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Gills and Liver Histology of *Clarias gariepinus* exposed to Aqueous Extract of *Carica papaya* Seed under Laboratory Condition

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Abstract: African Catfish, *Clarias gariepinus* were exposed to graded concentrations (0, 150, and 180, 210 and 240 mgL^{-1}) of aqueous extract of *Carica papaya* dry seeds in triplicates for 14 days. They were fed a 25 % crude protein diet at 1% biomass half at 800 and 1600 h. Experimental design was a completely randomized type. The control fish's gill histology revealed normal histomorphology with intact blood vessels, gill slits and supporting cartilage apparent. Exposed fish gill showed degeneration of the gill filament, hypertrophy, hyperchondrial of the gill arch and lesion. Normal tubules, haematopoietic structures, and a portal triad with a dilated portal vein were found in the livers of control fish. The exposed fish livers showed vacoulation, steatosis, necrosis and degeneration of hepatocyte degeneration. The findings revealed that histological studies of fish gill and liver could be useful biomarker for assessing aqueous extract of *C. papaya* dry seeds toxicosis.

Keywords: Histology, *Carica papaya*, gill, livery, plant extract.

1. Introduction

Botanical biocides contamination of natural water is widely reported in Nigeria because of their usage by local fisher forks. It was reported that the presence of macerated plant materials in rivers and streams stupefy fish for easy collection (Audu *et al.*, 2021). The alkaloid contents of the leaves and stem of *Melaleuca cajuputi* is active against microorganism and that the essential oil found in the leaves also contain P-cymene, linalool and beta-caryophyllene (Abd Wahab *et al.*, 2022). Furthermore, some plants contain chemicals which are traditionally used to harvest fish in most part of the world (Olamiposi *et al.*, 2022). Extracts of plants such as *Deris elliptica, D. trifoliate, Adeniace ssamploides, B. aegyptiaca, Carica papaya, Tephrosia candida, T. vogelii, Parkia folicoides* contain biocides which are used to catch fish in different parts of the world (Ekpendu *et al.*, 2020).

Carica papaya is a perennial tree with a crown of very large palmate leaves at the base of which the fruits are clustered. Its fruits are palatable feedstuff as well as its leaves that are widely used by the locals (Taher *et al.*, 2017). *C. papaya* seed contains piscicidal and anti-fertility properties which are widely used as fish poison for controlling the excessive breeding of tilapia in some part of Nigeria (Daagema *et al.*, 2020). The active ingredients of *C. papaya* are carpine, chymopapain, papain, aglycoside, sinigrin, the enzyme myrosin and carpasemine which are concentrated in the black seeds

(Daagema *et al.*, 2020). However, the presence of these botanicals in high concentrations may have adverse effects on aquatic organisms.

The African Catfish (*Clarias gariepinus*) is an ecologically important and commercially valued fish in Nigeria. The demand for this fish by large population of Nigerian has necessitated its harvest with the use of poisons by the locals (Abubakar *et al.*, 2022). In spite of the wide uses of *C. papaya* seeds as biocides in aquaculture, little toxicological studies have been done on its effects on the culture fishes. The mode in which the various organs respond to xenobiotic exposure has proven their useful as a tool in determining the health condition of fish, since histopathological biomarkers are considered the primary indicators of exposure to pollutants and show little temporal variation (Abubakar *et al.*, 2019). One of the great advantages of using histopathological biomarkers in environmental studies is that this category of biomarkers allows the examination of the target organs. Furthermore, the alterations found in these organs are clearer than functional ones, while revealing signs of damage to animal health (Sula *et al.*, 2020; Zhang *et al.*, 2021).

The gills being a respiratory organ of fish are frequently in contact with external environment and thus vulnerable to aquatic toxicants (Erdoğan *et al.*, 2022). The gills are particularly sensitive and can also be responsible for maintaining the optimal osmotic pressure and acid-base balance of body fluids. Because their large surface area contacts the external environment, the gills are sensitive to even minor chemical or physical changes in the surroundings and are the target organ for many contaminants in water (Sula *et al.*, 2020). The liver of fish can also be considered as a target organ to pollutants, alterations in its structure can be significant in the evaluation of fish health and the effects of a wide variety of environmental pollutants (Velkova-Jordanoska and Trajcevski, 2020). The metabolic rate of hepatocytes is certainly modulated by thyroid hormones. Thyroid dysfunction may perturb liver function, and liver disease effects thyroid hormone metabolism (Velkova-Jordanoska and Trajcevski, 2020). Hence, the structure, anatomy, physiology, morphology and health condition of fish gills and liver need to be studied more closely. The aim of this study was to examine the histological alterations in the gills and liver of *C. gariepinus* after being exposed to an aqueous extract of *C. papaya* dry seeds.

