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Impact of Sublethal Concentration of Detergents on Serum Biochemical Constituents of Freshwater Fish, *Mystus vittatus* (Bloch)

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ABSTRACT:
The 96 hours median lethal concentration (LC_{50}) of Tide detergent for *Mystus vittatus* by using probit analysis software (SPSS version 26) at 95% confidence limit was 17.981 mg/L. The current study has been carried out to investigate the impact of sublethal concentrations (4.5 mg/L or $1/4^{th}$ and 9.0 mg/L or $1/2^{th}$ of 96h LC_{50}) of detergent Tide on the serum biochemical constituents of *Mystus vittatus* after exposure to 7, 14, 21 and 28 days. The present study indicates a significant decrease in serum metabolites, including glucose and total protein, while triglyceride levels showed a significant increase. From the result of one way **ANOVA** analysis it was observed that the sublethal lethal concentrations were highly influenced the serum biochemical contents of fishes. The Post hoc test (Tukey) revealed that the serum glucose, serum total protein and serum triglyceride contents were altered with both sublethal concentrations of detergents and period of exposure. Thus, the results of this investigation provide an indication of how the fish *Mystus vittatus* can be used as a bioindicator for chemical pollution through alterations in biochemical parameters.

Keywords:
Tide, Detergent, Biochemical Metabolites, *Mystus vittatus*.

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INTRODUCTION

Detergents are a major source of pollution, quickly contaminating lakes, rivers, ponds, streams, and creeks. Detergents are the composition of organic compounds, collectively known as surfactants or surface-active agents that persist in the environment; however, their use remains essential because of their widespread applications as components in both domestic as well as industrial cleaners as soaps, shampoos, and washing powders. Modern detergents possess high cleaning efficiency, primarily due to the presence of surfactants, which reduce the surface tension of water (Singh and Pandey, 2023). Synthetic detergents were first introduced in the 1930s, but they did not gain widespread popularity until the 1950s and beyond, when large quantities began entering aquatic ecosystems. The use of detergents in households and industries can lead to contamination or pollution of aquatic environments, potentially causing serious environmental issues (Pickering, and Owen,

1994). Detergents are broadly classified into three principal chemical types, Anionic Detergents (e.g., alkylbenzenesulfonates), Cationic Detergents and Non-Ionic Detergents. These synthetic detergents are formulated using surface-active agents such as alkyl lauryl sulfonate, dodecylbenzene sulfonate, and alkylbenzene sulfonate. They also contain additional ingredients, including foam stabilizers, colorants, bleaching agents, protease enzymes, water softeners (carbonates, silicates, polyphosphates, and perborates), preservatives (sodium sulfate), and perfumes (Singh and Pandey, 2023). However, high concentrations of bleaching chemicals, cleaning agents or surfactants, and their constituents pose significant risks to aquatic life (Ankley and Burkhard, 1992). Only 15% of a detergent's synthetic composition is biodegradable, depending on the surfactants used. As a result, most detergents accumulate as pollutants into water bodies. Detergents and their byproducts pose a significant threat to aquatic organisms at concentrations ranging from 0.08 to 300 mg/L. The ill impacts of surface active and bleaching agents pose significant risks to aquatic organisms (Logeswari *et al*., 2021). The 'after wash' from detergents is either directly discharged into aquatic environments such as rivers, streams, lakes and ponds or enters these ecosystems through natural sewage systems (Ali *et al*., 2015). The reduction in air-water interaction, primarily due to water contamination by detergents, leads to oxygen deficiency for aquatic animals beneath the surface, ultimately causing the loss of aquatic flora and fauna (Arivizhivendhan *et al*., 2014). The presence of detergents in water exacerbates corrosion, hinders filtration, sedimentation, and coagulation processes, increases water oxygen saturation, and adversely affects the water's taste properties (Vasanthi *et al*., 2013). Furthermore, detergents harm fish by destroying their protective external layers, making them vulnerable to bacteria and parasites. They also damage the gill epithelium by altering tissue lipid composition and disrupting mucus production, which impairs breathing. Additionally, detergents affect peripheral nerve receptors, leading to changes in feeding and thermoregulatory behavior. In aquatic ecosystems, detergent pollution degrades water quality by reducing oxygen diffusion from the air into the water, causing oxygen intake failure in aquatic organisms. In the short term, detergent accumulation can impair fish vision and cause gill damage (Lal *et al*., 1983; Choudhary and Jha, 2013, Vasanthi *et al*., 2013, Arivizhivendhan, *et al*., 2014, Mousavi and Khodadoost, 2019, Ali, *et al*., 2015, Daniel *et al*., 2023). Considering the above points, the current investigation was conducted to determine the impacts of sublethal concentrations of detergent on the biochemical constituents of plasma of freshwater fish *Mystus vittatus* under laboratory conditions.

