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## Impact of Two Household Surfactants on Hepatic Lipid Peroxidation, Antioxidant Defense Responses and Hepatosomatic Index in Nile Tilapia, *Oreochromis niloticus*

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### Abstract

This study investigated the effects of two household surfactants, Morning Fresh Liquid Soap (MFLS) and Jik bleach (JIKB), on Nile tilapia (*Oreochromis niloticus*) by examining hepatic lipid peroxidation (Malondialdehyde, MDA), antioxidant defense responses (Superoxide dismutase, SOD; Catalase, CAT; reduced glutathione, GSH; Glutathione-S-transferase, GST), and the Hepatosomatic Index (HSI) over a 21-day exposure period. Juvenile *O. niloticus* were exposed to sublethal concentrations of MFLS (0.00096, 0.0024, 0.0048 ml/L) and JIKB (0.0007, 0.00175, 0.0035 ml/L) in a controlled laboratory setting. The physico-chemical parameters of the test environment; temperature (26.0-27.0°C), pH (6.8-7.0), and dissolved oxygen levels (6.0-7.4 mg/L), remained stable throughout the experiment. Exposure to both MFLS and JIKB led to increased lipid peroxidation as evidenced by elevated MDA levels, indicating oxidative stress in exposed fish compared to controls. Significant reductions in SOD activity were observed in fish exposed to both surfactants, particularly notable in the MFLS-exposed groups on days 7 and 14. CAT and GSH activities also decreased in a concentration-dependent manner, further indicating oxidative damage in liver tissues, while GST activity showed fluctuating patterns. Despite these biochemical responses, HSI values did not significantly differ among exposure groups, suggesting no acute liver damage over the study period. This study highlights the potential of household surfactants to induce oxidative stress in aquatic environments, underscoring the importance of monitoring and managing chemical discharges to safeguard aquatic ecosystems. Future research should explore longer exposure durations and higher concentrations to better assess potential chronic impacts on fish health and ecosystem stability.

**Keywords:** Antioxidants, Fish, Hepatosomatic index; Lipid peroxidation; Liver, *Oreochromis niloticus*, Surfactants

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### Introduction

In recent decades, global environmental challenges have escalated alongside rapid population growth and technological

advancements aimed at improving quality of life. However, these advancements have inadvertently increased health risks due to the release of toxic chemicals into the air,

water, and soil, which then enter the food chain and disrupt biochemical processes (Olushola *et al.*, 2014).

Similar to industrial and agricultural practices, domestic activities significantly contribute to environmental degradation, particularly in aquatic ecosystems. Household products such as detergents and disinfectants are major sources of pollutants in water bodies, stemming from their widespread use and improper disposal (Beey, 2001). Among these, surfactants—compounds that enhance cleaning efficiency—are prominent contributors to aquatic pollution (Lawal *et al.*, 2013; Samuel and Ojikutu, 2020).

While surfactants in household cleaning products are crucial for hygiene, their unregulated release into aquatic environments poses serious environmental concerns, especially for aquatic ecosystems. These surfactants have the potential to disrupt aquatic environments by interfering with biochemical processes in organisms. Detrimental effects caused by surfactants have been observed in various aquatic organisms (Sandbacka *et al.*, 2000; Cserhatiet *al.*, 2002; Panouillères *et al.*, 2007; Lawal *et al.*, 2013; Sobrino-Figueroa, 2013; Mustapha and Bawa-Allah, 2020; Samuel and Ojikutu, 2020).

Of particular concern is the ability of surfactants to induce oxidative stress by generating reactive oxygen species (ROS), which can damage essential cellular components such as lipids, proteins, and nucleic acids (Zhang *et al.*, 2003). One significant consequence of oxidative stress is lipid peroxidation, where ROS attack polyunsaturated fatty acids in cell membranes, resulting in the production of harmful by-products like malondialdehyde (MDA) (Kakkar and Jaffery, 2005). To counteract oxidative damage, organisms employ a complex system of antioxidant defenses, including enzymes like

superoxide dismutase (SOD), catalase (CAT), and glutathione-S-transferase (GST), as well as non-enzymatic antioxidants such as glutathione (GSH) (Hermes-Lima and Storey, 1993).

The liver plays a pivotal role in an organism's metabolism, detoxification, and maintenance of physiological balance. Exposure to pollutants like JIKB and MFLS can disrupt these functions, leading to oxidative stress characterized by an imbalance between ROS production and antioxidant defenses. The hepatosomatic index (HSI), which compares liver size to total body weight, serves as a critical indicator of physiological stress and pollutant exposure in aquatic organisms (Zhelev *et al.*, 2015). Changes in HSI reflect alterations in liver health and function, making it a key endpoint in studies evaluating the impacts of environmental contaminants on aquatic species (Rocha and Monteiro, 1999).

