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Subacute Effects of Sodium Lauryl Ether Sulfate on Oxidative Enzymes and Liver Responses in *Clarias gariepinus*: Dose and Time-Dependent Effects

Eneni Roberts Inala and Thomas Ohwofasa Ikpesu
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Abstract: *The present study was performed to determine the sublethal effects of sodium lauryl ether sulfate (SLES) on oxidative stress enzymes and liver response enzymes in Clarias gariepinus. The fish that was self-grown were exposed to the concentrations of SLES observed in the field (0.00, 1.00,1.50, 2.00, 2.50) mg/L for 30 days. Standard protocols were used to quantify superoxide dismutase and alanine aminotransferase activities in the blood of the fish. The hematoxylin and eosin histological examination procedure was used to determine the change in the treated fish's liver. Superoxide dismutase showed a remarkable initiatory increment followed by a descending pattern. Also, during exposure times, alanine aminotransferase activity increased markedly with increasing concentrations of SLES, and duration of the exposure. Vacuolar degeneration, severe necrosis, dilation of sinusoids, desquamation of the hepatocytes, lipid accumulation, clusters of lymphocytes, and fibroblasts are major pathological alterations observed in the fish exposed to SLES, and were dose and time-dependent. These results indicate that exposure to this anionic surfactant modifies changes in oxidative stress enzymes, and the induction of these enzymes correlated with the pathological changes in the liver of the fish. This study has shown that sodium lauryl ether sulphate can alter an organism's system even at very low doses, thus indiscriminately release these surfactants, especially anionic surfactants into the environment are admonished to be periodically monitored by environmental regulatory agencies. For a more informed public, environmental education and advocacy should be carried out regarding the effects on fisheries and, subsequently, human health through fish consumption and groundwater contamination.*

Keywords*: Sodium lauryl ether sulfate, Clarias gariepinus, Superoxide dismutase, Alanine aminotransferase,and Histology*

Eneni Roberts Inala
Department of Biology, Federal University Otuoke, Bayelsa State, Nigeria
Email: Emirob2000@yahoo.com
Orcid id: 0000-0002-8391-7820

Thomas Ohwofasa Ikpesu*
Department of Biology, Federal University Otuoke
Email: ikpesuto@fuotuoke.edu.ng
Orcid id: 0000-0002-0669-9727

1.0 Introduction

An extensive classification of the surfactants includes anionic, nonionic, cationic, and zwitterionic surfactants (Jackson *et al.*, 2016). The most common active ingredients in household cleaning products and laundry detergents are anionic surfactants (Ranji *et al*., 2019). One such highly regarded anionic surfactant with a broad range of applications as a foaming agent is sodium lauryl ether sulfate (SLES), which is made by ethoxylating sodium lauryl sulphate (SLS) (Barra Caracciolo *et al*., 2017).

The occurrence of SLES in the environment arises mainly from its presence of complex domestic and industrial effluents as well as its

release directly from some applications (eg oil dispersants and pesticides). Because it is one of the most rapidly biodegradable surfactants with 45-95 % degradable within 24 hours (Cserhati *et al*., 2002). However, the biodegradation of surfactants can change water column temperature and dissolved oxygen, since the process is exergonic and aerobic (Jurado *et al* ., 2011)

Rapid biodegradation can reduce the length of time aquatic organisms are in contact with the surfactants and subsequent reduction of direct toxicity, However, most aquatic organisms have minimum requirements for water quality to survive, and degradation of surfactants may reduce water quality below that minimum. Therefore, the existence of low toxicity and rapidly degrading surfactants not guarantee the safety of aquatic organisms (Yao, 2018).

Sodium lauryl sulfate can affect aquatic organisms, including phytoplankton, zooplankton, fish, microbes like yeast, and bacteria. However, the impacts differ depending on the type of organism (Ying, 2006). The surfactant is also toxic to mammals such as mice and humans, but to a lesser extent (Liwarska-Bizukojc, 2005). SLES was reported by Mortelmans (1986), to exhibit a negative result in *Salmonella* mutagenicity tests (Mortelmans,1986), and in the mouse lymphoma forward mutagenicity test (McGregor, 1988).

SLES is a substance that is used in some pharmaceutical industries to increase drug absorption through the skin, gastrointestinal mucosa, and other mucous membranes (Paulo *et al*.,2013). The surfactant can be utilised as a flocculant and de-inking agent in firefighting products, detergents, and soaps (Chaturvedi and Kumar, 2010). The amount of SLES in these items varies depending on the product and the producer, with biodegradation rates ranging from 45 to 95 percent in 24 hours (Fatma *et al*., 2015). Despite this, SLES continues to be introduced into the environment through home and industrial waste discharges, resulting in high levels of this pollutant (Cserháti *et al*., 2002).

