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PATHOLOGIC LESIONS AND ANTIOXIDANT RESPONSE IN THE LIVER OF AFRICAN CATFISH *CLARIAS GARIEPINUS* (BURCHELL, 1822) JUVENILE EXPOSED TO COMMERCIAL-GRADE DETERGENT

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ABSTRACT

This study was conducted to ascertain the toxicity of commercial-grade detergent (Viva) on fish antioxidant response and histopathological alterations using *Clarias gariepinus* juvenile. The Fish specimens were exposed to acute concentrations (0.0, 0.44, 48, 0.52, 0.56 and 0.60 mg/l) and sub-lethal concentrations (0.0, 0.0289, 0.0385 and 0.0577 mg/L) of the detergent (Viva) for 96hour lethal concentration (LC_{50}= 0.577 mg/l) and 21 days. Fish specimens were sampled for antioxidant response and histopathological lesions in the liver tissues at the end of the acute and sublethal. A significant increase in the Catalase (CAT), Melandealdehyde (MDA), and Superoxide dismutase (SOD) activities were observed during the 96 hours of exposure when compared with the control with an increase in concentration. While the sub-lethal exposure showed a significant decrease in the liver enzyme activities of CAT and SOD and an increase in MDA across the treatment groups. The liver histopathological alterations of the fish revealed normal hepatocytes with a central round nucleus in the control group to hepatic necrosis, vacuolation, hepatocytes degeneration, and congestion of sinusoid across the treatment groups. This study indicated that the alterations in liver antioxidant response and histopathological alterations were induced by the detergent, suggesting that these parameters can thus be used for ecotoxicological risk assessment of commercial-grade detergents on aquatic organisms.

Keywords: Detergent, Toxicity, Antioxidant, Histopathology and *Clarias Gariepinus*

INTRODUCTION

Water pollution is one of the most pressing issues facing the world in the twenty-first century, resulting from the introduction of numerous contaminants into water systems by a number of methods that alter water's natural characteristics (Mustafa *et al*., 2017). Depending on the dosage, nature, and of the toxin's, toxicity can be acute or (Ayofe *et al*., 2020). It is acute when the consequences of exposure to a relatively significant quantity of toxin provided frequently in a single dosage appear quickly (Essien-Ibok *et al*., 2019). Chronic toxicity occurs when an organism is exposed to low amounts of toxin on a regular or irregular basis over a long time, typically years. It is characterized by long-term consequences that can be lethargic or non-lethal (Essien-Ibok *et al*., 2019).

The contamination of the environment with detergent has become of concern, not only because of the threats that such pollutants provide to aquatic but also because of the public health implications. In Nigeria, Viva detergent is one of the most extensively used synthetic surfactants in cleaning goods (Isyaku and Solomon, 2016). Water pollution is one of the most pressing issues facing the world in the twenty-first century, resulting from the introduction of numerous contaminants into water systems by a number of methods that alter water's natural characteristics **(**Enemuadia and Emmanuel, 2017) and fishes bioaccumulate the contaminant leading to pathological changes and physiological dysfunctions. Exposure concentrations may not be lethal for the affected species, but they may affect internal functions and structures, which could affect the effectiveness of vital functions and processes such as competitive stress and environmental resistance, reproduction, growth, and so on under chronic exposure conditions (Ali *et al*., 2020).

Histopathological alterations have long been employed as indicators in the assessment of the health of contaminated fish and it gives a quick way of detecting the impacts of irritants in diverse tissues and organs (Abalaka, 2015; Ali *et al*., 2020). The gill, kidney, and liver in fish are responsible for important functions like respiration, excretion, and appropriate indicators to monitor xenobiotic bioaccumulation and biotransformation. One of the major benefits of utilizing histopathological biomarkers in environmental monitoring is that they allow for the examination of target organs (Sogbanmu *et al.,* 2019; Alimba *et al.*, 2019). Furthermore, pathological changes in fish tissues and organs are typically easier to detect than functional changes (Akinwande *et al*.,2016), and can act as early warning indications of animal health impairment (Islam *et al.*, 2018).

