Effects of diclofenac on the oxidative stress parameters of freshwater fish *Oreochromis niloticus*

Abstract

The indiscriminate use and abuse of pharmaceuticals have led to pharmaceutical residues in the aquatic environment which has been receiving great attention since significant levels of contamination have been found. The present study investigated the acute and sub-lethal effects of a pharmaceutical drug diclofenac (DCF) on oxidative stress parameters and the recovery ability in ~~*Oreochromis*~~ *O. niloticus*. The juveniles were exposed to different concentrations of diclofenac to determine the 96 h LC_{50}. The results indicated that diclofenac was toxic to ~~*Oreochromis*~~ *O. niloticus* with a 96 h $LC_{50}$of 0.489mg/L. The percentage mortality increased as the concentrations increased. Fish were exposed to a control (0.00 mg/L) and three sub-lethal concentrations of 0.48, 0.32, and 0.25 mg/L of diclofenac for 28 days and allowed to recover for 7 days. The result of the sub-lethal test indicated that the responses were always dose and duration dependent. The oxidative stress results showed significant concentration- and time-dependent increases in the values of lipid peroxidation, glutathione peroxidase, glutathione reductase and reduced glutathione but reduction in catalase and superoxide dismutase in the liver of the exposed fish. Many of the oxidative parameters were found to be restored after the 7-day recovery period. These results showed that DCF exposure had a profound negative influence on the selected indices of *Oreochromis niloticus*.

Key words: Diclofenac,toxicity, oxidative stress, *Oreochromis niloticus*, Nigeria

Introduction

All over the world, there has been growing concerns about environmental quality in recent years both locally and internationally. Pharmaceutical drugs have become the focus of environmental

concerns as some of these drugs are not eliminated from environment by conventional wastewater treatment processes. Unlike other contaminants, pharmaceuticals are biologically active compounds designed to interact with specific physiological pathways in the target organism. Thus, they represent a class of emerging compounds able to affect specific animal functions (e.g., development, growth and reproduction) at notable concentrations. In addition, these drugs also exit the organisms, either unchanged or as metabolites (Reis Filo *et al*., 2007; Sore´ *et al*., 2010). The extensive use of veterinary pharmaceuticals (especially in the treatment of multiple reinfections) and wastes resulting from direct disposal by manufacturing plants, hospitals, and homes contribute to the build-up of the drugs in the environment. The runoff of the pharmaceuticals and metabolites into surface waters stemming from the treatment of livestock and pets may result in the contamination of natural water systems and is becoming a potential risk to non-target organisms (Iglesias *et al*., 2012). The improper disposal of unused and expired drugs leads to considerable concentrations of various pharmaceuticals in municipal sewage. Discharge of sewage treatment plants is one of the main sources of pharmaceuticals in the aquatic environment (Fent *et al*., 2006).

Among the pharmaceutical drugs frequently detected in the aquatic environment is diclofenac. The use of pharmaceutical products is on the increase in our world today, and this is as a result of the rise in global population as well as the increasing need for geriatrics to depend on drugs (Arnold *et al.*, 2014). According to Daughton (2003) it is likely to increase further in developing countries such as Nigeria where pharmaceutical production companies are flourishing due to increasing dependence on pharmaceuticals drugs. In Nigeria, the presence of acetaminophen and diclofenac in groundwater and surface water body has been confirmed by Olaitan *et al.* (2014). Diclofenac belongs to the class of nonsteroidal anti-inflammatory drugs (NSAIDs) with

analgesic and anti-inflammatory properties and is a widely prescribed drug (Stu-lten *et al*., 2008). Diclofenac is found at concentrations ranging from 0.2 to 2.3 mg/L in Brazil and European countries such as Austria, Germany, Sweden, Finland, and Switzerland (WHO, 2012). It has been associated with hepatotoxicity (Van Leeuwen *et al*., 2011), severe haemorrhagic gastroenteritis, and kidney failure in birds (Oaks *et al.*, 2004). Saravanan *et al*. (2011) observed that the exposure of carp to diclofenac resulted in hematologic and biochemical alterations. Stepanova *et al*. (2013) reported exposure to early stages of common carp (*Cyprinuscarpio*) to 3 mg/L of diclofenac for 30 days observed mortality and oxidative stress.

