IJBC International Journal of Biological Chemistry

OPEN ACCESS

Changes in Biochemical Parameters in Clarias Gariepinus Induced with Sublethal Doses of Linear Alkylbenzene Sulfonates

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ABSTRACT

Background and Objective: Surfactants in the environment enhance the solubility of harmful organic compounds in soil due to their surface-active qualities, enhancing the mobility of toxic agents and further damaging the aquatic environment. This study investigates the chronic effects of Linear Alkylbenzene Sulfonates (LABs), a well-known surfactant, on biochemical alterations in freshwater culturable catfish, *Clarias gariepinus*, after 30 days of exposure to a sub-lethal dose of a detergent component. **Materials and Methods:** The fish experimentation was carried out following the applicable standards and legislation as the *C. gariepinus* utilized in this study was self-bred. Blood was collected from the caudal region following the standard procedures. The fish were exposed to the range of the concentrations of LABs reported in the field of 0.05, 0.10, 0.15 and 0.20 mg L^{-1} and the control for 30 days. The Acetylcholinesterase (AChE), Glutathione activities (GST) and hydro corticosteroid profiles were measured on days 2nd and 30th in the laboratory spectrophotometrically. **Results:** Findings revealed that the surfactant altered the enzymes in these organs and the effects were dosage and time-dependent. The activity of the GST and cortisol levels followed an increasing trend and a decline in the activity of AChE in all the concentrations throughout the experiment. **Conclusion:** Long-term exposure to LABs increased the production of ROS, which ultimately caused oxidative cell damage and reduced esterase activity in the fish tissues. This study shows that LAB poisoning can ultimately endanger life and have comparable negative consequences on human health. Since anthropogenic factors are the primary causes of surfactant exposure, concerned authorities must enact stringent mitigation measures to curb this underrated risk.

KEYWORDS

Linear alkylbenzene sulfonates, *Clarias gariepinus*, acetylcholinesterase, glutathione, hydro corticosteroid

INTRODUCTION

Surfactants are one of the most difficult emerging toxins that are released into the environment regularly by wastewater treatment plants. They are amphipathic molecules because they have both hydrophilic (polar charged or uncharged head group) and hydrophobic (non-polar hydrocarbon tail) properties[1]. Because of their particular physicochemical features, they are used in a variety of home and

ISSN: 2152-3509 (Online)
ISSN: 1819-1886 (Print)
https://doi.org/10.17311/ijbc.2022.8.19

Received: 13 Jun. 2022
Accepted: 28 Aug. 2022
Published: 01 Oct. 2022

industrial applications[2]. The persistence of changed products in the environment, on the other hand, is a major concern for environmental sustainability and healthy ecosystems[3-9].

Surfactants are used in the manufacturing of detergents, textiles, paints, plastics, medicines, insecticides, paper and personal care products, among other things. These surfactants can lower interfacial tension and stabilize foams and emulsions, making them important for particular tasks like oil recovery and mining[10-12]. Laundry detergents, personal care products and textile softeners, for example, use benzalkonium chloride and alkylbenzene linear sulfonate[13,14]. They are commonly employed in laundry detergents, industrial and institutional cleaners and textile processing wetting and dispersion agents[1,15].

Because of its outstanding cleaning qualities and great sudsing potential, Linear Alkylbenzene Sulfonate (LABs) is the most extensively used anionic surfactant in institutional and commercial laundry[1,16,17]. It has a hydrophobic tail and a hydrophilic head and it decreases surface tension between compounds in aqueous solutions[15,18].

The release of linear alkylbenzene sulfonate into the environment will occur primarily through sewage. Even though linear alkyl benzene sulfonate is easily biodegradable in aerobic conditions, it accumulates in aquatic environments as a result of its high consumption and low removal percentage in much anaerobic sewage treatment plants[19].

