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Toxicity Evaluation of Pharmaceutical Wastewater to the Nile Tilapia (*Oreochromis niloticus*)

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Abstract

This study assessed the levels of oxidative stress biomarkers in gills and liver, as well as the activities of transaminases in the liver of Nile tilapia (*Oreochromis niloticus*), exposed to pharmaceutical effluents. The pharmaceutical effluents were collected from two pharmaceutical industries in Lagos, Nigeria. The assessment of physicochemical characteristics of the effluents indicated that some parameters were not in accordance with NESREA limits. The acute toxicity studies showed that 96hrLC_{50} values of "effluent A" and "effluent B" were 27.0 ml/L and 18.0 ml/L respectively. The juveniles of *O. niloticus* were exposed to 1/100th and 1/10th LC_{50}s of the two effluents for a period of 14 and 28 days. These concentrations significantly increased the level of the lipid peroxidation product, malondialdehyde. There was also inhibition of the activities of superoxide dismutase and catalase as well as significantly lower levels of reduced glutathione after 28 days. The levels of the transaminases (aspartate aminotransferase and alanine transaminase) were elevated in the liver of the fish after the exposure to the effluents. The present findings showed that the wastewater caused oxidative stress and hepatocellular damage in the fish suggesting potential ecotoxicological risks of the wastewater to aquatic organisms.

Keywords

Pharmaceutical Effluents, *Oreochromis niloticus*, Oxidative Stress, Transaminases

1. Introduction

The pharmaceutical industry has been noted as one of the major sources of ef-

fluent discharge into aquatic ecosystems [1]. There are a few pharmaceutical companies in Nigeria of which most are located in Lagos and Ogun States and these companies discharge their wastewater into nearby creeks, rivers, and lagoons [2]. The effluents commonly known as pharmaceutical effluents are wastewater generated during drug production by these pharmaceutical industries [3]. The fate of pharmaceutical effluents in aquatic ecosystems is dependent on the physical and chemical characteristics of the individual components of the effluents and the nature of the receiving water body [4]. During wastewater treatment in sewage treatment plants (STPs), components with low adsorption coefficient tend to remain in the aqueous phase which enhances their mobility into the receiving water body [5]. Pharmaceutical effluents contain a vast number of chemicals and microorganisms depending on the drug that is being produced and are toxic to biological organisms due to the presence of salt, surfactants (detergents, emulsifiers and dispersant), ionic metals and their metal complexes, organic chemicals, biocides, unmetabolized drugs, toxic anions and microorganisms [6] [7] [8] [9]. Some of these components can combine with unsaturated fatty acids of phospholipids located in the cell membranes [10]. This leads to oxidative damage and the production of malondialdehyde and then the oxidative stress is countered by the action of antioxidative stress enzymes such as superoxide dismutase (SOD) and catalase (CAT) [11]. Results from a biochemical assay indicated that environmentally relevant concentrations of Benzo[b]fluoranthene increased aspartate aminotransferase and alanine transaminase levels in fish. Glutathione-S-transferase, superoxide dismutase and catalase were inhibited in the exposed fish, while malondialdehyde was significantly increased [12]. Several studies have reported that specific constituents of pharmaceutical effluents have deleterious effects on living organisms [13] [14]. It has been noted that the approach of investigating the effects of individual components of pharmaceutical effluents had a limitation of providing information on the effects of all components present as a mixture in the effluent [15]. Lots of research has assessed the toxicity of pharmaceutical effluents [16] [17]. It was established in a study that bacterial isolates from pharmaceutical waste water investigated revealed multi-drug resistant strains [18]. In that regard, this study investigated the acute and biochemical effects of pharmaceutical effluents on an ecotoxicologically relevant organism. The term waste water used in the study represents treated effluents collected from the discharge point before entry into the water body.

2. Methodology

2.1. Pharmaceutical Effluents Collection and Physicochemical Analyses

"Pharmaceutical effluent A" was collected from a pharmaceutical industry located at Victoria Island, Lagos, Nigeria while "Pharmaceutical effluent B" was from a pharmaceutical industry at Ikeja, Lagos, Nigeria. The physicochemical parameters of the effluents were assessed according to the methods of APHA [19].