Moreso, antioxidant enzymes are critical biomarkers in determining the level of toxicity in organisms. To protect against xenobiotics, organisms produce Reactive Oxygen Species (ROS), which react with biological macromolecules, causing tissue damage, lipid peroxidation, DNA damage, as well as cell death (Isibor,2017; Jaqueline and Leonardo, 2018). Most xenobiotics, including detergents, cause toxicity by stimulating the free radical generation, inducing lipid peroxidation, and disrupting the body's total antioxidant capability (Akinwande *et al*., 2016; Ejeha *et al*., 2019). As a result, antioxidant defences may be useful biomarkers for detergent, whereas increased lipid peroxidation, which occurs

as a result of oxidative degradation of membrane lipids, is commonly referred to as a measure of oxidative stress. Exposure to detergents also causes a variety of adaptive and deteriorating reactions in fish (Enemuadia and Emmanuel, 2017).

Antioxidant enzymes are also found in the biological system to protect tissue from oxidative damage when animals are subjected to dietary oxidative stress, they respond by inducing endogenous antioxidants to compensate (Islam *et al*., 2018). In recent decades, the contamination of aquatic ecosystems by detergents has gotten a lot of attention (Enemuadia and Emmanuel, 2017; Ayofe *et al*., 2020). Acute and chronic exposure to these chemicals, as well as their bioaccumulation, can cause tissue damage that poses a major putative effect not only for the organisms exposed but also for organisms such as humans during consumption (Ndome *et al*., 2013). Therefore, it appears necessary to research the negative effects of such detergents pollutants to develop strategies for protecting aquatic organisms and invariably public health.

The study of the fish liver is crucial in the field of fisheries Science, which has been impacted by a variety of issues such as aquatic pollution (Siti *et al*., 2017). The liver of a fish is thought to be primary storage, biotransformation, and excretion site for toxicants. The histopathological impact of Viva detergent on fish tissues is unknown due to paucity of experimental results. Among the most widely used biomarkers, those related to oxidative stress are being given priority in both environmental monitoring and laboratory studies (Jaqueline and Leonardo, 2018).

Clarias gariepinus, also known as African catfish, is a popular freshwater fish that is well distributed in water bodies in Nigeria and West Africa. The fish is tough, having a high tolerance for handling stress (Isibor, 2017). Low dissolved oxygen in water has a high adaption rate in *C. gariepinus*, especially in fish over 14 days old with functionally developed accessory respiratory organs (Abalaka, 2015). It can withstand drought for a long time, but it cannot survive in water temperatures below 9-10°C for a long time (Abalaka, 2015). Moreover, Catfish are important bio-indicators of contamination because of their widespread distribution, ability to respond to environmental pollution and role as a human food supply (Ayofe *et al*., 2020). As a result, the current study was conducted to analyse the effect of commercial-grade detergent (Viva) on African catfish (*Clarias gariepinus*) liver histology as well as the effect on antioxidant enzyme biomarkers such as Superoxide dismutase (SOD), Melandealdehyde (MDA), and Catalase (CAT) activities.

MATERIALS AND METHODS

Fish rearing and management

C. gariepinus of both sexes were obtained from a commercial fish seed farm in Zaria, Kaduna State. They weighed 10.01 ± 0.32 g (mean ± S.D.) and their length was in the range of 7–10.6 cm. The healthy juvenile of *C. gariepinus* were acclimatized to the laboratory conditions for 14 days in glass aquaria filled with dechlorinated water before the start of the toxicity bioassay. Water quality characteristics were determined using APHA (1995). The mean values for test water qualities were as follows: temperature 27.35 ± 0.06ºC, pH 8.23 ± 0.05, dissolved oxygen 2.02 ± 0.12 mg/l, electrical conductivity 1788.58 ±

100.25 s/m. The fish were fed twice daily with (45 % of crude protein) and were maintained on a 12:12 light/dark photoperiod.

Chemicals

The commercial grade "Viva plus" detergent powder used in this study is a product of Aspira Nigeria limited. It was purchased from a commercial store in Zaria, Nigeria.

Ethical Consideration

This experimental research was approved by the Ahmadu Bello University Ethical Committee on Animal Use and Care (ABUCAUC/2021/074) at Ahmadu Bello University, Faculty of Life Sciences, Biology Department, and consistent with its guidelines.

Experimental design and bioassay

Juveniles of *Clarias gariepinus* were randomly selected into the experimental groups for the 96 hours acute toxicity test concentrations (Control, 0.44, 0.48, 0.52, 0.56, and 0.60 mg/l). Each tank contains healthy 10 juveniles of *C. gariepinus* with a replicate. Fish were kept in 20 litres of de-chlorinated water. The method of APHA (1995) was used to determine the 96 h LC_{50} concentration after the pilot studies test. The 96-h LC_{50} for Viva detergent in *C. gariepinus* was found to be 0.577 mg/l. Four experimental group (control, 0.0289, 0.0385 and 0.0577 mg/l) concentrations were used for the sub-lethal 21 days bioassay. These concentrations were derived from the $1/5^{th}$, $1/10^{th}$ $1/15^{th}$ fraction of the LC_{50} to have a lower lethal concentration.

