



Original article

Influence of the Linear Alkylbenzene Sulfonate (LAS) on hematological and biochemical parameters of Nile Tilapia, *Oreochromis niloticus*Asmaa M.R. Gouda^a, Ahmed E. Hagrass^a, Mohamed A. Okbah^b, Maie I. El-Gammal^c^a Ecology Division, Zoology Department, Faculty of Sciences, Mansoura University, Egypt^b Marine Chemistry, National Institute of Oceanography and Fisheries, Egypt^c Environmental Sciences Department, Faculty of Science, Damietta University, Egypt

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ABSTRACT

The acute toxicity of household detergent (Ariel) on blood parameters and histology of *Oreochromis niloticus* was investigated using static bioassay for 96 h. **Linear alkylbenzene sulfonate (LAS) is an anionic surfactant widely used in detergents and cleaners, both in industrial and household applications. LAS contaminating aquatic ecosystems as a potential toxic pollutant, was investigated in the present study for acute toxicity. The fish samples were divided into six groups, including 20 fish in each group.** Normal feed was given to control group without detergents treatment. **Hematological parameters (RBC count, Hb, Ht and platelets) were significantly declined**, while WBC count showed a highly significant increase. **Compared with the control group, significant elevation of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) was recorded in fish treated with different concentrations of detergent.** Catalase (CAT), Superoxide dismutase (SOD) activities and Reduced Glutathione (GSH) concentration showed a highly significant reduction. Total proteins showed significant decrease, while total lipids, cholesterol and triglycerides significantly increased. The mean lethal concentration (LC₅₀) for 96 h of Ariel was at concentration 10 mg/L. Relative percentage of detergent residues in fish muscles was increased with higher detergent concentrations. In conclusion, exposure to detergents resulted in great alterations in the histological structure of liver and gills.

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1. Introduction

Detergents are derivatives of organic chemicals which persist in the environment, but their utilization is inevitable due to their participation in both cleaning agents and pesticides formalization as well as oil spills dispersal in seas (Isyaku and Solomon, 2016). The anionic surfactant linear alkylbenzene sulphonate (LAS) has high cleansing capacity because it decreases the surface tension of water, so it is the perfect ingredient in domestic and industrial detergents. The world annual usage of LAS exceeded other surfactants which attained 18.2 million ton. Unfortunately, 5 % of its pro-

duction reaches aquatic bodies and it is non-degradable in increased concentrations. Also, its toxicity occurs at a concentration ranging from 0.0025 ± 300 to 0.3 ± 200 mg/l (IHS 2015).

The Nile tilapia, *Oreochromis niloticus*, is a freshwater species which has a great importance in commercial aquaculture in Egypt. It has a rapid growth rate, high nutritional value, and good resistance to diseases and toxic compounds, so it is commonly used in the ecotoxicology field (Abd El-Gawad et al., 2016). Blood parameters are early alarms for pathophysiological alterations of the whole body due to toxicants exposure which exhibit pathological changes before the appearance of any external symptom of toxicity. Therefore, they are an efficient tool in monitoring health status of fish. Blood cell responses are important indicators of changes in the internal and external environment of the fish. Thus, the changes in the hematological parameters are good indicators of changes in the water quality (Liebel et al., 2013).

Modifications in the activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) lead to biochemical changes. It is an attempt by the fish to maintain its balance during exposure to toxic substances that impair biochemical and physiological performance (Abbas and El-Badawi, 2014). Once LAS absorbed

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through fish gills, it finds its way to the liver where metabolization and biotransformation processes occur, then conversion of LAS to hydrophilic compounds facilitates its excretion. Oxidative stress reactions induce the formation of reactive oxygen species (ROS), also known as oxidases, which binds with biomolecules causing their oxidative damage (Wibbertmann et al. 2011). Many surfactants produce oxidative stress which has negative impacts on the structural composition of tissue, permeability of the membrane and damage of biomolecules in organisms. Cellular metabolism and its regulation are disrupted when the ability of antioxidant defense mechanisms of organisms to neutralize these ROS is imbalanced which is achieved by specific enzymes such as Catalase (CAT), Superoxide dismutase (SOD), and Glutathione reductase (GSH) (Shukla and Trivedi, 2018). Biochemical biomarkers such as total proteins, total lipids, cholesterol, are powerful biomarkers which are usually utilized under stress conditions in assessing the general health status of fish. Due to the closed relation between the circulatory system of fish and the external environment, fish physiology including biochemical parameters and metabolic enzymes are favorable tools in monitoring water quality in aquatic environments (He et al., 2015).

The histopathological biomarkers are perfectly utilized as indicators for assessing both short- and long-term toxic impacts of water pollutants which causing tissues damage and histopathological degradations in fish (Liebel et al., 2013). The liver is the main organ of metabolism and detoxification of toxic compounds; however, when their concentrations are exceeded its detoxification ability is disturbed resulted in histological damage. So, liver histopathology in fish is an excellent monitoring tool (Gaber et al., 2014). Fish gills are a sensitive organ that is responsible for respiration, optimization of osmotic pressure, and excretion of unnecessary and harmful metabolic products. The large contact between the gills with the external environment increases their sensitivity to simple chemical or physical changes in the surrounding environment. Also, direct contact with toxic chemicals makes organs a target for many waterborne pollutants. This will lead to changes in their shape, impairing their performance and threatening the life of the fish, so assessment of gill morphology is key to monitoring the level of aquatic pollutants (Strzyewska et al., 2016).

