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Alkyl Benzene Sulfonate Induced Acute Toxicity and Potential Alteration of Growth, Hematological, Biochemical, Enzymological and Stress Biomarkers in *Oreochromis mossambicus* (Peters, 1852)

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Abstract | **Original Research Article**

The present study was intended to determine the toxic effect of Alkyl benzene sulfonate (ABS) on *Oreochromis mosambicus.* Probit analysis was employed to determine the 96h LC_{50} of surfactants for *O. mossambicus.* Moreover, the survivability of surfactant exposed was calibrated and validated using general threshold survival models (GUTS) in terms of required data sets and fit performance. Subsequently, fish were assigned to experimental groups exposed to 10% and 20% of 96h LC_{50} of surfactants for the period of 45 d to assess the changes in growth, hematological, plasma biochemical, and enzymological as well as stress enzyme parameters in gills and liver by employing standard protocol. The 24h, 48h, 72h and 96h LC_{50} values of ABS to *O. mossambicus* are 0.55mg/l, 0.28 mg/l, 0.09 mg/l and 0.06 mg/l respectively. Moreover, the GUTS- IT model better projected the survivability in *O. mossambicus* for ABS exposure. During sublethal exposure, a consequential reduction in specific growth rate (SGR), RBC, hemoglobin (Hb), hematocrit (Ht) value, plasma protein, albumin, and acetylcholinesterase (AChE) activities in gills and liver, as well as significant induction in gastrosomatic index (GSI), feed conversion ratio (FCR), plasma glucose, creatinine, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) levels and catalase (CAT), superoxide dismutase (SOD), malondialdehyde (MDA) in gills and liver were observed in exposed fish. Moreover, in both gills and the liver, GST and glutathione peroxidase (GPx) exhibited a significant initial increase followed by a subsequent decrease in exposed fish. The effects of ABS on fish were identified using the correlation matrix, integrated biomarker response (IBR) and biomarker response index (BRI). These findings show that exposure to surfactant affects multiple biomarkers in *O. mossambicus.*

Keywords: *Oreochromis mossambicus*, Alkyl benzene sulfonate, probit analysis, general threshold survival models, IBR, BRI.

1. INTRODUCTION

One of the most important requirements for maintaining life is access to water (Chaplin 2001). It is vital to the ecosystem's overall form and health (Bezerra Da Silva *et al.,* 2019). However, when contaminants like detergents are added to the system, it becomes polluted. Detergents have numerous uses in both commercial and residential settings, from laundry to car washes (Ivanković & Hrenović, 2010). However, the most significant way that detergents get into water is through sewage treatment plants that discharge into surface water (Scott & Jones 2000). Thus, excessive detergent runoff into waterways has negative consequences, including the accumulation of potentially hazardous chemicals that kills aquatic organisms (Faggio *et al.,* 2016; Faria *et al.,* 2021; Sula *et al.,* 2020; Susmi *et al.,* 2010). An imperative component of the detergent products is the surface active component of the detergent, known as surfactant, that poses a detrimental impact on the water bodies leading to a decline in water quality and increment in mortality of aquatic fauna (Mei-Hui Li, 2008). It has been documented that acute exposure to surfactants causes dose- and time- dependent mortality in the aquatic oligochaete worm *Tubifex tubifex* and the fish *Cyprinus carpio* (Bhattacharya *et al.,* 2019a, 2019b; Chatterjee, *et al.,* 2021a, 2021b).

Surfactant comprises a diversified class of chemical compounds comprising hydrophobic and hydrophilic sites which are crucial for organic contaminant solubilization (Lechuga *et al.,* 2016). Notwithstanding the way that most surfactants are degradable, their steady use in groundwater and perpetual disposal on the surface contributes to consistent occurrences in the aquatic environment (Mustapha & Bawa-Allah 2020). The average surfactant level for domestic wastewaters is from 1 to 10 mg/l, compared to over 300 mg/l in the surfactant-production industry (Rivera-Utrilla *et al.,* 2012). Most surfactants are perilous to macromolecules and alter their efficient functioning in the biological system by annexing them (Ivanković & Hrenović, 2010). Several studies have documented the toxic effect of surfactants in aquatic organisms (Freitas *et al.,* 2019, 2020; Hering *et al.,* 2020; Lechuga *et al.,* 2016; Mustapha & Bawa-Allah 2020).

Based on the classification, there are four types of surfactants: anionic, non-ionic, cationic, and zwitterionic (Jackson *et al.,* 2016). Out of these, anionic surfactants are widely utilized in a variety of industries, including textiles, emulsifiers, wetting agents, disinfectants, and cosmetics (Jardak *et al.,* 2016; Puchta, 1984). These compounds that have a lengthy, hydrophobic chain that connects to a positive nitrogen atom (Puchta, 1984). One such anionic surfactant is alkyl benzene sulphonate. It is basically used in household detergents as well as in numerous industrial applications (Mungray & Kumar, 2009)**.** Although a number of research have been done on the toxicity of ABS to aquatic plant (Wang *et al.,* 2011; Zhou *et al.,* 2018), there is as a dearth of evidence to suggest that ABS has a deleterious effect on the alterations of fish and other aquatic organisms.

O. mossambicus has been utilized as a model test organism in the present study as it has an excellent growth rate and high demand. It is also a tolerant and hardy fish for better endurance in a wide assortment of aquatic habitats (Chromcova *et al.,* 2015; Fiorino *et al.,* 2018; Forouhar Vajargah *et al.,* 2018; Hajam *et al.,* 2020; Hodkovicova *et al.,* 2019; Iswarya *et al.,* 2018; Liew *et al.,* 2013, 2015, 2020; Sehonova *et al.,* 2017; Woo & Chung 2020).

While the preliminary toxicity study investigates a lethal endpoint such as LC_{50}, it's substantially more apt to perform a sublethal toxicity investigation because the species are exposed to altogether lower, biologically pertinent toxic levels of inimical substances (Aliko *et al.,* 2019; Brahma & Gupta, 2020; Burgos-Aceves *et al.,* 2021; Fiorino *et al.,* 2018; Harikrishnan *et al.,* 2021; Petrovici *et al.,* 2020; Prokić *et al.,* 2019; Qyli *et al.,* 2020; Stara *et al.,* 2020). Fish growth is the crucial variable for their market success (Fazio 2019). But the presence of numerous toxicants in the aquatic system has resulted in a decrease in the growth rate of most fish (Bhunia *et al.,* 2003; Ko *et al.,* 2019; Majumder & Kaviraj, 2017). This decrease is attributable to the impaired activity of growth hormone (GH) as the function of GH is related to tissue and somatic growth (Wasinski *et al.,* 2019). Also, the pathophysiological reflexes in the entire organism are reflected by hematological and plasma biochemical biomarkers (Adhikari *et al.,* 2004). As blood is the main designator of whole-body health, a study on hematological and plasma biochemical parameters is essential to investigate the effects of toxicants on organisms (Burgos-Aceves, Cohen, Paolella, *et al.,* 2018; Burgos-Aceves, Cohen, Smith, *et al.,* 2018; Özok *et al.,* 2018). Xenobiotic metabolism in organisms contributes to the formation of ROS in immensely colossal quantities (Burgos-Aceves *et al.,* 2018; Faggio *et al.,* 2016; Hrycay & Bandiera 2015). This ROS triggers lipid peroxidation (LPO) that produces malondialdehyde (MDA) which causes rigorous damage to biomolecules such as DNA, protein, and membranes by inducing oxidative stress (Ansari *et al.,* 2019). Oxidative stress is induced as a result of disequilibrium between ROS formation and neutralization by antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), GST, and reduced glutathione (GSH) (Gobi *et al.,* 2018). In addition, AChE plays a component in signal terminations through expeditious hydrolysis of the neurotransmitter acetylcholine at cholinergic synapses. Cholinesterase suppression upon the integration of toxicant allows acetylcholine to accumulate in the synaptic cleft and blocks nerve transmission, which leads to paralysis and mortality of organisms (Trang & Khandhar, 2019). Therefore, a productive and auxiliary methodology for assessing the activities of stress enzymes could be a potential implement for aquatic toxicological investigations (Chatterjee *et al.,* 2021b).

Singular biomarkers cannot give a true and practical assessment of the toxicity of toxicants on aquatic life forms, and hence some existing literature recommended to utilize the amalgamated biomarker study to comprehend the reaction of an organism to toxic substances (Sanchez *et al.,* 2012; Stara *et al.,* 2020). IBR subsequently provides a comprehensive methodology that aggregately combines all the biomarker responses and plays a vital part in comparing the toxicity of contaminants (Beliaeff & Burgeot, 2002). Moreover, BRI has been widely utilized in recent years to integrate multiple biomarker responses. It is rudimentarily focused on the evaluation of the organism's overall health status (Hagger *et al.,* 2008).

According to Jager *et al.,* (2011), GUTS model serves as potential implements that can amend the environmental risk assessment of toxicants. To describe the death mechanism cognate to the damage, two causations of the process affecting survival are formalized: the stochastic death (SD) and IT approaches. The SD approach surmises that individuals are identical and have a probability to die upon chemical stress, which

increases with incrementing damage once some threshold damage has been exceeded. The IT approach postulates that individuals have differences in their sensitivity to chemical stress, and when the damage exceeds an individual's threshold, it dies instantly. Both approaches can lead to different data interpretations and presage for the time course of effect (Jager *et al.*, 2011). The significance GUTS model is that it has been found to be a solid method for the evaluation of effects of time-variable chemical exposure on the survival of aquatic organisms. It basically combines toxicokinetics and damage dynamics into a single compartment and therefore links external concentrations to the effect on survival (Jager *et al.*, 2011; Jager & Ashauer, 2018).