Tissue samples preparations

Analysis of antioxidant enzymes

The Liver of sacrificed fishes from the various treatments of the acute and sublethal concentrations were rapidly removed, cleaned from any extraneous materials, and immediately perfused with saline. The tissues were homogenized in phosphate buffer (0.1 M pH 7.4), and the homogenate was filtered and centrifuged at 1 500 rpm for 20 min. The supernatant was stored in a freezer until analysis Superoxide dismutase (SOD) activity was measured as described by McCord and Frodovich (1969). Superoxide radicals were formed when xanthine interacted with 2-[4-iodophenyl]-3- [4-nitrophenyl]-5-phenyltetrazolium chloride to generate a red-coloured formazan. The presence of SOD quickly associated with superoxide radicals, thereby limiting the availability of superoxide radicals and, as a result, blocking the synthesis of formazan, was utilized to measure the SOD activity in the tissues. At 505 nm, the estimated inhibition % of SOD formazan production was determined spectrophotometrically. Lipid peroxide was measured in tissues according to the method of Satoh, (1978) that based on the ability of thiobarbituric acid (TBA) to react with malondialdehyde (MDA) in an acidic medium at a temperature of 95°C for 30 min to form TBA reactive product the absorbance of the resultant pink product can be measured at 534 nm. Tissue CAT activities were measured according to Aebi *et al*., (1984). CAT activity was measured using the Beers and Sizer (1952) technique. A spectrophotometer set to 240 nm was used to reduce hydrogen peroxide (H_2O_2) in 1.0 Ml quartz cuvettes with a light path of 1.0 cm. CAT levels were measured in nanomoles of H_2O_2 consumed per minute per milligram of protein.

Histological analysis

Collected liver samples were immediately preserved for 48 hours in sample bottles containing 10% formalin and properly labelled against the various organs and treatments. The samples were then taken to Ahmadu Bello University Teaching Hospital, Pathology Unit, for histological analysis. The tissue samples were histologically examined using the Haematoxylin and Eosin method (Mamaduo *et al*., 2014). D.P.X (Dibutylphthalate polystyrene xylene) was used to mount the sections with a coverslip. Microphotographs were taken at a magnification of X120, and the segments were studied microscopically.

Statistical analysis

The obtained data were statistically analysed ANOVA test using Statistical Package for the Social Science (SPSS) Statistical software version 23 and data were reported as mean ± SEM. Duncan Multiple Range Test was used to further separate the significance difference across the means. Photomicrophraphs taken were presented in figures.

RESULTS

The acute and sublethal studies demonstrated that the physicochemical parameters observed when *C. gariepinus* were exposed to varying concentrations of the commercial-grade detergent (Viva)showed deviation from the detergent-free group. The temperature showed uniformity among the treated and control groups (). A significant decrease across the treatment groups when compared with the control was observed in dissolved oxygen. The electrical conductivity of the water in the various treatments showed decreased across the treatment groups while the pH value increased among the treatment group in the acute toxicity study. In the sub-lethal studies, across the treatment groups, a non-significant decrease was observed in the temperature while dissolved oxygen decreases significantly. The electrical conductivity and pH showed a significant increase across treatment groups in the sub-lethal study. Figure 1 & 2 showed that CAT, SOD and MDA levels were significantly increased in the liver of the fish treated with Viva detergent across the treatments for the acute study. The sub-lethal exposure showed a significant decrease across the treatment groups in CAT and SOD while MDA level (LPO) increased significantly in comparison the control. Activity of superoxide dismutase (SOD) exhibited a significant reduction ($p \leq 0.05$) by increasing the concentration, Also, a significant decline ($p \leq 0.05$) in catalase (CAT) activity was shown with the concentration gradient ion of the detergent.

