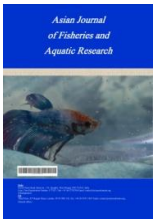

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# Toxicity of Crude Fruit Endocarp Extract of Calabash (*Lagenaria siceraria*) on African Catfish (*Clarias gariepinus*) Juveniles

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***Authors' contributions***

*This work was carried out in collaboration among all authors. Author JOA designed the study, performed the statistical analysis, managed the literature searches, experimental procedures and managed the analyses of the study. Author BSA wrote the protocol, managed the study analyses and supervised the entire process. Author TOO managed the study analyses and draft of the manuscript. Author AIU was involved in the laboratory bench work. All authors read and approved the final manuscript.*

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## ABSTRACT

**Background:** Illicit disposal of scraped out endocarp of bottle gourds as part of processing activity for domestic use into the aquatic environment pose a big threat to the aquatic ecosystem. This study investigated the toxicity effects of crude fruit endocarp extracts of *L. siceraria* on *C. gariepinus* juveniles.
**Methodology:** 120 mixed sex of *C. gariepinus* juveniles of mean weight and length (19.59 ±0.42 g; 14.6 ±0.80 cm) respectively were investigated. The fish were divided into 6 groups of 10 fish in each aquarium containing 5, 20, 35, 50 and 65 mg/L concentration of aqueous extract of *L. siceraria* and 0.00 mg/L (control) respectively with replicates for Four (4) days for acute toxicity analysis. The animals were sacrificed and blood samples collected for biochemical and isolation of the gills and liver for histopathological studies.

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**Results:** Experimental media pH, free carbon (iv) oxide, total alkalinity contents increased while dissolved oxygen decreased significantly ($P<0.05$) with increase in concentration in the acute bioassay. Temperature did not differ significantly ($P>0.05$) in all the groups thus, did not affect fish survival. Furthermore, AST, ALT and LDH profiles in blood serum of fish exposed to acute concentrations exhibited a significant increase ($P<0.05$) with increase in concentration. The exposed gills revealed progressive striking histological alterations viz. thickening and shortening of secondary lamellae, degeneration of connective tissue and complete loss of secondary lamella while the liver showed progressive histo-architectural distortions such as hepatic hypertrophy, cellular degeneration (necrosis), haemorrhage and intracellular infiltration with increase in concentration of the *L. siceraria* extract.

**Conclusion:** Aqueous extract of *L. siceraria* had adverse effect on juveniles of *C. gariepinus*, as evident in the negative modification of the body physiology via biochemical and histological investigations, thus toxic to the aquatic life. Further investigations on other vital organs - kidney, heart, GIT and the reproductive organs are recommended.

*Keywords: Acute toxicity; Lagenaria siceraria; gills; liver; blood; Clarias gariepinus.*

## 1. INTRODUCTION

The concern over increase reduction in the availability of aquatic animal protein and drastic extinction of same as a result of man's illicit activities has become a global phenomenon. Some of these are characterized by the indiscriminate washing of various substances into the aquatic ecosystem. This includes bitter bottle gourds (*Lagenaria siceraria)* when being processed into floats on water bodies, domestic utensils and wind musical instruments and pipes. Generally, plant is a store of wide range of structurally diverse bioactive substances [1]. Calabash like other members of the cucurbitaceae family contains cucurbitacins that are known to be cytotoxic at high concentration. The tetracyclic triterpenoid cucurbitacins present in fruits and vegetables of the cucumber family are responsible for the bitter taste [2]. High levels of cucurbitacin compounds are triggered by high temperature, wide temperature swings, low pH, very little water, low soil fertility and also due to improper storage of vegetables or over matured vegetables. These compounds are highly toxic to mammals and when absorbed into the blood, could cause hepatitis, pancreatitis, cholecystitis and renal damage [3]. Fishes are used as bio-indicators of pollution, because they have the potentials to retain significant dissolved contaminants that bioaccumulate in food chain and usually results to death in aquatic ecosystem [4]. *Clarias gariepinus* is a large cat fish that belongs to the family Clariidae [5]. The catfish is widely cultured throughout Africa in both natural and artificial habitats [6;7]. Owing to its fast growth rate and superior tolerance to deranged water quality, it remains the choice fish for research on aquatic ecotoxicity [8]. The aquatic ecosystem like the terrestrial environment, is continuously subjected to changes in quality that are due to the introduction of substances of diverse characteristics arising from man's cultural activities [9]. The accumulation of toxicants in an aquatic environment can result in reduced reproductive capabilities, alter growth rates and reduced ability to withstand variations in pH, temperature and dissolved oxygen [10]. Enzymes such carboxyesterase, lactate dehydrogenase (LDH) alanine and aspartate aminotransferases (ALT and AST) as well as alkaline (ALP) and acid phosphatases (ACP) have been determined in aquatic organisms for monitoring water pollution and are useful biomarkers to evaluate pollution levels in aquatic systems [11]. Cumulative effects of biochemical and physiological levels occur on exposure to toxicant and these effects depend on the nature of the toxicant, exposure time and environmental conditions [12]. Histopathology is an important component of several measures of fish health and histopathological markers have been recommended for field application, more often as a generalized, nonspecific response to severe stressful stimuli [13]. Gills are the most delicate structure of the teleost body that they are liable to damage by any irritant material in water whether dissolved or suspended. Therefore, gills are potentially useful to monitor the health of fish [14]. The liver is a very important organ performing vital functions such as detoxification, synthesis of several components of blood plasma, glycogen storage and release of glucose to the blood [15]. It also remains one of the organs that are sensitive to pollutants in aquatic environment [16]. The monitorization of histological changes in fish liver is a highly sensitive and accurate way to assess the effects

of xenobiotic compounds in field and experimental studies [17]. Fish injected with the dose 10ml of Cassava (effluent) waste water revealed severe necrosis, hypertrophy and vacuolation of hepatocytes [18]. Contamination of the water body may be linked with modification of the body physiology including the blood system leading to hyperaemia. Devi and Mishra [19] and Chamarthi et al [20] found leucocytes infiltration in *Channa punctatus* and *Cyprinus carpio* liver under chlorpyrifos and quinalphos exposure. In addition, Al-Mamoori et al [21] determined such histophatological changes, as well as, vasodilatation in small blood vessels and necrosis in *C. carpio* liver after acute and chronic exposure with 0.05 mg/L, 0.1 mg/L and 0.25 mg/L Chlorpyrifos. There was striking histological alterations such as, hepatocellular degeneration, central and sinusoidal congestions in the liver of *C. gariepinus* exposed to acute concentrated grades of *Vernonia amygdalina* [22].

Topal et al [23] reported lamellar oedema, cellulary infiltration, lamellar disorganization, degenerative changes in lamellar epithelium and lamellar thickening because of inflammation in the gill tissues of rainbow trout juveniles exposed to acute boric acid. *Clarias gariepinus* is used in the study because of its wide distribution in environment and easy acclimation to laboratory conditions [24]. This study was designed to investigate the effects of acute concentration of aqueous crude endocarp extracts of bottle gourd on the physiological wellbeing of the juveniles of African catfish via biochemical and histopathological investigation.