2. MATERIALS AND METHODS

2.1. *Test Chemicals and Organisms*

All the reagents used in this experiment were purchased from Sisco Research Laboratories (SRL), India. The test chemical, Alkyl benzene sulfonate was procured from Spectrum Indian Chemicals, India. It was discretely dissolved in pure distilled water to make a stock solution of 1 mL/100 mL (1% v/v). The test organism used in the bioassay was *O. mossambicus*. (Mean length 5.7 ± 0.65 cm, mean weight 8.5 ± 0.53 g). The specimens were obtained from the fish farm at Naihati, West Bengal, India, and given prophylactic treatment by bathing them in 0.05% potassium permanganate ($KMnO_4$) solution for 2 min to eschew any dermal infections.

2.2. *Maintenance Condition*

Fish were placed in outdoor cement tanks for acclimatization for 14 days and were provided with commercial feed (manufactured by CPF India Pvt. Ltd.) daily at 8.0 A.M. and 4.0 P.M. During this acclimation period, proper aeration (Aquaspeed AP-446) and daily partial renewal of water (20–25%) were performed for all the tanks. The values of the different physicochemical parameters of water used in the study were as follows: temperature 29.4 ± 0.9 ◦C, pH 7.6 ± 0.4, free CO_2 25.7 ± 2.8 mg L^{-1}, dissolved oxygen 6.1 ± 0.92 mg L^{-1}, total alkalinity 191 ± 8.2 mg L^{-1}, and hardness 134 ± 7.1 mg L^{-1} as $CaCO_3$.

2.3. *Acute Toxicity Bioassay*

After the completion of the acclimatization period, the static replacement bioassays were conducted in 15 L glass aquaria with 10 L of non-chlorinated tap water each containing 10 fish. The values of the different physicochemical parameters of were as follows: temperature 28.5 ± 0.8 ◦C, pH 7.5 ± 0.4, free CO_2 28.7 ± 2.5 mg L^{-1}, dissolved oxygen 6.9 ± 0.90 mg L^{-1}, total alkalinity 189 ± 8.4 mg L^{-1}, and hardness 139 ± 6.9 mg L^{-1} as $CaCO_3$. The details of the instrument are provided in the supplementary file (S1). The fish were not alimented for 24 h before the commencement of the test. Initial range-finding tests were conducted to estimate the spectrum of concentrations of the test chemical. The nominal concentrations of ABS (0.02, 0.04, 0.06, 0.08, 1.00, 1.02, 1.04 and 1.06 mg L^{-1}) were conclusively used to estimate the 24, 48, 72, and 96 h acute toxicity in terms of LC_{50} values to *O. mossambicus*. Mortalities were recorded every 24 h and dead fish were removed from tanks. No movement of fish even with a simple touch is considered as the indicator of mortality. Behavioral changes in terms of erratic, lateral, and circular movements, vertical hanging, caudal bending, opercular movement, body balance, fright response, and swimming rate were recorded during 96 h of exposure. A control set of experiment was done in parallel with the main experiment using regular tap water and fish, but without the addition of ABS at any point during the experiment. All experiments were done in triplicates.

2.4. *Chronic Toxicity Bioassay on Growth Parameters*

Experiments for chronic bioassays in fish were carried out in 15l glass aquaria, each containing 10l of water and five fish. Two sublethal concentrations of ABS (0.006 and 0.012 mg L^{-1}) were employed for the experiment along with control. There were three replicates for each concentration. Amid the experiment, a considerable proportion (20%) of the test medium was renewed and replaced with pulse treatment of surfactant. The experiment was continued for 45 days. The fish were fed with Osaka Green fish food (crude protein: 28%, crude fat: 3%, crude fiber; 4%, and moisture content 10%).

Fish were sampled at the end of 45 days, and the lengths (cm) and weights (g) of the sampled fish were recorded, and various growth parameters were calculated using standard formulae (Bhunia *et al.*, 2003).

a) Gastrosomatic index (GSI) = (V/W) ×100, where V is the visceral weight of the fish (g), and W is the observed bodyweight of fish (g).
b) Specific growth rate (SGR) %day = {(loge W_2 −loge W_1) / T} × 100, where $\log_e W^1$ is the natural logarithm of initial body weight of fish (g). LogeW_2 is the natural logarithm of the final body weight of fish (g), and T is the time interval.
c) Feed conversion ratio (FCR) = food given/weight gain where weight gain = final weight of fish (g) − the initial weight of fish (g).

2.5. *Chronic Toxicity Bioassay on Hematological Parameters*

Bioassays on hematological parameters were also conducted in 15 l glass aquaria, each containing 10 L of water and five fish (Majumder & Kaviraj, 2017). Two sublethal concentrations of ABS (0.006 and 0.012 mg L^{-1}) were employed for the experiment along with a control. There were three replicates for each concentration. Fish specimens were sampled after 15, 30, and 45 days of exposure. By the utilization of a disposable sterile syringe and a needle, blood was obtained by trans-fixing the heart. The collected blood sample was transferred immediately to vials containing an anticoagulant, EDTA, and was softly shaken to

eschew blood hemolysis. Red blood cell (RBC) counts were calculated using a hemocytometer (Mishra *et al.,* 1977). Hemoglobin (Hb) content was estimated by the cyanmethemoglobin method (Blaxhall & Daisley, 1973). The hematocrit value (Hct%) was determined with the standard microhematocrit method (Blaxhall & Daisley 1973).

2.6. *Chronic Toxicity Bioassay on Plasma Biochemical and Enzymological Parameters*

Experiments on plasma biochemical parameters were performed in a homogeneous type of experimental setup as depicted above in bioassay on hematological parameters. After the collection of blood, the blood samples were centrifuged at 3000 rpm for 20 min at 4 ◦C for the separation of plasma from the blood sample. Total glucose was quantified by the GOD-POD method utilizing a commercial kit. Total protein was quantified by (Lowry *et al.,* 1951). Albumin was estimated utilizing the BCG (bromocresol green) dye-binding method using a commercial kit (Tulip Diagnostics, India). Creatinine level was resolute according to modified Jaffe's method utilizing a commercial kit. Cholesterol was estimated by the CHOD-PAP method utilizing a commercial kit (Tulip Diagnostics, India). The activities of alanine aminotransferase (ALT/GPT) and aspartate aminotransferase (AST/ GOT) were determined according to the modified IFCC method (International Federation of Clinical Chemistry) utilizing a commercial kit (Tulip Diagnostics, India).

2.7. *Chronic Toxicity Bioassay on Stress Enzyme Parameters in Gills and Liver*

50^{-} mg of gills and liver tissue each were homogenized in 2 mL of phosphate buffer saline (PBS). The homogenized tissues were spun in a cold centrifuge (HERMLE Labortechnik) at 5000 rpm for 15 min at 4 ◦C. After centrifugation, the supernatants were stored at 20 ◦C till further analysis. The protein content in gills and liver tissue was quantified by utilizing the method of (Lowry *et al.,* 1951). Bovine serum albumin (BSA, Sigma) was utilized as a standard. Standard protocols have been used to quantify CAT, SOD, GST, GPx, and MDA activities (Akerboom & Sies, 1981; Beauchamp & Fridovich 1971; Beers & Sizer 1952; Habig *et al.,* 1974; Lawrence & Burk 1976; Ohkawa *et al.,* 1979). Effects of CAT, SOD, GST, and GPx were expressed as units per milligram of protein (U/mg protein), and MDA levels were expressed as nmol thiobarbituric acid reactive substance (TBARS) per min per milligram of protein (nmol TBARS/min/mg protein). Moreover, AchE activity was determined following the protocol of (Ellman *et al.,* 1961) and expressed as nmol/min/mg protein.

2.8. *Determination of IBR*

IBR was determined by utilizing the protocol of Beliaeff & Burgeot (2002). Each biomarker's IBR analysis and evaluation was carried out as follows:

a) Each treatment's mean as well as standard deviation (SD) are assessed.
b) Standardization of the results for each treatment as Fi′ = (Fi - mean F)/S, where Fi′ is the biomarker's standardized value, Fi is each treatment's mean value of a biomarker, F is the mean of the biomarker of all treatments and S is the treatment specific SD.
c) Based on the standardized data, X was calculated: + Fi′ in the case of activation and – Fi′ in the case of suppression,
d) The minimum value for each biomarker for all treatments was obtained and then added to X.
e) The score S was measured as B = |min Fi′| + Z, where B is the actual value of the minimum Fi′ and |min Fi′| is the actual value of the minimum Fi′.
f) Subsequently, the calculation of IBR is accomplished by multiplying the acquired value of each biomarker (Bi) by the value of its next biomarker, dividing each value by 2 and totaling the results. Results of the data were presented in a radar chart.

2.9. *Determination of BRI*

The BRI for determining the health status of the organism was performed utilizing the protocol of Hagger *et al.,* (2008). According to the protocol:

a) Alteration levels (AL) are measured from each biomarker's responses.
b) Each AL is given a score based on the proportion of deviation from the controls; ALs with a deviation greater than 100% were given a score of 1, ALs with a deviation between 50% and 100% were given a score of 2, ALs with a deviation between 20% and 50% were given a score of 3 and ALs with a deviation of less than 20% were given a score of 4.
c) Each biomarker's weightings are determined based on the underlying biological action (Piva *et al.,* 2011).
d) Using the following equation, the BRI is determined. BRI = {∑ (Sn X Wn)}/Wn e) Where Sn is the score and Wn is the weight of each biomarker "n."
e) Eventually, the BRI values are categorized: 1.0–2.5 (severe modification), 2.51– 2.75 (major alteration), 2.76–3.00 (moderate alteration) and 3.01–4.00 (slight alteration) (Hagger *et al.,* 2008).