2.2. Test Fish

Juveniles of Nile tilapia, *Oreochromis niloticus* (length 7.4 - 8.1 cm; weight 13.3 - 14.6 g) were acquired from a fish farm at Ikorodu Town, Lagos and transported to the laboratory. Upon arrival, they were transferred to holding tanks and acclimatized for a week, during which they were fed twice (morning and evening) daily with Coppens fish feed following established techniques [20].

2.3. Bioassay Procedure

A total of 90 acclimatized juvenile Nile tilapia specimens were exposed to sub-lethal concentrations ($1/100^{th}LC_{50}$ and $1/10^{th}LC_{50}$) of the effluents based on the results from an initial acute toxicity evaluation. The fish were divided into three groups of 30 per group for the control and effluent treatments in glass aquaria. Each test concentration along with the control was set in triplicates of 10 fish per replicate. The test media were renewed every 48 h to maintain the concentration and minimize oxygen stress for the duration of the exposure. Physical-chemical water parameters were monitored daily using appropriate digital instruments (Jenway). After days 14 and 28, fish samples were dissected to obtain tissues (liver and gill) required for biochemical assays. All procedures performed in studies involving the fish were in accordance with the ethical standards of the University of Lagos Committee on the use of animal subjects in scientific research.

2.4. Determination of Oxidative Stress Enzyme Biomarkers

The oxidative stress biomarker responses in the liver and gills of Nile tilapia evaluated after exposure to pharmaceutical effluents after the 14 and 28 days bioassay were reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT) and lipid peroxidation (MDA). The reduced glutathione (GSH) content of liver and gill tissues as non-protein sulphydryls was estimated according to the method described by Sedlak and Lindsay [21]. Superoxide dismutase activity was determined by its ability to inhibit the auto-oxidation of epinephrine by the increase in absorbance at 480 nm as described by Sun and Zigma [22]. Catalase activity was determined according to Sinha *et al.* [23]. It was assayed colorimetrically at 620 nm and expressed as micromoles of hydrogen peroxide (H_2O_2) consumed/min/ml/mg protein at 25˚C. Malondialdehyde (MDA) an index of lipid peroxidation was determined using the method of Buege and Aust [24].

2.5. Determination of Transaminase Enzymes Responses

Aspartate aminotransferase (AST) and alanine transaminase (ALT) levels were determined following the methods of Reitman and Frankel [25].

2.6. Data Analyses

Toxicological dose-response data involving quantal response was analyzed by Probit analysis. One-way analysis of variance (ANOVA) was used to determine

the differences ($p < 0.05$) among the various groups. Difference between each treatment group and the control was determined using Duncan multiple range test at $p < 0.05$.

3. Results

3.1. Physicocphemical Characteristics of the Pharmaceutical Effluents

The results of the physicochemical characterization of pharmaceutical effluent A indicated that suspended solids (90.1 mg/L), nitrate (225 mg/L), sulphate (450 mg/L), phosphate (164 mg/L), total hardness (600 mg/L) and cobalt (0.725 mg/L), colour (2490 PCU), turbidity (402 NTU), alkalinity (250 mg/L) were above NESREA limits [26] (**Table 1**).

Table 1. Physicochemical properties of pharmaceutical effluents.