Fish are widely recognized as indicators of aquatic environment health, exhibiting responses to various pollutants from human activities that mirror those observed in higher vertebrates. Studies have documented instances of induced oxidative stress in species like Senegalese sole (Álvarez-Muñoz *et al.*, 2009), zebrafish (Sobrino-Figueroa *et al.*, 2013), rainbow trout (Antunes *et al.*, 2016), spotted snakehead (Shukla and Trivedi, 2017), and common carp (Gheorghe *et al.*, 2022) due to surfactant exposure. Surfactant-exposed *Clarias gariepinus* has also shown changes in hepatosomatic index (Uedeme-Naa and Deekae, 2016).

Few studies (Chungsheng *et al.*, 2007; Gouda *et al.*, 2022) have assessed the impact of surfactants on lipid peroxidation, antioxidant defenses, and hepatosomatic index in *Oreochromis niloticus* (Nile tilapia). This research aims to investigate the effects of exposure to surfactants (MFLS and JIKB) on lipid peroxidation

levels (Malondialdehyde, MDA), oxidative stress markers (Catalase, Superoxide dismutase, Glutathione-S-transferase, Reduced glutathione), and the hepatosomatic index in Nile tilapia. By examining these parameters, we seek to enhance understanding of the environmental risks associated with household products and their implications for aquatic ecosystems. Such knowledge is essential for developing effective strategies to mitigate the impact of human-made pollutants on aquatic organisms and ecosystems worldwide.

*Oreochromis niloticus*, commonly known as Nile tilapia, is a freshwater fish species belonging to the Cichlidae family. It is native to Africa, particularly found in rivers, lakes, and other freshwater habitats across the continent. *Oreochromis niloticus* was selected as the model organism due to its economic importance in aquaculture and its sensitivity to environmental changes and pollutants (FAO, 2009).

## Materials and Methods

### Test Organism and Acclimatization

Healthy juvenile *Oreochromis niloticus* (6-8 weeks old, mean weight  $25.77 \pm 2.00$  g) were sourced from a private fish farm located in Isheri-Osun, Lagos. The fish were transported in oxygenated bags to the Aquatic Toxicology and Ecophysiology Laboratory at the Department of Marine Sciences, University of Lagos, and then acclimatized in a rectangular glass tank ( $113 \times 54 \times 18$  cm) equipped with artificial aeration and filled with dechlorinated tap water.

Upon arrival, the test animals underwent a 2-week acclimatization period to adjust to laboratory conditions before being used in the bioassays. During acclimatization, the fish were fed twice daily (morning and evening) to satiation with 1.5-2.0 mm Coppens<sup>®</sup> feed. Continuous aeration of the tank water was ensured using 220V air

pumps, and daily water changes were carried out to maintain water quality and prevent the accumulation of waste and decaying food particles.

### Test Chemicals

The test chemicals Jik<sup>®</sup> (JIKB) and Morning Fresh<sup>®</sup> (MFLS) were purchased from the University of Lagos Pharmacy. Morning fresh liquid soap (MFLS) removes grease and stains and is used more frequently in the kitchen. Ingredient in MFLS includes anionic surfactants, hydrotropes, salts, perfumes, colors and preservatives. JIK bleach (JIKB) that removes stains and kills germs is used more frequently for laundry and sanitization of household items such as toilets and sinks JIKB is composed of sodium hypochlorite (3.85% m/v), water and fragrance.

### Physico-Chemical Parameters of the Test Media

Physico-chemical parameters (dissolved oxygen, pH, salinity and temperature) of the test media were measured at least twice (at the beginning and at the end of experiment) for the various bioassays with the aid of digital instrument (Jenway Model 300 series for pH, Eutech 300 DO meter for dissolved oxygen and Refractometer for salinity).

### General Bioassay Procedure

Fish were exposed to sub-lethal concentrations;  $1/10^{\text{th}}$ ,  $1/20^{\text{th}}$ , and  $1/50^{\text{th}}$  of 96hLC<sub>50</sub> values of MFLS (0.048ml/L) and JIKB (0.0035ml/L) reported by Samuel and Ojikutu, (2020) for the duration of 7, 14 and 21days as follows

- MFLS: 0.00096, 0.00240, 0.00480 ml/L and Control (0.0000 ml/L)
- JIKB: 0.00070, 0.00175, 0.00350 ml/L and Control(0.0000 ml/L)

The test solution of chemicals used was prepared by dissolving 1ml of MFLS and

JIKB in 1L of distilled water to make a stock of 1000 ml each. The resultant stocks were then serially diluted to obtain required concentrations. The test chemicals (MFLS and JIKB) were made up to 15L of dechlorinated tap water in the plastic bioassay tanks.

Semi-static bioassay procedure was adopted in order to avoid drastic changes in concentration of test media via evaporation and excessive reduction in dissolved oxygen (DO) level. Test media were freshly prepared every 48hrs over the 21 days period of experiment. A total of 15 fish were exposed to each concentration of MFLS, JIKB and control in triplicate using (52 × 33 × 23 cm) plastic tanks. The volume was made to 20 litres.