The harmful effects of SLES may be linked to the disruption of the osmotic equilibrium and the creation of stress, as proven by Messina *et al.* (2014). The metabolism of xenobiotics within an organism greatly amplifies the generation of reactive oxygen species (ROS) (Burgos Aceves *et al*., 2018a; Faggio *et al*., 2018; Hrycay and Bandiera, 2015). This ROS essentially causes severe oxidative stress damage to biomolecules like DNA, protein, and membranes (Ansari et al., 2019). Disequilibrium between the production of ROS and its neutralization by antioxidant enzymes like SOD leads to oxidative stress (Kurutas, 2016). Oxidative stress has been demonstrated to be generated by a wide range of contaminants, including surfactants, which have been shown to have an impact on marine species (Almeida *et al*., 2015; Freitas *et al*., 2019; Messina *et al*., 2014; Nunes *et al*., 2005; Nunes *et al*., 2008).

As a result, an effective and auxiliary method of assessing the activity of antioxidant enzymes may be a useful tool for aquatic toxicological investigations (Bhattacharya *et al*., 2018). Quite a few reports have been documented regarding changes in oxidative stress in *C. gariepinus* upon exposure to pesticides (Mosleh *et al.,* 2014; Paris-Palacios *et al*., 2010). However, the adverse effects of surfactants on this fish regarding oxidative stress alterations as well as the histopathological aberration are deemed insufficient (Bhattacharya *et al*., 2018), despite evidence of SLES' effects on aquatic life (Freitas and Rocha, 2012; Rocha *et al*., 2007). This investigation was carried out to evaluate the long-term consequences of a low concentration of sodium lauryl ether sulfate on *C. gariepinus*, a common freshwater fish in Nigeria.

2.0 Materials and Methods

2.1 *Materials*

2.1.1 *Equipment*

Automatic weighing balance, refrigerator, Microscope, Leica rotatory microtome and spectrophotometer. glassware used include: test tubes, beakers, conical flask, reagents used include; distilled water, Ethyl 3-aminobenzoate methanesulfonate salt, Sigma, phosphate-buffered formalin, sodium phosphate buffer, methionine, nitro blue tetrazolium (NBT), riboflavin, and enzyme extract, alanine, a-keto glutarate,; arsenate, TRIS-HCl, dinitrophenyl hydrazine, formaldehyde, alcohol, xylene, paraffin wax, hematoxylin and eosin.

2.1.2 *Biological materials*

The biological materials used for this research were mainly the fish samples of *Clarias gariepinus*.

2.2 *Methods*

2.2.1 *Test fish and maintenance condition*

Fish experimentation was carried out following the applicable standards and legislation. The *C. gariepinus* used in this study was self-bred. They have high development potential, are more resistant to dissolved oxygen deprivation and poor water quality, have a voracious appetite, and feed herbivously. They were checked regularly for their health, maturity, and lack of parasite and disease infection. To preserve water quality, we replace the brood fish pond water regularly and refill it with fresh water.

Secondary sex features were used in mature adult sex determination. Body shape and colouring are examples of these features. Males tend to be larger and have wider skulls than females. Males become thinner, have larger, muscular heads, and turn a dark bluish to black colour as the spawning season approaches. When viewed from above, females' heads are narrower than their bodies. They grow soft, bulging bellies as the spawning season approaches. Their colour ranges from grey to olive.

Growth hormone and gonadotropin hormone released by the pituitary gland were utilized as a catalyst and inciting factors for the artificial breeding of fish. This gland is found beneath the brain. The pituitary gland was carefully extracted from the fish and stored in alcohol before being dried on filter paper and pulverised in a mortar. When the temperature and meteorological circumstances were favourable, the powdered pituitary gland was injected with a little amount of distilled water. The first injection contains 10-20% of the overall dosage. At the female's second injection, the male receives a single injection. The fish were injected in the evening for they to reproduce at night. However, for multiple injections, the first is given in the morning and the second after 6 hours. The injection was done intraperitoneally at the base of the pectoral or pelvic fin.

The brood fish were released into breeding tanks immediately after injection. One female and two male breeders make up a typical breeding pair. After the brood fish are discharged, the water flow in the spawning tank is kept at a low level. Usually, 4-6 hours after the second injection, spawning happens. The male's water-borne milt fertilizes the female's released egg, which is produced by the female. The fish egg takes 18 hours to develop; the fertilized egg has a dirty yellow colour and may be easily recognised from the unfertilized egg, which is white. The yolk sac that hangs below the fertilized eggs is what distinguishes them; this is where the egg gets its nutrients for two to three days. In 4 days at 28–31°C and 5 days at 20–22°C, the eggs hatch.

