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ALTERATION IN BIOCHEMICAL AND HAEMATOLOGICAL PARAMETERS ON EXPOSURE TO HOUSEHOLD DETERGENT (TIDE) OF THE ASIAN SNAKEHEAD FISH *CHANNA PUNCTATA*

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Abstract: Using a 96-hour static bioassay, authors looked at the effects of household Tide detergent on the blood parameters and energy metabolism of Asian snakehead fish (*Channa punctata*). The median 96-hour lethal dose (LC_{50}) of HHD was 60.0 mg/L. The control group received a non-detergent-treated standard meal. RBC count and haemoglobin considerably decreased; however, WBC count level noticeably increased at sublethal of dose 12.00 mg/L. There was a substantial up regulation of total protein. Organcholesterol and triglycerides rose, but total lipids decreased. Overall, the results showed that fish's biochemical and hematological parameters were significantly altered after exposure to detergent.

Keywords: Acute toxicity, Biochemical parameters, *Channa punctata*, Detergent, Fish.

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INTRODUCTION

Detergents are derived from organic compounds that endure in the environment; however, their use is unavoidable due to their application as components of household cleaners and pesticides as well as the scattering of oil pollution in the ocean (Isyaku and Solomon, 2016). Because of their components, such as surfactant, which decreases the surface tensions of water, currently available detergents have a high cleaning capacity. These detergents are synthesized by using surfactant substances, including alkyl lauryl sulfonate (LAS), dodecylbenzene sulfonate (DBS), and alkylbenzene sulfonate (ABS) (Abdel-Shafy *et al.*, 1988; Texter, 2001), and other ingredients such as foam stabilizers, colourants, bleaching agents, pigments, enzymes (proteases), water softeners (carbonates, silicates, poly-phosphates, and perborates), preservatives (sodium sulphate), and perfumes (Hauthal, 2016; Landeck *et al.*, 2020). The exact composition of commercial products is proprietary. However, 15% surfactant, 30% poly-phosphate, and 10% silicate are the relative amounts of the ingredients, along with 20% sodium perborate and 20% sodium sulphate, and the rest are fluorescent pigments and enzymes.

Higher concentrations of cleaning agents and their constituents are harmful to aquatic life. Ingredients such as surfactants and bleaching chemicals are hazardous at elevated concentrations (Ankley and Burkhard, 1992). Deleterious consequences resulting from surfactants were observed during investigations with micro-algae (Pavlić *et al.*, 2005; Aizdaicher and Markina, 2006); flatworms Turbellaria (Li, 2008), *Planorbella pilsbryi* snails (Prosser *et al.,* 2017), micro-crustaceans (Hodges *et al.*, 2006; Kizek *et al.*, 2017), and fish (Abel, 1974; Verma *et al.,* 1978; Lal *et al.,* 1983; Mousavi and Khodadoost, 2019). The metabolism of cells is affected by surfactants (Argese *et al.*, 1994). These influence the permeability of cell membranes at low doses, affecting the means by which aquatic organisms' respiratory systems behave. According to several studies (Hofer *et al.*, 1995; Nunes *et al.*, 2008), the metabolism of surfactants by aquatic creatures might result in highly reactive oxygen species (ROS) and bring organisms under oxidative stress.