Parameters	Effluent A	Effluent B	NESREA[a]
Temperature ˚C	24.4	25.9	<40
pH	6.65	6.81	6.5 - 9.0
DO (mg/L)	3.5	1.8	-
Conductivity (µS/cm)	333.5	526	500
Colour (PCU)	2490	100	3
Appearance	Yellow	Whitish	Clear
Alkalinity (mg/L)	250	212.5	200
Chloride	425.4	63.81	600
Turbidity NTU	402	102	5
Total Dissolved Solid (mg/L)	169	255	2000
Total Suspended Solid (mg/L)	90.1	24.5	30
Nitrate (mg/L)	225	148	20
Nitrite (mg/L)	53.4	42.5	-
Sulphates (mg/L)	450	0	200
Phosphate (mg/L)	164	40.5	5
Salinity (ppt)	0.16	0.24	0.6
Total Hardness (mg/L)	600	600	150
Sodium (mg/L)	120	100	200
Magnesium (mg/L)	200	150	200
Hydrogen Sulphide (mg/L)	0.1	0.15	0.2
TOC %	4.67	4.51	10
COD (mg/L)	80	100	80
BOD (mg/L)	20	31	30
Oil and Grease (mg/L)	17.1	11.2	-
Iron (mg/L)	0.547	1.753	20
Lead (mg/L)	<0.001	<0.001	0.05
Zinc (mg/L)	0.199	0.594	1
Copper (mg/L)	0.033	0.036	1.5
Cobalt (mg/L)	0.724	0.533	0.5
Manganese (mg/L)	0.144	0.226	5

Effluent A—Pharmaceutical effluent A; Effluent B—Pharmaceutical effluent B; [a]NESREA [26].

The results of pharmaceutical effluent B physicochemical analysis indicated colour (100), turbidity (102 NTU), nitrate (148 mg/L) and total hardness (600 mg/L) were higher than NESREA stipulated limits (Table 1). Equally, higher than the NESREA limits were the levels of conductivity (526.0 µS/cm), alkalinity (212.50 mg/L), phosphate (40.5 mg/L), cobalt (0.533 mg/L), chemical oxygen demand (100 mg/L) and biological oxygen demand (31 mg/L) (Table 1).

3.2. Acute Toxicities of the Pharmaceutical Effluents on *Oreochromis niloticus*

The 96-hour lethal concentration (LC_{50}) of pharmaceutical effluent A on juvenile tilapia fish was 27.0 ml/L (V:V) (Table 2) whereas that of pharmaceutical effluent B was 18.0 ml/L (V:V) (Table 3). The fish responded to the pharmaceutical effluents in a dose-dependent manner.

3.3. Oxidative Stress Enzymes Responses of Nile Tilapia (*Oreochromis niloticus*) to Pharmaceutical Effluent A

The results of the oxidative stress biomarkers in the gill and liver are presented in Table 4. The levels of GSH in the gills from the treated groups significantly ($p < 0.05$) decreased on days 14 and 28 in relation to the control. Activities of SOD in the gills of the treated groups were significantly ($p < 0.05$) lower than the control throughout the duration of exposure. $1/10^{th}LC_{50}$ of the effluent A inhibited ($p < 0.05$) the activities of gill CAT on days 14 and 28. The levels of MDA in the gills of the treated groups significantly significant ($p < 0.05$) increased throughout the period of exposure.

The levels of GSH in the liver of the treated groups were significantly lower ($p < 005$) than the control on day 14. However, there was no significant ($p > 005$) between the control and the $1/100^{th}LC_{50}$ group on day 28. After 28 days, the activities of SOD in the liver of the treated groups were significantly ($p < 0.05$) lower than the control. CAT activities in the liver of the treated groups significantly ($p < 0.05$) decreased after 14 and 28 days of exposure. The sub-lethal concentrations of effluent A caused a significant ($p < 0.05$) elevation in the levels of MDA.

Table 2. Sub-lethal dose determination for pharmaceutical A after 96 hours.

Effluent A	LC_5 (ml/L)	LC_{50} (ml/L)	LC_{95} (ml/L)	DF	SE	Equation of line
Concentration	1.9	27.0	38.84	3	0.36	Y = 1.2x ± 0.6
Confidence Interval	0.01 - 0.53	1.39 - 4.00	18.28 - 258.53			

Table 3. Sub-lethal dose determination for pharmaceutical B after 96 hours.

Effluent B	LC_5 (ml/L)	LC_{50} (ml/L)	LC_{95} (ml/L)	DF	SE	Equation of line
Concentration	7.00	18.00	42.63	3	0.37	Y = 2x ± 0.5
Confidence Interval	0.00 - 0.38	0.44 - 3.00	15.20 - 2211.00			

Table 4. Oxidative stress enzymes responses of the *Oreochromis niloticus* to pharmaceutical effluent A.