The fry emerged as soon as the hatchling's yolk sac was absorbed. Fry consume food on their own, take on the form of fish, and can reach a height of 1-2 cm. The fry is referred to as a fingerling as soon as it reaches a size of 10-15 cm, or around the size of a finger. The fingerling size of the fry might be reached in 45 to 60 days. The fry and fingerlings were fed a designed diet in the form of finely powdered cake or soybean powder, together with rice bran, 4-5 times each day. After two to three

weeks of stocking, the nursery pond is netted off, and advanced fries are moved to the raising pond for fingerlings production

To represent the fish's natural habitat, a greenhouse was built and maintained daily. Clayey loam soil was used to build fifteen earthen ponds, each measuring 60 gallons and measuring 27 1/4 x 24 1/8 x 29 12, Each of the final ponds received ten fingerlings that were fed on a finely powdered cake and rice bran three times a day for 12 weeks. After cleaning the aquariums using manual pumping equipment to siphon the old water, distilled water was replaced twice a week. No deaths were noted at the end of the 12 weeks. During the testing periods, no fish died in any of the groups.

2.2.2 *Test chemicals*

The technical grade of SLES, and other reagents were purchased from Sisco Research Laboratories Pvt. Ltd. (SRL), India. Following a conventional procedure, the stock solution of SLES (1% w/v) and subsequent dilutions were measured (APHA, 2005).

2.2.3 *Determination of oxidative stress parameters, and histological changes*

Throughout the experiment, the physicochemical properties of the water were measured daily. Biochemical/Histological profiles were measured in the laboratory on days 2, 9, 16, 23, and 30. At the end of each experimental period, a fish is picked from each pond, transported to the laboratory in a well-ventilated container, and anaesthetized with MS222 promptly (Ethyl 3-aminobenzoate methanesulfonate salt, Sigma). The blood samples were obtained from each fish's caudal vein, just behind the backbone, as described by (Congleton and LaVoie, 2001). This blood was drawn and centrifuged in anticoagulant-free centrifuge tubes. Blood was centrifuged at 3.000 speed for 10 minutes to obtain serum.

Serum samples were then kept at 80°C until they were analyzed. A fish was dissected immediately after the blood was collected, and the liver was removed and preserved in 10% phosphate-buffered formalin for histopathology.

2.2.4 *Superoxide dismutase assay*

Three (3) ml of the reaction mixture containing sodium phosphate buffer (pH 7.8), methionine, nitro blue tetrazolium (NBT), riboflavin, and enzyme extract. 50 mmol/L, 13 mmol/L, 75 mol/L, 2 mol/L, and 50 l, respectively, were the component concentrations. The reaction mixture was incubated for 15 minutes at 25 °C with fluorescent lighting. Meanwhile, a UV/vis spectrophotometer was used to measure the absorbance at 560 nm (Purkinje General Instrument Co., Ltd., Beijing, China). Non-illuminated solutions devoid of enzyme extract were employed as a control. Enzyme activity, which is measured in units per gram of fresh weight, was defined as the enzyme volume that equated to a 50% blockage of the reaction (FW)

2.2. 5 *Alanine amino transferase assay*

The enzyme activity was measured using the colorimetric method. Plasma was separated from red cells by centrifugation and used as an enzyme source. The alanine aminotransferase reaction mixture contained 400 mM alanine, 210 mM a-keto glutarate, 2.5mM arsenate; 20mM TRIS-HCl pH7.5 The reaction was terminated by 0.1% dinitrophenyl hydrazine in 2N HCl. After that, a suitable aliquot was transferred to NaOH 1.3N and the absorbance was read at 440nm. The Activities of ALAT are expressed in UI

2.2.6 *Histomorphology procedure*

*T*he histological examination procedure described by Gewaily and Abumandour (2020) was used. Tissue samples were dissected and cut into 0.5 cm3 pieces before being fixed for 24 hours in a 10% neutral buffered formaldehyde solution. After that, the samples were dehydrated in increasing amounts of alcohol before being cleared with xylene and embedded in paraffin wax. A Leica rotatory microtome was used to cut the slices to 5 m thickness and stain them with hematoxylin and

eosin (RM 20352035; Leica Microsystems, Wetzlar, Germany). The tissue sections were finally examined with a BX50/BXFLA microscope (Olympus, Tokyo, Japan).

2.3 Statistical analysis

To compare controls and exposed fish, two-way ANOVA was performed, followed by the Tukey post hoc test after verifying the normality using the Shapiro Wilk test. The results of the analyses are summarized as mean ± SE. $P \leq 0.05$ is considered statistically significant for mean values.