The Asian snakehead fish (*Channa punctata*) is a prominent member of freshwater habitats in the Indian subcontinent. It is an air-breathing, predatory fish. Asian snakehead fish (ASHF) is an ideal test model for toxicology research due to a number of ecotoxicological attributes, including ease of acclimation to laboratory conditions, affordability around the year, and economic relevance (Sharma and Chadha, 2017; Rüber *et al.*, 2020). Blood indicators are preliminary warning signs of pathophysiological alterations throughout the entire body. Those are brought on by toxicant exposure because they show up before any visible symptoms of contamination. As a result, they serve as an effective tool for assessing the well-being of fish. Significant markers for alterations in the fish's internal and external context are blood cell reactions. So, according to Liebel *et al.* (2013), modifications to the hematological parameters are reliable markers of changes in the water's character. Fish ingest detergent through their gills, from where it then travels to the liver for biotransformation and metabolization. The liver subsequently transformed detergent and its constituents, particularly the surfactant, into hydrophilic molecules, which aid in excretion. Reactive oxygen species (ROS), often referred to as oxidases, are produced as a result of oxidative stress events and attach to biomolecules to cause oxidative damage (Wibbertmann *et al.*, 2011). As previously indicated, numerous HDDs and surfactants cause oxidative stress, resulting in detrimental effects on the cellular structure of tissue, membrane permeability, and damage to macromolecules in living organisms. When the capacity of fish like organisms' antioxidant protective mechanisms to neutralize these oxidative stresses were lacking, that is accomplished by certain enzymes like catalase (CAT), superoxide dismutase (SOD), and MDA, cellular metabolism and its control get impacted (Shukla and Trivedi, 2018). RBC, WBC, Hg, total lipids, and cholesterol are significant biochemical indicators that are frequently used in stressful situations to evaluate the well-being state of fish. Fish physiology, including biochemical markers and metabolic enzymes, is advantageous for assessing the condition of water in aquatic habitats because of the intimate connection between the circulatory arrangement of fish and their surroundings (He *et al.*, 2015).

The primary objective of the present investigation, which investigated the ways in which HHD contaminants affected ASHF, turned out to be to determine the mean lethal concentration (LC_{50}) of household detergent (Tide) and assess the effects of medium-term exposure on biochemical indicators for alterations in biochemical, serological and haematological developments.

MATERIALS AND METHODS

In the current investigation, authors used normal/common household detergent (Tide) . The following assumed starting HHD

concentrations (mg/L) were employed to describe the treatments for the acute toxicity tests: 0, 30, 50, 70, and 100. A total of 100 mature adult ASH fishes (AASHFs) were kept in stagnant dechlorinated water (50 L) that was continually oxygenated for a week to acclimatize the fish. The specimens had average weights of 28.0–32.0 g and lengths of 13.5–15.5 cm. Every two days, their water supply was replaced to get rid of excrement and leftover food. Every day, a dry traditional seafood meal containing 40% crude protein was provided to the AASHFs; however, it was stopped 24 hours prior to the investigation's commencement. Dead fish were promptly eliminated from the experiment. The fish were allocated into five testing sets, with one group serving as a control after seven days. Each set of 20 AASHFs was shifted distinctly to a 50-L aquarium and subjected to different HHD (Tide) concentrations-30.0 mg/L, 50.0 mg/L, 70.0 g/L, and 100.0 g/L-as well as the control group that received no HHD. Each set received three replicas. Additionally, AASHFs were exposed to doses of 12.0 mg/L, 6.0 mg/L, and 4.0 mg/L for a short period of time (7 to 21 days). These numbers were fractions ($1/5^{th}$, $1/10^{th}$, and $1/15^{th}$, respectively) of the HHD's 96-hour LC_{50} value, which was found to be 60.00 mg/L.

AASHFs were gently taken out at random from each set. A sample of blood was retrieved from the caudal vein, which is downward to the spinal column, and divided into two halves using uncontaminated, one-milliliter polypropylene syringes: For the purpose of measuring the complete blood count (CBC), the first fraction was transferred to an EDTA anticoagulation vial. The remaining half of the blood sample was placed into Eppendorf vessels, which do not contain an anticoagulant, and centrifuged for 10 minutes at 3000 rpm. The serum was subsequently separated in a different tube for further biochemistry examinations. Red blood cell count (RBCs), white blood cell count (WBCs), and colorimetric micro-method haemoglobin (Hb) were measured for every specimen employing the well-established methods described by Fuller (1974), Medway *et al.* (1969), Turk's fluid dilution method (Moroff *et al.*, 1994), and acid haemoglobin method (Mgbenka *et al.*, 2003), respectively.