At predetermined time intervals (days 7, 14 and 21), two (2) fish from each triplicate concentrations of MFLS, JIKB and control were randomly selected, weighed and dissected. The test organism liver was extracted, weighed and kept in separate universal bottles and refrigerated for homogenization (for biochemical assay).

#### **Biomarker of Oxidative Stress Study**

The liver tissue of the animal was rinsed in an ice-cold 1.15% KCl solution, gently dried, and weighed. Subsequently, it was homogenized in 0.1M phosphate buffer (pH 7.2) by placing the organs in a mortar, adding acid-washed laboratory sand, and grinding with a pestle. The resulting homogenate was then centrifuged at 2500 rpm for 15 minutes. After centrifugation, the supernatant was carefully decanted and stored at -21°C until further analysis of antioxidant enzyme activity using a UV-VISIBLE spectrophotometer, as described by Habbu *et al.* (2008).

Superoxide Dismutase (SOD) activity was determined by its ability to inhibit the auto-oxidation of epinephrine, which was quantified by the increase in absorbance at 480 nm, following the methods described by Beauchamp and Fridovich (1971) and

Sun and Zigma (1978). Catalase (CAT) activity was assessed by measuring the decrease in absorbance at 240 nm due to the decomposition of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), as outlined by Aebi (1984).

Reduced glutathione (GSH) levels were determined using Ellman's reagent (5,5'-dithiobis (2-nitrobenzoic acid), DTNB), which reacts with sulfhydryl groups of GSH to produce a yellow compound measured spectrophotometrically at 412 nm, as described by Ellman (1959) and Sedlak and Lindsay (1968). Glutathione S-Transferase (GST) activity was evaluated by its ability to conjugate reduced glutathione (GSH) with 1-chloro-2, 4-dinitrobenzene (CDNB), with the formation of the CDNB-GSH conjugate measured spectrophotometrically at 340 nm, following the methodology detailed by Habig *et al.* (1974).

Malondialdehyde (MDA), an indicator of lipid peroxidation, was measured by its reaction with thiobarbituric acid (TBA) to form a colored complex, which was quantified spectrophotometrically at 532 nm, based on the procedures outlined by Buege and Aust (1978) and Ohkawa *et al.* (1979).

#### **Hepatosomatic Index**

Following the exposure period, the fishes were taken out and rinsed with distilled water. Both control and treated groups were euthanized by decapitation, and their weights were recorded. Subsequently, each fish was dissected to extract the liver, which was gently dried using blotting paper, and its weight was measured in grams. The Hepatosomatic Index (HSI) was calculated by dividing the liver weight by the total body weight of the fish. The formula for HSI calculation was based on the equation provided by Parameswaran *et al.* (1974).

$$\text{HSI} = \frac{\text{Weight of the liver}}{\text{Weight of the body}} \times 100$$

#### **Statistical Analysis**

The data were expressed as mean  $\pm$  standard error of the mean (SEM) and presented in graphical format. Statistical analysis was conducted using one-way Analysis of Variance (ANOVA) followed by the Student Newman-Keuls (SNK) test for post hoc comparisons. A significance level of 5% was used for all analyses. Microsoft Excel and SPSS 20.0 software were utilized for data analysis.

## Results

During the experimental period, the physico-chemical parameters of the test media were stable. The temperature remained consistently between 26.0°C and 27.0°C, while pH levels ranged from 6.8 to 7.0, indicating alkaline conditions. Dissolved oxygen content varied slightly, ranging from 6.0 to 7.4 mg/L throughout the sub-lethal evaluations.

Tables 1 and 2 present the results of hepatic lipid peroxidation (Malondialdehyde, MDA) and antioxidant defense responses [Superoxide dismutase (SOD), Catalase (CAT), reduced glutathione (GSH), and Glutathione-S-transferase (GST)] in *Oreochromis niloticus* exposed to sub-lethal concentrations of MFLS and JIKB, respectively.

For MFLS exposure (Table 1), the mean SOD activity in the liver of *O. niloticus* ranged from 1.19 to 5.303 U/mg protein. The lowest SOD activity occurred on day 7 in fish exposed to 0.0048 ml/L, while the highest was on day 14 in the control group (0.00 ml/L of MFLS). SOD activity generally decreased with increasing MFLS concentration. ANOVA revealed significant differences ( $p < 0.05$ ) in mean SOD levels at days 7 and 14 but not at 21 days. The Student-Newman Keuls (SNK) test indicated significant differences ( $p < 0.05$ ) between the control and groups exposed to 0.00240 and 0.00480 ml/L of MFLS at days 7 and 14.

