

HISTOPATHOLOGY OF GILLS AND LIVERS OF JUVENILE *Clarias gariepinus* (BURCHELL, 1822) EXPOSED TO AQUEOUS EXTRACTS OF *Caricapapaya* DRY SEEDS UNDER LABORATORY CONDITIONS

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Abstract

African Sharptooth, *C.gariepinus* (mean weight 11.81g±0.23g and mean length 9.8cm ± 0.2) were exposed to graded concentration (0, 150, and 180, 210 and 240mgL⁻¹) of aqueous extract of *C.papaya* dry seeds in triplicates for 4 days. They were fed a 25% crude protein diet at 1% biomass half at 800 and 1600h. 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 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 vacuolation, steatosis, necrosis and degeneration of hepatocyte degeneration. The findings suggest that pathology of *Clarias gariepinus*' gill and liver could be useful biomarker for assessing aqueous extract of *Carica papaya* dry seeds toxicosis.

Keywords:Gill,liver,histopathology, *Caricapapaya*andhepatocytes

Introduction

Botanical biocides contaminations of natural water are widely reported in Nigeria because of their usage by locals. Piscicidal plants such as *Blighia sapida*, *Kigelia Africana*, *Tetrapleura tetraptera*, *Raphia vinifera*, *Parkia biglobosa* and *Tephrosia vogelii* are widely used by local fisherforks (Ojoguet *al.*, 2017).Extracts ofplants such as*Deris elliptica*, *D. trifoliata*, *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.*, 2016). Adaramoye *et al.* (2010) reported that the presence of(tspace.library.utoronto.ca) macerated plant materials in rivers and streams stupefy fish for easy collection. Desai (2009) observed that the alkaloid contents of the leaves and stem of *Croton zambesicus* is active against microorganism and that the essential oil found in the leaves also contain P-cymene, linalool and beta-caryophyllene. Ajani and Ayoola (2010) added that most of the plants contain chemicals which are traditionally used to harvest fish in almost all part of the world.

Carica papaya is a perennial tree with a crown of very large palmate leavesat the base of which the fruits areclustered. Its fruits arepalatable feedstuff as well as its leaves that are widely used by the locals (Acquaye *et al.*, 2000). *C.papaya* seed contain piscicidal and anti fertilityproperties which are widely used as fish poison for controlling the excessive breeding of tilapia in some part of in Nigeria (Akah *et al.*, 2001). The active ingredients of *carica papaya* are carpine, chymopapain, papain, bactericidal a glycone of lucotropaeolin benzyl isothiocyanate, aglycoside, sinigrin, the enzyme myrosin and carpasemine and are concentrated are in the black seeds (Akahet *al.*, 2001). However, the presence of these botanicals in high concentrations may have adverse effects on aquatic organisms.

The sharptooth catfish (tspace.library.utoronto.ca) (*Clarias gariepinus*) is an ecologically important and commercially valued fish in Nigeria (Abubakar, 2013). These catfish are frequently and widely culture in fish farms all over Nigeria (Ojogu *et al.*, 2017).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.*, 2019).

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 (Idi-Ogede *et al.*2016). Furthermore, the alterations found in these organs are clearer than functional ones (Fanta *et al.*, 2003). It also reveals signs of damage to animal health (Hinton and Lauren, 2016).The gills being a respiratory organ of fish (Abubakar *et al.*, 2019) are frequentlyin contact with external environment and thus vulnerable to aquatic toxicants(Abubakar,2013).Thegillsareparticularlysensitiveandcanalsobe responsiblemaintainingtheoptimalosmoticpressureandacid-basebalanceof body fluids. Due to the high level of vascularization present in the gills, they arehighlyvulnerableto mechanical injury(Abubakar *et al.*, 2019).Theyplay an important role in excreting unnecessary and harmful metabolic products and in supporting food drainage (Genten *et al.*, 2009). 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 (Hinton *et al.*, 2016). Water flowing through the gills exposes the organ to constant contact with potentiallyharmful chemical and biological substances, which is reflected in morphological changes in the organ and impairment of its functions. Moreover, even minor mechanical damage to the structure of gills may be dangerous to fish. Lesions resulting from any of these influences may be a direct cause of increased mortality in reared fish (Abubakar *et al.*, 2019). Studies have been conducted on histopathological changes in the gills andliver of fish exposed to various substances (Auta, 2001).The liver of fish can be considered as a target organ to pollutants, alterations in its structure can be significant in the evaluation of fish health (Kolbasiet *al.*, 2009) and the effects of a wide variety of environmental pollutants. The liver plays a major role in complex enzymatic processs of tetraiodothronine (thyroxine)-tri- iodothyronine(T₄-T₃)conversion(Kolbasietal.,2009).Themetabolicateof hepatocytes is certainly modulated by thyroid hormones. Thyroid dysfunction may perturb liver function, and liver disease effects thyroid hormone metabolism(Kolbasietal.,2009).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.

Materials and methods

Procurement of *Carica papaya* seed

Pawpaw (*Carica papaya*) fruits were obtained from Gammon market in Ifelodun, Kwara State. The seeds were extracted from the fruits using Ayotunde and Ofem (2005) methods which involved 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 seeds 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.

Extract preparation

Aqueous extraction of the coarsely grinded *C. papaya* seed powder was obtained by crushing the seeds into coarsely powdery form using mortar and pestle, 1200g powdered *C. papaya* seeds was soaked in 4800ml of distilled water for 48 hours in closed container at room temperature with occasional shaking, then filtered through muslin cloth and finally the filtrate was water bath for 6 hours till the filtrate dried. The weight of extract obtained after subjecting to water bath was 34.05g. A stock solution was prepared with the extract obtained by adding 1.0g of the *C. papaya* extract in 1 Litre of distilled water using the method of Olaifa *et al.* (2003).

Collection of fish and Acclimatization

Two hundred and fifty (250) African sharptooth catfish juveniles with mean weight of $11.81\text{g} \pm 0.23\text{g}$ and mean length $9.8\text{cm} \pm 0.2$ 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 50Litres capacity jerry-can. They were held in a 1000Litres 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 2days interval. Feeding of fish was stopped 24hours prior to exposure to acute toxicity bioassay media (Abubakar (2016).

