2O_2 decomposition following decrease in absorbance at 240 nm. The unit of expression was in U/mg protein (Aebi 1984). SOD was measured spectrophotometrically at 420 nm and was expressed as the quantity of enzyme in mg^{-1} protein required to inhibit 50% of epinephrine auto-oxidation. The activity was expressed as units/mg protein (Misra and Fridovich 1972). Making use of bovine serum as a standard, the methods of Lowry et al. (1951), was employed spectrophotometrically to estimate total protein in the tissues. The glucose level was estimated according to the method described by Cooper and McDaniel (1970).

Lipid peroxidation

Sharma and Krishna-Murti (1968) method was used in the estimation of tissue lipid peroxidation (LPO). The concentration of TBARS was measured by the absorption at 535 nm at molar extinction coefficient of 156 mM/cm . Nanomoles was used as the unit of expression for specific activity of TBARS/mg protein.

Statistical analysis

The statistical package (IBM SPSS version 20), was used in the analysis of the data generated. One-way analysis of variance (ANOVA) was used to determine whether there were any significant differences between the means of the lethal

concentrations at the different exposure durations, and this was followed by a Duncan multiple range tests. Similarly, a two-way ANOVA was performed to understand if there was an interaction effect between concentrations of IVMT and the duration of exposure of the drug on LPO, antioxidant enzymes, and serum biochemical parameters assayed. Significant difference was set at ($p < 0.05$) level.

Ethical statement

All experimental procedures were approved by the institutional ethics clearance committee (Ref: FUNAI/SEN/ EBC/17/ VOL.1/5) and performed in compliance with the standards described by the institution of animal welfare act in line with the National Environmental Standard Regulations Enforcement Agency (NESREA) Act of Nigeria on the protection of animals against cruelty.

Results

Median lethal concentration (LC₅₀) and safe level

The cumulative mortality of fishes treated with various concentrations of IVMT is presented in (Table 1). Increase in IVMT concentration, led to the increase in fish mortality simultaneously. The control number 1 and 2 recorded no mortality during the experimental period. The 96-h LC₅₀ values of IVMT was estimated to be 32 μg L⁻¹, 25 μg L⁻¹, 17 μg L⁻¹, and 15 μg L⁻¹, for 24 h, 48 h, 72 h, and 96 h, respectively. A concentration-dependent increase and duration-dependent decrease were observed in mortality rate, such that as the exposure duration increased from 24 to 96 h, the median lethal concentration was reduced. Also the estimated safe level using different standard methods showed variations as presented in (Table 2).

Toxicity effects of ivermectin on LPO and antioxidant enzymes

Ivermectin affected the cell membrane of hepatocytes/erythrocytes of treated fish, and the result was estimated as LPO as presented in (Fig. 1). LPO increased significantly ($p < 0.05$) in the treated fish when compared to control. Equally there was significantly ($p < 0.05$) LPO increase in 24- and 48-h durations of exposure. In the 72- and 96-h durations, there were significant increase of LPO in the lower concentrations of 9 μg L⁻¹ and 13 μg L⁻¹ and subsequent significant reductions at the higher concentrations of 21 μg L⁻¹ and 25 μg L⁻¹. LPO maximum value (10.94 ± 0.19 nmol mg protein⁻¹) was recorded in 9 μg L⁻¹ concentrations after 96-h duration of exposure, while minimum value was in 25 μg L⁻¹ concentrations after 24-h exposure. The control and solvent control displayed not to be affected. Ivermectin elicited significant ($p < 0.05$) dose-dependent SOD increase when compared to control. But with increased duration of exposure, SOD significantly increased with a mixed trend, with the highest value (33.65 ± 0.48 U mg protein⁻¹) recorded in 25-μg L⁻¹ concentration after 96-h duration of exposure. The lowest value was recorded in 96-h duration at 9 μg L⁻¹ concentration. The antioxidant enzymes CAT, GR, and GPx significantly increased in 9–25 μg L⁻¹ concentrations in 24–96-h exposure durations when compared to control. However, with increasing duration of exposure, GPx significantly decreased at the higher concentrations 21 μg L⁻¹ and 25 μg L⁻¹ in 48- and 96-h exposure durations. Highest value (15.45 ± 0.17 nmol min⁻¹ mg protein⁻¹) was recorded at 96-h duration and in 9 μg L⁻¹ concentration. Minimal value (10.99 ± 0.18 nmol min⁻¹ mg protein⁻¹) was recorded at 72-h duration in 21 μg L⁻¹ concentration. Maximum value of GR was recorded in 72-h duration and 21 μg L⁻¹ concentration, while lowest value was at 24-h duration and 9 μg L⁻¹ concentration. Two-way ANOVA results showing interaction between concentration of IVMT and exposure duration on LPO and antioxidant enzymes parameters SOD, CAT, GR, and GPx of *Clarias*

Table 1 Cumulative mortality of *C. gariepinus* exposed to various concentrations of IVMT

Concentration μg/L	Number exposed	Cumulative mortality of fish at different durations (hours)				Survival (%)	Mortality (%)
		24 h	48 h	72 h	96 h		
Control	30	0	0	0	0	100	0
DMSO	30	0	0	0	0	100	0
09	30	0	2	4	6	80	20
13	30	0	2	6	10	67	33
17	30	6	8	12	16	47	53
21	30	6	12	16	20	33	67
25	30	10	18	30	30	0	100
96-h LC ₅₀		32 μg L ⁻¹ , 25 μg L ⁻¹ , 17 μg L ⁻¹ , 15 μg L ⁻¹					