Catalase (CAT) activity ranged from 15.73 to 36.01 U/mg protein after 21 days of MFLS exposure. The lowest CAT activity was observed on day 7 in fish exposed to 0.0048 ml/L, while the highest was on day 14 in those exposed to 0.00096 ml/L. CAT activities generally decreased with MFLS concentration, except for higher values recorded on days 14 and 21 compared to the control. ANOVA showed no significant differences ( $p > 0.05$ ) in mean CAT levels on days 7 and 21. SNK test indicated a significant difference ( $p < 0.05$ ) in CAT activity between the control and fish exposed to 0.00096 ml/L on day 14.

The mean GSH levels ranged from 0.22 to 0.75 U/mg protein in *O. niloticus* liver tissue after 21 days of MFLS exposure. The lowest GSH level occurred on day 7 in fish exposed to 0.0048 ml/L, while the highest was on day 14 in the control group (0.00 ml/L). GSH levels decreased with increasing MFLS concentration, with significant differences ( $p < 0.05$ ) observed among days 7, 14, and 21 according to ANOVA. SNK analysis showed no significant difference ( $p > 0.05$ ) between the control and fish exposed to 0.00096 and 0.0024 ml/L on day 7.

Glutathione-S-transferase (GST) activity ranged from 0.35 to 1.60 U/mg protein in *O. niloticus* liver tissue after 21 days of MFLS exposure. The lowest GST activity was recorded on day 7 in fish exposed to 0.0048 ml/L, while the highest was on day 14 in the control group (0.00 ml/L). Except for fluctuations on day 21, GST activity generally decreased with increasing MFLS concentration. ANOVA indicated significant differences ( $p < 0.05$ ) in mean liver GST activity across days 7, 14, and 21. SNK analysis revealed significant differences ( $p < 0.05$ ) in GST activity between the control and all other MFLS concentrations on days 7 and 21.

Malondialdehyde (MDA) activity in *O. niloticus* liver tissue exposed to MFLS

ranged from 0.002 to 0.970 U/mg protein over 21 days. The lowest MDA activity occurred on day 7 in the control group (0.00 ml/L), while the highest was on day 21 in fish exposed to 0.0048 ml/L. MDA values increased with increasing MFLS concentration, with significant differences ( $p < 0.05$ ) observed on days 7 and 14 according to ANOVA. SNK analysis showed no significant difference ( $p > 0.05$ ) in mean MDA levels between the control and fish exposed to 0.00096 and 0.0024 ml/L on day 7.

For JIKB exposure (Table 2), SOD activity in *O. niloticus* liver tissue ranged from 1.46 to 6.26 U/mg protein over 21 days. The lowest SOD activity (1.46 U/mg protein) was recorded on day 7 in fish exposed to 0.00175 ml/L, while the highest (6.26 U/mg protein) was on day 14 in those exposed to 0.0007 ml/L. SOD activity generally decreased with increasing JIKB concentration, although ANOVA showed no significant differences ( $p > 0.05$ ) on days 7 and 14. SNK analysis indicated no significant difference ( $p > 0.05$ ) in mean SOD activity among the control and groups exposed to 0.00, 0.0007, and 0.00175 ml/L on day 21.

Catalase (CAT) activity ranged from 17.03 to 33.82 U/mg protein in *O. niloticus* liver tissue after 21 days of JIKB exposure. The lowest CAT level was observed on day 7 in fish exposed to 0.00175 ml/L, while the highest was on day 14 in those exposed to 0.0007 ml/L. CAT activity fluctuated without a clear pattern of increase or decrease. ANOVA indicated no significant differences ( $p > 0.05$ ) in mean CAT activity among different JIKB concentrations on days 7, 14, and 21.

Reduced glutathione (GSH) levels ranged from 0.22 to 0.75 U/mg protein in *O. niloticus* liver tissue after exposure to JIKB for 21 days. The lowest GSH level occurred on day 21 in fish exposed to 0.0035 ml/L, while the highest was on day

14 in the control group (0.00 ml/L). GSH levels decreased with increasing JIKB concentration, with significant differences ( $p < 0.05$ ) observed on days 14 and 21 according to ANOVA. SNK analysis indicated no significant difference ( $p > 0.05$ ) in mean GSH levels between the control and fish exposed to 0.0007 ml/L on days 14 and 21.

Glutathione-S-transferase (GST) activity ranged from 0.44 to 1.87 U/mg protein in *O. niloticus* liver tissue after 21 days of JIKB exposure. The lowest GST activity was recorded on day 7 in fish exposed to 0.00175 ml/L, while the highest was on day 14 in those exposed to 0.0007 ml/L. GST activity fluctuated without a discernible pattern. ANOVA indicated no significant differences ( $p > 0.05$ ) in mean GST activity among different JIKB concentrations on days 7, 14, and 21.

Malondialdehyde (MDA) activity in *O. niloticus* liver tissue exposed to JIKB ranged from 0.002 to 0.272 U/mg protein over 21 days. The lowest MDA activity occurred on day 7 in the control group (0.00 ml/L), while the highest was on day 14 in fish exposed to 0.0035 ml/L. MDA values increased with increasing JIKB concentration, with significant differences ( $p < 0.05$ ) observed across days 7, 14, and 21 according to ANOVA. SNK analysis showed significant differences ( $p < 0.05$ ) in mean MDA levels between the control and all other JIKB concentrations on days 7 and 14.

