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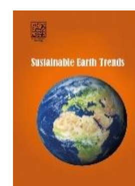

Ecotoxicological effects of linear Alkylbenzene sulfonate on biochemical indices in *Oreochromis niloticus* exposed invitro

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A B S T R A C T

The anionic surfactant linear alkyl benzene sulfonate (LABS) is a synthetic surfactant, frequently utilized in domestic detergents as well as for a variety of industrial purposes. The effects of this ubiquitous pollutant on freshwater fish *Orechromis niloticus* were investigated. For 30 days, the fish were treated with the concentration ranges of LABS reported in the field (0.05,0.1,0.15, and 0.2) mg/L, including the control. Fish in both the control and experimental groups received twice-daily meals at 3% of their body weight throughout the experiment. A fish was removed from each plastic tub after each experimental period (2,9,16,23, and 30th) and was punctured in the heart to obtain blood samples. Blood was drawn and placed in sample bottles and analyzed for lipid peroxidation, aspartate aminotransferase, alanine aminotransferase, catalase, protein, and superoxide dismutase using standard methods. No deaths were noticed in either the control fish or the fish subjected to different sublethal concentrations of linear alkylbenzene sulfonate. After exposure to the surfactants, there was a noticeable alteration in the activities of the marker's indices in the blood of the fish. Tissue-specific, clear-cut time and dose dependence characterized the rate of changes in the assessed markers in the fish.

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1. Introduction

The most common sulfonated alkylbenzene salt, linear alkyl benzene sulfonate (LABS), is a synthetic surfactant with the highest volume utilized in both home detergents and a wide range of industrial uses. With an aromatic ring sulfonated in the para-position and an alkyl chain in each, they are a mixture of isomers and homologs (Cserhati et al., 2002). The surfactant, which is a member of the anionic surfactant family, has great detergency and is widely employed in liquid detergent compositions because of its high solubility. Like soap, LABS is sensitive to water hardness, and as water hardness increases, LAS performs less effectively as a deterrent (Verge et al., 2001)

According to studies, linear alkylbenzene sulfonate is a form of ubiquitous pollution that, even at low concentrations, can harm aquatic animals and aquatic plants by toxically harming them. Similar to this, LAS may hinder the development and reproduction of invertebrates and other species of the soil ecosystem at 40–60 mg kg^{-1} dry weight of soil[2]. All around the world, amphibian numbers have been drastically declining. One of the main reasons for this decrease, especially in the aquatic environment, is due to aquatic pollutants. The fall of bullfrog tadpoles has also been attributed to LAS because it alters their cardiac function, which can harm their development and make them more sensitive to predators and less competitive in terms of successful reproduction (Nusrat Bibi et al., 2014)

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The amount of LABS that accumulates in the sludge is dependent on the kind of sludge and the procedures that occur throughout the treatment process. In most cases, primary sludge retains 10–20 percent of the LABS present in the raw wastewater. Numerous surfactants have been found to induce oxidative stress, resulting in adverse effects on tissue integrity, membrane permeability, and biomolecular integrity in living organisms. The cellular processes of metabolism and regulation experience alterations when the antioxidant defense mechanisms of organisms fail to entirely eradicate reactive oxygen species (ROS). This elimination process is facilitated by specific enzymes, including Catalase (CAT), Superoxide dismutase (SOD), and Glutathione reductase (He et al., 2015). Total proteins, total lipids, and cholesterol are biochemical indicators that are routinely employed in assessing the general health status of fish under stress situations. Because of the strong relationship between a fish's circulatory system and its surroundings, fish physiology and biochemical indices such as metabolic enzymes are useful instruments for evaluating water quality (Di Giulio and Hinton, 2008). The *Oreochromis niloticus,* commonly known as the Nile tilapia, holds significant importance in the realm of commercial aquaculture due to its watery habitat. Due to its notable attributes like as its rapid growth rate, high nutritional value, and resilience against diseases and harmful substances, this organism is extensively employed in the field of ecotoxicology (Ibrahim et al., 2012). Furthermore, it holds considerable importance in food chains and serves as a bio-indicator for monitoring pollution in aquatic systems. Blood parameters are early warning indications of pathophysiological changes throughout the body induced by toxicant exposure, which exhibits pathological changes before any external poisoning symptom is displayed. As a result, they're a useful tool for tracking fish health. Blood cell responses are important indicators of changes in the fish's internal and external environment (Kumar et al., 2007). As a result, changes in biochemical parameters are excellent indicators of water quality. Integrated biomarker response provides an all-encompassing inquiry that integrates all biomarker responses and plays a critical role in identifying the toxicity of pollutants (Samanta et al., 2015) An amalgamated biomarker study gives a detailed understanding of an organism's response to toxic substances because individual biomarkers cannot provide an accurate and useful assessment of the toxicity of toxicants in an organism (Kumar et al., 2014). Therefore, the objective of this research was to employ the freshwater fish *O. niloticus* as a model to evaluate the concentration-dependent effects of LABS on a protein level, lipid peroxidase, and aminotransferase activity using long-term static bioassays.