Gills				
Days Concentrations	GSH (μmol/ml/mg pro)	SOD (μmol/ml/mg pro)	CAT (μmol/ml/mg pro)	MDA (μmol/ml/mg pro)
14 days				
Control	18.90 ± 0.88	4.52 ± 0.14	30.71 ± 1.80	0.78 ± 0.10
$1/100^{th}LC_{50}$	16.10 ± 0.61*	3.92 ± 0.12*	26.08 ± 1.06	3.49 ± 0.68*
$1/10^{th}LC_{50}$	13.99 ± 0.30*	3.21 ± 0.02*	23.21 ± 1.41*	4.47 ± 0.35*
28 days				
Control	17.25 ± 0.67	4.62 ± 0.36	30.36 ± 1.42	0.88 ± 0.05
$1/100^{th}LC_{50}$	13.79 ± 0.85*	3.06 ± 0.05*	27.83 ± 0.75	4.03 ± 0.36*
$1/10^{th}LC_{50}$	11.02 ± 0.43*	2.94 ± 0.03*	25.55 ± 0.50*	5.44 ± 0.61*
Liver				
Days Concentrations				
14 days				
Control	15.74 ± 0.22	4.06 ± 0.61	35.54 ± 1.15	0.73 ± 0.05
$1/100^{th}LC_{50}$	12.91 ± 0.56*	2.69 ± 0.32	23.36 ± 0.54*	2.00 ± 0.29*
$1/10^{th}LC_{50}$	9.82 ± 0.49*	2.27 ± 0.04*	22.03 ± 0.95*	4.41 ± 0.06*
28 days				
Control	16.10 ± 1.00	4.36 ± 0.20	34.12 ± 1.07	0.76 ± 0.04
$1/100^{th}LC_{50}$	12.41 ± 1.42	3.17 ± 0.41*	19.32 ± 1.20*	1.820 ± 0.38*
$1/10^{th}LC_{50}$	9.31 ± 0.75*	1.85 ± 0.33*	14.50 ± 0.43*	5.69 ± 0.34*

*Denotes treatments that are significantly ($p < 0.05$) different from the control within the same duration of exposure.

3.4. Oxidative Stress Enzymes Responses in the Liver of Nile Tilapia (*Oreochromis niloticus*) to Pharmaceutical Effluent B

The results of the oxidative stress biomarkers in the gills and liver of the fish exposed to effluent B are presented in Table 5. GSH levels in the gills of the treated groups were significantly ($p < 0.05$) lower than the control after the duration of exposure. The SOD activities in the gills of the treated groups were lower than the control, however, only those in the $1/10^{th}LC_{50}$ group were significant ($p < 0.05$) after 14 days of exposure. On day 28, the SOD activities in the treated-groups were markedly ($p < 0.05$) lower than the control. CAT activities in the gills of were markedly ($p < 0.05$) lower throughout the duration of the exposure. The levels of MDA in the gills of the treated groups were significantly ($p < 0.05$) higher than the control throughout the period of exposure.

The GSH levels in the liver of the treated groups were significantly ($p < 0.05$) lower than the control on day 28. The activities of the SOD in the liver of the treated groups were significantly lower than the control but did not vary significantly ($p > 0.05$). CAT activities of the liver of the treated groups were significantly

Table 5. Oxidative stress enzymes responses in the liver of the Tilapia Fish (*Oreochromis niloticus*) to pharmaceutical effluent B.