The hepatosomatic indices (HSI) in *O. niloticus* liver exposed to MFLS ranged from 1.8 to 3.4, showing fluctuations without a specific pattern of change over 21 days (Table 3). ANOVA indicated no significant differences ( $p > 0.05$ ) in HSI values among different MFLS concentrations (control, 0.00096, 0.0024, and 0.0048 ml/L) on days 7, 14, and 21.

For JIKB exposure (Table 4), HSI in *O. niloticus* liver ranged from 2.28 to 3.00 over 21 days, with the lowest value

recorded on day 7 in the control group (0.00 mL/L) and the highest also on day 7 in fish exposed to 0.0007 ml/L. ANOVA showed no significant differences ( $p>0.05$ ) in HSI among different JIKB concentrations on days 7, 14, and 21.

## DISCUSSION

This study investigated the effects of two household surfactants, Morning Fresh Liquid Soap (MFLS) and Jik Bleach (JIKB), on hepatic lipid peroxidation (MDA), antioxidant defense responses (SOD, CAT, GSH, GST), and hepatosomatic index (HSI) in Nile tilapia (*Oreochromis niloticus*). These chemicals are commonly used in households for cleaning and disinfection purposes, containing different active ingredients which could potentially impact aquatic organisms if discharged into aquatic environments.

The experimental conditions were carefully controlled to maintain stability in physico-chemical parameters. Water temperature ranged between 26.0°C and 27.0°C, pH levels were maintained between 6.8 and 7.0 (indicating slightly alkaline conditions), and dissolved oxygen levels were adequate (6.0 to 7.4 mg/L) throughout the study period. Such stable conditions are crucial in aquatic toxicology experiments to ensure that observed effects are attributable to the test substances rather than environmental fluctuations.

Environmental pollutants induce oxidative stress by generating reactive oxygen species (ROS) (Almroth, 2008). When ROS production exceeds the antioxidant defense system's capacity for decomposition, it leads to cellular damage. In *O. niloticus*, exposure to sublethal concentrations of surfactants (MFLS and JIKB) increased lipid peroxide levels, measured as malondialdehyde (MDA), indicating oxidative stress compared to control animals. The MDA levels in exposed *O. niloticus* rose with increasing

surfactant concentrations, showing significant differences on days 7 and 14. These findings align with previous studies by Achuba and Osakwe (2003), Avci *et al.* (2005), which observed similar increases in MDA levels under pollutant exposure. Olushola *et al.* (2014) also reported elevated MDA levels in the livers of *Clarias gariepinus* and *O. niloticus* exposed to Pb and Zn, while Otitolaju and Olagoke (2011) noted significant MDA increases in *C. gariepinus* exposed to polycyclic aromatic hydrocarbons. Additionally, Chungsheng *et al.* (2007) observed a dose-dependent rise in MDA in cultured hepatocytes of *O. niloticus* exposed to perfluorooctanoic acid (PFOA).

Superoxide Dismutase (SOD), Catalase (CAT), and Glutathione (GSH) are critical biomarkers of oxidative stress and key components of the antioxidant defense system. They play crucial roles in detoxifying reactive oxygen species (ROS) and protecting cells from oxidative damage (Livingstone, 2001; Halliwell, 2007; Gouda *et al.*, 2022). Depending on the concentration and type of pollutants, the activities of SOD and CAT can either increase or decrease (Sedeño-Díaz *et al.*, 2012). They are typically activated under mild stress conditions but may decline under more severe stress (Abd El-Gawad *et al.*, 2016).

In this study, the activity of SOD was found to be inhibited in the liver of *O. niloticus* exposed to MFLS and JIKB after 7, 14, and 21 days compared to the control group. This finding is consistent with previous research by Faramobi *et al.* (2007). Specifically, significant inhibition of SOD activity was observed in fish exposed to JIKB on day 21 and in those exposed to MFLS on days 7 and 14. Statistical analysis using Student Newman Keul's (SNK) test indicated significant differences ( $p<0.05$ ) between the control group and fish exposed to higher concentrations of MFLS on days 7 and 14,

while no significant differences ( $p>0.05$ ) were found among the control and all JIKB concentration groups on day 21.

SOD plays a crucial role in protecting cells from damage by converting superoxide radicals ( $O_2^-$ ) into hydrogen peroxide ( $H_2O_2$ ), which is then further detoxified by CAT into water and molecular oxygen ( $O_2$ ). The inhibition of SOD activity by the tested chemicals results in increased oxidative stress in tissues due to the accumulation of superoxide radicals ( $O_2^-$ ).