## 2. MATERIALS AND METHODS

### 2.1 Procurement and Preparation of Experimental Plant (*Lagenaria siceraria)*

Dried bitter gourd fruit (Calabash: *L. siceraria*) was purchased from a local market in Jos, Plateau State, Nigeria. The dried fruit was carefully opened into two halves using a hand saw and then endocarp was scraped out, ground into powder then sieved with 90 $\mu$ m mesh size plastic sieve and stored in airtight polyethylene bag for use according to Audu et al [25].

### 2.2 Phytochemical Analyses of Crude Fruit Endocarp Extract of *L. siceraria*

Phytochemical screening for alkaloid, flavonoid, tannin, saponin, cardiac glycosides and steroid was carried out using standard qualitative procedures as described by Solowora [26] and Trease and Evans [27].

### 2.3 Collection and Preparation of Animals (*Clarias gariepinus*)

A total number of 180 live and apparently healthy, mixed sex Juveniles of *C. gariepinus* were purchased from Catfish Experts Global Ventures in Zarmaganda, Jos, Plateau State, Nigeria. The fish were transported to Hydrobiology and Fisheries Research laboratory of University of Jos, Nigeria and were transferred into six (6) plastic tanks and were allowed to acclimatize to laboratory conditions for a period of two weeks. The animals were fed with 2mm extruded floating (Top feeds®) twice daily at 3% of their body weight. Three quarters of the water in the tank was siphoned out on daily basis to remove left over feed and faecal matter and replaced with fresh water. Mortality observed during acclimation were replaced and allowed to stabilize to zero. Feeding was stopped 24 hours prior to exposure to the bioassay media to avoid interference of faeces [28].

### 2.4 Preparation of Stock Solution of *L. siceraria*

Range finding test was conducted for accurate $LC_{50}$ (Median lethal concentration that causes 50% mortality of exposed animals). Based on the range finding test (RFT), five definitive concentrations (65, 50, 35, 20 and 5mg/L) of crude fruit endocarp extracts of *L. siceraria* were obtained [22]. The five test concentrations were weighed and macerated in 10 litres of water each for 24 hours. The solution was filtered using a funnel choked with non-absorbent cotton wool to obtain stock solutions.

### 2.5 Experimental Design

A total of twelve (40 x 25 x 23 cm) transparent rectangular glass tanks of 22.5 L capacity each with 10 liters of de-chlorinated municipal tap water were divided into 6 groups. Each test tank was replicated. Tanks were designated as A1 – A2, B1 – B2, C1 – C2, D1 – D2, E1 – E2 and control F1 – F2. Fish were sorted into cohort and distributed in batches of ten fish/per experimental tank including the control [29].

### 2.6 Acute Toxicity

The animals were weighed and length measured while the means of weight (19.59 ± 0.42g) and

length (14.6 ± 0.80cm) were determined. Acute toxicity of *L.siceraria* was carried out according to the methods described by EPA [30]. After acclimation period of 7 days, feeding was discontinued 24hours before the commencement of the experiment to minimize the production of wastes in the test containers. Ten juveniles of the test organisms *(C. gariepinus)* were randomly distributed into each of the six (6) aquaria and their replicates containing 5, 20, 35, 50 and 65 mg/L concentration of *L. siceraria* and 0.00mg/L (control) kept devoid of the plant extract. During the 96hrs static non-renewal bioassay, temperature was measured every 24 hours while pH, dissolved oxygen, free carbon dioxide total alkalinity were measured at the begi and end of the 96hrs bioassay using star methods [31]. The test was carried out unde natural photoperiod of 12:12 light –dark c Mortality was observed hourly for the first and recorded at 12, 24, 48, 72 and 96$^{th}$ intervals. The criteria for mortality include fa to respond to touch stimuli using a glass rod

## 2.7 Biochemical Examination

Blood samples were collected from the surv fish into non–heparinized tubes through ca puncture. This was centrifuged immediate 1500 rpm for five minute to obtain plasma Alkaline phosphatase (ALP), Aspa aminotransferase (AST), Ala aminotransferase (ALT), Total protein (TP), Bilirubin (TB), Direct Bilirubin (DB) and La dehydrogenase (LDH) were assayed with th of GENESYS-20 spectrophotometer (GENE 20. Thermo Electron Corporation USA) follo the manufacturer's instruction of the of Ran Spectrum and Agape reagent kits Biochemistry Department of National Veter Research Institute Vom, Plateau State Ni according to Audu et al. [25].

## 2.8 Histopathological Examination

Fish from the different groups were sacri dissected after which the gills and liver isolated. The tissues were carefully washe remove all traces of blood and then put histological bottles containing ten percent fo saline solution [22]. The bottles were car labeled with their corresponding concentrat Histopathological examinations systemically carried out in the central diagn unit of National Veterinary Research Ins Vom, Plateau State Nigeria. Analysis was b on the conventional method proposed Avwioro [32]. After which the fixed slides were microscopically examined and effects of the toxicant on the tissues were observed and recorded. Micrographs of exposed tissues of the different concentrations were taken and concluded based on the difference in their physiology drawn [32].

## 2.9 Statistical Analysis

Data were analyzed using SPSS 18.0. All data were expressed as mean ± SEM. Values were expressed as significance ($P$<0.05).

## 3. RESULTS AND DISCUSSION

Phytochemicals are plant derived chemicals which are beneficial to human health and disease prevention [33]. From this study, result of the phytochemical screening of aqueous fruit endocarp extract of *L. siceraria* showed that the endocarp of the plant contained varied proportions of some bioactive substances which are; alkaloids, flavonoids, cardiac glycosides and saponins in an aqueous solution (*Table 1*). This result is similar to the findings of Alamgir et al [34] who reported the presence of phytoconstituents including alkaloid, glycosides, steroids, tannins, saponin and flavonoid in different crude extracts of *Lagenaria siceraria*, *Cucumis sativus* and *Cucurbita maxima*. It is important to mention that phytochemical constituent analyses vary with the geographical location [35]. On the impact of bioactive substance on fish health, studies have incriminated saponin and alkaloid in fish intoxication [36];[37]. Saponins are recognize dichthyotoxins that are capable of inducing respiratory asphyxiation and erythrocytes damage [36], while alkaloids can partake in the inhibition of oxidative phosphorylation thereby blocking the mitochondria enzyme, NADH ubiquinonereductase and could culminate in oxygen consumption compromise [37]. Tiwari and Singh [38] reported that at very high concentration, flavonoids chelate metals such as iron and zinc and reduce the absorption of these nutrients. They inhibit digestive enzymes and may also precipitate proteins, while cardiac glycosides have a highly toxic effect on the vertebrate heart and also activate the nerve centres in the brain that causes vomiting. The phytoconstituents of the fruit endocarp extract of *L. siceraria* may be responsible for the alteration of the physico-chemical parameters of the water in the experimental tanks, the blood biochemistry and the histo- architectural pattern of the gills and liver.