The physicochemical features of linear alkylbenzene sulfonate have been observed to have a high solid adsorption coefficient, which is attributed to the surfactants' physicochemical properties. Linear alkylbenzene sulfonate molecules adsorb to suspended solids in water bodies and hence end up in sediments or sludge in treatment facilities[20]. The recommended linear alkylbenzene sulfonate, which has been claimed by some researchers to biodegrade perfectly and has also been reported to biodegrade poorly in rivers, lakes, ponds and even soils, which may be toxic to aquatic faunas and bras and cause severe damage to vital organs as well as haematological, hormonal and enzyme disturbances[21-23]. Nonylphenol, a chemical used in detergent production, has been found in wastewater streams all over the world, which is a worry due to its toxicity to many aquatic creatures. Microbial populations are also increased by surfactants[23].

Environmental risks to freshwater habitats are developing at a quicker rate as industrialization, urbanization and agricultural activities intensify. Billions of kilos of industrial chemicals end up in freshwater bodies across the world[24]. Surfactant exposure has been linked to consequences on non-target creatures such as nervous system disturbances, oxidative stress and other physiological effects. Inhibiting Acetylcholinesterase activity in the synapse as well as a few other enzymes, has become the gold standard in assessing the impact of these compounds on biota.

Toxicologists believe detergents to be a necessary evil and as with many discoveries or breakthroughs, humans have been eager to reap their advantages but slow to grasp and cope with their harmful implications. These impacts are sometimes permanent and detrimental to persons and the environment. Because their characteristics fluctuate, fish toxicity varies with each surfactant group[25].

Clarias gariepinus is one of the most prolific and extensively dispersed fish in Nigeria as well as the main Clarias catfish in Africa[26]. Pond-reared African catfish are especially vulnerable to detergent exposure because they are frequently grown in crop-producing areas, using the resultant wastewater from houses[27]. The amount of Linear Alkylbenzene Sulfonates in Nigeria's Waste Water is unknown. Many scientists have utilized *C. gariepinus* as a laboratory fish model to study microbiological, pathogenic or environmental research[28]. Unfortunately, there is little or no information on the toxicological and pathological effects of *C. gariepinus* exposure to LABs.

The objective of this study was to investigate the impact of exposing *C. gariepinus* to sublethal dosages of LABs by monitoring behavioural and clinical changes.

MATERIALS AND METHODS

Study area: This study was carried out at the Department of Biology, Federal University Otuoke Nigeria between June, 2021 to May, 2022. The fish experiments were carried out following the appropriate norms and regulations and were approved by the appropriate authority in the country.

Fish selection: *Clarias gariepinus* used in this study was self-bred. The brood stock/fish used in this study exhibited a rapid growth potential, a higher tolerance to dissolved oxygen deficiency and poor water quality, a strong appetite and an omnivorous feeding regimen. They were frequently tested for health, maturity and the absence of parasite and disease infestation. The brood fish pond water was routinely replaced and replenished with new water to maintain water quality.

Sex determination: In determining the sex of mature adults, secondary sex traits were considered. Body shape and colouration are examples of these characteristics. Typically, males are larger and have wider skulls than females. As spawning season approaches, males become thinner, have larger, more muscular heads and acquire a dark blue to black hue. When viewed from above, the female head is thinner than the female body. As the spawning season approaches, they develop soft, swollen bellies. There is a range of shades from grey to olive in their appearance.

Controlled breeding: The pituitary gland secretes growth hormone and gonadotropin hormone, which regulate sexual organ growth and function. It is used as a catalyst and stimulating factor in fish artificial breeding. This gland is located under the skull. The gland was separated and put in a Petri dish containing 100% ethanol. The pituitary gland was dried on filter paper and crushed in a mortar after being kept in alcohol. The powdered pituitary gland was mixed with a small amount of distilled water when the temperature and meteorological circumstances were suitable. The entire dosage is injected, females are given double doses. The initial injection accounts for 10-20% of the total dosage. The male receives a single shot during the female's second injection. To enable the fish to breed at night, they were injected in the evening. In the case of repeated injections, the first is administered in the morning and the second after 6 hrs. The injection was administered intraperitoneally at the pectoral or pelvic fin base.

Spawning: After injecting the brood fish, they were immediately released into breeding tanks. One female and two males make up a typical breeding pair. To maintain a steady flow of water in the spawning tank, the brood fish are released. After the second injection, spawning usually occurs within 4-6 hrs. Fertilization takes place in the water after the female lays an eggs.