Bioassay

The following concentrations of aqueous extract of *C. papaya* dry seed were prepared using the method of Abubakar (2016).

1Litre=1000g; 1ml

= 1mg;

1500mlofthetoxicantin10litresofwater=150mg/L

1800mlofthetoxicantin10litresofwater=180mg/L

2100mlofthetoxicantin10litresofwater=210mg/L

2400mlof thetoxicant in10litres ofwater =240mg/L

Preliminary 24 hours range finding test was conducted for the juvenile catfish (*C. gariepinus*) following static bioassay procedures described by Abubakar (2016) to determine the toxic range of aqueous extracts of *C. papaya* seeds on juvenile catfish.

Ten (10) juvenile fish were selected at random into five (5) plastic aquaria with holding capacity of 20Litres.

It was filled with 10Litres 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.

Experimentaldesign

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.

Acutebioassay 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 APHA (1998) method. African sharp tooth juvenile mean weight $11.81\text{g} \pm 0.23\text{g}$ and mean length $9.8\text{cm} \pm 0.2\text{cm}$ 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 150ml, 180ml, 210ml, 240ml and a control with no toxicant. Each concentration level was in triplicate. The desired stock solution was measured using 50ml calibrated beaker and introduced into 10Litres of borehole water inside the plastic aquaria. The mixture was allowed to stand for 30 minutes for proper mixing before randomlyintroducing test fishes. One hundred and fifty(150) juveniles of African sharptooth were randomly distributed into the toxicant concentrations to give a stocking density of 10 fish per plastic aquaria using the method of Abubakar (2013). Each plastic tank was covered with nylon mesh screen to prevent the fish from leaping out of the tank. Feeding of fish stopped 24hours prior to and during the 96h 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 24h (0h, 3h, 6h and 12h) 48h, 72h and 96h exposure period.

Histopathological Analysis

One dead fish from each exposed group was chosen at random and their gills andliverswereremovedusingadissectingsetaccordingtoAbubakar(2016).

One live fish from each control group was chosen at random and euthanized according to the method of Abubakar *et al.* (2019). The gills and liver were sectioned 1-2mm, in thickness and fixed in 10% formalin (doi.org) and processed according to standard paraffin procedures. Five micrometer thick paraffin sections were placed on glass slides and stained with (doi.org) haematoxylin and eosin. Approximately 2-4 paraffin sections of each fish were histopathologically analyzed. Photomicrographs were then taken at low power ($\times 100$) 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.

Results

Histopathological 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 hepatocytes and necrosis of the liver of the exposed fish. 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. The control gills and livers had normal morphology and internal arrangement components (Plates 1 to 10) as revealed in their structures.

A.HISTOPATHOLOGICALSTUDYOFGILLS

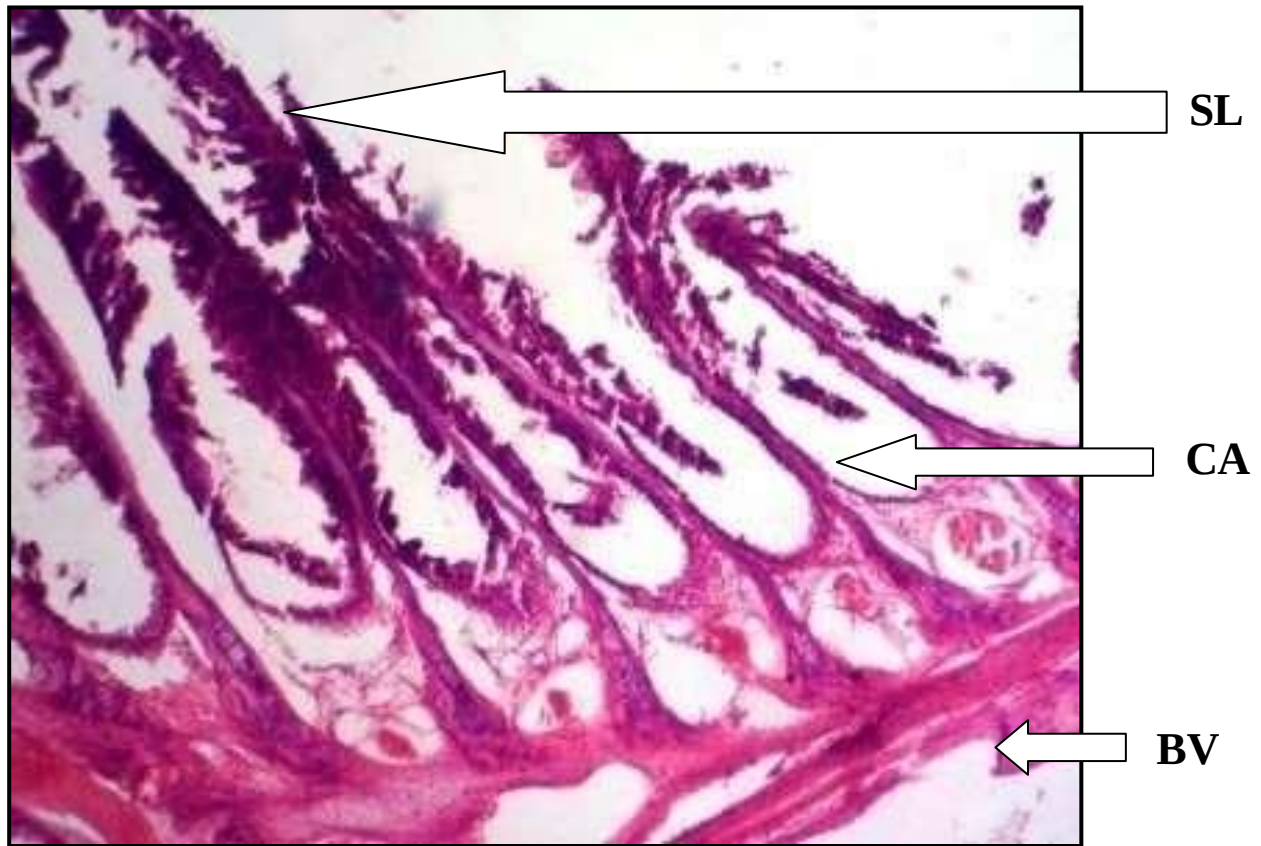


Plate 1: Photomicrograph of the gill of a control *C.gariepinus* showing normal gill histomorphology with intact blood vessels, gill slits,normal vascular secondary lamella and supporting cartilage apparent stained with H&EMag. X100.The SL, CA and BV inside the image stand for:
SL-Secondarylamella,
CA-Cartilage apparent
BV-Blood vessel



