Asian Journal of Environment & Ecology

3(4): 1-9, 2017; Article no.AJEE.34778
ISSN: 2456-690X

Toxicity and Histological Effects of Two Liquid Soaps on African Mud Catfish (*Clarias gariepinus*, Buchell, 1822) Fingerlings

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Authors' contributions

This work was carried out in collaboration between all authors. Authors IE and OI designed the study, wrote the protocol and the first draft of the manuscript. Authors AB and JA managed the analyses of the study and performed the statistical analysis. Author NC managed the literature review and searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJEE/2017/34778
Editor(s):
(1) Egbuonu, Anthony Chinedum Cemaluk, Department of Biochemistry, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike, Nigeria.
Reviewers:
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(2) U. D. Enyidi, Umudike Umuahia Abia State, Nigeria.
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(6) A. B. A. Mustapha, Morocco.
Complete Peer review History: http://www.sciencedomain.org/review-history/20453

Original Research Article

Received 10th June 2017
Accepted 15th July 2017
Published 10th August 2017

ABSTRACT

Aim: To determine the toxicity and histological effects in *Clarias gariepinus* fingerlings exposed to different concentrations of liquid soaps.
Study Design/Methodology: A total of 500 *C. gariepinus* fingerlings with a mean weight of 1.7 ± 0.2 g were purchased from the University of Calabar fish farm. Samples were carefully collected and transferred to a transparent rectangular plastic container of 60 × 30 cm^3 and transported to the postgraduate research laboratory, Department of Zoology and Environmental Biology, University of Calabar, Calabar, Nigeria. Fish specimen were transferred into a laboratory aquarium (80 x 30 x 30 cm^3), each containing 80 litres of water and allowed to acclimate under laboratory conditions of

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30.02 ± 0.09℃ and a pH of 8.0 for two weeks. Twent y-five (25) fingerlings were used for each aquarium exposed to four (4) different concentrations of the two soaps (GBC car wash and morning fresh) and the control group. The fingerlings were exposed to 0.00, 400, 450, 500 and 550 ppm of the two soaps and the experiment was done in duplicate. The mean weight of fingerlings used for the study was 1.7 ± 0.2 g. The mortality data trend of fingerlings exposed to different concentrations of the soaps were dependent on the concentration and exposure duration.

Results: The result of the probit transformation revealed that mortality rate increased linearly with increase in concentration of the soaps. The regression equations for the probit transformation of *Clarias gariepinus* fingerlings exposed to different concentration of GBC and morning fresh were y = 4.7546x – 0.6244 and y = 6.2557x – 0.6175 respectively. The 96 hours LC_{50} value of GBC car wash and morning fresh liquid soap on *C. gariepinus* fingerlings were 5.40 and 5.80 respectively. The low LC_{50} value for the fingerlings exposed to the soaps showed a high toxicity of the two liquid soaps. Distress behaviour was observed; they showed gasping of air, erratic swimming, piping and frequent surfacing.

Conclusion: The study revealed that GBC and morning fresh liquid soaps resulted in several histological alterations in the gills ranging from erosion of secondary gill lamellae, fusion of gill lamellae and hyperplasic /erosion of gill lamellae. GBC car wash liquid soap was slightly more toxic to the test organism than morning fresh liquid soap, suggesting that the chemical constituents of GBC car wash may have more effects in other exposed non target organisms in the environment. Government should enlighten and sensitize the public on the lethal and sub-lethal effects of soap effluents to our eco-system as well as enforce against their discharges into our environment, in order to maintain a healthy aquatic environment.

Keywords: Toxicity; histological; soaps; Clarias gariepinus; fingerlings.