Table 2 Estimate of safe levels of IVMT at 96-h exposure duration

Drug	96-h LC ₅₀ (µg/L)	Method	AF	NOEC (µg/L)
Ivermectin	15	Hart et al. (1948)*	–	0.59
		Sprague (1977)	0.1	1.5
		CWQC (1972)	0.01	0.15
		NAS/NAE (1973)	0.1–0.00001	1.5 × 10 ^{−4}
		CCREM (1991)	0.05	0.75
		IJC (1977)	5% LC ₅₀	0.75

*C = 48-h LC₅₀ × 0.03/S², where C = presumable harmless concentration and S = 24-h LC₅₀/48-h LC₅₀

CWQC Committee on Water Quality Criteria, NAS/NAE National Academy of Sciences/National Academy of Engineering, IJC International Joint Commission

gariiepinus are presented in (Table 3). The LPO and all the antioxidant enzymes showed significant ($p < 0.05$) interaction effect between the concentration and duration of exposure.

Toxicity effects of ivermectin on serum biochemical parameters of *C. gariiepinus*

Effects of IVMT exposure on serum biochemical parameters of *C. gariiepinus* are shown in (Fig. 2). There was significant ($p < 0.05$) concentration and duration-dependent increase in ALT, AST, and ALP when compared with solvent control. Highest values of ALT occurred at 48-h duration and 25 µg L^{−1} concentration, while minimum value was recorded

at 96-h duration and 9 µg L^{−1} concentration. Maximum value of ALP was recorded in 96-h duration and 21 µg L^{−1} concentration, while the least value was recorded in 9 µg L^{−1} concentration after 24-h duration of exposure. AST maximum value was recorded in 21 µg L^{−1} concentration in 96-h duration of exposure. The minimum value was recorded in 13 µg L^{−1} concentration in 24-h duration of exposure. There was significant ($p < 0.05$) concentration and duration-dependent decrease in protein, with maximum value recorded in 9 µg L^{−1} concentration after 72-h duration of exposure. Glucose significantly ($p < 0.05$) increased in the fish treated to 9–21 µg L^{−1} IVMT concentrations in 24-h duration. Glucose response was variable with increase in the exposure

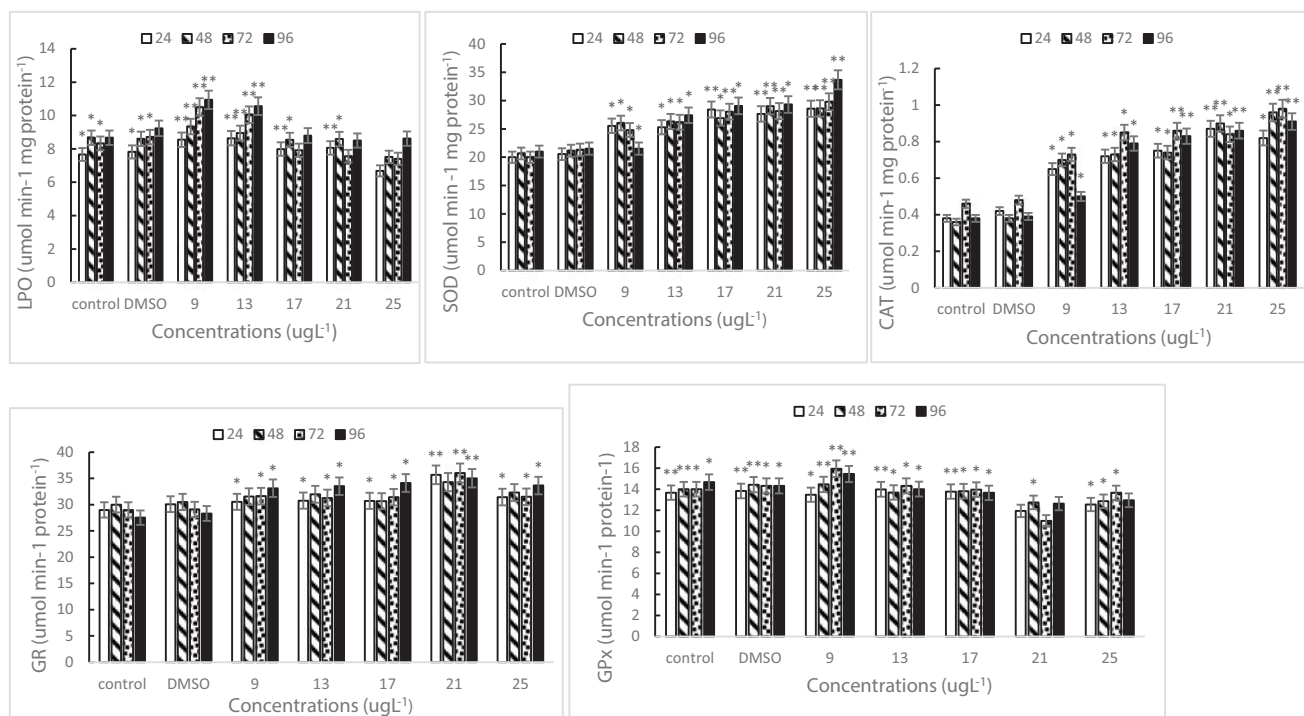


Fig. 1 Changes in lipid peroxidation (LPO), superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), and glutathione peroxidase (GPx) (µmol min^{−1} mg protein^{−1}) activities in liver tissues of *C. gariiepinus* exposed to 9, 13, 17, 21, and 25 µg L^{−1} concentrations of ivermectin for 24-, 48-, 72-, and 96-h durations. Bars represent means

and vertical lines in the SE of three (3) individual observations. Bars with asterisk symbol between the control and different concentrations differ significantly ($p < 0.05$) within the same exposure duration. Significant difference was set at ($p < 0.05$) level