**Table 1. Result of phytochemical screening of *L. siceraria* fruit endocarp crude extract**

| Bioactive substances | Remarks | Colour |
|---|---|---|
| Alkanoid | + | Orange |
| Flavanoid | + | Yellow |
| Tannins | - | Blank |
| Saponins | + | Froth |
| Cardiac glycosides | + | Redish brown |
| Steroid | - | No ring formation |

*Key: + Present, - Absence*

Results of mean water quality parameters measured during the 96 hours experimental period showed that the pH of the experimental tanks were exhibited some degree of variations. The pH were observed to increase with increase in toxicant concentrations with the highest at 65mg/L (pH 7.88), while the lowest pH value of 7.65 was recorded from the control tank (0.0mg/L) – (Table 2). Noga [39] recommended pH range of 6.5- 8.5 for fresh water fish. This study however showed that the pH of the experimental tanks were within the recommended range, hence, could not have affected the well-being of the experimental animal. Similarly, free carbondioxide ($CO_2$) and total alkalinity increased with increase in toxicant concentration. The highest free $CO_2$ value of 10.8mg/L was obtained in the tank with concentration 65.0mg/L, while the least value of 5.98mg/L was recorded in 0.0mg/L tank. The lowest toxicant concentration (0.0mg/L) recorded the lowest alkalinity value of 30.95mg/L, while the highest alkalinity value of 42.60mg/L was recorded from highest toxicant concentration (65.0mg/L). This is similar to the findings of Audu et al [25]. According to Capkin et al [40], total alkalinity above 20mg/L can significantly increase the survival rate of fishes, thus, the higher total alkalinity recorded in this study was ideal for fish survival. However, Dissolved oxygen (DO) values were observed to decrease with increase in concentration of the toxicant. The least DO value of 1.9mg/L was obtained in the tank with the highest concentration of 65.0mg/L while the highest value for DO was obtained in the control tank(0.0mg/L) with DO value of 5.8mg/L. Prasad et al [41] reported that the reduction in dissolved oxygen content in a bioassay media as toxicant concentration increased, may be due to antioxidant property of the toxicant. Similar reason must have been responsible for the decrease in DO with increased in intoxicant concentration as observed in this research. Correspondingly, Temperature ranged from 23.75–23.38$^0$C and was observed to have decreased with increase in toxicant concentration. The least temperature of 23.38$^o$C was recorded in tank with the highest concentration of 65.0mg/L while the highest temperature of 23.75$^o$C was recorded in the control tank (0.0mg/L).The temperature range recorded in this study were within the range for the fish as Madevi et al [42] reported 20 – 28$^0$C for the culture of test fish. Therefore, the temperature was within acceptable limits for fish culture [43]. (Table 2).

Blood enzymes of *C.gariepinus* juveniles treated with acute concentration of *L. siceraria* fruit endocarp extract showed AST, ALT and LDH values as 42±1.0, 29±0.0 and 178.52±1.88 µ/l respectively, with the highest at 65mg/L. However, higher ALP, TB, DB and TP with the values (141.00±1.0 µ/l, 0.213±0.001, 0.165±0.005 mg/dl and 52.93±1.13 g/dl respectively) were observed in the control tanks than other concentrations – Table 3.

**Table 2. Mean water quality parameters in tanks for acute bioassay of *Clarias gariepinus* juveniles exposed to fruit endocarp crude extracts of *Lagenria siceraria***

| Parameters | Concentration (mg/L) | | | | | |
|---|---|---|---|---|---|---|
| pH | 0.00 | 5.0 | 20.0 | 35.0 | 50.0 | 65.0 |
| | 7.65 ±0.12 | 7.73 ±0.14 | 7.78 ±0.17 | 7.85 ±0.15 | 7.85 ±0.15 | 7.88 ±0.16 |
| Free $CO_2$ (mg/L) | 5.98 ±1.59 | 6.38 ±1.52 | 7.85 ±1.78 | 8.68 ±2.15 | 9.90 ±2.55 | 10.80 ±3.00 |
| Alkalinity(mg/L) | 30.95 ±14.29 | 33.53 ±15.78 | 35.40 ±15.84 | 37.80 ±15.54 | 41.20 ±17.97 | 42.60 ±18.25 |
| Dissolved Oxygen (mg/L) | 5.80 ± 0.32 | 5.38 ±0.28 | 3.88 ±0.32 | 3.25 ±0.24 | 2.43 ±0.08 | 1.90 ±0.06 |
| Temperature($^o$C) | 23.75 ±0.60 | 23.75 ±0.60 | 23.63 ±0.66 | 23.63 ±0.66 | 23.63 ±0.66 | 23.38 ±0.80 |

*Values mg/L ± standard mean error*

**Table 3. Mean concentration of biochemical parameters in blood serum of *Clarias gariepinus* juveniles exposed to acute concentration of crude fruit endocarp extract of *Lagenaria siceraria***

| S/No | CONC. g/L | AST u/l | ALT u/l | ALP u/l | TB mg/dl | DB mg/dl | TP g/dl | LDH u/l |
|---|---|---|---|---|---|---|---|---|
| 1. | 0.0 | 32 ±1.0 | 23 ±1.0 | 141.00 ±1.0 | 0.213 ±0.001 | 0.165 ±0.005 | 52.93 ±1.13 | 155.08 ±1.02 |
| 2. | 5.0 | 35 ±0.0 | 25 ±0.0 | 140.65 ±1.15 | 0.213 ±0.0 | 0.108 ±0.01 | 51.11 ±0.89 | 156.53 ±0.12 |
| 3. | 20.0 | 35 ±1.0 | 27 ±1.0 | 75.81 ±2.49 | 0.195 ±0.001 | 0.098 ±0.004 | 50.60 ±0.98 | 168.42 ±2.79 |
| 4. | 35.0 | 38 ±2.0 | 27 ±0.0 | 69.75 ±0.95 | 0.183 ±0.001 | 0.097 ±0.001 | 49.59 ±0.99 | 168.04 ±2.96 |
| 5. | 50.0 | 38 ±1.0 | 28 ±1.0 | 69.03 ±1.03 | 0.185 ±0.009 | 0.085 ±0.004 | 49.65 ±0.45 | 175.22 ±2.98 |
| 6. | 65.0 | 42 ±1.0 | 29 ±0.0 | 60.01 ±1.99 | 0.171 ±0.001 | 0.076 ±0.004 | 48.14 ±0.98 | 178.52 ±1.88 |

*Values ± standard mean error. ALP= alkaline phosphatase, TP = total protein, DB= direct bilirubin, AST= aspartase aminotransferase, TB= total bilirubin LDH=lactate dehydrogenase & ALT = alanine aminotransferase*

Alteration of fish blood biochemistry is indicative of unsuitable environmental conditions or the presence of stress factors [44]; [45]; [46]. Therefore, the measurement of serum biochemical parameters is useful biomarkers in toxicology [47]. In this present study, AST, ALT and LDH profiles in blood serum of *C. gariepinus* juveniles exposed to acute concentration of fruit endocarp crude extract of *L. siceraria* exhibited a significant increase ($P$<0.05) with increase in concentrations as compared with the control. Similar results were previously recorded in the blood of Nile tilapia and African catfish after exposure to polluted water [48]. Increased levels of ALT indicate an adaptive response to its leakage into the blood stream due to the presence of water toxicity (*Table 3*). According to Wright and Plummer [49], ALP is employed to assess the integrity of plasma membrane and endoplasmic reticulum. In this present study, ALP activity decreased significantly ($P$<0.05) with increase in acute concentrations. ALP activity observed in the acute bioassay revealed that the plant extract altered the integrity of the serum of the test fish. This agrees with the findings of Audu et al [25,] who reported a decrease in ALP activity of Common carp (*Cyprinus carpio*) exposed to crude leaf extract of *C. sativa*.