3.0 Results and Discussion

3.1 *Biochemical assessment*

The activity of SOD in the erythrocytes of the fish that had been subjected to varying concentrations of sodium lauryl ether sulphate demonstrated that the marker's enzyme reacts in a certain way in response to increasing amounts of the toxicant. On day 2, the activity of the SOD is equivalent to that of the control, ranging between 3.82 and 4.19 u/mgHb. Although there was a modest increase in enzyme activity in response to a higher concentration of the toxicant, this did not constitute a statistically significant change ($p > 0.05$). On day 9, however, there was an increase in activity, and this activity continued to decline as the period of exposure increased. The highest and lowest enzyme activities (8.422 u/mgHb and 0.134 u/mgHb, respectively) were recorded at a concentration of 2.5 mg/l, which were highly significant ($p \leq 0.05$ and $p \leq 0.01$) when compared with the control (Fig. 1). To identify the incidence and effects of xenobiotics, biochemical biomarkers are increasingly being used in ecological risk assessment of the ecosystem. This is due to their potential as a rapid early warning signal against potentially harmful stressor effects. Biochemical biomarkers, ideally, will detect effects at the subcellular level before they become visible at higher levels of implosion. In this investigation, SOD activity increases on days 2 and 9 and subsequently decreases afterward. SOD is an important antioxidant defense in nearly all living cells exposed to oxygen. It is an enzyme that deals with the superoxide radical by either adding or removing an electron from the superoxide molecules it encounters, thus changing the O2− into one of two less damaging species: either molecular oxygen (O_2) or hydrogen peroxide (H_2O_2). SOD out-competes damaging reactions of superoxide, thus protecting the cell from superoxide toxicity. The reaction of superoxide with non-radicals is spin-forbidden. In biological systems, this means that its main reactions are with itself (dismutation) or with another biological radical such as nitric oxide (NO) or with a transition-series metal. The superoxide anion radical (O_2^-) spontaneously disputes to O_2 and hydrogen peroxide (H_2O_2) quite rapidly. In this investigation, an initial increase and later decrease in superoxide dismutase activity were observed when *C. gariepinus* was subjected to sodium lauryl ether sulfate. The initial increase can be attributed to the fact that the toxicant-induced damage in the fish, a similar result observed by Farombi and Adelowo (2007) on *Clarias gariepinus* reported SOD activities increases in the liver and kidney treated with butachlor. Alteration in the SOD activity was supported by Stara *et al.* (2012) who noticed similar changes in the muscle of common carp *Cyprinus carpio* treated with the pesticide simazine. These findings are also supported by Andy (2014) who observed a time-dependent elevation in superoxide dismutase activity in *Channa striatus* exposed to 2, 4-D pesticide. Hemalatha *et al*. (2015) reported the sublethal effect of quinalphos on SOD activity of freshwater fish *Cyprinus carpio*.

Alanine aminotransferase Activity in the plasma of C. *gariepinus* induced with various concentrations of sodium lauryl ether sulfate showed that the toxicant affected the physiology and biochemical activities in the fish. No visible changes in the control, but the responses in the treated fishes are obvious, and

the enzyme activity increased with the toxicant concentrations and duration of the exposure (Figure 1). On day 2, at concentrations of 1mg/l, 1.5, 2.00 and 2.50mg/l of SLES, the enzyme activity ranges between (1.65 – 2.13) U/ml, and varies significantly ($p \leq 0.05$) and ($p \leq 0.01$) only at 2.0 and 2.50mg/l. on day 9 the range was (3.20 – 6.70) U/ml. Day 16 (4.50 - 8.30) U/ml, day 23 (7.10- 13.20) U/ml, and day 30 (7.10 – 15.23) U/ml. The activity of the enzyme on days 9, 16 ,23 and 30 was highly significant ($p \leq 0.01$) when compared with the control in all the treatments (Fig. 2). Alanine aminotransferase is one of the most significant enzymes involved in protein and amino acid metabolism (Zikic *et al*., 2001). AAS is a sensitive indicator of even minor cellular damage and is considered an indicator of stress-based tissue impairment (Palanivelu *et al*., 2005). ln the present study, it was observed that exposure of *C. gariepinus* to sublethal doses of sodium lauryl ether sulfate resulted in increased ALT activities in all the blood of the fish. An enzyme such as alanine aminotransferase is usually found in low blood concentrations and can be elevated as observed in this investigation. The increase indicated degenerative changes in the tissue system by disrupting the physiological and biochemical processes caused mainly due to leakage of the enzyme from the liver cytosol to the bloodstream. If chemical aggressors damage some organs, they will release those enzymes toward the plasma followed by an increase in their catalytic activity. The results were in agreement with the observations of Jee *et al*. (2005) who observed increased activity of serum ALT in Korean rockfish (*Sebastes schlegeli*) exposed to cypermethrin and with Begum (2004), Herman and Geraldine (2009), and Ibrahim *et al.* (2012),

