Histological Changes in the Gills and Liver of African Catfish (*Clarias gariepinus*) Juveniles Exposed to Acute Concentrations of Aqueous Crude Fruit Extract of Gum Arabic Tree (*Acacia nilotica*)

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Abstract

Histological alterations are used as indicators of aquatic pollutants and overall health of the entire population of organisms in an ecosystem. These histological parameters are related to other biomarker of stress since many pollutants have to undergo metabolic activation in order to be able to provoke cellular changes in the affected organism.

Apparent healthy One hundred and twenty (120) juvenile of Clarias gariepinus, mean weight 9.77 ± 0.42g and mean length of 13.8 ± 0.80cm were used. Fish were placed in twelve (12) circular plastic tanks (40 × 30 × 20cm) and ten (10) fish were placed in each of the tanks and were allowed to acclimatize tolaboratory conditions for a period of 2 weeks during which the fish were fed with Vital feed® once daily at 3% of their body weight. The gills and liver were excised and processed, by using routine histological screening.

Histological section through the gills and liver of C. gariepinus juveniles exposed to acute concentrations of aqueous crude fruit extract ofAcacia nilotica revealed some severe histological alterations such as complete loss of secondary lamellae, massive presence of inflammatory cells, and degeneration of connective tissue, hypertrophy and hyperplasia which increased as the toxicant concentration increased. The acute bioassay of aqueous crude fruit extract of A. nilotica on C. gariepinus juveniles revealed that the substance is toxic to the exposed fish at various concentrations and the result showed the 96hrLC_{50} caused 50% mortality. The toxicant had adverse effects on the water qualities, behavioral signs & histological parameters of the exposed fish compared to the control groups. The disruption in these parameters could have led to the degrees of impairment in the gills and livers of the exposed fish and the inability of the exposed fish to withstand infections, thereby causing a great concern for fish survival. Therefore, the use of Acacia niloticain water bodies could lead to contamination of aquatic ecosystem by causing alteration in the physiological and general health of fish and other aquatic fauna.

Keywords: Histological, Gills, Liver, Clarias gariepinus, Acute, Acacia nilotica

INTRODUCTION

Aquatic pollution is a dangerous environmental threats facing man and his environment impacting various levels of biological organizations (Al-Zaidan, 2017). The effects of aquatic pollution have become a matter of urgent concern because it threatens both public water supply and aquatic organism (Vinodhini & Narayanan, 2009). Pollutants are released by anthropogenic activities without the sufficient measure to remove harmful constituents (Agrawal et al., 2010).

Vulnerability of Aquatic system is due to their tendency to accumulate high concentrations of chemicals from a variety of point and nonpoint sources to water bodies such as rivers, streams, marine ecosystem and groundwater (Di Giulio & Hinton, 2008). The increased socio-economic activities as resulted in introducing toxicants to the aquatic ecosystems thereby, causing harm to the aquatic biota.

Histology acts as an integrated parameter, providing a complete evaluation of the organism' health, effectively monitoring the effects of exposure of environmental pollutants (Vander Oost *et al*., 2003). Fish are sensitive indicators of pollutants present in water. These pollutants cause severe physical and physiological alternation in fishes by Trivedi, Kumur et al. (2002). Histological alterations can be used as indicators for the effects of pollutants on organisms, and a reflection of the overall health of the entire population in an ecosystem (Fatima, 2009).

Histological biomarkers are valuable indicators of the harmful effect of pollutants and potential pathogens (Vander Oost *et al*., 2003). Biomarkers have been proposed as sensitive tools for the early detection of environmental exposure to pollutants and their adverse effects on aquatic biota (Van Der Oost et al., 2003; De la Torre, Ferrari &Salibian, 2005). They are link between environmental pollutants and its effects, providing unique information on the general health status of an ecosystem (Maria et al., 2009). These markers are intermediate biomarkers in terms of ecological importance response, time and level to biological organism and as such are very suitable for the assessment of potentially harmful effects of various pollutants (Vander Oost *et al*., 2003).

Histological screening help to establish casual relations between exposure of contaminants and various biological responses and have proven to be a sensitive tool used in detecting direct effects of chemical compounds within target organs (Altinok&Capkin, 2007). A study of histopathology provides very important and useful data concerning changes in cellular or sub-cellular structure of an organ much earlier than external notification. Histological biomarkers help to examine target organs and the alterations found in these organs are easier to identifying than functional ones in environmental studies (Fanta et al., 2003).

METHODOLOGY

EXPERIMENTAL PLANT (*Acacia nilotica*) Fruit.

The dry fruit of *A. nilotica*(Plate 1) was obtained from Jos North Local Government Area, Plateau State, Nigeria. The fruit was scientifically identified at Plant Science Technology Department, University of Jos,Plateau State.

COLLECTION AND ACCLIMATION OF EXPERIMENTAL FISH (*C. gariepinus*) JUVENILES

Apparently healthy One Hundred and twenty (120) juvenile of *Clarias gariepinus*, mean weight 9.77 ± 0.42g and mean length of 13.8 ± 0.80cm were used forthis investigation. Fish were placed in twelve (12) circular plastic tanks (40 × 30 × 20cm) and ten (10) fish were placed in each of the tanks and were allowed to acclimatized tolaboratory conditions, fish were fed with Vital feed® once daily at 3% of their body weight. Three quarters of the water in the tank were siphoned out on daily basis to remove left over feed and faecal matter and replaced with fresh borehole water. Mortality observed during acclimation period was removed, replaced and allowed to stabilize to zero. Photo period was natural (12hr day: 12hr night) and feeding was stopped 24 hours prior to exposure to the bioassay media(Audu et al., 2020).