Plate2:Photomicrograph of the gill of *C.gariepinus* exposed to 150mg/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) stained with H&E Mag . X100. The SL, CA and BV inside the image stand for:
SL-Secondary lamella,
CA-Cartilage apparent
BV-Blood vessel

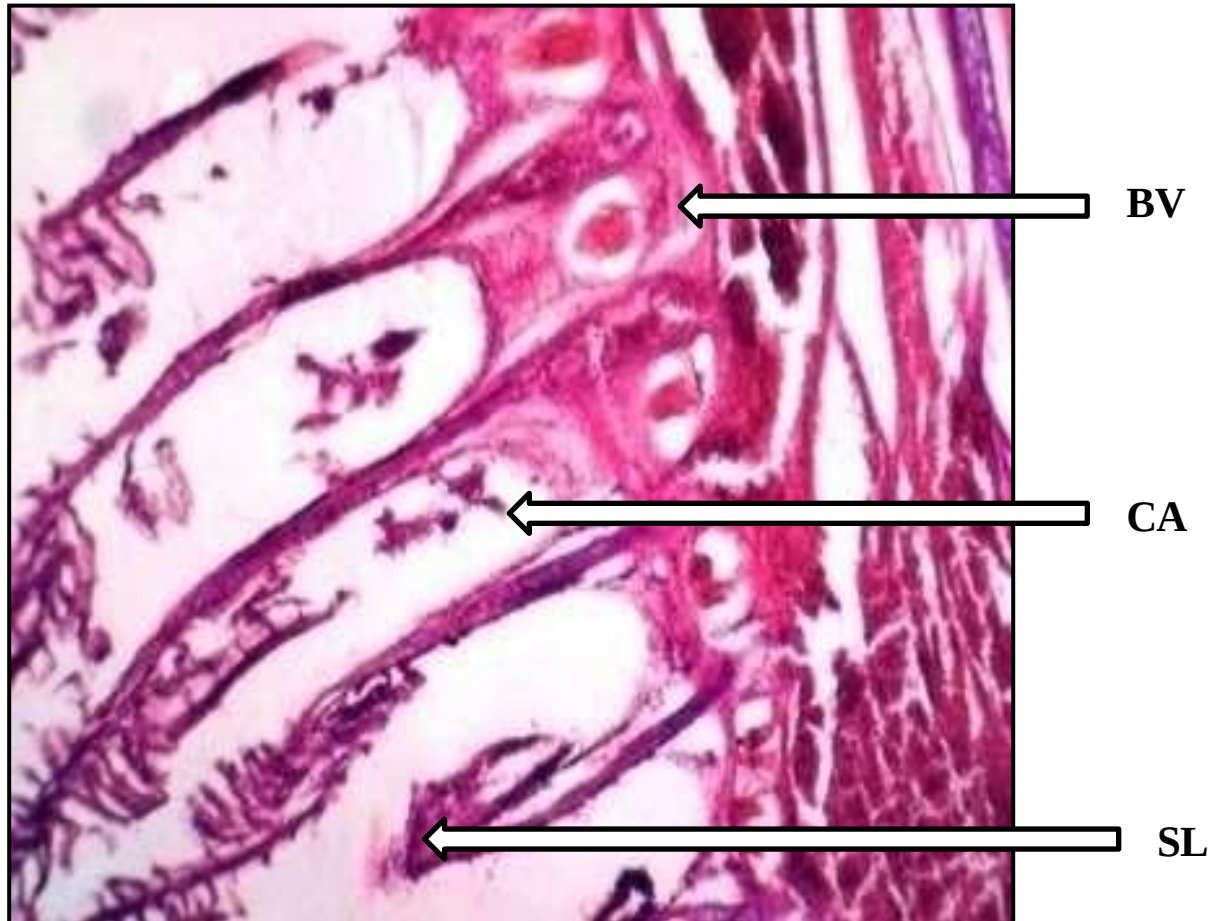


Plate 3: Photomicrograph of the gill of *C. gariepinus* juvenile exposed to 180 mg/L⁻¹ of aqueous extract of *C. papaya* dry seed showing hypertrophy of blood vessel (BV), supporting cartilage (CA) and secondary lamellae (SL) stained with H&E Mag . X100. The SL, CA and BV inside the image stand for:
SL-Secondary lamella,
CA-Cartilage apparent
BV-Blood vessel