1. INTRODUCTION

Soap is a washing agent made by reacting animal or vegetable oils with potassium or sodium hydroxide [1]. Soaps act by emulsifying grease and other dirt and lowering the surface tension of water so that it more readily penetrates open materials such as textiles [1]. Nigeria and other developing nations of the world have in recent times, experienced alarming rates of water pollution. This era of globalization and industrialization has the rate of aquatic pollution due to the increasing number of industrial and domestic effluents that are discharged into aquatic systems thus altering the balance of the aquatic ecosystem. However, the range of alterations related to physiological abnormalities have been observed. These effects have been attributed to various chemicals known to be present within treated or/and untreated industrial effluents. Indeed, extensive laboratory-based studies have confirmed that chemicals contained in industrial effluents can induce many effects seen in effluent exposed fish [2]. It is unhygienic for people to wash cloths, utensils, cars, apparels, working tools etc. directly into water bodies using various types of soaps without the effects and aquatic life and other users. It has been established that sensitivity of fishes and other water organism towards soaps depends on type of soap, its concentration and organism species [3]. Different types of soap are used globally according to [4], soap has the capacity to pose environmental problems to fish and invertebrates. Fish is usually affected by toxicant in aquatic environment the moment it is exposed to it. Toxicity of soap depends to a high degree, on its chemical structure, water, pH and hardness, oxygen concentration and temperature of water [4]. This study is aimed at assessing the toxicity and histological effects of *Clarias gariepinus* fingerlings exposed to different concentrations of liquid soaps.

2. MATERIALS AND METHODS

2.1 Study Area

Geographically, University of Calabar fish farm is located at latitude 4°56’ 021” North, 8°20’ 045’’ East [5], close to University of Calabar staff quarters, in Cross River state. The temperature of the area ranges from 21.05° to 33.15° Celsius, with maximum within the months of January to April. Rainfall is not stable and consistent as it rains through the year. It rains more between the months of April and November with the peak in May to October. An average annual precipitation of 2718 mm has been recorded in the area.

2.2 Collection and Acclimation of the Fish Specimen

A total of 500 *C. gariepinus* fingerlings with a mean weight of 1.7 ± 0.2 g were purchased from the University of Calabar fish farm. Samples were carefully collected and transferred to a transparent rectangular plastic container of 60 × 30 cm^3 and transported to the postgraduate research laboratory, Department of Zoology and Environmental Biology, University of Calabar, Calabar, Nigeria. Fish specimen were transferred into a laboratory aquarium (80 x 30 x 30 cm^3), each containing 80 litres of water and allowed to acclimate under laboratory conditions of 30.02 ± 0.09℃ and a pH of 8.0 for two weeks. During this period the fingerlings were fed once daily with commercial feed pellets, Copen, at 5% of their body weight. The unconsumed feeds and faeces were removed regularly from the holding tank and the water in the tank was changed every 24 hours.

2.3 Purchase of Soap and Preparation of the Stock Solutions

Morning fresh soap and GBC car wash soap used for the study was purchased from Watt market. The stock solution was prepared by dissolving 1 g of each soap (GBC car wash and morning fresh) in 1 liter of clean water and the prepared stock solutions of the soaps were stored in separate 1 liter beaker and corked. Serial dilutions of 400, 450, 500 and 550 ppm concentrations were made from the stock solution and utilized as exposure concentrations.

2.4 Range Finding Tests

Range finding tests was carried-out before the commencement of the experiment in order to determine the appropriate concentration range of the soaps that had effects on *Clarias gariepinus* fingerlings. A wide range of concentrations like; x_1, x_2 and x_3 ppm was tested; including one which killed all organisms within 96 hours and another concentration which did not kill the organisms within 24 hours.

2.5 Toxicity Tests

Toxicity experiment were carried out with five treatments i.e. 0.00 (control), 400, 450, 500 and 550 ppm for each of the soap (GBC car wash and Morning fresh) in duplicate. Twenty-five (25) fingerlings of *C. gariepinus* were stocked each in the five glass aquaria of 25 x 15.5 x 15.5 cm^3 for the experiment and a total of 500 *Clarias gariepinus* fingerlings and twenty (20) aquaria were used in total for the study (250 fingerlings for each liquid soap).