EXPERIMENTAL DESIGN

A 24hour preliminary test was conducted to obtain a realistic toxic concentration for the acute toxicity test in a static nonrenewable bioassay. The test was carried out to determine the 96hr LC_{50} (lethal concentration that will cause 50% mortality of the test animal in 96hr). A total of six (6) circular plastic test tanks (40 × 30 × 20cm) were used, each duplicate replicated in a randomized block design. The tanks were labelled Al - A2, Bl - B2, Cl - C2, Dl - D2, El - E2 and F1 –F2 which served as the control tank. Each tank contained ten (10) mixed sex juveniles of *C. gariepinus* of mean weight 9.77g ± 0.4g and mean length 13.8g ± 0.42g. Each of tanks was filled with 20L (twenty liter) of borehole water. Photo period was natural (12hrs day: 12hrs night)(Audu et al., 2020).

PREPARATION OF STOCK SOLUTION

Acacia nilotica fruit was air-dried, weighed using Camry Premium weighing machine. The dried sample was crushed using a mortar and pestle. Known weight 0.30, 0.25, 0.20, 0.15 and 0.10g of *A. nilotica* powered was macerated in 1L of distilled water each and allowed to stand for 24hrs (Audu et al., 2014). Mixture was filtered using 0.5mm sieve containing a funnel choked with non-absorbent cotton wool and filtrate forms the stock solution (Audu et al., 2020).

RANGE FINDING TEST (RFT)

Range finding test (RFT) was conducted to determine the concentrations of aqueous crude extract of *Acacia nilotic*a that would cause 50% mortality of the test fish after 96 hours (4 days). The Range finding test involved exposing five (5) fish to different concentrations 0.40, 0.35, 0.30, 0.25 and 0.2g/L of aqueous crude fruit extract of *A.nilotica* with 0.00g/L which served as the control without the test material. Based on the results of the RFT, a definitive concentrations of 0.3, 0.25, 0.20, 0.15 and 0.1g/L of the aqueous crude fruit extract of *A.nilotica* and 0.00g/L (control) were prepared in six (6) circular plastic tanks and replicated for bioassay (UNEP, 1989).

WATER QUALITY PARAMETERS

During the bioassay which lasted for 96 hours (4 days), water quality parameters such as temperature, was measured every 24hours while the dissolved oxygen, total alkalinity, pH, free carbon dioxide, nitrite, total hardness, conductivity and total ammonia were all determined using standard laboratory methods as described by APHA (1985) at the beginning and end of the bioassay.

HISTOLOGICAL ANALYSIS

The fish (live) were dissected and the gill and liver were carefully excised and fixed in 10% formal saline solution. They were prepared for histological analysis using the routine histological methods and haematoxylin-eosin staining techniques described by Drury & Wallington (1980).

DATA ANALYSIS

Data ontained were analysed using statistical package for social science (SPSS) Statistical differences between and within groups and were subjected to One Way Analysis of Variance (ANOVA) and Duncan multiple range test.Regression analysis was conducted according to the method of Sprague,(1972). Post Hoc Test was used to compare differences between treatment means at probablity level P=0.05.

RESULTS

The result of water quality parameter during acute toxicity tests showed that the mean values of water quality parameters varied significantly ($P<0.05$) compared to the control. The temperature value showed slight increase in all the test tank range from 18 to 19.5^{0}C. Hydrogen ion concentration pHshowed a decreasing trend with increase in the concentration of the test material i.e. this ranged between 3.7 and 7.05. Dissolved oxygen content decreased proportional with increase in the concentrations of the aqueous crude fruit extract of *Acacia nilotica* in the test tanks with values in the range of 2.00 to 5.90g/L. Free carbon dioxide showed an increase pattern with increase in concentration of the fruit extract in the test tanks with values in the range of 2.00 to 5.90g/L. Free carbon dioxide showed an increase pattern with increase in concentration of the toxicant with a range of 30.0 to 56.5 ppm while decrease with increase in concentration of the toxicant in all the test tanks with a range of 31.0 to 73.0 g/L. Nitrite range from 0.02 to 0.07 as the toxicant concentrations increased.Unionized ammonia(NH_3) showed a slight variation as the concentration of the test tanks increase with range of 0.20 to 0.45 g/L. Hardness increased proportionally with increased the concentrations of all the experimental tanks with values in the range of 5.50 to 9.15g/L while the control tank recorded the lowest value of 5.50g/L

TABLE 1: MEAN VALUES OF WATER QUALITIES PARAMETERS OF TANKS WITH *Clarias gariepinus* JUVENILES EXPOSED TO ACUTE CONCENTRATIONS OF AQUEOUS CRUDE FRUIT EXTRACT OF *Acacia nilotica*

Conc(g/L)	pH	DO (mg/L)	Free CO_2 (ppm)	Alkalinity (mg//L)	Nitrite (mg/L)	NH_3 (mg/L)	T^0C	Hardness (mg/L)
0.30	3.70	2.00	56.50	31.00	0.07	0.45	19.50	9.15
	±0.10	0.00	`0.50	1.00	0.00	0.05	0.00	0.05
0.25	4.45	2.40	49.50	33.50	0.07	0.40	18.50	8.40
	±0.25	0.10	2.50	0.50	0.01	0.00	0.50	0.00
0.20	5.40	4.35	39.50	35.00	0.05	0.39	18.25	7.45
	±0.30	0.15	1.50	3.00	0.01	0.01	0.25	0.05
0.15	6.30	5.05	38.00	55.50	0.05	0.35	18.10	7.10
	±0.60	0.15	2.00	1.50	0.01	0.01	0.10	0.10
0.10	6.85	5.65	34.50	65.50	0.04	0.28	18.10	6.50
	±0.05	0.15	0.50	3.50	0.01	0.03	0.10	0.00
0.00	7.05	5.90	30.00	73.00	0.02	0.20	18.00	5.50
	±0.05	0.10	0.00	1.00	0.00	0.00	0.00	0.00

