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Acute toxicity of ibuprofen on selected biochemical and oxidative stress parameters of liver in *Clarias gariepinus* Juveniles (Burchell, 1822)

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Abstract
The present study evaluated the acute toxicity of Ibuprofen (IBU) on lipid peroxidation, antioxidant enzyme system and biochemical parameters of *Clarias gariepinus* juveniles. The 96 h LC_{50} of IBU was 0.38 mg·L^{-1}, in a semi static system with significant difference ($p< 0.05$) in LC_{10-90} values obtained for different durations of exposure. Catalytic activities of glutathione reductase (GR), Superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT) and lipid peroxidation (LPO) were evaluated. Biochemical parameters such as serum glucose, protein, alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were also measured. LPO (130.62%), SOD (76.95%), CAT (69.09%), GR (47.04%) and the liver function parameters ALP (37.70%), AST (35.55%) and ALT (69.45%) were all significantly ($p<0.05$) elevated when compared with control indicating oxidative stress and hepatic damage. Glucose increased in the lowest concentration of 0.28mg/L (9.20%) and decreased in the higher concentrations 0.33mg/L-0.48mg/L (21.10%). Protein decreased (46.38%) and GPx decreased dose dependently after 24h exposure. However, decreases in day 2-4 appeared with mixed trend. Formation of oxygen radicals may have contributed to the toxicity of IBU.

Keywords: Ibuprofen; toxicity; *C. gariepinus*; oxidative stress; biochemical parameter; LC_{10-90}.

1. Introduction
Increasing environmental awareness and regulations have resulted in decreasing concentrations of most of the "conventional" pollutants (heavy metals, organic contaminants, pesticides). There is, however, a large group of chemicals that have not been studied or regulated well, namely pharmaceuticals [1]. Fate of pharmaceuticals after use is only scarcely known, and already low concentrations of these bioactive compounds may have significant effects on aquatic organisms [2].

Pharmaceuticals are excreted after application in their native form or as metabolites and enter aquatic systems via different ways. The main pathway from humans is ingestion following excretion and disposal via wastewater. Municipal wastewater is therefore the main route that brings human pharmaceuticals after normal use and disposal of unused medicines into the environment [3]. Hospital wastewater, wastewater from manufacturers and landfill leachates may contain significant concentrations of pharmaceuticals [3]. Pharmaceuticals not readily degraded in the sewage treatment plant (STP) are being discharged in treated effluents resulting in the contamination of rivers, lakes, estuaries and rarely, groundwater and drinking water [4]. Where sewage sludge is applied to agricultural fields, contamination of soil, runoff into surface water and also drainage may occur. In addition, veterinary pharmaceuticals may enter aquatic systems via manure application to fields and subsequent runoff, but also via direct application in aquaculture [4].

IBU is a non-steroidal, anti-inflammatory, analgesic and antipyretic drug. It is an important non-prescription drug that is widely used. Its persistence, resistance to biodegradation and water solubility favour the entry of this drug into the environment, where it has been detected worldwide [5]. Non-steroidal anti-inflammatory drugs ($NSAID_S$), such as Ibuprofen are often employed in the treatment of severe headaches (such as migraine), dysmenorrheal (menstrual pain), rheumatoid arthritis, osteoarthritis and many other causes of pain [6]. They act by inhibiting cyclooxygenase (COX) enzyme which is involved in the production of

prostaglandins that normally protect the epithelial lining of the stomach wall from acid secretion, and they also have an important role in the production of pain, inflammation and fever [7]. IBU is classified as hazardous compound based on its inherent properties like hydrophobic crystal structure [8, 9] reports that IBU's persistence, bio-accumulative nature, water solubility, low volatility, and low tendency for adsorption to organic matter, helps it to remain in the aquatic environment for prolonged duration, consequently enhancing its absorption and bio-concentration by hydrobionts present in the environment [10]. also reported that IBU in surface water has a half-life of 32 days. Only 15% of ingested IBU is excreted unmetabolised by humans and fish, while 26 % is excreted as hydroxy-IBU and 43% as carboxy-IBU, including conjugates [11].

A study conducted by [12] revealed that traces of pharmaceutical compounds including ibuprofen were found in 12 rivers across United Kingdom. This development calls for concern as it could cause serious damage to aquatic life (flora and fauna) [12]. In aquaculture, IBU has been used as an intervening agent for prostaglandin biosynthesis of marine shrimp (*Litopenaeus vannamei* Boone) [7]. The author also reported that IBU was used to treat sperm abnormalities in male marine shrimp, (*L. stylirostris).* Although various cases of the detection of pharmaceuticals in water are described in the literature, a comprehensive impact of ibuprofen on non-target organisms has not been described yet.

In Nigeria, information on the concentrations of this group of contaminants in aquatic systems is scarce. Biochemical characteristics of blood are among the important indices of the status of the internal environment of the fish organism. Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) are enzymes frequently used in the diagnosis of damage caused by pollutants in various tissues, such as liver, muscle and gills [13]. A cellular oxidative stress is established when the pro-oxidant forces overwhelm the antioxidant defenses (enzymatic and non-enzymatic), leading to the generation of reactive oxygen species (ROS) [14, 50]. reported that fish has an inbuilt defensive mechanism to neutralize the effect of ROS generated by metabolism of xenobiotics.