Pharmaceutical drugs represent a major group of substances of emerging concern and diclofenac, categorised in the therapeutic group of anti-inflammatory and analgesics, is a widely used pharmaceutical drug in the world and one of the most common pharmaceutical drug currently detected in the environment (UNESCO and HELCOM, 2017). Diclofenac remains one of the most used and widely sold anti-inflammatory and analgesics in the world and it has been utilised for an extended period of time. It has been widely detected in aquatic environments (e.g. 50 countries) and at concentrations that can be indicative of detrimental environmental effects (Zhang *et al*., 2008; Fick*et al*., 2009). In addition to its inclusion on the EU first watch list a recent data analysis indicated that diclofenac was among the 20 most sold pharmaceuticals in the Baltic Sea catchment area. Furthermore, it was also among the 20 pharmaceuticals with the highest concentrations in WWTP influent and effluent, showed very low levels of removal in conventional WWTP systems (circa 1%), and was among the 20 highest concentrations of measured pharmaceutical drugs detected in river water (UNESCO and HELCOM, 2017). It has also been detected in Baltic Sea biota at levels above threshold values (e.g. in Perch) (Karlsson

and Viktor, 2014; Hallgren and Wallenberg, 2015) and previous studies have linked toxic effects in marine organisms to high concentrations of diclofenac.

Diclofenac was included on the EU first watch list (2013/39/EU) withthe stated aim being to gather monitoring data for the purpose of facilitating the determination of appropriate measures to address the risk posed by those substances. Inclusion on such watch list is done when there is insufficient data to assess potential negative impacts on the environment, the assertion being based on results from the prioritization process of hazardous substances under the WFD, research results and similar reports.

There are no current restrictions on the use of diclofenac in the Nigeria, though in India for example, its uses being phased out due to the documented detrimental effects on vultures. Furthermore, the monitoring of diclofenac and the development of the indicator may have direct relevance to policies related to WWTPs and pharmaceutical disposal/take-back initiatives.

The strongest evidence of the detrimental effects of diclofenac stems from the terrestrial environment. Residues of diclofenac causing kidney failure is considered to be the main cause for a decline of >95 % in the population of oriental white-backed vulture, one of the (previously) most common raptors in India and Pakistan (Oaks *et al.*, 2004, Reddy *et al.*, 2006; Swan *et al.*, 2006; Cuthbert *et al.*, 2006,2007).

In the aquatic environment diclofenac has been shown to bioaccumulate in fish (Brown *et al.* 2007; Schwaiger*et al.*, 2004; Brozinski*et al.*, 2013), including diclofenac metabolites (Kallio *et al.*, 2010), and mussels (Ericson *et al.*, 2010). Toxic effects have also been recorded, including kidneys disruption (Schwaiger *et al.*, 2004; Triebskorn*et al*, 2004; Hoeger*et al.*,2005), damage to eggs and embryos (Hallare*et al.*, 2004), and altered gene expression (Cuklev *et al.*, 2011). In

crabs diclofenac has been shown to cause disruption of osmoregulation (Eades and Waring 2010) and in broadcast spawning marine invertebrates it may have consequences for reproductive success (Zanuri*et al.*, 2017). In mussels diclofenac has been shown to have a number of impacts. Early studies indicated that byssus strength (i.e. the ability to attach to substrates) was impaired and that energy was potentially diverted from growth and reproduction, with possible long term population effects (Ericson *et al.*, 2010). More recent studies using biomarkers have shown a range of alterations indicative of oxidative stress, gill and digestive gland damage, altered protein folding, impaired immunological response, and energy metabolism changes due to diclofenac or pollutant cocktail exposure (Schmidt *et al.*,2011; Schmidt *et al.*, 2013; Turja *et al.*, 2014; Gonzalez-Rey and Bebianno, 2014; Turja *et al.*, 2015; Mezzelani*et al.*, 2016). It has also been shown that mussels from more pristine environments were more strongly influenced and that recovery time differed (Kumblad*et al.*, 2015) and suggested that this biomarker approach may offer promise as an environmental status indicator component (Löf *et al.*, 2016).