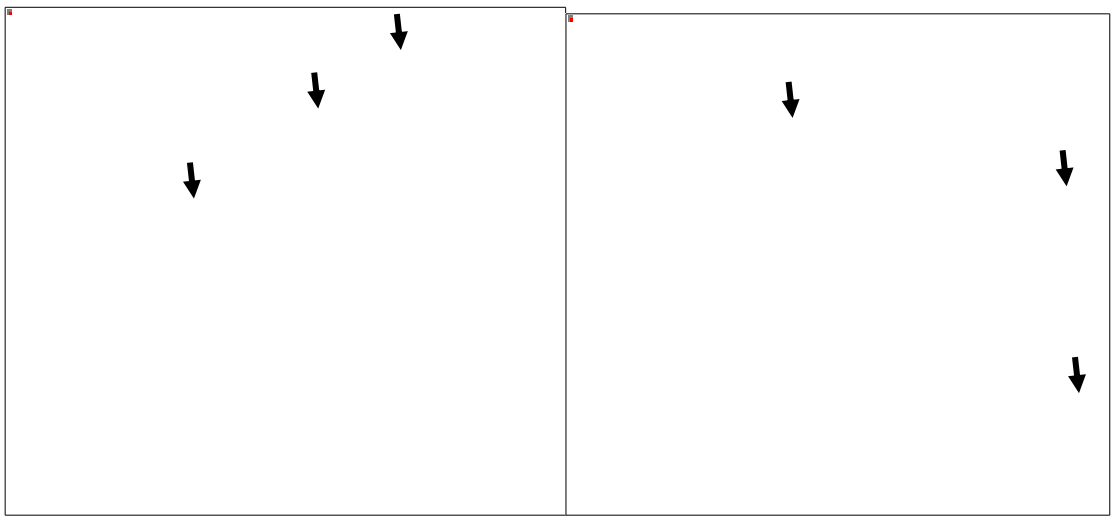
Values in ± are standard error

HISTOPATHOLOGICAL ANALYSIS

The histological sections of the gills and liver of fish exposed to 96hrs LC_{50} of aqueous crude fruit extract of *A.nilotica* was observed through light microscope and those of the control fish (0.00g/L) (Plate 1-12).

The gill of the exposed fish (*C. gariepinus*) juveniles show histopathological signs compared to the control group (0.00g/L) which shows normal morphology with intact secondary lamellae (Black arrows) attached to intact primary lamellae (Plate 1). The fish exposed to 0.10g/L of aqueous crude fruit extract of *A.nilotica*, shows massive loss of secondary lamellae (Black arrows) making the primary lamellae to appear as rough sticks in some portion of the section due to the presence of remnants of the lost secondary lamellae, while some area appear smooth (White arrows) due to complete loss of secondary lamellae (Plate 2). Similarly, the gills of *C.gariepinus* juveniles exposed to 0.15g/L of aqueous crude fruit extract of*A.nilotica* shows complete loss of secondary lamellae making the (Black arrows) to appear as smooth stalks (Plate 3).

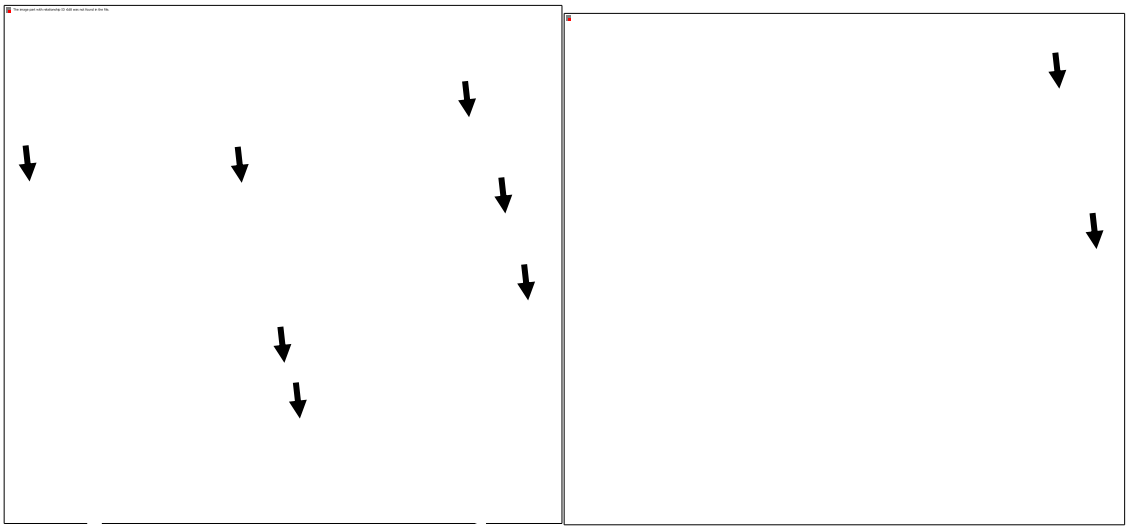
The gill of the *C.gariepinus* juveniles exposed to 0.20 - 0.25g/L of aqueous crude fruit extract of *A. nilotica* shows complete loss of secondary lamellae severely inflamed evident by the massive presence of inflammatory cells within the tissue (hyperplasia) and complete loss of secondary lamellae, a massive hypertrophy, hyperplasia of the lamellae respectively. (Plate 4 and 5). The gill of the *C. gariepinus* juveniles exposed to 0.3g/L of aqueous crude fruit of *A. nilotica* shows complete loss of secondary lamellae, massive hypertrophy and hyperplasia of the cells (Plate 6). Similarly, the liver of the *C. gariepinus* juveniles (Control) exposed to 0.00g/L of aqueous crude fruit extract of *A. nilotica* shows normal morphology i.e. the hepatocytes present and Intact cytoplasmic membrane (White arrows) surrounding cell nuclei (Plate 7). Liver of *C. gariepinus* exposed to 0.10g/L of aqueous crude fruit of *A. nilotica* shows mild loss of both nuclear and cytoplasmic material (Plate 8). The liver of *C. gariepinus* juvenile exposed to 0.15g/L of *A. nilotica* shows complete loss of nuclear material in most of the cells making the hepatocytes to appear as empty polygonal shaped cells (Black arrows) without nuclei, shown by the massive presence of chronic inflammatory cells (White arrow heads) within the tissue (Plate 9). The liver of fish exposed to 0.2 and 0.25g/L of the toxicant shows tissue degeneration (necrosis) with conspicuous loss of nuclei and necrosis with complete loss of nuclear material respectively (Plate 10 and 11). The liver of *C. gariepinus* juvenile exposed to 0.3g/L of aqueous crude fruit extract of *A. nilotica* shows massive cellular degeneration (necrosis) (Plate 12).