2.6 Histology of the Gills

After 96 hours exposure period, the gills were removed and fixed in 10% buffered formalin for 48 hours, dehydrated in graded ethanol for 2 hours and xylene, impregnated in paraffin wax to enhance sectioning with the microtome [6]. The samples were then blocked on wooden blocks to aid microtomy and sectioning was performed with a rotary knife at 5 µm and stained with haematoxylin and eosin. Photomicrography of sections was mounted on glass slides and pictures were taken with digital motic image capture, Laser microtomy model.

2.7 Probit Analysis

The mean of the mortality for the duplicate of each toxicant concentration group and the control group was computed. The mortality-concentrations data was subjected to probit transformation, regression and LC_{50} was also computed using Predictive Analytical Software (PASW) version 20. The significance of the slope were tested using chi-square. Graphs were drawn using Microsoft excel version 2013.

3. RESULTS

3.1 Toxicological Effect of Soap

During the period of exposure of *C. gariepinus* fingerlings to the different concentrations of the two soap types (GBC car wash and Morning fresh), distress behavioural responses due to the effects of soap were observed. Distress behavioural responses such as gasping for air, erratic swimming and piping or frequent surfacing were observed with increase in concentration of the two liquid soaps used in this study. As the experiment progressed, the experimental fish (*C. gariepinus*) became weaker and those that could not tolerate the higher concentrations of the toxicant went comatose. In the control, normal behavioural responses were observed. The result of the probit transformation revealed that mortality rate increased linearly with increase in concentration of the soaps (Fig. 1a and 1b). The 96 hours LC_{50} for GBC and Morning fresh liquid soaps were 5.40 ± 6.64 and 5.80 ± 8.89, having a lower and upper interval of 3.017 to 9.665 for GBC liquid soap and 1.062 to 9.958 for morning fresh liquid soap respectively (Table

5a and 5b). The regression equations for the probit transformation of *Clarias gariepinus* fingerlings exposed to different concentration of GBC and morning fresh were y = 4.7546x – 0.6244 and y = 6.2557x – 0.6175 respectively (Table 2a and 2b).

Table 1a and 1b. Probit transformation/analysis of mortality data of *Clarias gariepinus* exposed to different concentrations of GBC car wash and morning fresh liquid soap

GBC car wash liquid soap								
Conc (ppm)	**Log Conc (x)**	**N**	**R**	**P**	**M_R**	**Y**	**R_P**	***P***
0.00	0.00	25	0	0	0	0.0	0.00	0.00
400	5.991	25	9	0.36	36.0	10.95	-1.95	0.438
450	6.109	25	19	0.76	76.0	18.07	0.93	0.723
500	6.215	25	20	0.80	80.0	22.40	-2.40	0.896
550	6.310	25	25	1.00	100.0	29.2	0.78	0.969
Morning fresh liquid soap								
Conc (ppm)	**Log Conc (x)**	**N**	**R**	**P**	**M_R**	**Y**	**R_P**	***P***
0.00	0.00	25	0	0	0	0.0	0.00	0.00
400	5.991	25	13	0.52	52.0	11.98	1.014	0.499
450	6.109	25	15	0.60	60.0	16.86	-1.862	0.694
500	6.215	25	19	0.76	76.0	20.47	-1.468	0.819
550	6.310	25	24	0.96	96.0	22.69	1.302	0.908

n = Number of fish fingerling tested at each concentration, r = Number of fish fingerling responding, p = Response rate, r/n, M_R = Mortality rate, Y = Expected probit from visual regression line, R_P = Residual probit, P = Probability

Table 2a and 2b. Results of regression analysis of log concentration – probit relationship of *Clarias gariepinus* fingerlings exposed to concentration of GBC car wash and morning fresh liquid soap