The acute and sublethal studies showed that the physicochemical parameters observed when C. gariepinus were exposed to varying concentrations of the commercial-grade detergent (Viva) differ significant. The physicochemical parameters recorded are shown in Table 1 and 2. The temperature showed an increase within the treatment groups in the acute studies Table 1 and a uniform decrease within the treatment groups in the sublethal studies as shown in Table 2. A significant decrease across the treatment groups when compared with the control was observed in dissolved oxygen.
The electrical conductivity of the water in the various treatments showed decreased across the treatment groups while the pH value increased among the treatment group in the acute toxicity study. In the sub-lethal studies, across the treatment groups, a non-significant decrease was observed in the temperature while dissolved oxygen decreases significantly. The electrical conductivity and pH showed a significant

increase across treatment groups in the sub-lethal study.

Table 2 showed that CAT, SOD, and MDA levels (LPO Statistically significantly different with an increased level of antioxidant enzymes in the liver, of the fish treated with Viva detergent across the treatments for the acute study. The sub-lethal exposure showed a significant decrease across the treatment groups in CAT and SOD while MDA level (LPO) increased significantly in comparison to the control.

Table 1: Acute Effect of Viva Detergent on Physicochemical Parameters (96hours Exposure)

Conc. (mg/l)	**DO (mg/l)**	**EC (s/m)**	**Temp (°C)**	**pH**
0	3.05 ± 0.05^{a}	1921.0 ± 33.0^{a}	25.0 ± 0.00^{b}	6.90 ± 0.02^{c}
0.44	2.35 ± 0.00^{b}	1886.5 ± 1.50^{a}	25.0 ± 0.00^{b}	8.61 ± 0.08^{a}
0.48	$1.83\pm0.13b^{c}$	1846.5 ± 142.5^{a}	26.85 ± 1.85^{ab}	8.59 ± 0.09^{b}
0.52	$1.83\pm0.13b^{c}$	1739.0 ± 166.0^{a}	28.90 ± 0.20^{a}	8.48 ± 0.01^{ab}
0.56	1.68 ± 0.23^{c}	1716.0±209.0a	29.10 ± 0.1^{a}	8.39 ± 0.0^{3b}
0.6	1.40 ± 0.15^{c}	1622.5 ± 49.5^{a}	29.25 ± 0.1^{a}	8.38 ± 0.12^{ab}

Mean with different superscripts along the same column are significantly different at $p<0.05$

Note: Concentration (Conc.), dissolve oxygen (DO), electrical conductivity (EC), temperature (Temp) and power of hydrogen (pH)

Table 2: Sub-lethal Effect of Viva Detergent on Physicochemical Parameters.

Conc. (mg/l)	**DO (mg/l)**	**EC (S/m)**	**Temp (℃)**	**pH**
0	3.1 ± 0.1a	1303 ± 0.5^{b}	25.3 ± 0.0^{a}	7.89 ± 0.1^{d}
0.0289	1.8 ± 0.05b	1271 ± 0.5^{b}	25.3 ± 0.0^{a}	8.36 ± 0.02^{c}
0.0385	1.4 ± 0.05b	1316 ± 0.5b	25.25 ± 0.0^{a}	8.56 ± 0.03^{b}
0.0577	1.25 ± 0.00b	1767 ± 140a	25.05 ± 0.0^{a}	8.67 ± 0.1^{a}

Mean with different superscripts along the same column are significantly different at $p< 0.05$

Note: Concentration (Conc.), dissolve oxygen (DO), electrical conductivity (EC), temperature (Temp) and power of hydrogen (pH)