Clarias gariepinus has remained an important candidate for research. The farming of *C. gariepinus* is important in Nigeria and Africa because it provides source of income, create employment opportunities and above all, provides animal protein to the majority of African populace and has low cholesterol content [15, 16]. reported that ROS such as hydrogen peroxide (H_2O_2), superoxide anion O2- and hydroxyl radical (OH·) at higher concentrations can react with biological macromolecules potentially leading to enzyme inactivation, lipid peroxidation (LPO), DNA damage and even cell death. Oxidative stress may cause many diseases such as cancer, respiratory problems, and neurological problems [17].

The aim of this study was to evaluate the acute toxicity of IBU on the liver of freshwater African catfish *C. gariepinus* using oxidative stress biomarkers and other biochemical parameters to assess the potential risk posed by waterborne pharmaceuticals to the fish physiology and survival.

2. Materials and methods

2.1 Experimental fish specimen and drug

This research was carried out March 2015 at the wet laboratory of the Department of Fisheries and Aquaculture, Federal University Ndufu Alike Ikwo, Ebonyi State, Nigeria. Ebonyi State is located between latitudes 6.24^{0}N and 6.28 ^{0}N and longitudes 7.00 ^{0}E and 7.06 ^{0}E on the South-Eastern part of Nigeria. Juveniles of Freshwater African catfish *C. gariepinus*, of mean length of 27.36±0.23 cm and weight of 197.39±2.34g were soured from the Department of Fisheries and Aquaculture farm complex, Federal University Ndufu-Alike Ikwo Ebonyi State. The fish were transported to the fisheries wet laboratory, which is a bit distant from the farm complex and treated with 0.05% potassium permanganate (KMnO4) for 2 minutes in order to avoid any dermal infections. Fish were acclimatized for 7 days in plastic tank of 1000 L capacity each and fed ad libitum daily with commercial feed (Coppens commercial feed of 4mm, Coppens International Helmond Netherlands). IBU stock solutions were prepared using commercial formulations of BRUSTAN-N manufactured by RANBAXY Laboratories Ltd India, Industrial Area-3, DEWAS-455001, with Mfg. Lic. No.25/24/83. (Each film-coated tablet contains Ibuprofen BP 400mg as the active ingredients).

2.2 Experimental design for acute exposure and tissue preparation

Methods of [18, 19], was followed for the acute 96-h static bioassays. 180 fish (197.39 ± 2.34 g and 27.36 ± 0.23 cm) were randomly introduced in the glass aquarium of 60x30x30cm capacity at the rate of 10 fish per aquarium. Each aquarium was covered with wooden framed nylon mesh to prevent jumping out by fish. The fish were treated to the following different concentrations of IBU: 0.28mg/L, 0.33mg/L, 0.38mg/L, 0.43mg/L, 0.48mg/L and a control containing 40liters of water and no drug. Three replicates were maintained for each concentration group and control. The percentage mortality/survival of fish in control and treated groups was recorded at 24, 48, 72 and 96 hours duration intervals respectively. Fishes were considered dead when their operculum stops beating and such was promptly removed to prevent surviving ones cannibalizing it. One fish from each replicate (in all the treatment groups and control) was sacrificed after anesthetizing with tricaine methane sulfonate (MS-222) to minimize stress. This was carried out on days 1, 2, 3, and 4 during exposure. The fish were dissected and liver tissue were removed, quickly rinsed in 0.9% NaCl solution, and homogenized 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 LPO while the other part was centrifuged for20 min at 10,500 rpm under 4^{o}C to obtain the supernatant which was stored at 4^{o}C for enzyme assay.

The physicochemical properties of the test water were analyzed daily [19].

2.3 Assessment of lipid Peroxidation

Estimation of tissue lipid peroxidation (LPO) was done by the measurement of thiobarbituric acid reactive substances (TBARS) according to [20]. The TBARS concentration was measured by the absorption at 535 nm at molar extinction coefficient of 156 mM/cm. The specific activity was interpreted in nanomoles of TBARS/mg protein.

2.4 Evaluation of antioxidants and biochemical enzymes.

The activity of glutathione peroxidase (GPx) was estimated by monitoring the rate of NADPH oxidation at 340 nm by the coupled reaction with glutathione reductase. Specific activity of glutathione reductase was estimated using the extinction coefficient 6.22mMcm^{-1} [21]. Values were expressed in unit/min/mg protein. Tissues catalase (CAT) activities were

determined spectrophotometrically by measuring the rate of H_2O_2 breakdown based on decrease in absorbance at 240 nm [22] and the values were expressed in U/mg protein. The superoxide dismutase (SOD) activity was assayed at 420 nm following the procedure of [23]. The assay is based on the oxidation of epinephrine-adrenochrome transition by the enzyme and expressed in U/mg protein. Tissue AST and ALT levels were estimated in the different homogenates following the procedure of [24]. ALP was estimated using commercially available kit (KEE GAD Biogen Pvt. Ltd., India). Total protein in the tissues were estimated spectrophotometrically following the methods of [25] and using bovine serum as a standard while the glucose levels were estimated by the method of [26].