Table 3 Two-way ANOVA results showing interactions between concentration of IVMT and exposure duration on LPO and antioxidant parameters of *C. gariepinus*. Significant effects ($p < 0.05$) in bold

LPO/antioxidant parameters	Main effects				Interaction	
	Concentration		Exposure duration		Conc. × exposure duration	
	<i>F</i>	<i>p</i> value	<i>F</i>	<i>p</i> value	<i>F</i>	<i>p</i> value
LPO	66.085	0.000	81.488	0.000	7.784	0.000
SOD	169.716	0.000	0.947	0.424	9.861	0.000
CAT	251.006	0.000	13.073	0.000	7.245	0.000
GR	25.473	0.000	5.738	0.002	2.066	0.020
GPx	110.404	0.000	26.066	0.000	9.888	0.000

duration. Maximum value was recorded in $9 \mu\text{g L}^{-1}$ concentration after 24-h duration of exposure, while minimum value was recorded in $17 \mu\text{g L}^{-1}$ concentration after 72-h duration of exposure. Two-way ANOVA results showing interactions between concentration of IVMT and exposure duration on serum biochemical parameters; Protein, glucose, ALT, AST, and ALP of *C. gariepinus* are presented in (Table 4). All the serum biochemical parameters recorded significant ($p < 0.05$) interaction effect between the concentration and durations of exposure.

Discussion

The 96-h LC_{50} of IVMT in the present study was $15 \mu\text{g L}^{-1}$ and as such highly toxic to juveniles of

C. gariepinus. Similar findings have been reported for other fish species including channel catfish (*Ictalurus punctatus*), *Cyprinus carpio*, *Oncorhynchus mykiss*, and *Cyprinodon variegatus* with 96-h LC_{50} values of $32 \mu\text{g L}^{-1}$, $56 \mu\text{g L}^{-1}$, $6 \mu\text{g L}^{-1}$, and $20 \mu\text{g L}^{-1}$, respectively (KKegeley et al. 2016). Generally, the toxic effects of xenobiotics to fish and other organisms in aquatic ecosystem have been reported to be influenced by dose, duration of exposure, bioaccumulation, sex, strain of species, temperature, pH, dissolved oxygen, formulation of test drug, biotransformation, and excretion (Saravanan et al. 2012; Rauf and Arain 2013; Somdare et al. 2015). The interspecies differences observed in literature may be connected to the heterogeneous metabolism of individual fish species. The observed mortality in present study may be due to one or a combination of effects caused by IVMT (e.g., neurotoxicity, hepatotoxicity, and oxidative stress). Previous