2.10. *Statistical Analysis*

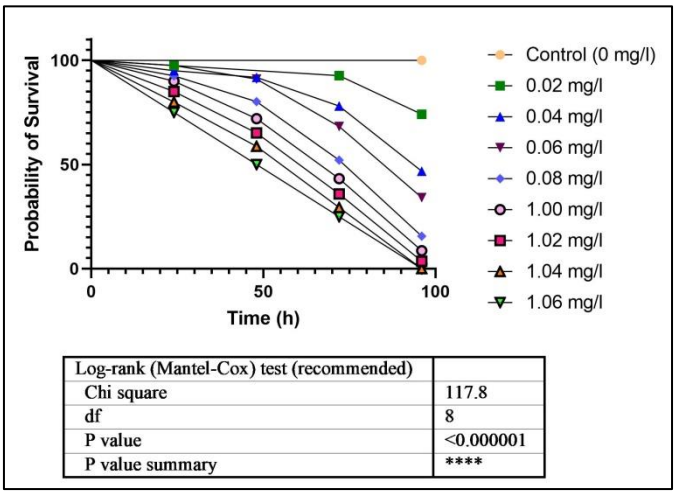
Utilizing MS Excel 2016, Finney's probit analysis has been performed for calculating the LC_{50} values (Finney 1971). Utilizing Kaplan-Meier analysis, survival curves were determined and constructed using Graphpad Prism (ver. 9). After performing the normality check utilizing the Shapiro Wilk test, two-way ANOVA followed by the Tukey post hoc test had been used to determine the comparison between controls and exposed

fish using Graphpad Prism (ver. 9). The correlation matrix plot for the determination of the correlations of biomarkers was determined and constructed using PAST (ver. 4.03). The level of statistical significance was accepted as being $p < 0.05$. Data are presented as mean ± SEM. GUTS-SD/IT modeling was conducted in open GUTS software to predict the LC_{50} values of ABS during long- term exposure (100 days) and to predict the mode of action of the surfactant.

3. RESULTS

No mortality of *O. mossambicus* was recorded in control during the experiment. The mortality rate (%) of the test animals significantly increased ($p < 0.05$) with increasing concentration of the ABS and exposure times (24, 48, 72, 96h)**.**

The survivability curve also depicts that ABS significantly affected the overall survival rates of *O. mossambicus* in a dose and duration-dependent manner with respect to control (Mantel log-rank test; $p < 0.05$). It is observed that 100 % survivability of *O. mossambicus* exists in control in all exposure periods (24, 48, 72, and 96 h). However, with the increment of concentration ABS as well as periods of exposure (24, 48, 72, and 96 h, the survivability rate of *O. mossambicus* decremented significantly (Mantel log-rank test; $p < 0.05$) (Fig. 1).



Log-rank (Mantel-Cox) test (recommended)	
Chi square	117.8
df	8
P value	<0.000001
P value summary	****

Fig 1: **Kaplan Maier survivability curve of *O. mossambicus* exposed to ABS**

The 24, 48, 72, and 96 h LC_{50} values of ABS to fish are reported to be 0.554 ± 0.147, 0.280 ± 0.130, 0.094 ± 0.139, and 0.069 ± 0.161mg/l (Table 1).

Table 1: **The LC_{50} values and 95% confidence limits of ABS to *O. mossambicus* at different exposure periods (24, 48, 72, and 96 h)**

Exposure period (h)	LC_{50} ± SE (mg/l)	95% confidence limit	
		Lower	Upper
24	0.554 ± 0.147	0.286	1.075
48	0.280 ± 0.130	0.155	0.504
72	0.094 ± 0.139	0.050	0.175
96	0.069 ± 0.161	0.033	0.142

The GUTS model parameters, as well as the fitted performance of GUTS (SD or IT), are given in Table 2. The fitted performance of GUTS-IT was better than that of GUTS-SD in the case of ABS predicated on AIC values (A smaller AIC value indicates the best fit). Thus, the model simulation illustrated that the GUTS-IT model can better predict the survivability of ABS-exposed *O. mossambicus.*

Table 2: Model parameters in case of ABS [Kd indicates Dominant rate constant; m_w indicates Threshold for mortality; b_w indicates Killing rate; h_b indicates ABSkground hazard rate & F_s indicates Spread factor of the threshold distribution]

Symbol	GUTS-RED		unit	AIC Value	
	SD	IT		SD	IT
k_d	143.8 (0.001641 - 143.8)	0.001641 (0.001641 – 143.8)	d^{-1}	199.38	**195.57**
m_w	0.9895 ($5.471e^{-6}$-1.049)	0.0006192 ($5.471e^{-6}$-2.12)	mg/l		
b_w	29.82 (0.02485 – 315519)	-	L/mg/d		
h_b	0.07 ($1e^{-6}$ – 0.07)	0.0102 ($1e^{-6}$ – 0.07)	d^{-1}		
F_s	1	20 (1.05 – 20)			

Moreover, the forecasted LC_{50} values from GUTS-IT models are given in Table 3.

Table 3: Forecasted LC_{50} values of ABS to *O. mossambicus*

Time [d]	LC_{50} GUTS-IT (mg/l)
1	0.3776 (0.2335 – 0.7625)
2	0.189 (0.1169 – 0.3815)
3	0.1261 (0.07797 – 0.2546)
4	0.9464 (0.05853 – 0.1954)
7	0.05421 (0.03353 – 0.1314)
14	0.02726 (0.01686 – 0.1005)
100	0.004091 (0.00253 -0.09391)

During the test period in our study, the control group behaved normally. However, irregular swimming, loss of balance, increased surface grasping, progressive motionlessness, and increased opercular movement were seen in ABS-treated fish after 96 hours of exposure (Table 4).

Table 4: Impact of ABS on behaviors of *O. mossambicus at* 96h exposure (-: absent; +: mild; ++: moderate; +++: strong)

Concentration (mg/l)	Erratic swimming	Surface grasping	Motionlessness	Loss of Equilibrium	Opercular Movement
0.00	-	-	-	-	-
0.02	-	+	+	-	+
0.04	+	+	+	+	++
0.06	++	++	++	++	++
0.08	+++	+++	+++	+++	+++

The indicators of the growth performance of *O. mossambicus* upon exposure (45 days) to sublethal concentrations of ABS are demonstrated in Fig. 2. The chronic exposure of fish to 10% and 20% of LC_{50} of ABS (0.006 and 0.012 mg/l) showed a significant reduction ($p<0.05$) in SGR and FCR and a significant increase ($p<0.05$) in gastrosomatic index (GSI) in compare to control. No significant differences were observed in the condition factor of fish exposed to 10% and 20% of LC_{50} of ABS (0.006 and 0.012 mg/l) with respect to control.

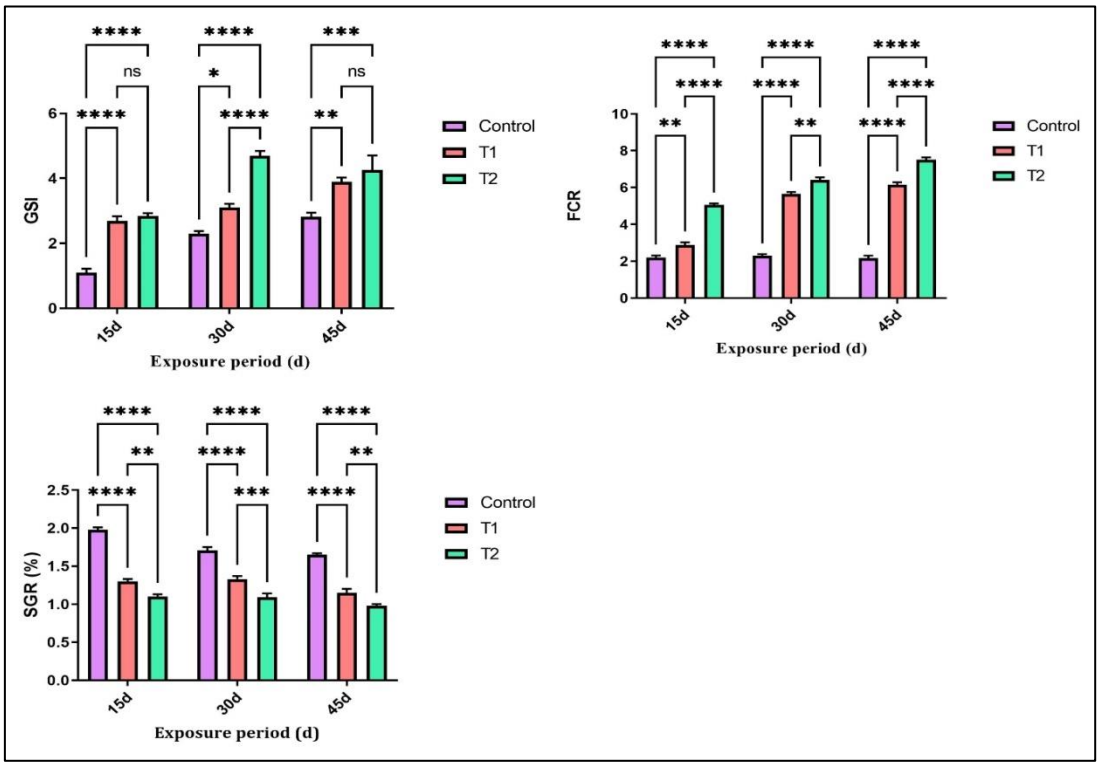


Fig. 2: Changes in GSI, SGR, and FCR of *O. mossambicus* upon addition of ABS. * indicates the level of significance Control indicates 0 mg/l of ABS, T1 indicates the concentration of 10% of 96h LC_{50} values of ABS (0.006 mg/l), T2 indicates the concentration of 20% of 96h LC_{50} values of ABS (0.012 mg/l)

The effect of ABS on hematological parameters is depicted in Fig. 3. A significant decline in RBC, Hb, and Ht (%) ($p < 0.05$) was visually examined in the fish exposed to concentrations of 10% and 20% of LC_{50} of ABS (0.006 and 0.012 mg/l) at 15, 30, and 45 d exposure with reference to control.

