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The effect of short term treatment with ivermectin on the oxidative stress parameters in the tissues of *Clarias gariepinus* (Burchell, 1822), juvenile

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Abstract: The effect of short term treatments with ivermectin on the oxidative stress parameters in the tissues of *Clarias gariepinus* juveniles was studied between July and September 2015. Three hundred healthy juveniles of fresh water African catfish, *Clarias gariepinus* were used for the study. The present study investigated the behavioural responses, lethal concentration of ivermectin and the effect of sublethal concentrations on lipid peroxidation , antioxidant parameters and acute toxicity in the tissue of C. gariepinus. All animals were divided into 7 groups: Gp 1 (Tap water) served as the untreated control (0) while groups 2- 7 were treated with graded levels of ivermetin 0.15, 0.30, 0.45, 0.60, 0.75, 0.90mg/l body weight and was done for 28 days. The percentage mortality increased as the concentrations increased. The 24, 48, 72 and 96h Lc_{50} of ivermectin were: 0.75, 0.62, 0.45, 0.38mg/L respectively indicating four groups. The tissues of fish were exposed to various sublethal levels of ivermectin. Catalase, lipid peroxidation, glutathione peroxidase and glutathione reductase, superoxide dismutase activities of liver, and gill was highest in the control when compared to other groups.

Keywords: *Clarias gariepinus*, Ivermectin, oxidative stress, catalase superoxide dismutase, glutathione peroxidase, glutathione reductase

Introduction

Living systems encounter a variety of stresses during their continuous interaction with environment. Environmentally induced stresses frequently activate the endogenous production of reactive oxygen species (ROS), most of which are generated as side products of tissue respiration. Hence, constant exposure to stressors may enhance ROS mediated oxidative damage. Oxidative damage may be minimized by antioxidant defence mechanisms that protect the cell against cellular oxidants and repair systems that prevent the accumulation of oxidatively damaged molecules. Oxidative stress is also said to be the presence of reactive oxygen species in excess of the buffering capacity of variable antioxidants as recorded by Yong et al. (2015) from a biological view point, various active oxygen species are generated in the body during the process of utilizing of oxygen, the body is furnished with elaborate mechanisms, to remove active oxygen species and free radicals. These by products of oxygen metabolism are not necessarily a threat to the body under physiological conditions. However, if active oxygen species or free radicals are generated excessively or at abnormal sites the balance between formation and removal is lost, resulting in oxidative stress, thus inducing various diseases. The major ROS that are of physiological significance are superoxide anion (O^-_2), hydroxyl radical (OH), and hydrogen peroxide (H_2O_2). Peroxidation of lipids disturbs the integrity of cell membranes and leads to rearrangement of membrane structure. Glutathione peroxidase (GSH-Px) is a protective antioxidant that acts along with catalase (CAT) in scavaging out hydrogen peroxide to ensure optimum protection against oxidative stress and tissue specific damage. Glutathione reductase (GSH-R) catalyzes the reduction of glutathione disulfide (GSSG) to the sulfhydryl form glutathione (GSH), which is a critical molecule in resisting oxidative stress and maintaining the reducing environment of the cell. The ratio of GSSH/GSH present in the cell is a key factor in properly maintaining the oxidative balance of the cell, that is, it is critical that the cell maintains high levels of the reduced glutathione and a low level of the oxidized glutathione disulfide. This narrow balance is