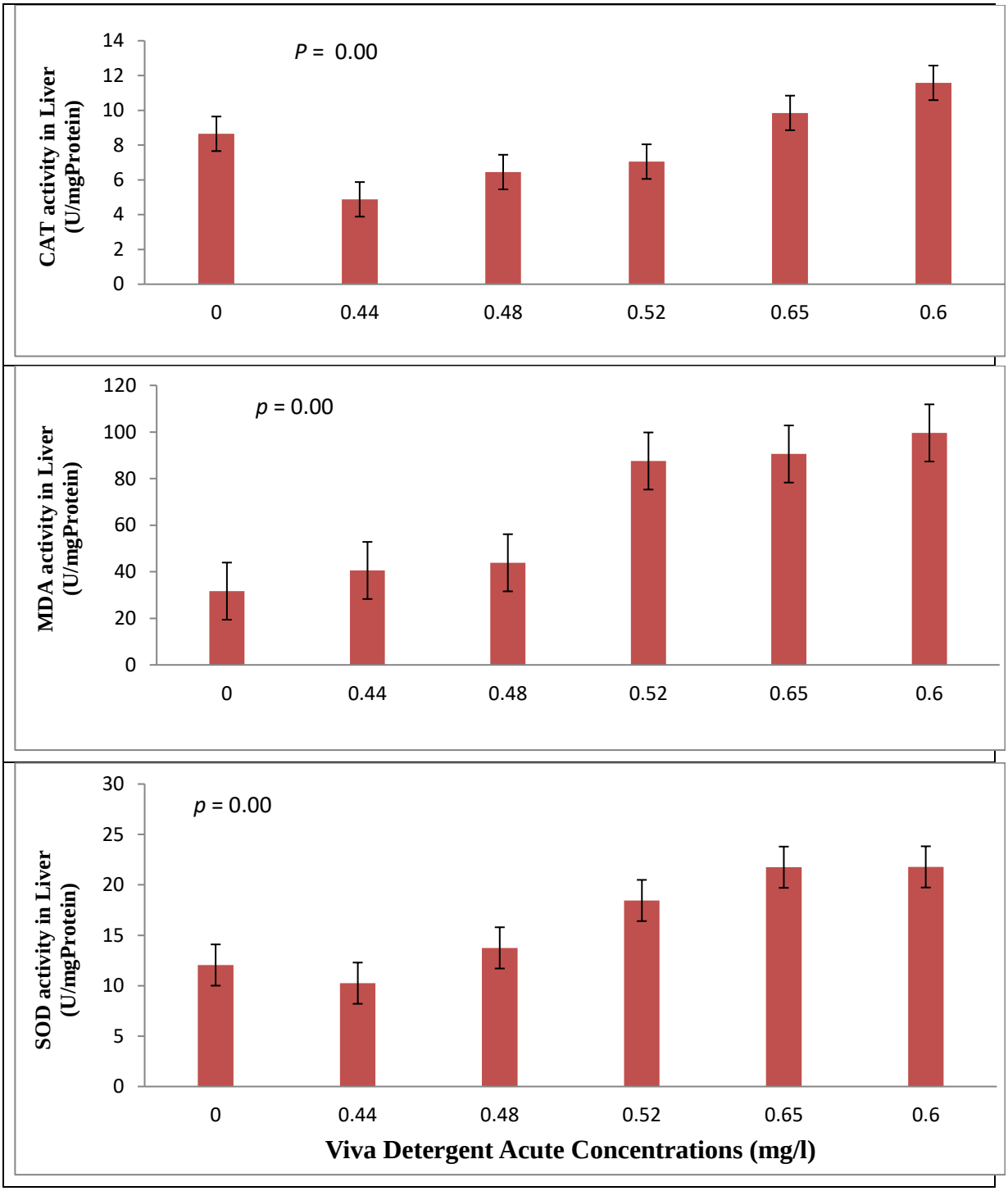


Figure 1: Antioxidant parameters of *Clarias gariepinus* exposed to Acute concentrations of Viva detergent