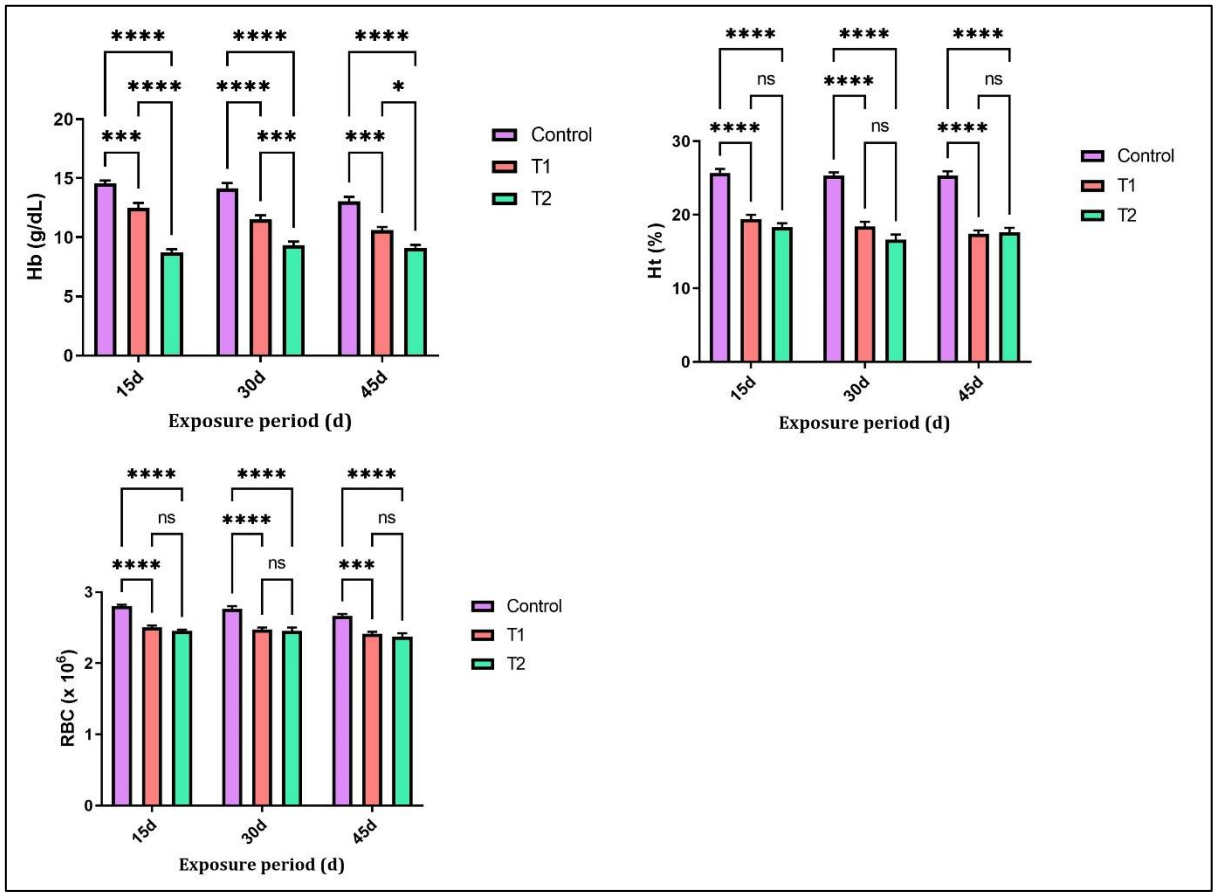


Fig. 3: **Changes in RBC, Hb, and Ht value of fish upon the addition of ABS. The values are represented as mean ±SE. ns indicates non-significant differences and the * indicates the level of significance. Control indicates 0 mg/l of ABS, T1 indicates the concentration of 10% of 96h LC_{50} values of ABS (0.006 mg/l), T2 indicates the concentration of 20% of 96h LC_{50} values of ABS (0.012 mg/l)**

The effect of ABS on plasma biochemical parameters is depicted in Fig. 4. The result showed that glucose levels in the exposed fish incremented significantly ($p < 0.05$) with reference to the control group when exposed to 10% and 20% of LC_{50} of ABS (0.006 and 0.012 mg/l). Total protein decremented significantly ($p < 0.05$) with reference to the control group when fish are exposed to 10% and 20% of LC_{50} of ABS (0.006 and 0.012 mg/l). Albumin levels decremented significantly ($p < 0.05$) with reference to the control group when exposed to ABS concentration of 20% of 96h LC_{50} value (0.006 mg/l) at 30 and 45 d exposure period. Creatinine levels in exposed fish incremented significantly ($p < 0.05$) in all exposure periods (15, 30, and 45 d) with reference to control when exposed to 10% and 20% of 96h LC_{50} of ABS (0.006 and 0.012 mg/l). Plasma ALT and AST enzyme activities significantly incremented ($p < 0.05$) in the fish exposed to 10% and 20% of LC_{50} of ABS (0.006 and 0.012 mg/l) compared to the control group at all exposure periods (15, 30, and 45 d).

Results of stress enzyme parameters in gills and liver of *O. mossambicus* upon addition of sublethal concentrations of ABS (0.006 and 0.012 mg/l) are depicted in Fig. 5 and 6 respectively. CAT activity in the gills and liver of exposed fish incremented significantly ($p < 0.05$) at 10% and 20% of LC_{50} of ABS (0.006 and 0.012 mg/l). SOD activity incremented significantly ($p < 0.05$) in the gills and liver of ABS-exposed fish at all exposure periods except during the 15d exposure period where no consequential increase in SOD activity is visually examined in the gills of ABS-exposed fish (10% of 96h LC_{50}). GST activity in the gills increased significantly ($p < 0.05$) at 10% and 20% of LC_{50} of ABS (0.006 and 0.012 mg/l) during 15d and 30d exposure periods but consequentially declined ($p < 0.05$) during 45d exposure period. Moreover, GST activity in the liver increased significantly ($p < 0.05$) at 10% and 20% of LC_{50} of ABS (0.006 and 0.012 mg/l) during 15d and 30d exposure periods. However, no consequential decrease in GST activity is visually examined in the liver of ABS-exposed fish during the 45d exposure period. GPx activity incremented significantly ($p < 0.05$) at 10% and 20% of LC_{50} of ABS (0.006 and 0.012 mg/l) during 15

and 30 d exposure periods but decremented significantly at 45d exposure period at 10% and 20% of LC_{50} of ABS (0.006 and 0.012 mg/l) in both gills and liver. MDA activity incremented significantly ($p < 0.05$) at 10% and 20% of LC_{50} of ABS (0.006 and 0.012 mg/l) during all exposure periods (15, 30, and 45 d).

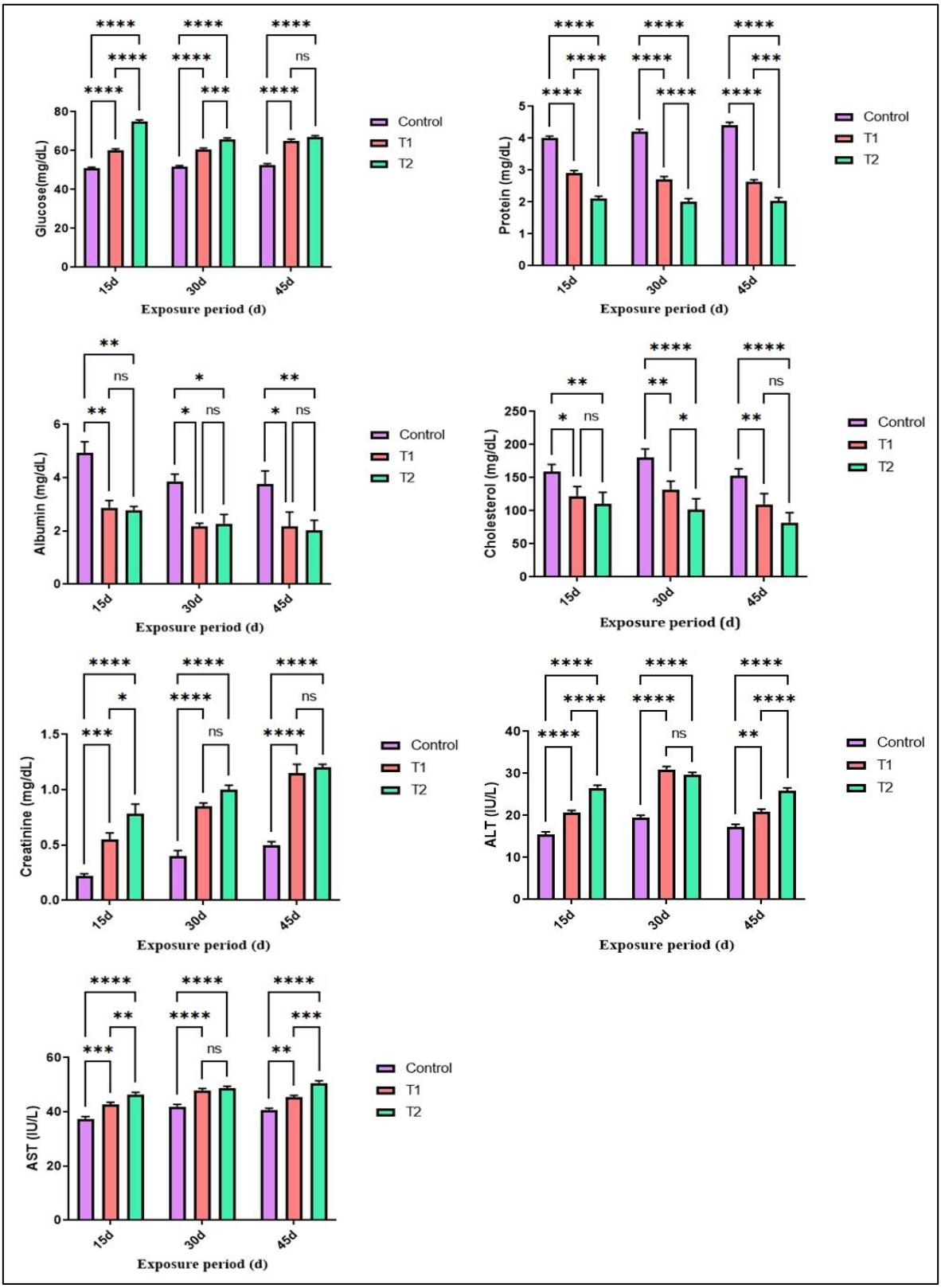


Fig. 4: Changes in glucose, protein, albumin, creatinine, ALT, and AST levels of fish upon addition of ABS. The values are represented as mean ±SE. ns indicates non-significant differences and the * indicates the level of significance. Control indicates 0 mg/l of ABS, T1 indicates the concentration of 10% of 96h LC_{50} values of ABS (0.006 mg/l), T2 indicates the concentration of 20% of 96h LC_{50} values of ABS (0.012 mg/l)