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maintained by glutathione reductase, which catalyzes the reduction of GSSG to GSH as advocated by Deponte, (2013). Ratio between oxidized and reduced glutathione (2GSH/GSSG) is one of the important determinants of oxidative stress in the body. Higher production of ROS in body may change DNA structure, result in modification of proteins and lipids, activation of several stress-induced transcription factors, and production of proinflammatory and anti-inflammatory cytokines. Significant decreases in the activity of tissue superoxide dimutase (SOD) and brain GSH-Px have been reported after lead exposure (Tripathi *et al*., 2001). Superoxide dimutase plays an important role and helps to convert superoxide radical to hydrogen peroxide for possible conversion to water and molecular oxygen by CAT as advocated by Shao *et al*. (2012). Nwani *et al*., (2013) reported on stress as a cofactor in the progression and severity of several diseases. The principal effect of stress on the liver is related solely to changes in hepatic blood flow with resultant vasospasm, centrilobular hypoxia and ultimately liver damage. Oxidative stress contribute to many pathological conditions such as chronic fatigues syndrome, chronic obstructive pulmonary disease was reported by Ahmed *et al*. (2014), Dut *et al*.(2008), Jenner (2003) and Sharma *et al*. (2006). Ivermectin is a broad spectrum antiparasitic drug, white yellowish-white nonhygroscopic, crystalline powder and semi-synthetic antihelmintic with a melting point of about 155°C administered orally, through subcutaneous injection or as a pour-on formulation and which is effective in controlling nematode and parasitic arthropods in a wide variety of host species were reported by Davies *et al*. (1998); Mladineo *et al*. (2006) and Tremblay *et al*. (2012). The use of ivermectin in the agricultural industry has increased their presence in the environment, because ivermectin administered to animals and humans are excreted in the faeces. The efficacy of this drug is dependent on the toxic concentrations presented to the parasite and the duration to cause an irreversible damage that may cause harm to the organism. The presence of ivermectin in water therefore, even at sublethal dose can elicit behavioural, morphological, haematological, histological and biochemical alterations as fishes are generally very sensitive to contamination of their environment. Ahmed *et al*., (2014) investigated the physiological and oxidative stress biomarkers in the Freshwater Nile Tilapia, *Oreochromis Niloticus* L., exposed to sublethal doses of Cadmium. The need to investigate whether short term exposure to sublethal concentrations of the antiparasitic drug (ivermectin), has an effect on the antioxidant status and oxidative stress of African catfish (*Clarias gariepinus*) becomes imperative. There is therefore need to determine the acute toxicity of the drug (ivermectin) and its effect on the behaviour of *C. gariepinus* under laboratory exposure. The fish presents a good model for ecotoxicology studies due to the fact that it can readily withstand extreme environmental conditions. In Nigeria, there is paucity of scientific documentation on toxicological effects of ivermectin on most indigenous fish species of Africa, hence *Clarias gariepinus* has been selected for the study because, it is widely distributed in Africa and other tropical countries of the world. *Clarias gariepinus* inhabits a wide variety of areas and are known to thrive where many other fishes cannot. Studies on *Clarias*, have shown that it is very hardy and adaptable animal, sufficiently able to exploit a wide variety of animal under environmental condition hence its use for experimental model to investigate on the lethal concentrations of ivermectin on *C. gariepinus* based on the time of exposure and sublethal levels of ivermectin on lipid peroxidation and oxidative stress. This article's objectives therefore are to investigate behavioural responses of fish to ivermectin. Determine the cumulative mortality caused by different doses of ivermectin. Determine the behavioral characteristics of *C. gariepinus* at different concentrations of ivermectin. Assess the lethal concentration (Lc_{50}) of ivermectin on *C. gariepinus* on (95% confidence intervals) based on the time of exposure. Evalute the end points of acute toxicity testing in *C. gariepinus* exposed to ivermectin for different durations (24, 48, 72 and 96 h). Determine the effects of exposure to various sublethal levels of ivermectin on lipid peroxidation and oxidative stress parameters in *C. gariepinus*.

Materials and methods

Animal Stress Procedure and Treatments

Experimental Fish

Three hundred healthy juveniles of fresh water African catfish, *Clarias gariepinus* (Family: Clariidae: Order, Siluriformes; Genus: *Clarias*) with the mean weight of 159.8 ± 42g and standard mean length of 30.18 ± 5cm were obtained from a reputable fish farm in Nsukka, Enugu State, Nigeria and transported to the Fisheries laboratory, Department of Zoology and Environmental Biology, University of Nigeira, Nsukka. They were acclimatized for three weeks in a 3001

capacity plastic tank, during which they were fed 3% of their body weight in divided rations, twice daily (7.00am and 7.00pm) with locally prepared floating pelleted diet, containing 35% crude protein as recommended by Eyo *et al.* (2013). All animals were cared for according to guidelines of the Institutional Animal Ethics. The experiment duration was 4 weeks. The fishes were subjected on a bath treatment with tetracycline to avoid possible dermal infection as a result of injuries sustained from the stress of transportation. The faecal matter and other waste materials were siphoned off daily to reduce ammonia content in water. Dead fish were immediately removed with forceps to prevent deterioration of water quality. Feeding of the fishes was terminated 24h before the range finding and acute toxicity test to avoid interference of faeces as recorded by Ward and Parrish (1982) and Reish and Ohida (1987).