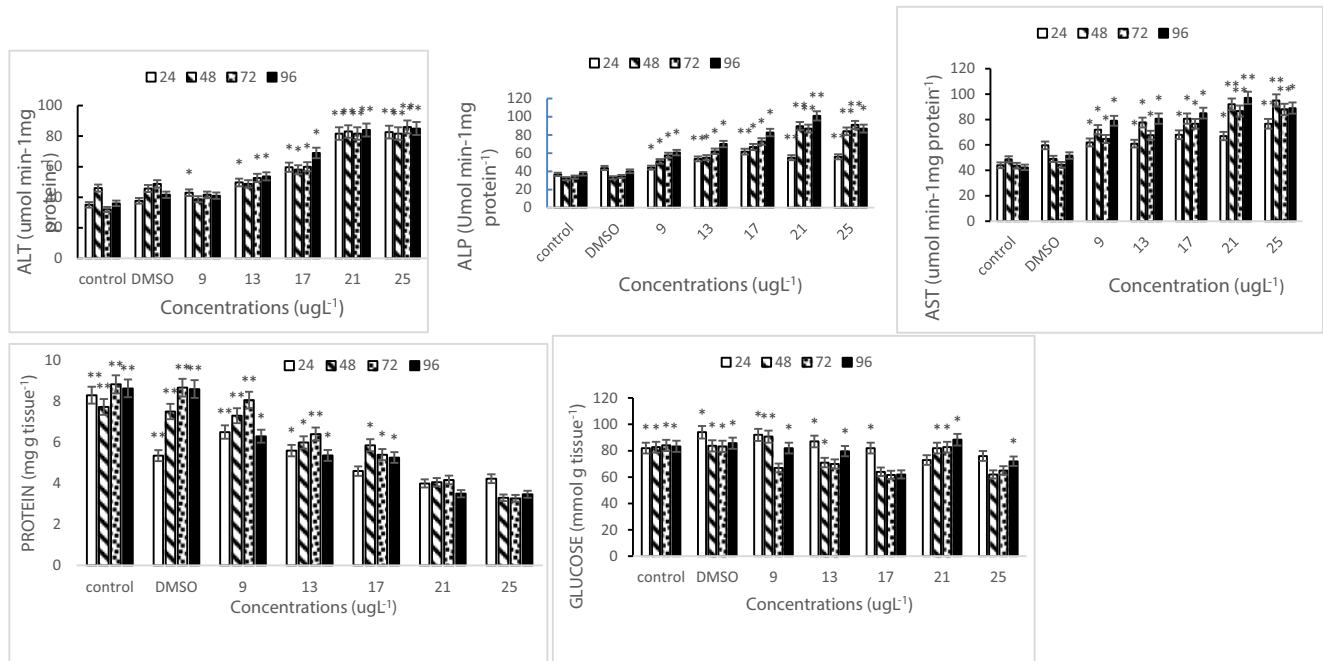


Fig. 2 Change in serum enzymes (alanine aminotransferase = ALT, alkaline phosphatase = ALP, aspartate aminotransferase = AST), protein (mg g tissue^{-1}) level and glucose ($\text{mmol g tissue}^{-1}$) in the liver juvenile *C. gariepinus* exposed to 9, 13, 17, 21, and 25 $\mu\text{g L}^{-1}$ concentrations of ivermectin for 24-, 48-, 72-, and 96-h durations. Bars represent means and

vertical lines in the SE of three (3) individual observations. Bars with asterisk symbol between the control and different concentrations differ significantly ($p < 0.05$) within the same exposure duration. Significant difference was set at ($p < 0.05$) level

Table 4 Two-way ANOVA results showing interactions between concentration of IVMT and exposure duration on biochemical parameters of *C. gariepinus*. Significant effects ($p < 0.05$) in bold

Biochemical parameters	Main effects				Interaction	
	Concentration		Exposure duration		Conc. × exposure duration	
	<i>F</i>	<i>p</i> value	<i>F</i>	<i>p</i> value	<i>F</i>	<i>p</i> value
ALT	781.896	0.000	12.263	0.000	9.966	0.000
AST	620.544	0.000	214.670	0.000	22.265	0.000
ALT	378.592	0.000	148.447	0.000	20.099	0.000
Protein	266.359	0.000	16.062	0.000	12.973	0.000
Glucose	33.305	0.000	31.483	0.000	8.429	0.000

studies using Atlantic salmon (*Salmo salar*) have reported significantly higher acetylcholine esterase (AChE) activity in the brain after exposure to IVMT, suggestive of the compound ability to cross the fish blood-brain barrier (Hoy et al. 1990; Ucan-Marin et al. 2012). Lumeret (2012) reported that the P-glycoproteins in fish blood-brain barrier are not as efficient as that of mammals. Ivermectin has been reported to cause toxic effects on the liver proteome of *Sparus aurata* at a dose of 0.2 mg/kg of fish (Shoeb 2013). Wistar albino rats treated with ivermectin (5–15 mg/kg) induced significant biochemical changes in plasma and hepatic cells (Didier and Loor 1995). Thiripurasundari et al. (2014) reported species-specific neurotoxic and hepatotoxic changes in zebra fish and catla fish. Even though the present work did not examine the neurotoxicity of IVMT, mortality recorded may have been due to general physiological stress and neurotoxic effect of the drug on the fish central nervous system.

Increase in the population and activities of cellular antioxidant enzymes helps to depopulate ROS, and oxidative stress will occur when the population of ROS outnumbers the antioxidant defense system (Zhang et al. 2004). LPO activity is an acceptable biomarker for measurement of environmental pollution, and xenobiotics with their metabolites have been shown to induce oxidative stress, by producing free radicals that leads to damage to membrane lipids, DNA, and proteins (Gutteridge 1995; Islas-Flores et al. 2014). The present study recorded significant LPO increase in 9 $\mu\text{g L}^{-1}$ concentration after 48-, 72-, and 96-h durations of exposure. This indicates that ROS may be associated with the metabolism of IVMT and that there was increased production of ROS in the 48-, 72-, and 96-h durations so much that the antioxidant enzymes were not able to depopulate them, therefore causing peroxidation of membrane lipids. However, at the higher acute concentrations and durations, LPO due to IVMT exposure to the fish significantly decreased. This decrease may be associated with elevation in the population and activities of cellular antioxidant enzymes which consequently helped to depopulate ROS and reduce oxidative stress. The pattern of oxidative stress recorded in the present study is comparable with Gonzalez-Rey and Bebianno (2012) who reported significant lipid

peroxidation in mussels (*Mytilus galloprovincialis*) by ibuprofen in the first week of exposure indicating damage due to oxidative stress. Subsequently in the second week, there was a significant decrease in lipid peroxidation by the end of the 2 weeks. Serafini et al. (2019) reported increased hepatic LPO and ROS levels in two highest concentrations and all concentrations (0.0, 1.124, 1.809, and 3.976 $\mu\text{g L}^{-1}$) of eprinomectin, respectively, after 24-h exposure of silver catfish.