According to Oluah et al [50], LDH catalyze the biochemical process of converting pyruvate to lactate with the attendant oxidation of NADPH. Also, an increased LDH activity serum are indications of a shift in the carbohydrate metabolism from the glucose and glycogen catabolism to lactate synthesis which reflects the possible dependence of *C. albopunctatus* on anaerobic pathway during exposure to sub-lethal Actellic 25 EC and Gammalin 20. This is in tandem with the result of the present studies in which the highest level of LDH was recorded in the highest toxicant concentration with low level of dissolved oxygen (Table 3). The protein synthesis of an organism is of important diagnostic significance because of its involvement in enzymes, hormones, and antibodies. Thus, the influence of toxicants on total protein concentration of fish has been taken into consideration to evaluate the response to stressors and consequently the increasing demand for energy [51]. In the present study, the total protein concentration in the blood of African catfish were significantly decreased ($P$<0.05) as the concentration increases. This is not in line with the findings of Osman et al [48], who reported a significant increase in total protein concentration from upstream (clean water) to downstream (polluted water) sites. Such increase in total protein reflects liver dysfunction due to heavy toxicant concentrations [52].

Histological changes in gill, liver and kidney of fishes are useful in assessing toxic effects of toxicant in fish [17]. The gills are among the most vulnerable structures of the teleost fish because of their external location and intimate contact with the water. So, they are liable to damage by any irritant materials whether dissolved or suspended in the water [53].

Sections of the gills of *C. gariepinus* juveniles exposed to acute concentrations of *L. siceraria*

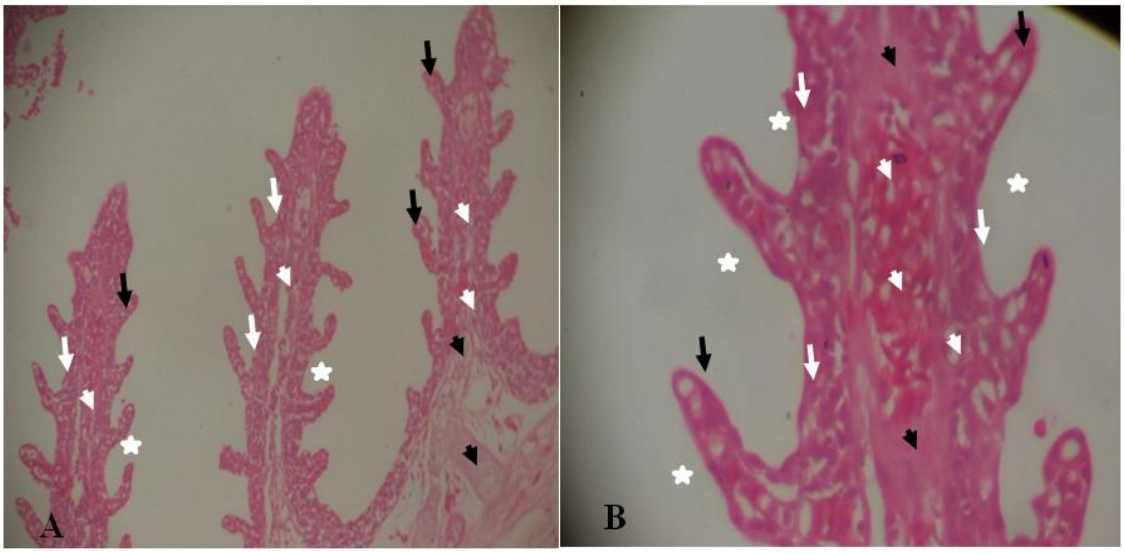


**Plate 1. Gills of *Clarias gariepinus* exposed to 0.00 mg/L of Crude Fruit Endocarp Extract of *Lagenaria siceraria for* 96 hours**

*Key: Showing normal morphology. The Secondary lamellae (black arrows) appear straight and attached to the primary lamellae (white arrows) at approximately equal intervals, white arrowheads= red blood cells, black arrowheads= connective tissue, white stars= inter-lamellae spaces. H&E A: X100 B: X400*

revealed progressive histological alterations such as thickening and shortening of secondary lamellae, increase in inter- lamellae spaces, degeneration of connective tissue and complete loss of secondary lamellae were observed in the gill of the exposed fish with increase in toxicant concentration *(Plates 1-6)*. Therefore, *L. siceraria* have noxious effect on the gill of *C. gariepinus*. This observation was similarly reported by Audu et al [22], who reported a progressive moderate to severe histo-architectural changes (lamellar hyperplasia and occluded water channels) observed in the gills of *C. gariepinus* exposed to concentrated grades of *V. amydalina* depicts a dose-dependent distortion especially with marked severity in those given the higher concentration of the extract. Several authors also reported changes in gill of different fish species treated with different toxicant [54]. Gills of *Tilapia zillii*, exposed to aluminum resulted in several forms of histopathological changes such as cellular hyperplasia in the epithelial layer of primary filaments and fusion of secondary lamellae, epithelial lifting, interstitial edema and blood congestion in the vascular axis of primary filaments and few telangiectasis were also observed at gill lamellae [17].

Liver is known to be associated with detoxification, biotransformation and is the most organ affected by toxicants in water [55]; [56]. In this study, liver of *C. gariepinus* juveniles exposed to acute concentrations of *L. siceraria* showed progressive histo-architectural distortions such as hepatic hypertrophy, cellular degeneration (necrosis), haemorrhage and intracellular infiltration with increase in concentration of the toxicant further substantiate the toxic potential of this plant *(Plates 7-12)*.This observations is in line with the work of Audu et al [22] who reported histo-architectural changes in the liver (moderate to severe hepatocellular degeneration, central and sinusoidal congestions) tissues of *C. gariepinus* exposed to grades of *V. amygdalina* with the exception of the low concentration of this extract every other concentration appeared to be toxic and run a concentration dependent histological disruption. This study also agrees with findings of Hadi and Alwan [17], who reported that *C. gariepinus* fingerlings exposed to aluminium for 96hrs showed important alterations in the liver comprise hypertrophy of hepatocyets, nuclear hypertrophy, blood congestion in the central veins, cytoplasmic vacuolation, cellular degeneration, damage of nuclei, bile stagnation, congestion in the blood sinusoids, cellular necrosis in the parenchymal tissues and decreasing in the number of hepatocytes nuclei of hepatic tissue compared with the control. The result of this study

is not parallel with the work of Rajesh et al. [57], which reported that endoscopy showed esophagitis, gastric erosions, ulcers and duodenitis in patients who developed gastrointestinal toxicity due to drinking bitter bottle gourd juice.