The organs that were examined were instantly taken out, sterilized in a frozen 1.15% KCl buffer, immediately drenched in Whatmann filter paper, and swiftly measured in an 8% ice-cold single-pan balance. The organ tissue was incorporated with TCA and homogenized for about two minutes at medium speed using a homogenizer and Teflon pestle. The homogenized tissue was subsequently spun at 3000 rpm for 30 minutes. The resulting liquid was often stored in a freezer after that step was carried out twice to ensure that extraction was comprehensive. The resulting substance was blended with ether, chloroform, and ethanol before being centrifuged at 3000 rpm. The remaining substance was then dissolved in a 4% NaOH solution for protein quantification after the supernatant was eliminated. Using Folin's phenol reagent, the protein compositions of the organs were determined using the Lowry *et al.* (1951) approach. According to Zlatkis *et al.* (1953), the approach used for the quantification of cholesterol was based on a reagent utilizing ferric chloride, glacial acetic acid, and concentrated sulfuric acid. With the aforementioned reagents, cholesterol becomes purple. The final colour complex's strength as determined by UV-vis spectroscopy at 560 nm (Agilent, Cary 60 UV-Vis Spectrophotometer). The Anthrone approach was used to determine the amount of glycogen in the dried tissue (Carroll *et al.*, 1956). Employing a chloroform-methanol combination as the solvent, the total quantity of lipids contained in the sample (muscle) was determined.

RESULTS

Haematological parameters

The blood's Hb concentration began increasing throughout the course of the first

four days. AASHF exposed to 12.0 mg/L of house hold detergent showed a minimum Hb value of 8.75±1.15 g/dL, which was significantly lower (p≥0.05) than the control group's 13.15±1.12 g/dL (Table 1, Fig. 1c). Additionally, the RBC count significantly decreased (p≥0.05) with concentration progression, with the minimum value being 2.60±0.08 cells/mm^3 at 12.0 mg/L of HHD, contrary to 3.16±0.16 cell/mm^3 for the control (Table 1, Fig. 1a). The WBC count showed a statistically considerable rise (p≥0.05) with increasing HHD concentrations. On the other hand, this amount decreases after 96 hours of sub-lethal HHD exposure, while the RBC number elevates in parallel. WBC count values ranged from 5.15±0.75×10^3 per mm in the control AASHF to 5.55±0.75×10^3 per mm in the AASHF exposed to 12.0 mg/L of house hold detergent (HHD) (Table 1, Fig. 1b).

Table 1: Total RBC count, Hemoglobin percentage and total leukocytes count in blood of AASHFs at a sublethal concentration of HHD (12.0 mg/L) and control experiments at various exposure periods.

Exposure period (days)	4	7	14	21
Total RBC count				
Control	3.19±0.16	3.18±0.18	3.18±0.23	3.16±0.16
Sub-lethal dose	3.23±0.15	3.94±0.12	2.98±0.10	2.60±0.08
Hemoglobin percentage				
Control	13.19±1.16	13.18±0.16	13.18±0.16	13.15±1.12
Sub-lethal dose	14.75±0.85	12.75±1.12	10.05±0.81	8.75±1.15
Total leukocytes count				
Control	5.25±0.72	5.33±0.55	5.18±0.41	5.15±0.75
Sub-lethal dose	4.95±0.45	5.20±0.70	5.35±0.75	5.55±0.75

Table 2: Total serum protein, total serum cholesterol, total serum triglyceride and total serum lipids in blood of AASHFs at a sublethal concentration of HHD (12.0 mg/L) and control experiments at various exposure periods.