SOD is the first line of defense, acting as a free radical scavenger in nearly all living cells exposed to oxygen, and it catalyzes the partitioning of the superoxide anion radical into molecular oxygen, water, or hydrogen peroxide. If SOD is not regulated, it leads to various types of cell damage. It has been reported that the buildup and binding of toxicants/drugs in cytoplasm, cell membranes, and mitochondria may lead to degeneration of cells and consequent discharge of SOD enzyme into the blood circulation (Iqbal et al. 2003; Saravanan et al. 2012). In this study, result indicated a dependent concentration; we report a significant increase in SOD compared to control, and among the treated group, increase in SOD was concentration dependent. However, with increase in the duration of exposure, SOD activity occurred with a mixed trend. The concentration-dependent increase in SOD suggests that with the increase in superoxide radicals arising from higher dose of IVMT, increased SOD is released to contain the activity of the radicals and helps maintain cellular membrane integrity. In a duration where the SOD is reduced, it is suggested that the activity of superoxide radicals may have overwhelmed SOD ability to contain them, thereby compromising cell membrane integrity. Ogueji et al. (2017b) also reported that reduction of SOD activity in diazepam-treated fish may be a sign of abridged ability to protect cells against superoxide radicals due to increased hydrogen peroxide production. Tissue-specific dose and duration-dependent increase in SOD activity due to sublethal exposure of *C. gariepinus* juveniles to diazepam have been reported (Ogueji et al. 2017b; Ogueji et al. 2017a). Shortfall in SOD after sublethal exposure of Nile tilapia (*Oreochromis niloticus*) to the verapamil was attributed to buildup of hydrogen peroxide in fish tissues (Ajima et al. 2017).

CAT is an intracellular antioxidant enzyme that promotes the removal of hydrogen peroxide (H_2O_2) and its conversion to molecular oxygen (O_2) and water. CAT activity therefore is directly regulated by the buildup of H_2O_2 in the tissues (Ogueji et al. 2017a; Nwani et al. 2017). In this investigation, we report that CAT and GR activity in IVMT-treated *C. gariepinus* significantly increased in a concentration-dependent pattern. But with increasing duration of exposure, both enzymes increased with mixed trends. The CAT activity may have increased because of the harmful effects of H_2O_2 which came from the breakdown of anion superoxide by SOD. The increase may be an adjustment to defend fish from harmful free radical toxicity induced by IVMT. It may also be due to other factors such as age, concentration of IVMT, sex, etc. However, decrease of CAT activity in some durations of exposure could stem from decreases in reaction rates resulting from the excess production of H_2O_2 . This could have arisen because of the flux of superoxide radicals or activated metabolites generated by IVMT on the cell membrane of treated fish. Similar findings on reduced CAT activity have been reported after exposing *C. gariepinus* juveniles to acute concentrations of ibuprofen and sublethal concentrations of diazepam (Ogueji et al. 2017a; Ogueji et al. 2017b; Nwani et al. 2017). GR catalyzes the reduction of GSSG to GSH which functions as a scavenger for hydroxyl radicals and singlet oxygen and consequently reduces oxidative stress. The increase in GR activity suggests that the enzyme has the ability to maintain the reduction process of GSSG to GSH. A similar finding was reported when *C. gariepinus* was exposed to sublethal concentrations of fenthion (Nwani et al. 2017). Significant increase ($p < 0.05$) was seen in GR activity from day 14 to 28 in the liver and day 7 to 28 in the gill of treated *C. gariepinus* to diazepam when compared to control (Ogueji et al. 2017b).

GPx prevents oxidative stress by catalyzing the reduction of hydrogen peroxide to water and oxygen. When GPx is inhibited, more hydrogen peroxide becomes available, which leads to tissue damage and oxidative stress. GPx activity is also directly connected with the concentration of reduced glutathione GSH. This is because it makes use of reduced glutathione to eliminate hydrogen peroxide and leading to the formation of oxidized glutathione (GSSG).

IVMT reduced significantly the activity of GPx in the exposed fish. This showed that its capacity to reduce hydrogen peroxide was compromised as it may have subdued the reduction of GSH to GSSG. Our report is comparable to Altinok et al. (2012) who recorded similar observation in rainbow trout treated to carbosulfan. Tissue-specific response in *C. gariepinus* to diazepam with respect to GPx activity was reported (Ogueji et al. 2017b).

GPx increased in the liver tissue in day 7 but was inhibited in day 14–28. However, in the gill tissue, GPx activity increased throughout the duration of exposure.

Binding of xenobiotics to hepatocyte cell membrane may lead to cell damage and subsequent leakage of liver function enzymes into blood circulation (Saravanan et al. 2012). Acute exposure of fish to IVMT elicited significant concentration and duration-dependent increases in ALT, ALP, and AST. Damage in hepatic cells or liver cirrhosis may have been responsible for the significant increases in the enzymes. Similar findings have been reported after acute concentration exposure of ibuprofen drug to *C. gariepinus* juveniles (Saravanan et al. 2012; Ogueji et al. 2017a).

Serum protein decreased significantly with the increase in the concentration of IVMT; the decrease may be attributed to cell damage by the drug and consequent inability of cell to produce protein or the possible conversion of the available protein as source of energy for repair of the damaged cells caused by the IVMT. Similar decrease in serum protein level was reported by Saravanan and Ramesh (2013) in *Cirrhinus mrigala* exposed to acute and sublethal clofibrac acid and diclofenac.