2.5 Statistical Analysis

The data obtained were analyzed using statistical package SPSS (IBM version 20). The data were subjected to one way ANOVA and means were separated by Duncan's multiple range tests. Significant difference was declared at 5% level. The safe level estimates of the Ibuprofen were drawn based on [18, 27-31]. The LC_{10-90} of IBU at different durations of treatment were compared using one way ANOVA, followed by a Duncan's multiple range tests.

3. Results

3.1 Physico-Chemical Parameters of the Test Water

Water quality parameters of the test water are presented in Table 1. The temperature of the experimental water varied from 29.6 to 31.20ºC and the pH ranged from 6.19 to 6.76. The dissolved oxygen concentration ranged from 3.2 to 5.4 $mg \cdot L^{-1}$; conductivity values ranged from 142 to 196 $\mu M \cdot cm^{-1}$ while total hardness varied from 28–48 $mg \cdot L^{-1}$ during the experimental period.

Table 1: Physicochemical Parameters of dissolution water monitored during the experiment with Ibuprofen

Parameters	Range	Mean ± SE
Temperature (ºC)	29.6 – 31.2	30.44±0.09
pH	6.19 – 6.76	6.44±0.07
Dissolved Oxygen (mgl^{-1})	3.2 – 5.4	3.74±0.29
Conductivity (μscm^{-1})	142 – 196	162±10.15
Hardness (mgl^{-1} CaCO3)	28 – 48	37.6±0.89
Alkalinity (ml^{-1})	10 – 72	38.6±3.36

Table 2: Cumulative mortality of Clarias gariepinus exposed to various concentrations of Ibuprofen

		Cumulative mortality of fish at different intervals (hours)					
Conc. Mg/L	**Number exposed**	**24h**	**48h**	**72h**	**96h**	**Survival (%)**	**Mortality (%)**
Control	30	0	0	0	0	100	0
0.28	30	0	2	2	2	93	7
0.33	30	0	4	4	8	73	27
0.38	30	2	4	8	12	60	40
0.43	30	8	10	14	18	40	60
0.48	30	14	14	20	30	0	100

Table 3: Lethal concentrations (LC_{10-90}) of Ibuprofen on *C. gariepinus* (95% confidence intervals) as a factor of exposure duration*.

	Concentrations ($mg \cdot L^{-1}$) at various exposure	**durations (95% confidence intervals)**		
Lethal concentration	**24**	**48**	**72**	**96**
LC_{10}	0.395^{a} (0.369-0.422)	0.319^{b} (0.286-0.353)	0.310^{b} (0.230-0.381)	0.302^{b} (0.256-0.348)
LC_{20}	0.423^{a} (0.411-0.436)	0.375^{b} (0.337-0.414)	0.349^{bc} (0.301-0.398)	0.328^{c} (0.290-0.365)
LC_{30}	0.445^{a} (0.443-0.446)	0.421^{b} (0.379-0.463)	0.381^{c} (0.352-0.409)	0.347^{d} (0.316-0.379)
LC_{40}	0.463^{a} (0.452-0.475)	0.465^{a} (0.418-0.512)	0.409^{b} (0.389-0.428)	0.366^{c} (0.341-0.390)
LC_{50}	0.481^{a} (0.459-0.504)	0.508^{a} (0.449-0.567)	0.438^{b} (0.408-0.468)	0.383^{c} (0.341-0.390)
LC_{60}	0.501^{b} (0.467-0.535)	0.559^{a} (0.504-0.615)	0.469^{b} (0.417-0.522)	0.402^{c} (0.393-0.411)
LC_{70}	0.522^{b} (0.474-0.571)	0.618^{a} (0.555-0.679)	0.506^{b} (0.421-0.591)	0.423^{c} (0.423-0.423)
LC_{80}	0.549^{b} (0.483-0.615)	0.693^{a} (0.622-0.764)	0.552^{b} (0.423-0.681)	0.448^{c} (0.436-0.459)
LC_{90}	0.587^{b} (0.495-0.679)	0.814^{a} (0.727-0.901)	0.624^{b} (0.422-0.826)	0.488^{c} (0.456-0.519)

*Values with different alphabet superscripts in rows differ significantly ($p < 0.01$) between exposure durations within lethal concentrations.

Table 4: Estimate of safe levels of Ibuprofen at 96 h exposure duration

Drug	96h LC50 (mg/L)	Method	AF	Safe level (mg/L)
Ibuprofen	0.38	Hart *et al*. (1948)*	-	0.16
		Sprague (1977)	0.1	3.8×10^{-2}
		CWQC (1972)	0.01	3.8×10^{-3}
		NAS/NAE (1973)	$0.1\text{-}1.0^{-5}$	3.8×10^{-2} - 3.8×10^{-6}
		CCREM (1973)	0.05	1.9×10^{-2}
		IJC (1977)	5% LC_{50}	1.9×10^{-2}

*C = 48 h LC50 × 0.03/S2, where C = presumable harmless concentration and S = 24 h LC_{50}/48 h LC50.