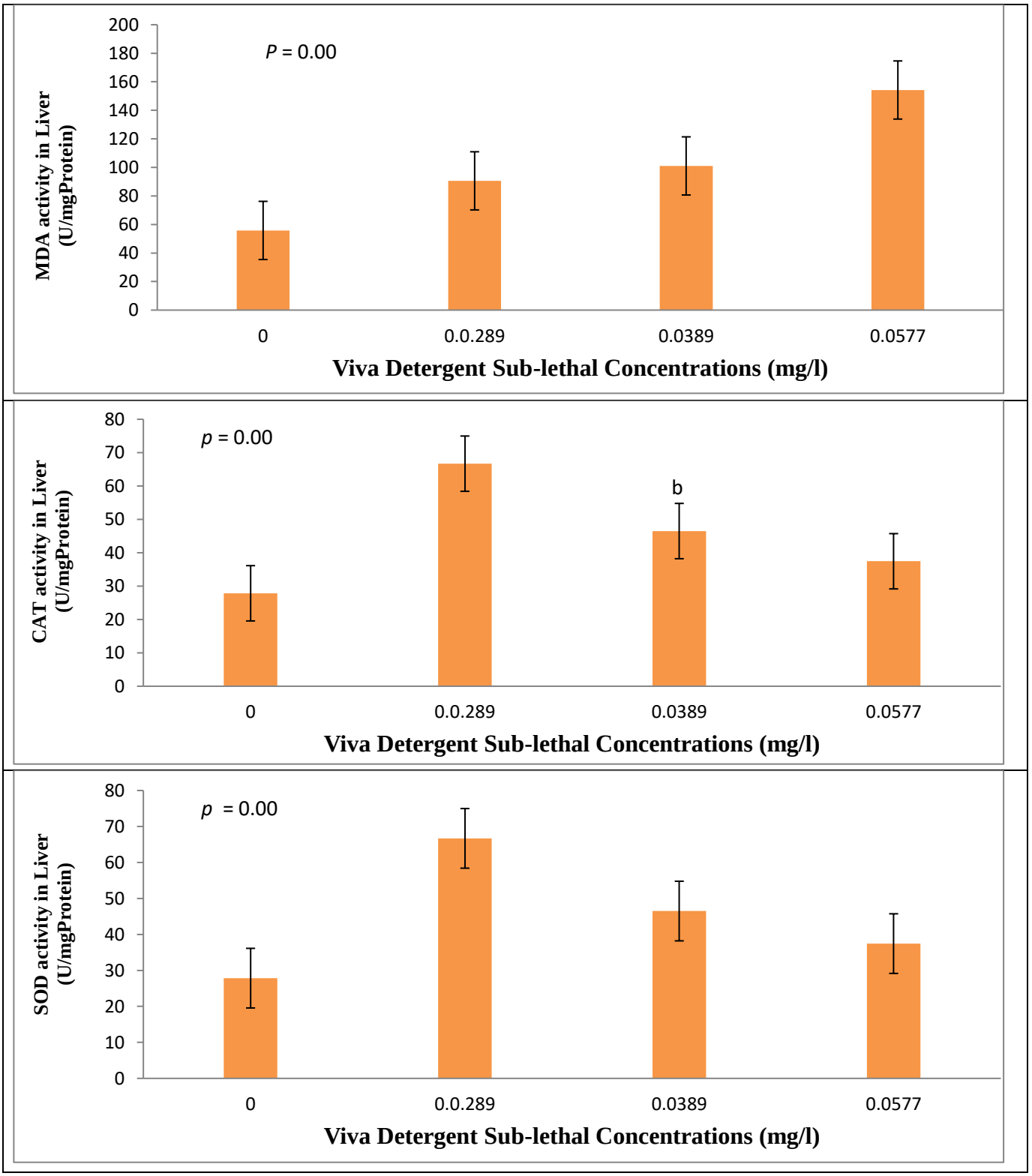


Figure 2: Antioxidant parameters of *Clarias gariepinus* exposed to Sub-lethal concentrations of Viva detergent