Incubation and hatching: Incubation lasts 18 hrs. The fertilized egg is filthy yellow and easily distinguished from the unfertilized egg, which is white. It is distinguished by the presence of a yolk sac hanging below the fertilized egg, from which it obtains nutrition for 2-3 days. Hatching takes 4 days at temperatures ranging from 28-31°C and 5 days at temperatures ranging from 20-22°C.

Fry and fingerling: As soon as the hatchling's yolk sac was absorbed, the fry emerged. Fry eat on their own, grow to be 1-2 cm long and take on the form of a fish. A fingerling is defined as a fry that is 10-15 cm long or around the size of a finger. The fry will reach fingerling size in 45-60 days. The fry and fingerlings were fed a specially prepared diet with a 1:1 ratio of soybean powder or finely powdered cake and rice bran 4-5 times each day. The nursery pond is netted out after 2-3 weeks of stocking and advanced fry is moved to the rearing pond for fingerling production.

Construction of experimental ponds: A greenhouse was constructed to mimic the fish's natural environment and it was cleaned regularly. Clayey loam soil was used to construct fifteen earthen ponds, each weighing 60 gallons and measuring 27 1/4×24 1/8×29 12 ten fingerlings were placed in each of the final ponds for 12 weeks and fed a finely powdered cake and rice bran thrice daily. The distilled water was refreshed twice a week after cleaning the aquaria using manual pumping equipment. After the 12th week, there was no mortality.

Toxicological investigation: After the 12th week, each of the final ponds with their repetitions was exposed for 30 days to the range of linear alkylbenzene sulfonates concentrations recorded in the field 0.05, 0.10, 0.15 and 0.20 mg L^{-1} and the control. Throughout the tests, both control and experimental fish were fed twice daily at around 3% of their body weight. Water and toxicants were fully replaced every 72 hrs and the earthen ponds were kept as clean as feasible.

Throughout, the experiment, the physicochemical properties of the water were measured regularly. The unused fish were returned to the main pond and after each trial period, a fish is removed from each pond, transported to the laboratory in a well-ventilated container and anaesthetized with MS222 promptly (ethyl 3-aminobenzoate methanesulfonate salt, sigma).

According to previous study[29], blood samples were collected from the caudal vein directly behind the backbone of each fish. This blood was taken and held at 80°C in two anticoagulant-free centrifuge tubes for AChE, GST activity and hydro corticosteroid levels. The liver and brain of a fish were removed immediately after the blood was collected. The liver was frozen in liquid nitrogen and stored at -25°C until the analysis, while the brain was carefully removed, weighed, washed with 50 M Tris-HCl buffer and homogenized in a container using a tissue homogenizer (Polytron PT-6100, USA). HCl-Tris buffer (1% Triton X and 0.1 percent PMSF). As a homogenizing solvent, Sigma Aldrich was utilized. For 20 min, the sample was centrifuged at 12,000×g. (GRACE High-Speed Refrigerated Centrifuge, India). The supernatant was separated into its tube and utilized as an enzyme source[30].

The acetylcholinesterase, glutathione and hydro corticosteroid profiles were measured in the laboratory on days 2 and 30.

Assessment of the acetylcholinesterase activities: Acetylcholinesterase activity was determined using a commercial kit manufactured by Bohringer Mannheim that was based on the Ellman spectrophotometric assay procedure, adapted for microplates[31] using acetylthiocholine iodide as a substrate.

Blood was collected from fish via tail puncture with a 1 mL insulin syringe and placed in heparinized tubes containing 66 L of 0.1 M sodium-EDTA. Following centrifugation at 4°C for 1 hr, the erythrocytes were separated and washed twice with 0.9% NaCl before being resuspended in 0.5 mL potassium phosphate (12.5 mM, pH 7.4) for the enzyme test.

The liver and brain tissues were homogenized (1:10, w/v) at 9500 rpm for 1.5 min in homogenization buffer [100 m KCL and 1 m EDTA (pH 7.4)]. The supernatants were separated by centrifuging homogenates at 10,000 g for 30 min at a temperature of 4°C.