This study is the first investigation in Egypt which showed the impact of detergents residues on Nile Tilapia, and was conducted to determine the mean lethal concentration (LC₅₀) of household detergent, Ariel (Linear Alkylbenzene Sulfonate), and assess its exposure effects on hematological parameters, biochemical, oxidative stress biomarkers, and histopathological histology of *Oreochromis niloticus*.

2. Materials and methods

Linear Alkylbenzene Sulfonate (LAS) Standard 1000 ppm 120 ml Amber Glass was used in the present study (RICCA Chemical Company, Texas), with standard solution (by mass) 3.4%, 8.8%, 7.3%, and 5.1% for the homologues C10, C11, C12, and C13, respectively. The concentration of LAS in fish tissues is the sum of homologues C10, C11, C12, and C13. The resulting treatments were characterized by the following nominal initial concentrations of LAS (mg L⁻¹): 0, 10, 40, 60, 80, 100, respectively. A total of 120 healthy Nile tilapia, *Oreochromis niloticus*, with an average weight of 6.6–7.8 g and an average length of 7.2–8.3 cm was held in static dechlorinated water (50 L) that was continuously aerated for 7 days to acclimatization. Their water was changed every two days to remove fecal and non-consumable feed. Fish were fed ad libitum daily with commercial feed which was discontinued 24 h before the start of the experiment with immediately removing of dead fish. After 7 days,

the fish were divided into five test groups and a control group; each group consists of 20 Nile Tilapia fish was transferred separately to aquaria of 50-liter volume and exposed to various concentrations of household detergents (Ariel; 10 mg/L, 40 mg/L, 60 mg/L, 80 mg/L, 100 mg/L) as well as the control without any treatment. Three replicates were made for each concentration.

Fish were caught gently from each groups randomly. With 3 ml sterile plastic syringes, a blood sample was withdrawn from the caudal vein located ventrally of the vertebral column then divided into two parts: The first portion was transferred to an EDTA anticoagulant tube to measure the complete blood count (CBC). The second portion of the blood sample was transferred to tubes without an anticoagulant (Eppendorf tube), then centrifuged at 3000 rpm for 10 min. Finally, the serum was isolated in another tube for further biochemical analyzes. Blood picture (red blood cell count (RBCs), white blood cell count (WBCs), hemoglobin (Hb), hematocrit (Ht), and platelets count) for each sample was determined using a hematological Analyzer (Sysmex Kx, Japan; Dacie and Lewis, 1984).

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were estimated by colorimetric method using RAM diagnostic kit according to Reitman and Frankel (1957) at 530–550 nm. Catalase (CAT) activity in serum was determined by the colorimetric method of Aebi (1984). Superoxide dismutase (SOD) activity in serum was evaluated by the procedure of Nishikimi et al. (1972) at 560 nm. Reduced glutathione (GSH) concentration was estimated in serum by the colorimetric method of Beutler et al. (1963) at 405 nm. Level of serum total proteins, total lipids, total cholesterol (TC), and triglyceride (TG) were estimated according to Gornall et al. (1949), Zollner and Kirsch (1962), Richmond (1973), and Fossati and Prencipe (1982), respectively.

Survival observations of fish samples in each group were reported after 24, 48, 72, and 96 h. Then, LC₅₀ value (The concentrations of the chemical that kills 50% of the test animals) was calculated graphically. After the fish were dissected, a weight of 100 mg of fish muscle from each set was extracted according to Knepper et al. (1999) within 30 ml methanol for 16 h in extraction vessels which evaporated to dryness then the dry residues were dissolved in 100 ml of warm deionized water in a water bath. The dissolved solutions were used to determine levels of surfactants by the procedure described by APHA (1995) and Zaporozhets et al. (1998). The liver and gills of Nile tilapia were fixed in neutralized formalin, dehydrated, embedded in paraffin wax, and sectioned at 5 μm then stained with Hematoxylin and Eosin, according to Carleton et al. (1967).

3. Results

3.1. Hematological parameters

The blood hemoglobin content (Hb) showed a significant decrease ($p \leq 0.05$) with increased concentrations of detergent, reaching its minimum value of 7.75 ± 0.23 g/dl in fish exposed to 100 mg/L of detergent (Ariel) compared to control (12.43 ± 0.02 g/dl). Also, RBC count exhibited a significant decrease ($p \leq 0.05$) with concentration gradient; the lowest value 1.19 ± 0.06 cell/mm³ was recorded at 100 mg/L of detergent (Ariel) compared to control 2.59 ± 0.04 cell/mm³. Furthermore, Ht % showed a significant decrease ($p \leq 0.05$) by increasing detergent concentrations. The minimum value of Ht being at 100 mg/L of detergent (Ariel) and the mean value being 20.67 ± 0.53 %, while Ht in control fishes was 41.5 ± 2.63 %. WBC count indicated a significant increase ($p \leq 0.05$) with increased concentrations of detergent. The highest value of WBC count was $220.04 \pm 15.61 \times 10^3$ cell/mm³ in fish exposed to 100 mg/L of detergent (Ariel) while the lowest value

was $92.29 \pm 6.05 \times 10^3$ cell/mm in control fish. Blood platelets count exhibited significant decrease ($p \leq 0.05$) with concentration gradient, reaching a minimum value of 10.95 ± 0.31 cell/mm³ in fish at 100 mg/L of detergent (Ariel) compared to control with mean value 29.17 ± 0.40 cell/mm³ (Table 1).