MATERIALS AND METHODS

The required number of test fish, *Mystus vittatus* (8.5±0.2 cm; 9.0±1g in weight) were collected from neighboring fresh waterbodies and bring to the Ichthyology Lab of Zoology department in plastic container. The collected fishes were dipped into 1.0 % solution of $KMnO_4$ for 5 minutes to confiscate dermal contamination. Fishes were stocked and acclimated to laboratory conditions for 15 days at 26 ± 1.5°C prior to experimentation. For the first week, the acclimatized fishes were given artificial air by aerator. All of the fish were given commercial food pellets on a regular basis, and the medium (tap water) was changed every two days to get rid of food remnants and faeces. Feeding was stopped 24 hours before the toxicity test.

Table 1: 96h Lethal Concentrations of Detergent Tide for *Mystus vittatus*

95% Confidence limits for different Lethal Concentrations values						
Lethal Value	**Estimated**	**Lower limit**	**Upper limit**	**Chi-Square**	**df**	**Sig.**
LC_{10} (mg/L)	3.761	1.184	6.277	5.290	6	0.507*
LC_{50} (mg/L)	17.981	13.870	22.390			
LC_{99} (mg/L)	85.965	54.445	238.309			
* Significance level is greater than .050, no heterogeneity factor is used in the calculation of confidence limits						

96 hr LC_{50} value was found to be 17.981 mg/L concentration of the Tide detergent for *Mystus vittatus* by probit analysis calculated by SPSS version 26. The Chi-Square test revealed a statistically significant difference (X2= 5.290, P=.507, Df 6). Further, the fish were exposed to chronic toxicity study. On the basis of 96 hours LC_{50} the fish, *Mystus vittatus* were exposed to sublethal concentrations (1/4th of 96 hours LC_{50} = 4.5 mg/L and 1/2th of 96 hours LC_{50} = 9.0 mg/L) for the period of 7, 14, 21 and 28 days. The chronic toxicity study was conducted to examine the effects of sub-lethal detergent concentrations on the serum biochemical parameters (glucose, protein, and triglyceride) of the fish *Mystus vittatus*. A control group was maintained under similar environmental conditions. The test fishes were fed with commercial food, and the water was exchanged daily to remove feces and food remnants. Blood samples were collected from the caudal vein and transferred into glass tubes, then centrifuged at 3500 rpm for 10 minutes. The resulting serum was carefully collected into Eppendorf tubes for further analysis. Serum nutrients like glucose, protein and triglycerides were analysed by ISO certified digital Glucometer, Lawery's method as Glutamate pyruvate described by David (1992) and Barnes and Blackstock (1973) methods, respectively.

RESULTS AND DISCUSSION

Any alterations in serum biochemical parameters reflect changes in the physiological state of fish. Although no mortality was found in current investigation but we observed some adverse physiological impacts on the detergent exposed fishes. Table 2 presents the quantitative estimation results of serum metabolites, including glucose, total protein, and triglycerides, in control and detergent-exposed *Mystus vittatus*.

The amount of glucose content in blood reduces with raising the concentration of detergent and exposure period. The serum glucose levels were significantly reduced in detergent-exposed fishes as compared to the control group (Table 2-5). This decrease in glucose levels (hypoglycemia) suggests its rapid consumption by the fish, likely as a response to metabolic toxic stress. Chemical stress increases opercular beating and surfacing activity in detergent-exposed fish, requiring higher glucose and oxygen levels to meet the rising energy demands. Similar reduction in serum glucose level has also been reported by Singh and Pandey (2023) in detergent exposed *Channa punctatus*.

Proteins, the fundamental building blocks of cells, play a crucial role in various biological processes and are greatly responsive to toxicants, making them one of the initial indicator of chemical stress. In the current investigation significant reduction in the total plasma proteins contents was found in detergent treated fish, *Mystus vittatus* as compared to control groups (Table 2-5). The serum protein content reduces with enhancing the concentration of detergent and exposure period. Proteins, the fundamental building blocks of cells, play a crucial role in various biological processes and are highly sensitive to toxicants, making them one of the earliest indicators of chemical stress. The interruption in protein content in the present study suggests that blood proteins undergo proteolysis, leading to the release of free amino acids. Decrease in the protein level may also indication of reduced rate in their synthesis. Variations in protein levels under stress condition may result from extensive proteolysis, disintegration, or reduced protein synthesis. During stress, proteins also serve as an energy source in the tricarboxylic acid cycle. Additionally, fluctuations in serum protein content may be attributed to the increased synthesis of the sequestering enzyme metallothionein. Due to their relatively low carbohydrate reserves, fish utilize protein as an alternative energy source to meet elevated energy demands under stress (Naveed *et al*., 2010). The protein level reduction observed in the present investigation align with the findings of other researchers (Prakash and Verma, 2020; Singh and Pandey, 2023; Singh et al., 2023).