2. Material and Methods

2.1. Chemicals

Sodium phosphate buffer (pH 7.8), methionine, nitro blue tetrazolium (NBT), riboflavin, 400 millimolar (mM) alanine, 210mM α-keto glutarate, 2.5mM arsenate, 20mM thiobarbituric, hydrochloric acid, trichloroacetic acid, H_2O_2, sodium phosphate, and linear alkyl benzene sulfonate.

2.2. The Fish

Fish experimentation was done following all applicable rules and regulations. A greenhouse was constructed to depict the natural habitat of the fish and was cleaned daily. Clayey loam soil was used in constructing a mini pond with the dimensions of; 270 1/2 x 24 1/4 x 29 ½. The following conditions and physicochemical parameters of the water in the pond were maintained; Temperature 27.50 ± 0.25°C, pH 7.2 ± 0.03, dissolved oxygen 7.20 ± 0.10mg/l, total alkalinity 148 ± 2.1mg/l as $CaCO_3$, hardness 112 ± 1.5mg/l as $CaCO_3$.
The *O.niloticus* used in this study was tracked from the egg stage until the desired stage of maturation was reached. The fish were fed with fodder rich in proteins and vitamins twice daily. The fish produced from this setup was allowed to grow for 20 weeks to obtain the desired size for toxicological investigation

2.3. Experimental Design

A total of 150 juvenile *O.niloticus,* with an average weight of 32.60 ± 0.10gm and an average length of 11.20 ± 0.03cm, were acquired from a private farm in Otuoke, Bayelsa State, Nigeria. The cichlids were meticulously transported and acclimatized to laboratory settings in glass tanks filled with distilled water for fourteen days before to being used in the experiment. The fish were given two daily

feedings of TAIYO feeds, which consisted of fish meal, wheat flour, soybean meal, maize meal, yeast, vitamins, and mineral salt. The feed had a proximate analysis that showed a crude protein content of 32%, crude fat content of 4%, maximum crude fiber content of 5%, and maximum moisture content of 10%. The feeds and fecal wastes that were not consumed were taken out, and water was refilled three times per week, following the recommendation of Sahito et al. (2015). The water used for toxicity studies was dechlorinated tap water. The water was rendered free of chlorine by leaving it uncovered for 36 hours (Audu and Yola, 2020, Kolawole and Mustapha, 2023). This water was utilized for acclimatization, conducting control tests, and preparing the different concentrations of the test chemicals. Thirty glass aquaria were utilized, three for each treatment. Fish were treated for 30 days to the linear alkyl benzene sulfonate concentration ranges reported in the field (0.05, 0.1, 0.15, and 0.20 mg/L) (Kwikiriza et al., 2016). Through successive diluting, the stock solution yielded the concentrations of linear alkyl benzene sulfonate needed for the test. Both the control and experimental groups of the fish were fed twice a day at 3% of their body weight throughout the trial. Every day the glass aquaria were cleaned as best they could, and the water and surfactant were changed. After every experimental period—the second, ninth, sixteenth, twenty-third, and thirtyth—a fish was taken out of each glass aquarium, and its heart was punctured to collect blood samples for biochemical analysis. Using centrifuge tubes devoid of anticoagulant, the blood samples were drawn. Serum was obtained via centrifugation, in which blood was spun for ten minutes at a speed of 3,000 revolutions per minute. The serum samples were then kept, until the analysis, at -80°C. A fish specimen was quickly dissected after the blood was drawn, and the kidney, gills, and liver were meticulously removed. Then, to keep the temperature at -25^{0}C, these removed organs were moved to a container filled with liquid nitrogen to study lipase peroxidase