GBC car wash liquid soap				
Conc. (Log Unit)	**Response rate, p**	**Equation**	**Co-efficient of determination, r^2**	**Significant level, α**
0.00	0.00			
400	5.991			
450	6.109	Y = 4.7546x – 0.6244	0.70	0.05 (S)
500	6.215			
550	6.310			
Morning fresh liquid soap				
Conc. (Log Unit)	**Response rate, p**	**Equation**	**Co-efficient of determination, r^2**	**Significant level, α**
0.00	0.00			
400	5.991			
450	6.109	Y = 6.2557x – 0.6175	0.81	0.05 (S)
500	6.215			
550	6.310			

Table 3a and 3b. Chi-square tests of *Clarias gariepinus* fingerlings exposed to concentration of GBC car wash and morning fresh liquid soap

GBC car wash liquid soap			
	Chi square	**df[a]**	**Sig.**
PROBIT Pearson Goodness-of-FitTest	4.072	1	0.044[a]
Morning fresh liquid soap			
	Chi square	**df[a]**	**Sig.**
PROBIT Pearson Goodness-of-FitTest	2.188	1	0.139[a]

Table 4a and 4b. Covariance's and correlation of *Clarias gariepinus* fingerlings exposed to concentration of GBC car wash and morning fresh liquid soap

GBC car wash liquid soap		
Probit	**Concentration**	**Natural Response**
Concentration	2.876	0.509
Natural Response	0.279	0.104
Morning fresh liquid soap		
Probit	**Concentration**	**Natural Response**
Concentration	7.903	0.89
Natural Response	2.021	0.66

Table 5a and 5b. LC_{50} with 95% confidence limits of *Clarias gariepinus* fingerlings exposed to concentrations of GBC car wash and morning fresh liquid soap

GBC car wash liquid soap		
LC_{50} with ± 95%CL	**Confidence limits**	
	Lower	**Upper**
5.40 ± 6.64	3.017	9.665
Morning fresh liquid soap		
LC_{50} with ± 95%CL	**Confidence limits**	
	Lower	**Upper**
5.80 ± 8.89	1.062	9.958

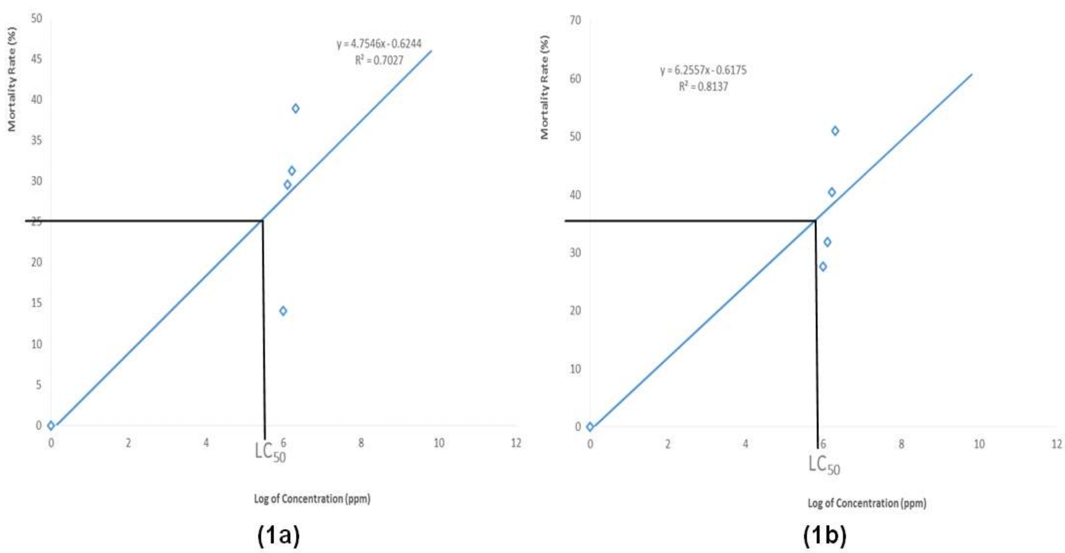

Fig. 1a, b. 96 hours LC_{50} of African Mud Catfish (*Clarias gariepinus*) fingerlings exposed to different concentrations of GBC car wash (1a) and morning fresh (1b) liquid soap in static system