3.2. Liver enzymes

Serum ALT showed a tendency to increase by increasing detergent concentrations. Fish exposed to 100 mg/L concentration of (Ariel) had significantly ($p \leq 0.05$) increased serum ALT (199.20 ± 5.45 U/L) compared to control (22.15 ± 1.61 U/L). Also, serum AST showed a significant increase ($p \leq 0.05$) in fish exposed to 100 mg/L concentration of detergent (Ariel) with a mean value of 460.48 ± 7.76 U/L compared to control with a mean value of 40.1 ± 1.67 U/L (Table 1).

3.3. Oxidative stress biomarkers

Activity of superoxide dismutase (SOD) exhibited a significant reduction ($p \leq 0.05$) by increasing the concentration of the detergent. The lowest value (161.91 ± 8.63 U/L) was recorded in fish exposed to a concentration of 100 mg/L of detergent (Ariel), while the highest value (357.39 ± 2.74 U/L) was in control fish. Also, a significant decline ($p \leq 0.05$) in catalase (CAT) activity was shown with the concentration gradient. The minimum value was 93.48 ± 7.84 U/L at 100 mg/L of detergent (Ariel) compared to control with a mean value of 223.12 ± 3.05 U/L. Furthermore, reduced glutathione (GSH) concentration exhibited a significant decrease ($p \leq 0.05$) with elevated detergent concentrations. The minimum value (12.00 ± 1.75 mg/dl) was recorded at 100 mg/L compared to control with a mean value of 42.97 ± 0.21 mg/dl (Table 1).

3.4. Biochemical biomarkers

Serum total proteins content showed a significant decline ($p \leq 0.05$) in fish treated with 100 mg/L of detergent (Ariel) with a mean value of 2.61 ± 0.27 g/dl in comparison with that of control fish with a mean value of 4.90 ± 0.02 g/dl. Total lipids level showed a significant increase ($p \leq 0.05$) in detergent-treated fish, the highest reading 1174.17 ± 9.05 mg/dl was registered at 100 mg/L of detergent (Ariel) while the control reading represented the lowest value 196.96 ± 3.21 mg/dl. Cholesterol concentration exhibited a significant increase ($p \leq 0.05$) in the blood of detergent-treated fish. The maximum value (479.28 ± 2.97 mg/dl) revealed at 100 mg/L of detergent (Ariel) while the minimum value (79.37 ± 1.72 mg/dl) was obtained in control fish. Triglyceride concentra-

tion showed significant elevation ($p \leq 0.05$) in serum of detergent exposed fish. The highest value 199.97 ± 6.80 mg/dl was at 100 mg/L of detergent (Ariel) whilst the lowest value 82.21 ± 6.45 mg/dl was registered in control fish (Table 1).

3.5. LC₅₀ (Concentration of 50 % Mortality) Value:

The 96 h LC₅₀ value of LAS concentration is 10 mg/L (Table 2 and Fig. 1). It was also noticed changes in behavioral responses of Nile Tilapia such as opercular movement of fish exposed to the detergent was faster than controlled, frequent surfacing, loss of nervous control, try to jump out of media in detergent treated fish. In dead fishes, opercular region became blackish, there was hemorrhage at lower lip, along mid ventral line behind the mouth and between pectoral fin and at the base of anal and pelvic fins).

3.6. LAS concentrations in fish muscles

In the present study, LAS concentration in fish muscles showed high significant increase ($p \leq 0.05$) in fish treated with 100 mg/L of detergent (Ariel) with mean value 22.41 ± 0.40 compared to muscles in fish of control group with mean value 0.36 ± 0.03 . The concentrations of LAS and relative percentage of the different homologues (C10, C11, C12 and C13) in muscles was linearly increased with higher treated detergent concentrations (Table 3 and Figs. 2 and 3).

(A) is representing the liver of control group showing normal hepatic and hepatopancreatic tissues (arrow and arrowhead respectively). (B) is representing the liver of fish treated with 10 mg/L detergent showing moderate degree of hepatic vacuolar changes (arrow). (C) is representing the liver of fish treated with 40 mg/L detergent showing degenerative changes of hepatic tissues associated with marked fatty degeneration and degeneration (arrow), necrosis and atrophy of pancreatic cells (arrowhead). (D) is representing the liver of fish treated with 60 mg/L detergent showing increase the fatty degenerative changes of hepatocytes (arrow) and necrosis and atrophy of the pancreatic cells (arrowhead). (E) is representing the liver of fish treated with 80 mg/L detergent showing marked increase the degenerative fatty changes in diffuse manner (arrow) and severe degenerative changes within hepatopancreas (arrowhead). (F) is representing the liver of fish treated with 100 mg/L detergent showing large necrotic foci within the hepatic tissue associated with complete necrosis of the hepatopancreas (arrow). H&E stain, bar = 50 μm (Fig. 4).

(A) is representing the gill of control group showing normal primary and secondary gill lamellae (arrow). (B) is representing the gill of fish treated with 10 mg/L detergent showing mild

Table 1
Effect of LAS Concentrations on Hematological Parameters, Serum Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Catalase (CAT), Superoxide dismutase (SOD), Reduced glutathione (GSH), Total proteins (TP), Total lipids (TL), Total Cholesterol (TC) and Triglycerides (TG) of Nile tilapia, *Oreochromis niloticus*.