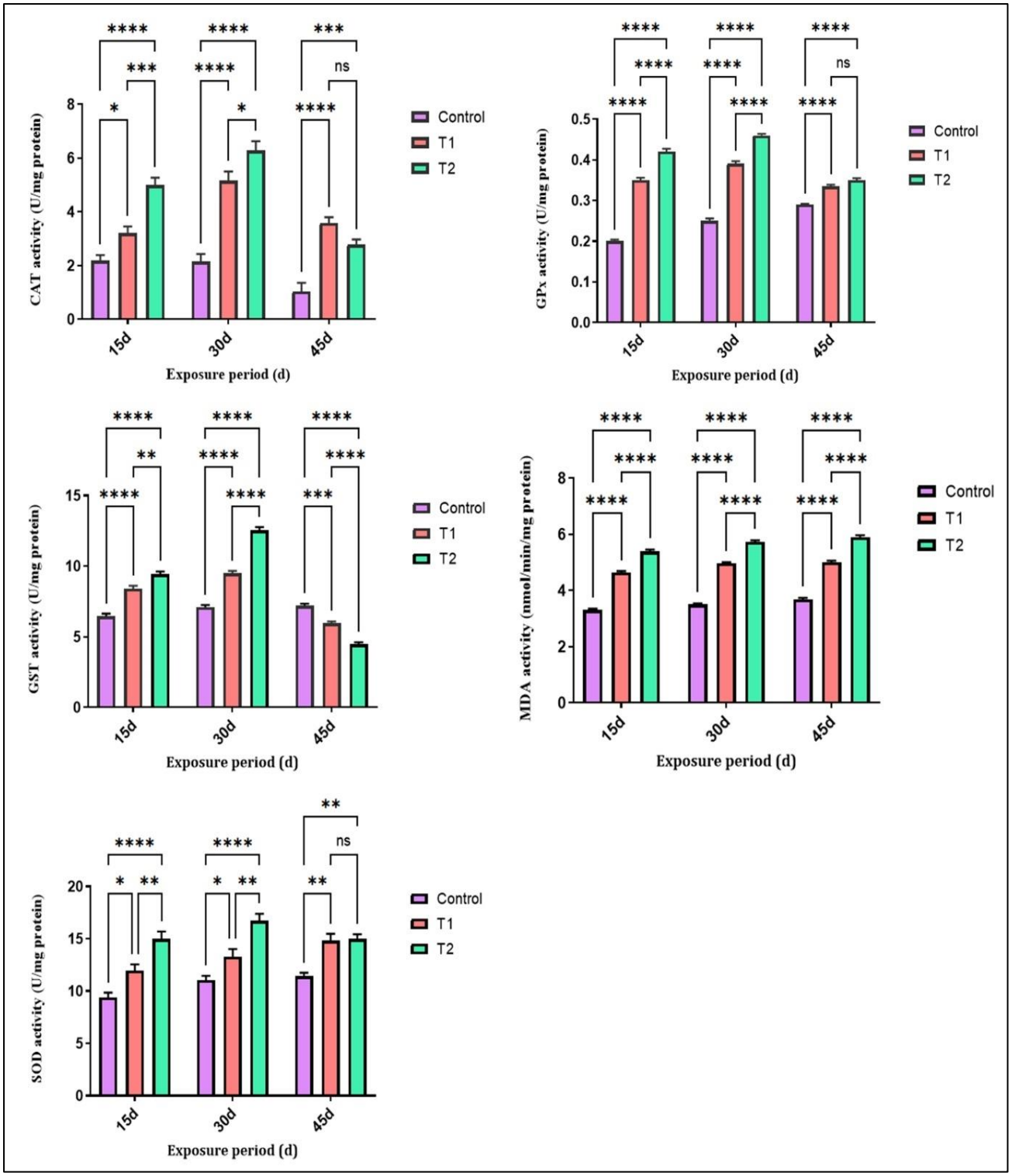


Fig. 5: Changes in CAT, SOD, GST, GPx, and MDA levels in gills of fish upon the addition of ABS. The values are represented as mean ±SE. ns indicates non-significant differences and the * indicates the level of significance. Control indicates 0 mg/l of ABS, T1 indicates the concentration of 10% of 96h LC_{50} values of ABS (0.006 mg/l), T2 indicates the concentration of 20% of 96h LC_{50} values of ABS (0.012 mg/l)

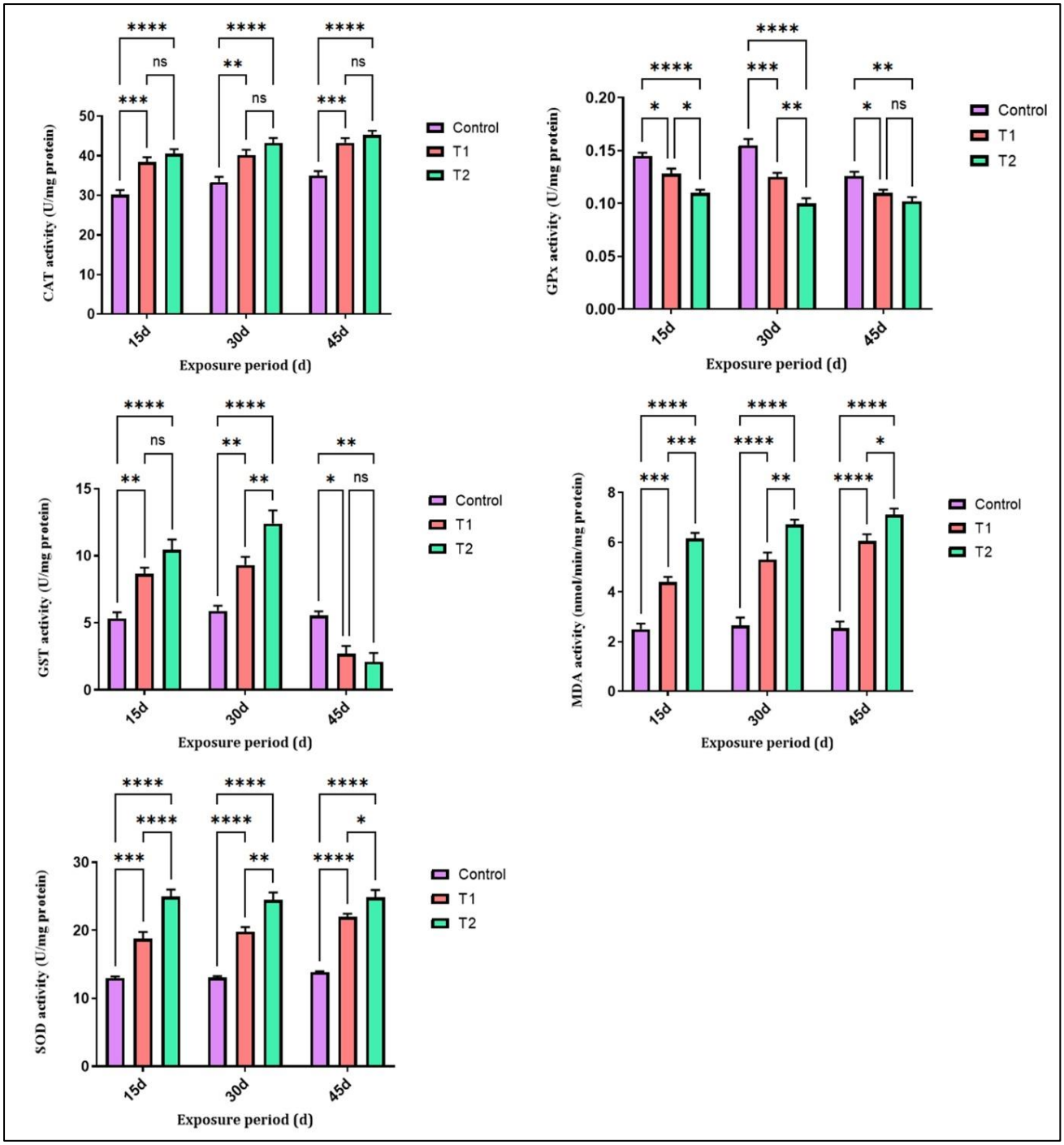


Fig. 6: **Changes in CAT, SOD, GST, GPx, and MDA levels in the liver of fish upon the addition of ABS. The values are represented as mean ±SE. ns indicates non-significant differences and the * indicates the level of significance. Control indicates 0 mg/l of ABS, T1 indicates the concentration of 10% of 96h LC_{50} values of ABS (0.006 mg/l), T2 indicates the concentration of 30% of 96h LC_{50} values of ABS (0.012 mg/l).**

AChE activity decreased at 10% and 20% of LC_{50} of ABS (0.006 and 0.012 mg/l) in the gills and liver of *O. mossambicus* as compared to the control (Fig. 7).