3.2 Histological Examination

Histology examination showed that the gill lamellae of fish in the control group were well distributed without any observed fusion or erosion (Plate 1a). In the gills of *C. gariepinus* exposed to GBC car wash, erosion of secondary gill lamellae was observed in 400 ppm (Plate 1b) and 450 ppm (Plate 1c), while fusion of gill lamellae was observed in 500 ppm (Plate 1d). In the gills of *C. gariepinus* exposed to morning fresh, erosion of secondary lamellae was observed in 450 ppm (Plate 2b) and hyperplasic/erosion of gill lamellae was observed in 500 ppm (Plate 2d).

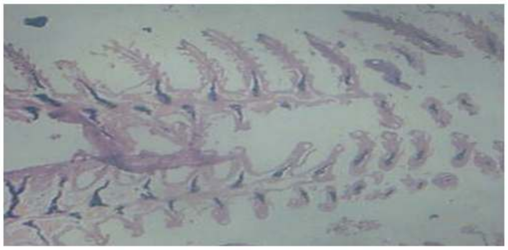

Plate 1a (control)

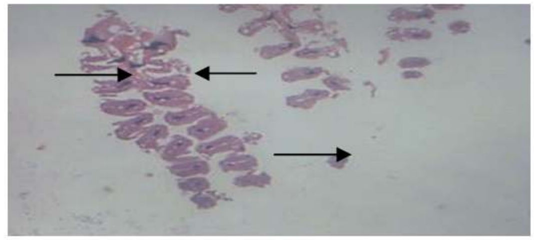

Plate 1b (400ppm)

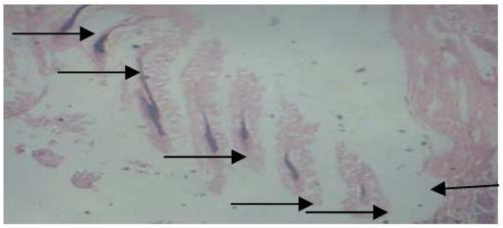

Plate 1c (450ppm)

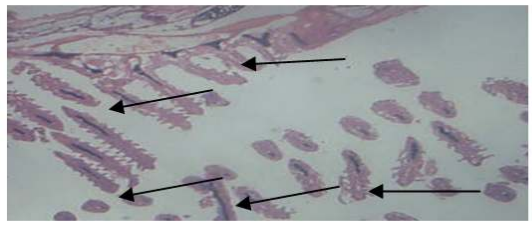

Plate 1d (500/550ppm)

Plate 1a-d. Micrograph of the gills tissues of *Clarias gariepinus* showing histological effects at different concentrations (0.00 – 550ppm) of GBC car wash liquid soap. X100

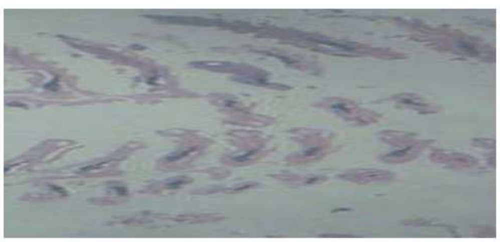

Plate 2a. (Control)- Morning fresh liquid soap)

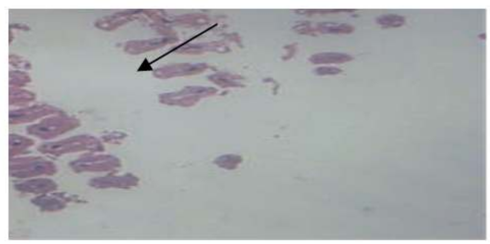

Plate 2b. (400 ppm)