One of the adaptive mechanisms by aquatic organisms to reduce stress is to increase their rate of metabolism (Saravanan et al. 2012). One common response of fish to acute toxic effects is elevation in blood glucose level (Luskova et al. 2002). In this investigation, glucose levels were elevated significantly ($p < 0.05$) in the fish treated to 9 to 21 $\mu\text{g L}^{-1}$ concentrations after 24-h exposure to IVMT but decreased significantly with increased concentration and durations of exposure. The increase blood sugar may just be an initial adrenergic effect of the drug which has been associated with increase in blood sugar level. Das and Mukherjee (2003) argued that the adrenergic effects may lead to elevated blood sugar immediately after the fish is exposed to stress induced by pesticides or xenobiotics. Changes in carbohydrate metabolism can occur in fish exposed to various stressful conditions. For example, the secretion of catecholamines and adrenocorticoid by fish in stressful conditions has been reported (Ogueji et al. 2017a). This leads to marked changes in carbohydrate reserves which according to Wedemeyer et al. (1984) caused hyperglycemia. Significant increase ($p < 0.05$) was reported in glucose level of *C. gariepinus* after oral administration of ciprofloxacin, amoxicillin, and ampicillin (Fraisal 2003). The observed dose-dependent decrease in glucose from 48 to 96 h in this study may be due to kidney failure, where damaged kidneys release glucose into the urine or inhibition of glucose biosynthesis (glycogenolysis and gluconeogenesis) as a result of liver cells damage (Ogueji et al. 2017b). Authors, however, clearly state these claims have not been specifically investigated in current study and therefore may not be concluded.

Conclusion

The current findings revealed that IVMT negatively affected the health of the fish, by inducing oxidative stress and altering the activities of the antioxidant enzymes, serum metabolites (protein and glucose), and intercellular enzymes (ALT, AST, ALP) of the fish. The use of IVMT in aquaculture and other veterinary services should be highly regulated to guard against toxicological effects on nontarget organisms especially fish in water basins/areas surrounded by aquaculture.

Funding information This work was supported by the Tefund Nigeria and Alex Ekwueme Federal University Ndufu-Alike Ikwo (AE-FUNAI) under grant (Ref. No. 002).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

- Aebi H (1984) Catalase in vitro. *Methods Enzymol* 105:121–126
- Ajima MNO, Pandey PK, Kumar K, Poojary N (2017) Neurotoxic effects, molecular responses and oxidative stress biomarkers in Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758) exposed to verapamil. *Comp Biochem Physiol C* 196:44–52
- Altinok I, Capkin E, Boran H (2012) Mutagenic, genotoxic and enzyme inhibitory effects of carbosulfan in rainbow trout *Oncorhynchus mykiss*. *Pestic Biochem Physiol* 102:61–67
- APHA [American Public Health Association] American Water Works Association and Water Pollution Control Federation (APHA/AWWA/WEF) (2005) Standard methods of examination of water and wastewater, 21st edn. APHA, Washington
- Arise RO, Malomo SO (2009) Effects of ivermectin and albendazole on some liver and kidney function indices in rats. *Afr J Biochem Res* 3(5):190–197
- Athanassopoulou FV, Ragias V, Roth M, Liberis N, Hatzinnikolau S (2002) Toxicity and pathological effects of orally and intraperitoneally administered ivermectin on sea bass *Dicentrarchus labrax*. *Dis Aquat Organ* 52:69–76
- BIO [Intelligence Service] (2013) Study on the environmental risks of medicinal products, Final Report prepared for Executive Agency for Health and Consumers. Pp 184–200
- Black KD, Fleming S, Nickell TD, Pereira PMF (1997) The effects of ivermectin, used to control sea lice on caged farmed salmonids, on infaunal polychaetes. *ICES J Mar Sci* 54:276–279
- Blahova J, Pihalova L, Hostovsky M (2013) Oxidative stress responses in Zebra fish *Danio rerio* after sub-chronic exposure to atrazine. *Food Chem Toxicol* 61:82–85. <https://doi.org/10.1016/j.fct.2013.02.041>