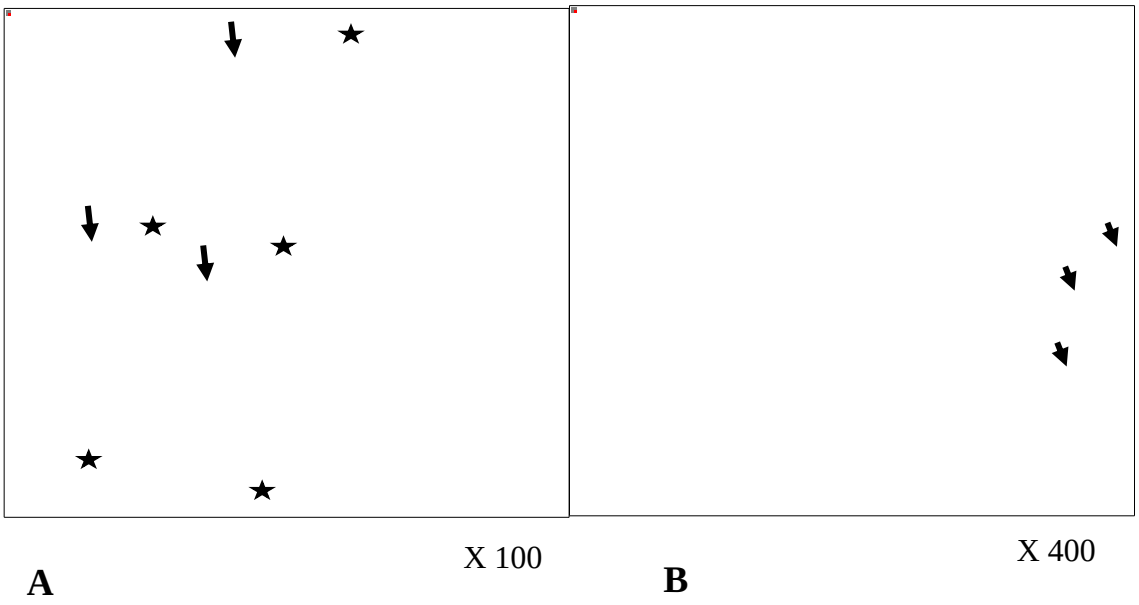
X 100 X 400

A **B**

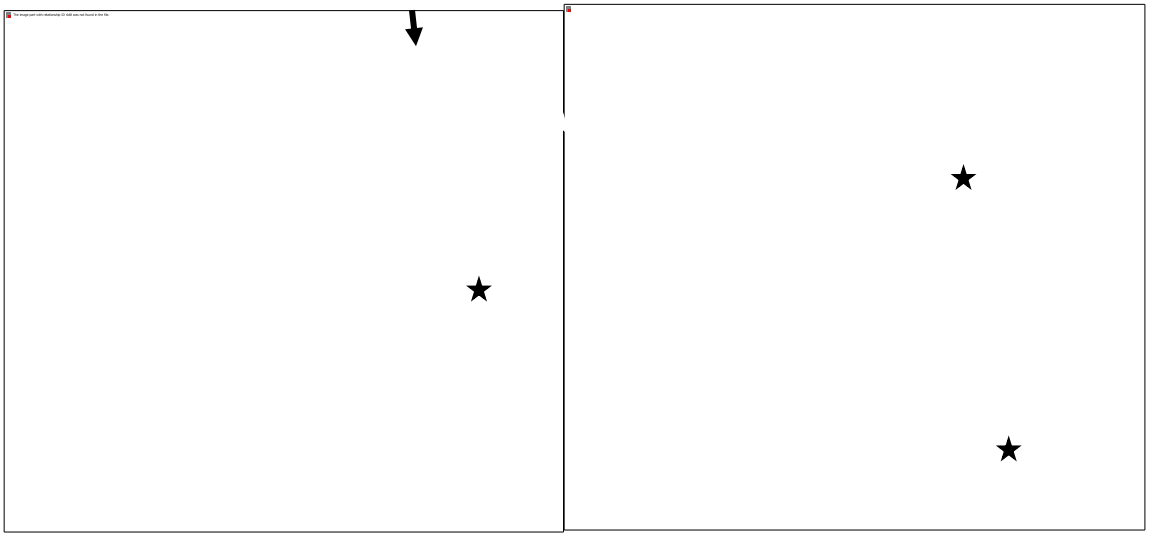
Plate 1. The Gills of the *Clarias gariepinus* Juveniles exposed to 0.00g/L (control)Aqueous Crude Fruit Extract of *Acacia nilotica*, showing normal morphology of the gill with intact secondary lamellae (white arrow) and intact primary lamellae (black arrow).



(Plate 2) Gill exposed to 0.10g/L showing mild loss of secondary and primary lamella



(Plate 3) Gill exposed to 0.15g/L showing complete loss of secondary lamellae making the primary lamellae (Black arrows) to appear as smooth stalks.