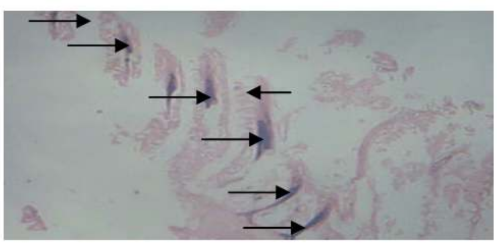

Plate 2c. (450 ppm)

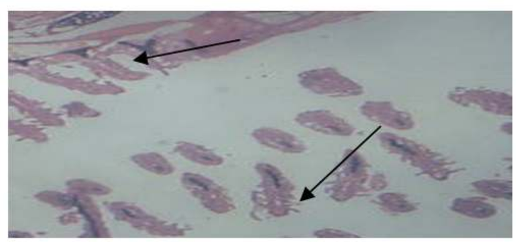

Plate 2d. (500/550 ppm)

Plate 2a-d. Micrograph of the gills tissues of *Clarias gariepinus* showing histological effects at different concentrations (0.00 – 500ppm) of morning fresh liquid soap. X100

4. DISCUSSION

Soap effluents which end up in the aquatic environment through indiscriminate use, careless handling, accidental spillage or discharge of untreated effluents into natural water-ways have harmful effects on the fish population and other forms of aquatic life. Humans make use of this water for various purposes such as drinking, cooking, bathing, washing [7]. In the present study, all the soap used was observed to induce severe damage to some organs in *C. gariepinus* such as the gills and skin although some soap caused more damages than others of the same concentration. The observation is similar to [8] who reported that soap and detergent effluents induce severe damage to vital organs in *C. gariepinus* like the gills, kidney, liver, skin, heart and the brain. The toxicological effects of the soaps were concentration dependent and similar observations were reported by [9,10]. The 96 hours LC_{50} value of the soaps used in this study was 5.40 ppm for GBC liquid soap and 5.80 ppm for Morning fresh. These values fell within the concentrations ranges given by other authors such as [3] and [11] for *C. gariepinus* respectively. The 96h LC_{50} value of GBC liquid soap was lower than that of morning fresh liquid soap, indicating that the GBC liquid soap is more toxic than the morning fresh soap. The variation observed in the toxicity of the two soaps to *C. gariepinus* in this study may be attributed to the difference in physical and chemical compositions of the soap surfactants. For example, GBC car wash is known to consist of chemical constituents ranging from ethylenediaminetetraacetic acid, polysorbate 20 and some surfactants, while morning fresh consist of; alkyl benzene sulfonate, tripolyphosphate, $NaCO_3$ and $NaSO_4$. This observation is similar to findings of [1,3,7,12,13] who reported that the level of toxicity of any toxicant depends on its bioaccumulation, the differential physical and chemical compositions of the compound forming the toxicant, solubility of the soap and the reaction of the exposed organism. At the lethal concentrations, distress behavioral response was observed to increase with increase in concentration of the soaps used. Such distress behavioral responses include incessant jumping and gasping for breath, erratic swimming, piping or frequent surface to bottom movement, restlessness, loss of skin coloration, sudden change of direction during movement, resting at the bottom, loss of equilibrium and gradual onset of inactivity. However, such distress behavior was not found in the control experiment throughout the period of the bioassay, rather, the fish were swimming normally and actively. These behavioural abnormalities observed in this study are similar to the observation of [3,7,11,12,14,15,16], behavioural abnormalities such as nervous and respiratory impairment resulted in blockage of nervous transmission between the nervous systems and various effectors sites, enzyme dysfunctions that may induce paralysis of respiratory muscles and/or depression of respiratory center and disturbances in energy or metabolic pathways which results in depletion of energy. According to [7], soaps and detergents, including the biodegradable ones may induce poisonous effects and osmo-regulatory imbalances in aquatic organisms especially if present in concentrations that exceed metabolic demand. In this study, little changes were observed in the lower sub-lethal concentrations (400 ppm) of the soap used, and this may be attributed to the avoidance behaviour of the test organisms to the soap [3]. According to [7], fish exposed to low concentration of toxicant do not reach the stage of exhaustion; rather they quickly become adapted to the stressor. At increased lethal concentration (450 ppm and 500 ppm), the behavioural responses of the experimental fish increased simultaneously and the organisms later became inactive. According to [17,18], this observation is a normal situation in acute and sub-acute toxicity experiments. This observation obtained in this study could possibly be due to energy depletion resulting from the