Gills				
Days Concentrations	GSH (µmol/ml/mg pro)	SOD (µmol/ml/mg pro)	CAT (µmol/ml/mg pro)	MDA (µmol/ml/mg pro)
14 days				
Control	18.04 ± 0.72	5.76 ± 0.616	31.33 ± 0.65	0.83 ± 0.07
$1/100^{th}LC_{50}$	15.09 ± 0.60*	4.296 ± 0.648	27.76 ± 0.75*	1.41 ± 0.04*
$1/10^{th}LC_{50}$	13.90 ± 0.32*	2.82 ± 0.51*	26.48 ± 0.94*	2.91 ± 0.50*
28 days				
Control	17.18 ± 0.57	5.28 ± 0.33	33.87 ± 0.60	0.82 ± 0.04
$1/100^{th}LC_{50}$	14.49 ± 0.77*	3.75 ± 0.058*	26.26 ± 1.27*	1.30 ± 0.20*
$1/10^{th}LC_{50}$	13.50 ± 0.39*	3.506 ± 0.15*	22.67 ± 0.83*	2.61 ± 0.28*
Liver				
Days Concentrations				
14 days				
Control	19.70 ± 0.232	4.10 ± 0.07	35.28 ± 0.61	0.74 ± 0.05
$1/100^{th}LC_{50}$	18.85 ± 0.90	3.46 ± 0.28	28.47 ± 1.12*	1.88 ± 0.08*
$1/10^{th}LC_{50}$	14.83 ± 0.26*	3.82 ± 0.52	21.39 ± 0.62*	2.31 ± 0.10*
28 days				
Control	21.04 ± 0.89	4.18 ± 0.36	24.47 ± 0.23	1.27 ± 0.30
$1/100^{th}LC_{50}$	18.14 ± 0.65*	3.61 ± 0.30	22.82 ± 0.87	1.85 ± 0.18*
$1/10^{th}LC_{50}$	15.54 ± 0.39*	3.52 ± 0.37	18.82 ± 0.65*	2.78 ± 0.19*

Denotes treatments that are significantly ($p < 0.05$) different from the control within the same duration of exposure.

($p < 0.05$) lower than the control after 14 days of exposure. After 28 days of exposure, CAT activities in the liver of $1/10^{th}LC_{50}$ group were significantly ($p < 0.05$) lower than the control whereas the activities in the liver in the $1/100^{th}LC_{50}$ group did not markedly ($p > 0.05$) differ from the control. The levels of MDA in the liver of the treated groups were significantly ($p < 0.05$) higher than the control throughout the period of exposure.

3.5. Transaminase Enzymes Responses

Figure 1 & Figure 2 display the levels of transaminase enzymes in the fish exposed to sub-lethal concentrations of pharmaceutical effluent A. AST and ALT levels in the liver of the treated groups were significantly ($p < 0.05$) higher than the control after 14 and 28 days. AST levels in the liver of the fish ranged from 76.27 ± 1.02 to 98.99 ± 1.00 IU/L on day 14 whereas on day 28 they ranged from 74.08 ± 0.788 to 88.78 ± 1.22. ALT levels ranged from 26.78 ± 1.24 to 36.33 ± 0.56 IU/L and 24.85 ± 0.87 to 34.32 ± 1.00 IU/L on day 14 and 28 respectively.

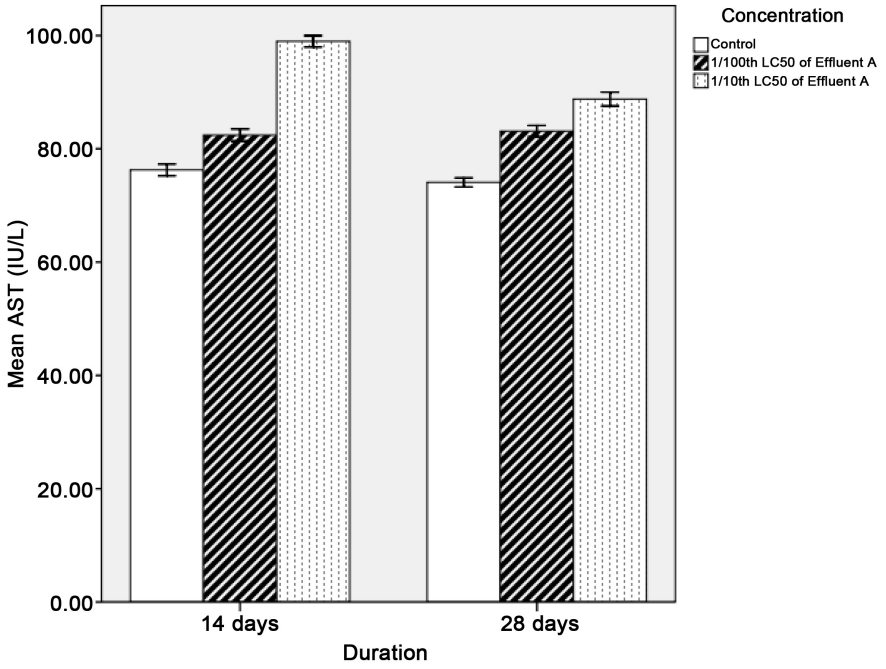

Figure 1. AST alterations in *O. niloticus* during chronic exposure to pharmaceutical effluent A.