3.2 Median Lethal Concentration (LC_{50}) and Application Factor

The cumulative mortality of C. gariepinus exposed to various concentrations of Ibuprofen is presented in Table 2. An increase in IBU concentration as observed in the study (Table 2) led to the increase in fish mortality simultaneously. No mortality was observed in the control during the experimental period. The LC_{50} values (95% confidence limits) of different concentrations of Ibuprofen (Table 3) were found to be 0.48, 0.51, 0.44 and0.38mg·L^{-1} for 24, 48, 72 and 96 h LC_{50} respectively following [32] method and using SPSS (IBM version 20). A dose dependent elevation and duration dependent decrease were observed in mortality rate, such that as the exposure duration increased from 24 to 96 h, the median concentration was reduced. There were significant differences ($p < 0.05$) in $LC_{10\text{-}90}$ values obtained for different durations of exposures (Table 3). There were variations in safe level estimated by different methods at 96 h of exposure of the fish as presented in Table 4.

3.3 Effects of Ibuprofen (IBU) on Lipid Peroxidation and Antioxidant Enzymes

The effects of Ibuprofen on tissue membrane LPO measured as the TBARS in the liver of C. gariepinus (Burchell) is presented in Table 5. There was both duration and concentration dependent induction of LPO in the liver, with the lowest TBARS formation observed on day 1 of the exposure. When compared to control, TBARS formation only increased significantly ($p<0.05$) in the higher concentrations of 0.38-0.48mg/L in day 1. However, in day 2-4, the formation of TBARS in all treated fish significantly ($p<0.05$) elevated. There was significant ($p<0.05$) duration dependent elevation in SOD activity in all IBU treated fish. In days 1 and 2, the significant ($p<0.05$) increases where dose dependent while in days 3 and 4, the activity of SOD in fish treated to IBU elicited significant ($p<0.05$) concentration induction with mixed trend. The activity of CAT in IBU treated fish in day 1 and 2 was comparable, but significantly ($p<0.05$) deferred from days 3 and 4, where significant elevation where recorded. However there was a biphasic response as percentage inhibitions were recorded in day 2 and increases in day 3. When compared with control, CAT activity in IBU treated fish also elicited significant ($p<0.05$) concentration inductions with mixed trend in day 1, 2 and 3. The activity of CAT in day 4 showed significant ($p<0.05$) concentration dependent elevation.

IBU did not elicit any significant ($p<0.05$) duration effect on the activity of GR in 0.33 and 0.38mg/L concentrations. The activity of GR in IBU treated fish in day 1 and 2 was comparable, but significantly ($p<0.05$) differed from day 3 and 4, where significant increases were recorded. There was also significant ($p<0.05$) concentration induction of GR activity in IBU treated fish when compared to control, with the highest activity recorded in the highest concentration of 0.48mg/L in all durations. GPx activity in IBU treated fish was highest in day 1. There was significant ($p<0.05$) duration dependent inhibitions of GPx activity with mixed trend. When compared to control, there was significant ($p<0.05$) inhibition in GPx activity in day 1, with the lowest activity recorded in the highest concentration of 0.48mg/L. However, in day 2, 3 and 4, the activity of GPx in all concentrations was comparable to control.

3.4 Effect on Liver function parameters

Effects of Ibuprofen exposure on tissue biochemical enzymes, glucose and protein levels in C. gariepinus (Burchell) are presented in Table 6 and 7. At day 1, there was significant ($p<0.05$) concentration dependent increase in ALT and concentration dependent significant ($p<0.05$) elevations in AST of treated fish when compared to control. In day 2-4 durations, IBU elicited significant ($p<0.05$) ALT concentration elevations with mixed trend. In day 2, the induction of AST significantly ($p<0.05$) differed from control in 0.38-0.48mg/L concentrations, while the values of AST in 0.28-0.33mg/L was comparable to control. In the higher durations of 72h and 96h, IBU elicited significant ($p<0.05$) inductions AST in treated fish. Fish treated to 0.28mg/L concentration of Ibuprofen, elicited significant ($p<0.05$) duration dependent increase in ALP. IBU also elicited significant ($p<0.05$) decreases in plasma protein, in the higher concentrations of 0.43mg/L-0.48mg/L in all durations of exposure. Glucose increased in the lowest concentration of 0.28mg/L (9.20%) and decreased in the higher concentrations 0.33mg/L-0.48mg/L (21.10%).

Table 5: Activity of lipid peroxidation (TBARS, nmol TBARS mg $protein^{-1}$), glutathione peroxidase (GPx, nmol min^{-1}mg $protein^{-1}$), catalase (CAT, mmol min^{-1}mg $protein^{-1}$) and superoxide dismutase (SOD, U mg $protein^{-1}$) in the liver of *C. gariepinus* exposed to acute concentrations (0.28, 0.33, 0.38, 0.43 and 0.48 mgL^{-1}) of Ibuprofen.