Parameters	LAS Concentration (mg/L)					
	0	10	40	60	80	100
Hb(g/dl)	12.43 ± 0.02	12.27 ± 0.09	10.49 ± 0.16	9.17 ± 0.19	8.08 ± 0.09	7.75 ± 0.23
RBCs(cell/mm ³)	2.59 ± 0.04	2.34 ± 0.11	2.26 ± 0.05	1.84 ± 0.10	1.58 ± 0.04	1.19 ± 0.06
Ht (%)	41.5 ± 2.63	38.35 ± 1.65	32.58 ± 0.57	27.57 ± 1.43	22.77 ± 1.64	20.67 ± 0.53
WBCs(×10 ³ cell/mm ³)	92.29 ± 6.05	112.98 ± 9.41	143.91 ± 0.26	168.96 ± 8.19	191.00 ± 4.26	220.04 ± 15.61
Platelet(cell/mm ³)	29.17 ± 0.40	28.27 ± 1.09	26.20 ± 0.98	17.45 ± 2.17	14.43 ± 1.31	10.95 ± 0.31
ALT(U/L)	22.15 ± 1.61	29.23 ± 0.78	157.28 ± 3.03	178.54 ± 6.47	187.30 ± 6.93	199.20 ± 5.45
AST(U/L)	40.1 ± 1.67	61.88 ± 0.88	336.89 ± 3.75	353.01 ± 3.17	449.71 ± 6.43	460.48 ± 7.76
SOD(U/L)	357.39 ± 2.74	334.52 ± 15.77	281.42 ± 3.06	241.13 ± 9.25	219.25 ± 5.93	161.91 ± 8.63
CAT(U/L)	223.12 ± 3.05	206.98 ± 6.90	166.71 ± 1.46	152.13 ± 7.90	126.68 ± 4.59	93.48 ± 7.84
GSH (mg/dl)	42.97 ± 0.21	37.59 ± 3.04	33.79 ± 3.64	25.25 ± 1.08	22.01 ± 1.24	12.00 ± 1.75
TP(g/dl)	4.90 ± 0.02	4.61 ± 0.24	4.09 ± 0.09	4.03 ± 0.42	3.57 ± 0.26	2.61 ± 0.27
TL (mg/dl)	196.96 ± 3.21	207.09 ± 1.45	616.35 ± 13.79	758.21 ± 6.07	1045.2 ± 29.94	1174.17 ± 9.05
TC (mg/dl)	79.37 ± 1.72	87.72 ± 2.94	100.42 ± 3.54	215.03 ± 10.85	443.43 ± 9.22	479.28 ± 2.97
TG (mg/dl)	82.21 ± 6.45	97.49 ± 3.14	153.42 ± 6.67	170.41 ± 6.24	198.41 ± 5.75	199.97 ± 6.80

Table 2
Acute Toxicity of Det-Arial on Nile tilapia, *Oreochromis niloticus*.

No. of Fish Exposed	Detergent Conc. (mg/L)	Mortality of Fishes Noted After Time Intervals of				% Mortality Within 96 h
		24 h	48 h	72 h	96 h	
20	100	6	4	5	5	100 %
20	80	4	5	4	5	90 %
20	60	3	4	5	6	90 %
20	40	3	2	4	5	70 %
20	10	1	1	4	4	50 %
20	Control	0	0	1	2	15 %

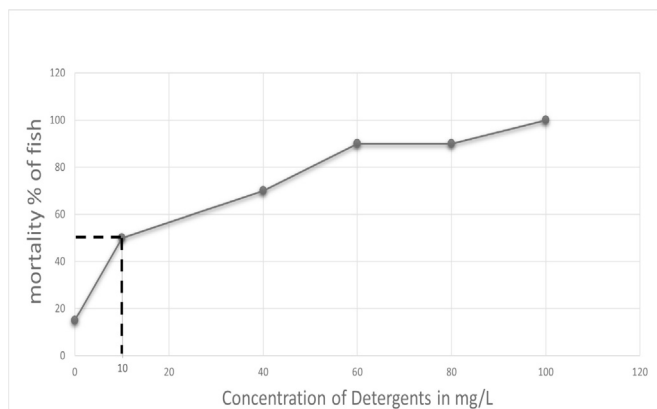


Fig. 1. Graphical Presentation of LC₅₀ Value in Nile tilapia, *Oreochromis niloticus* with Linear Alkylbenzene Sulfonate (LAS).

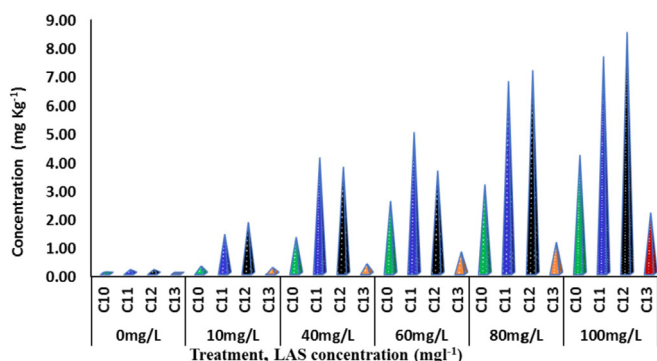


Fig.2. Treatment, The different Homologues Concentration (C10, C11, C12 and C13 in mg.kg⁻¹) of LAS Concentration.