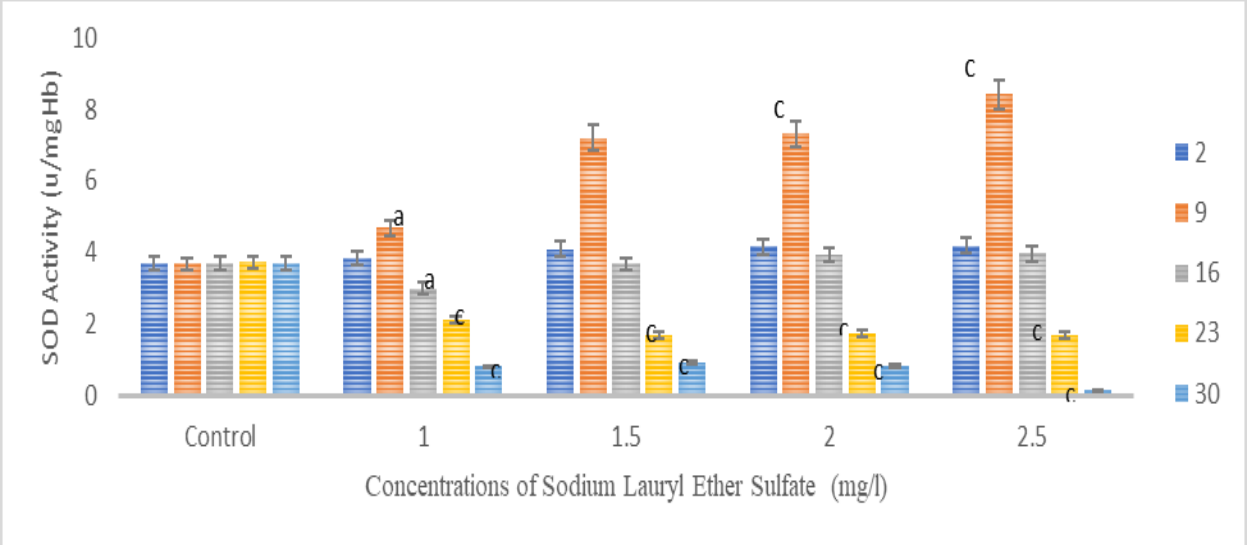

Fig. 1: SOD activity in the erythrocytes of *C. gariepinus* exposed low concentrations of sodium lauryl ether sulfate; dose and time-dependent effects; data presented as mean ± SE. SE. A letter above bars indicates significant differences between the control and the treatments [a] ($p \leq 0.05$); [c] ($p \leq 0.01$)