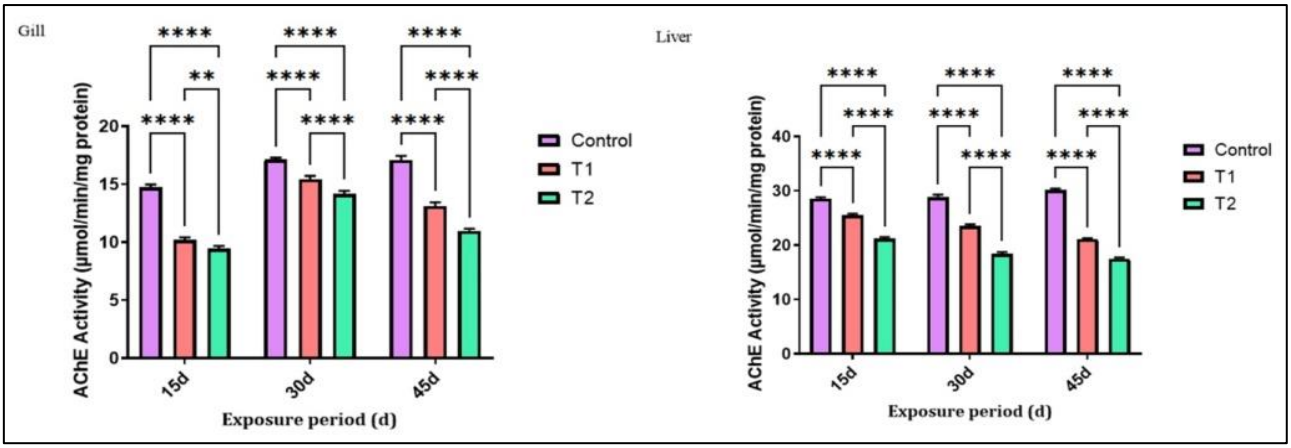


Fig. 7: Changes in AChE activities in gills and liver of fish upon the addition of ABS. The values are represented as mean ±SE. ns indicates non-significant differences and the * indicates the level of significance. Control indicates 0 mg/l of ABS, T1 indicates the concentration of 10% of 96h LC_{50} values of ABS (0.006 mg/l), T2 indicates the concentration of 20% of 96h LC_{50} values of ABS (0.012 mg/l).

Two-way ANOVA results revealed significant variations ($p<0.05$) in each biomarker with various experimental conditions (Table 5).

Table 5: Two-way ANOVA for ABS concentration in mg/l (ABS) and period of exposure in days (exposure period) on oxidative stress biomarkers in *O. mossambicus* after chronic exposure to ABS

Source	SS	DF	MS	F (DFn, DFd)	P-value
GSI					
Interaction	2.006	4	0.5016	F (4, 18) = 4.720	P=0.0088
Exposure period	15.89	2	7.943	F (2, 18) = 74.74	P<0.0001
ABS	10.44	2	5.220	F (2, 18) = 49.12	P<0.0001
SGR					
Interaction	0.08613	4	0.02153	F (4, 18) = 5.521	P=0.0044
Exposure period	2.505	2	1.252	F (2, 18) = 321.1	P<0.0001
ABS	0.1817	2	0.09083	F (2, 18) = 23.29	P<0.0001
FCR					
Interaction	10.20	4	2.551	F (4, 18) = 56.31	P<0.0001
Exposure period	77.78	2	38.89	F (2, 18) = 858.5	P<0.0001
ABS	17.43	2	8.715	F (2, 18) = 192.4	P<0.0001
RBC					
Interaction	0.003733	4	0.0009333	F (4, 18) = 0.2545	P=0.9032
Exposure period	0.05287	2	0.02643	F (2, 18) = 7.209	P=0.0050
ABS	0.5401	2	0.2700	F (2, 18) = 73.65	P<0.0001
Hb					
Interaction	4.926	4	1.231	F (4, 18) = 3.729	P=0.0222
Exposure period	4.811	2	2.405	F (2, 18) = 7.285	P=0.0048
ABS	107.2	2	53.59	F (2, 18) = 162.3	P<0.0001
Ht					
Interaction	4.420	4	1.105	F (4, 18) = 1.123	P=0.3766
Exposure period	6.243	2	3.121	F (2, 18) = 3.172	P=0.0660
ABS	340.6	2	170.3	F (2, 18) = 173.1	P<0.0001
Glucose					
Interaction	165.6	4	41.40	F (4, 18) = 28.12	P<0.0001
Exposure period	33.28	2	16.64	F (2, 18) = 11.30	P=0.0007
ABS	1404	2	702.0	F (2, 18) = 476.7	P<0.0001
Protein					
Interaction	0.3885	4	0.09713	F (4, 18) = 4.442	P=0.0113
Exposure period	0.01627	2	0.008133	F (2, 18) = 0.3720	P=0.6946
ABS	21.85	2	10.92	F (2, 18) = 499.5	P<0.0001
Albumin					
Interaction	0.3083	4	0.07708	F (4, 18) = 0.1965	P=0.9370
Exposure period	4.079	2	2.040	F (2, 18) = 5.200	P=0.0165
ABS	19.52	2	9.760	F (2, 18) = 24.88	P<0.0001
Creatinine					
Interaction	0.07893	4	0.01973	F (4, 18) = 2.340	P=0.0942
Exposure period	0.8467	2	0.4233	F (2, 18) = 50.20	P<0.0001

Source	SS	DF	MS	F (DFn, DFd)	P-value
ABS	1.896	2	0.9482	F (2, 18) = 112.4	P<0.0001
ALT					
Interaction	70.40	4	17.60	F (4, 18) = 14.45	P<0.0001
Exposure period	185.9	2	92.94	F (2, 18) = 76.30	P<0.0001
ABS	464.6	2	232.3	F (2, 18) = 190.7	P<0.0001
AST					
Interaction	17.01	4	4.254	F (4, 18) = 2.504	P=0.0787
Exposure period	82.64	2	41.32	F (2, 18) = 24.32	P<0.0001
ABS	335.3	2	167.6	F (2, 18) = 98.69	P<0.0001
CAT-gill					
Interaction	8.653	4	2.163	F (4, 18) = 9.188	P=0.0030
Exposure period	19.04	2	9.522	F (2, 18) = 40.44	P<0.0001
ABS	41.02	2	20.51	F (2, 18) = 87.11	P<0.0001
CAT-liver					
Interaction	2.377	4	0.5943	F (4, 18) = 0.1340	P=0.9678
Exposure period	103.1	2	51.55	F (2, 18) = 11.62	P=0.0006
ABS	505.8	2	252.9	F (2, 18) = 57.00	P<0.0001
SOD-gill					
Interaction	9.815	4	2.454	F (4, 18) = 2.414	P=0.0868
Exposure period	15.21	2	7.606	F (2, 18) = 7.483	P=0.0043
ABS	109.5	2	54.74	F (2, 18) = 53.85	P<0.0001
SOD-Liver					
Interaction	9.027	4	2.257	F (4, 18) = 1.253	P=0.3245
Exposure period	9.095	2	4.548	F (2, 18) = 2.525	P=0.01080
ABS	599.3	2	299.7	F (2, 18) = 166.4	P<0.0001
GST-gill					
Interaction	53.17	4	13.29	F (4, 18) = 148.3	P<0.0001
Exposure period	66.77	2	33.39	F (2, 18) = 372.5	P<0.0001
ABS	16.29	2	8.143	F (2, 18) = 90.85	P<0.0001
GST-liver					
Interaction	90.74	4	22.69	F (4, 18) = 20.23	P<0.0001
Exposure period	169.0	2	84.49	F (2, 18) = 75.33	P<0.0001
ABS	33.49	2	16.74	F (2, 18) = 14.93	P=0.0002
GPx-gill					
Interaction	0.02480	4	0.006200	F (4, 18) = 75.30	P<0.0001
Exposure period	0.01085	2	0.005425	F (2, 18) = 65.89	P<0.0001
ABS	0.1255	2	0.06273	F (2, 18) = 761.8	P<0.0001
GPx-liver					
Interaction	0.0007620	4	0.0001905	F (4, 18) = 3.550	P=0.0265
Exposure period	0.001266	2	0.0006330	F (2, 18) = 11.80	P=0.0005
ABS	0.006522	2	0.003261	F (2, 18) = 60.76	P<0.0001
MDA –gill					
Interaction	0.03693	4	0.009233	F (4, 18) = 0.9142	P=0.4769
Exposure period	0.8105	2	0.4052	F (2, 18) = 40.12	P<0.0001
ABS	21.82	2	10.91	F (2, 18) = 1080	P<0.0001
MDA-liver					
Interaction	1.933	4	0.4833	F (4, 18) = 2.463	P=0.0822
Exposure period	3.562	2	1.781	F (2, 18) = 9.077	P=0.0019
ABS	77.50	2	38.75	F (2, 18) = 197.5	P<0.0001
AchE-gill					
Interaction	10.94	4	2.735	F (4, 18) = 40.94	P<0.0001
Exposure period	109.3	2	54.63	F (2, 18) = 818.0	P<0.0001
ABS	76.52	2	38.26	F (2, 18) = 572.9	P<0.0001
AchE-liver					
Interaction	32.97	4	8.244	F (4, 18) = 76.00	P<0.0001
Exposure period	465.7	2	232.8	F (2, 18) = 2147	P<0.0001
ABS	22.52	2	11.26	F (2, 18) = 103.8	P<0.0001

Moreover, the results of the correlation matrices between the concentrations of ABS are depicted in Fig. 8.