2. Methodology

2.1 Extract preparation

Pawpaw (*C. papaya*) fruits were obtained from Gammon Market at Ifelodun, Kwara State. The seeds were harvested or extracted from the fruits by cutting the fruits into half with a sterilized knife and rinsing the seeds in distilled water to remove the adhering fruit membrane. After that, the seed were spread out on a paper and allowed to air dry at room temperature in an enclosed environment. Using a mortar and pestle, the dried seed was ground into a coarse powder, weighed and placed into a container, stored in a cold, dry area until needed. Approximately, 1200 g powdered *C. papaya* seeds was soaked in 4800 ml of distilled water for 48 hrs in closed container at room temperature with occasional shaking, then filtered through muslin cloth and finally the filtrate was water bath for 6 hrs till the filtrate dried. The weight of extract obtained after subjecting to water bath was 34.05 g. A stock solution was prepared with the extract obtained by adding 1.0 g of the *C. papaya* extract in 1 Litre of distilled water (Opute and Oboh, 2021).

2.2 Collection of fish

Two hundred and fifty (250), African Catfish juveniles with mean weight of 11.81±0.23 g and mean length 9.8±0.2 cm, were purchased from National Centre for Agriculture and Mechanization Integrated Farm Project (NIFAP), Idofian, Ifelodun Local Government, Kwara State. They were

transported early in the morning inside a black 50 Litres capacity jerry-can. They were held in a 1000 Litres capacity plastic tank for 14 days for acclimation. The tank was filled up to 25 Litres level with borehole water. They were fed a 25% crude protein diet at 1% biomass half at 800 and 1600h, respectively. The water in the acclimation tank was replenished at 2-days interval. Feeding of fish was stopped 24 hrs prior to exposure to acute toxicity bioassay media.

2.3 Bioassay

The following concentrations of aqueous extract of *C. papaya* dry seed were prepared based on the method described by Abubakar (2016):

1 Litre = 1000 g;

1 ml = 1 mg;

1500 ml of the toxicant in 10 litres of water = 150 mg L^{-1}

1800 ml of the toxicant in 10 litres of water = 180 mg L^{-1}

2100 ml of the toxicant in 10 litres of water = 210 mg L^{-1}

2400 ml of the toxicant in 10 litres of water = 240 mg L^{-1}

Preliminary 24 hrs range finding test was conducted for the juvenile catfish (*C. gariepinus*) following static bioassay procedures to determine the toxic range of aqueous extracts of *C. papaya* seeds on juvenile catfish (Abubakar., 2016). Ten (10) juvenile fish were selected at random into five (5) plastic aquaria with holding capacity of 20 Litres. It was filled with 10 Litres of water. Only test fish was put at a time and observed to determine the time mortality would occur. The concentrations from which serial dilutions will begin were arrived at after the survival of fish beyond 2 hours on exposure to the toxicant. The toxicants were introduced in a single dose. The mortality of the test fishes in the aquaria were monitored and recorded every five (5) minutes for the first hour, every fifteen (15) minutes for the next second hour, once, every thirty (30) minutes for the next three (3) hours and every four hours for the twenty-four (24) hours period.

Completely randomized design was used, consisting of three triplicate treatments including a control, 15 plastic aquaria of 20 litres capacity containing 10 Litres of water each was used. Three plastic aquaria serve as control while the other twelve serve as treatments for the test fish. Ten juveniles of African sharp tooth catfish were randomly distributed to each of the plastic aquaria and impound with 10 litres of borehole water.

2.4 Acute bioassay test

In order to estimate lethal concentrations (LC50), a 96- hour static acute toxicity bioassay was performed in the Central Research laboratory, Faculty of Agriculture, University of Ilorin using the method described by Joshi *et al.* (2022). African sharp tooth juvenile mean weight 11.81±0.23g and mean length 9.8±0.2cm) were exposed to different concentrations of aqueous extract of *C. papaya* dry seed. The test fish were exposed to four concentrations of the toxicant (aqueous extract of C. papaya dry seed) and the fifth had no toxicant which served as the control. The nominal concentrations were 150 ml, 180 ml, 210 ml, 240 ml and a control with no toxicant. Each concentration level was in triplicate. The desired stock solution was measured using 50 ml calibrated beaker and introduced into 10 Litres of borehole water inside the plastic aquaria. The mixture was allowed to stand for 30 minutes for proper mixing before randomly introducing test fishes. One hundred and fifty (150) juveniles of African Catfish were randomly distributed into the toxicant concentrations to give a stocking density of 10 fish per plastic aquaria (Abubakar., 2016). Each plastic tank was covered with nylon mesh screen to prevent the fish from leaping out of the tank. Feeding of fish stopped 24 hours prior to and during

the 96 hr exposure period. This was to prevent interference with the absorption and metabolism of the extract by wastes in reconstituted extracts. The exposed and control fish were observed for signs of toxicity with prompt recordings at 24 hr, 48 hr, 72 hr and 96 hr exposure period.