As a buffer, 50 M Tris-HCl with a pH of 7.4 was utilized throughout the experiment. In 96-well plates containing 5,5-dithio-bis-2-nitrobenzoic acid (DTNB), acetylcholine (ATC) was produced in 50 M Tris-HCl. I. 210 L of Tris-HCl (pH 7.4) buffer, 20 L of 0.1 M DTNB and 10 L of acetylcholinesterase enzyme from each tissue. Fifty-four mg mL^{-1} of supernatant from each tissue were transferred separately to another 96-well plate II and incubated at 28 °C for 15 min. Then, 10 L of ATC (2.5 M) was added to the mixture in 96-well plate II, followed by a 10-min incubation. Thiocholine is produced when the substrate acetylthiocholine

is hydrolyzed by the cholinesterase enzyme. The reaction between thiocholine and Ellman's reagent (DTNB) produces 2-nitrobenzoate-5-mercaptothiocholine. Using a microplate reader (Tecan Multimode Microplate, United Kingdom) with fluorescence excitation at 485 nm and emission at 535 nm, a reading was taken at 405 nm[30]. The erythrocyte's enzymatic activities were measured in (mol/min % hematocrit) and tissue enzymatic activities were measured in mol/min/mg protein. Each unit of activity equated to the hydrolysis of 1 nmol of substrate per minute. Capillary hematocrit tubes were used to test the hematocrit level.

Determination of glutathione-s-transferase in the erythrocytes: Blood that was preserved in a heparin vial was centrifuged at 4°C for 10 min at 3,000 rpm. The top yellow plasma layer was gently pipetted into a vial without disrupting the white buffy layer. The total GST activity in the fish plasma was measured using the GST assay kit. The assay kit uses CDNB, which is suitable for the widest spectrum of GST isozymes. The GST catalyzes the conjugation of L-glutathione to CDNB by using glutathione's thiol group:

$$GSH+CDNB \rightarrow GS\text{-}DNB\ Conjugate+Hcl$$

The reaction product, GS-DNB Conjugate, absorbs at 340 nm. The rate of increase in the absorption is directly proportional to the GST activity in the sample.

Determination of glutathione-s-transferase activities in the liver and the brain: Using spectrophotometry, the activities of the GST were assessed in the liver and brain of the fish. The protocols were modified. In brief, 50 mg of tissue was homogenized at 4°C using a FastPrepR in K-phosphate buffer (1 mL, 0.1 M pH 7.8, 100 mM K-phosphate, 20% (v/v) glycerol and 0.2 mM fluoride phenylmethylsulphonyl. At 4°C, homogenates were centrifuged for 15 min at 10,000 g and 4°C) and the supernatants separated. At 340 nm for 5 min, the conjugation between 1-chloro-2,4-dinitrobenzene and reduced glutathione was measured to determine the GST activity. The enzyme activity was quantified in terms of units per mg of proteins (U mg^{-1}).

Hydro corticosteroid measurement: An ELISA human cortisol test kit was used to quantify plasma cortisol in the investigated fish. For standard curve calibration, seven standard solutions of cortisol in human plasma (0, 20, 50, 100, 200, 400 and 800 ng mL^{-1}) were utilized. Cortisol standard solutions of 50, 100 and 200 ng mL^{-1} were used in the recovery test and four dilutions of the fish plasma samples (1/2, 1/4, 1/8 and 1/16) were created for the linearity test. Plasma samples and standard solutions were put to each well fish and the content was conjugated to peroxidase before the enzyme substrate was added. By adding 0.5 M phosphoric acid, the enzymatic reaction was stopped and the absorbance was read at 450 nm.

Statistical analysis: SPSS Version 16 software was used for statistical analysis (Systat Software Inc., CA, USA). The results were presented as Means±SE. The normality of the data was determined using normal Q-Q plots and the Shapiro-Wilk Normality Test. T-tests were used to identify which individual groups of various treatments were significantly different from the control group at ($p<0.05$, $p<0.01$).

RESULTS

AChE activities in the brain and the erythrocyte: The activities of the acetylcholinesterase activity in the brain and red blood cells of *C. gariepinus* subjected to sublethal doses of LABs are illustrated in Fig. 1-4. The data revealed that the surfactant altered the enzymes in these organs and the effects were dosage and time-dependent.