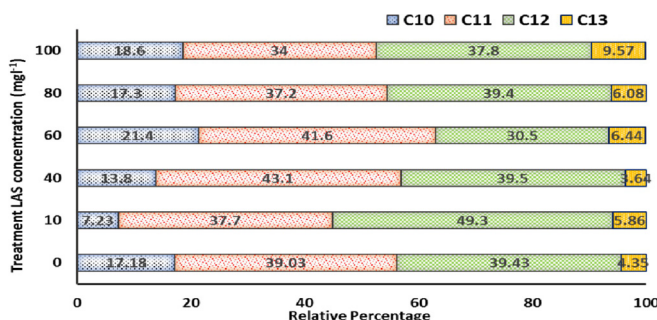


Fig.3. Treatment, Relative % of The Different Homologues (C10, C11, C12 and C13 in mg.kg⁻¹) of LAS Concentration, Histopathological Investigations.

degenerative changes within the secondary lamellae (arrow). (C) is representing the gill of fish treated with 40 mg/L detergent showing degenerative and desquamative changes of secondary gill

lamellae associated with proliferation of chloride cells (arrow). (D) is representing the gill of fish treated with 60 mg/L detergent showing marked adhesion of the secondary gill lamellae accompanied with marked leukocytic cells infiltration (arrows). (E) is representing the gill of fish treated with 80 mg/L detergent showing severe degree of adhesion of the secondary gill lamellae due marked leukocytic cells infiltration and associated with marked desquamative changes (arrows). (F) is representing the gill of fish treated with 100 mg/L detergent showing severe degree of secondary gill lamellae adhesion (arrow) accompanied with marked telangectasis of the blood capillaries (arrowheads). H&E stain, bar = 50 μm (Fig. 5).

4. Discussion

Hematological components investigations including hemoglobin content, hematocrit, red blood cells, white blood cells and platelets counts are widely used in ecotoxicology studies because they reflect the physiological alterations and health status of fish that reflect the quality of inhabitant water (Zaghloul et al. 2007).

The noticed decline in Hb, RBCs and Ht values in the current investigation may be attributed to the exposure of fish to pollutants that cause either inhibition of erythrocyte production or damage and bleeding in the gills. Destruction of mature RBCs may also occur due to hypoxic condition which resulted from declined Hb content in the cellular medium due to the cytotoxic effects of pollutants on the hematopoietic tissue that induce the production of reactive oxygen species (ROS). ROS destroy the cell membrane of the erythrocyte and inhibit its functions making fish suffering from anemia (Srivastava and Reddy, 2020).

The declined RBCs count, Hb content and Ht in the present study are in agreement with earlier investigations proceeded on *Oreochromis niloticus* (Baki et al., 2015) exposed to Perfluorooctane sulfonate (PFOS), a persistent organic pollutant, *Cichlasoma dimerus* (Vázquez and Lo Nostro, 2014) treated with Sublethal Concentrations of 4-tert-Octylphenol, *Oreochromis niloticus* (Ada et al., 2012) exposed to paraquat, *Clarias albopunctatus* Fingerlings (Oluah et al., 2018) and *Labeo rohita* (Alaguprathana and Poonkothai, 2021) exposed to methyl orange dye solution. Further, Osman et al. (2018) showed a great reduction in Hb, RBCs and Ht of fish after exposure to different pollutants under both field and lab conditions.

White blood cells have an important role in the fish defense system. Under stress conditions or exposure to toxicants, changes in the number of white blood cells are considered natural responses (Narra et al., 2017). In the current study, the significant elevation in WBC count which linearly increased with detergent concentrations gradient clarified that the presence of the detergent induces the defense mechanism of the fish to counteract the stress of toxicant. The elevated WBCs in the current study agrees with studies performed on *Cichlasoma dimerus* (Vázquez and Lo Nostro, 2014) exposed to sublethal concentrations of 4-tert-Octylphenol, *Clarias albopunctatus* Fingerlings (Oluah et al., 2018) treated with sublethal concentrations of Ronstar and *Labeo rohita*