In the case of ABS:

a) Based on hematological parameters ABS is negatively correlated with RBC, Hb, and Ht (%).

b) Based on biochemical parameters, ABS is positively correlated with creatinine, AST, and ALT, but negatively correlated with protein and albumin.

c) Based on stress parameters in gills ABS is positively correlated with CAT, SOD, GPx, and MDA ($p < 0.05$).

d) Based on stress parameters in the liver, the concentration of ABS is positively correlated with CAT, SOD, and MDA ($p < 0.05$) but negatively correlated with GPx and AChE.

e) Based on acetylcholinesterase (AChE) activity in gills and the liver, ABS is negatively correlated with AChE.

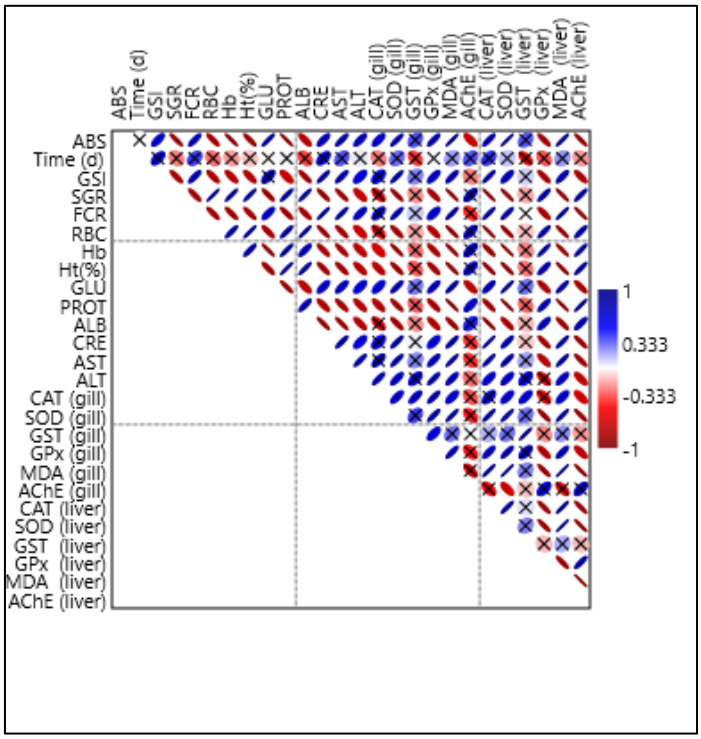


Fig. 8: Correlation matrix plot on stress parameters of *O. mossambicus* after exposure to ABS. p<0.05 are boxed

To compute the overall stress of ABS on *O. mossambicus*, the IBR index was applied. Greater IBR values denote adverse circumstances for the organisms, while low IBR scores reflect favorable environmental conditions for the organisms. According to the finding of the study, T2-30d is by far the most affected group in the case of surfactant exposed fish (Fig. 9).

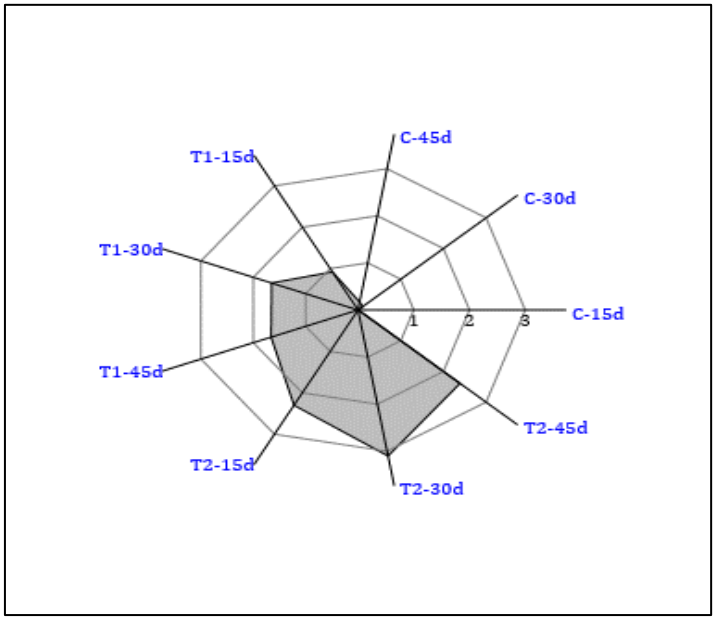


Fig 9: **Dose and duration dependent IBR values**

BRI values representing the overall general health status of the fish are shown in Fig. 10. It is observed that the BRI values of ABS, exposed fish are within the range of 0-2.5.

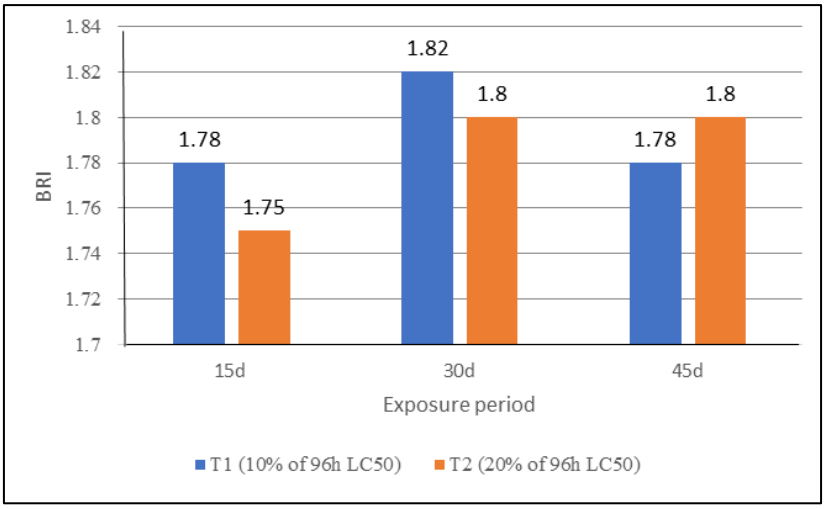


Fig 10: BRI values of ABS exposed fish at different exposure periods. T1 indicates 10% of 96h LC50 of ABS (0.006 mg/l) and T2 indicates 20% of 96h LC50 of ABS (0.012 mg/l).

4. DISCUSSION

The 24, 48, 72 and 96 h LC_{50} values of ABS to *O. mossambicus* are 0.55mg/l, 0.28 mg/l, 0.09 mg/l and 0.06 mg/l based on Finney's probit analysis and 0.3776, 0.189, 0.1261, and 0.9464 mg/l as per GUTS-IT model. However, as indicated by the LC50 values, based on EPA guidelines (Chatterjee *et al.*, 2021b), it is highly toxic to fish, *Oreochromic mossambicus*. Nonetheless, its toxicity may change with species size and quality, as well as with shifts in the water's physiochemical characteristics (Chatterjee *et al.*, 2021a). Moreover, irregular swimming, loss of balance, increased surface grasping, progressive motionlessness, and increased opercular movement were observed in ABS-treated fish after 96 hours of exposure. Similar behavioural changes were reported in *Cyprinus carpio* after exposure to the type-II pyrethroid pesticide alpha-cypermethrin (Bej *et al.*, 2021), and in *Oreochromis mossambicus* after exposure to mancozeb (Saha *et al.*, 2016).

The growth parameter serves as a designator of populations' life conditions that could be utilized to detect stress due to contamination (López Siangas *et al.*, 2012). The sublethal exposure of fish to 10% and 20% of LC_{50} of ABS (0.006 and 0.012 mg/l) showed a significant reduction ($p<0.05$) in SGR and a significant increase ($p<0.05$) in GSI and FCR in comparison to control. A decrease in the SGR of fish occurs due to disruption of the metabolic processes in the fish's body (Kim *et al.*, 2018). The increase in the value of the FCR is probably due to the toxic effect of toxicants in the fish's body, which interfere with the function of respiration and inhibit the metabolic activity of the fish's body so that the process of digestion of food is disrupted (Padmanabha *et al.*, 2015; Sunanda *et al.*, 2016). As a result, the decreased growth rate of *O. mossambicus* enlisted in our current study is most likely due to decreased appetite resulting in reduced feed intake or increased expenditure of energy in the presence of toxicant for continuing normal metabolic process, leaving less energy available for growth (Abdel-Tawwab *et al.*, 2013). Moreover, dose-depend decrease in SGR and increase in the FCR in our study might be related to altered stability or downregulation of GH (Guo *et al.*, 2021). Declines in growth parameters were reported in *O. mossambicus* upon exposure to phenol and aniline (Bhunia *et al.*, 2003; Saha *et al.*, 1999). The growth of *O. niloticus* was reported to be reduced when exposed to sublethal concentrations of cypermethrin (Majumder & Kaviraj, 2017) and abamectin (Mahmoud *et al.*, 2021).

Hematological indices are paramount biomarkers for evaluating physiological alterations in animals (Ogueji *et al.*, 2020). In our investigation a significant decline in RBC, Hb, and Ht values ($p < 0.05$) was observed in concentration and the duration-dependent manner in the fish exposed to concentrations of 10% and 20% of LC_{50} of ABS (0.006 and 0.012 mg/l) (Fig 3). This decrease in hematological parameters might be attributed to the surfactant's harmful effect on hematopoietic tissues. Lower levels of RBC and hemoglobin may also be attributed to erythrocyte destruction in blood- forming tissues, aberrant heme synthesis, increased free radical generation, and inadequate oxygen delivery by gills (Ghaffar *et al.*, 2021). The decline of Ht levels in the fish could also be attributed to the lysing of erythrocytes. Hence the reduction in Hb and Ht levels along with reduced RBC levels is an evident indication of anemia (Ololade & Oginni 2010). Moreover, the reduction in the levels of RBC, Hb, and Ht values might be attributable to the altered erythropoiesis activity (Ghaffar *et al.*, 2021). Consequential alterations in hematological parameters have also been reported in several fish species exposed to different toxicants like *Heteropneustes fossilis* exposed to nickel(II) oxide nanoparticles (Samim &

Vaseem 2021), *Ctenopharyngodon idella* exposed to copper, chromium, and lead (Shah *et al.,* 2020), *Osteobrama belangeri* (Valenciennes, 1844) exposed to unionized ammonia (Mangang & Pandey, 2021) and *O. mossambicus* exposed to arsenic (Tuteja *et al.,* 2021).