Exposure period (days)	4	7	14	21
Total serum protein				
Control	3.19±0.16	3.18±0.18	3.18±0.23	3.16±0.16
Sub-lethal dose	3.23±0.15	3.94±0.12	2.98±0.10	2.60±0.08
Total serum cholesterol				
Control	13.19±1.16	13.18±0.16	13.18±0.16	13.15±1.12
Sub-lethal dose	14.75±0.85	12.75±1.12	10.05±0.81	8.75±1.15
Total serum triglyceride				
Control	5.25±0.72	5.33±0.55	5.18±0.41	5.15±0.75
Sub-lethal dose	4.95±0.45	5.20±0.70	5.35±0.75	5.55±0.75
Control	32.62±2.15	31.51±2.20	31.35±2.28	32.12±2.24
Sub-lethal dose	36.12±2.25	32.12±2.30	28.12±2.21	25.12±2.56
Total serum lipids				
Control	129.34±5.22	129.95±5.05	130.05±5.12	130.34±5.55
Sub-lethal dose	132.65±4.95	127.45±4.75	120.35±4.15	115.27±4.34

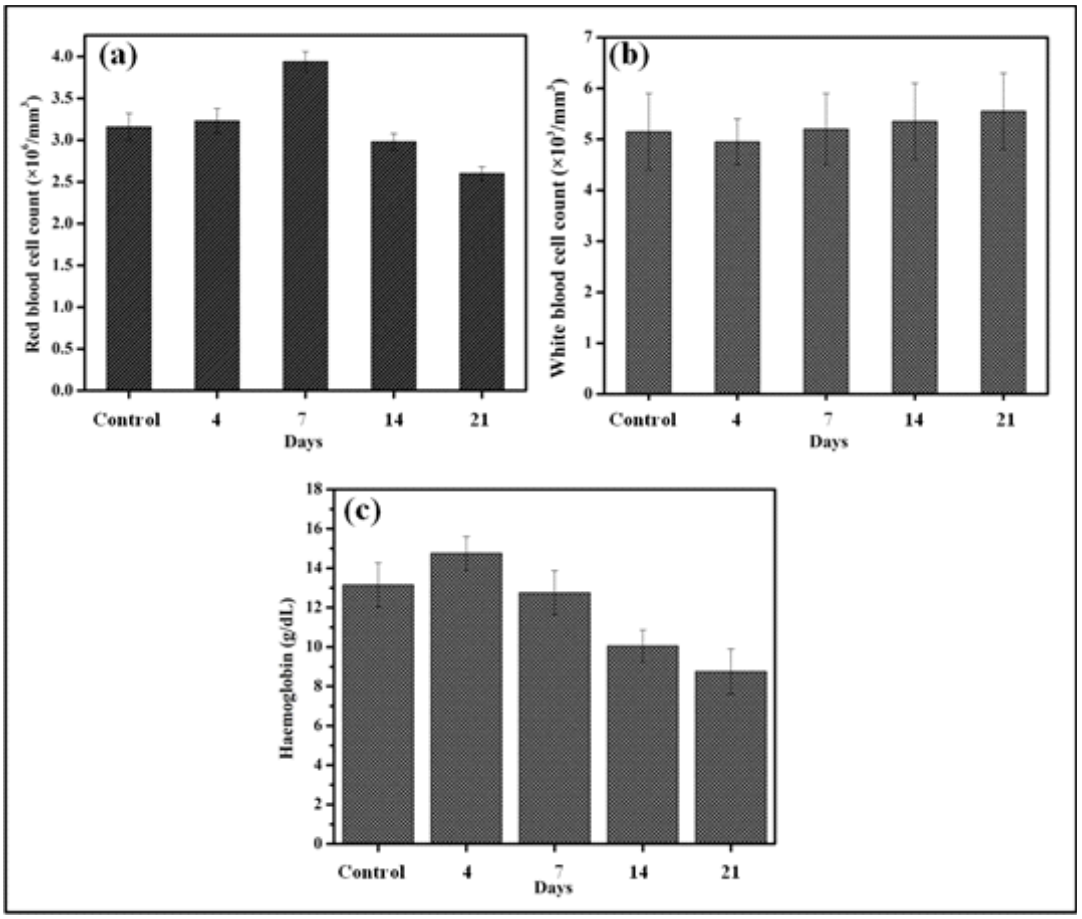

Fig. 1: Haematological parameters of Asian Snakehead Fish exposed to household detergent Tide to sublethal dose 12.0 mg/L (a) Red blood cell count, (b) White blood cell count (c) Hemoglobin level.