On day 2nd, AChE activity in the brain were 47.20, 44.70, 41.90 and 40.20 mol/min/mg protein at 0.05, 0.10, 0.15 and 0.20 mg L^{-1} of LABs, respectively, compared to 58.80 mol/min/mg protein in the untreated fish. Except for the 0.20 mg L^{-1} treatment, statistical analysis indicated that the AChE activities were not significant ($p>0.05$) when compared to the control in Fig. 1.

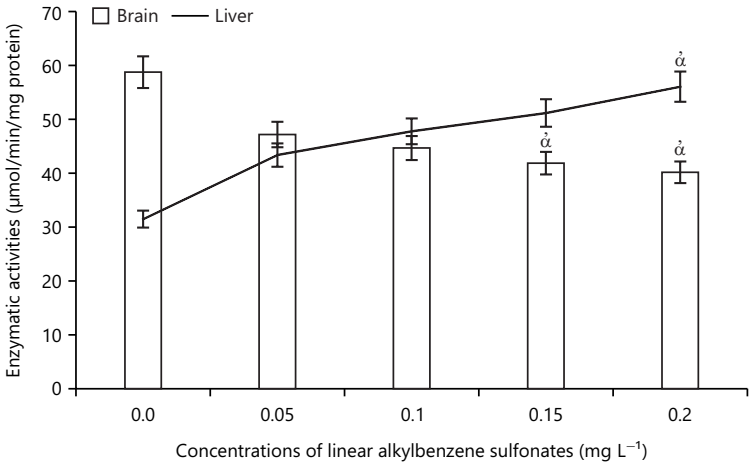

Fig. 1: Acetylcholinesterase activity in the brain and glutathione-s transferase activity in the liver of *C. gariepinus* on day 2nd exposure to linear alkylbenzene sulfonates

Data presented as Mean±SE, symbols above the bars indicate significant differences between the control and the experimental groups ($p<0.05$)

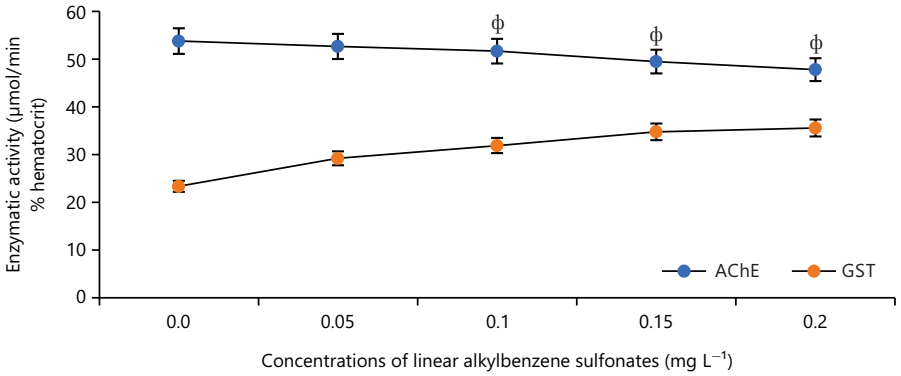

Fig. 2: Acetylcholinesterase and glutathione-s transferase activity in the erythrocyte of *C. gariepinus* on day 2nd exposure to linear alkylbenzene sulfonates

Data presented as Mean±SE, symbols above the bars indicate significant differences between the control and the experimental groups ($p<0.05$)