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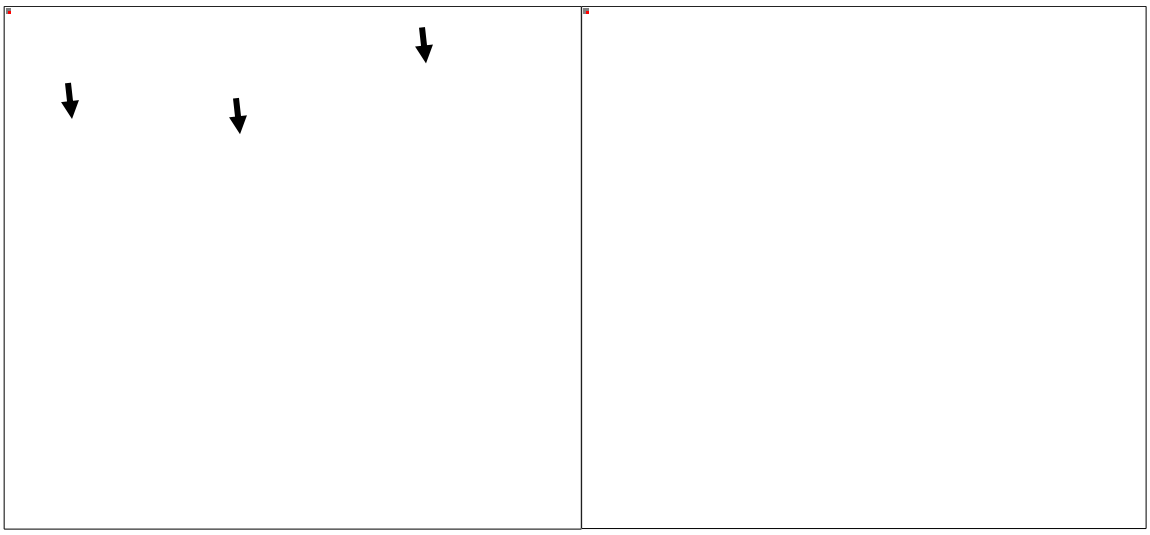
(Plate 4) Gill exposed to 0.20g/L showing complete loss of Secondary lamellae, severely inflamed evident by the massive presence of inflammatory cells within the tissue (hyperplasia).



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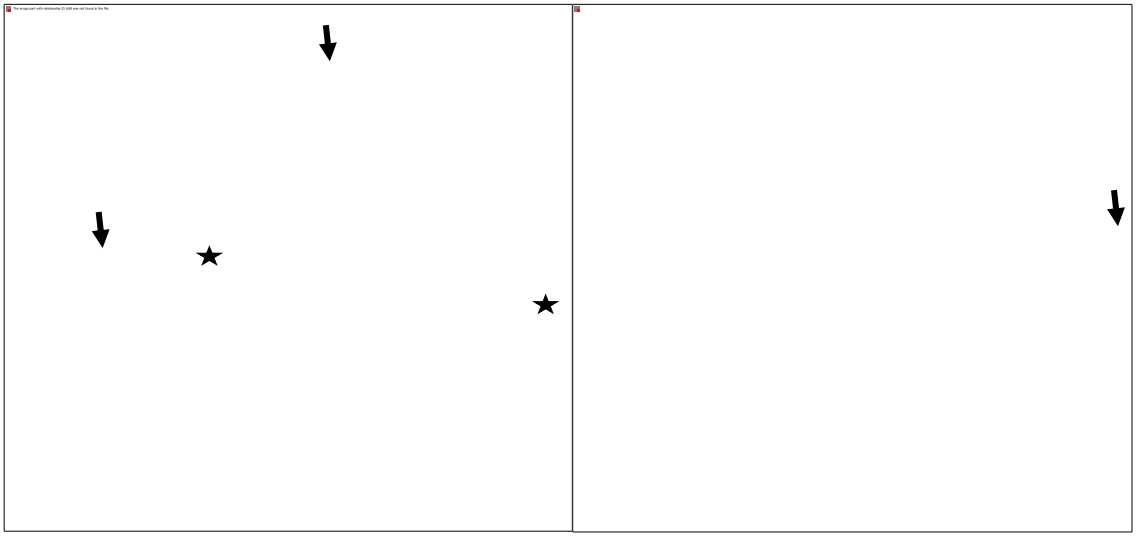
A **B**

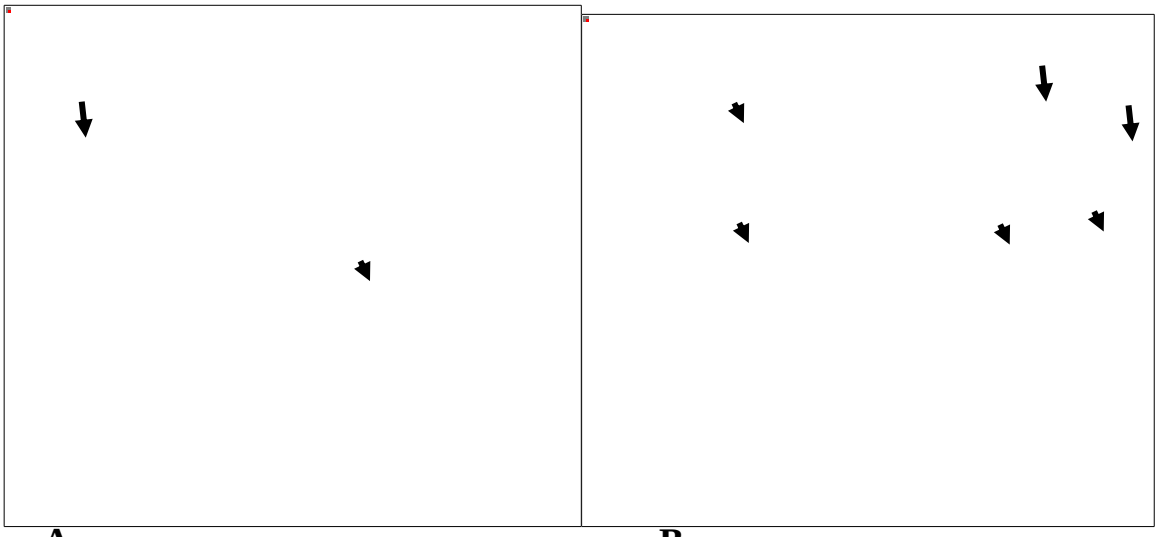
(Plate 5) Gill exposed to 0.25g/L showing complete loss of secondary lamellae, a massive hypertrophy of the lamellawith complete loss of secondary lamellae hence all appear smooth.