Determination of Lc_{50} concentration

Acute toxicity test was carried out in a plastic tank (25 liter capacity each), which contained 10liters of well de-chlorinated and aerated tap water each, which was used in determining the Lc_{50} of ivermectin. All animals were divided into 7 groups. Seven different concentrations (Tap water (0), 0.15, 0.30, 0.45, 0.60, 0.75 and 0.90 mg/1 body weight) were selected for definitive exposure after a series of range finding tests. Group 1serves as the controls (received tap water as drinking source), groups 2-7 were given graded levels of ivermectin. The test was set in triplicate along with the control. A set of 10 fish specimen were randomly exposed to each test concentration, another set of 10 fish were kept on a normal tap water that does not have the test drug (ivermectin) and it is considered as the control. The water in the tanks was changed on daily basis and at same time, the test solution was changed in alternate day inorder to counter-balance the decreasing drug concentration. The behavioural changes such as fin and opercula movements, equilibrium status, swimming rate, feeding frequency and skin coloration, during the test period were observed. The percentage mortality was recorded at intervals of 24, 48, 72 and 96 hours. The Lc_{50} value (with 95% confidence limits) of different concentrations of the test drug in *C. gariepinus* was determined using the probit analysis method (Finney, 1971), and it was found to be; 0.75 (0.70-0.83), O.62 (0.58-0.65), O.45 (0.31-0.59) and 0.3. The safe level of the test drug was estimated by multiplying the 96h Lc_{50} with different application factors (AF) as was proposed by Sprague, (1971), Committee on Water Quality Criteria (CWQC, 1972), and the International Joint Commission IJC, (1977). Dead fishes were removed with forceps to avoid contamination of the water. The mean water quality of the test solution determined in the experimental tanks following the standard method (APHA *et al*., 2005) were: dissolved oxygen 7.02 $\pm$ 0.46mg/1, temperature 25.70 $\pm$ 0.86°C, pH 7.04 $\pm$ 0.34, conductivity 275 $\pm$ 2.30µScm-1 and total hardness 202.5 $\pm$ 4.45mg/1 as $CaCO_3$.The experiment was carried out in an indoor system under normal photoperiod of day/night (12:12) illumination prevalent around July and August at Nsukka, Nigeria during the period under study.

Determination of sub-lethal concentration and in vivo exposure experiment

The 96h Lc_{50} value of ivermectin on *C. gariepinus* based on the probit analysis was determined to be 0.38mg/L. On the basis of this value, two sub-lethal concentrations of 19µg/L and 38µg/L which corresponds to the 1/20 and 1/10 of the 96h Lc_{50} of ivermectin were used for the in vivo exposure. The sum total of 90 acclimatized fish specimens were exposed to the sub-lethal concentration in 3 replicates having 10 fish per replicate. The selection was made randomly regardless of the sex. Some fish specimen was subjected to normal tap water and is regarded as the control. The exposure lasted for a period of 21days and in order not to starve the fish; they were being fed daily with small quantity of food which was approximately 1% of their body weight about an hour before the test solution was renewed. The organs from each experimental group and control were collected at intervals of 1,7,14 and 21 days. Prior to the collection of the tissues, the experimental fish were anesthetized with tricaine methanesulfonate (MS 222) in order to minimize stress.

Determination of Liver and gill Malondialdehyde (MDA) Contents

The fish specimens were dissected and the liver and gill tissues were collected and rinsed in a washing buffer (1.15% KCl) after which the tissues were homogenized immediately in a homogenizing buffer (50mM Tris-HCl, 1.15% KCl). Five parameters of the antioxidants and oxidative stress were analyzed and the averages were being recorded as mean $\pm$ SE. The various organs (liver and gills) excised from the animals were washed differently in 1.15% KCL, dried

on filter paper, weighed and recorded. The organs were differently homogenized in four parts of homogenizing buffer that is, one gram of organ in 4ml of buffer and centrifuged at 10,000revolution per minute (rpm) for 15minutes in an ultracentrifuge at temperature ≤ 2 degree Celsius. The supernatant was collected after centrifugation and kept of the freezer ≤ 2 degree Celsius. Each time the supernatant was outside the freezer, it was kept in ice bags.

Determination of Antioxidants enzymes

1) Lipid peroxidation Assay content was estimated according to the method of Buge and Aust, (1978); Halliwell and Chirico (1993). Malondialdehyde (MDA) is formed during oxidative degeneration as a product of free oxygen radicals, which is accepted as an indicator of lipid peroxidation. 2.0 ml of TCA-TBA-HCl reagent was added to 1.0ml of the sample and mixed thoroughly. The solution was heated for 15mins in a boiling water bath. After cooling, the flocculent precipitate was removed by centrifuging at 1000rpm for 10minutes. The pink chromogen was detected spectrophotometrically at 535nm against a blank that contains the entire reagent except the sample. The results were expressed as nmoles of MDA formed/min/mg protein using an extinction coefficient of the chromophore 1.56×105 Mcm and expressed as nmoles of MDA formed/min/mg of protein.