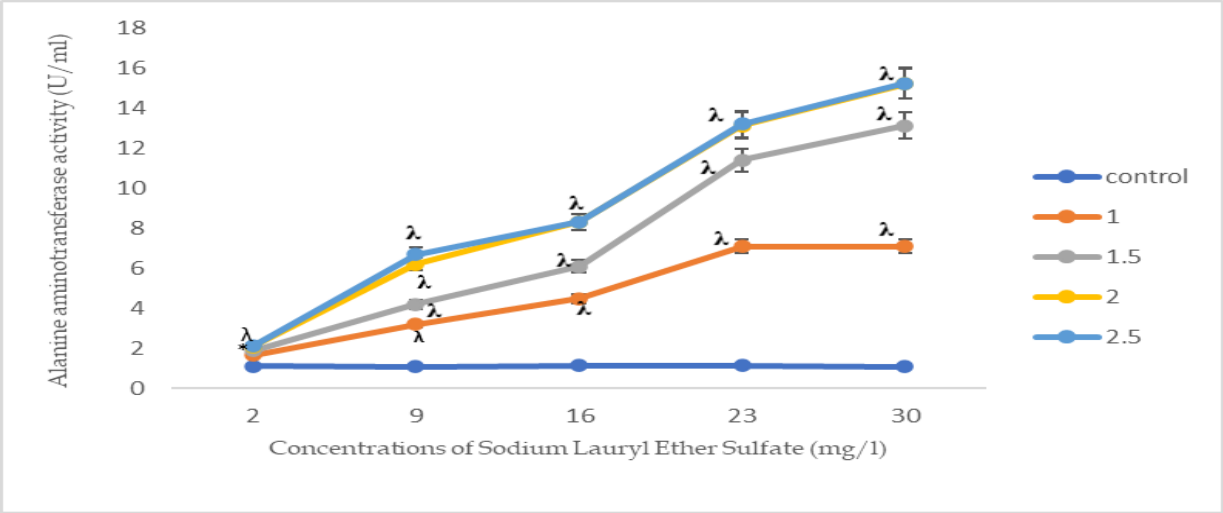

Fig. 2: Activity of alanine aminotransferase in the plasma of *C. gariepinus* exposed to sublethal concentrations of sodium lauryl ether sulfate; Data presented as mean ± SE. The symbol above bars indicate significant differences between the control and the experimental groups *(p<0.05); $^{\lambda}$ (p < 0.01

3.2 Liver Histology

The fish (*C. gariepinus*) was subjected to sodium lauryl ether sulfate at field quantities. The liver of the control fish revealed no histological changes, and the hepatic parenchyma exhibits a consistent distribution of hepatocytes surrounding the vascular system (sinusoids), as seen in Fig. 3Q.

Compared to the control fish, various histological changes were identified in the livers of the fish exposed to SLES and the severities were time and dose-dependent. Figure 3R showed vacuolar degeneration, dull patches, and a focal area of necrosis on day 2 of exposure to the surfactant at 1.00 mg/l of the SLES. Severe dilation of the sinusoid and desquamation of the hepatocytes with the increase in the concentrations of the toxicant to 1.50mg/l of the SLES, within the same period of exposure (Figure 3X); At the concentrations of 2.00mg/l, the fish's liver showed lipid accumulation, and clusters of lymphocytes and fibroblast (Figure 3Y). The liver was seriously affected at the concentrations of 2.50mg/l within the exposure durations, as there were coagulative necrosis and hepatocyte swelling (Fig. 3Z). The organ most associated with the detoxification and biotransformation process is the liver, and due to its function, position, and blood supply, and, its defect may be useful as markers that indicate exposure to environmental stressors (Van der Oost *et al*., 2003; Velmurugan *et al*.,2007).

In this study different structural alterations were observed in the fish exposed to SLES, such as vacuolar degeneration, dull patches and focal area of necrosis, dilation of the sinusoid desquamation of the hepatocytes, lipid accumulation, clusters of lymphocytes, fibroblast, coagulative necrosis, hepatocyte swelling, intravascular hemolysis, congestion of sinusoid, hepatocyte degeneration, melanocyte, enlarged cytoplasm, hepatocyte necrosis, endothelial linings of blood vessels, demarcated fatty vacuoles, periportal inflammatory infiltrate. Congestion of sinusoid, shrinkage of hepatocytes, and vacuolar degeneration have been reported in the liver of fishes after chronic exposure to a surfactant LAS (Hampel *et al*., 2008). Congestion is a blood circulation disturbance due to the increased volume of blood in the blood capillary. Vacuolar degeneration is

known as an acute swelling of the organ (Rejeki *et al*.,2008). Endothelial lining of blood vessels and sinusoid dilatation are considered indicators of severe alteration in the environment (Brito *et al*., 2012; Sayed and Younes, 2017).