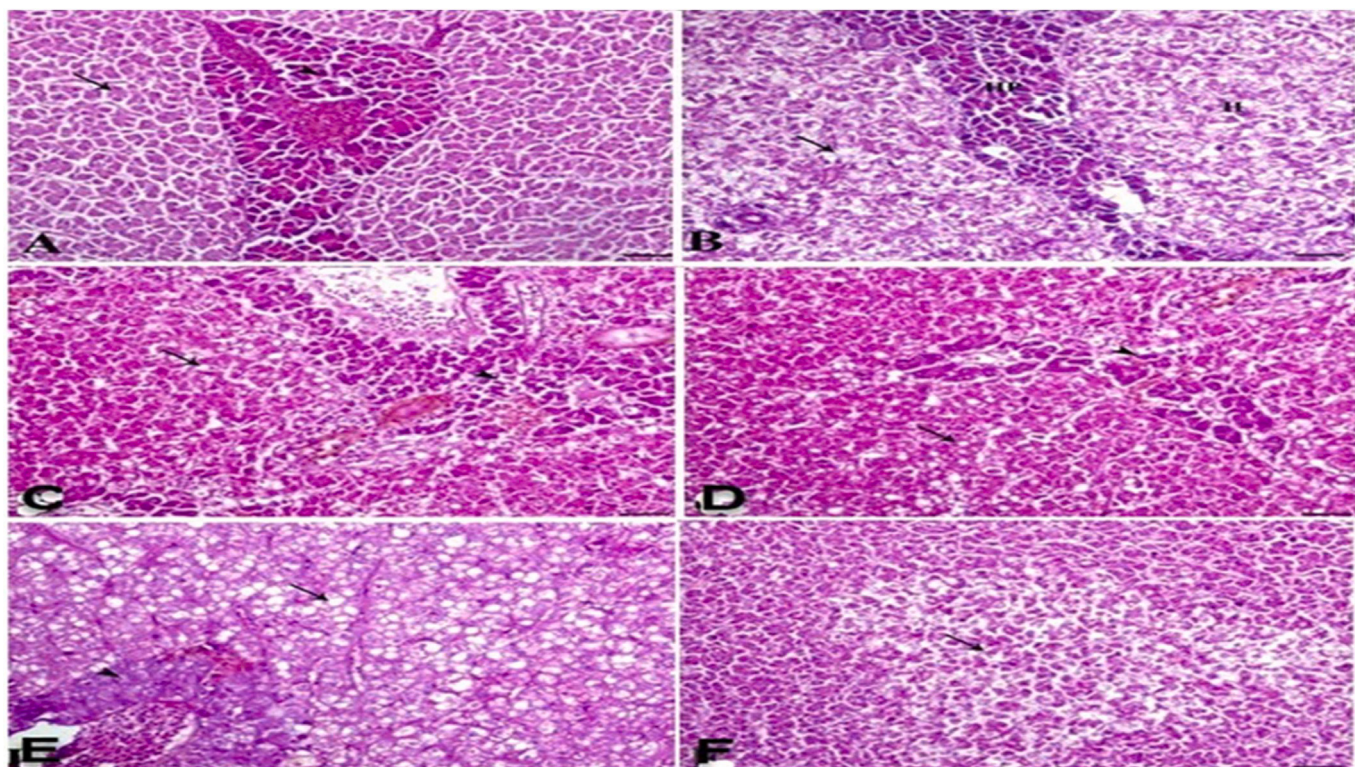


Fig. 4. Photomicrograph of Liver Sections Stained with Hematoxylin and Eosin in Fish of Different Groups.

Table 3
The Different Homologues (C10, C11, C12 and C13 in mgL⁻¹) of the LAS Concentration in Muscles of Nile tilapia, *Oreochromis niloticus*.

Conc. mgL ⁻¹	Conc. mgkg ⁻¹ (W/Wt.)				Total Conc. (mg.kg ⁻¹)	Relative percentage %			
	C10	C11	C12	C13		C10	C11	C12	C13
0	0.06 ±0.01	0.14 ± 0.03	0.14 ± 0.01	0.15 ± 0.01	0.36 ±0.03	17.18 ± 4.03	39.03 ± 5.70	39.43 ± 0.04	4.35 ± 1.72
10	0.27 ±0.05	1.39 ±0.16	1.81 ± 0.04	0.22 ± 0.04	3.68 ± 0.03	7.23 ± 1.55	37.66 ± 3.96	49.26 ± 1.42	5.86 ± 0.99
40	1.28 ± 0.17	4.09 ± 0.44	3.76 ± 0.51	0.35 ± 0.04	9.47 ± 0.81	13.77 ± 2.97	43.06 ± 0.91	39.54 ± 2.01	3.64 ± 0.06
60	2.56 ± 0.33	4.95 ±0.17	3.63 ± 0.04	0.77 ± 0.03	11.90 ± 0.49	21.40 ± 1.94	41.63 ± 0.27	30.54 ± 1.62	6.44 ± 0.06
80	3.14 ± 0.05	6.75 ± 0.09	7.13 ± 0.12	1.10 ± 0.01	18.11 ± 0.18	17.32 ± 0.42	37.25 ± 0.15	39.37 ± 0.27	6.08 ± 0.00
100	4.18 ± 0.11	7.62 ± 0.44	8.48 ± 0.00	2.15 ± 0.06	22.41 ± 0.40	18.65 ± 0.81	33.96 ± 1.34	37.83 ± 0.65	9.57 ± 0.12

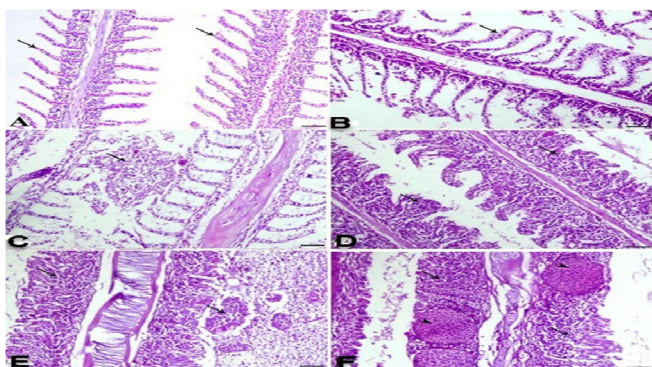


Fig. 5. Photomicrograph of Gills Sections Stained with Hematoxylin and Eosin in Fish of Different Groups.