2.5 Histological analysis

One dead fish from each exposed group was chosen at random and their gills and livers were removed using a dissecting set. One live fish from each control group was also chosen at random and euthanized. The gills and liver were sectioned 1-2 mm, in thickness and fixed in 10% formalin and processed according to standard paraffin procedures. Five micrometer thick paraffin sections were placed on glass slides and stained with haematoxylin and eosin. Approximately 2-4 paraffin sections of each fish were histologically analyzed (Abd Elmeged and Alghamdi, 2022). Photomicrographs were then taken at low power (×100) objective with a digital camera (Olympus C4-0 AB-4). Photomicrography of control groups were compared with those of exposed groups under the guidance of a pathologist from the Veterinary Medicine Department, University of Ilorin. Kwara State, Nigeria.

3. Results and discussion

Histological changes observed in the gills and liver of experimental fish showed different lesions, which ranged from desquamation of epithelia lining of cartilage of the gills to degeneration of the hepathocytes and necrosis of the liver of the exposed fish. The control gills and livers had normal morphology and internal arrangement components as revealed in their structures: figures 1 to 5 show the photomicrograph of the gills while figures 6 to 10 revealed the photomicrograph of the livers.

Histological study of the gills showing a typical structural organization of the lamella in the control fish while the exposed fish however showing progressive architectural distortion (hyperplasia and hyperthrophy of secondary lamella, degeneration of gill filament, hyperchondrial of gill arch and lesions at various concentration levels depict a dose-dependent distortion especially in the higher concentration of the extract. The functional implication of these lesions portends serious threat to respiration more specifically the obstruction of the interlamellar space (water channel) which has a direct effect on gaseous exchange across the lamellar epithelium of the gill (Opute and Oboh, 2021). This might be due to the potent and toxicological efficacy of the phytochemicals (such as alkaloids, carpaine, tannins, papains) present in *Carica papaya* extracts. This corroborates with the observation on *C. gariepinus* accumulate toxicants through the lamella surface (Abubakar., 2016). Accumulation in the gills is considerable because of their external location and necessarily intimate contact with the water that allows for dissolved or suspended materials in the water to be absorbed through the delicate epithelium. The lamella epithelial lining reacts to the toxicants creating tissue osmoregulatory imbalance. Prolonged exposure results in marked degeneracy of the epithelium followed by reduction in lamellae. Asphyxiation occasioned by insufficient gaseous exchange was responsible for the death observed at the highest concentrations of the *C. papaya* extracts. The gill histology alterations recorded by *C. papaya* extracts in this study are similar to the work documented on gill lesions as a result of the effect of *Adenia cissampeloides* on the gill of tilapia (Audu *et al*., 2021). Lesions were also reported on African catfish as a result of toxicity of extracts of *Piptadenas triumaficanumbak* (Abidin *et al*., 2022). It is also similar to the finding on the histopathological alterations in the gills and liver of *C. gariepinus* juveniles exposed to acute concentrations of *Anogeissus leiocarpus* (Sani *et al*., 2020).

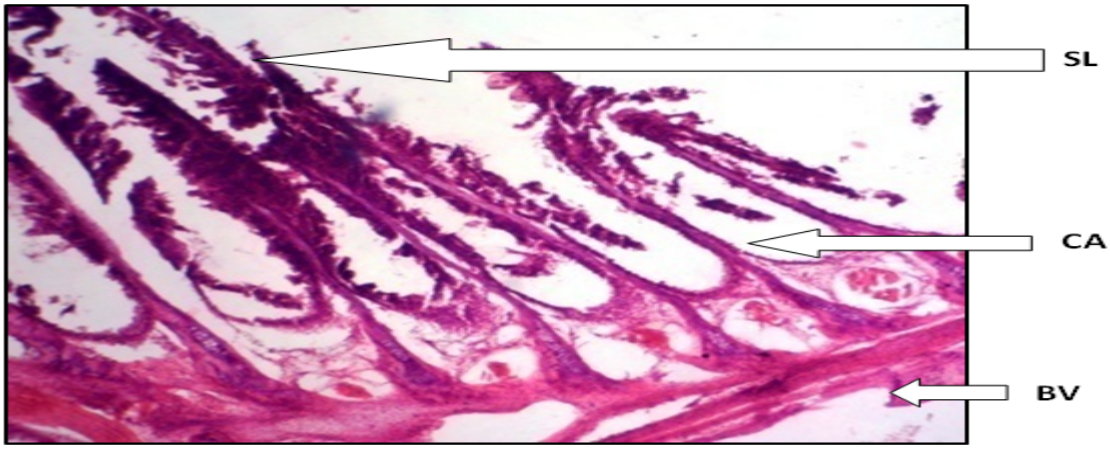

Figure 1. Photomicrograph of the gill of a control *C. gariepinus*s showing normal gill histomorphology with intact blood vessels, gill slits, normal vascular secondary lamella and supporting cartilage. CA-Cartilage apparent, BV-Blood vessel and SL- Secondary lamella (Hematoxylin and eosin, x 100)