Biochemical biomarkers

AASHF subjects exposed to 12.0 mg/L of HHD had significantly different serum total protein levels ($p \geq 0.05$). Compared to control AASHF, which had a value of 6.72±0.50 mg/dL, it had a level of 5.80±0.75 mg/dL (Table 2, Fig. 2c). In the HHD-subjected AASHF, the value of total blood lipids significantly decreased ($p \geq 0.05$); the maximum value, 132.65±4.95 mg/dL, was found at 12.0 mg/L of HHD after 4 days, and the lowest level was 115.27±4.34 after 21 days (Table 2, Fig. 2d). On the contrary, the control measurement indicated that the value was 130.34±5.55 mg/dL. In the serum of HHD-treated AASHF, cholesterol levels increased significantly ($p \geq 0.05$) with increasing dose and exposure duration. After 21 days of contact, the highest value (72.68±3.41 mg/dL) was found at 12.0 mg/L of HHD, and after 4 days, 58.12±2.35 mg/dL was obtained. Meanwhile, the control trial had an outcome of 52.68±2.45mg/dL (Table 2, Fig. 2a). The serum of HHD-exposed AASHF exhibited substantial decreases in triglyceride content ($p \geq 0.05$). After 4 days of contact, the maximum result, 36.12±2.25 mg/dL, was determined at 12.0 mg/L of HHD. After 21 days, the lowest measurement of 25.12±2.56mg/ dL was recorded. The level was found to be 32.12±2.24 in the control fish experiment (Table 2, Fig. 2b).

DISCUSSION

When contaminants are introduced, fish swimming and movement swiftly slow down (Chandanshive *et al.*, 2007). Earlier, similar results were also observed. Along with these observations, the acute LC_{50} for household detergents such as Surf, Besto, and Key detergents, respectively, for weed fish was around 12, 77, and 32 mg/L (Palanichamg, 1991). LC_{50} for Omo and Ariel, respectively, were from 33.03 to 35.19 ppm and 37.43 to 39.79 ppm. Fingerlings of *Heterobranchus longifilis* and *Clarias gariepinus* were employed in the experiments (Ndome *et al.,* 2013). According to