2.4. Biochemical parameters

Total protein: Total protein concentrations in terrestrial and aquatic species are utilized as a broad measure of their clinical health, stress, and well-being. The refractometry method of analysis was used to evaluate the total protein concentration in fish, following George and O'Neill's (2001) protocol because of its ease, rapidity, and minimal amount of material required. To separate the serum, the blood sample was centrifuged at 600 $\times$ g for 5 minutes. The measurement of total serum protein content was conducted using a portable refractometer manufactured by JSCP-Uridens(r) in Sao Paulo, Brazil. A volume of 10µl of serum was added to the portable refractometer, and the concentration of total serum protein was measured and reported in grams per deciliter (gdL^{-1}). The calibration of the refractometer was verified by employing distilled water before conducting each set of readings. All measurements were conducted at a temperature of 20°C. The mean ± standard error (mg/100mL) was reported for the concentration of serum protein

2.5. Oxidative Biomarkers

Aspartate aminotransferase: Using Randox kits, the aspartate aminotransferase was determined using the Reitman and Frankel colorimetric method. In a nutshell, plasma was separated from red blood cells by centrifugation and used as an enzyme source. There were 210mM aspartate, 210mM a-keto glutarate, 2.5mM arsenate, and 20mM TRIS-HCl pH 7.5 in the ASAT reaction mixture. 0.1% dinitrophenyl hydrazine in 2N HCl was used to stop the reaction. Following that, an appropriate aliquot was transferred to 1.3N NaOH, the absorbance was read at 440nm, and its Activity was expressed in UI.

Alanine aminotransferase: A colorimetric assay was used to determine the enzyme activity. In a nutshell, the plasma was isolated from red cells through centrifugation and utilized as a source of enzymes. The reaction mixture for alanine aminotransferase consisted of 400 millimolar (mM) alanine, 210mM α-keto glutarate, 2.5mM arsenate, and 20mM TRIS-HCl at pH 7.5. The process was halted by the addition of 0.1% dinitrophenyl hydrazine in a solution of 2N hydrochloric acid. Subsequently, an appropriate portion was transferred to a solution of NaOH with a concentration of 1.3N, and the absorbance was measured at a wavelength of 440nm. The activities of ALAT are expressed in UI.

Lipid peroxidation: Total malondialdehyde (MDA) an index of lipid peroxidation was measured using the method as described by

Otitoloju and Olagoke (2008). A volume of 1.0 milliliter of the supernatant was combined with 2 milliliters of a TCA-TBA HCL reagent, which consisted of thiobarbituric acid at a concentration of 0.37%, hydrochloric acid at a concentration of 0.24N, and trichloroacetic acid at a concentration of 15%, in a ratio of 1:1:1. The tricarboxylic acid-thiobarbituric acid-hydrochloric acid reagent was subjected to boiling at a temperature of 100°Cfor 15 minutes, followed by a cooling period. The flocculent materials were separated using the process of centrifugation, which involved spinning at a speed of 3000 revolutions per minute (rpm) for 10 minutes. The liquid portion was extracted and the optical density was measured at a wavelength of 532nm relative to a control sample. The calculation of MDA involved the utilization of the molar extinction coefficient for MDATBA, which is a complex with a value of 1.56×105 M/Cm.

Catalase: The procedure outlined by Montavon et al. (2007) was used to ascertain the catalase activity. The amount of CAT that resulted in the breakdown of 1 mol of H_2O_2 per minute under the experimental conditions is defined as the enzyme activity per unit. The activity was measured in units per gram of fresh weight (U/g FW).

Superoxide Dismutase: The activity of superoxide dismutase (SOD) was determined in a solution of sodium phosphate buffer (pH 7.8), methionine, nitro blue tetrazolium (NBT), riboflavin, and enzyme extract at concentrations of 50 mmol/L, 13 mmol/L, 75 mol/L, 2 mol/L, and 50 L, respectively. The reaction mixture was subjected to incubation for 15 minutes at a temperature of 25 degrees Celsius while being exposed to fluorescent illumination. The UV/vis spectrophotometer (Purkinje General Instrument Co., Ltd., Beijing, China) was used to measure the absorbance at 560 nm. For comparison, solutions lacking enzyme extract were used as a control and where not lit. Enzyme activity was determined by the volume of the enzyme that caused a 50% inhibition of the reaction, measured in units per gram of fresh weight (FW).

2.6. Data analysis

The statistical differences between the control and the various treatments were compared using a paired t-test. $P < 0.05$ was considered significant. For the analysis, GraphPad InStat (version 3.00, GraphPad InStat Software Inc. 200) was used.