One common mechanism of toxicity shared among a variety of different toxicant classes is the induction of oxidative stress (Birben*et al.*, 2012). Oxidative stress is the result of an imbalance between reactive oxygen species (ROS) and the antioxidant systems of the body. Metabolic processes are responsible for the formation of certain ROS such as hydrogen peroxide (H_2O_2), superoxide radical (O_2 -) and the hydroxyl radical (OH·) anion (Kinnula *et al.*, 2002). Reactive oxygen species cause toxicity through binding to proteins, lipids and DNA/RNA. Due to their reactive nature, they bind to the DNA bases causing structural alterations that go on to affect translation and transcription resulting in inhibition of protein and enzyme formation (Valko *et al.*, 2005; Ghelfi *et al.*, 2016).

Diclofenac has been known to cause oxidative damage through binding to lipids resulting in an increase in lipid peroxidation (Gomez-Olivan*et al.*, 2014). Consequently, oxidative stress can be measured as either an increase in ROS that cause effects, an increase in oxidative damage, or as a change in the activity of anti-oxidant defence mechanisms. Diclofenac has been known to react with glutathione (GSH) indicating it is metabolised to prevent damage to cells. Hepatic protein adducts have been detected in liver cells in mice resulting in the diclofenac-GSH conjugate becoming a useful biomarker for the hepatotoxicity of diclofenac (Valko *et al*., 2005).

It has been established that pharmaceutical drugs induce oxidative stress. Oxidative stress is the disturbances in the balance between the production of Reactive Oxygen Species (ROS) and oxidative defences. It usually results to tissue damage and disturbances in the normal redox state of cells. Oxidative stress can also cause base damage as well as unwanted gaps in the DNA (Cadet *et al* 2003). This is as a result of the ROS generated in the cell. Examples of Reactive Oxygen Species include: hydrogen peroxide (H_2O_2), superoxide radical (O_2) and hydroxyl radical (OH). All these radicals make up the Reactive Oxygen Species (Santos, 2014). There cause diseases that are associated with oxidative stress in humans. These include-Vitiligo (patchy loss of skin pigmentation), autism (neurological disorder), chronic fatique, Asperger's syndrome (having social defect), sickle cell disease (Tejada, *et al.,* 2007).

Nwani *et al.* (2016) noted that the level of damage caused to the cell is dependent on the level of the stress caused to cell. While severe oxidative stress causes death, mild stress causes slight changes which can normalize when the cell recovers.

An antioxidant defense system (ADS) is needed to protect bio-molecules from the harmful effects of ROS (Kumar, 2013). Fish are endowed with defensive mechanisms to neutralize the impact of Reactive Oxygen Species (ROS) resulting from metabolism of various chemicals.

These include various antioxidant defense enzymes such as superoxide dismutase (SOD) Catalase (CAT), glutathione peroxidase (GPx), glutathione S-transaferase (GST) and glutathione reductase (GR). Low molecular weight antioxidants such glutathione reductase (GSH), ascorbate (Vitamin C), Vitamin A and E are also reported to contribute in the reduction of oxy radicals. ROS which is not neutralized by this antioxidant defense system damages bio-molecules. One of the most important targets of ROS is membrane lipids which undergo peroxidation (LPO). Thus LPO estimation has also been successfully employed to signify oxidative stress induced in aquatic animals by such chemicals (Folarin *et al.*, 2018). The present study was designed to investigate the acute and sub-lethal effects of a pharmaceutical product diclofenac on the oxidative stress parameters of Tilapia fish (*O~~reochromis~~. niloticus)*. The study will also investigate the recovery ability of the fish after exposure to the drug.

Methods

Experimental fish and Maintenance

Three hundred healthy juveniles of fresh water Tilapia, *Oreochromis niloticus* (Family: Cichlidae; Order: Cichliformes; Genus: Oreochromis) with the mean weight of 29 ± 4.1g were collected from a private fish farm and acclimatized for 14 days in concrete pond using non-chlorinated tap water at biology department laboratory of University of Nigeria, Nsukka. They were fed 3% of their body weight in divided rations, twice daily (7.00am and 7.00pm) with Coppens commercial feed, containing 35% crude protein. The fish were subjected to 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 maintain hygienic conditions. Dead fishes were removed with forceps to prevent deterioration of water quality. The physicochemical properties of the test water were analysed