2) Catalase Assay

Catalase activity assayed according to the method of Beers and Sizer, (1952) with peroxide (30mM) as substrate. One unit of catalase activity is defined as the micromoles of peroxide consumed per minute per milligram of protein sample.

3) Superoxide Dimutase Assay

The liver superoxide dismutase activity was assayed according to the method of Misra and Fridovich, (1972). This procedure depends upon the antioxidation of epinephrine (1.8mM) in the presence of 1.0 of 0.1M carbonate buffer (pH 10.2). The absorbances was measured at 480nm in a Shimadzu UV spectrophotometer. One unit of superoxide dismutase activity is the amount of protein required for 50% of inhibition of epinephrine antioxidation/minute/mg of protein.

4) Glutathione peroxidase Assay (GSH-Px)

Hepatic glutathione peroxidase was assayed according to the method of Habig *et al*. (1974) using DTNB [5,5 dithiobis (2-nitrobenzoic acid)] reagent (1.0ml) as substrate and 3.0ml of 0.3M disodium hydrogen phosphate added to the supernatant. Enzyme activity is measured by following the increase in absorbance at 420nm. The reaction mixture consisting of 0.2ml of 0.8mM EDTA, 0.1ml of 10mM sodium azide, 0.1ml of 2.5mM H_2O_2, 0.2ml of GHS, 0.4ml of 0.4mM phosphate buffer (PH 7.0) and 0.2ml of homogenate was incubated at 37°C for 10minutes. The reaction was arrested by the addition of 0.5ml of 10% TCA and tubes were centrifuged at 2000rpm. The activity of GSH-Px was expressed as μM of glutathione oxidized/minute/mg of protein

5) Glutathione Reductase Assay (GSH-R)

The glutathione Reductase was assayed by adding 1ml of 1mM NADPH and 1.0ml of 0.5mM sodium phosphate buffer (PH 7.6), 0.5ml of the tissue homogenate was added and mixed thoroughly. The absorbance was measured at 540nm. The activity of GSH-R was expressed as μM of glutathione reduced/min/mg of protein.

Statistical Analysis

The values obtained were expressed as mean ± SD. All data were analysed with SPSS 17.0 computer program. The hypothesis testing method include three-way analysis of variance (ANOVA) to determine to significant differences between tissues, concentrations and sampling durations. The values of $p<0.05$ were considered to be statistically significant.

Results

Behavioral responses and Mortality

Fish exposed to different acute concentrations of ivermectin, display some kind of behavioural and physiological abnormalities such as;loss of equilibrium, fin movement, decrease in feeding frequency, swimming rate and skin discoloration (Tab. 1). As the exposure duration increases, it leads to exhaustation, weakness and general decrease in the responses of the fish. Normal behavior was observed in the control fish. Fish exposed to 0.15mg/L, showed normal behavior all through the time of exposure. Also, fish exposed to 0.3mg/L, showed normal behavior for the first 48h but afterwards the alert fish stopped their movement and remained static in response to the changes in the surrounding environment. Generally, fish exposed to higher concentration of the drug, showed abnormal behavior and try to avoid the test water by displaying an obnoxious movement such as; faster swimming, jumping and other erratic movements.

Tab. 1: Behavioral characteristics of *C. gariepinus* at different concentrations of ivermectin.

Duration (h)	Concentration (mg/1)	Equilibrium status	Skin discoloration	Feeding frequency	Swimming rate	Fin movement
24h	Control	+++	-	+++	+++	+++
	0.15	+++	-	+++	+++	+++
	0.30	+++	-	+++	+++	+++
	0.45	+++	-	++	++	+++
	0.60	++	+	+	++	++
	0.75	+	++	+	+	++
	0.90	+	+++	-	+	+
48h	Control	+++	-	+++	+++	+++
	0.15	+++	-	+++	+++	+++
	0.30	+++	-	++	+++	++
	0.45	++	+	++	++	++
	0.60	++	++	+	++	+
	0.75	+	+++	-	+	+
	0.90	+	+++	-	+	+
72h	Control	+++	-	+++	+++	+++
	0.15	+++	-	+++	+++	+++
	0.30	++	+	++	+++	++
	0.45	++	++	+	++	+
	0.60	+	++	+	++	+
	0.75	+	+++	-	+	+
	0.90	-	+++	-	-	-
96	Control	+++	-	+++	+++	+++
	0.15	+++	-	+++	+++	+++
	0.30	++	+	+	++	+
	0.45	+	++	+	+	+
	0.60	+	+++	-	+	+
	0.75	-	+++	-	-	-
	0.90	-	+++	-	-	-