3. Results and discussion

Fish that are exposed to environmental pollutants demonstrate a range of physiological reactions, such as disruptions in blood homeostasis (Pimpao et al., 2007). Blood biochemical profiles offer valuable insights into the internal conditions of an organism (Bagnyukova et al., 2005), and linear-chain alkylbenzene sulfonates are the most popular used synthetic anionic surfactants. They have been successfully used in biotechnological, and other industrial processes, and to remove petrochemical products from polluted soil (Okechukwu and Auta, 2007; Serafim et al., 2012). In the present investigation, no mortality was observed in the control fish as well as the fish exposed to various concentrations of linear alkylbenzene sulfonate. The activity of the marker's indices in the blood of the fish exposed to the surfactants showed marked alterations. The measurement of total protein in plasma is employed as a means of monitoring the progression of immunological diseases, liver dysfunction, and compromised kidney function (Banaee et al., 2011; Chen et al., 2006). No significant difference ($P > 0.05$) between the protein level in the control and the various treatments on day 2 irrespective of the concentration. The experimental results demonstrated a notable alteration in the concentrations of plasma total protein in comparison to the control group of fish. The protein content of the fish subjected to LABs declines as the concentrations of the surfactant and the duration of exposure increase. The level of protein, when the fish was exposed to 0, 0.05, 0.10, 0.15, and 0.20 mg/L LABs were 19.126, 19.110, 19.32, 19.130, and 19.156mg/100ml respectively. The same trends were observed on days 9th and 16th in all the treatments. However, on days 23rd and 30th the protein level decreases with an increase in the concentrations of the toxicant. The protein levels in the treatments 0, 0.05, 0.10, 0.15 and 0.20mg/L LABs were 19.032, 12.107,11.100,9.012, and 9.011mg/100ml respectively on day 23rd, 19.156, 12.014, 11.000, 9.102 and 8.901mg/100ml on the 30th. And were significantly different ($P < 0.05$) at higher concentrations of 0.15 and 0.20 mg/l of LABS for both days. The decrease in the protein level

is proportional to the exposure duration with the concentration of the toxicant (Fig. 1). Protein level varies significantly ($P < 0.05$) between the control and the treatment on days 23 and 30. According to recent investigations, it has been observed that the presence of pollutants has a substantial impact on the overall protein content found in the tissues of medium-sized marine fin fishes originating from the Thoothukudi Coast of India (Dobsikova et al., 2006; Fırat et al., 2011). The protein content in several tissues of fish exhibited a decrease when the concentration of lambda-cyhalothrin increased. Notably, the liver protein content had the most significant decline compared to other tissues (Liebel et al., 2013). It has been observed that exposure to Lambda Cyhalothrin, a type II synthetic pyrethroid, in *Clarias gariepinus* resulted in elevated protein levels due to the induction of toxic stress. A comparable finding was documented by Otitoloju and Olagoke (2008) in a study where *Cirrhinus mrigala* was subjected to diazinon exposure.

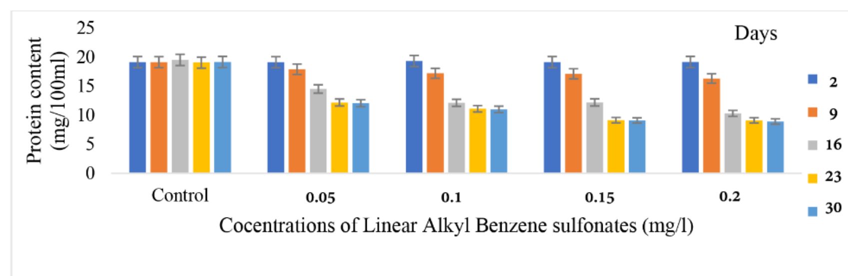

Fig. 1. Changes in the protein content in *O.niloticus* exposed to different concentrations of Linear Alkyl Benzene sulfonates