Additionally, the reduced activity of SOD is expected to lead to a decrease in CAT activity, as there would be less  $H_2O_2$  generated for CAT to break down (Otitolaju and Olagoke, 2011). This was observed in *O. niloticus* exposed to MFLS, where CAT activity generally decreased with higher concentrations of MFLS, except for elevated values on days 14 and 21 compared to the control. However, CAT activity in fish exposed to JIKB showed fluctuating levels without a clear trend across different concentrations and time points. Analysis of variance indicated no significant differences ( $p>0.05$ ) in CAT activity among different JIKB concentrations on days 7, 14, and 21, although a significant difference ( $p<0.05$ ) was noted on day 14 between the control group and fish exposed to 0.00096 ml/L of MFLS.

The decrease in CAT activity following inhibition of SOD has been documented in previous studies (Fatima and Ahmad, 2005; Saliu and Bawa-Allah, 2012), further supporting the interconnected roles of these enzymes in combating oxidative stress induced by environmental pollutants.

Reduced Glutathione (GSH) serves as a crucial non-protein thiol within cells, playing a vital role in defending against oxidative stress by neutralizing free radicals and other reactive species (Gouda *et al.*, 2022). The cellular levels of GSH in

fish can fluctuate in response to exposure to oxidative pollutants over varying concentrations and durations (Gouda *et al.*, 2022). In this study, the GSH levels in *O. niloticus* decreased significantly ( $p<0.05$ ) on days 7, 14, and 21 as the concentrations of MFLS and JIKB increased. Similar findings were reported by Chunsheng *et al.* (2007), who observed decreased GST-GSH activity in the livers of *O. niloticus* exposed to Perfluorooctane sulfonate (PFOS) and Perfluorooctanoic acid (PFOA) over a 30-day exposure period. Additionally, Gouda *et al.* (2022) noted a reduction in GSH levels in *O. niloticus* exposed to linear alkylbenzene sulfonate (LAS), attributing it to severe oxidative stress induced by xenobiotics, which either suppress GSH levels or oxidize GSH to its oxidized form (GSSG) to eliminate ROS production (Bradai *et al.*, 2014).

The decrease in CAT and SOD activities, along with GSH levels observed in this study, aligns with findings from Alvarez-Munoz *et al.* (2007), Atli and Canli (2010), Sobrino-Figueroa (2013), and Gouda *et al.* (2022). Specifically, GST activity significantly decreased ( $p<0.05$ ) on days 7, 14, and 21 with higher concentrations of MFLS, whereas the activity of GST in *O. niloticus* exposed to JIKB did not exhibit consistent patterns across different concentrations. This observation regarding MFLS is consistent with the results of Otitolaju and Olagoke (2011), who investigated lipid peroxidation and antioxidant defense enzymes in *C. gariepinus* as biomarkers for monitoring exposure to polycyclic aromatic hydrocarbons, noting significant increases in MDA levels alongside decreases in SOD, CAT, and GST activities. These findings underscore the impact of environmental pollutants on oxidative stress biomarkers in aquatic organisms, highlighting the interconnected roles of antioxidant enzymes and GSH in cellular defense mechanisms against oxidative damage.



The liver serves as a crucial metabolic organ in fish, making the Hepatosomatic Index (HSI) a valuable biomarker for assessing environmental stressors (Pait and Nelson, 2003). HSI reflects the proportion of liver weight relative to total body weight and is indicative of the metabolic state and health of the liver. Decreases in HSI often suggest heightened detoxification activities in response to toxic substances (Pereira and Kuch, 2005). Conversely, a decrease in HSI levels can indicate chemical stress on fish populations (Kopecka *et al.*, 2006). In this study, the HSI values fluctuated across the 21-day exposure period to both MFLS and JIKB surfactants, but no significant differences were observed among the various concentrations tested. This suggests that the surfactants did not induce acute liver damage sufficient to cause significant alterations in HSI over the short term.

While HSI is a sensitive indicator of liver health and metabolic conditions in fish, its stability in this study indicates that the surfactant exposures did not cause immediate severe liver damage detectable through changes in HSI. Long-term studies or higher exposure concentrations may be necessary to assess potential chronic effects on liver health more comprehensively.

The findings of this study indicate that both MFLS and JIKB can induce oxidative stress in Nile tilapia, as evidenced by changes in antioxidant enzyme activities (SOD, CAT, GSH, GST) and lipid peroxidation (MDA levels). These responses varied with surfactant type, concentration, and exposure duration. Notably, MFLS appeared to exert more pronounced effects on antioxidant defenses compared to JIKB, particularly in terms of significant alterations in SOD, CAT, GSH, and GST activities. The hepatosomatic index also showed a non-

significant increase and decrease in *O. niloticus* exposed to MFLS and Jik. These can serve as biomarker for early detection of pollution during biomonitoring programs.