(Alaguprathana and Poonkothai, 2021) methyl orange dye solution treated.

Blood platelets participate in blood clotting and general defense mechanisms. Exposure of fish to different water pollutants usually inhibits the production of thrombocytes (Kayode and Shamusideen, 2010). The current findings are harmonized with that of investigations conducted on *Oreochromis niloticus* (Osman et al., 2018) which recorded a remarkable reduction in thrombocytes of fish exposed to pollutants and *Cichlasoma dimerus* (Vázquez and Lo Nostro, 2014) which recorded a decline in blood platelets count exposed to sublethal concentrations of 4-tert-Octylphenol.

Alanine transaminase (ALT) and aspartate transaminase (AST) are very useful biomarkers in toxicological studies which are naturally located in the liver and other organs. They are enzymes that play a significant role in proteins and amino acid metabolism in

different body organs; they belong to the serum non-functional or tissue-specific enzymes. Therefore, their increased presence in the serum of fish may give information and evidence for injury of the tissue or organ dysfunction (Osman et al., 2018). The recorded high levels of serum ALT and AST after exposure to different concentrations of detergents can be explained as the enzymes leaked into the extracellular fluid due to cell damage which increase the permeability of membranes and/or the increased synthesis of these enzymes by the liver following the hepatic cell damage (Fagbuaoro et al., 2016). The increase in levels of ALT and AST in the present study agrees with the studies performed on juvenile *Clarias gariepinus* (Nkpondion et al., 2016) exposed to commercial detergent (Ariel), *Oreochromis niloticus* exposed to pollutants (Helal et al., 2018), pesticides (Adedeji et al., 2009), heavy metals (Mekawwy et al., 2010), nonylphenols (Mekawwy et al., 2011) and *Labeo rohita* (Alaguprathana and Poonkothai, 2021) treated with methyl orange dye solution.

SOD, CAT and GSH are sensitive oxidative stress biomarkers that are parts of antioxidant defense. They represent the major weapons of the antioxidant system due to their inhibitory effects on the formation of oxyradicals, so they detoxify and scavenge the ROS and protect cells from damage by free radicals. They are activated under mild adverse stress and declined under more intense stress (Abd El-Gawad et al., 2016). The reduced SOD and CAT in the present study might be attributed to exposure of fish to high concentrations of the detergent for 96 h that cause oxidative stress leading to tight junction disruption followed by cytotoxicity which increases the production of ROS which exploited SOD, CAT and GSH in the detoxification processes (Kumar et al., 2016).

Glutathione is the major non-protein thiol of cells with low molecular mass, so it is easily oxidized to rescue cells from free radicals and other reactive species and protect them from oxyradicals toxicity. Thus, cellular glutathione content in fish varies with the concentration and period of exposure to oxidant pollutants. Reduction in GSH concentration in this study might be explained by the increased concentrations of detergents that induce severe oxidative stress may be either due to suppression of GSH levels in response to xenobiotics due to loss of adaptive mechanisms or oxidation of GSH to GSSG to eliminate the produced ROS (Bradai et al., 2014). Decrease in the activity of CAT and SOD and concentration of GSH in the present study was harmonized with the investigations proceeded on *Ruditapes philippinarum* treated with non-ionic surfactant NPEO (Alvarez-Munoz et al., 2006), *Solea senegalensis* (Alvarez-Munoz et al., 2007), *Anabas testudineus* (Nair et al., 2017), *Danio rerio* (Sobrinho-Figueroa, 2013) exposed to commercial detergents and LAS, *Prochilodus lineatus* (Modesto and Martinez, 2010) exposed to Roundup Transorb (RDT) which is a glyphosate-based herbicide containing a mixture of surfactants, *Oreochromis niloticus* (Atli and Canli, 2010) exposed to sublethal concentrations of metals and *Melanotaenia fluviatilis* (Miranda et al., 2020) subjected to perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA), which are a group of persistent anthropogenic organic surfactants. However, the present investigation conflicted with the findings of Shukla and Trivedi (2018) which showed elevation in both SOD and CAT activities in *Channa punctatus* and the stimulating effect of LAS whether was alone or additively with detergents on oxidative stress.

Protein plays a vital role in several biological functions and serves as building blocks for cells. Decreased protein content might be attributed to protein loss by either excessive proteolysis or degradation or reduced protein synthesis during stress conditions. Proteins are also used in the tricarboxylic acid (TCA) cycle for energy production in stress conditions. Changes in the plasma protein concentrations may be a result of increased production of metallothionein which is a sequestering agent. Due to less amount of carbohydrates in fish, it resorts to the utilization of protein as an

alternative source of energy to fulfill its increased needs of energy during stress (Prakash and Verma, 2019).

The reduction in protein content in the current study coincided with results of Prakash and Verma (2018) who showed depletion in protein levels in fish exposed to detergents and with studies carried out on *Labeo rohita* subjected to detergent industry effluent (John et al., 2019) and methyl orange dye solution (Alaguprathana and Poonkothai, 2021), *Oreochromis niloticus* exposed to copper sulfate (Mutlu et al., 2015), *Clarias gariepinus* and *Oreochromis niloticus* exposed to textile dye industry wastewater (Agbon et al., 2014), *Cyprinus carpio* with textile industrial effluent (Dhanalakshmi et al., 2018) and in arsenic exposed fish (Prakash and Verma, 2020) respectively.