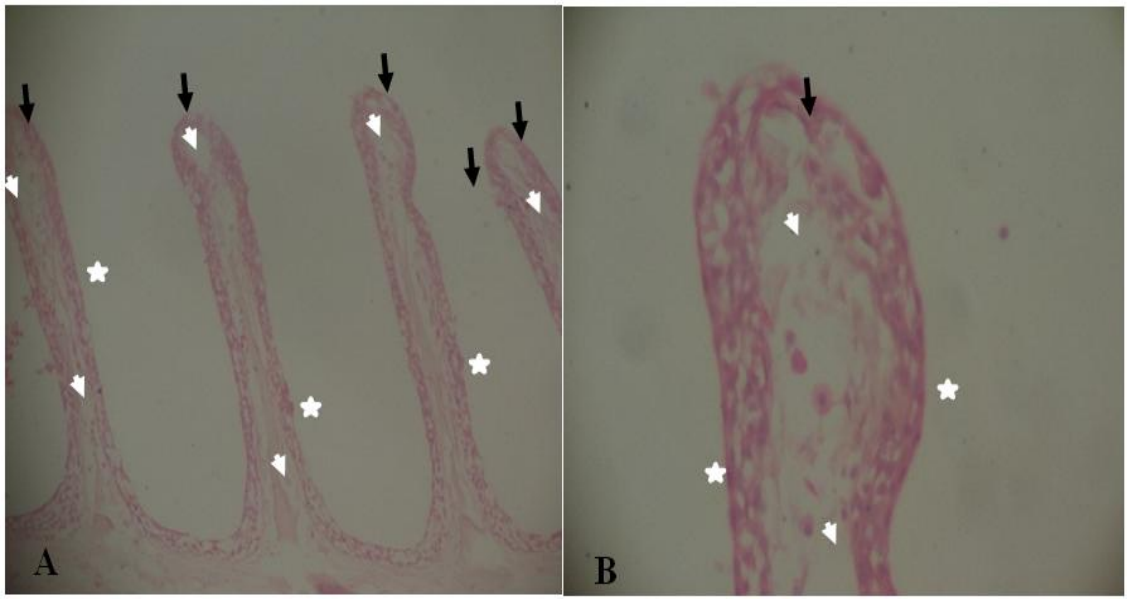


**Plate 2. Gills of *Clarias gariepinus* exposed to 5.0 mg/L of Crude Fruit Endocarp Extract of *Lagenaria siceraria for* 96 hours**

*Key: White arrow heads shows total loss of secondary lamellae and severe degeneration of connective tissue. The primary lamellae (black arrows) appear as smooth stakes with no sign of secondary lamellae attached. The inter-lamellae spaces (white stars) appear smooth and increased due to the absence of secondary lamellae. H&E A: X100 B: X400*

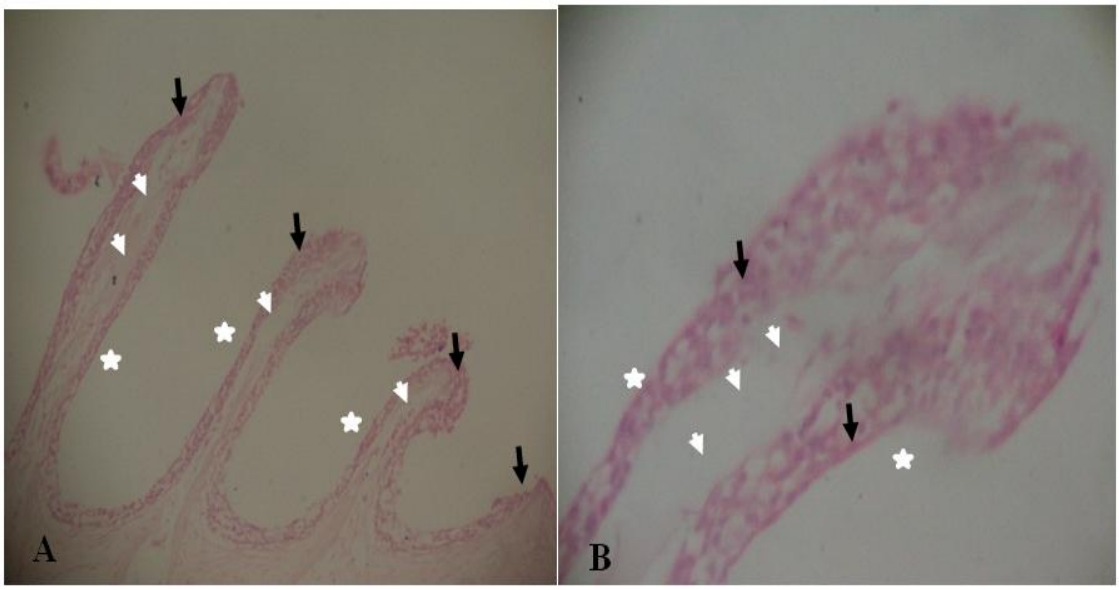


**Plate 3. Gills of *Clarias gariepinus* exposed to 20.0 mg/L of Crude Fruit Endocarp Extract of *Lagenaria siceraria* for 96 hours**

*Key: showing complete loss of secondary lamellae and tissue atrophy. The Primary lamellae (black arrows) appear atrophied and smooth with no trace of secondary lamellae attached. There is massive degeneration of connective tissue (white arrowheads). The inter-lamellae spaces (white stars) appear increased in size due to the absence of secondary lamellae. H&E A: X100 B: X400*

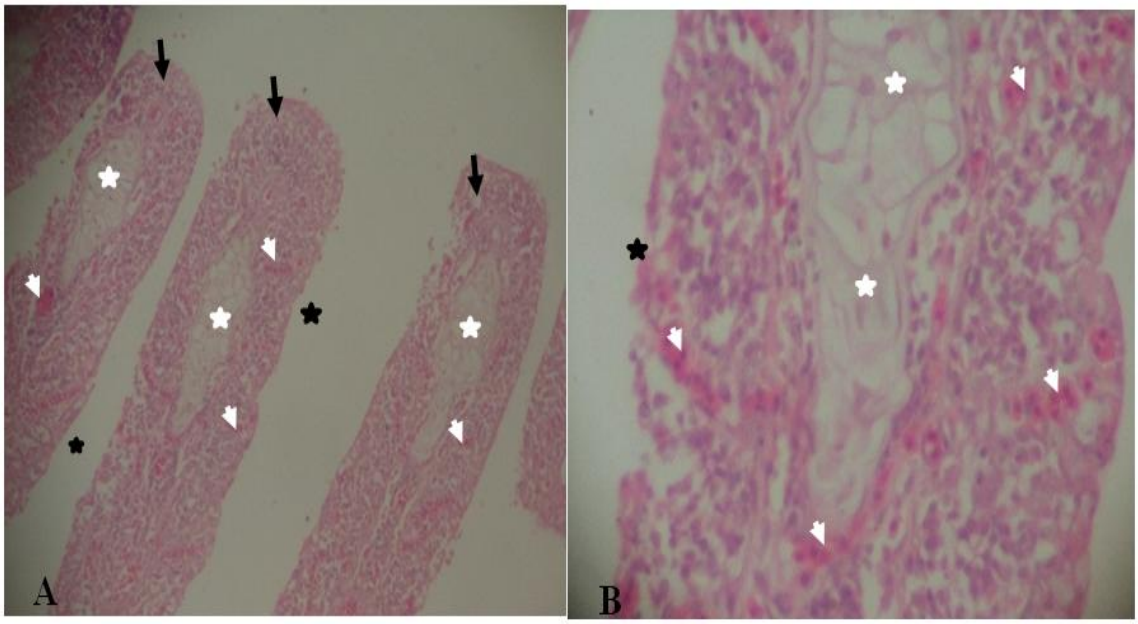


**Plate 4. Gills of *Clarias gariepinus* exposed to 35.0 mg/L of Crude Fruit Endocarp Extract of *Lagenaria siceraria* for 96 hours**

*Key: Shows total loss of secondary lamellae with mild tissue congestion evident by the congestion of red blood cells (white arrowheads) within the tissue. The chondrocytes (white stars) are atrophied evident by the decrease in their components. Black arrows= primary lamellae lacking the secondary lamellae, black stars= The inter-lamellae spaces. H&E A: X100 B: X400*