- Boonstra H, Reichman EP, vanden Brink PJ (2011) Effects of the veterinary pharmaceutical ivermectin in the indoor aquatic microcosms. *Arch Environ Contam Toxicol* 60:77–89
- Boxall ABA (2010) Veterinary medicines and the environment. *Comp Vet Pharmacol Handb Exp Pharmacol* 199:291–313
- Cabello FC (2006) Heavy use of prophylactic antibiotics in aquaculture: a growing problem for human and animal health and for the environment. *Environ Microbiol* 8(7):1137–1144
- Campbell WC, Fisher MH, Stapley EO, Albers-Schonberg G, Jacob TA (1983) Ivermectin: a potent new antiparasitic agent. *Science* 221:823–828
- Campbell WC (1989) Ivermectin and abamectin. Springer-Verlag, New York
- Carlsson G, Patring J, Kreuger J, Norrgren L, Oskarsson A (2013) Toxicity of 15 veterinary pharmaceuticals in zebrafish (*Danio rerio*) embryos. *Aquat Toxicol* 126:30–41
- CCREM (1991) Canadian water quality guidelines. Ottawa, Canadian Council of Resources and Environmental Ministry, Inland Waters Directorate, Environment Canada
- Cooper GR, McDaniel V (1970) The determination of glucose by the O-toluidine method. *Stand Method Clin Chem* 6:159–162
- CWQC [Committee on Water Quality Criteria] (1972) A report of the committee on water quality research series, EPA-R3-73-003, US Environmental Protection Agency report. Cincinnati, CWQC
- Das BK, Mukherjee SC (2003) Toxicity of cypermethrin in *Labeo rohita* fingerlings: biochemical enzymatic and haematological consequences. *Comparative biochemistry physiology part C. Toxicol Pharmacol* 134:109–121
- Didier AD, Loor F (1995) Decreased biotolerability for ivermectin and cyclosporin A in mice exposed to potential P-glycoprotein inhibitors. *Int J Cancer* 63:263–267
- Dybas RA (1989) Abamectin use in crop protection. In: Campbell WC (ed) Ivermectin and abamectin. Springer-Verlag, New York, pp 287–310
- Duce IR, Scott RH (1985) Actions of dihydro-avermectin B1a on insect muscle. *Brit J of Pharmacol* 85(2):395–401
- FAO (2018) The State of World Fisheries and Aquaculture 2018 - Meeting the sustainable development goals. Rome. Licence: CC BY-NC-SA 3.0 IGO
- Finney YT (1971) Probit analysis. Cambridge University Press, Cambridge
- Forbes AB (1993) A review of regional and temporal use of avermectins in cattle and horses worldwide. *Vet Parasitol* 48:19–28
- Fraisal ASR (2003) Adverse effects of some antimicrobial agents used in fish. Unpublished Ph.D. Thesis. Faculty of Veterinary Medicine, Cairo University Cairo. pp238
- Geary TG, Moreno (2011) Macrocyclic lactone anthelmintics: Spectrum of activity and mechanism of action. *Curr Pharmaceut Biotech* 13(6):8662–872
- Gonzalez-Rey M, Bebianno MJ (2012) Does non-steroidal anti-inflammatory (NSAID) ibuprofen induce antioxidant stress and endocrine disruption in mussel *Mytilus galloprovincialis*? *Environ Toxicol Pharmacol* 33:361–371
- Gutteridge JMC (1995) Lipid peroxidation and antioxidants as biomarkers of tissue damage. *Clin Chem* 41:1819–1828
- Hally BA, Jacob TA, Lu AYH (1989) The environmental impact of the use of ivermectin: Environmental effect and fate. *Chemos* 18:1543–1563
- Halley BA, VandenHeuvel WFA, Wislocki PG (1993) Environmental effects of the usage of avermectins in livestock. *Vet Parasitol* 48(1–4):109–125
- Halliwell B, Gutteridge JMC (1999) In: free radicals in biology and medicine, Oxford University press, Oxford; pp. 543
- Hart WB, Weston RF, Demann JG (1948) An apparatus for oxygenating test solution in which fish are used as test animals for evaluating toxicity. *Trans Am Fish Soc* 75:288–295
- Hoy T, Horsberg TE, Nafstad I (1990) The deposition of ivermectin in Atlantic salmon (*Salmo salar* L). *Basic Clin Pharmacol Toxicol* 67(4):307–312
- Iglesias A, Nebot C, Miranda JM, Vazquez BI, Cepeda A (2012) Detection and quantitative analysis of 21 veterinary drugs in river water using high-pressure liquid chromatography coupled to tandem mass spectrometry. *Environ Sci Pollut Res* 19:3235–3249