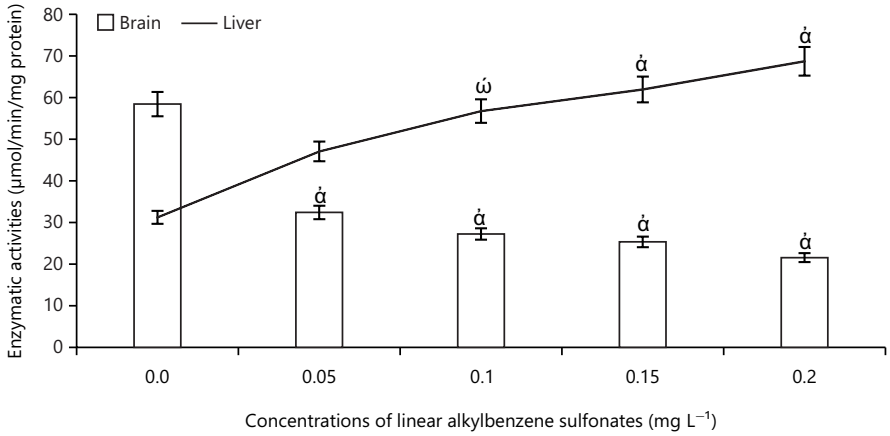

Fig. 3: Acetylcholinesterase activity in the brain and glutathione-s transferase activity in the liver of *C. gariepinus* on day 30th exposure to linear alkylbenzene sulfonates

Data presented as Mean±SE, Symbols above the bars indicate significant differences between the control and the experimental groups ($p<0.05$)

On the same day, at the same doses, the enzyme activity in red blood cells was 53.12, 52.20, 49.80 and 48.11 mol min^{-1} percent hematocrit, while, the enzyme activity in the control fish was 54.13 mol min^{-1}

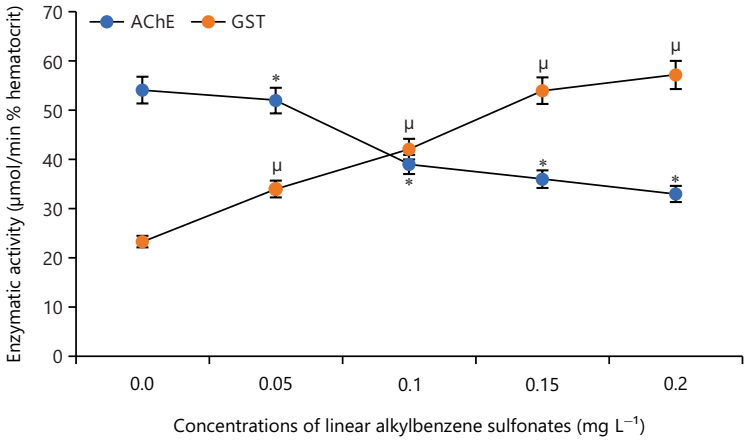

Fig. 4: Acetylcholinesterase and glutathione-s transferase activity in the erythrocyte of *C. gariepinus* on day 30th exposure to linear alkylbenzene sulfonates

Data presented as Mean±SE. Symbols above the bars indicate significant differences between the control and the experimental groups ($p<0.05$)

Table 1: Fluctuations in hydrocortisone (ng mL^{-1}) in *C. gariepinus* exposed to low concentrations of linear alkyl benzene sulfonates (mg L^{-1})

	Mean±SE				
Days	Control	0.05	0.1	0.15	0.2
2	11.10±1.02[a]	13.20±0.06[a]	14.20±0.21[a]	14.30±0.50[a]	15.90±0.10[a]
30	11.22±0.71[a]	15.90±2.10[b]	17.30±1.15[c]	18.20±0.32[c]	20.30±1.00[c]

*[a]Not significant ($p<0.05$), [b]Significant ($p<0.05$) and [c]Highly significant ($p<0.01$)

percent hematocrit. When compared to the control, the inhibition of AChE activity was highly significant ($p<0.05$) in all treatments except 0.05 mg L^{-1} of LABs treated fish in Fig. 2.

On day 30th, the enzyme activity in the brain and erythrocytes, with the same conditions of exposure were 58.60, 32.50, 27.30, 24.40, 21.60 mol/min/mg protein and 54.12, 52.00, 39.10, 36.20, 33.10 µmol min^{-1} % hematocrit at 0.00, 0.05, 0.10, 0.15 and 0.20 mg L^{-1} of LABs, respectively in Fig. 3 and 4. When comparing the treated and control fish, the lengthy exposure caused a significant ($p<0.05$) decrease in enzyme activity in all treatments and matrices.