disturbances in the metabolic state of the test organism [19]. According to [6] and [19], the impairment of the carbohydrate metabolism in *C. gariepinus* resulted in the depletion of energy, causing lethargies and loss of equilibrium so that those organisms that cannot tolerate the toxicant will enter into a state of coma and later die. In this study, it was observed that, mortality rate increased simultaneously with increase in the concentration of the different soap used. This is consistent with findings of other authors that as regards all categories of toxicant; a threshold is reached at which there is no drastic survival of animal and animal therefore lies within a tolerable zone only below the threshold, but above the tolerance zone are the zone of resistance [3,7,11]. Gills are partially sensitive to adverse environmental conditions. Owing to the direct and continuous contact with the aquatic environment, fish gills which are organs for respiratory gas exchange, osmoregulation, excretion of nitrogenous waste products and acid base regulation, are directly affected by contaminants [19]. Findings of this study revealed haemorrhageing of the gill filaments on the dead fish and this is a clear indication of intoxication, and this was in agreement with the observations of [5,14]. In the gills of the control fishes used in this study, there was no recognizable changes and each gill consists of primary filament and secondary lamellae which agrees with findings of [19] for *C. gariepinus* exposed to lethal and sub-lethal concentration of soap and detergents. The histopathological studies of the sectioned gills of *C. gariepinus* during this study showed marked histological alterations. In the gills of *C. gariepinus* exposed to the various detergents, erosion of secondary gill lamellae, fusion of gill lamellae and hyperplasic gill lamellae cells were observed. This agrees with the findings of [20,21,22] who reported that such anomalies in the gill structure of *O. niloticus* on exposure to glyphosate herbicidal, waterborne copper and lead effects respectively. This could be attributed to the activities of the test organisms exposed to which is as result of changes in the environment, diffusion distance from surrounding water to capillaries and at the same time and increase in the amount of tissue (blood corpuscles) in the blood spaces of secondary lamellae [19]. Also, the cellular hypertrophic condition observed, led to a decrease in the respiratory capacity between the lamellae, impairs the diffusion of oxygen across the gills due to the swollen condition of the epithelium and decrease in free gas exchange which in turn limits the compensatory changes that makes organism in question to become in adaptive when the duration of exposure and the concentrations of the effluents exceeds biological tolerance limits [19]. Therefore, the histological effects observed in this study indicate that the fish were responding to the effects of the concentrated liquid soap. Such information confirms that histological alterations are good biomarkers for laboratory assessment, particularly in areas that are naturally subjected to environmental variations or depletion due to chemical pollutants.

5. CONCLUSION

The study revealed that both liquid soaps (GBC car wash and morning fresh) resulted in several histological alterations in the gills ranging from erosion of secondary gill lamellae, fusion of gill lamellae and hyperplasic /erosion of gill lamellae. The 96 hours LC_{50} values indicates that GBC car wash liquid soap was slightly more toxic than morning fresh liquid soap in *Clarias gariepinus* fingerlings, suggesting that the chemical constituents of GBC car wash may have more effects in other exposed non target organisms in the environment.

ETHICAL CONSIDERATION

The authors ensured that all the ethical and other basic principles underlying behavior and advancing welfare for the use of animals in research, including handling, relevant laws and regulations were considered before proceeding with the research.

ACKNOWLEDGEMENTS

Authors acknowledge the Head of Department, Fisheries and Aquaculture, Professor Albert Ekanem, Institute of Oceanography, University of Calabar, for providing the laboratory facilities for carrying out this research work.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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