Aspartate aminotransferase (ASAT), although a liver-specific enzyme, is usually found in low quantities in blood concentrations, and high amounts in skeletal muscle cells and promotes gluconeogenesis from amino acids in association with alanine aminotransferase (Haider and Rauf, 2014). Hence, if chemical aggressors damage some organs, they will release those enzymes toward the plasma followed by an increase in their catalytic activity. Besides the liver, the red blood cells, kidneys, and heart tissues are other sources of ASAT. The present results revealed a significant ($P < 0.05$) increase in ASAT in the serum of linear alkyl benzene sulfonates treated fish compared with the control group, Fig. 2 shows the ASAT activities in the plasma of *O. niloticus* exposed to various concentrations of LABS (mg/L). The enzyme activity was induced in all the treatments irrespective of the durations and the concentrations of the toxicant. The exposure concentrations in mg/L and induction of ASAT (U/ml) at different periods are: Day 2, 0.05(17.87),0.1(19.23), 0.15 (22.50),0.20(26.70), and the enzyme activity only varies significantly ($p \leq 0.05$) between the control and treatment at 0.15 and 0.20mg/L of the toxicant. On day 9, 0.05(17.99), 0.10(23.10), 0.15(26.20), 0.20 (26.80). The level of significance on day 9 is similar to day 2. On day 16, 0.05(19.20), 0.10 (25.10),0.15(28.90), 0.20(28.50), and the inducement varies significantly ($p \leq 0.05$) between the treatments and the control at 0.1mg/L, 0.15mg/l and 0.20mg/L of the surfactant. Day23,0.05 (19.80),0.10(27.30), 0.15(31.90), 0.20(32). Also, on day 30, 0.05(17.22),0.1(29.30),0.15(33.30),0.20(32.90) . The enzyme activity on days 23 and 30 is the same. It varies significantly ($p \leq 0.05$, $p \leq 0.01$) at concentrations of 0.1mg/l, 0.15mg/L and 0.20mg/L.

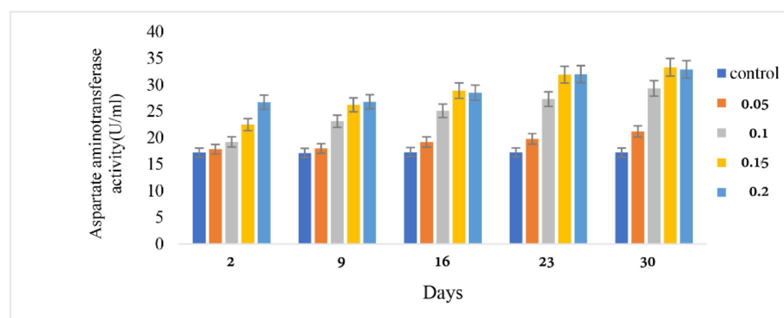

Fig. 2. Activity of aspartate aminotransferase in the plasma of *O. niloticus* exposed to sublethal concentrations of linear alkyl benzene sulfonates. A symbol on the bars indicates significant differences between the control and the experimental groups $^{@}$ ($P < 0.05$); $^{\beta}$ ($P < 0.01$)

The alteration in ASAT content is a sign of tissue or organ damage that activates the enzyme's release into the serum or bloodstream. An uptick in these enzyme activities may benefit fish by facilitating gluconeogenesis and energy generation through the structural reorganization of proteins and the inclusion of keto acids into the TCA cycle. A similar observation was reported by Wu et al. (2019) in mice induced with Malva sylvestris extract. A comparable result was also observed by Begum (2004) in Carbofuran insecticide-induced biochemical alterations in liver and muscle tissues of the fish *Clarias batrachus.* Alanine aminotransferase plays a crucial role in the synthesis and deamination of amino acids during stressful conditions to meet the organism's high energy demand (Huseini et al., 2005). The hepatic cells contain a high concentration of aminotransferase due to the liver's crucial role in converting food substances. In this investigation, the plasma alanine aminotransferase activity in *O. niloticus* subjected to sublethal quantities of linear alkylbenzene sulfonate is shown in Figure 3. The activity of the enzyme is dependent on both time and concentration. The enzyme activity in (U/ml) at 0.05mg/l of LABS on the 2nd, 9th, 16th, 23rd, and 30th days are as follows: 10.40 ± 0.30, 5.11, 15.50 ± 0.41, 21.40 ± 1.70, 32.90 ± 2.11, and 43.50 ± 1.89 respectively. The activity levels on the 2nd to 30th days are as follows: 10.80 ± 1.10, 18.40 ± 0.60, 24.20 ± 3.10, 42.10 ± 0.70, and 57.32 ± 2.47, with a concentration of 0.10mg/l. At a concentration of 0.15mg/l of the toxicant, the enzyme activity on the respective days is as follows: 11.90 ± 1.12, 22.40 ± 0.04, 32.10 ± 0.50, 54.20 ± 0.50, and 63.80 ± 1.13. The ALAT activity on days 2 to 30 at an inducer chemical concentration of 0.20 mg/l is as follows: 12.40 ± 2.60, 28.12 ± 3.10, 36.10 ± 0.58, 59.10 ± 3.10, and 70.23 ± 4.60 (Fig. 3). On day 2 and day 9, the enzyme activity at a concentration of 0.05mg/l showed significant variation ($P < 0.05$) between the different treatments and the control. However, in other treatments, regardless of the day, the enzyme activity was extremely significant ($P < 0.01$) compared to the control (Fig. 3). The observed rise in ALT levels in the plasma of fish treated with LABs, as compared to the control group, suggests tissue damage. This damage may be a result of disruptions in normal physiological and biochemical processes, such as the Krebs' cycle and TCA cycle. Consequently, the enzyme ALT may have leaked from the liver cytosol through the membrane into the bloodstream. The current findings align with the observations of Jee et al. (2005), who reported elevated blood ALT activity in Korean rockfish, *Sebastes schlegeli,* following exposure to cypermethrin. Multiple publications have also noted an elevated level of ALT activity in teleostean fishes as a result of pesticide exposure (Begum, 2004). The enhanced activity of alanine aminotransferase (ALT) supplied the necessary oxaloacetic acid and pyruvate to meet the heightened energy requirements during the stressful conditions caused by carbofuran in the fish. The species referred to as *Clarias batrachus* was studied by Samanta et al. (2015).