GST activities in the liver and the erythrocyte: GST activity in the liver and red blood cell tissues rises with toxicant concentrations and periods of exposure. On day 2, the GST activity in the liver and the erythrocytes at 0.00, 0.05, 0.10, 0.15 and 0.20 mg L^{-1} of LABs treated fish were: 31.50, 43.40, 47.60, 51.20, 56.11 mol/min/mg protein and 23.50, 29.40, 32.10, 35.00 and 35.80 µmol min^{-1} % hematocrit, respectively. Except for the fish treated with 0.05 mg L^{-1} LABs, GST activity in the liver differs significantly ($p<0.05$) between each treatment and the control. But there was no significant ($p>0.05$) difference between the control and different treatments in the fish that were induced in Fig. 1 and 2.

On the 30th day, the enzyme activity in both tissues at the same concentrations was as follows: Liver: 31.30, 47.20, 56.90, 62.10, 68.90 mol/min/mg protein, erythrocytes: 23.30, 34.00, 42.10, 54.00, 57.20 µmol min^{-1} % hematocrit. The induction of the enzyme in the liver varies significantly ($p<0.05$) between each treatment and the control, except for the fish treated with 0.05 mg L^{-1} in Fig. 1, while, in the erythrocytes, it was significant ($p<0.05$) in each of the treatments, when compared with the control in Fig. 3 and 4.

Hydro corticosteroid secretion: The observable change in hydrocortisone secretion in *C. gariepinus* in the control fish at different times of exposure and at different concentrations is shown in Table 1. In the

treated fish, the change is consistent with the exposure durations and concentrations. The cortisol concentrations in the fish on day 2nd day of exposure at 0.0, 0.05, 0.10, 0.15 and 0.20 mg L^{-1} of LABs are 11.10±1.02, 13.20±0.06, 14.20 ±0.21, 14.30±0.50 and 15.90±0.10 ng mL^{-1}. No significant ($p>0.05$) difference between each of the treatments and the control when compared.

On day 30th, at the same concentrations of the toxicant, the cortisol level in the fish are: 11.22±0.71,15.90±2.10, 17.30±1.15, 18.20±0.32 and 20.30±1.00 ng mL^{-1}. The level of secretions of the enzymes in the treated fish was highly significantly ($p<0.05$ and $p<0.01$) when compared with the control fish in Table 1.

DISCUSSION

Esterases, also known as cholinesterase or choline esterase are the biochemical catalysts that neutralise choline base esters[32]. Catalysis and transformation of acetylcholine or acetylcholine-like substances to choline and acetic acid are the primary functions of these enzymes. When the cholinergic neuron is stimulated, the changes in this pathway are critical for it to go back to resting mode[33]. Acetylcholine is released at neuromuscular junctions when muscles contract, which aids in the movement of body organs by promoting muscle contraction. The enzyme cholinesterase neutralises acetylcholine, allowing the muscles to relax from their contractile state for a short period of time[34]. Hepatic synthesis and blood plasma transport are the primary modes of transport for this neurotransmitter[35]. Neuronal Acetylcholinesterase shares a high degree of resemblance with this enzyme, which is also known as erythrocyte cholinesterase and propionylcholinesterase is another pseudo-cholinesterase that can be found in various body organs and tissues, including the plasma[36,37]. AChE activity is commonly utilized as an environmental biomarker. This enzyme's activity is critical for several physiological activities, including prey localization, predator avoidance and food orientation and when AChE activity falls, ACh is not broken and accumulates within synapses, preventing them from functioning normally[38].

Changes in brain, liver and blood AChE activity found in surfactant-exposed fish are likely mirrored in movement disruptions, with the fish sluggish and stationary in the swimming habit, which helps to explain surfactant-induced behavioural modifications. Because AChE participates in neuronal and neuromuscular transmissions, inhibiting cholinesterase in the brain, liver and erythrocyte hurts movement[39]. In these tissues, the surfactant caused significant AChE inhibition and inhibiting AChE causes excessive ACh accumulation at synapses and neuromuscular junctions, resulting in overstimulation of ACh receptors and, potentially, death due to respiratory failure[40]. A similar report was presented when carbamate insecticides inhibited AChE activity in various freshwater fish species[41]. Chronic exposure of the fish *Rhamdia queen*[42] to lower concentrations of Roundup® (0.45 and 0.95 mg L^{-1}) reduced the activity of this enzyme. Similar observations were also reported after exposure of *Anguilla* to fenitrothion and thiobencarb[39]. Also, erratic swimming, convulsions and lethargy were noticed in silver catfish (*Rhamdia queen*) fingerlings treated with 10 mg L^{-1} clomazone for 96 hrs[40]. This study had shown that erythrocyte AChE inhibition was found to be more susceptible to surfactant adverse effects than liver AChE inhibition. As a result, it may serve as a more accurate environmental monitoring tool. Similar findings were made with carbosulfan-exposed rainbow trout[43].