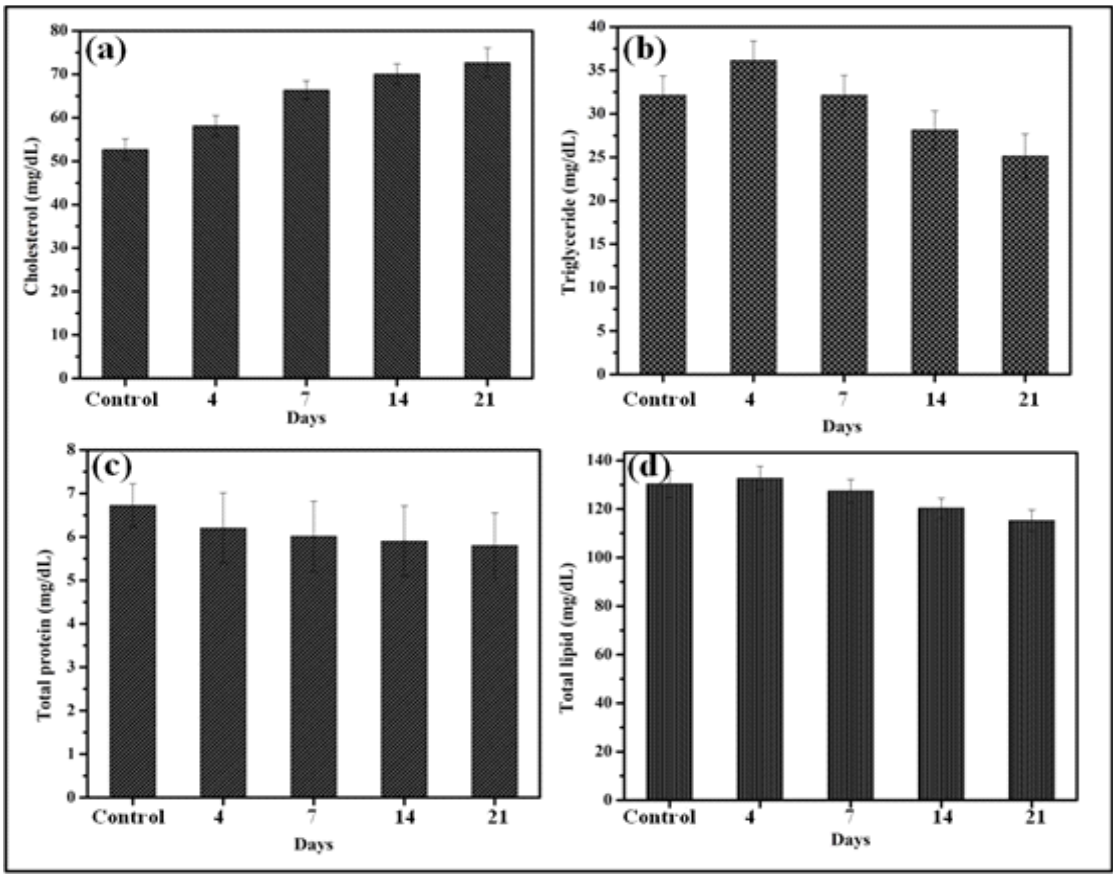

Fig. 2: Biochemical parameters of Asian Snakehead Fish exposed to household detergent Tide to sub lethal dose 12.0 mg/L (a) total serum cholesterol (b) total serum triglyceride (c) total serum protein (d) total serum lipid.

Kumar *et al.* (2007), the LC_{50} concentrations of the surfactants LAS were 0.48, 0.28, 0.18, and 0.03 mL/L for the various exposure times (24 h, 48 h, 72 h, and 96 h). According to Eknath (2013), the 96-hour LC_{50} for two sample HHDs exposed to *Mystus montanus* was 120 mg/L and 23.5 mg/L for two different detergents, respectively.

Studies of hematological aspects, such as haemoglobin, RBCs, and WBCs, are frequently utilized in ecological toxicology investigations since they represent fish well-being and physiological changes that are a reflection of the state of the surrounding water (Zaghloul *et al.*, 2007).

The continuous exposure of AASHF to contaminants that both prevent erythrocyte formation or harm and cause haemorrhage in the gills may be responsible for the observed drop in Hb and RBC levels in the current investigation. Decreased Hb concentration in the cellular media as a result of the cytotoxic effects of contaminants on the hematological tissue that cause the generation of ROSs might occur in hypoxic conditions, which can also lead to the disintegration of developed RBCs. Fish experience anaemia as a result of ROS damaging the erythrocyte's cell membrane and impairing its functioning (Kavitha and Venkateswara Rao, 2007; Narra *et al.*, 2017). The decreased RBC count and haemoglobin level observed in the current investigation are consistent with previous studies conducted on *Labeo rohita* to observe the impact of domicile sewage and industrial effluents (Zutshi *et al.*, 2010), acute and chronic sublethal lindane exposure on aquatic teleost fish *Cyprinus carpio* (Saravanan *et al.*, 2011), glyphosate-based herbicide on common carp *Cyprinus carpio* (Kondera *et al.*, 2018). Additionally, Osman *et al.* (2018) observed significant decreases in fish Hb, RBCs, and Ht following exposure to various contaminants in both outdoor and lab settings.