weekly using the standard methods according to American Public Health Association (APHA 2005), American Water Works Association (AWWA) and Water Pollution Control Federation (WPCF 2005) were: temperature (28.7 ± 0.15 °C), conductivity (234.10 ± 0.35 µM cm^{-1}), dissolved oxygen (7.9 ± 0.26 mg l^{-1}), pH (7.5 ± 0.04) and hardness in terms of CaCO3 was (135.50 ± 0.60 mg l^{-1}).Feeding was terminated 24 h before the commencement of the range finding and acute toxicity tests to avoid interference by faeces (Ward and Parrish 1982). The experiment was conducted according to the approved guidelines of the Animal Ethics Committee of the Enugu State University of Science and Technology (ESUT). The experiment was carried out in an indoor experimental system under normal photoperiod of day/night (12:12) cycle prevalent at Nsukka, Nigeria.

Determination of sub-lethal concentrations

The LC_{50} of DCF to *O. niloticus* was determined by exposing a set of 10 fish specimens each to five different (0.35, 0.45, 0.55, 0.65 and 0.75mg/1) DCF concentrations and control in 40 l glass aquaria (60 × 30 × 30 cm size) for 96 h. Each experimental set up was set in triplicate and the mortalities were recorded. The mortality data (Table 1) were used to obtain the 96 h LC_{50} following the probit analysis method as described by Finney (1971). The 96-h value of DCF that was 0.489 mg/L. Based on the 96 h $LC_{50,}$ three different sublethal concentrations (0.25, 0.32, and 0.48 mg/L) were selected for the sublethal exposure. A total of 120 acclimated fish were used in the sublethal experiment. The sample was divided into four groups (Groups 1, 2, 3 and 4) in separate 40-L glass aquaria. The fish in groups 1, 2, and 3 were exposed to 0.25, 0.32, and 0.49 mg/L of DCF, respectively. The fish in group 4 were designated as the control and only exposed to tap water. A total of 30 fish were randomly distributed to each of the four groups of the experimental set up without regard to the sex. Each experimental group was further divided into

three with ten fish per replicate. The experimental set up was semi-static and the test solution was changed every alternate day to counter balance the decreasing drug concentration. The experiment set up lasted for 21 days and another 7-days recovery during which the fish were fed small quantity of feed (approximately 1% of the body weight) to avoid mortality arising from starvation. There were no mortalities during the 28–day exposure period. Three fish from each of the experimental and control groups were removed for sampling at the end of every week. The fish were anesthetised with a solution of tricainemethanesulfonate (MS 222) at a concentration of 0.1 g l^{-1} to minimise stress. The liver was dissected out, carefully washed in an ice-cold 1.15% KCl solution, blotted and weighed. The live samples were homogenized in prechilled phosphate buffer (0.1M, pH 7.2). Some parts of the homogenate were used for the estimation of thiobarbituric acid reactive substances (TBARS), while the other part was further centrifuged at 12,500 × g for 10 min at 4°C for estimation of other oxidative stress biomarkers.

Assay of oxidative stress and antioxidant enzymes

The LPO was assessed by measuring malondialdehyde (MDA) formation, as described by Wallin *et al.* (1993). The activity of CAT was assayed, as described by Sinha (1972). The SOD activity was determined spectrophotometrically by measuring the inhibition of antioxidation of epinephrine at pH 10.02 at 30 °C, as described by Arthur and Boyne (1985). The activity of glutathione reductase (GR) was assayed by measuring NADPH oxidation at 340 nM (Tayarani et al. 1989), the activity being expressed as U/mg protein. The activity of glutathione peroxidase (GPx) was measured by the method of Lawrence and Buck (1976), with the specific activity being determined using the extinction coefficient of 6.22 mM/cm.The activity of glutathione (GSH) was assayed as described by King and Wootton (1959).

Statistical analysis

The median lethal concentration was calculated following the probit analysis method of Finney (1971). The LC_{50} was converted to toxic units using the method of (Michniewicz*et al.*, 2000). One way analysis of variance using (SPSS version 16.0) was used to analyse the data followed by Duncan multiple range post-hoc test at 95% significant level to separate the means of treatment. Analysis and sample percentages were also used where applicable.