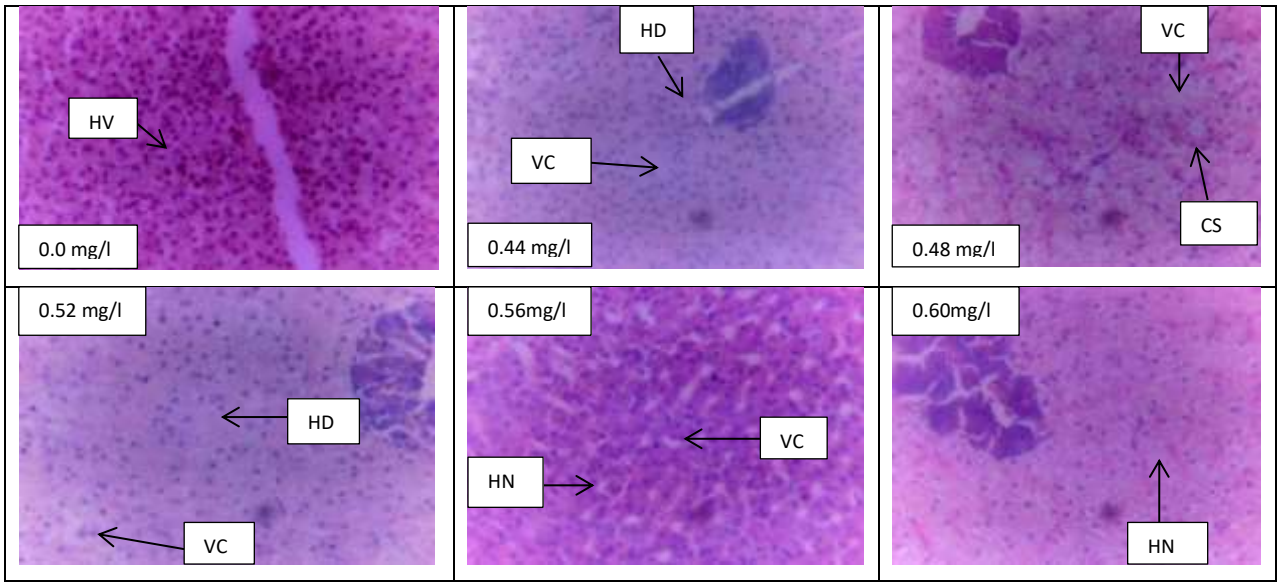


Figure 3: Photomicrograph of liver tissue of fish exposed to acute concentrations of Viva detergent (mg x 100). **Key: NH-** normal hepatocytes with central round nucleus, HN-hepatic necrosis, VC- Vacuolation, HD- hepatocytes degeneration, HV- hepatic vacuolation, CS- Congestion of Sinusoid

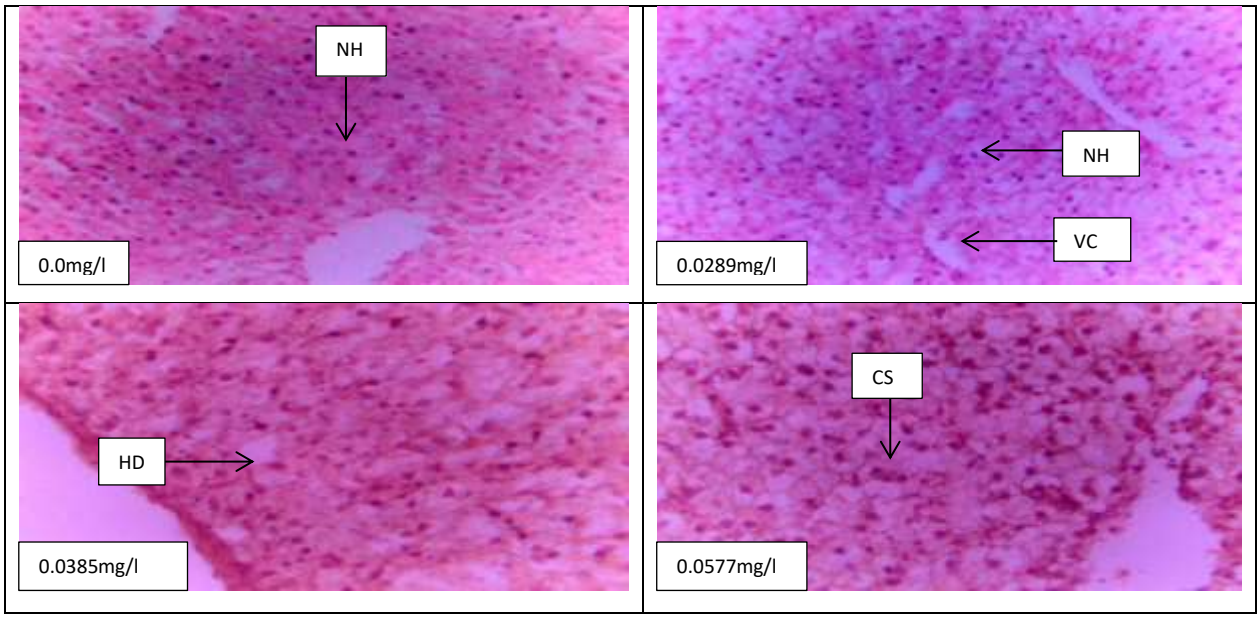


Figure 4: Photomicrograph of liver tissue of fish exposed to sub-lethal concentrations of Viva detergent (mg x 100). **Key: NH-** normal hepatocytes with central round nucleus, HN-hepatic necrosis, VC- Vacuolation, HD- hepatocytes degeneration, HV- hepatic vacuolation, CS- Congestion of Sinusoid.

DISCUSSION

Liver in fish is the place for metabolism, which has an important role in the detoxification of pollutants from the whole body (Sonam and Chaube, 2019). The histopathological alterations observed in liver of *C. gariepinus* exposed to Viva detergent included congestion of blood vessels and sinus and degeneration of hepatocytes and necrosis vacuolation, except in the control groups, indicating the toxic effect of the detergent. The severity of these injuries increased with increasing concentrations of the Viva detergent in both the acute and sublethal exposure. In the liver of fish exposed to Viva detergent, vacuolization of hepatocyte necrosis was observed. It seems that the formation of vacuoles in hepatocytes is a defensive action in the face of the detergent and to prevent the release of the toxicant (Chaube, 2019).