Overall, both MFLS and JIKB induced oxidative stress in Nile tilapia, as evidenced by alterations in antioxidant enzyme activities and lipid peroxidation levels. MFLS appeared to exert more pronounced effects on antioxidant defenses compared to JIKB. These biochemical responses can serve as early indicators of pollution in biomonitoring programmes. Understanding the ecological risks associated with household chemicals entering aquatic environments is crucial for effective environmental management. While the concentrations used in this study were sub-lethal, prolonged exposure or higher concentrations could potentially lead to more severe impacts on fish health and ecosystem integrity. In conclusion, this research provides valuable insights into the biochemical responses of Nile tilapia, *Oreochromis niloticus* to household surfactants, contributing to our understanding of aquatic toxicology and environmental risk assessment.

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**Table 1:** Lipid peroxidation and antioxidant defense responses (U/mg protein) in the liver of *Oreochromis niloticus* exposed to sub-lethal concentrations of MFLS for 21 days.

MFLS (ml/L)	Period of Exposure (Days)		
	7	14	21
<b>Superoxide Dismutase (SOD)</b>			
0.00000 (Control)	2.05±0.11 <sup>C</sup>	5.30±0.05 <sup>B</sup>	3.01±0.44 <sup>A</sup>
0.00096	1.57±0.06 <sup>B</sup>	5.19±0.29 <sup>B</sup>	2.36±0.03 <sup>A</sup>
0.00240	1.43±0.05 <sup>AB</sup>	3.82±0.50 <sup>A</sup>	2.42±0.11 <sup>A</sup>
0.00480	1.19±0.04 <sup>A</sup>	3.72±0.23 <sup>A</sup>	2.48±0.19 <sup>A</sup>
<b>Catalase (CAT)</b>			
0.00000 (Control)	22.04±3.23 <sup>A</sup>	27.26±0.74 <sup>A</sup>	20.34±1.52 <sup>A</sup>
0.00096	17.95±2.44 <sup>A</sup>	36.01±0.75 <sup>B</sup>	21.05±0.48 <sup>A</sup>
0.00240	17.01±2.46 <sup>A</sup>	27.78±2.55 <sup>A</sup>	17.66±2.10 <sup>A</sup>
0.00480	15.73±1.48 <sup>A</sup>	27.75±2.26 <sup>A</sup>	16.89±1.13 <sup>A</sup>

Reduced glutathione (GSH)			
0.00000 (Control)	0.59±0.03 <sup>B</sup>	0.75±0.05 <sup>B</sup>	0.63±0.06 <sup>C</sup>
0.00096	0.36±0.12 <sup>AB</sup>	0.70±0.04 <sup>B</sup>	0.43±0.04 <sup>B</sup>
0.00240	0.37±0.05 <sup>AB</sup>	0.41±0.08 <sup>A</sup>	0.49±0.03 <sup>BC</sup>
0.00480	0.22±0.04 <sup>A</sup>	0.29±0.07 <sup>A</sup>	0.23±0.05 <sup>A</sup>
Glutathione S-Transferase (GST)			
0.00000 (Control)	0.63±0.04 <sup>C</sup>	1.60±0.01 <sup>B</sup>	1.00±0.112 <sup>B</sup>
0.00096	0.48±0.02 <sup>B</sup>	1.58±0.07 <sup>B</sup>	0.71±0.008 <sup>A</sup>
0.00240	0.42±0.02 <sup>AB</sup>	1.18±0.16 <sup>A</sup>	0.73±0.032 <sup>A</sup>
0.00480	0.35±0.01 <sup>A</sup>	1.16±0.07 <sup>A</sup>	0.75±0.050 <sup>A</sup>
Malondialdehyde (MDA)			
0.00000 (Control)	0.002±0.0009 <sup>A</sup>	0.06±0.008 <sup>A</sup>	0.07±0.0079 <sup>A</sup>
0.00096	0.005±0.0003 <sup>A</sup>	0.15±0.008 <sup>B</sup>	0.08±0.0009 <sup>A</sup>
0.00240	0.007±0.0006 <sup>A</sup>	0.17±0.033 <sup>B</sup>	0.08±0.0073 <sup>A</sup>
0.00480	0.045±0.0024 <sup>B</sup>	0.22±0.028 <sup>B</sup>	0.10±0.0087 <sup>A</sup>

Means (± S.E, standard error) with same superscript letter in a column are not significantly different ( $p>0.05$ ) in the SNK test

**Table 2:** Lipid peroxidation and antioxidant defense responses (U/mg protein) in the liver of *Oreochromis niloticus* exposed to sub-lethal concentrations of JIKB for 21 days.