Results

Fish mortalities and safe levels of diclofenac

The mortality rate of fish in the treatment group during the acute exposure, increased with increasing concentration and the duration of exposure to DCF (Table 1). No mortality was observed in the control group after 96 h of the test. For the group exposed to 0.35 mg/l concentration of DCF, the fish mortality rate was 20% after 96 h. However, at a higher concentration of 0.75 mg/l, the mortality rate after 96 h increased to 100%. The safe level of DCF as obtained by multiplying the LC_{50} by various application factors, ranged between 4.89 $\times 10^{-2}$to 4.89 $\times 10^{-6}$mg/l (Table 2).

Table 1. Cumulative mortality of *~~Oreochromis~~O. niloticus* exposed to various concentrations of Diclofenac

Concentration (mg/L)	Cumulative mortality 24 h	48 h	72 h	96 h	% Mortality	% Survival
Control	00	00	00	00	00	100
0.35	02	04	05	06	20	70
0.45	03	06	08	10	34	60
0.55	04	08	12	16	54	46
0.65	08	15	21	26	87	13
0.75	10	20	25	30	100	0

Table 2: Estimated safe levels of diclofenac for ~~*Oreochromis*~~ *O. niloticus* after 96 h exposure

Drug	96 h LC_{50} (mg/L)	Method	AF	Safe level (mg/L)
Diclofenac	0.489	Hart et al. (1948)*	-	8.13×10^{-3}
		Sprague (1971)	0.1	4.89×10^{-2}
		CWQC (1972)	0.01	4.89×10^{-3}
		NAS/NAE (1973)	0.1 – 0.00001	4.89×10^{-2} – 4.89×10^{-6}
		CCREM (1991)	0.05	2.445×10^{-2}
		IJC (1977)	5 % LC_{50}	2.445×10^{-2}

*C = 48h LC_{50} x $0.03/S^2$, where C = presumable harmless concentration and S = 24 h LC_{50}/48h LC_{50}

Effects of lipid peroxidation and antioxidant enzyme

The effect of different sub-lethal concentrations of diclofenac on lipid peroxidation in the form of TBARS formation and the responses of other antioxidants enzymes (CAT, SOD, GPx, GSH-R and GSH) in the liver of tissue of ~~*Oreochromis*~~ *O. niloticus* are presented in Table 3. Diclofenac was associated with oxidative stress in ~~*Oreochromis*~~*O. niloticus* in a manner dependent on the drug concentration in the aquatic medium and the duration of exposure. The activity of LPO increased significantly on exposure to the drug ($p < 0.05$) and the effects of the drugs appeared more pronounced at higher concentration on prolonged exposure. There was slight recovery after

the 7-day withdrawal. The activities of the oxidative stress biomarkers SOD and CAT where significantly reduced by diclofenac as the concentration increases throughout the exposure period. Reduction in SOD and CAT activities were more on day 21 compared to previous noted days and effects diclofenac on the activities of SOD and CAT were similar in magnitude. The GR and GPx activities increased significantly in fish exposed to the drug, the effect of the drug concentrations was significant for the duration of exposure. The was significant recovery after the 7-day withdrawal.

There was significant increase in GSH activity in fish exposed to diclofenac as the concentration increases throughout the exposure time. There was recovery after the withdrawal phase in the exposed fish.

Table 3: Changes in oxidative stress biomarkers of ~~*Oreochromis*~~ *O. niloticus* on 21-day exposure to Diclofenac.