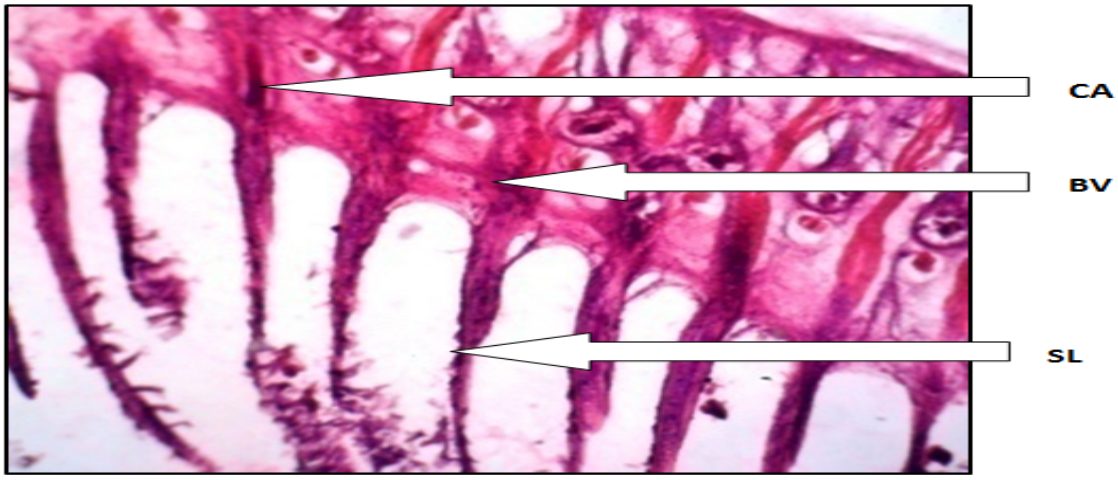

Figure 2. Photomicrograph of the gill of *C. gariepinus* exposed to 150 mg L^{-1} of aqueous extract of *C. papaya* dry seed showing desquamation of the epithelial lining of supporting cartilage (CA), secondary lamellae (SL) and blood vessel (BV) (Hematoxylin and eosin, x 100)

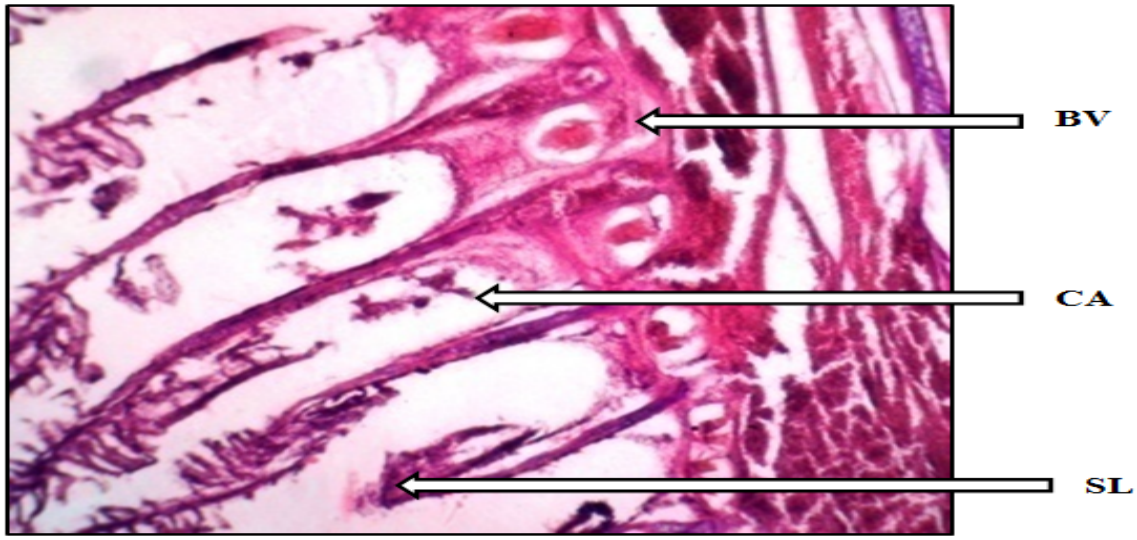

Figure 3. Photomicrograph of the gill of *C. gariepinus* juvenile exposed to 180 mg L^{-1} of aqueous extract of *C. papaya* dry seed showing hyperthrophy of blood vessel (BV), supporting cartilage (CA) and secondary lamellae (SL) (Hematoxylin and eosin, x 100)