None (-), Mild (+), Moderate (++) and Strong (+++)

The mortality of fish in the treatment group during the acute exposure, increased with increasing concentration and the duration of exposure to the drug (Tab. 2). The mortality rate of 17% was observed after 96h in fish exposed to 0.15mg/l of ivermectin while 100% mortality was observed in fish exposed to 0.90mg/l at the same period of time.

Tab. 2: Cumulative mortality of *C. gariepinus* exposed to various concentrations of ivermectin.

Concentration (mg/l)	Number of deaths						
	Exposed	24h	48h	72h	96h	Mortality (%)	Survival (%)
Control	30	0	0	0	0	0	100
0.15	30	0	0	0	5	17	83
0.3	30	0	0	10	10	33	67
0.45	30	0	0	1	15	50	50
0.6	30	10	15	20	67	67	33
0.75	30	15	25	25	25	83	17
0.9	30	20	30	30	30	100	100

Median lethal concentration (Lc_{50}) and Application factors (AF): As the exposure time increased from 24 to 96h, the median lethal concentration (Lc_{50}) required in killing the fish also reduced to that at 24, 48, 72 and 96h, the Lc_{50} with confidence intervals was; 0.75 (0.70-0.83), 062 (0.59-0.65), 0.45 (0.31-0.59) and 0.38 (0.25-0.15) respectively (Tab. 3). The estimated safe levels of the drug were determined by multiplying the 96h Lc_{50} with different AF (Tab. 3) The values of the safe level of the drug (ivermectin) in *C. gariepinus* varied from 3.8x10-2 to 3.8x10-6mg/L. The results of statistical end points of toxicity testing (No effective concentration (NOEC), Low effective concentration (LOEC) and Lethal concentration (Lc_{50}) for the drug are shown in Figure 1. *C. gariepinus* exposed to ivermectin, NOEC, LOEC and Lc_{50} showed as minimal variation except in Lc_{50} which showed variations with respect to the exposure time.

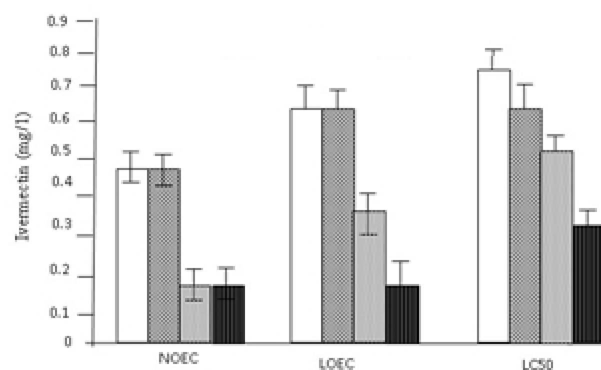

Fig. 1: Statistical end points of acute toxicity testing in *C. gariepinus* exposed to ivermectin for different durations (24, 48, 72 and 96h respectively) Where NOEC: No effective concentration; LOEC: Low effective concentration; Lc_{50}: Lethal concentration.

Effects of lipid peroxidation and antioxidant enzyme

The effect of different sub-lethal concentrations of ivermectin of lipid peroxidation in the form of TBARS formation and the responses of other antioxidants enzymes (CAT, SOD, GSH-Px and GSH-R) in the liver and gill of tissues of *C. gariepinus* are presented in Table 4. There was a reduction in LPO in both tissues of *C. gariepinus* exposed to ivermectin throughout the duration of the experiment. Also, there was no significant difference ($p<0.05$) in the values of LPO when both tissues were compared on each of the days of exposure.There was no significant difference ($p>0.05$) in the values of CAT in both tissues of *C. gariepinus* exposed to ivermectin throughout the

Tab. 3: Lethal concentrations of ivermectin on *C. gariepins* (95% confidence intervals) based on the time of exposure