Abnormal accumulation of triglycerides and other fats may lead to the creation of vacuoles in hepatocytes and ultimately necrosis is associated with tissue damage (Justin and Joseph, 2017; Edori *et al*., 2019). Total damage observed in the liver led to inhibition of the release of toxins in the body of fishes (Mustafa et al., 2017). Liver tissue damage in fish exposed to detergent and other toxicants has been reported by several authors (Dong et al., 2017; Mohebbi *et al*., 2017; Enemuadia and Emmanuel 2017; Siti et al., 2017 Justin and Baby, 2017 and Ejeha *et al* 2019). Similar changes in the liver on some commonly culture species in Niger Delta exposed to detergent have been reported by (Enemuadia and Emmanuel 2017). Abalaka, (2017) reported on the effect of pond water pollution on the gills and liver of *Oreochromis mossambicus* Naeemi *et al*., (2013) also reported on the effect of liver alterations when *Caspian kutum* was treated with Linear Alkybenxene Sulfonte.

According to Ali *et al*., (2011), oxidative stress generated by toxicants in aquatic organisms can result in the generation of reactive oxygen species (ROS) and changes in antioxidant enzymes. The inducement of reactive oxygen species (ROS) can attack neighbouring molecules, causing damage to their chemical structure and function, as well as dysfunction in a variety of organs and systems (Lukaszewicz-Hussain, 2010). The cellular antioxidant systems can reduce the generation of reactive oxygen species (ROS) and their harmful effects (Akinwande *et al.,* 2016). Antioxidant enzymes are used by organisms under stress to adapt to environmental stress, and their activity varies depending on the dose, species, and route of exposure (Heba, 2016). SOD and CAT enzymes play a key role in removing ROS created during xenobiotic bioactivation, and the stimulation of the SOD/CAT system may be the first line of defense against ROS (Nwani *et al*., 2010). Furthermore, SOD and CAT are very sensitive and respond quickly, allowing organisms to avoid oxidative stress (Heba, 2016). In mammals, SOD is the first enzyme to respond to oxidative stress throughout any stressful situation (Winston and Di Giulio, 1991; Isibor, 2017). The increase in SOD and CAT levels in the liver of *C. gariepinus* treated with Viva detergent suggests a detoxification mechanism to counteract the toxicity. A similar discovery was made in Bryconcephalus tissues treated with MP (Monteiro et al., 2009; Modesto and Martinez, 2010). Sharbidre *et al*., (2011), recorded those changes in antioxidant enzyme activity in *Poecilia reticulata*

subjected to sublethal doses of MP and chlorpyrifos show an adaptive response to ROS. The greatest levels of SOD and CAT activity in the livers of *C. gariepinus* suggest that both enzymes are vital in combating the formation of superoxide radicals (O_2) and hydrogen peroxide (H_2O_2) caused by the high metabolic activity of the liver (Isibor 2017). SOD, MDA, and CAT levels in *Catla* increased after exposure to methyl parathion, according to Abhijith *et al*., (2016).

As mentioned in the current study, the produced ROS on exposure may have caused damage to the liver, culminating in the leakage of these enzymes into plasma.CAT is responsible for the detoxification of H_2O_2 produced by SOD activity, the gradual rise in catalase (CAT) activity in the liver seen in the current study could be an adaptive response to H_2O_2 created by SOD activity (Nwani *et al.*, 2010; Islam *et al*., 2018; Ejeh *et al.,* 2019). The change in SOD and CAT activity during the exposure period in the current investigation could have been due to the organ's various response patterns, such as sensitivity, accumulation, and detoxification processes. The current study's findings suggest that the fish uses enzymatic methods to cope with the impacts of produced ROS caused by *C. gariepinus* during Viva detergent exposure build-up.

CONCLUSION

Histopathological observation in the study indicates that exposure to acute and sub-lethal concentrations of detergent (Viva) caused deteriorative effects in the liver tissues of *C. gariepinus*. The severity of histopathological changes depends on toxicant dose and time of exposure of fish to Viva detergent. The study also found that Viva detergent has a high potential for inducing oxidative stress in *C. gariepinus,* which is sensitive in both acute and sub-lethal concentrations. It shows the activities of antioxidant enzymes of SOD and CAT, indicating the different rates of free radical generation and antioxidant potentials of the liver tissue. These parameters can be very useful biomarkers in assessing the toxicity in the aquatic ecosystem. However, I strongly recommend further studies for a better understanding of its deleterious effects.

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