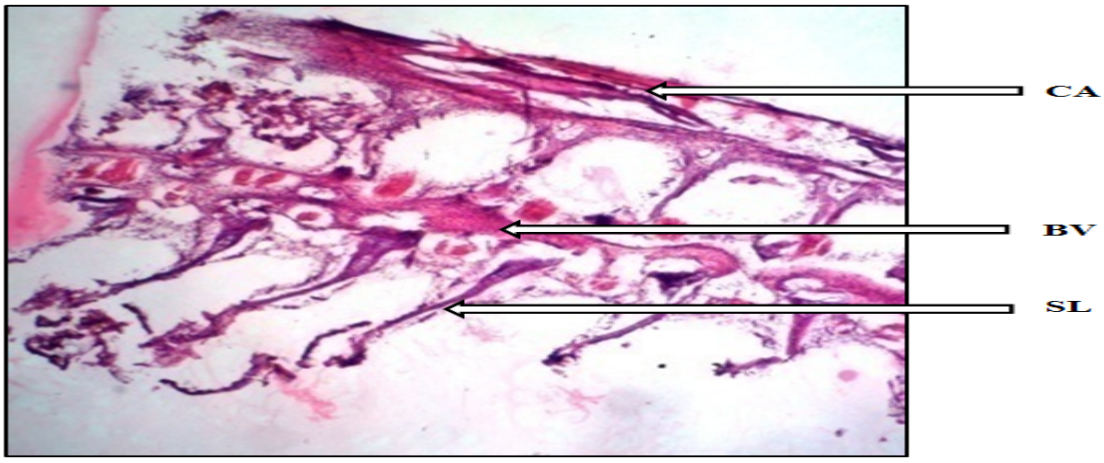

Figure 4. Photomicrograph of the gills of *C. gariepinus* juvenile exposed to 210 mg L^{-1} of aqueous extract of *C. papaya*dry seed showing less vascularized secondary lamella in form of hyperchondrial and hyperthrophy of the gill arch. CA-Cartilage apparent, BV-Blood vessel and SL- Secondary lamella (Hematoxylin and eosin, x 100)

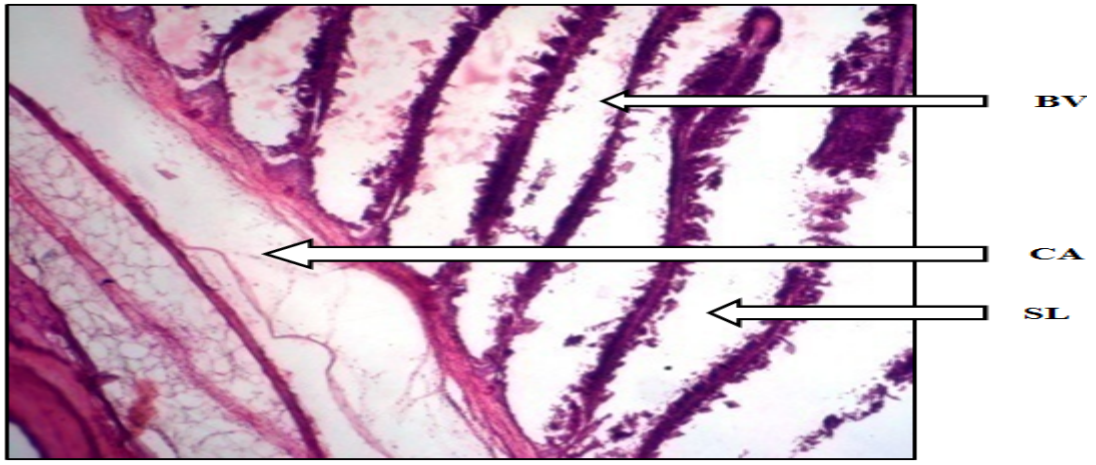

Figure 5. Photomicrograph of the gill of *C. gariepinus* exposed to 240 mg L^{-1} of aqueous extract of *C. papaya* dry seed showing alterations as the secondary lamella appears hyperchondrial and less vascularised. High degenerated lesion observed at the gill arch, filament, and lamellae. CA-Cartilage apparent, BV-Blood vessel and SL- Secondary lamella (Hematoxylin and eosin, x 100)