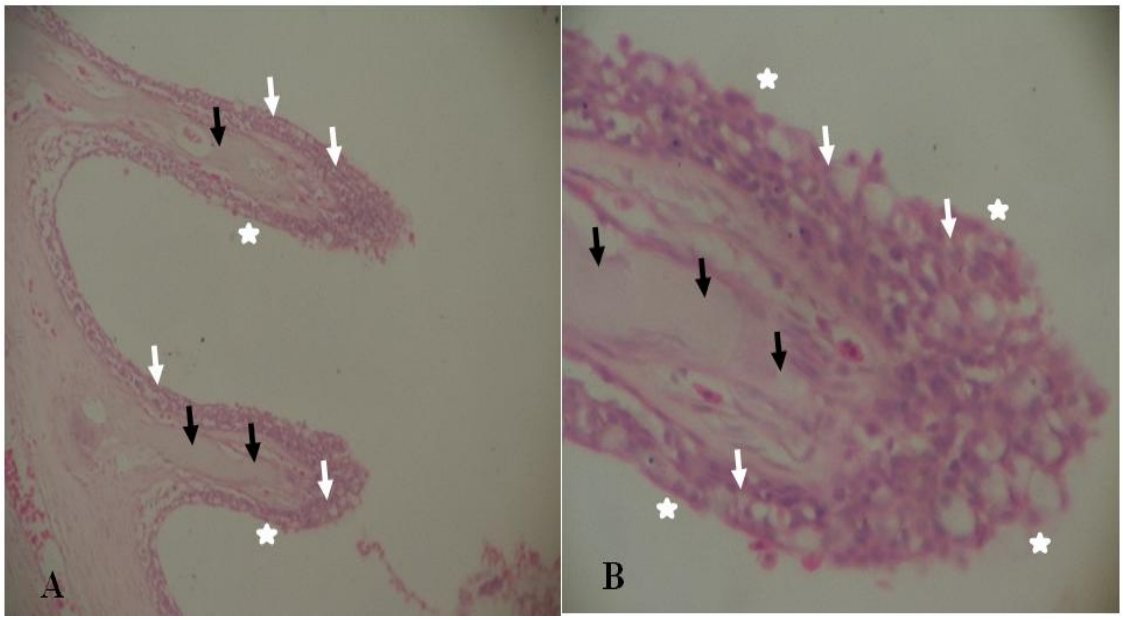


**Plate 5. Gills of *Clarias gariepinus* exposed to 50.0 mg/L of crude fruit endocarp extract of *Lagenaria siceraria* for 96 hours**

*Key: Shows complete loss of secondary lamella making the primary lamellae (white arrows) to appear as smooth stalks. The cartilaginous layer (black arrows) present with mild atrophy. White stars= inter-lamellae spaces. H&E A: X100 B: X400*

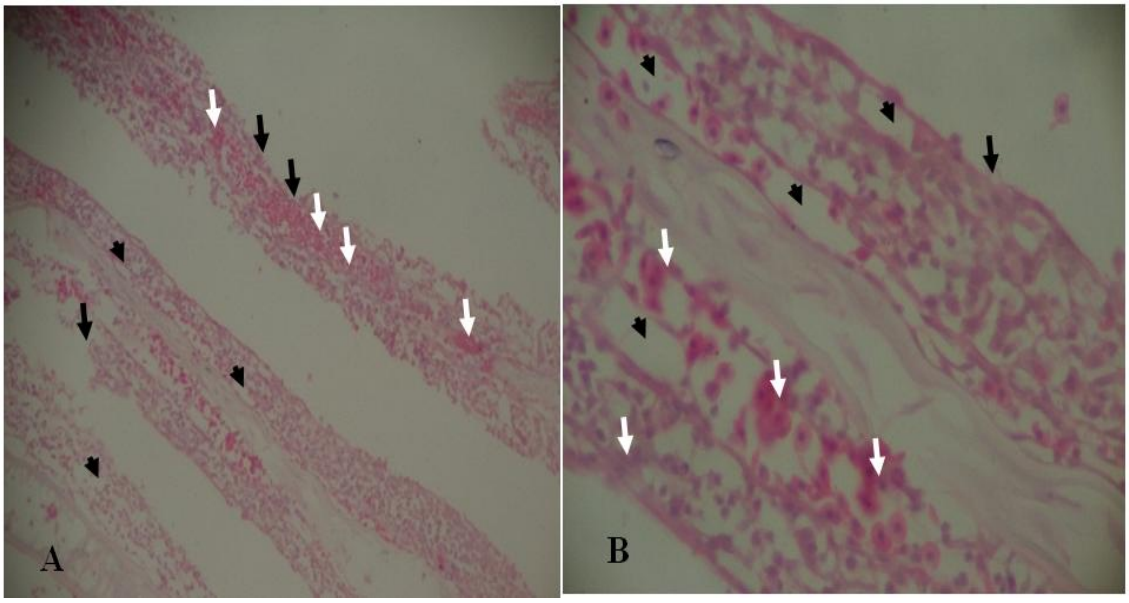


**Plate 6. Gills of *Clarias gariepinus* exposed to 65.0 mg/L of crude fruit endocarp extract of *Lagenaria siceraria* for 96 hours**

*Key: shows complete loss of secondary lamellae, primary lamellae erosion (black arrows) is shown by massive loss of the outermost layers of the lamellae. White arrows indicate mild tissue congestion evident by the cluster of red blood cells within the tissue. Generalized atrophy is shown by the increase in interstitial spaces (black arrowheads). H & E A: X100 B: X400*

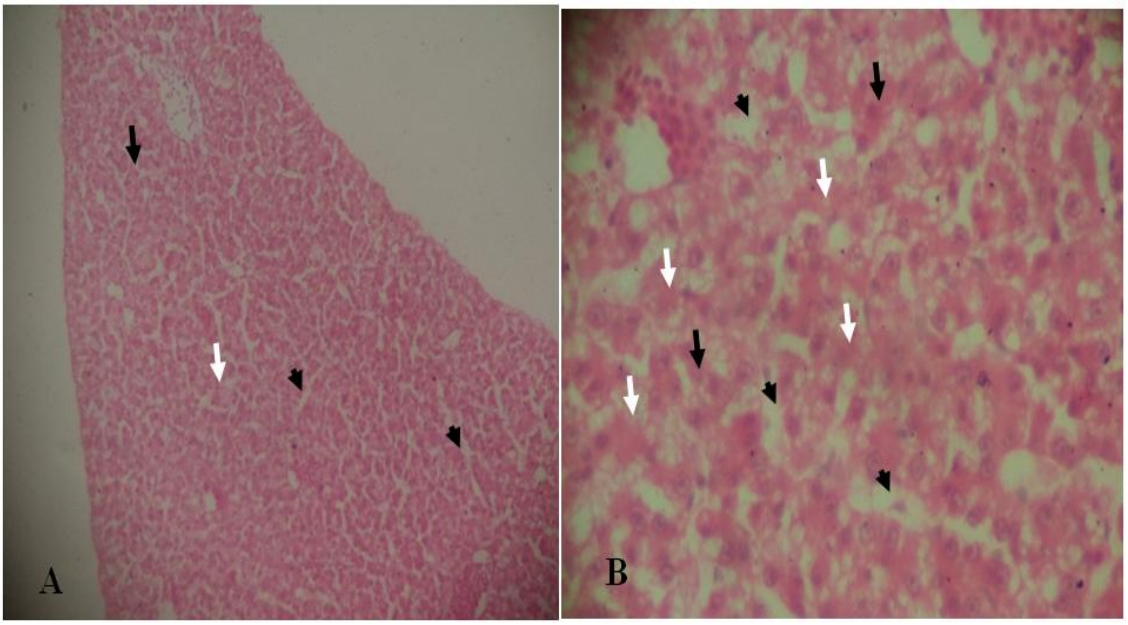


**Plate 7. Liver of *Clarias gariepinus* exposed to 0.00 mg/L of crude fruit endocarp extract of *Lagenaria siceraria* for 96 hour**