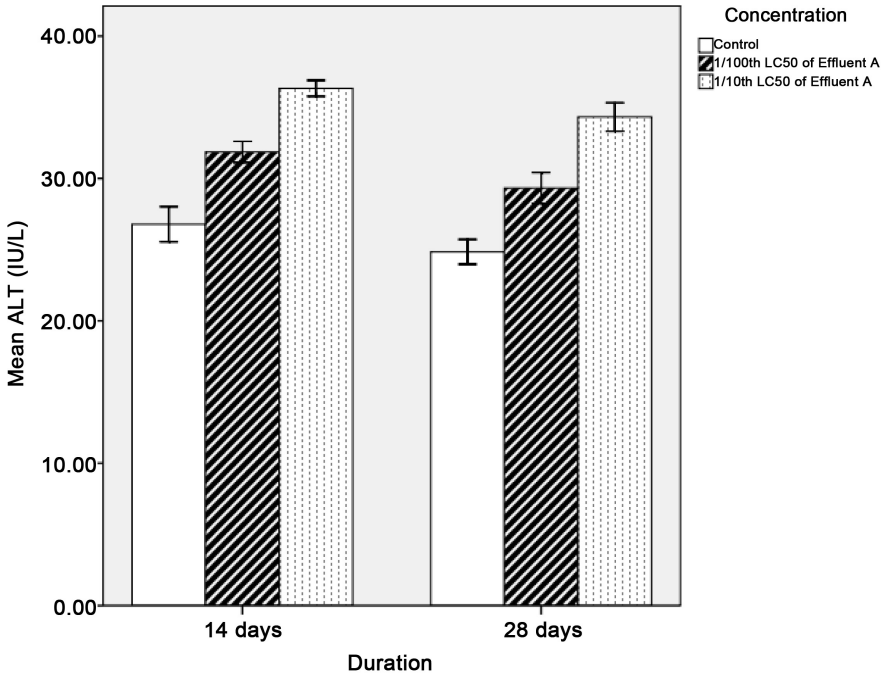

Figure 2. ALT alterations in *O. niloticus* during chronic exposure to pharmaceutical effluent A.

Figure 3 & **Figure 4** illustrate the levels of transaminase enzymes in the fish exposed to sub-lethal concentrations of pharmaceutical effluent B. AST and ALT levels in the liver of the effluent B treated groups were significantly ($p < 0.05$) higher than the control after 14 and 28 days. The observed levels of AST in the