The levels of total lipids, triglycerides and total cholesterol in fish plasma are considered moderate sensitive biomarkers to water pollution. The degree of sensitivity relies on types and levels of contaminant, its mode of action and period of exposure (Sabae and Mohamed, 2015). Cholesterol is the most remarkable component of the body, considering an important constituent of cell membranes. It also participates in the synthesis of both bile acids and steroid hormones. Alterations in levels of cholesterol and triglyceride in the blood indicate malfunction of the liver as the main role of the liver is lipids homeostasis (Sayed et al., 2011).

The increased concentrations of total serum lipids, cholesterol and triglyceride in the present study indicate disturbance of fat metabolism which may be due to one or more of the following reasons: infiltration of cholesterol and other lipids constituents due to damage of cell membrane, retarded excretion of cholesterol by the liver, increased production by the liver and other tissues due to the effect of the pollutants, and thyroid dysfunction which blocks conversion of cholesterol to sex steroids as a result of gonad dysfunction causing the release of cholesterol into the blood. Exposure of fish to pollutants increases cholesterol and triglyceride concentrations in the blood due to liver damage which inhibits enzymes that convert cholesterol into bile acid. The rise in plasma triglycerides is possibly due to the hypoactivity of lipoprotein lipase in blood vessels which breaks up triglycerides (Metwally, 2009). Increased serum lipid, cholesterol and triglyceride levels in the present study reconciled with the investigations executed in *Tor putitora* (Yousafzai and Shakoory, 2011), *Clarias gariepinus* (Osman, 2012), *Oreochromis niloticus* (Osman et al., 2018) caught from polluted areas, *Oreochromis niloticus* exposed to copper sulfate (Mutlu et al., 2015), pesticides and heavy metals (Firat et al., 2011).

Fish swimming and movement slow down immediately with the addition of toxicants (Chandanshive and Kamble, 2006). The 96 h LC₅₀ for the Surf, Besto and Key detergents are 12.734, 77.624 and 32.292 ppm respectively to Rasboralonga (Palanicham and Murugan, 1991). The LC₅₀ values of Ariel detergent was 35 ppm for 48 h to freshwater teleost *Oreochromis mossambicus* (Anilkumar et al., 1994). Mortality rate of fish *Tilapia* sp. was 80 % at 50 ppm, while 100 % mortality was in 51 ppm of detergent water (Prakash, 1996). The LC₅₀ values of LAS for different exposure periods (24 h, 48 h, 72 h and 96 h) were 0.48, 0.28, 0.18 and 0.03 ml/l respectively (Kumar et al., 2007). The 96 h LC₅₀ for two samples detergents were 120 mg /L and 23.5 mg /L respectively to Det-I and Det-II (Chandanshive, 2013).

Study of liver histology considered the guideline for environmental stress causing histopathological alterations which depend on nature and concentration of contaminants and duration of exposure. These alterations are probably due to cell necrosis and degeneration of structural proteins in the membrane of the hepatocytes resulted from vascular congestion of the blood vessels and sinusoids that impair blood flow to all tissues. also, ROS which produced due to exposure to surfactants resulted in hepatotoxicity and hepatocyte necrosis (Ismail et al., 2017). The results agree with Priya et al. (2016) who recorded hepatocyte morphological

changes in fish, *Tilapia Mossambica* exposed to detergents represented in nuclei displacement and vacuolization. Also, with Abbas et al. (2007) reported vascular degeneration with congestion of main hepatic blood vessels and diffuse vascular degeneration in *Oreochromis niloticus* treated with thiobencarb. As well as thinner vascular walls, congestion and early fibrosis and slight degenerative changes in some of the hepatocytes were noticed in liver of *Oreochromis niloticus* exposed to *Moringa oleifera* seed extract for 96 h (Abbas and El-Badawi, 2014). While Doyins and Kafilat (2020) recorded no histological alterations in liver histology of *Clarias gariepinus* treated with sublethal concentrations of the anionic and nonionic surfactants for 28 days.

Gills are a vital organ that performs important functions which maintain fish life including ion osmoregulation and gas exchange, but are usually susceptible to environmental pollutants due to their direct contact. Therefore, gills are particularly sensitive to adverse environmental conditions and different pollutants which damage them (Mohamed et al., 2017). The current results agree with Doyins and Kafilat, 2020 who observed erosion of the secondary lamellar in the gills of *Clarias gariepinus* treated with sublethal concentrations of both AES and LAS for 28 days. And Abbas et al. (2007) also demonstrated that *Oreochromis niloticus* treated with thiobencarb showed clear edema of the lining epithelium in cells of secondary lamellae and separation of the lining epithelial cells from their capillary beds. Furthermore, hyperplasia and lamellar fusion between the secondary lamellae and degeneration changes and necrosis in the epithelial lining of the secondary lamellae associated with congestion were noticed in gills of *Oreochromis niloticus* exposed to *Moringa oleifera* seed extract for 96 h (Abbas and El-Badawi, 2014).

5. Conclusion

The present study revealed that exposure of Nile Tilapia, *Oreochromis niloticus* to sub-lethal concentrations of detergent induce various toxicological effects represented in enzymatic degradation, biochemical parameters impairments, and histopathological alterations of liver and gills. These findings confirm that the existence of detergents in aquatic environments has severe impacts on fish health making it liable to diseases and threaten its life.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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