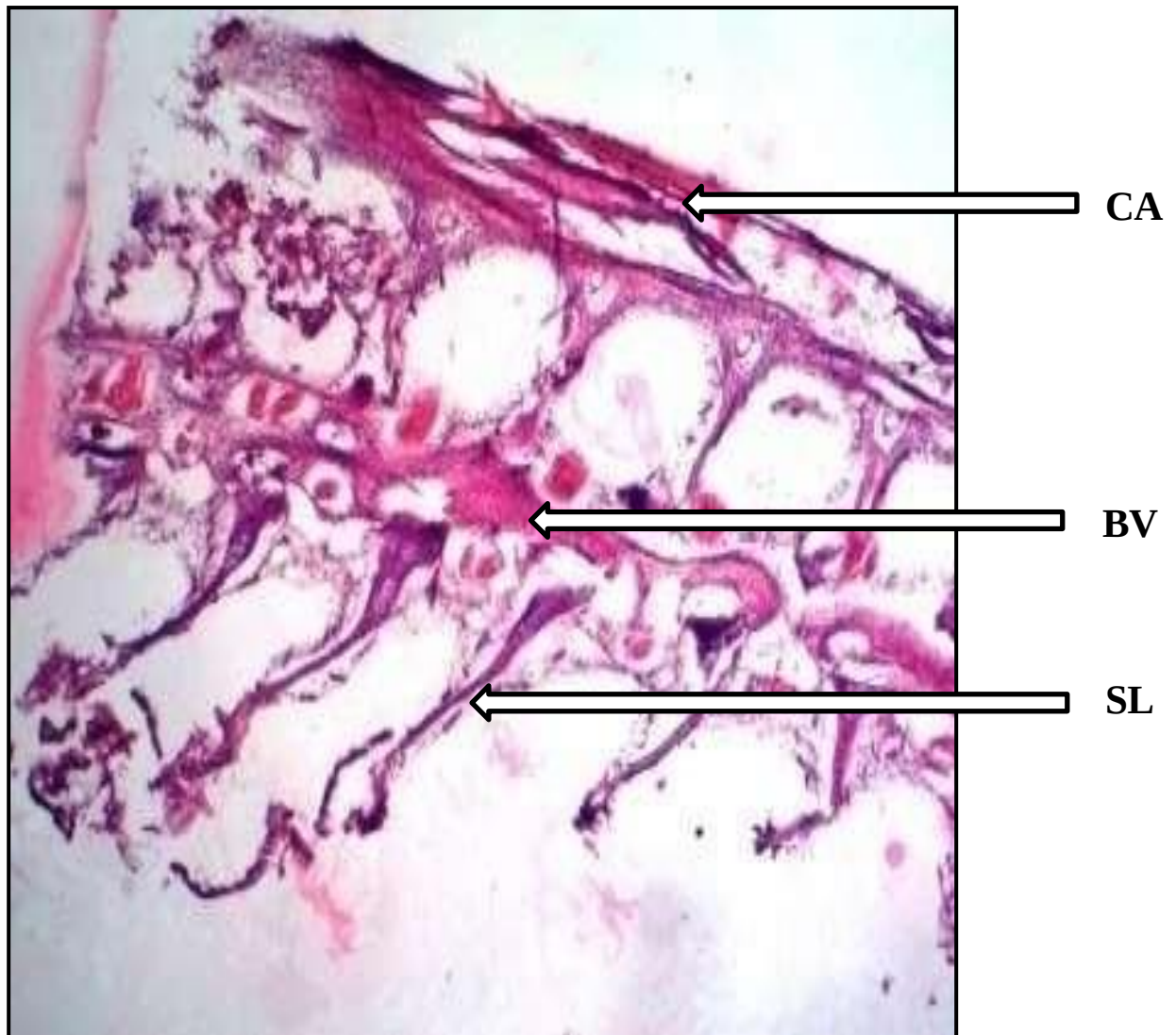


Plate4: Photomicrograph of the gills of *C. gariepinus* juvenile exposed to 210 mg/L^{-1} of aqueous extract of *C. papayadry* seed showing less vascularized secondary lamellae in the form of hyperchondria

hyperthrophyof the gill arch stained with H&EMag. X100. The SL,
CA and BV inside the image stand for:
SL-Secondarylamella,
CA-Cartilageapparent
BV-Blood vessel