Lethal Concentration	24h	48h	72h	96h
LC_{10}	0.50 (0.41-0.57)^{a}	0.51 (0.45-0.55)^{a}	0.25 (0.08-0.34)^{a}	0.15 (0.04-0.24)^{b}
LC_{20}	0.58 (0.51-0.64)^{a}	0.54 (0.49-0.58)^{a}	0.30 (0.13-0.40)^{a}	0.21 (0.08-0.30)^{b}
LC_{30}	0.64 (0.58-0.69)^{a}	0.57 (0.53-0.60)^{a}	0.35 (0.19-0.45)^{a}	0.26 (0.12-0.35)^{b}
LC_{40}	0.70 (0.64-0.76)^{a}	0.60 (0.56-0.63)^{a}	0.40 (0.25-0.51)^{a}	0.31 (0.18-0.42)^{b}
LC_{50}	0.75 (0.70-0.83)^{a}	0.62 (0.59-0.65)^{a}	0.45 (0.31-0.59)^{a}	0.38 (0.25-0.51)^{b}
LC_{60}	0.82 (0.75-0.92)^{a}	0.65 (0.61-0.68)^{a}	0.51 (0.38-0.71)^{a}	0.45 (0.32-0.65)^{b}
LC_{70}	0.89 (0.81-1.04)^{a}	0.67 (0.64-0.72)^{a}	0.59 (0.45-0.89)^{a}	0.55 (0.41-0.88)^{b}
LC_{80}	0.98 (0.87-1.20)^{a}	0.71 (0.67-0.76)^{a}	0.68 (0.53-1.23)^{a}	0.69 (0.51-1.34)^{a}
LC_{90}	1.12 (0.97-1.46)^{a}	0.76 (0.71-0.83)^{a}	0.84 (0.63-1.98)^{a}	0.95 (0.66-2.53)^{a}

Values with different alphabetic superscripts differ significantly ($p<0.05$) between exposure durations within lethal concentrations.

Tab. 4: Effects of exposure to various sublethal levels of ivermectin on lipid peroxidation and oxidative stress parameters in *C. gariepinus*.

Parameter	Tissue	Ivermectin (ugl^{-1})	1 day	7 days	14 days	21 days
LPO	Liver	Control	2.96 (±0.87)^{a1A}	2.90 (±0.35)^{a1A}	2.88 (±0.93)^{a1A}	2.44 (±0.86)^{a1A}
		19	2.53 (±0.77)^{a1A}	2.30 (±0.55)^{a1A}	1.60 (±0.42)^{a1A}	1.32 (±0.87)^{a1A}
		38	2.44 (±0.96)^{a1A}	2.53 (±0.64)^{a1A}	1.68 (±0.45)^{a1A}	1.53 (±0.94)^{a1A}
	Gill	Control	2.89 (±0.54)^{a1A}	2.76 (±0.77)^{a1A}	2.48 (±0.68)^{a1A}	2.39 (±0.89)^{a1A}
		19	25.52 (±0.33)^{a1A}	1.90 (± 0.67)^{a1A}	1.47 (±0.94)^{a1A}	1.32 (±0.66)^{a1A}
		38	2.18 (±0.64)^{a1A}	1.69 (±0.74)^{a1A}	1.68 (±0.76)^{a1A}	1.40 ±1.40)^{a1A}
CAT	Liver	Control	1.67 (±0.83)^{a1A}	1.68 (±0.89)^{a1A}	1.69 (±0.64)^{a1A}	1.88 (±089)^{a1A}
		19	1.46 (±0.71)^{a1A}	1.67 (±0.71)^{a1A}	1.69 (±0.64)^{a1A}	1.83 (±0.38)^{a1A}
		38	1.38 (±0.94)^{a1A}	1.56 (±0.65)^{a1A}	1.68 (±0.87)^{a1A}	1.99 (±0.91)^{a1A}
	Gill	Control	1.66 (±0.86)^{a1A}	1.67 (±0.54)^{a1A}	1.73 (±0.81)^{a1A}	1.89 (±0.87)^{a1A}
		19	31.35 (±1.54)^{a1A}	1.47 (±0.64)^{a1A}	1.57 (±0.79)^{a1A}	1.75 (±0.80)^{a1A}
		38	1.38 (±0.83)^{a1A}	1.43 (±0.73)^{a1A}	1.59 (±0.77)^{a1A}	1.89 (±0.78)^{a1A}
SOD	Liver	Control	80.60 (±4.45)^{a1A}	78.24 (±3.76)^{a1A}	74.35 (±2.95)^{a1A}	69.89 (±3.09)^{a1A}
		19	64.56 (±2.31)^{a2A}	63.23 (±2.87)^{a2A}	66.21 (±1.99)^{a2A}	64.15 (±1.83)^{a2A}
		38	65.03 (±2.46)^{a2A}	65.29 (±2.86)^{a2A}	64.83 (±2.71)^{a2A}	67.81 (±2.76)^{a2A}
	Gill	Control	79.85 (±3.14)^{a1A}	75.86 (±3.05)^{a1A}	72.88 (±2.11)^{a1A}	70.11 (±1.86)^{a1A}
		19	65.01 (±2.08)^{a2A}	63.06 (±2.18)^{a2A}	62.28 (±1.88)^{a2A}	64.01 (±1.11)^{a2A}
		38	64.50 (±2.86)^{a2A}	63.58 (±2.66)^{a2A}	63.09 (±1.94)^{a2A}	66.67 (±1.55)^{a2A}