Parameter	Conc.(mg/L)	Duration (day) 1	7	14	21	7-day withdrawal
LPO (U/L)	Control	1.56 ± 0.09^{a1B}	1.91 ± 0.29^{b1B}	2.68 ± 0.45^{b1A}	1.80 ± 0.38^{b1B}	2.41 ± 0.52^{b1A}
	0.48	2.08 ± 0.41^{a1B}	4.68 ± 0.12^{a2B}	5.45 ± 0.44^{a3A}	6.85 ± 0.46^{a4A}	4.04 ± 0.74^{a2A}
	0.32	1.66 ± 0.43^{a1B}	4.13 ± 0.20^{a2B}	5.41 ± 0.27^{a3A}	6.77 ± 0.42^{a4A}	4.33 ± 0.11^{a2A}
	0.25	1.43 ± 0.17^{a1B}	4.31 ± 0.18^{a2B}	5.70 ± 0.20^{a3A}	6.34 ± 0.22^{a4A}	4.30 ± 0.31^{a2A}
SOD(U/L)	Control	8.25 ± 0.32^{a1A}	8.62 ± 0.57^{a1A}	9.76 ± 0.53^{a1A}	8.76 ± 0.38^{a1A}	8.33 ± 0.32^{a1A}
	0.48	6.91 ± 1.18^{b2A}	4.47 ± 0.23^{c1A}	5.74 ± 0.34^{b1A}	4.18 ± 0.16^{b1A}	8.39 ± 0.26^{a3A}
	0.32	8.32 ± 0.58^{a3A}	5.90 ± 0.35^{b2A}	5.47 ± 0.30^{b2A}	4.54 ± 0.17^{b1A}	7.88 ± 0.51^{b3A}
	0.25	8.08 ± 0.68^{a3A}	5.82 ± 0.35^{b2A}	5.74 ± 0.30^{b2A}	4.68 ± 0.28^{b1A}	7.69 ± 0.40^{b2A}
CAT(U/L)	Control	0.65 ± 0.01^{a2A}	0.77 ± 0.07^{a3A}	0.60 ± 0.04^{a1A}	0.60 ± 0.02^{a1A}	0.62 ± 0.03^{a1A}
	0.48	0.59 ± 0.07^{a4A}	0.39 ± 0.06^{b2A}	0.24 ± 0.02^{b12A}	0.16 ± 0.06^{c1A}	0.44 ± 0.03^{b3A}
	0.32	0.63 ± 0.04^{a2A}	0.37 ± 0.08^{b1A}	0.31 ± 0.08^{b1A}	0.30 ± 0.15^{b1A}	0.58 ± 0.03^{ab2A}
	0.25	0.67 ± 0.04^{a2A}	0.36 ± 0.05^{b1A}	0.27 ± 0.04^{b1A}	0.22 ± 0.01^{b1A}	0.54 ± 0.02^{ab2}
GR (U/L)	Control	11.10 ±0.57^{a1A}	11.50 ±0.67^{b1A}	10.82 ± 0.56^{b1A}	10.89 ± 0.52^{b1A}	11.30 ±0.34^{a1A}
	0.48	11.60 ±0.64^{a1B}	14.83 ±0.34^{a2B}	15.89 ± 0.90^{a2A}	16.25 ± 0.57^{a2A}	12.58 ±0.44^{a1A}
	0.32	12.15 ±0.46^{a1A}	15.41 ± 0.41^{a2A}	15.96 ± 0.28^{a2A}	15.71 ± 0.61^{a2A}	12.73 ±0.79^{a1A}
	0.25	12.02 ±0.46^{a1B}	14.38 ± 0.73^{a2A}	16.11 ± 0.63^{a23A}	15.37 ± 0.60^{a2A}	12.18 ±0.23^{a1A}
GPx (U/L)	Control	4.87 ± 0.08^{a1A}	6.14 ± 0.20^{b1A}	4.80 ± 0.58^{b1A}	5.98 ± 0.42^{b1A}	4.92 ± 0.24^{a1A}
	0.48	5.30 ± 0.33^{a1A}	9.03 ± 0.33^{a2A}	8.44 ± 0.48^{a2A}	9.88 ± 0.40^{a2A}	6.37 ± 0.32^{a1A}
	0.32	5.50 ± 0.23^{a1A}	8.37 ± 0.07^{a2A}	8.40 ± 0.20^{a2A}	9.07 ± 0.45^{a2A}	5.17 ± 0.17^{a1A}
	0.25	4.60 ± 0.43^{a1A}	8.59 ± 0.35^{a2A}	8.70 ± 0.21^{a2A}	9.16 ± 0.19^{a2A}	5.69 ± 0.52^{a1A}
GSH (U/L)	Control	2.68 ± 0.28^{b1A}	3.57 ± 0.08^{a1B}	3.56 ± 0.40^{b1A}	2.94 ± 0.27^{b1A}	2.77 ± 0.31^{a1A}
	0.48	3.37 ± 0.92^{a1B}	3.86 ± 0.17^{a1B}	4.63 ± 0.50^{a1B}	3.86 ± 0.19^{a1B}	3.64 ± 0.37^{a1A}
	0.32	3.65 ± 0.33^{a1B}	3.77 ± 0.53^{a1B}	4.69 ± 0.22^{a1B}	3.67 ± 0.25^{ab1B}	3.74 ± 0.15^{a1A}
	0.25	3.91 ± 0.23^{a1B}	3.88 ± 0.58^{a1B}	4.31 ± 0.17^{b1B}	3.76 ± 0.17^{ab1B}	3.62 ± 0.17^{a1A}

