

## Original Research Article

# Toxicity and Histopathological effects of African Clariid Mud Catfish, *Clarias gariepinus* (Buchell, 1822) fingerlings Exposed to liquid soaps (GBC and morning fresh liquid soap)

## ABSTRACT

The toxicologic and histopathological effects of two soaps on *Clarias gariepinus* was evaluated. Twenty-five (25) fingerlings were used for each aquarium exposed to five (5) different concentrations of the two soaps (GBC and morning fresh soap) 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. A total of 500 *Clarias gariepinus* fingerlings were used throughout the study for each soap toxicant. The mean weight of fingerlings used for the study was  $1.7 \pm 0.2$  g. The mortality data trend of fingerlings exposed to different concentrations of the soaps were dependent on the concentration and exposure duration. The 96 hours  $LC_{50}$  value of GBC 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 soaps. The study revealed that GBC and morning fresh liquid soaps resulted in several histopathological alterations in the gills ranging from erosion of secondary gill lamellae, fusion of gill lamellae and hyperplastic /erosion of gill lamellae. GBC liquid soap was slightly more toxic to the test organism than morning fresh liquid soap. As a result of the high toxicity of the two soaps, indiscriminate discharge of soap effluents should be avoided. The Government should also 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; Histopathological; 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 sky rocked 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 [2]. The effects of these activities are often outrageous with corresponding immediate or later consequences to both direct and indirect dependents of these water bodies [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 minding the effects and implication to aquatic life and other users. Soap is salt of fatty acid produced by treating vegetable oil or animal oils and fats with a strong alkaline solution [3]. 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]. Toxicity of soap depends to a high degree, on its chemical structure, water, pH and hardness, oxygen concentration and temperature of water [4]. The study is aimed at assessing the toxicity and histopathological 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 of the study fish

A total of 500 *C. gariepinus* fingerlings with a mean weight of  $1.7 \pm 0.2$  g were purchased from the University of Calabar fish farm. Samples were carefully collected and transferred to a plastic container and transported to the postgraduate research laboratory, Department of Zoology and Environmental Biology, University of Calabar, Calabar, Nigeria.

### 2.3 Acclimation of fingerlings

Fish specimen were transferred into a laboratory aquarium (80 x 30 x 30 cm<sup>3</sup>), each containing 80 litres of water and allowed to acclimate under laboratory conditions of  $30.02 \pm 0.09$  °C 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.

### 2.4 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 1g of each soap (morning fresh and GBC car wash) in 1L of clean water and the prepared stock solutions of the soaps were stored in separate 1litre beaker and corked. Serial dilutions of 400, 450, 500 and 550 ppm concentrations were made from the stock solution and utilized as exposure concentrations.

### 2.5 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  $x_3$  mg/l was tested; including one which killed all organisms within 96 hours and another concentration which did not kill the organisms within 24 hours.

### 2.6 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<sup>3</sup> for the experiment and a total of 500 *Clarias gariepinus* fingerlings were used in total for the study (for each soap) .

### 2.7 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 5µm and stained with haematoxylin and eosin. Photomicrography of sections was mounted on glass slides and pictures taken with digital motic image capture, Laser microtomy model.

## 2.8 Probit analysis

The mortality-concentrations data was subjected to probit transformation, regression analysis and  $LC_{50}$  and was also computed using Predictive Analytical Software (PASW) version 20.

## 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 effects of soap were observed. Distress behavioural responses such as gasping for air, erratic swimming and piping or frequent surfacing was observed to increase 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 mortality trends of *Clarias gariepinus* fingerlings exposed to the soaps were dependent on the concentrations. Mortality data were transformed into probits that were plotted against the concentration. The percentage mortality – concentration relationship was determined by linear regression analysis, and 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  $LC_{50}$  (the concentration that will kill 50% of the fingerlings) for GBC and Morning fresh liquid soaps were  $5.40 \pm 6.64$  (Fig. 1a) and  $5.80 \pm 8.89$  (Fig 1b) (Table 5a and 5b), having a lower and upper interval of the of 3.017 to 9.665 for GBC liquid soap and 1.062 to 9.958 for morning fresh liquid soap (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  $y = 4.75x - 0.6244$  and  $y = 6.2557x - 0.6175$  respectively (Table 1a and 1b).