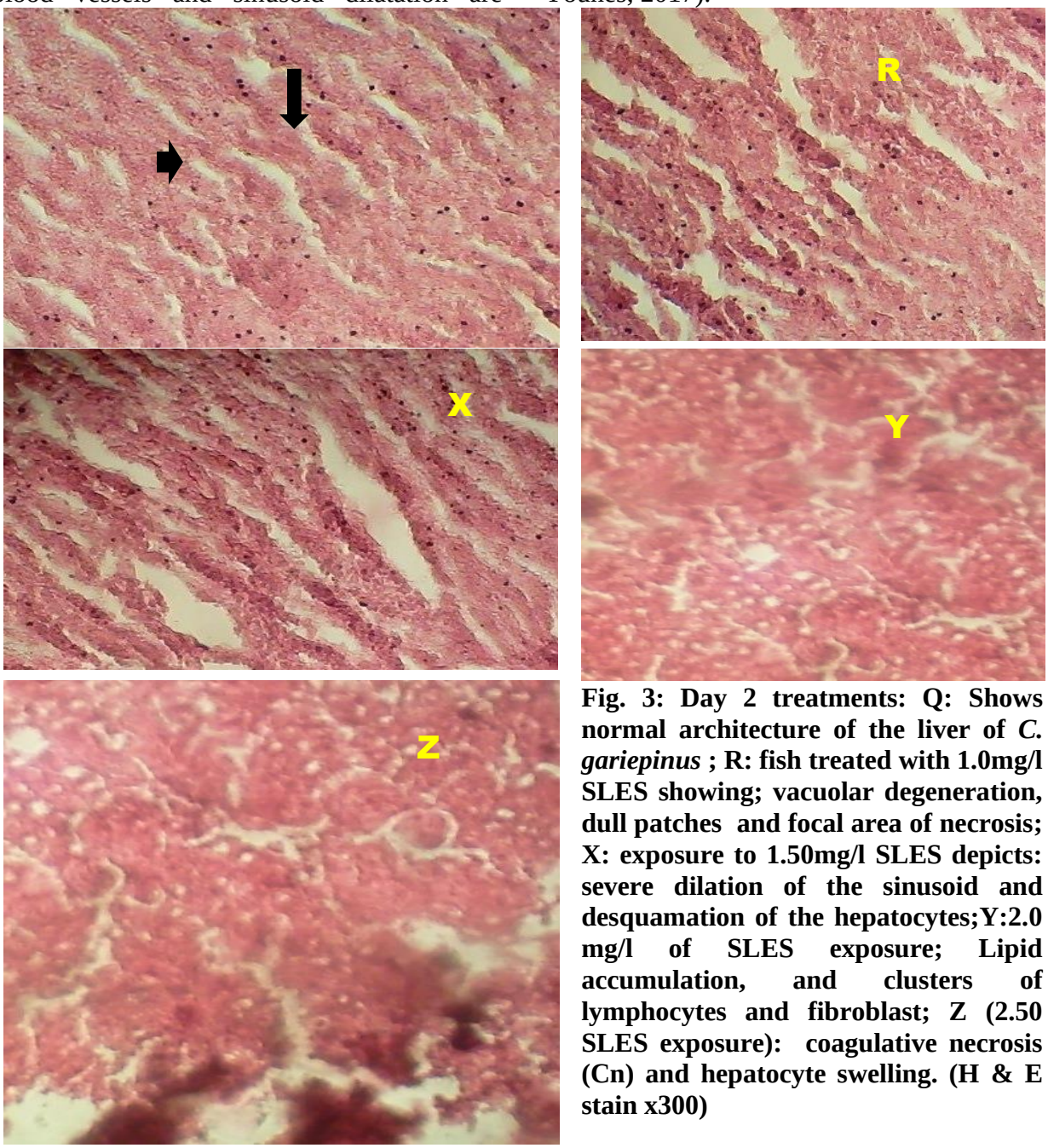

Fig. 3: Day 2 treatments: Q: Shows normal architecture of the liver of *C. gariepinus* ; R: fish treated with 1.0mg/l SLES showing; vacuolar degeneration, dull patches and focal area of necrosis; X: exposure to 1.50mg/l SLES depicts: severe dilation of the sinusoid and desquamation of the hepatocytes;Y:2.0 mg/l of SLES exposure; Lipid accumulation, and clusters of lymphocytes and fibroblast; Z (2.50 SLES exposure): coagulative necrosis (Cn) and hepatocyte swelling. (H & E stain x300)

On day 30, with the same concentrations as day 2, the liver of the treated fish showed different responses and was more severe. At 1.00mg/l, there was severe intravascular hemolysis in blood vessels (Fig $4R_1$). When the concentration was increased to 1.50mg/l, the fish's liver showed congestion of sinusoid and hepatocyte degeneration (Figure $4X_1$). At 2.00mg/l of SLES, fish's liver showed melanocytes with enlarged cytoplasm and hepatocyte necrosis (Fig. $4Y_1$), and at 2.50mg/l SDA exposure, endothelial linings of blood

vessels, large well-demarcated fatty vacuoles, and periportal inflammatory infiltrate were observed (Fig. $4Z_1$). Degeneration and desquamation of the hepatocytes may be attributed to the fact that the small lipids droplets in hepatocytes increase in the induced *C. gariepinus* due to adipocyte infiltration. These changes are common in carnivorous species such as *Anguilla anguilla* (Rodriquez *et al*., 2005). Conversely, a few, and small lipid droplets are found in herbivorous species such as *Gambusia affinis* (Giari *et al*., 2008).