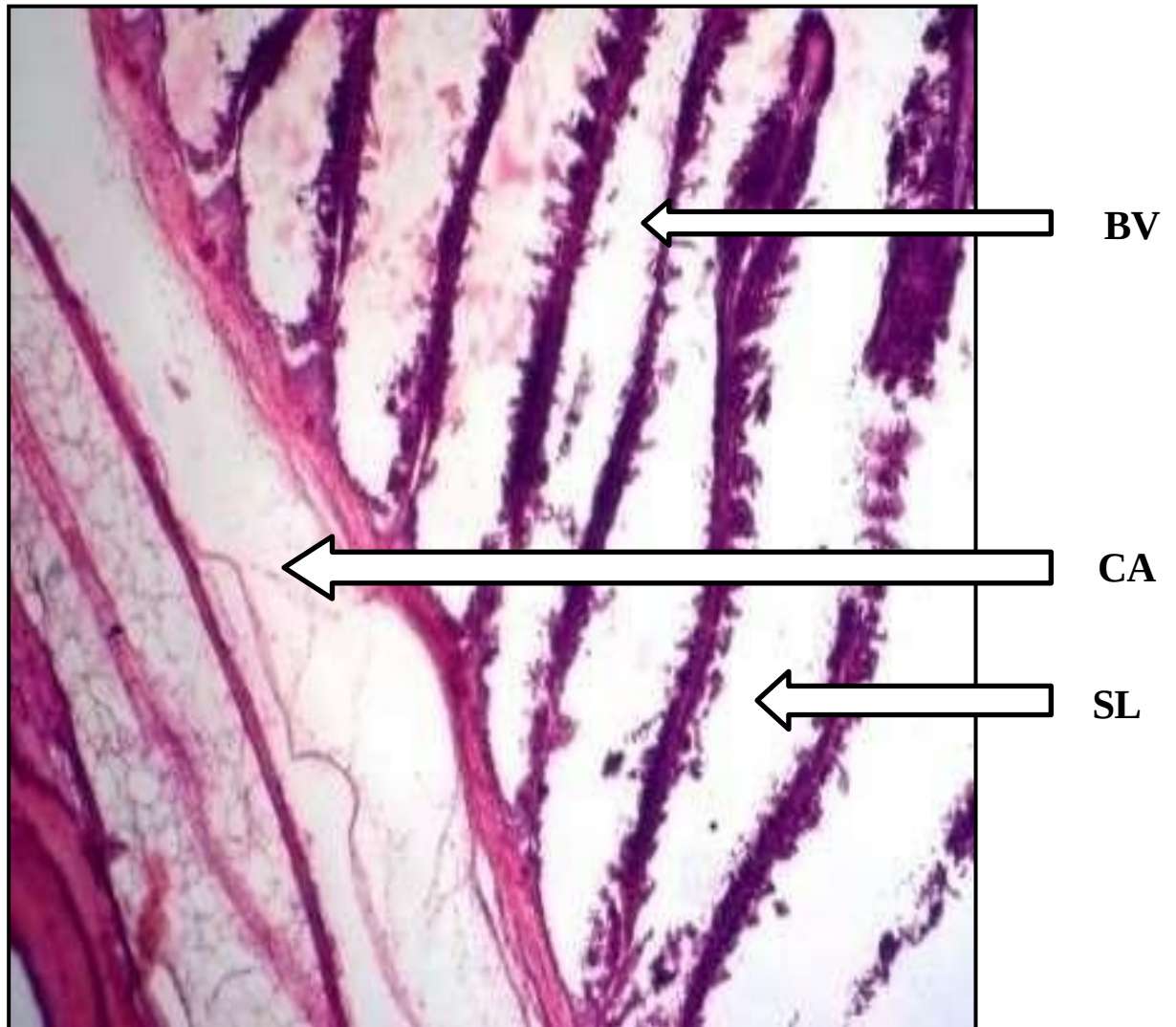


Plate5: Photomicrograph of the gill of *C.gariepinus* exposed to 240mgL^{-1} of aqueous extract of *C. papayadry* seed showing alterations as the secondary lamella appears hyperchondrial and less vascularised. High degenerated lesion observed at the gill arch, filament, and lamellae stained with H&EMag. X100. The SL, CA and BV inside the image stand for:
SL-Secondary lamella,
CA-Cartilagelapparent
BV-Blood vessel

B.Histopathologicalstudyoflivers

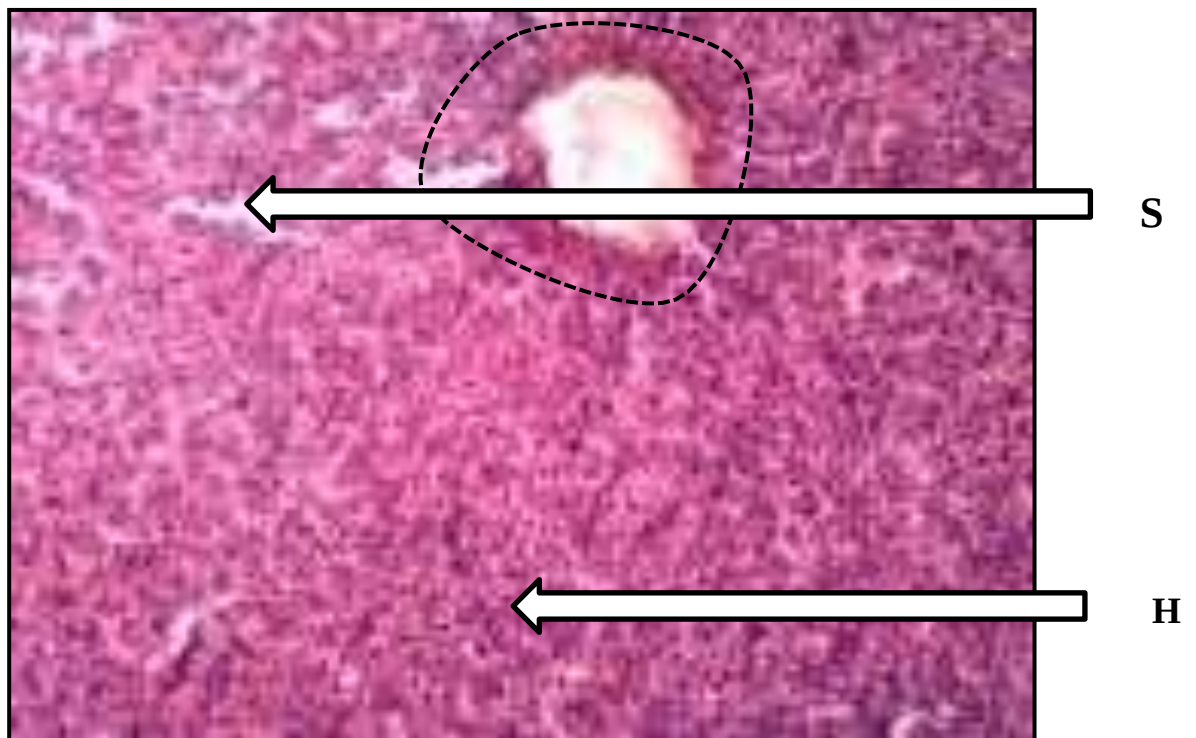


Plate 6: Photomicrograph of the liver from a control *C. gariepinus* showing typical sized spaced central veins surrounded by densely distributed

hepatocytesincords.Mildsinusoidal dilatedapparentspace stained with
H&EMag . X100. The S and H inside the image stand for:
H-Hepatocyte
S-Sinusoidal