**Table 1a. Probit Transformation/analysis of mortality data of *Clarias gariepinus* exposed to different concentrations of GBC 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 |

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 1b. Probit Transformation/analysis of mortality data of *Clarias gariepinus* exposed to different concentrations of morning fresh**

| 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. Results of regression analysis of Log Concentration – probit relationship of *Clarias gariepinus* fingerlings exposed to concentration of GBC liquid soap**

| Conc. Unit) | (Log | Response rate, p | Equation               | Co-efficient of determination, $r^2$ | Significant level, $\alpha$ |
|-------------|------|------------------|------------------------|--------------------------------------|-----------------------------|
| 0.00        |      | 0.00             |                        |                                      |                             |
| 400         |      | 5.991            | $Y = 4.7546x - 0.6244$ | 0.70                                 | 0.05 (S)                    |
| 450         |      | 6.109            |                        |                                      |                             |
| 500         |      | 6.215            |                        |                                      |                             |
| 550         |      | 6.310            |                        |                                      |                             |

**Table 2b. Results of regression analysis of Log Concentration–probit relationship of *Clarias gariepinus* fingerlings exposed to concentration of morning fresh liquid soap**

| Conc. Unit) | (Log | Response rate, p | Equation               | Co-efficient of determination, $r^2$ | Significant level, $\alpha$ |
|-------------|------|------------------|------------------------|--------------------------------------|-----------------------------|
| 0.00        |      | 0.00             |                        |                                      |                             |
| 400         |      | 5.991            | $Y = 6.2557x - 0.6175$ | 0.81                                 | 0.05 (S)                    |
| 450         |      | 6.109            |                        |                                      |                             |
| 500         |      | 6.215            |                        |                                      |                             |
| 550         |      | 6.310            |                        |                                      |                             |

**Table 3a. Chi-square Tests of *Clarias gariepinus* fingerlings exposed to concentration of GBC soap**

|                                    | Chi square | df <sup>a</sup> | Sig.               |
|------------------------------------|------------|-----------------|--------------------|
| PROBIT Pearson Goodness-of-FitTest | 4.072      | 1               | 0.044 <sup>a</sup> |

**Table 3b. Chi-square Tests of *Clarias gariepinus* fingerlings exposed to concentration of Morning fresh liquid soap**

|                                    | Chi square | df <sup>a</sup> | Sig.               |
|------------------------------------|------------|-----------------|--------------------|
| PROBIT Pearson Goodness-of-FitTest | 2.188      | 1               | 0.139 <sup>a</sup> |

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

| PROBIT           | Concentration | Natural Response |
|------------------|---------------|------------------|
| Concentration    | 2.876         | 0.509            |
| Natural Response | 0.279         | 0.104            |

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

| PROBIT           | Concentration | Natural Response |
|------------------|---------------|------------------|
| Concentration    | 7.903         | 0.89             |
| Natural Response | 2.021         | 0.66             |

**Table 5a. LC<sub>50</sub> with 95% confidence limits of *Clarias gariepinus* fingerlings exposed to concentrations of GBC liquid soap**

| LC <sub>50</sub> with ± 95%CL | Confidence limits |       |
|-------------------------------|-------------------|-------|
|                               | Lower             | Upper |
| 5.40 ± 6.64                   | 3.017             | 9.665 |

**Table 5b. LC<sub>50</sub> with 95% confidence limits of *Clarias gariepinus* fingerlings exposed to concentrations of morning fresh soap**

| LC <sub>50</sub> with ± 95%CL | Confidence limits |       |
|-------------------------------|-------------------|-------|
|                               | Lower             | Upper |
| 5.80 ± 8.89                   | 1.062             | 9.958 |

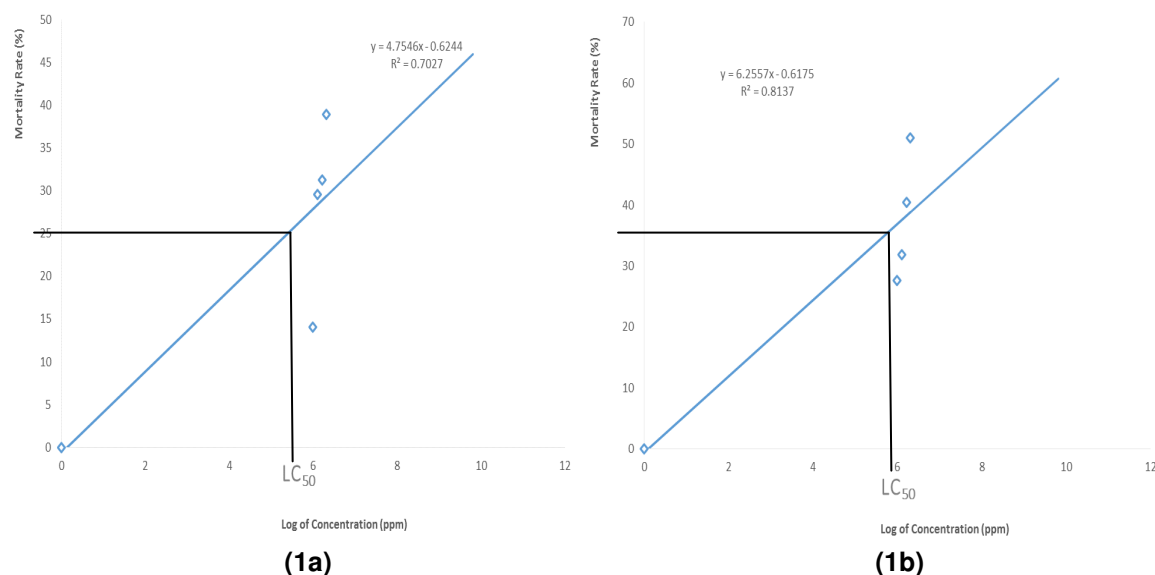


**Fig. 1a, b. Probit graph of African Mud Catfish (*Clarias gariepinus*) fingerlings exposed to different concentrations of GBC liquid (1a) and Morning fresh Soap (1b)**

### 3.2 Histopathological changes

Histopathology 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 hyperplastic/erosion of gill lamellae was observed in 500 ppm (Plate 2d).