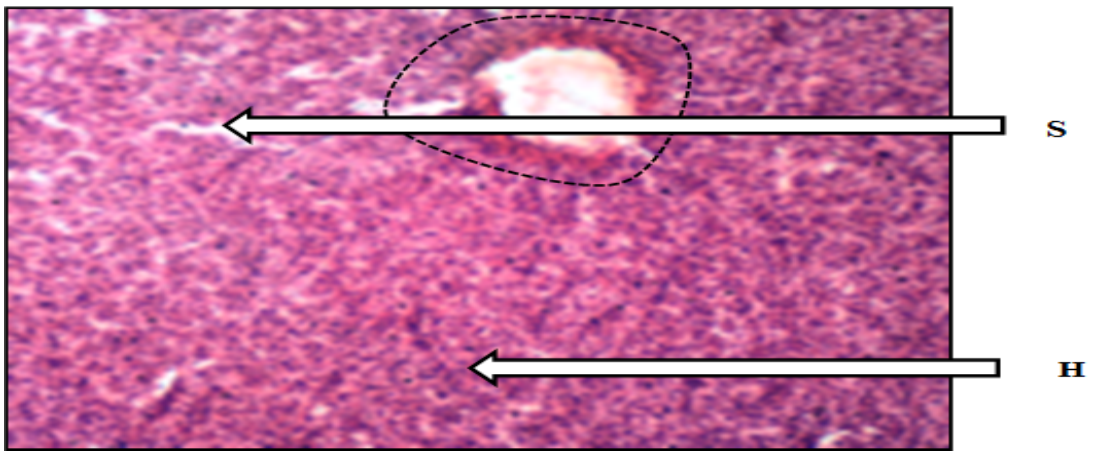

Figure 6. Photomicrograph of the liver from a control *C. gariepinus* showing typical sized spaced central vein surrounded by densely distributed hepatocytes in cords. Mild sinusoidal dilated apparent space. S-Sinusoidal and H- Hepatocyte (Hematoxylin and eosin, x 100)

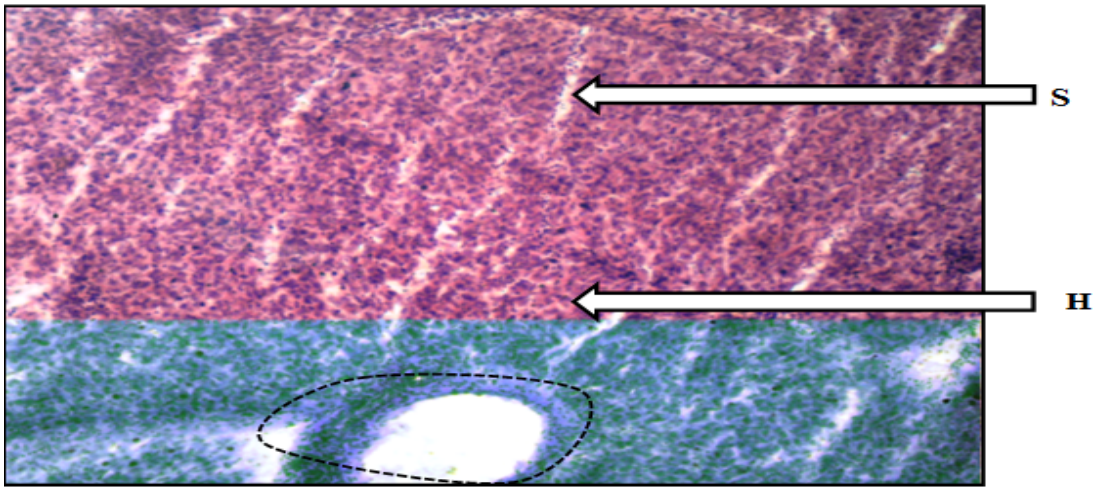

Figure 7. Photomicrograph of the liver of *C. gariepinus* juvenile exposed to 150 mg L^{-1} of aqueous extract of *C. papaya* dry seed showing vacoulation of hepatocyte. S-Sinusoidal and H- Hepatocyte (Hematoxylin and eosin, x 100)