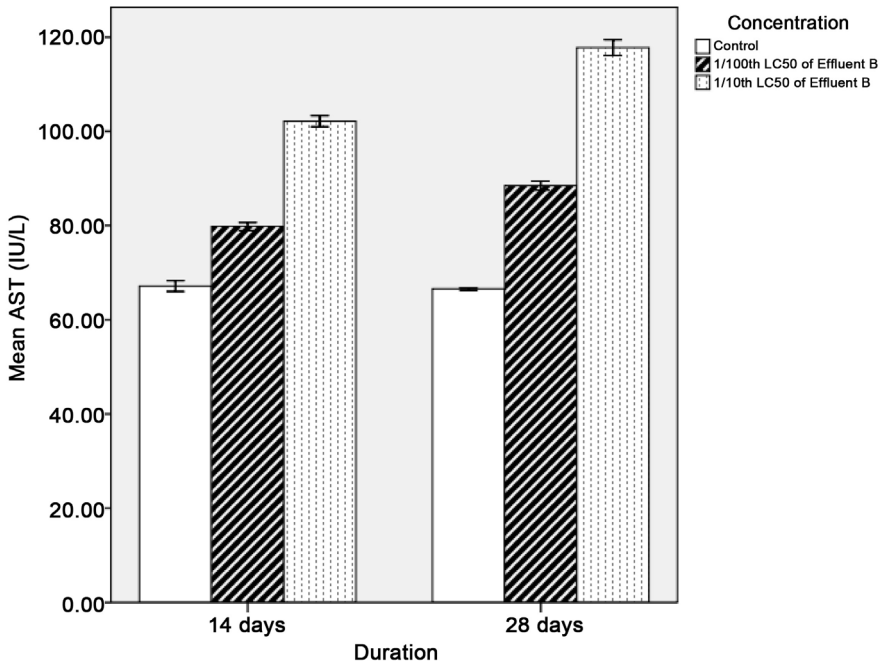

Figure 3. AST alterations in *O. niloticus* during chronic exposure to pharmaceutical effluent B.

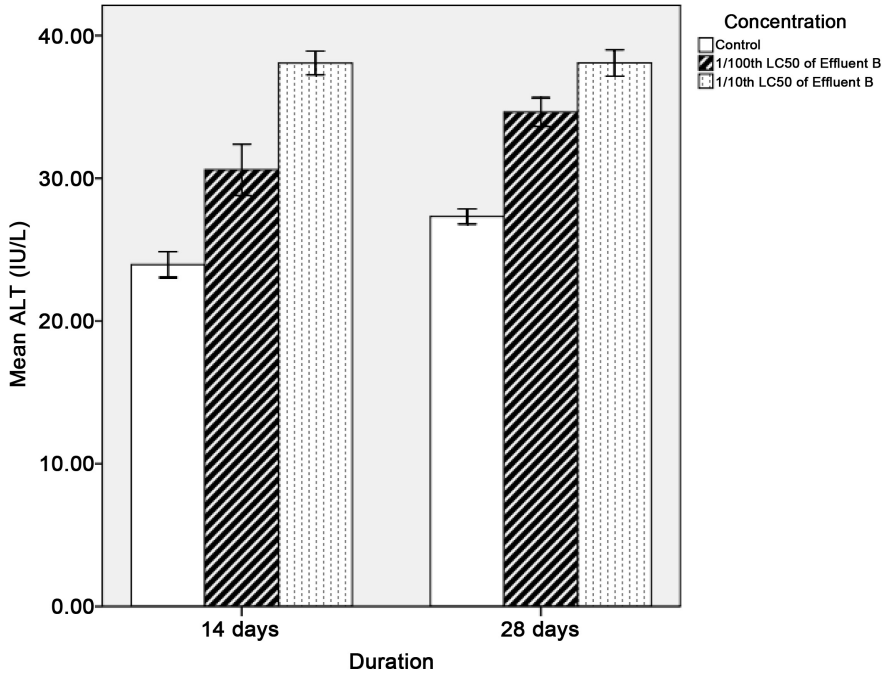

Figure 4. ALT alterations in *O. niloticus* during chronic exposure to pharmaceutical effluent B.

liver of the fish ranged from 67.16 ± 1.18 to 102.17 ± 2.06 IU/L on day 14. By day 28, the levels of AST ranged from 66.57 ± 0.26 to 117.79 ± 1.68 IU/L. ALT levels ranged from 23.95 ± 0.90 to 38.07 ± 0.83 IU/L and 27.32 ± 0.53 to 38.08 ± 0.92 IU/L on day 14 and 28 respectively.

4. Discussion

The monitoring of pharmaceutical effluents before they are released into water bodies is pertinent to the protection of aquatic organisms that come in contact with their constituents. The physicochemical results of the effluents in the present study indicated that some of their parameters were not in accordance with NESREA limits. This agrees with a previous study [27] that said that the effluents were not treated properly before they were discharged into the nearby water bodies.

Specifically, the levels of nitrates and phosphates were higher than the stipulated limits. Nitrates and phosphates are important in aquatic ecosystems because they provide food for algae and plants which serve as food for fishes. Thus, an increase in nitrates and phosphates may lead to an increase in the fish population. However, if the concentrations of nitrates and phosphates increase beyond safe limits this may lead to an exponential increase in algal and plant growth (eutrophication). This phenomenon is associated with decreased levels of dissolved oxygen, thus, impairing the life functions of fishes.