Parameter		Exposure duration (hours)			
	Concentration (mg/L)	24	48	72	96
LPO	**Control**	**2.09 ± 0.01^{a1}**	**3.71 ± 0.22^{a2}**	**4.73 ± 0.29^{a3}**	**3.69 ± 0.18^{a2}**
(unit/mg protein)	0.28	2.49 ± 0.61^{a1}	4.53 ± 0.12^{ab2}	5.79 ± 0.33^{b3}	7.23 ± 0.19^{b4}
	0.33	2.64 ± 0.30^{a1}	4.87 ± 0.40^{ab2}	6.35 ± 0.22^{bc3}	7.70 ± 0.36^{bc4}
	0.38	6.07 ± 0.39^{b1}	6.17 ± 0.26^{c1}	6.28 ± 0.72^{bc1}	8.13 ± 0.36^{bc2}
	0.43	5.92 ± 0.25^{b1}	5.67 ± 0.74^{bc1}	6.87 ± 0.48^{c12}	8.16 ± 0.37^{bc2}
	0.48	6.56 ± 0.18^{b1}	6.36 ± 0.24^{c1}	7.00 ± 0.16^{c2}	8.51 ± 0.78^{c3}
SOD	Control	9.45 ± 0.89^{a1}	12.00 ± 0.73^{a2}	11.59 ± 0.73^{a2}	12.93 ± 0.25^{a3}
(unit/mg protein)	0.28	10.73 ± 0.31^{b1}	14.19 ± 0.28^{b2}	19.16 ± 0.64^{b3}	19.13 ± 0.24^{b3}
	0.33	10.99 ± 0.31^{b1}	14.22 ± 0.31^{b2}	18.83 ± 0.31^{b3}	19.89 ± 0.36^{bc4}
	0.38	11.59 ± 0.85^{b1}	14.99 ± 0.33^{b2}	19.58 ± 0.65^{b3}	18.15 ± 0.88^{b3}
	0.43	12.84 ± 0.26^{c1}	15.62 ± 0.69^{bc2}	18.48 ± 0.98^{b3}	21.49 ± 0.59^{cd4}
	0.48	13.66 ± 0.58^{c1}	16.90 ± 0.81^{c2}	20.89 ± 0.89^{b3}	22.88 ± 0.93^{d3}
CAT	Control	0.38 ± 0.01^{a1}	0.39 ± 0.01^{a12}	0.49 ± 0.36^{a23}	0.55 ± 0.07^{a3}
(unit/mg protein)	0.28	0.57 ± 0.73^{b1}	0.55 ± 0.70^{ab1}	0.77 ± 0.39^{bc2}	0.77 ± 0.35^{b2}
	0.33	0.55 ± 0.65^{b1}	0.59 ± 0.78^{b1}	0.82 ± 0.46^{bc2}	0.82 ± 0.39^{bc2}
	0.38	0.61 ± 0.95^{bc1}	0.56 ± 0.73^{ab1}	0.74 ± 0.55^{b12}	0.84 ± 0.01^{bc2}
	0.43	0.68 ± 0.49^{bc1}	0.67 ± 0.01^{b1}	0.90 ± 0.12^{c2}	0.89 ± 0.24^{bc2}
	0.48	0.79 ± 0.51^{c1}	0.72 ± 0.02^{b1}	0.82 ± 0.41^{bc12}	0.93 ± 0.20^{c2}
GR	Control	24.54 ± 0.28^{a1}	27.55 ± 1.44^{a1}	27.15 ± 1.48^{a1}	27.32 ± 1.56^{a1}

(unit/mg protein)	0.28	30.90± 0.88^{b12}	32.90 ±0.89^{b23}	29.22 ± 0.31^{a1}	35.34 ±1.53^{bc3}
	0.33	32.55 ± 1.22^{bc1}	32.17 ± 0.91^{b1}	36.17 ±1.40^{b1}	35.16 ± 1.78^{b1}
	0.38	34.22 ± 1.23^{bc1}	34.56 ±1.07^{bc1}	36.81 ±1.40^{bc1}	37.15 ±1.79^{bc1}
	0.43	35.15 ±1.70^{cd12}	33.56 ± 1.59^{b1}	38.83 ±0.30^{bc2}	38.63 ±1.22^{bc2}
	0.48	37.83 ± 0.71^{d1}	37.41 ± 0.58^{c1}	39.59 ± 0.89^{c2}	40.17 ± 0.38^{c2}
GPx	Control	24.16 ± 0.52^{d3}	15.69 ± 2.37^{a1}	18.79 ±0.88^{a12}	20.48 ±0.52^{b23}
(unit/mg protein)	0.28	22.99 ± 1.21^{cd2}	16.84 ± 0.2^{a1}	18.13 ± 0.36^{a1}	16.01 ± 0.33^{a1}
	0.33	21.45 ± 0.52^{bc3}	17.04 ±0.30^{a12}	17.68 ± 0.16^{a2}	15.89 ± 0.49^{a1}
	0.38	20.59 ±1.07^{abc2}	16.94 ± 0.88^{a1}	18.84 ±0.21^{a12}	16.79 ± 0.33^{a1}
	0.43	19.84 ± 0.29^{ab3}	15.69 ± 0.64^{a1}	18.19 ±0.99^{a23}	16.30 ±0.38^{a12}
	0.48	18.86 ± 0.26^{a3}	15.82 ± 0.29^{a1}	17.74 ± 0.34^{a2}	16.17 ± 0.38^{a1}

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

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Table 6: Changes in ALP, AST and ALT (umol min^{-1}mg protein^{-1}) activities in liver of *C. gariepinus* exposed to acute concentrations (0.28, 0.33, 0.38, 0.43 and 0.48 mgL^{-1}) of Ibuprofen.