Plasma biochemical parameters are typically used to govern the general health status of an organism (Kavitha *et al.,* 2012). Among these parameters, glucose is the most sensitive index denoting the stress state of an organism (Serdar 2019). In our present investigation induction in glucose level upon the integration of ABS might be a designation of carbohydrate metabolism disruption, possibly due to the enhancement of glucose 6-phosphatase activity in the liver or incremented liver glycogen breakdown (Ajima *et al.,* 2015). Similar hyperglycemic responses have been reported in *O. mossambicus* after exposure to dimethoate and chlorpyrifos (Qayoom *et al.,* 2016). Total protein serves as a potential indicator of the immune system as well as liver and kidney dysfunction (Ghelichpour *et al.,* 2019). In our study, the consequential decline of total protein content upon the addition of ABS might have resulted from a decrementation in the rate of protein synthesis or incremented rate of amino acid degradation (Ramesh & Saravanan, 2008). Similar results related to reduced total protein content were reported after exposure of *O. mossambicus* to the pesticide chlorpyrifos (Ghayyur *et al.,* 2019). Albumin plays a crucial role in preserving the osmotic balance between the circulating blood and the tissue membrane (Khan *et al.,* 2016). In the present study, abbreviated albumin content upon the addition of ABS may be attributed to the inhibitory effect of toxicants on the biosynthesis of albumin in the liver, liver dysfunction, and malnutrition (Ujowundu *et al.,* 2016). Significant reductions in albumin concentration were documented in *O. mossambicus* upon exposure to the pesticides chlorpyrifos and monocrotophos (Narra *et al.,* 2017). Creatinine is utilized as a sensitive indicator of glomerular filtration rate and kidney functions (Hamed & El-Sayed, 2019). In our study incremented creatinine levels with the addition of surfactants might be due to kidney dysfunction by structural damage (Faheem *et al.,* 2019). A similar result was reported with textile dyeing effluent exposure in *O. mossambicus* (Joseph and John 2020).

An incrementation in blood enzyme levels categorically AST and ALT are considered to be stress designators leading to tissue impairment (Akbary *et al.,* 2018). In our study, the incrementation in the levels of ALT and AST upon the integration of the surfactants might be because of the relinquishment of these enzymes into the bloodstream leading to an alteration in liver function and subsequently causing liver damage (Banaee *et al.,* 2011; Vali *et al.,* 2020). Similar outcomes regarding the incrementation in AST and ALT levels were reported when *O. mossambicus* was exposed to novel organophosphorus insecticide (RPR-V) (Rao 2006).

CAT is an antioxidant enzyme mainly active in detoxifying ROS and degrading H_2O_2 to molecular oxygen and water (Ighodaro & Akinloye 2018). In our investigation, CAT activity in the exposed fish increased significantly ($p < 0.05$) at 10% and 20% of LC_{50} of ABS (0.006 and 0.012 mg/l) at all exposure periods (15d, 30d, and 45d) Increased CAT activity in the gills and liver of *O. mossambicus* after surfactant treatment in the current study is presumably a result of a neutralizing impact on the detrimental effect of the incrementing ROS generation caused by the toxicant (Kumari *et al.,* 2014a). Moreover, this induction in CAT probably results in the augmentation of nuclear Nrf2 expression which defends the cells from H_2O_2-induced alterations ultimately leading to the generation of oxidative stress (Ma, 2013).

Among the stress enzymes, SOD is a category of metalloenzymes that initially prevent the toxicity caused by ROS against injuries (Hansel & Diaz, 2021). These enzymes catalyze the dismutation of superoxide anion-free radical (O^2-) into molecular oxygen and hydrogen peroxide (H_2O_2) thereby damaging the cells (Bhattacharya *et al.,* 2021). In our study SOD activity increased significantly ($p < 0.05$) at the liver of fish exposed 10% and 20% of LC_{50} of ABS (0.006 and 0.012 mg/l) of ABS at all exposure periods (15d, 30d, and 45d). These surfactants induced ascent in SOD activity in the gills and liver of *O. mossambicus* might be due to the initiation of superoxide radical that shields the cell from oxidative stress (Kumari *et al.,* 2014b).

GST is a major bio-processing enzyme in phase II that is commonly considered to be a key player in the detoxification mechanism (Allocati *et al.,* 2018; Livingstone, 1998). GST activity in the liver increased significantly ($p < 0.05$) at 10% and 20% of LC_{50} of ABS (0.006 and 0.012 mg/l) during 15d and 30d exposure periods. The higher formation rate of glutathione disulfide (GSSG) probably contributes to this incremented activity of GST (Kaur 2017). However reduction in GST activity occurred during the 45d exposure period with respect to the 15d and 30d exposure period indicating the compromised detoxification process of the organism under long-term exposure (Sreejai & Jaya, 2010).

By catalyzing the conversion of hydrogen peroxide to water and oxygen, GPx reduces possible oxidative stress. More hydrogen peroxide, prompting tissue disintegration and oxidative stress, is accessible when GPx is obstructed (Kaur, 2017). The activity of GPx is associated with the concentrations of GSH. This is because it makes utilization of reduced glutathione to expel hydrogen peroxide and provokes the development of oxidized glutathione (Ogueji *et al.,* 2020). In our investigation, GPx activity increased significantly ($p < 0.05$) at all concentrations of all the surfactants 10% and 20% of LC_{50} of ABS (0.006 and 0.012 mg/l) during 15 and 30 d exposure periods but decreased significantly at 45d exposure period at all concentrations 10% and 20%

of LC_{50} of ABS (0.006 and 0.012 mg/l). This incremented GPx activity in the liver of *O. mossambicus* appeared to have a consequential role in protecting the cell against antioxidants (Xiong *et al.,* 2014). However, at the end of the exposure period, diminishing GPx activity may have been a reaction to the failure of the antioxidant defense system to prevent the induction of toxicant-induced ROS (Özok, 2020). This decrease in GPx may indicate that the toxicant-induced ROS developed through lipid peroxidation exceeded the antioxidant capacity (Serdar, 2019).

Lipid peroxidation (LPO) is a fundamental aspect of oxidative stress, which is predominantly generated by the oxidative breakdown of polyunsaturated lipids in cell and organelle membranes (Grotto *et al.,* 2009). Bi-product of LPO such as malondialdehyde, (MDA) is utilized as a designation for increased concentration of ROS and cellular injury (Ayala *et al.,* 2014; Grotto *et al.,* 2009). MDA activity increased significantly ($p < 0.05$) at all concentrations of surfactants 10% and 20% of LC_{50} of ABS (0.006 and 0.012 mg/l) during all exposure periods (15, 30, and 45 d). This increment in MDA levels alters the permeability of the cell membrane, causing toxicants to enter the cell ultimately leading to DNA damage and conclusively apoptosis (Ayala *et al.,* 2014).

IBR is commonly utilized as a designator of environmental stress to determine the toxicological implications of sundry xenobiotic compounds towards fish. It is withal an efficacious method in evaluating the organism's health status (Dey *et al.,* 2016; Li *et al.,* 2011). In this analysis, IBR results suggest that T2-30d is the most affected group, followed by T2-45d, T2-15d, T1-30d, T1-15d, T1-45d, C-45d, C-15d, and C-30d. Similar trends in alterations in duration- dependent IBR values were reported after cationic surfactants were applied to *Cyprinus carpio* ((Bhattacharya *et al.,* 2021a; Bhattacharya *et al.,* 2021). In integration BRI is used to assess the health status of fish (Hagger *et al.,* 2008; Magni *et al.,* 2017). The BRI values of ABS are 1.83 in case of T1-15d, 1.76 in case of T1-30d, 1.76 in case of T1-45d, 1.80 in case of T2-15d, 1.78 in case of T2-30d, and 1.82 in case of T2-45d. Thus the BRI values are within 0-2.5 which portrays paramount alterations from the normal (Hagger *et al.,* 2008). Similar directional changes in BRI values were reported after administration of pesticide lambda-cyhalothrin to *Cyprinus carpio* (Chatterjee *et al.,* 2021a). Thus, it is conspicuous from our finding that ABS impacts fish health adversely.

5. CONCLUSION

According to the results of our research, it is possible to draw the conclusion that ABS produces severe changes in survival as well as changes in behaviour at the acute level during short-term exposure and abatement of haematological, biochemical, and stress parameters at the sublethal level during long-term exposure in *O. mossambicus*. Consequently, the present findings on the toxicity of the ABS to *O. mossambicus* may be employed as a potential implement for increasing awareness among individuals to restrict the indiscriminate utilisation of surfactants. Hence, surfactants used in certain large-scale processes, should be properly disposed of and directed to a treatment facility, without depending on degradation in natural environmental systems following uncontrolled disposal. Furthermore, a significant focus should be placed on the development of biosurfactants or microbial surfactants, which are extremely biodegradable and have lower toxicity than chemical surfactants. However, additional research is necessary in order to determine their hazardous effect on fish on a molecular and ultrastructural level. Moreover, additional areas for exploration in future research include hormonal and histopathological studies.

ETHICAL APPROVAL

The test bioassay was directed according to the rules endorsed by Institutional Animal Ethics Committee.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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