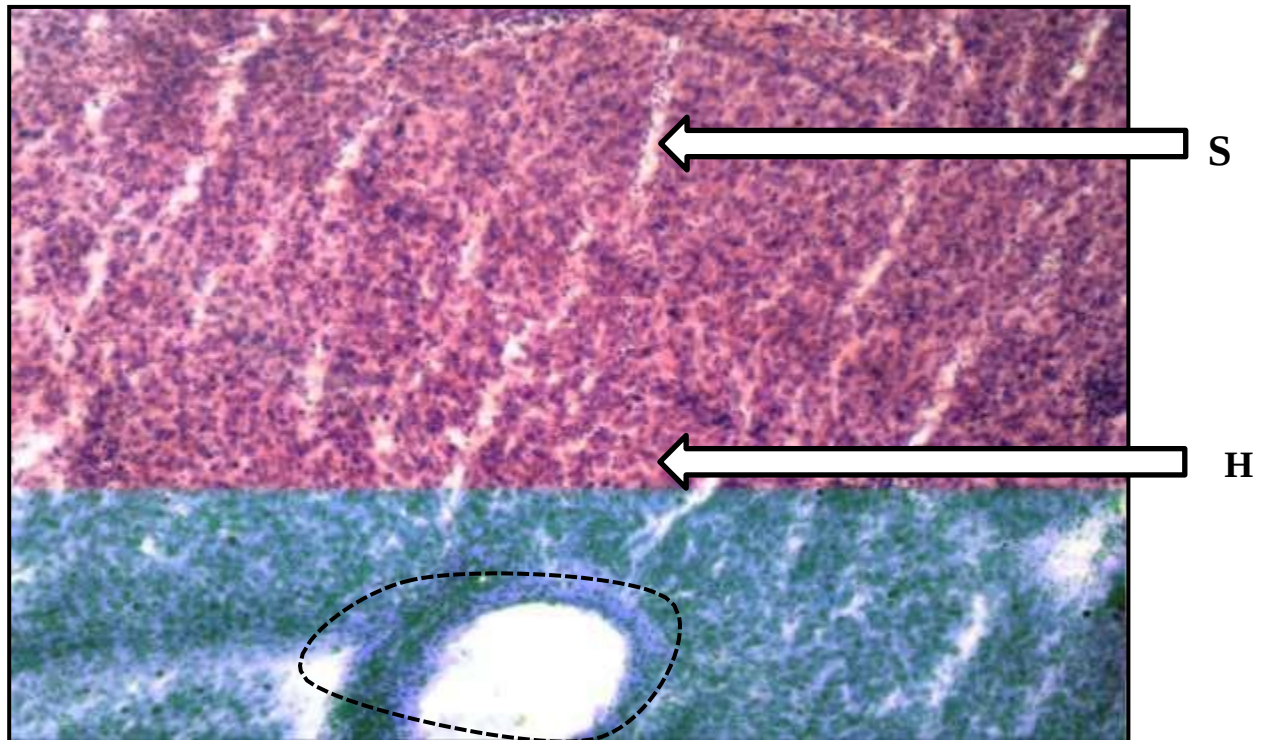


Plate7:Photomicrographoftheliverof*C.gariepinus*juvenileexposedto
150 mgL⁻¹ of aqueous extract of *C. papaya* dry seed showing
vacoulation of hepatocyte stained with H&EMag . X100. The S and H
inside the image stand for:
H-Hepatocyte
S-Sinusoidal

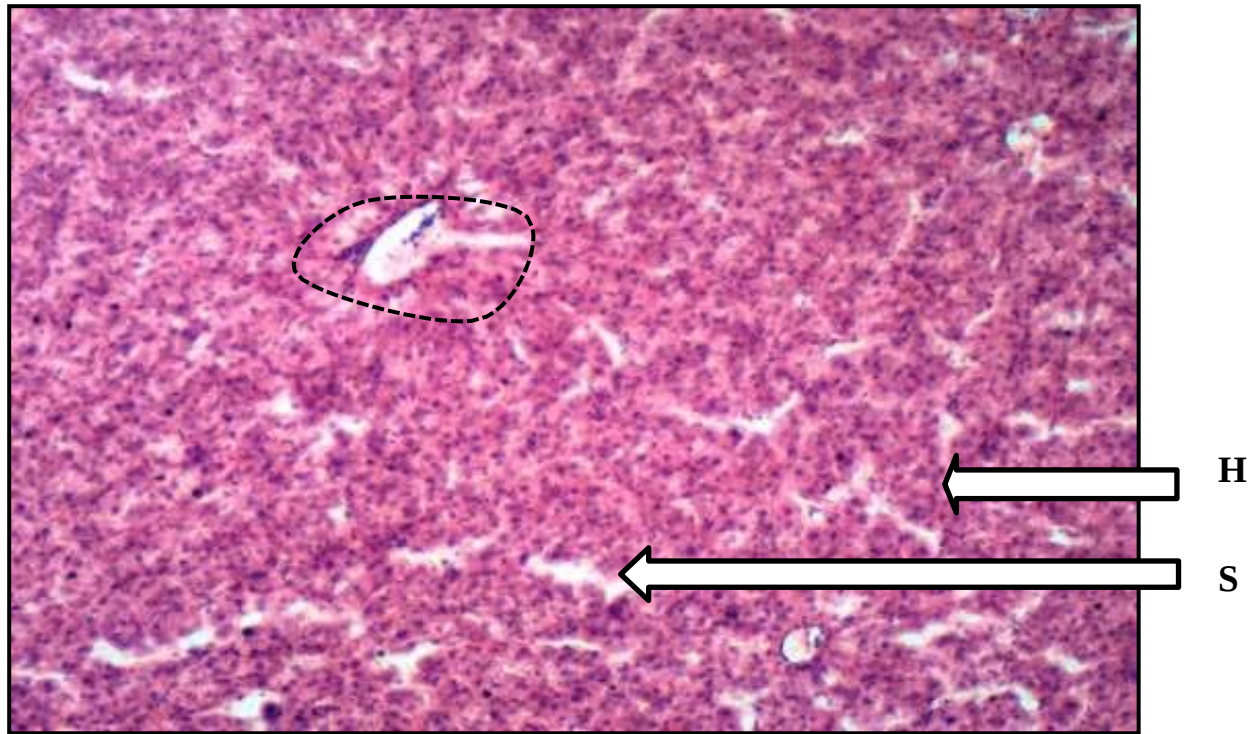


Plate8: Photomicrograph of the liver of *C. gariepinus* juvenile exposed to 180 mgL^{-1} of aqueous extract of *C. papaya* dry seed showing hyperplasia and necrosis of hepatocyte stained with H&E Mag . X100. The S and H inside the image stand for:
H-Hepatocyte
S-Sinusoidal