Parameter	Concentration (mg/L)	Exposure duration (hours)			
		24	48	72	96
ALT	Control	54.67 ± 1.45^{a2}	48.00 ± 1.73^{a1}	59.67 ±0.88^{a23}	61.00 ± 2.08^{c3}
	0.28	62.33 ± 1.20^{a12}	56.67 ±4.81^{bc1}	72.33 ± 3.84^{b2}	84.00 ± 2.31^{a3}
	0.33	71.33 ± 3.53^{b2}	53.00 ±2.52^{ab1}	78.00 ±2.08^{b23}	82.00 ± 2.00^{b3}
	0.38	72.00 ± 1.16^{b12}	66.00 ± 1.16^{d1}	76.33 ±3.18^{b12}	80.00 ± 5.29^{b2}
	0.43	76.00 ± 4.36^{b23}	63.33 ±1.67^{cd1}	74.00 ± 2.87^{b2}	83.67 ± 1.45^{b3}
	0.48	77.00 ± 3.51^{b1}	68.67 ± 0.67^{d1}	77.00 ±5.69^{b12}	82.67 ± 0.33^{b2}
AST	Control	44.00 ± 2.98^{a1}	55.67 ± 2.96^{a2}	62.33 ± 1.45^{a2}	60.00 ± 5.78^{a2}
	0.28	52.33 ± 2.03^{b1}	55.67 ± 2.85^{a1}	71.00 ± 1.53^{b2}	72.00 ± 1.16^{b2}
	0.33	56.33 ± 3.28^{bc1}	54.67 ± 2.91^{a1}	79.00 ± 1.16^{b2}	78.67 ± 0.88^{b2}
	0.38	54.33 ± 2.60^{bc1}	67.00 ± 1.00^{b2}	74.00 ±2.31^{b32}	77.00 ± 2.52^{b3}
	0.43	62.00 ± 3.06^{cd1}	66.33 ± 0.33^{b1}	78.67 ± 4.37^{b2}	80.00 ± 2.08^{b2}
	0.48	65.00 ± 2.65^{d1}	67.67 ± 1.45^{b1}	80.33 ± 4.01^{b2}	81.33 ± 1.20^{b2}
ALP	Control	33.33 ± 2.40^{a1}	44.67 ± 2.03^{a2}	45.00 ± 2.08^{a2}	43.67 ± 0.88^{a2}
	0.28	34.67 ± 2.40^{ab1}	53.00 ± 2.52^{b2}	62.67 ± 1.76^{b3}	70.33 ± 1.86^{b4}
	0.33	32.67 ± 0.88^{a1}	63.33 ± 1.76^{c2}	68.00 ± 0.58^{c3}	68.00 ±1.16^{b3}
	0.38	34.33 ± 2.33^{ab1}	52.33 ± 2.85^{b2}	68.00 ± 1.16^{c3}	74.00 ±2.89^{b3}
	0.43	40.00 ± 0.58^{bc1}	58.33 ±1.20^{bc2}	73.33 ± 1.76^{d3}	73.00 ±1.53^{b3}
	0.48	42.00 ± 1.16^{c1}	57.00 ±1.53^{bc2}	71.67 ±1.45^{cd3}	74.00 ±2.31^{b3}

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

Table 7: Mean values of protein (mg g tissue^{-1}) and glucose (mmol g tissue^{-1}) levels in the liver of *C. gariepinus* exposed to acute concentrations (0.28, 0.33, 0.38, 0.43 and 0.48 mgL^{-1}) of Ibuprofen.