X 100 X 400

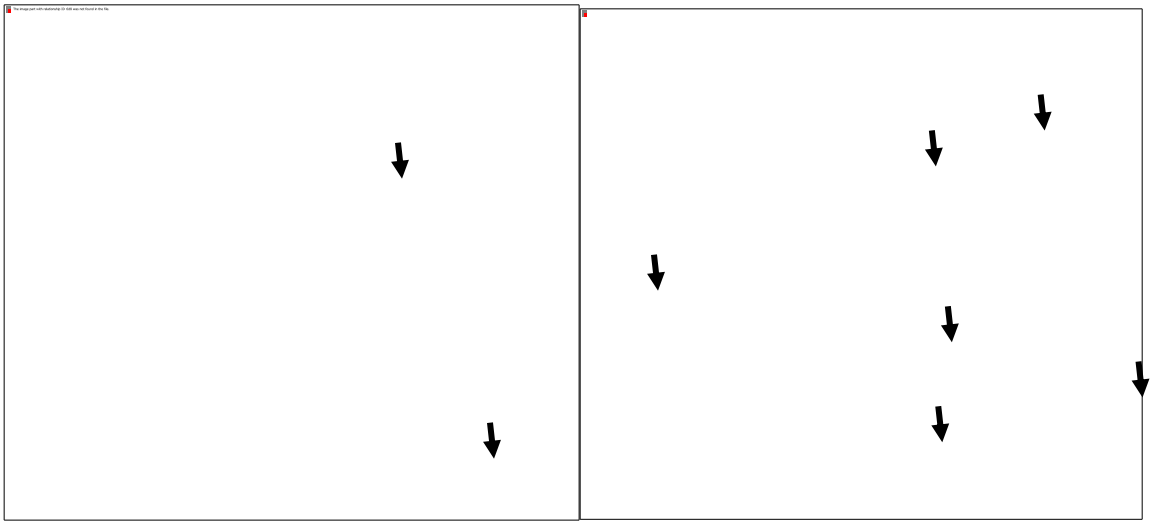
A **B**

(Plate 6) Gill exposed to 0.30g/L showing complete loss of secondary lamellae, massive hypertrophy of the cells with complete loss of Primary lamellae and secondary lamellae. The Primary lamellae present with abnormal midline cartilage (White stars. Blood vessels in the midline shows blood congestion (white arrowheads).



A X 100 **B** X 400

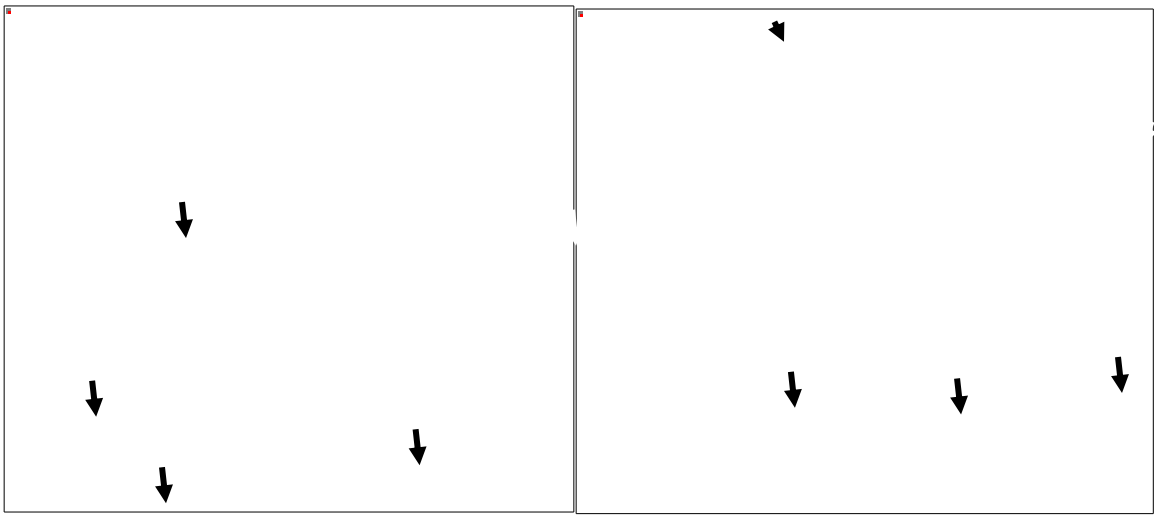
(Plate7) Liver of the *Clarias gariepinus* juveniles Exposed to0.00g/L (Control) Aqueous Crude Fruit Extract of *Acacia nilotica* showing normal morphology. The hepatocytes present intact cytoplasmic membrane (white arrows) surrounding cell nuclei (black arrowheads).