Table 2: Effects of Sublethal concentrations of detergent on Some Serum Biochemical Parameters of *Mystus vittatus* (N=5) (Mean±SD)

Biochemical Parameters	Concentration (mg/L)	Exposure Period (Co-efficient of variance)			
		7 Days	14 Days	21 Days	28 Days
Serum Glucose (mg/dl)	Control	1.034±0.0114 (0.0110)	1.050±0.0100 (0.0100)	1.034±0.0114 (0.0110)	1.032±0.0084 (0.0081)
	4.5 mg/L	0.934±0.0114 (0.0122)	0.836±0.0114 (0.0136)	0.722±0.0192 (0.0266)	0.672±0.0192 (0.0286)
	9.0 mg/L	0.810±0.0187 (0.0187)	0.720±0.158 (0.0220)	0.636±0.0182 (0.0286)	0.580±0.158 (0.0273)
Total Protein (mg/dl)	Control	3.060±0.0100 (0.0033)	3.050±0.0082 (0.0027)	3.046±0.0114 (0.0037)	3.042±0.0084 (0.0028)
	4.5 mg/L	2.920±0.0158 (0.0054)	2.492±0.0164 (0.0066)	2.358±0.0192 (0.0082)	2.278±0.2315 (0.1102)
	9.0 mg/L	2.094±0.0195 (0.0093)	1.824±0.0114 (0.0063)	1.700±0.0158 (0.0093)	1.616±0.0114 (0.0071)
Triglyceride (mg/dl)	Control	80.084±0.590 (0.0007)	80.288±0.4818 (0.0060)	80.044±0.2070 (0.0003)	80.054±0.0623 (0.0008)
	4.5 mg/L	88.148±0.022 (0.0003)	93.956±0.688 (0.0073)	98.39±0.523 (0.0053)	101.970±0.4266 (0.0042)
	9.0 mg/L	92.984±0.453 (0.0049)	99.590±0.244 (0.0025)	105.208±0.530 (0.0050)	111.068±0.6995 (0.0063)

Table 3: Serum Biochemical Profile in detergent exposed fishes, *Mystus vittatus*

Biochemical Parameters	Concentration (mg/L)	Minimum (mg/dl)	Maximum (mg/dl)	Mean	SD	SE
Serum Glucose (mg/dl)	Control	1.034	1.050	1.03800	0.008000	0.004000
	4.5 mg/L	0.672	0.934	0.79100	0.117468	0.058734
	9.0 mg/L	0.584	0.810	0.68750	0.099040	0.049520
Total Protein (mg/dl)	Control	3.042	3.060	3.04950	0.007724	0.003862
	4.5 mg/L	2.278	2.920	2.51200	0.285970	0.142985
	9.0 mg/L	1.616	2.094	1.81300	0.209269	0.104634
Triglyceride (mg/dl)	Control	80.044	80.288	80.11750	0.114930	0.057465
	4.5 mg/L	88.148	101.970	95.61600	5.960845	2.980422
	9.0 mg/L	92.984	111.068	102.21250	7.733814	3.866907

Triglycerides serve as the primary energy reserve in fish, and their serum levels are commonly used to assess an organism's metabolic status. In the present investigation, detergent-exposed *Mystus vittatus* exhibited a significant increase in serum triglyceride levels compared to the control groups (Table 1-4), leading to a hypertriglyceridemic condition. The amount of triglyceride content in blood increases with an increase in concentration of detergent and period of exposure. Hadi *et al*. (2009) stated that a hypertriglyceridemic condition in fish under stress may result from liver dysfunction. Similarly, Srivastava and Prakash (2018) noted that the degeneration of liver cells can lead to the release of various lipolytic enzymes into the bloodstream, which convert triglycerides into fatty acids and glycerol. This disruption leaves triglycerides unprocessed, and ultimately, the reduced rate of lipolysis leads to elevated serum triglyceride levels.