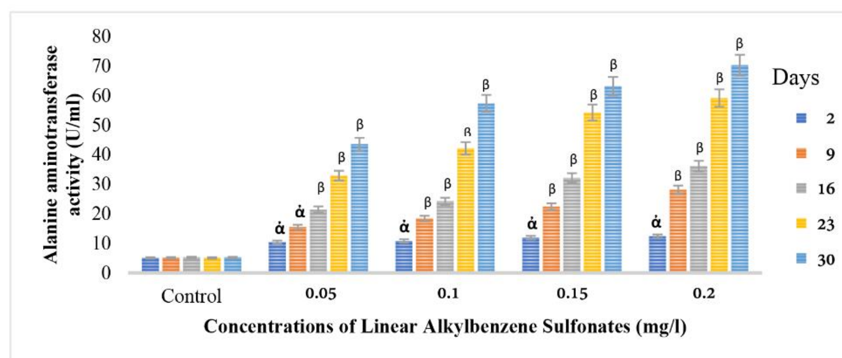

Fig. 3. Activity of alanine aminotransferase in the plasma of *C.gariepinus* exposed to sublethal concentrations of linear alkylbenzene sulfonates; Data presented as mean ± SE. The symbol above bars indicates significant differences between the control and the experimental groups $^{\alpha}$ ($P < 0.05$); $^{\beta}$ ($P < 0.01$).

MDA is commonly employed as a biomarker to assess lipid peroxidation, a process that is considered a valuable sign of oxidative damage in tissues. During this study, the liver, gills, and kidneys of the fish were subjected to exposure to LABS for 48 hours. There were notable increases in the LPO activity in these organs when compared to the control group,

specifically at the lower concentration of the toxicant. The activity of lipase peroxidase in the liver, gills, and kidney of *O.niloticus* revealed that in 48 hours of exposure, there was inhibition in the activity of the enzyme when compared with the control. In this period, at concentrations of 0.00, 0.05, 0.10, 0.15, and 0.20mg/L of LABS the enzyme activities in the liver, gills, and kidney were; liver: 0.702, 0.218, 0.215, 0.216, and 0.213 micromoles/100g of wet tissue; gills: 0.403, 0.389, 0.361, 0.321 and 0.311 micromoles/100 g of wet tissue; kidney: 0.515,0.438, 0.432,0.423 and 0.411 micromoles/100 g of wet tissue. The enzyme activity in the liver on day 2 was significantly ($P \leq 0.05$) inhibited in all the treatments. On day 30, the peroxidase activities in these organs were significantly ($P \leq 0.05$, $P \leq 0.01$) affected in all the treatments (Fig. 4). The range of inductions in the liver, gills, and kidney of the treated fish in order of increasing concentration of the toxicants were; 2.54 - 3.25, 1.20 -1.98, and 1.48 - 2.67 respectively. These increases were statistically significant with a p-value of less than 0.05 across all treatment groups. Likewise, by the 30th day of exposure, there was a statistically significant increase ($P < 0.01$) in the amount of lipid peroxidation (LPO) across all treatment groups. The significance of peroxidase in aquatic organisms can be linked to its crucial role in initiating cellular membrane damage induced by pro-oxidants and xenobiotics as peroxidase serves as an indication for assessing the oxidative damage of cellular components (Kumar et al., 2014). The observed increase in MDA concentrations suggests that the antioxidative mechanisms successfully eliminated reactive oxygen species (ROS) and mitigated oxidative harm within the tissues (Kumar et al., 2014). The enzyme typically exhibits a higher concentration of polyunsaturated fatty acids compared to other enzymes, which has been shown as a significant factor in mitigating cellular dysfunction.