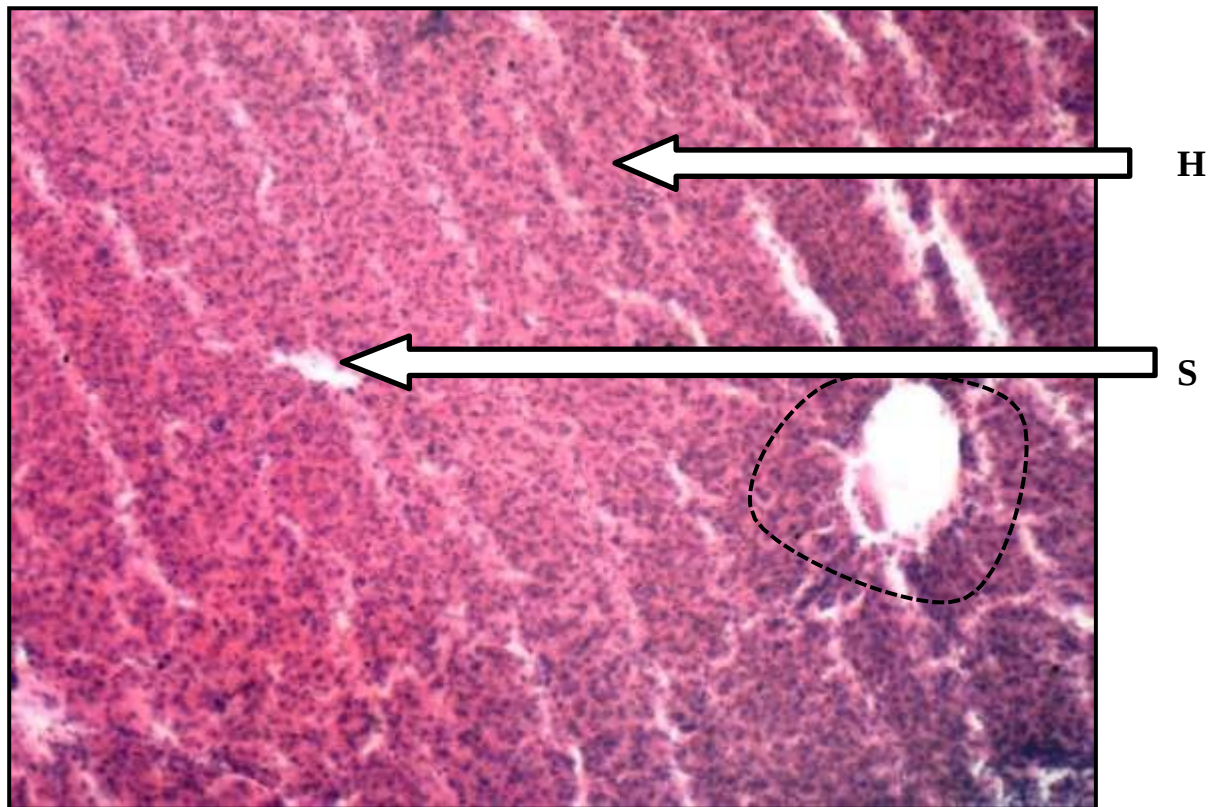


Plate9: Photomicrograph of the liver of *C. gariepinus* juvenile exposed to 210 mgL^{-1} of aqueous extract of *C. papaya* dry seed showing hyperplasia, degeneration of hepatocyte and necrosis stained with H&E Mag . X100. The S and H inside the image stand for:
H-Hepatocyte
S-Sinusoidal

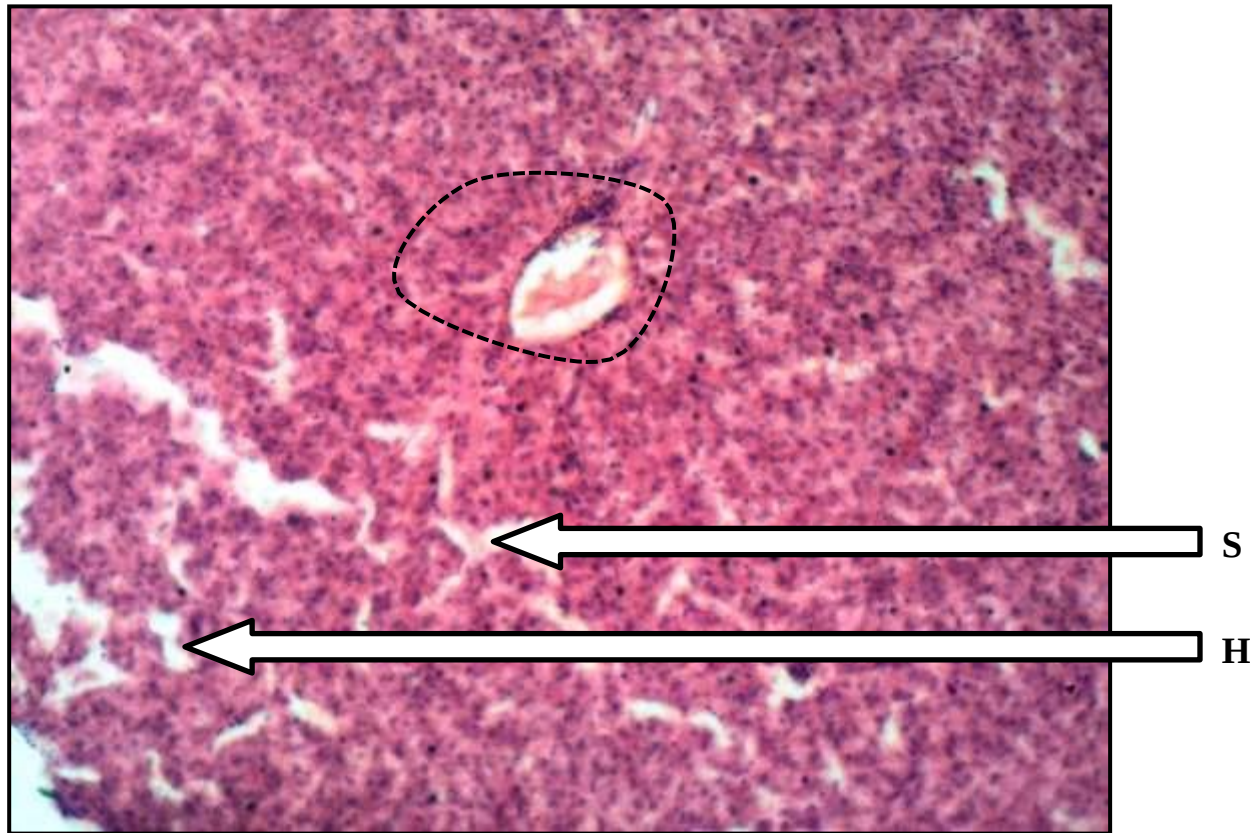


Plate 10: Photomicrograph of the liver of *C. gariepinus* juvenile exposed to 240mgL^{-1} of aqueous extract of *C. papaya* dry seed showing steatosis, vacuolation, necrosis and degeneration of hepatocyte stained with H&E Mag . X100. The S and H inside the image stand for: H- Hepatocyte; S-Sinusoidal

Discussion.