Table 4: One way ANOVA for testing impact on Serum Biochemical Profile of detergent exposed fishes, *Mystus vittatus*

Biochemical Parameters	Source	df	Sum of Squares	Mean sum of Squares	F	P
Serum Glucose	Between Groups	0.259	2	0.130	16.439	0.001
	Within Groups	0.071	9	0.008		
	Total	0.330	11			
Total Protein	Between Groups	3.075	2	1.538	36.717	0.000
	Within Groups	0.377	9	0.042		
	Total	3.452	11			
Triglyceride	Between Groups	1029.208	2	514.604	16.190	0.001
	Within Groups	286.070	9	31.786		
	Total	1315.279	11			

When the experimental data was subjected to statistical analysis (Table 2-4), the values show significant difference and some values did not show relative significant difference and this may mean that during chronic toxicity detergent exposed fish have been might be adapt in moderate polluted water. The data was tested statistically the result revealed that the One way analysis of variance showed a significant alterations among the serum glucose, total protein and triglyceride contents of various experimental group fishes (F=16.439; P=0.001 for glucose; F=36.717; P=0.000 for protein and F=16.190; P=0.001 for triglyceride) Table 3. The Post hoc test (Tukey) revealed that the serum glucose, total protein and triglyceride contents in fishes were significantly differed (P<0.05) Table 4. The Tukey test revealed that the serum glucose, total protein and triglyceride content were altered by sublethal concentrations of detergent (P <0.05). From the result of one way ANOVA analysis it was observed that the sublethal lethal concentrations were highly influenced the serum biochemical contents of fishes (Table-3). The Post hoc test (Tukey) revealed that the serum glucose, serum total protein and serum triglyceride contents were altered with both sublethal concentrations of detergents and period of exposure (Table-4). Many researchers reported a similar trend of alterations in serum biochemical parameters (Prakash and Verma, 2020; Singh and Pandey, 2023). The marked alterations in the serum biochemical parameters of the detergent treated fishes as compared to the control fishes confirms the earlier report of Singh and Pandey (2023) who reported blood parameters alterations in fish *Channa punctatus* exposed to household detergent Tide. The decrease in serum biochemical constituents resulted from the destruction of fish cells by the surfactant and trace amounts of heavy metals present in detergents (Idowu *et al*., 2017).

Table 5: Post hoc test (Tukey) for individual effect of sublethal concentration of detergent in the freshwater fish *Mystus vittatus*

Biochemical Parameters	Concentration (a)	Concentratio (b)	Mean Difference (a-b)	Std. Error (SE)	Sig. (P)
Serum Glucose	Control	6 mg/L	-15.498500*	3.986577	0.009
		12 mg/L	-22.095000*	3.986577	0.001
	4.5 mg/L	Control	15.498500*	3.986577	0.009
		12 mg/L	-6.596500^{NS}	3.986577	0.274
	9.0 mg/L	Control	22.095000*	3.986577	0.001

		6 mg/L	6.596500 NS	3.986577	0.274
Total Protein	Control	6 mg/L	0.537500*	0.144702	0.012
		12 mg/L	1.236500*	0.144702	0.000
	4.5 mg/L	Control	-0.537500*	0.144702	0.012
		12 mg/L	0.699000*	0.144702	0.002
	9.0 mg/L	Control	-1.236500*	0.144702	0.000
		6 mg/L	-0.699000*	0.144702	0.002
Triglyceride	Control	6 mg/L	-15.498500*	3.986577	0.009
		12 mg/L	-22.095000*	3.986577	0.001
	4.5 mg/L	Control	15.498500*	3.986577	0.009
		12 mg/L	-6.596500NS	3.986577	0.274
	9.0 mg/L	Control	22.095000*	3.986577	0.001
		6 mg/L	6.596500NS	3.986577	0.274
*. The mean difference is significant at the 0.05 level. NS = Non-Significant.					

CONCLUSIONS

Experimental studies on the toxicity of detergent to fishes suggest that some synthetic household detergents are more toxic to fishes than the biological harder detergents. Blood is the important indicators of changes in the internal and/or external environment of the fish. The present investigation is to understand the effect of Tide, detergent on serum biochemical parameters such as glucose, protein, and triglyceride of the freshwater fish, *Mystus vittatus*. A decreasing trend in glucose and protein levels, along with an increase in blood triglyceride content, was observed with rising detergent concentrations and prolonged exposure, this adjustment likely serves to meet the heightened energy demands during stress conditions. In fish, exposure to chemical pollutants induces either increase or decrease in the some biochemical parameters. In view of the chronic toxicity impact of tide detergent, it can be inferred; indiscriminate discharge of detergent effluents would affect the survival of freshwater fish fauna and ultimately leads to death. The results presented in this investigation highlight the harmful effects of detergents in aquatic environments, posing serious health risks to fish and threatening their survival. Thus, the alterations in the serum biochemical parameters are good indicators of changes in the water quality.

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