Tab. 4: Continued

Parameter	Tissue	Ivermectin (ugl^{-1})	1 day	7 days	14 days	21 days
GSH-PX	Liver	Control	37.45 (±1.19)^{a1A}	37.86 (±2.11)^{a1A}	39.42 (±2.06)^{a1A}	39.88 (±1.94)^{a1A}
		19	29.88 (±1.24)^{a2A}	30.42 (±1.64)^{a2A}	33.27 (±1.63^{b2A}	33.74 (±1.17)^{b2A}
		38	28.66 (±1.08)^{a2A}	29.96 (±1.19)^{a2A}	34.61 (±1.9)^{b2A}	35.27 (±1.65)^{b2A}
	Gill	Control	32.89 (±1.84)^{a1A}	33.24 (±2.07)^{a1A}	35.66 (±1.72)^{a1A}	33.48 (±2.07)^{a1A}
		19	31.35 (±1.54)^{a1A}	9.27 (±1.79)^{a1A}	30.66 (±1.15)^{b1A}	31.48 (±1.46)^{b2A}
		38	27.38 (±1.76)^{a1A}	29.29 (±1.83)^{a1A}	33.17 (±1.73)^{a1A}	31.48 (±2.73)^{a2A}
GSH- R	Liver	Control	4.86 (±0.85)^{a1A}	4.24 (±0.91)^{a1A}	3.97 (±0.91)^{a1A}	3.81 (±0.77)^{a1A}
		19	2.47 (±0.67)^{a2A}	2.54 (±0.68)^{a2A}	3.17 (±0.55)^{b1A}	3.70 (±0.47)^{b1A}
		38	2.77 (±0.73)^{a2A}	2.47 (±0.83)^{a2A}	4.04 (±0.83)^{b1A}	3.58 (±0.64)^{b1A}
	Gill	Control	4.54 (±0.19)^{a1A}	4.16 (±0.39)^{a1A}	3.75 (±0.27)^{a1A}	3.81 (±0.55)^{a1A}
		19	2.41 (±0.26)^{a1A}	2.20 (±0.22)^{a1A}	3.18 (±0.31)^{b1A}	3.47 (±0.61)^{b1A}
		38	2.13 (±0.34)^{a1A}	2.21 (±0.34)^{a1A}	3.63 (±0.28)^{b1A}	3.50 (±0.67)^{b1A}

Values with different alphabetic (lower case) superscripts differ significantly ($p < 0.05$) between exposure durations and tissue. Values with different numeric superscripts differ significantly ($p < 0.05$) between concentrations within exposure duration and tissue. Values with different alphabetic superscripts (upper case) differ significantly ($p < 0.05$) between tissues within exposure duration and concentration. Units of measurements are:lipid peroxidation ie MDA was assayed by the measurement of of thiobarbituric acid-reactive substances (TBARS, nmol TBARS mg protein-1), catalase (CAT, mmol min-1 mg protein-1) superoxide dismutase (SOD, U mg protein-1), glutathione reductase (GSH-R, nmol min-1mg protein-1) and glutathione peroxidase (GSH-Px, nmol min-1mg protein-1).

duration of the experiment. Significant difference in CAT did not also exist when both tissues (liver and gill) were compared.There was a concentration dependent significant decrease in the activity of SOD in both tissues of *C. gariepinus* exposed to ivermectin. In the liver for example, SOD decreased from 80.60 ± 4.45µmol min-1 in control to 65.03±2.46µmol min-1mg protein-1 in fish exposed to 38µgl-1 ivermectin.

GSH-Px: There was duration dependent significant increase in GSH-Px in the gills of *C. gariepinus* exposed to38µg/l of ivermectin from day 14 of the experiment. In the liver, there was significant increased in GSH-Px in *C. gariepinus* exposed to both concentration of ivermectin from day 14 of the experiment.Also, there was significant difference ($p>0.05$) in GSH-Px when both tissues were compared.