**Plate 1a (control)**

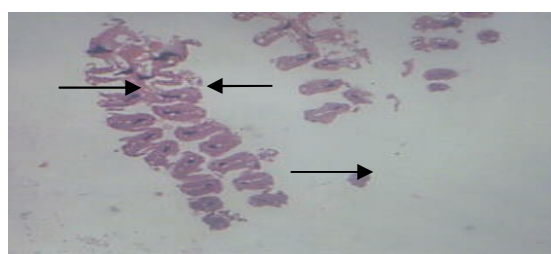


**Plate 1b (400ppm of GBC liquid soap)**

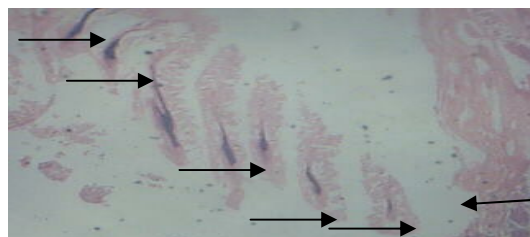


**Plate 1c (450ppm of GBC liquid soap)**



**Plate 1d (500/550ppm of GBC liquid soap)**

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

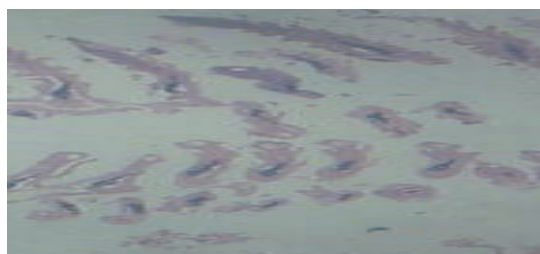


**Plate 2a. (Control)- Morning Fresh Liquid Soap)**

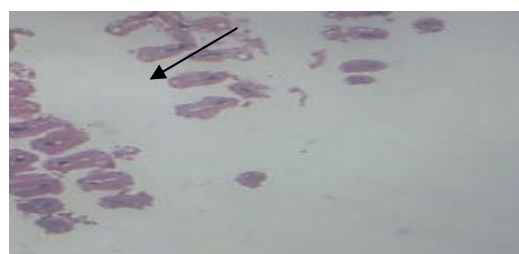


**Plate 2b. (400 ppm)**

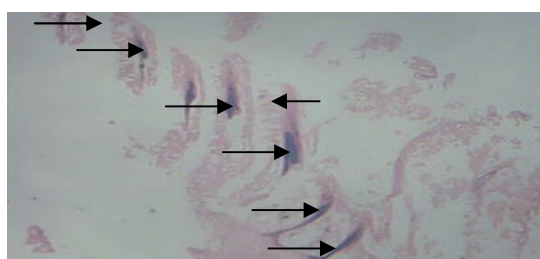


**Plate 2c. (450 ppm)**

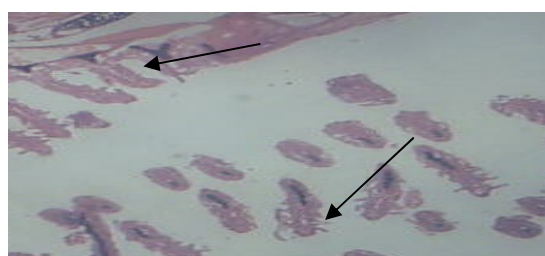


**Plate 2d. (500/550 ppm)**

**Plate 2a- d. Micrograph of the gills tissues of *Clarias gariepinus* showing histopathological changes 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]. The 96h LC<sub>50</sub> 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 [10] for *C. gariepinus* respectively. The 96h LC<sub>50</sub> 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. This observation is similar to findings of [3, 7, 11, 1] 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 [12, 13, 14, 11, 3, 10, 7], attributed such behavioural abnormalities observed in this study to nervous and respiratory impairment as a result of 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 [15], 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 [16]. According to [16] and [6], 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 [10, 3, 7]. 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 [16]. Findings of this study revealed haemorrhage 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 [12]. 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 [16] 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 hyperplastic gill lamellae cells were observed. This agrees with the findings of [17, 18, 19] 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 increase in the activities of the test organisms exposed to the changing 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 [16]. 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 [16].

## 5. CONCLUSION

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

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