Parameter	Concentration (mg/L)	Exposure duration (hours)			
		24	48	72	96
Protein	Control	8.13 ±0.19^{c12}	9.06 ± 0.34^{c2}	7.73 ± 0.44^{d1}	8.57 ± 0.41^{b12}
	0.28	6.60 ± 0.11^{b23}	5.40 ± 0.15^{b1}	6.90 ± 0.32^{cd3}	5.87 ± 0.26^{a12}
	0.33	6.43 ± 0.33^{b12}	5.03 ± 0.59^{ab1}	6.83 ± 0.51^{cd2}	6.07 ±0.34^{a12}
	0.38	5.80 ± 0.30^{a12}	4.63 ±0.14^{ab1}	5.83 ± 0.82^{bc12}	6.50 ±0.17^{a2}
	0.43	5.60 ± 0.12^{a2}	4.43 ± 0.08^{ab1}	4.33 ± 0.03^{a1}	6.63 ± 0.15^{a3}
	0.48	5.80 ± 0.17^{a2}	4.33 ± 0.03^{a1}	4.60 ± 0.25^{ab1}	6.63 ± 0.22^{a3}
Glucose	Control	76.67 ± 1.20^{a1}	79.00 ±4.58^{a12}	89.00 ± 2.60^{c2}	83.33 ±3.33^{ab12}
	0.28	77.67 ± 4.49^{a12}	81.00 ±1.53^{b12}	70.00 ±5.03^{ab1}	91.00 ± 4.04^{b2}
	0.33	69.33 ± 0.67^{a1}	62.33 ± 2.58^{a1}	70.33 ±4.09^{ab1}	83.33 ±1.76^{b2}
	0.38	75.33 ± 4.06^{a12}	69.67 ±2.72^{ab1}	71.33±3.53^{ab12}	82.33 ± 2.85^{ab2}
	0.43	74.33 ± 3.18^{a2}	71.00 ±1.53^{ab2}	59.67 ± 0.33^{a1}	81.33 ± 1.33^{a3}
	0.48	68.33 ± 6.01^{a1}	71.67± 6.94^{ab1}	76.33 ± 4.18^{b1}	81.67 ± 1.20^{a1}

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

4. Discussion

Ecotoxicity procedure for the mode of action of pharmaceuticals on non-target organisms is presently receiving attention by researcher's worldwide [33]. The presence of pharmaceuticals in the aquatic environment may act on molecules, cells and organs of organisms through unexpected mode of action [4, 34]. suggested 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. Knowledge on the acute toxicity of pharmaceuticals could provide valuable information on the mode of action and toxicity of these compounds. The present study reports the acute toxicity of IBU on a freshwater African catfish *C. gariepinus* using selected biochemical and antioxidant parameters as an end points test and to evaluate the potential use of these

parameters as biomarkers of response to IBU.

We report IBU to be toxic to juveniles of *C.gariepinus* with 96-h LC_{50} value of 0.38mg L^{-1}. Similar findings have been reported for other fish species e.g bluegill sunfish (*Lepomis macrochirus* Illinois), with 96-h LC_{50} value of 173 mg L^{-1} [2]. IBU toxicity varies between species and even strains of same species: the LC_{50} of IBU was found to be 132.6 mg L^{-1} in *Daphnia magna* (Straus, 1820) at 48 h [35], 17.1 mg L^{-1} in the mollusk *Planorbis carinatus* (Muller, 1774) [36], 22.36 mg L^{-1} in *Hydra attenuata* (Pallas, 1766) [37] at 72 h, and 142 mg L^{-1} in the fish *Cirrhinus mrigala* (Hamilton, 1822) at 24 h [38]. These data and that reported in literature therefore indicate that IBU toxicity varies between species.

Reactive oxygen species such as superoxide (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH^-) are free radicals that contain oxygen atoms that are highly reactive due to the presence of unpaired electrons [39]. The inability of the body to eliminate the ROS timely by the antioxidant system would lead to oxidative stress [40, 41]. The IBU-induced elevation of LPO and subsequent oxidative stress as obtained in the present study is in agreement with [43] who reported increase in LPO due to IBU induced oxidative stress in liver, blood, and gill of *C. carpio* (Linnaeus, 1758). Increase in LPO have also been reported in *C.gariepinus* by other xenobiotics like fethion [50]

SOD is the first mechanism of antioxidant defense and the main enzyme responsible for offsetting the effects of ROS, particularly the superoxide ion [42]. In the present study, SOD activity increased in liver. This is in agreement with [43] who reported highest increase in SOD activity in liver after 12 h of exposure of *C. carpio* (Linnaeus, 1758) to IBU. Also the findings are consistent with [44], who reported an increase in SOD activity after exposure to 7,098 mg L^{-1} of DCF. The increase in SOD activity found in liver may be due to IBU oxidative metabolism that favours the formation of anion radical O^{2*}, which is responsible for the increase of SOD activity [45]. The accumulation and binding of IBU in cell membranes, cytoplasm, and mitochondria may cause structural damage and disintegration of cells, which results in the release of SOD enzyme into the blood circulation [46, 47].

CAT is a hematin-containing enzyme located in peroxisomes and facilitates the removal of hydrogen peroxide (H_2O_2), which is metabolized to molecular oxygen (O_2) and water. Therefore, the SOD–CAT system provides the first defense against oxygen toxicity. CAT activity is directly regulated by the concentration of H_2O_2 [48]. The present result showed a biphasic response with a decrease in CAT activity in day 2 and an increase in day 3 of fish treated to IBU in all concentrations. In 0.28, 0.33, 0.38mgL^{-1} concentrations of IBU was obtained 3.51%, 7.27% and 8.19% decreased activity of CAT in day 2 and 35.09%, 49.09% and 21.31% elevated activity in day 3 respectively. The increase may be an adaptive response to protect fish from IBU induced free radical toxicity. However, depletion in day 2 could stem from decreases in reaction rates resulting from the excess production of H_2O_2. This could have been because of the flux of superoxide radicals or activated metabolites generated by IBU on the cell membrane of treated fish, which have been shown to inhibit CAT activity [49]. The present result is consistent with [50] who reported a biphasic response with an increase in CAT activity from day 7 to 14 of *Clarias gariepinu*s (Burchell, 1822) treated to sublethal concentration of fenthion at 8.0mg/L in two tissues (liver and gill), and a decline of its activity in day 21.