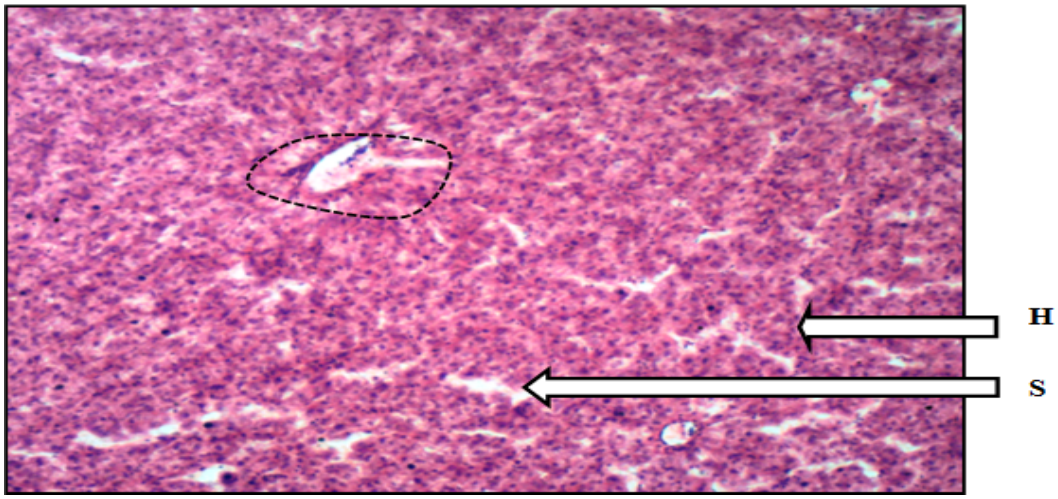

Figure 8. Photomicrograph of the liver of *C. gariepinus* juvenile exposed to 180 mgL^{-1} of aqueous extract of *C. papaya* dry seed showing hyperplasia and necrosis of hepatocyte. S-Sinusoidal and H-Hepatocyte (Hematoxylin and eosin, x 100)

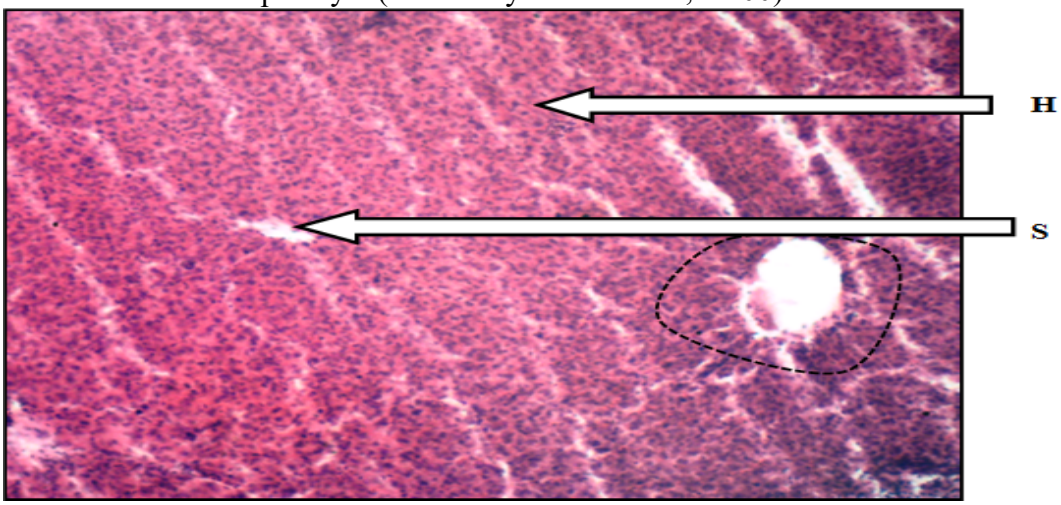

Figure 9. Photomicrograph of the liver of *C. gariepinus* juvenile exposed to 210 mg L^{-1} of aqueous extract of *C. papaya* dry seed showing hyperplasia, degeneration of hepatocyte and necrosis. S-Sinusoidal and H- Hepatocyte (Hematoxylin and eosin, x 100)