The fish immune system heavily relies on WBCs. Variations in the quantity of WBCs are regarded as normal reactions under stress or when exposed to toxins (Narra *et al.*, 2017). The present investigation having wide rise in WBC count, which climbed linearly after four days with an increase in HHD amounts, made it clear that the introduction of HHD stimulates the AASHF's defence mechanism in order to combat the stress of toxicants. The higher WBCs in the present research are consistent with those found in studies on Chanchita *Cichlasoma dimerus* (Vázquez and Nostro, 2014), *Clarias* cat fish exposed to doses of Ronstar (Oluah *et al.*, 2018), and *Labeo rohita*, which were subjected to sublethal and methyl orange dye solution (Alaguprathana and Poonkothai, 2021).

Proteins, the fundamental units of cells, are essential for many biological processes. Extensive proteolysis, disintegration, or low protein formation while under stress could all be explanations for protein level variations. In the tricarboxylic acid (TCA) cycle, proteins are also employed to produce energy when under stress. Enhanced synthesis of the sequestering enzyme metallothionein may be the cause of variations in plasma protein content. Fish uses protein as an alternate source of energy since it contains fewer carbohydrates, which helps it meet its higher energy demands under stress (Naveed *et al.*, 2010). The variation in the amount of protein in the present research is consistent with findings from Prakash and Verma (2019). They demonstrated that fish that were exposed to detergents had variation in protein amounts, as well as studies on *Oreochromis niloticus* subjected to copper sulphate (Mutlu *et al.*, 2015), *C. gariepinus* and *O. niloticus* exposed to textile dye solution (Alaguprathana and Poonkothai, 2021), *Cyprinus carpio* exposed to textile industry effluent (Roopadevi and Somashekar, 2012). Similarly, the fishes showed harmful effects when exposed to arsenic (Prakash and Verma, 2020), which is also harmful to humans (Prakash and Verma, 2021).

The fluctuation in cholesterol, triglyceride, and total serum lipids in the current study indicates an alteration in fat metabolism that could be caused by one or more of the following factors: percolation of cholesterol and other lipid elements because of damage to cell membrane, extended liver excretion of cholesterol, raised creation by the liver and other tissues due to the impact of environmental contaminants, and thyroid dysfunction that prevents the transformation of triglycerides to fatty acids. In addition to harm to the liver that prevents the conversion of cholesterol into bile acid, fish exposed to pollution have higher blood levels of cholesterol and triglycerides. The hypoactivity of the enzyme lipoprotein lipase, which breaks down triglycerides in blood vessels, may be the cause of the increase in plasma triglycerides (Metwally, 2009; Hamdy *et al.*, 2018). The aforementioned current study's fluctuation in serum lipid, cholesterol, and triglyceride content is consistent with studies conducted on freshwater fish *Tor putitora* collected from polluted portion of River Kabul (Yousafzai and Shakoori, 2011), Nile tilapia *Oreochromis niloticus* and African Catfish *Clarias gariepinus* exposed to River Nile (Osman *et al.*, 2018), and exposed to copper sulphate (Mutlu *et al.*, 2015), pesticides, heavy metals, and other pollutants (Fırat *et al.*, 2011; Alm-Eldeen *et al.*, 2018).

CONCLUSION

The results of the current study showed that AASHF, *Channa punctata*, being subjected to sub-lethal detergent doses results in a variety of toxicological consequences, including enzymatic degradation, biochemical characteristic alteration, and histological abnormalities of the serum. The results presented here demonstrate that the presence of detergents in aquatic locations has detrimental effects on fish health, putting species at risk for medical conditions and jeopardizing their lives.

STATEMENTS AND DECLARATIONS

The authors have no competing interests to declare that are relevant to the content of this article.

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