Glutathione reductase (GR) is an enzyme which catalyzes the reduction of oxidized glutathione (GSSG) to glutathione (GSH) [51]. Glutathione redutase is important for the glutathione redox cycle that maintains substantial levels of reduced cellular GSH. In the present study, there was significant concentration induction of GR activity in IBU treated fish when compared to control, with the highest activity recorded in the highest concentration of 0.48mg/L in all durations. However, there was no significant difference from day 1 to day 4 durations of exposure. This result is consistent with [52] who reported induced IBU elevated GR activity in mussel *Mytilus galloprovincialis* (Lamark, 1819) during first week of exposure. Also In the study of [53], the activity of GR increased significantly in experimental groups of zebra fish after 28-day exposure to atrazine at the concentrations of 0.0003 and 0.03mg.L^{-1}. [54] found a significant increase in GR activity in embryo-larval stages of common carp (*Cyprinus carpio* Linnaeus) exposed for 30 days to 0.52 mg.L^{-1} of terbuthylazine and 0.9, 4, and 14 mg.L^{-1} of metribuzin compared to control.

GPx, responsible for enzymatic defense against hydrogen peroxide (H_2O_2), is strictly linked with the concentration of GSH because it catalyses the reaction between glutathione and hydrogen peroxide, resulting in the formation of glutathione disulphide (GSSG) [55]. In this study, IBU caused an inhibition in the GPx activity the decreased activity of GPx indicated its reduced capacity to scavenge H_2O_2 and lipid hydroperoxides. The decreased activity of GPx may be the result of $^{\bullet}O^-_2$ production [56] or a direct action of the drug on the synthesis of the enzyme [57]. It has been reported that trichlorfon and methidathion caused a decrease in the GPx activity [58].

The significant elevation of glucose in the lowest concentration may be associated with the metabolism of the drug. Effective metabolism at this concentration may have induced hyperglycaemia due to increased rate of glycogenolysis or gluconeogenesis. However, with increase in concentration, higher percentage of the drug may have remained unmetabolised leading to increased mortality of fish, Similar finding was reported by [59] who studied the toxicity of 15 veterinary pharmaceuticals in zebrafish (*Danio rerio*) embryos. The authors reported that Albendazole was metabolized efficiently into albendazole sulfoxide at lower exposure concentrations resulting in reduced toxicity, but at higher concentrations, increasing proportions of albendazole remained un-metabolized leading to increased mortality of embryos.

These findings are similar to those reported by [47] on Indian major carp *Cirrhinus mrigala* (Hamilton, 1822) exposed to Ibuprofen. During stress condition, there is an increase in the concentration of circulating catecholamines or corticosteroids which results in hyperglycemic condition [60]. The observed reduction in plasma protein level may be as a result of the inhibition of protein biosynthesis and the possible utilization of the available protein as an energy source for repair of the damaged cells caused by the drug. In addition, detoxification and excretion pathways during xenobiotic biotransformation through cytochrome P_{450}-dependent oxidative metabolism are known to be energy demanding [61] and may cause depletion of protein in the liver. Liver disorder due to toxicant stress may also lead to decrease in protein levels [62, 63] noted that during xenobiotic stress, proteins are mobilized and degraded into amino acids to feed the tricarboxylic acid cycle and increase adenosine triphosphate synthesis so as to satisfy the high energy demands of the cells. Other authors have similarly reported protein reduction in tissues of fish exposed

to various environmental contaminants [64, 65].Changes in protein and carbohydrate metabolism during stress conditions may also affect the activity of AST and ALT and the elevation of transaminases can be taken as a measure of compensatory mechanism to impaired metabolism [66]. Determinations of AST and ALT enzymes in blood plasma are a sensitive indicator of cellular damage, organ dysfunction and also indicate water pollution [67, 68]. In this study, plasma AST, ALP and ALT activities were increased indicating the disorder in Kreb's cycle caused by the drug IBU. Damage in hepatic cells may have been responsible for the significant duration dependent increase in ALP. Severe histopathological alterations in gill and liver and inhibition of cyclooxygenase activity were observed in diclofenac treated fish *Salmon trutta* [69].

5. Conclusion

The present study showed that IBU elicited oxidative stress as indicated by increase in LPO. The increase in the activities of biochemical enzymes (ALT, AST) is evident that IBU induced hepatic damage, and the change in the antioxidant enzymes (SOD, CAT, GR) profile under IBU stress, suggest that ROS may be involved in the toxic effect of IBU. The integrated use of biochemical and oxidative stress biomarkers such as that shown here with the African catfish may be useful to regulatory agencies in determining the risk that polluting pharmaceuticals have on an aquatic ecosystem.

6. Conflict of interest statement

None declared.

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