*Key: Shows normal morphology evident by hepatocytes with intact nuclei (black arrows) surrounded by intact cytoplasm (white arrows). The hepatocytes are interspersed by hepatic sinusoids (black arrowheads). A: X100 B: X400*

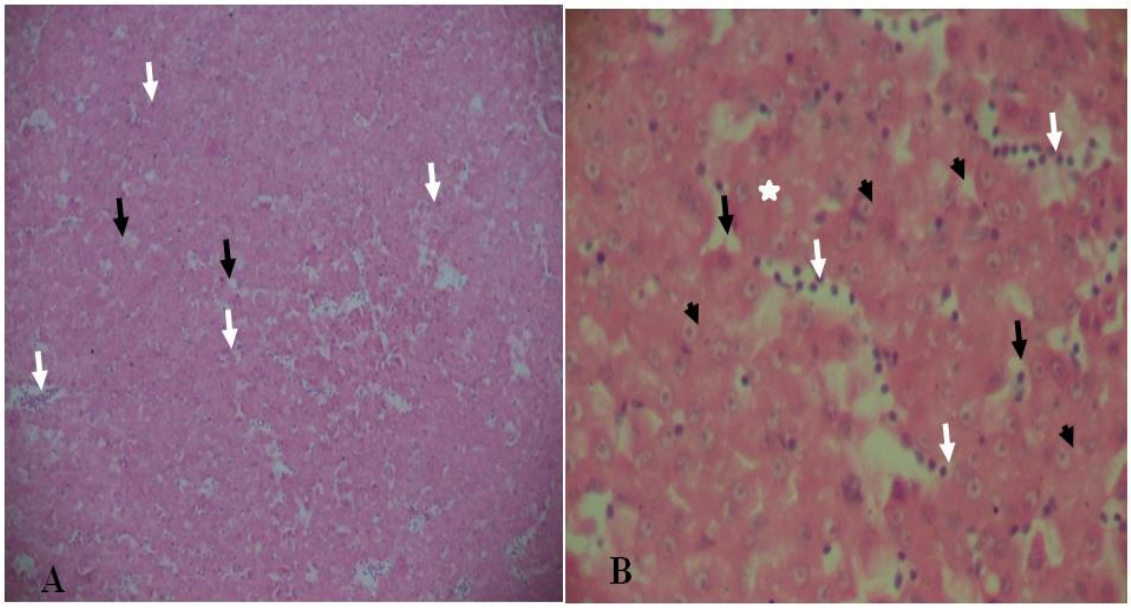


**Plate 8. Liver of *Clarias gariepinus* exposed to 5.0 mg/L of crude fruit endocarp extract of *Lagenaria siceraria* for 96 hours**

*Key: Showing inflammation evident by the presence of inflammatory cells (white arrows) within the tissue. There is evidence of hepatic hypertrophy shown by the reduction in sinusoidal spaces (black arrows). Cell nuclei (black arrowheads) appear surrounded by a clear zone. H & E A: X100 B: X400*

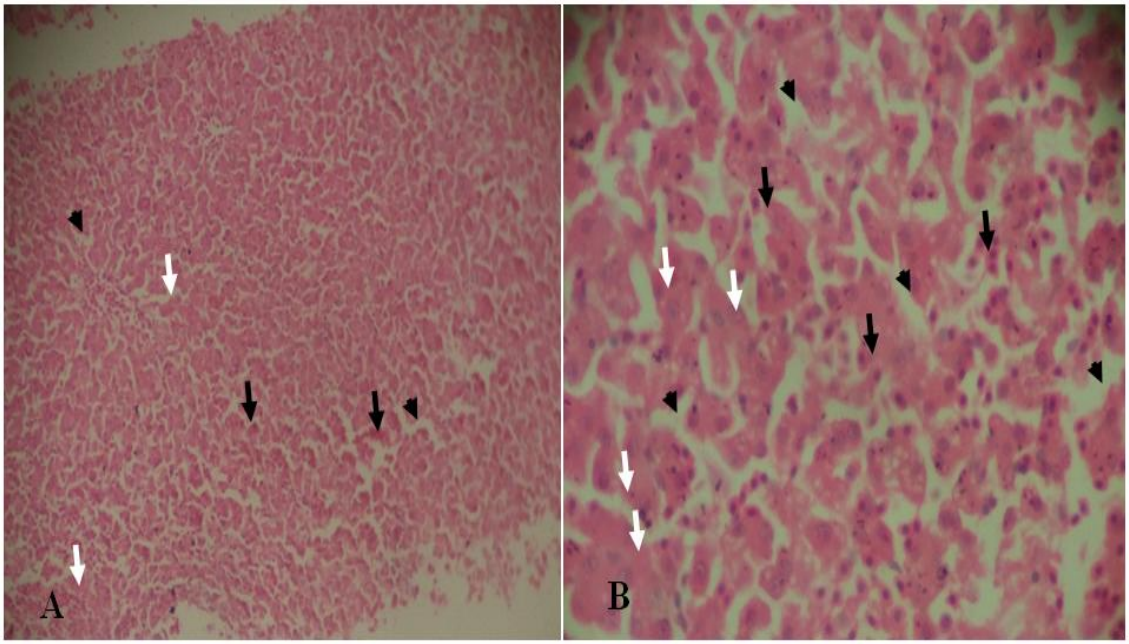


**Plate 9. Liver of *Clarias gariepinus* exposed to 20.0 mg/L of crude fruit endocarp extract of *Lagenaria siceraria* for 96 hours**

*Key: Showing generalized haemorrage evident by the presence of red blood cells (black arrows) within the interstitial spaces. The hepatocytes (white arrows) appear morphologically abnormal and interspersed by hepatic sinusoids (Black arrowheads). H&E A: X100 B: X400*