Histological study of the gills shows a typical structural organization of the lamella in the (tspace library.utoronto.ca) control fish while the exposed fish however show progressive architectural distortion(tspace library.utoronto.ca) (hyperplasia and hypertrophy of secondary lamella, degeneration of gill filament, hyperchondrial of gill arch and lesions) at various concentration levels depicts 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 (Camargo and Martinez, 2007; Kumar *et al.*, 2010). 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 of Abubakar (2013) who reported that *C. gariepinus* accumulate toxicants through the lamella surface. 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. The change in physiological property is evident in shrinkage observed at the lamella thus suggesting that extract of *carica papaya* intake mostly occurs via the gills. Prolonged exposure results in marked degeneracy of the epithelium followed by (tspace library.utoronto.ca) reduction in lamellae. Respiratory distress caused by the degeneration of gills is a sign that the total (tspace library.utoronto.ca) surface area of the gill has been drastically reduced. Asphyxiation occasioned by insufficient gaseous exchange was responsible for the death (tspace library.utoronto.ca) observed at the highest concentrations of the *carica papaya* extracts. The gill histopathology alterations recorded by *C. papaya* extracts in this study are similar to the works of Ajani and Ayoola (2010) who documented gill lesions as a result of the effect of *Adeniacissampeloides* on the gill of tilapia. Abalaka *etal.* (2010) also reported lesions on African catfish as a result of toxicity of extracts of *P. biglobosa*. It is also similar to the findings of Idi-Ogede *et al.* (2016) who studied the effects of sniper 1000E on the histology of *Oreochromis niloticus* under laboratory conditions. 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 of Olurin *et al.* (2006) who reported fused secondary lamellae, hyperplasia and oedema in the gills of *C. gariepinus* (academicjournals.org) exposed to glyphosate. Hyperplasia in some situations represents adaptations (academicjournals.org) by the organism to protect underlying tissues from toxicants (Abubakar *et al.*, 2019). However, increase in thickness of epithelial layers (academicjournals.org) 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 (Idi-Ogede *et al.*, 2016). Thus, as hyperplasia might indeed have a protective role, it may also inhibit the respiratory, secretory and excretory functions of the gills (doi.org). It lowers circulation at the gills, widens the blood spaces and thus contracts the pillar cells (Idi-Ogede *et al.*, 2016). Epithelial and Hyperplasia were also observed by Abubakar (2013). Variations in the epithelial surface of gills show important physiological adaptations relating to the area available for increased gaseous exchange (Olurin *et al.*, 2006). The vulnerability of the gill is considered because of their external location and (academicjournals.org) intimate contact with water which made them liable to damage by toxicants. The lamellar 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 (Olurin *et al.*, 2006). This can cause asphyxiation, tissue necrosis and final death. Apparent lamellae oedematous changes were observed and were probably due to increased capillary permeability which could lead to difficulty in respiration and osmo regulation stress may occur (Olurin *et al.*, 2006).

The liver of fish may not show the diversity (academicjournals.org) of pathology as seen in higher animals due to lack of Kupffer cells in the liver sinusoid (academicjournals.org) (Abubakar, 2013). However, its susceptibility to a number of toxicants cannot be overemphasized (Olurin *et al.*, 2006). The high proportion of fibrotic tissue within the lobules and peribiliary connective tissue of the treated fish showed hepatic cirrhosis. It is thus believed that the most dramatic cirrhosis found in fish is the peribiliary cirrhosis of the hepato-renal syndrome associated with dietary toxicity (Abubakar, 2006). The most frequent of the degeneration was hepatocyte enlargement with large vacuoles and sinusoid congestion (Abubakar, 2013). 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 (academicjournals.org) (Olurin *et al.*, 2006). 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 blockage of the sinusoids makes the blood flow from the hepatic artery and veins into the central vein rather difficult (Olurin *et al.*, 2006). The sinusoids widened to make up the right volume of blood in the central vein. The function of the canaliculi that form the bile duct is

hampered and as such, bile secreted from gall bladder cannot adequately get into them. The exposure of *C. gariepinus* to glyphosate caused histopathological alterations both in gills and liver tissues (academicjournals.org). The high accumulation of *c. papaya* extract in the liver of the fish signifies a key role in accumulation and detoxification. Although agent such as metallothionein in fishes when bioaccumulate in the liver tissue may reach a proportion in which the function of the liver may be impeded, thus resulting in gradual degeneration of the liver cells (Idi-Ogede *et al.*, 2016). 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. Oxygen required to support the intense metabolic activity of the liver is supplied in arterial blood via the hepatic artery (Abubakar *et al.*, 2019) which results in necrosis of parenchyma cells. Sinusoids clog (doi.org) up thereby blocking the blood from the hepatic artery and the interbiliary portal vein which will have to pass through the sinusoids (tspace.library.utoronto.ca) to get to the central vein. The inability of blood to get to the central vein on time makes the liver pump blood harder resulting in liver stress (Olurin *et al.*, 2006). The (tspace.library.utoronto.ca) kupffer cells are attached to the walls of the sinusoids (phagocytic cells of the liver which participate (with the spleen) in the removal of spent erythrocytes and other particulate debris from circulation. The function of the kupffer cells will be impeded due to the clogging up of the sinusoids (Olurin *et al.*, 2006). This makes old erythrocytes to accumulate in the blood (tspace.library.utoronto.ca). These erythrocytes are weak and their binding power to oxygen is seriously reduced such that less oxygen is taken into the body system (doi.org). The canaliculi surrounding the liver cells are also clogged by the syncytial arrangement of the degenerated liver cells and bile secretion into the canaliculi is impaired. Also lipid regulations by the liver are affected.

Conclusion

In conclusion, aqueous extract of *Carica papaya* dry seed brought distortion in structure, anatomy, physiology, morphology and health condition of exposed fish' gills and livers. It was recommended that continual introduction of (tspace.library.utoronto.ca) botanical extracts into the streams, rivers and ponds should be checked order to sustain the life of aquatic biotas.

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