GSH-R: There was duration significant increase in GSH-R from day 14 in both tissues of *C. gariepinus* exposed to both sub-lethal concentration of ivermectin. In the gill for example, GSH-R increased from 2.13±0.34 nmol min/mg protein-1 on day 1 in *C. gariepinus* exposed to 38µg/l ivermectin to 3.50±0.67 nmol min-1mg protein-1 on day 21.

Discussion

This study demonstrated the toxic effect of ivermectin in the juveniles of the fresh water fish *C. gariepinus*. Toxicity of compounds to organisms has been known to be dependent on concentration, sex, development stages and exposure periods as recorded by Nwani *et al.* (2014); Pandey *et al*. (2011). This study showed that the abnormal behavioral alteration in ivermectin exposed fish indicated disturbances in the internal physiology of the fish and this may be attributed to the neurotoxic property of the drug. Behavioral changes are said to be among the most sensitive indicators of potential toxic effects of toxicants. This in line with the report of Banaee *et al*. (2011) and Nwani *et al*. (2014). The cumulative mortality increase as the concentration increased.The stressful behaviour observed in *C. gariepinus* was higher as the concentration of the drug increased and this may be said to be due to increased ache which eventually resulted to the death of the fish.

This in line with the study of Adedeji *et al.,* 2008). The present study showed that 0.3mg/l Lc_{50} in 96h obtained from ivermectin, indicates that the drug was toxic to *C. gariepinus* juveniles. In this study, on exposure to sub-lethal concentration of ivermectin to various concentration and time there was reduction in LPO in both tissues of *C. gariepinus,* there was also no significant difference in LPO when both tissues were compared on each of the days of exposure thus reflecting that there was reduction in oxidative stress and lipid peroxidation.This in line with the report of Davies *et al.,* 2010. The level of LPO and GSH in *Clarias gariepinus* treated group was found to be low when compared to the controls. During this study, the antioxidant system of *Clarias gariepinus* was severely impaired, causing a low level of LPO and high GSH. During the process of inflammation, oxidative stress occurs which leads to a significant decrease in antioxidant enzyme system. The main target of oxidative stress is the polyunsaturated fatty acids in cell membranes causing lipid peroxidation. The decreased LPO level in tissues indicated the decreased lipid peroxidation activity in the tissues. This agreed with the report of Sakin *et al*. (2012). The reduced values of LPO obtained in the present study, are not in agreement with previous report in fish exposed to primextra herbicide as recorded by Nwani *et al*., (2014) and Thamolwan *et al*.,(2016). The oxidative tissue damage in cirrhosis, causes a significantly low level of catalase. In the study, there was a concentration dependent significant decrease in the activity of SOD in both tissues of *C. gariepinus* exposed to ivermectin. According to Puerto *et al*. (2010), decrease in SOD is attributed to direct damage of its protein structure by the drug and increasing amounts of hydrogen peroxide produced. The low level of SOD when compared to the control indicates that the high risks of cell injuries. This study is in line with the study of Shao *et al*. (2012).There was time dependant significant increase in GSH-R of both tissues of *C. gariepinus*. GSH-R deficiency predisposes to oxidative damage which is thought to contribute to onset and progression of many disease states as advocated by Daniela *et al*. (2015).Thus, GSH-Px depletion in stress–treated fish may be correlated with increased susceptibility of the plasma membrane to peroxide attack, as reflected in changes in MDA levels. This reduction might have resulted from the oxidation of GSH-Px to stress-induced generation of free radicals. The depletion of GSH-Px further enhances the susceptibility of the lymphoid tissues to oxygen metabolites and acid-mediated cell damage.

In conclusion, the result of this study implies that increase concentration of ivermectin led to increases in cumulative mortality. Behavioral response such as feeding frequency, swimming rate and fin movement of *C. gariepinus* at highest concentrations of ivermectin were totally non-functional. Lethal concentrations of ivermectin on *C. gariepins* based on the time of exposure was felt mostly at highest concentration. Effects of exposure to various sub-lethal levels of ivermectin on lipid peroxidation and oxidative stress parameters in *C. gariepinus* showed the level of LPO and GSH, SOD in *Clarias gariepinus* treated groups were found to be low when compared to the controls. However, there were increases as the time progresses in GSH and SOD. This resulted to oxidative damage and stress which is thought to contribute to onset and progression of many disease states.

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