X 100 X 400

A **B**

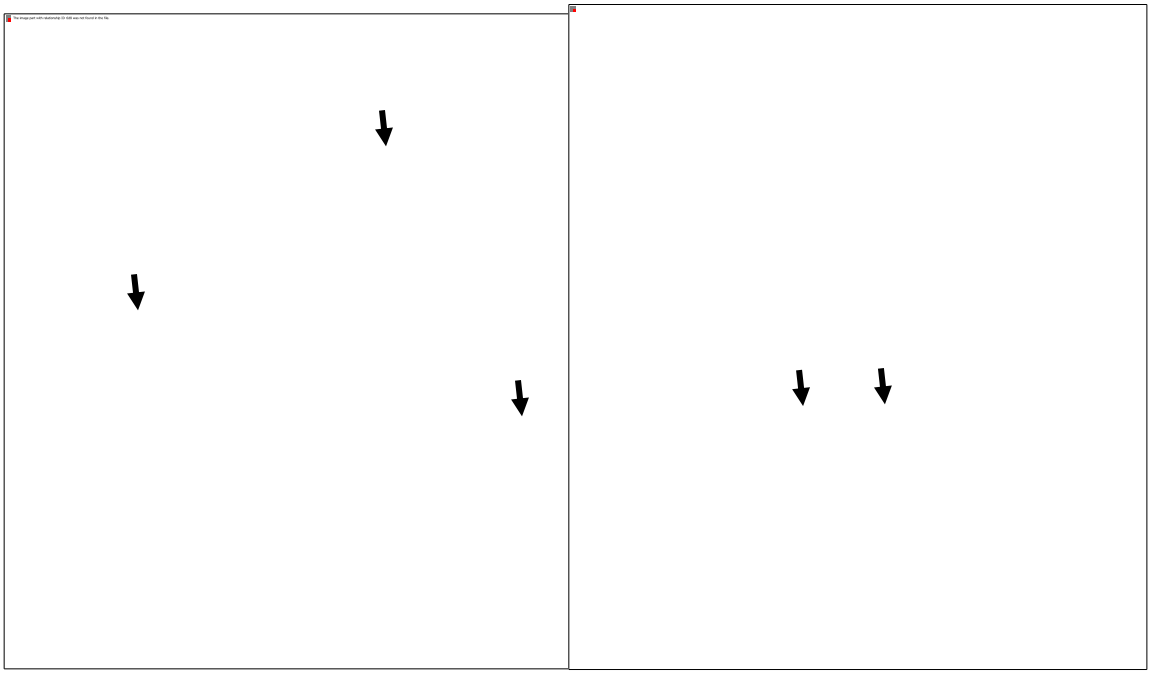
(Plate 8)exposed to 0.10g/L showing mild loss of both nuclear and cytoplasmic material, some nuclei appear as naked (white arrows) nuclei due to the loss of cytoplasmic materials while the nuclei in some cells appear as empty spaces (Black arrows).



X 100 X 400

A **B**

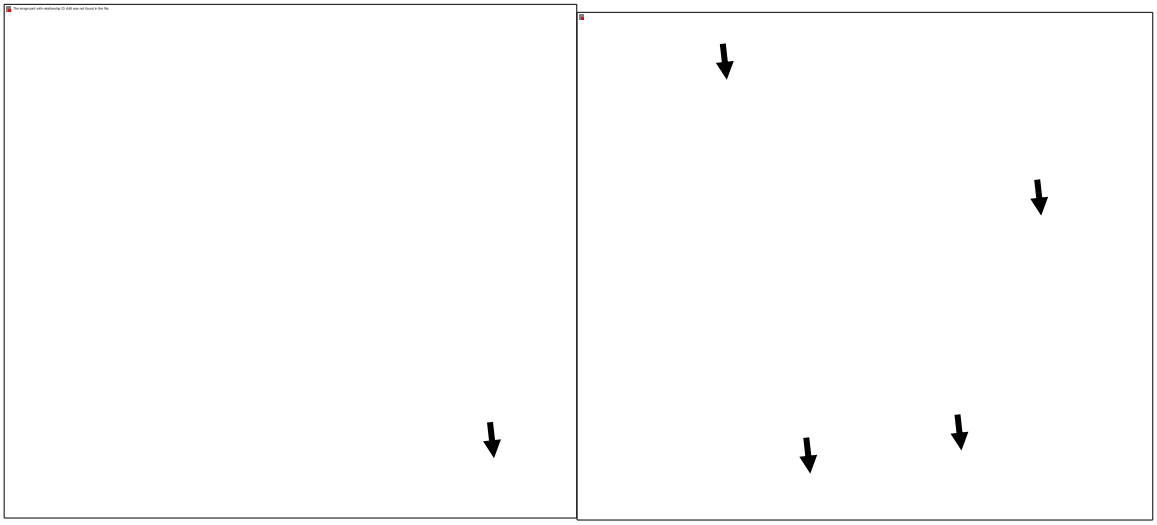
(Plate 9) Liver exposed to 0.15g/L showing complete loss of nuclear material in most of the cells making the hepatocytes to appear as empty polygonal shaped cells (black arrows) without nuclei, shown by the massive presence of chronic inflammatory cells (White arrowheads) within the tissue.



X 100 X 400

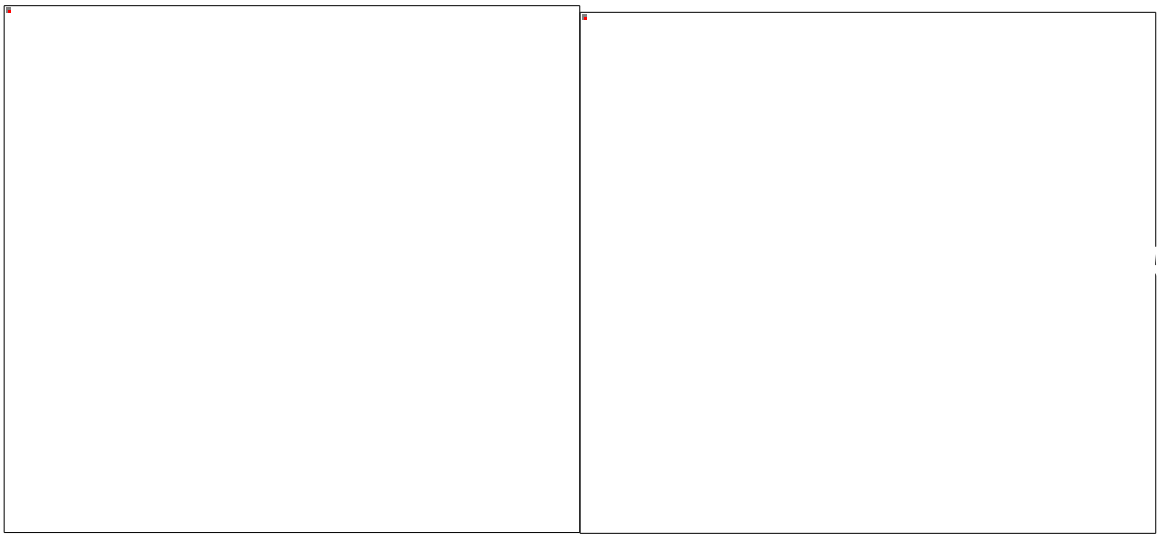
A **B**

(Plate 10): Liver exposed to 0.20g/L showing severe tissue degeneration (necrosis) as shown by naked nuclei (white arrows) hepatocytes with complete loss of nuclear material (white arrowheads). Chronic inflammation is shown by massive presence of inflammatory cells (white arrows) both within and outside the blood vessels.