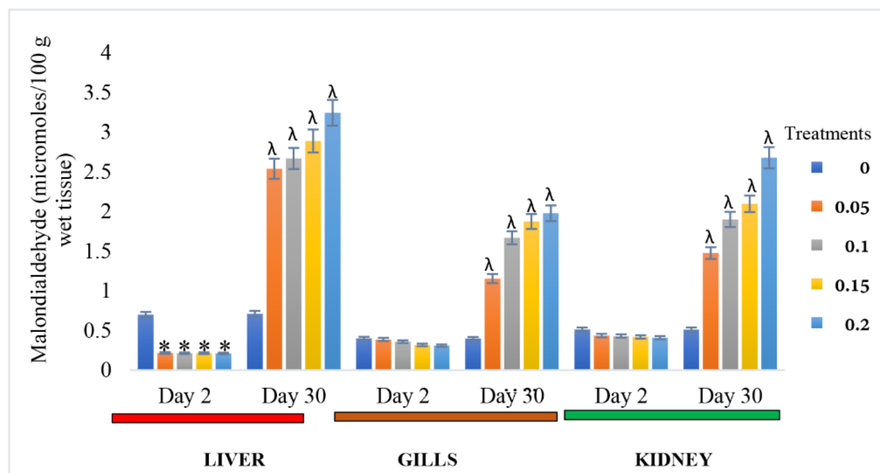

Fig. 4. Variation in lipase peroxidase in the tissues of *O. niloticus* exposed to sublethal concentrations of linear alkylbenzene sulfonates (mg/); Data presented as mean ± SE. A symbol above the bars indicates significant differences between the control and the experimental groups *($P < 0.05$); $^{\lambda}$($P < 0.01$)

Erythrocytes, often known as red blood cells, play a significant role in the antioxidant capacity of the blood. One crucial component of their antioxidant defense system is the enzyme catalase (Bagnyukova et al., 2005). In this investigation, the catalase activities increased with the increase in the concentration of the toxicant on day 2, and were significant between the control and various treatments ($P \leq 0.05$) except with the lower concentration of 0.055mg/L. the catalase activities decrease after day 2, in the order day 9< 16< 23< 30. At the higher concentration of 0.20mg/L, on days 16,23 and 30, the catalase activities were highly significant ($P < 0.1$) when compared with the control .Elevated CAT activities were observed in the fish in this study that were exposed to low concentrations of linear alkyl benzene sulfonates. As part of its detoxifying process, ROS are reduced by catalase (Regoli et al., 2011; Regoli and Giuliani, 2014). Fig. 5 shows the catalase activity in erythrocytes of *O.niloticus* sublethal exposed to LABs, and it shows that LABs inhibited catalase in investigated fish.

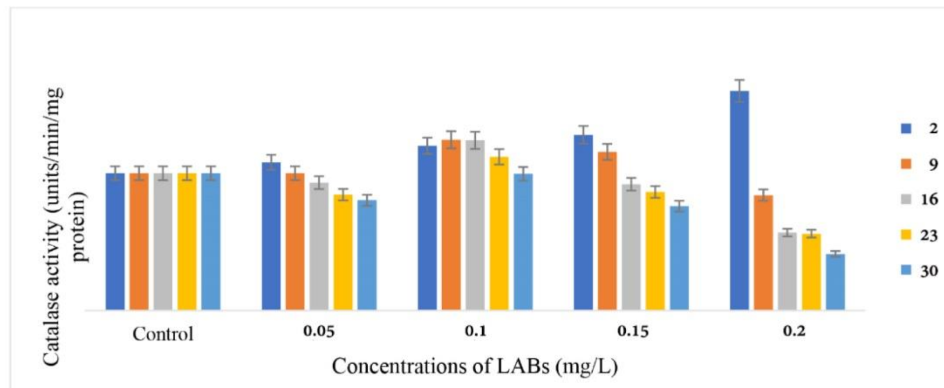

Fig. 5. Catalase activities in the erythrocytes of *O.niloticus* exposed low concentrations of LABs. Dose and Time-Dependent Effects; Data presented as mean ± SE.