An essential detoxifying enzyme called glutathione helps the body get rid of harmful toxins and pollutants. It is crucial for the conjugation of xenobiotics and for defending against peroxidative damage because it functions as a protective antioxidant. The cell is shielded from free radicals by the induction of glutathione-S transferase, a significant antioxidant produced by the cell. If left unchecked, these highly reactive substances will harm or destroy important cell components (e.g., membranes, DNA). The increase of glutamine in the fish's liver can be seen as an important response to a chemical stressor given their wide range of crucial roles in cell function and antioxidant defences. The fact that GST activity was generally induced in the plasma and liver homogenates at different surfactant concentrations in this study

indicates the role of this enzyme in defence against the toxicity of xenobiotics[44]. The test fish in the current study displayed comparable inductions in GST activity at various surfactant concentrations and times. These findings are consistent with research done on *Oreochromis mossambicus* exposed to two different hydrogen sulphide concentrations[45]. The reactive oxygen species produced during surfactant metabolism in the fish liver can increase the permeability of hepatocytes, kidneys, gills, spleen and heart cells, resulting in a leakage of GST and other enzymes into the plasma[46]. Similarly, the high level of GST in the plasma of the fish may be attributed to the stress imposed by the surfactant on the liver. The excessive increase in GST activities in the plasma of the investigated fish after a long period of toxicity in this study indicated that long-term surfactant exposure caused tissue damage in fish; a similar observation has also been reported in *O. mykiss* and *Channa punctata* exposed to diazinon and monocrotophos[47]. The induction of GST activity observed in this study indicates that this biomarker is important in the ecological risk assessment of the surfactant-contaminated environment.

Cortisol levels fluctuated in the control group but there was no significant difference ($p>0.05$). However, plasma cortisol levels increased dramatically in all treatments and the increase was proportional to the exposure period and concentrations. Toxins have been shown to disrupt hormone signalling cellular pathways and change the secretory pattern of cortisol, supporting the current findings[48]. Similar research had found that toxicants like copper influenced endocrine regulation in *Oncorhynchus mykiss*[49]. Similarly, elevated plasma cortisol levels in the current study could be attributed to increased ACTH levels caused by toxicant exposure as previously reported by Brazilian adult adults[50].

CONCLUSION

This study showed that exposure to surfactants can lead to important alterations in the biochemical enzymes of fish. These enzymes can be used as valuable biomarkers for the identification of surfactant pollution in aquatic bodies. Results also indicated that the existence of surfactants in water bodies may be injurious to the health of aquatic animals. In addition, research is necessary to explain the specific differences in the relationship between AChE inhibition, GST induction hydro corticosteroid disruption and physiological perturbation associated with alterations of marker's enzymes in fish and other aquatic inhabitants.

SIGNIFICANCE STATEMENT

Surfactants are emerging environmental contaminants and their toxicity poses a substantial threat to ecological, evolutionary, nutritional and environmental balances. The presence of surfactants such as linear alkyl benzene sulfonates, sodium dodecyl sulfate and α-olefine sulfonate in freshwater reservoirs could cause deleterious effects on aquatic life. Their physiological alterations may potentially decrease the survival rate of aquatic life in nature. Therefore, measures should be taken to mitigate the possible contamination of the aquatic ecosystem by many contaminants carelessly empty into water bodies and to strengthen the current findings, further continuation research should be made. Additionally, more studies on their potential residual effects are required to be performed to completely understand their hazardous impacts on aquatic ecosystems, with the requirement of using environmentally safe surfactants.

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