- Iheanacho SC, Ikwo TN, Igweze NO, Chukwurdha C, Ogueji EO, Onyeneke R (2018) Effects of different inclusion levels of melon seed (*Citrullus canatus*) peel on growth, haematology and histology of *Oreochromis niloticus* juvenile. *Turk J Fish Aquat Sci* 18(3):379–384
- IJC (1977) New and Revised Great Lakes Water Quality Objectives. Great Lake Basin, Windsor. IJC, Ottawa
- Iqbal S, Suhel P, Suwarna P, Bilal B, Rizwanul H, Sheikh R (2003) Oxidative stress biomarkers of exposure to deltamethrin in freshwater fish, *Channa punctatus*. *Ecotoxicol Environ Saf* 56:295–299
- Islas-Flores H, Gómez-Oliván LM, Galar-Martínez M, García-Medina S, Neri-Cruz N, Dublán-García O (2014) Effect of ibuprofen exposure on blood, gill, liver, and brain on common carp (*Cyprinus carpio*) using oxidative stress biomarkers. *Environ Sci Pollut Res* 21:5157–5166. <https://doi.org/10.1007/s11356-013-2477-0>
- Johnson SC, Margolis L (1993) Efficacy of ivermectin for control of the salmon louse *Lepeophtheirus salmonis* on Atlantic salmon. *Dis Aquat Org* 17:101–105
- Kegley SE, Hill BR, Orme S, Choi AH (2016) *PAN Pesticide Database*, pesticide action network, North America (Oakland, CA.). <http://www.pesticideinfo.org>
- Lawrence RA, Burk RF (1976) Glutathione peroxidase activity in selenium deficient rat liver. *Biochem Biophys Res Commun* 71:952–958
- Lowry OH, Resebrough NJ, Farr AL, Randall RJ (1951) Protein measurements with folin phenol reagent. *J Biol Chem* 193:265–275
- Lumeret JP (2012) A review on the toxicity and non-target effects of macrocyclic lactones in terrestrial and aquatic environments. *Curr Pharm Biotechnol* 13:1004–1060
- Luskova V, Svoboda M, Kolarova J (2002) The effects of Diazinon on Blood Plasma Biochemistry in Carp (*Cyprinus carpio* L). *Acta Veterinaria Brno* 71:117–123
- Misra HP, Fridovich I (1972) The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *J Biol Chem* 247:3170–3175
- NAS/NAE (1973) Water quality criteria, EPA-R3–033. US Government Printing Office, Washington
- Nwani CD, Somdare PO, Ogueji EO, Nwani JC, Ukonze JA, Nwadinigwe AO (2017) Genotoxicity assessment and oxidative stress responses in freshwater African catfish *Clarias gariepinus* exposed to fenthion formulations. *Drug Chem Toxicol* 40(3):273–280
- Ogueji EO, Nwani CD, Iheanacho SC, Mbah CE, Okeke OC, Ibrahim BU (2017a) Acute toxicity of ibuprofen on selected biochemical and oxidative stress parameters of liver in *Clarias gariepinus* juveniles (Burchell, 1822). *J Entomol Zool Stud* 5(4):1060–1068
- Ogueji EO, Nwani CD, Iheanacho SC, Mbah CE, Okeke OC, Ibrahim BU (2017b) Toxicity of diazepam on lipid peroxidation, biochemical and oxidative stress indicators on liver and gill tissues of African catfish *Clarias gariepinus* (Burchell, 1822). *Int J Fish Aquat Stud* 5(3):114–123
- Oliveira R, Grisolia CK, Monteiro MS, Soares AMVM, Domingues I (2016) Multilevel assessment of ivermectin effects using different zebrafish life stages. *Comp Biochem and Physiol, Part C* 187:50–61. <https://doi.org/10.1016/j.cbpc.2016.04.004>
- Pereira L, Fernandez MN, Martinez CBR (2013) Hematological and biochemical alterations in the fish *Prochilodus lineatus* caused by the herbicide clomazone. *Environ Toxicol Pharmacol* 36:1–8
- Prasse C, Loffler D, Ternes TA (2009) Environmental fate of the anthelmintic ivermectin in an aerobic sediment/water system. *Chemosphere* 77:1321–1325
- Rauf A, Arain N (2013) Acute toxicity of diazinon and its effects on hematological parameters in the Indian carp, *Cirrhinus mrigala* (Hamilton). *Turk J Vet Anim Sci* 37:535–540
- Reish DL, Oshida P S (1987) Manual of methods in aquatic environment research Part 10. Short term static bioassay FAO fisheries. Technical paper 247 FAO Rome 1- 62pp
- Roberts RJ, Johnson KA, Casten MT (2004) Control of *Salmincola forniensis* (Copepoda: Lernaeopodidae) in rainbow trout, *Oncorhynchus mykiss* (Walbaum): a clinical and histopathological study. *J Fish Dis* 27(2):73–79
- Saravanan M, Ramesh M (2013) Short and long-term effects of clofibrac acid and diclofenac on certain biochemical and ionoregulatory responses in an Indian major carp, *Cirrhinus mrigala*. *Chemosphere* 93:388–396
- Saravanan M, Devi KU, Malarvizhi A, Ramesh M (2012) Effects of ibuprofen on hematological, biochemical and enzymological parameters of blood in an Indian major carp, *Cirrhinus mrigala*. *Environ Toxicol Pharmacol* 34:14–22
- Serafini S, Sauza FC, Baldisserotto DM, Picoli F, Segat JC, Baretta D, Silva AS (2019) Fish exposed to eprinomectin show hepatic oxidative stress and impairment in enzymes of the phosphotransfer network. *Aquaculture* 508:199–205
- Sharma SK, Krishna-Murti CR (1968) Production of lipid peroxides by brain. *J Neurochem* 15:147–149
- Shoeb Q (2013) Biochemical toxicity of Ivermectin in Wistar albino rats. *Am Eurasian J Toxicol Sci* 5(1):15–19
- Shoop W, Soll M (2002) Ivermectin, abamectin and eprinomectin. In: Vercruyse J, Rew RS (eds) *Macrocyclic lactones in antiparasitic therapy*. Cabi, Oxon, pp 1–29
- Somdare PO, Nwani CD, Nwadinigwe AO, Nwani JC, Odo GE, Ugbor ON, Ezeibe AB (2015) Fenthion induced toxicity and histopathological changes in gill tissue of freshwater African catfish, *Clarias gariepinus* (Burchell, 1822). *Afr J Biotechnol* 14(25):2103–2113
- Sprague JB (1977) Measurement of pollutant toxicity to fish: in: bioassay methods for acute toxicity. *Water Res* 3:793–821
- Thiripurasundari M, Sathya K, Uma A, Srinivasan MR, Rajasekar P (2014) A comparative study on the toxicity of ivermectin in zebra fish and catla fish models. *Indo Am J Pharm Res* 4(09):87–89
- Ucan-Marin F, Ernst W, Keith O'Dor R, Sherry J (2012) Effect of food borne ivermectin on juvenile Atlantic salmon (*Salmo salar* L). Survival, growth, behavior and physiology. *Aquaculture* 334(337): 169–175
- Varo I, Rigos G, Navarro JC, Delramo J, Calduchginer J, Hernandez A, Pertusa J, Torreblanga A (2010) Effect of ivermectin on the Gilthead seabream *Sparus aurata*: a proteomic approach. *Chemosphere* 80(5):570–577
- Wedemeyer G, Mcleay DJ, Goodyear CP (1984) Assessing the tolerance of fish and fish population to environmental stress. The problems and methods of monitoring. In: Cairns WV, Hodson PV, Nriagu JO (eds) 164-195pp *Contaminant effects on fisheries*. John Wiley and son Inc, New York
- Zhang JF, Shen H, Wang XR, Wu JC, Xue YQ (2004) Effects of chronic exposure of 2, 4-dichlorophenol on the antioxidant system in liver of freshwater fish *Carassius auratus*. *Chemosphere* 5:167–174