The rise in the activities of the enzyme could be attributed to the elimination of reactive oxygen species from the cell caused by the surfactant's exposure, as observed in the study conducted by Stara et al. (2012). This finding aligns with the outcomes of earlier research on fish exposed to various pollutants, including herbicides, as reported by Guilherme et al. (2012) and Stara et al. (2012). Contrary to this discovery, Oruc and Usta (2007) found that CAT activity in the muscle of *C. carpio* was suppressed after being exposed to diazinon. Similarly, Tripathi and Shasmal (2010) observed a notable decrease in CAT activity in the skeletal muscle and brain of Heteropneustes following exposure to chlorpyrifos. Additionally, Kaur and Jindal (2017) discovered that exposure to chlorpyrifos resulted in reduced CAT activity in the gills, kidneys, and liver of *Ctenopharyngodon idellus.* The study on the erythrocytes of fish exposed to different concentrations of LABS revealed that the activity of SOD, an enzyme used as a marker, responds in a specific manner to rising levels of the toxicant. On the second day, the activity of the Superoxide Dismutase is comparable to that of the control, with values ranging from 3.82 to 4.19 units per milligram of hemoglobin (u/mgHb). While there was a slight rise in enzyme activity when exposed to a higher concentration of the surfactant this change was not statistically significant ($P > 0.05$). On the ninth day, there was a rise in the enzyme activity, which subsequently decreased as the duration of exposure increased. The enzyme activities reached their peak and lowest levels at a concentration of 0.20mg/l, with values of 8.422 u/mgHb and 0.134 u/mgHb, respectively. These differences were very significant ($P < 0.05$ and $P < 0.01$) compared to the control (Fig. 6).

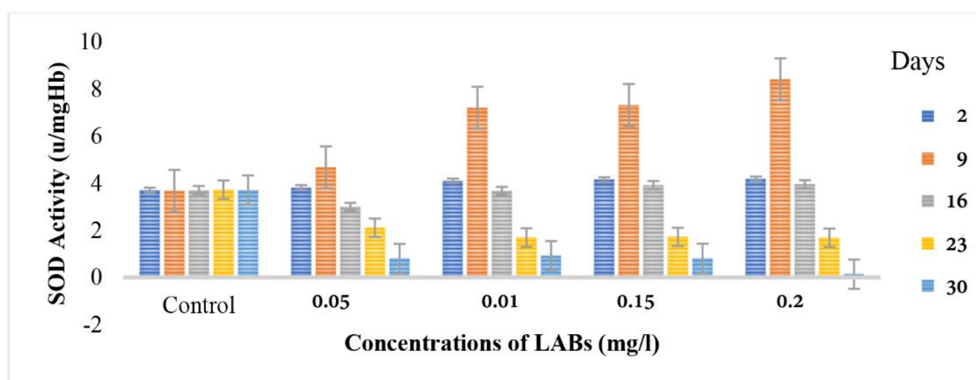

Fig. 6. SOD activity in the erythrocytes of *O.niloticus* exposed low concentrations of LABs; Dose and Time-Dependent Effects; Data presented as mean ± SE.

The rise in SOD activities can be ascribed to the interaction between SOD and vulnerable and crucial cellular components, such as nitrogen oxide radicals and harmful chemicals. Yu et al. (2021) observed an increase in the activity of Superoxide Dismutase in the Gill, Liver, and Plasma of *Catla Catla* fish that were exposed to Methyl Parathion. Naz (2017) reported a rise in the amount of superoxide dismutase activity in three species of Indian main carps, namely *Catla catla, Cirrhinus mrigala*, and *Labeo rohita*, following exposure to a cocktail of

pesticides. The heightened Superoxide Dismutase activity signifies an escalated antioxidant status, aiming to counteract the effects of Reactive Oxygen Species. The induction of Superoxide Dismutase observed in this investigation aligns with the findings of earlier studies conducted on fish that were exposed to pesticides (Nwani et al., 2013; Sinhorin et al., 2014).

4. Conclusion

Surfactants are significant chemicals whose consumption is rising as a result of the rising standard of living. Nearly all of the surfactants are discharged to the sewers following cleaning operations. The release of sewage effluents into surface waters and the use of sewage sludge as a soil conditioner on land both release surfactants into the environment. This study has shown that sublethal concentrations of linear alkylbenzene sulfonates have a direct impact on *O. niloticus* protein levels, phosphatase, and aspartate aminotransferase activities. After exposure, the marker enzymes in the tissues of the fish exposed to the surfactant were significantly affected. Tissue-specific, clear-cut time and dose dependence characterized the rate of changes in the assessed markers in the fish.

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