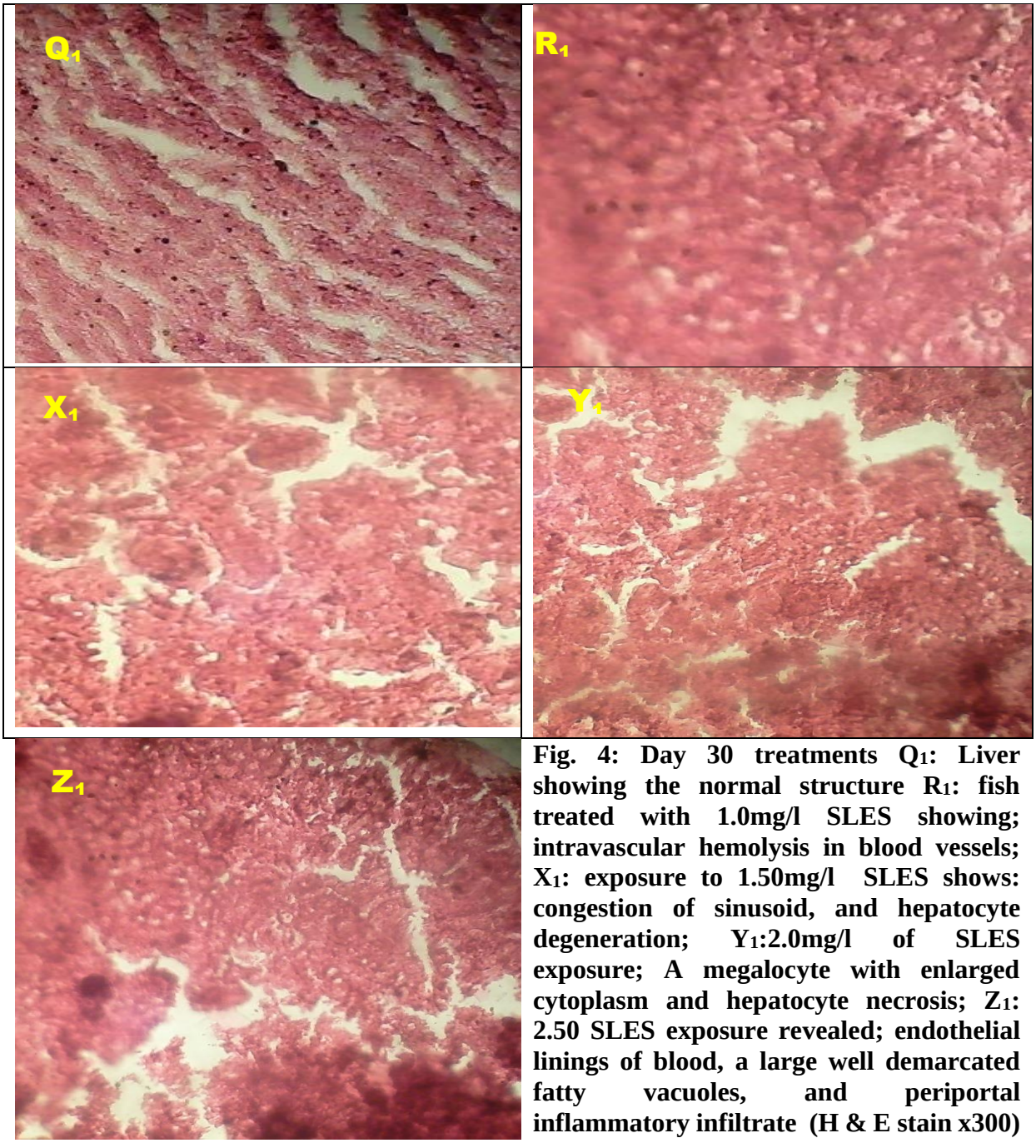

Fig. 4: Day 30 treatments Q_1: Liver showing the normal structure R_1: fish treated with 1.0mg/l SLES showing; intravascular hemolysis in blood vessels; X_1: exposure to 1.50mg/l SLES shows: congestion of sinusoid, and hepatocyte degeneration; Y_1:2.0mg/l of SLES exposure; A megalocyte with enlarged cytoplasm and hepatocyte necrosis; Z_1: 2.50 SLES exposure revealed; endothelial linings of blood, a large well demarcated fatty vacuoles, and periportal inflammatory infiltrate (H & E stain x300)

4.0 Conclusion

This study has shown that sodium lauryl ether sulphate can alter an organism's enzymatic activities even at very low doses. Similar to this, the changes in the liver's histology showed how deadly SLES is. As a result, the environment, particularly aquatic resources, can experience numerous physiological changes as a result of low surfactant concentrations. To reduce runoff from diffuse sources, improved wastewater treatment, management strategies, and intervention are urgently required. Similarly, for a comprehensive assessment of risk, additional research is advised to identify additional toxicity biomarkers at various levels of biological organization in various animal species. Small- to medium-sized businesses that indiscriminately release these surfactants into the environment are urged to be periodically monitored by environmental regulatory agencies. For a more informed public, environmental education and advocacy should be carried out regarding the effects on fisheries and, subsequently, human health through fish consumption and groundwater contamination.

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