A X 100 **B** X 400

(Plate 11): Liver exposed to 0.25g/L showing tissue degeneration (necrosis) with conspicuous loss of nuclei (perivascular and intravascular inflammation) evident by the presence of inflammatory cells (white arrowhead) due to tissue degeneration.



A X 100 **B** X 400

(Plate 12): Liver exposed to 0.30g/L showing massive cellular degeneration (necrosis) shown by complete loss of nuclear material. Chronic inflammatory cells within the central vein (liver cirrhosis) due to cellular degeneration, Inflammation.

DISCUSSION

Dissolved oxygen content was observed to decrease with increase concentration of the toxicant.

Dissolved oxygen values of the test media varied significantly ($P < 0.05$). Low dissolved oxygen

concentration can increase the susceptibility of fish to toxic effects of toxicant. Decreased

dissolved oxygen observed may have resulted to increase in free carbon dioxide, this is in

agreement with FAO (Food and Agricultural Organization) that in water of low O_2 and high CO_2,

where gaseous exchange at the respiratory surface is limited, the fish increase their ventilation

rate and become restlessness, loss of equilibrium and may die. The extent of the reduction in pH

depends on the amount of CO_2 present in the water (Zdenka *et al*., 1993).Free carbon (IV) oxide content increased with increase in the amount of the aqueous crude fruits extract of *Acacia nilotica*. There was significant difference ($P<0.05$) in the amount of carbon (IV) oxide in the test tanks compared to the control in the 96 hours acute toxicity test. Because of the anesthetic properties of carbon dioxide, it has the ability to disrupt the normal physiological activities of fish (Malcom, 1994). This could induce stress in fish by its ability to combine with water to form a weak acid (Malcom, 1994). And where the water is alkaline, it neutralizes but if the water is acidic, it becomes more acidic (Malcom, 1994). This acidity could result to physiological distress, affect the pH of the blood and cause severe imbalance in fish (Malcom, 1994) which could have been responsiblefor the instability observed in test fish.

The total alkalinity content in this experiment was observed to decrease with increase in the amount of the toxicant concentration. Total alkalinity recorded was significant difference ($P <0.05$) between test tanks and the control. Capkin, Altinok& Karahan (2006) reported that alkalinity (as $CaC0_3$) levels above 20mg/L can increase the survival rate of fishes significantly. High acidity or alkalinity can cause damages to skin, gills and eyes of fish directly (Capkin et al.2006). Hydrogen ion concentration (pH) of the experimental media were observed to decrease with increase toxicant

concentrations in the acute bioassay. There was significant difference ($P<0.05$) in pH values between treatment tanks and control. This could have resulted to respiratory distress, disturbance of ion/osmoregulatory performance and acid-base balance of body fluids. The severity of such disturbance have been reported to be related to the extent of acidification of the water (as was seen in this experiment) and the calcium ion concentration of the water (Malcom, 1994). Patin (2004), reported that acute effects of toxicants were greatly affected by temperature. However, temperature was within acceptable limits in this experiment, varied significantly ($P<0.05$) in all the test tanks including the control thus, did not affect fish survival.

HISTOPATHOLOGICAL CHANGES

Histological alteration in gill and liver of fishes are useful in assessing toxic effects of toxicant in fish (Hadi & Alwan, 2012). Sections of the gills of *C. gariepinus* juveniles exposed to aqueous crude fruit extract of*A.nilotica* revealed some severe histological alterations such as complete loss of secondary lamellae, massive presence of inflammatory cells, degeneration of connective tissue, hypertrophy and hyperplasia with increased in toxicant concentration. This observation is in agreement with Audu *et al*. (2017) 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* depicting a dose-dependent distortion especially with marked severity in those given the higher concentration of the extract.Wade et al. (2002) also reported damages ranges from oedema,telangiectasis of gill lamella and gill hyperplasia to vacuolation of liver of *Oreochromis niloticus*when exposed to toxicity of Cassava (*Manihot esculenta* Crantz) effluent.

Liver is known to be associated with detoxification and biotransformation and is the major organ affected by toxicants in water (Van der Oost et al., 2003). In this study, liver of *C. gariepinus* juveniles exposed to acute concentrations ofaqueous crude fruit extract of *A.nilotica* showed histological distortions such as hypertrophy, hemorrhage and cellular degeneration (necrosis) which increased as the toxicant concentration increased which further substantiates the toxic potential of this plant.This observations is in line with the work of Audu et al. (2017) 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 & Alwan (2012) who reported that *C. gariepinus* fingerlings exposed to aluminum for 96hrs showed important changes in the liver such as hypertrophy of hepatocytes, 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.

CONCLUSION

The toxicant had adverse effects on the water qualities, behavioral & histological parameters of the exposed fish compared to the control groups. The disruption in these parameters could have led to the degrees of impairment in the gills and livers of the exposed fish and the inability of the exposed fish to withstand infections, thereby causing a great concern for fish survival.Therefore, the use of *Acacia nilotica*in water bodies could lead to contamination of aquatic ecosystem by causing alteration in the physiological and general health of fish and other aquatic fauna.

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