JIKB (ml/L)	Period of Exposure (Days)		
	7	14	21
Superoxide Dismutase (SOD)			
0.00000 (Control)	2.05±0.11 <sup>A</sup>	5.30±0.05 <sup>A</sup>	3.01±0.44 <sup>B</sup>
0.00070	1.75±0.11 <sup>A</sup>	6.26±0.68 <sup>A</sup>	2.15±0.19 <sup>AB</sup>
0.00175	1.46±0.13 <sup>A</sup>	4.80±0.37 <sup>A</sup>	2.03±0.07 <sup>AB</sup>
0.00350	1.50±0.19 <sup>A</sup>	4.49±0.23 <sup>A</sup>	1.60±0.04 <sup>A</sup>
Catalase (CAT)			
0.00000 (Control)	22.04±3.23 <sup>A</sup>	27.26±0.74 <sup>A</sup>	20.34±1.52 <sup>A</sup>
0.00070	18.76±1.03 <sup>A</sup>	33.82±3.59 <sup>A</sup>	18.36±1.56 <sup>A</sup>
0.00175	17.03±2.04 <sup>A</sup>	29.36±2.41 <sup>A</sup>	19.16±2.82 <sup>A</sup>
0.00350	19.73±2.03 <sup>A</sup>	29.99±1.92 <sup>A</sup>	18.27±1.32 <sup>A</sup>
Reduced glutathione (GSH)			
0.00000 (Control)	0.59±0.03 <sup>A</sup>	0.75±0.05 <sup>B</sup>	0.63±0.06 <sup>B</sup>
0.00070	0.55±0.05 <sup>A</sup>	0.67±0.10 <sup>B</sup>	0.54±0.10 <sup>B</sup>
0.00175	0.47±0.07 <sup>A</sup>	0.35±0.05 <sup>A</sup>	0.55±0.05 <sup>B</sup>
0.00350	0.46±0.05 <sup>A</sup>	0.23±0.08 <sup>A</sup>	0.22±0.03 <sup>A</sup>
Glutathione S-Transferase (GST)			
0.00000 (Control)	0.63±0.04 <sup>A</sup>	1.60±0.01 <sup>A</sup>	1.00±0.11 <sup>A</sup>
0.00070	0.54±0.04 <sup>A</sup>	1.87±0.20 <sup>A</sup>	0.57±0.05 <sup>A</sup>
0.00175	0.44±0.04 <sup>A</sup>	1.43±0.11 <sup>A</sup>	0.56±0.04 <sup>A</sup>
0.00350	0.46±0.06 <sup>A</sup>	1.36±0.09 <sup>A</sup>	0.47±0.01 <sup>A</sup>

Malondialdehyde (MDA)			
0.00000 (Control)	0.002±0.001 <sup>A</sup>	0.055±0.01 <sup>A</sup>	0.07±0.01 <sup>A</sup>
0.00070	0.015±0.003 <sup>B</sup>	0.167±0.02 <sup>B</sup>	0.08±0.00 <sup>AB</sup>
0.00175	0.020±0.003 <sup>B</sup>	0.165±0.04 <sup>B</sup>	0.10±0.00 <sup>BC</sup>
0.00350	0.038±0.005 <sup>C</sup>	0.272±0.02 <sup>C</sup>	0.11±0.01 <sup>C</sup>

Means (± S.E, standard error) with same superscript letter in a column are not significantly different ( $p>0.05$ ) in the SNK test.

**Table 3:** Hepatosomatic Index of *Oreochromis niloticus* exposed to sub-lethal concentrations of MFLS for 21 days.

MFLS (ml/L)	Period of Exposure (Days)		
	7	14	21
0.00000 (Control)	2.85±0.54 <sup>A</sup>	2.65±0.13 <sup>A</sup>	2.28±0.51 <sup>A</sup>
0.00096	2.75±0.13 <sup>A</sup>	2.82±0.36 <sup>A</sup>	2.15±0.31 <sup>A</sup>
0.00240	2.58±0.04 <sup>A</sup>	2.73±0.26 <sup>A</sup>	1.80±0.50 <sup>A</sup>
0.00480	2.85±0.53 <sup>A</sup>	3.40±0.29 <sup>A</sup>	2.72±0.20 <sup>A</sup>

Means (± S.E, standard error) with same superscript letter in a column are not significantly different ( $p>0.05$ ) in the SNK test.

**Table 4:** Hepatosomatic Index of *Oreochromis niloticus* exposed to sub-lethal concentrations of JIKB for 21 days.

JIKB (ml/L)	Period of Exposure (Days)		
	7	14	21
0.00000 (Control)	2.85±0.54 <sup>A</sup>	2.65±0.13 <sup>A</sup>	2.28±0.51 <sup>A</sup>
0.00070	3.00±0.21 <sup>A</sup>	2.91±0.26 <sup>A</sup>	2.82±0.35 <sup>A</sup>
0.00175	2.30±0.47 <sup>A</sup>	2.53±0.30 <sup>A</sup>	2.51±0.10 <sup>A</sup>
0.00350	2.57±0.25 <sup>A</sup>	2.62±0.38 <sup>A</sup>	2.49±0.54 <sup>A</sup>

Means (± S.E, standard error) with same superscript letter in a column are not significantly different ( $p>0.05$ ) in the SNK test.