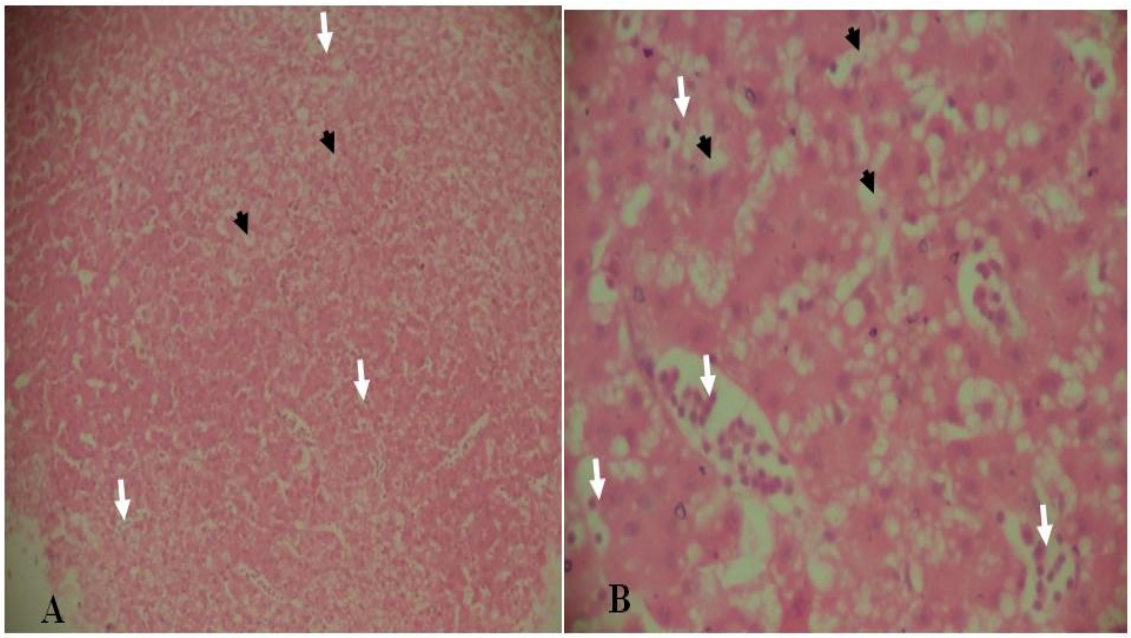


**Plate 10. Liver of *Clarias gariepinus* exposed to 35.0 mg/L of crude fruit endocarp extract of *Lagenaria siceraria* for 96 hours**

*Key: Shows massive inflammation evident by the presence of inflammatory cells (white arrows) and tissue atrophy evident by the increase in intracellular spaces (black arrowheads). H&E A: X100 B: X400*

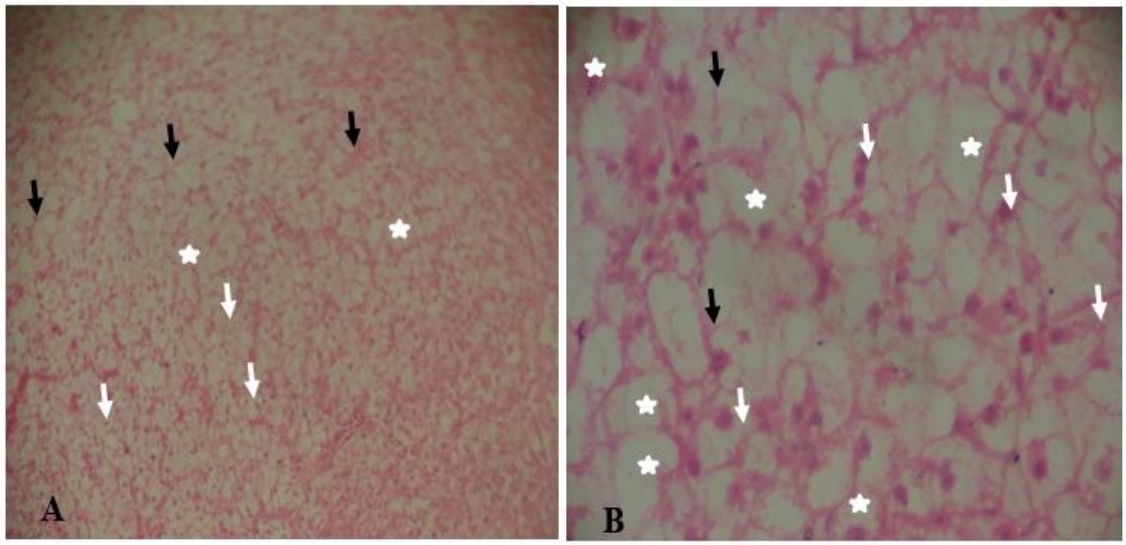


**Plate 11. Liver of *Clarias gariepinus* exposed to 50.0 mg/L of crude fruit endocarp extract of *Lagenaria siceraria* for 96 hours**

*Key: Shows massive cellular degeneration (necrosis) and loss or tissue architecture, making the cellular demarcations (black arrows) to appear as strands surrounding empty spaces (white stars). These empty spaces are cytoplasmic components that have been completely lost hence few nuclear material (white arrows) left appear as naked nuclei. H&E A: X100 B: X400*

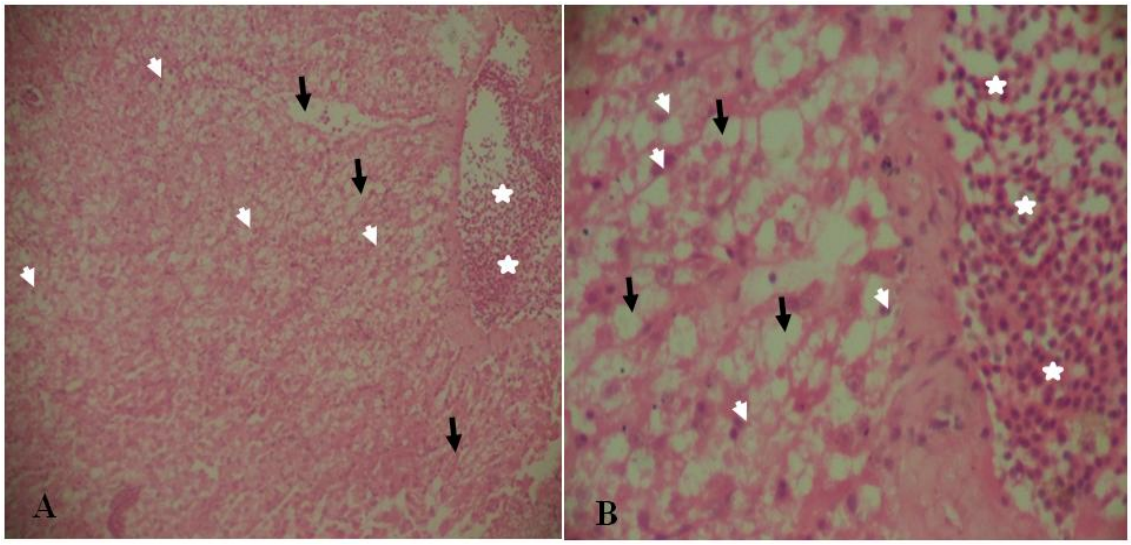


**Plate 12. Liver of *Clarias gariepinus* exposed to 65.0 mg/L of crude fruit endocarp extract of *Lagenaria siceraria* for 96 hours**

*Key: Shows severe intracellular infiltration evident by the presence of inflammatory cells (white stars) and generalized tissue necrosis evident by the presence of vacuolated cells (black arrows) and naked nuclei (white arrowheads). Tissue atrophy is evident by the increase in intercellular spaces. H&E A: X100 B: X400*

## 4. CONCLUSION

Aqueous extract of *L. siceraria* had adverse effect on juveniles of *C. gariepinus, as evident in the negative modification of the body physiology via biochemical and histological investigations,* thus toxic to the aquatic life. Further investigations on other vital organs - kidney, heart, GIT and the reproductive organs are recommended.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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