The concentrations of cobalt in the effluents were also found to be higher than the limits. A previous report [28] observed that the chronic exposure of aquatic organisms to cobalt was a causal factor of growth reduction.

In this study, the biochemical responses of Nile tilapia (*O. niloticus*) to sub-lethal concentrations of pharmaceutical effluents from two industries in the most industrialized city (Lagos) in Nigeria were also investigated. The oxidative stress biomarkers including superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH) and malondialdehyde (MDA) as well as transaminase enzymes were monitored in Nile tilapia (*O. niloticus*) exposed to the pharmaceutical effluents.

GSH is an endogenous antioxidant that is directly involved in the neutralization of free radicals and reactive oxygen species [29]. Reduced glutathione donates a reducing equivalent to ROS to neutralize them [30]. This reaction converts GSH to glutathione disulfide [31]. The increase in the oxidized state of glutathione in relation to the reduced state indicates oxidative stress. This implies that the depletion of GSH could sensitize the organism to the toxicity of xenobiotics that induce oxidative stress [32]. However, the elevation of GSH levels could be an adaptive mechanism to moderate oxidative stress [33]. In the present study, depletion of GSH was observed in the gills and liver of *O. niloticus* exposed to sub-lethal concentrations of pharmaceutical effluents. Heavy metals, one of the constituents of pharmaceutical effluents can substantially alter GSH levels in the tissues of fishes, either by causing depletion or elevation in its levels [34] [35] [36].

Catalase is an antioxidant enzyme that catalyzes the conversion of H_2O_2 into O_2 and H_2O [37]. Therefore, depletion of this enzyme may lead to an increase of ROS but an increase in the levels of the enzyme may be a counteractive or adaptive response to the production of ROS. CAT levels were generally lower in the

treated fish. Furthermore, the activities of SOD in the tissues of the test organism were inhibited by the pharmaceutical effluents. SOD works with CAT to break down H_2O_2 [11]. SOD also catalyzes the dismutation of superoxide, one of the major ROS in the cell, into O_2 or H_2O_2 [38].

Ultimately, there was a general significant elevation of MDA in the tissues of the test fish exposed to the pharmaceutical effluents. MDA is normally used as a lipid peroxidation marker. Lipid peroxidation occurs when free radicals or ROS oxidatively degrades lipids in cell membranes thereby causing cellular damage [10]. The high levels of MDA in the tissues of the test fish are indicative of pharmaceutical effluent-induced peroxidative damage.

Transaminase enzymes' activities are sensitive measures of hepatotoxicity and histopathologic changes in the liver [39]. AST and ALT levels were elevated in the liver of the fish exposed to the pharmaceutical effluents. A study [40] has reported that increased ALT and AST levels suggest increased proteolysis, enhanced protein catabolism and hepatocellular damage in the organism. Another set of studies [41] observed a significant increase in the levels of AST and ALT in animals treated with lead and nickel respectively. Similarly, it was reported that praziquantel, a pharmaceutical drug, caused an elevation in the levels of AST and ALT in *C. gariepinus* [42]. This indicates that the synergistic or additive interactions among the toxic components of the effluents may be the causal factor of the elevation in the levels of the transaminase enzymes.

5. Conclusion

The present findings have established that sub-lethal concentrations of pharmaceutical effluents induce biochemical effects in Nile tilapia, in terms of oxidative stress and hepatotoxicity. This raises concerns about the impact of pharmaceutical effluents on the health of fishes that inhabit aquatic environments that receive these effluents. Therefore, there is a need for proper legislation that makes it mandatory for pharmaceutical industries to effectively treat their effluents before discharging them into the environment. Their effluents should be monitored frequently in order to protect the rich biodiversity of the aquatic ecosystems. This study has shown that oxidative stress and transaminase enzymes' activities can be effectively used to monitor the effects of treated pharmaceutical effluents in aquatic organisms.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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