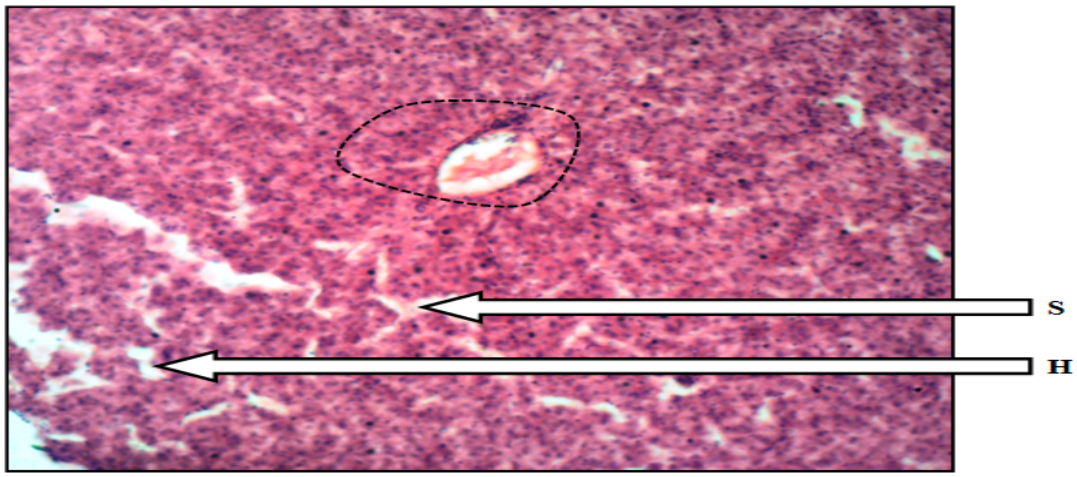

Figure 10. Photomicrograph of the liver of *C. gariepinus* juvenile exposed to 240 mg L^{-1} of aqueous extract of *C. papaya* dry seed showing steatosis, vacoulation, necrosis and degeneration of hepatocyte. S-Sinusoidal and H- Hepatocyte (Hematoxylin and eosin, x 100)

The 240 mg L^{-1} concentration of aqueous extract of *C. papaya* dry seed was observed to alter the secondary lamella of the gill and thus, made it appeared hyperchondrial and less vascularised. This result is in agreement with the study reported on fused secondary lamellae, hyperplasia and oedema in the gills of *C. gariepinus* exposed to glyphosate (Jia *et al*., 2022). Hyperplasia in some situations represents adaptations by the organism to protect underlying tissues from toxicants (Abubakar *et al*., 2019). However, increase in thickness of epithelial layers and fusion of adjacent secondary lamellae as a result of hyperplasia will not only decrease the surface area available for oxygen extraction, it will also increase the oxygen diffusion distance between water and blood (Abubakar., 2016). Thus, as hyperplasia might indeed have a protective role, it may also inhibit the respiratory, secretory and excretory functions of the gills. It lowers circulation at the gills, widen the blood spaces and thus contract the pillar cells. Variations in the epithelial surface of gills show important physiological adaptations relating to the area available for increased gaseous exchange (Islam *et al*., 2022). The vulnerability of the gill is considered because of their external location and intimate contact with water which made them liable to damage by toxicants. The lamella reduction of the treated fish must have been caused by respiratory stress. Fused lamellae can disrupt the circulation of the deoxygenated blood via the branchial arteries into the secondary lamellae in a direction opposite to that of water flow, as a result, oxygen uptake is hampered (Jia *et al*., 2022). This can cause asphyxiation, tissue necrosis and final death. Apparent lamellae oedematous changes were observed and were probably due to increase capillary permeability which could lead to difficulty in respiration and osmo regulation stress may occur (Akinbadewa *et al*., 2021).

The liver of fish may not show the diversity of pathology as seen in higher animals due to lack of Kuppfer cells in the liver sinusoid (Michalopoulos and Bhushan, 2021). However, its susceptibility to a number of toxicants cannot be overemphasized. The high proportion of fibrotic tissue within the lobules and peribilliary connective tissue of the treated fish showed hepatic cirrhosis. It is thus believed that the most dramatic cirrhosis found in fish is the peribilliary cirrhosis of the hepato-renal syndrome associated with dietary toxicity (Abd-Elhakim *et al*., 2021). The most frequent of the degeneration was hepatocytes enlargement with large vacuoles and sinusoid conjection. The shrinkage of the hepatic cells can result in cirrhosis - the contracting of the blood vessels thereby greatly impeding the portal flow through the liver (Jia *et al*., 2022). The functions of the liver such as the conversion of glucose to glycogen for storage, regulation of lipids and deamination of amino acids are

impaired. The exposure of *C. gariepinus* to glyphosate caused histopathological alterations both in gills and liver tissues. The surface area of the liver cell is also decreased which may be due to the increase in the intrabiliary fibre-connective tissue. The intercellular spaces seen are zones of total cell degeneration. Thus cirrhosis, the outcome of prolonged hepato-cellular injury is manifested by fibrosis of hepatic cords and peribiliary connective tissue.

Conclusion

In conclusion, aqueous extract of *Carica papaya* dry seed brought distortion in structure, anatomy and health condition of the exposed fish' gills and livers. The observed histological changes were as a result of various clinical factors. The higher the concentration of aqueous extract of *C. papaya*, the more severe the degree of damage to fish gill and liver. It is recommended that continual introduction of botanical extracts into the streams, rivers and ponds should be checked in order to sustain the life of aquatic biotas.

Disclosure statement: *Conflict of Interest:* The authors declare that there are no conflicts of interest.
Compliance with Ethical Standards: This article does not contain any studies involving human subject.

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