Values with different small letter alphabet superscript between different drug concentrations along a column were significantly different; while values with different numeric superscript across a row were significantly different; and values with different capital letter superscript along a column were significantly different between same concentrations ($p < 0.05$). of Diclofenac.

Discussion

The effect of pharmaceutical drugs on non-target organisms has been on the increase due to the ever growing population. Fent *et al*.(2006)reported that although pharmaceutical drugs are usually in low concentration, and are also considered to be non-toxic compounds, they can exert toxic effects on non-target species. The increase in LPO suggests that there is increase in production of reactive oxygen species (ROS). The interaction of ROS with biological molecules may cause increase in LPO, DNA damage and protein oxidation resulting in the disturbance of the physiological processes (Tejeda, 2007). The elevation in LPO associated with oxidative stress has also been reported in rats administered various concentrations of albendazole (~~AbdEl~~Abdel-Rahman *et al*.,1999;Locatelli *et al.,* 2004;Nwani *et al.,* 2016). Some related pharmaceuticals, notably benznidazole (Pedrosa *et al*., 2001) and mebendazole (Für *et al*., 2012), have been reported to stimulate the production of ROS and to cause oxidative damage to animals.

Antioxidant enzymes play significant roles in preventing cellular damage in animals (Pedrosa *et al*. 2001). The inhibition of SOD and CAT activity in the liver tissues contributed to higher LPO values in the exposed fish, indicating that in aquatic environment DCF could induce oxidative stress in fish. Inhibition of SOD and CAT that lead to oxidative stress was also reported in *Clarias gariepinus* exposed to primextra herbicide (Nwani *et al*.,2016). Ahmed (2015) also reported that simultaneous treatments with vitamin E and/or lycopene resulted in a significant decrease in the tissue SOD activities. This decrease in SOD activity can be attributed to the inhibition of superoxide radical formation or the potential free radical scavenging activity of vitamin E and/or lycopene (Ural, 2013). According to Puerto *et al* .(2010), decrease in SOD and CAT were attributed to direct damage of its protein structure by the drug and increasing amounts

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of hydrogen peroxide produced. The low level of SOD and CAT when compared to the control indicates the high risks of cell injuries.

The GPx depletion in the stress–treated fish may be connected with increased exposure of the plasma membrane to peroxide attack, as reflected in changes in LPO levels. The depletion of GPx further enhances the susceptibility of the lymphoid tissues to oxygen metabolites and acid-mediated cell damage. These effects may subject livers to higher risk of damage from oxidative stress and more limited antioxidant responses. The continuous oxidative damage caused to the cells could paralyse them and eventually degrade completely the self defence mechanisms of the cells (Birben*et al.,* 2012). Our result is in agreement with Ahmad *et al*. (2012) who reported that structural and functional alterations in the liver result in changes in the levels of these enzymes in circulation.

Conclusion

This present study shows that diclofenacis toxic and may cause significant alterations in the oxidative stress of ~~*Oreochromis*~~ *O. niloticus*. Thus it can be said that DCF at various doses and duration of study can cause adverse effects on liver resulting in oxidative stress. High concentration of DCF above its safe level is highly toxic to tilapia fish and could be toxic to non-target organisms. Thus, caution should be exercised in the clinical use of the~~e~~is drug for therapeutic purpose, which should be limited to the lowest dose and treatment duration required to achieve the best therapeutic effect to avoid being toxic to non- target organisms. It is also clear that there is a need for further studies to determine the accurate effects of this drug on several other biological organisms, and also to determine whether the effects are similar when fish are subjected to longer exposures to lower concentrations; a combined toxicity study will satisfy this need.

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