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Histopathological Changes in the Gills and Liver of the African Catfish (*Clarias gariepinus*) Juveniles Exposed to Crude Stem Bark Extracts of *Adenium obesum* (Desert Rose)

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Abstract

Histopathological alterations in fish organs are among the biomarkers used to measure the impact of toxicants, providing insights into the effects of environmental contamination on aquatic organisms. This study investigated the effects of acute concentrations (0.00, 6.5, 8.0, 9.5, 11.0 and 12.5 mg/L) of stem bark crude extract of *Adenium obesum* on the histopathological changes in the liver and gills of *Clarias gariepinus* juveniles over a period of 96 hours in a static non-renewable bioassay. Mean water quality parameters (free carbondioxide, dissolved oxygen content, pH and total alkalinity levels) and histo-architectural integrity of liver and gills were determined using standard methods. Statistically significant differences ($p<0.05$) were found in all the studied physico-chemical parameters of the water compared with control. There was marked pieces of evidence of concentration-dependent alteration to the gills and liver histological organizations. The acute investigations revealed that acute exposure of catfish juveniles to *A. obesum* stem bark crude extract culminated in both water quality parameters and histological alterations in gills and liver more particularly the higher concentrations of extract in the acute exposure.

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1.0 Introduction

Aquaculture is the fastest-growing food sector in the world, accounting for an estimated 43 % of all fish consumed by humans globally (Ibrahim & Ambali, 2014). Synthetic chemicals have been the major pesticides in the control of pests and undesirable fish species in aquaculture but these chemicals have harmful effects on non-targeted fish species (Audu *et al.*, 2019). Thus, causing serious environmental hazards (Audu *et al.*, 2019). Environmental hazards occur when pollutants are discharged directly or indirectly into water bodies without adequate treatment to remove harmful constituents (Ahmed *et al.*, 2021). Water pollutants (plant extracts, heavy metals, and industrial effluents) have two poisoning mechanisms on fish (Audu *et al.*, 2020).

One occurs at high concentrations and provoking a rapid suffocation by destruction of the gill epithelium and the other prevails at low concentrations which inhibits the main metabolic pathways such as gills, kidney and liver (Audu, *et al.*, 2020).

The plant *A. obesum* is locally known as Kariya in Hausa ethnic group of Northern Nigeria (Samson, *et al.*, 2014). The plant is an important medicinal plant

which shows a wide range of biological activities (Akhtar *et al.*, 2016). Medicinal plants constitute a natural reservoir for medicines worldwide (Sofowora *et al.*, 2013). They serve mainstream therapeutics and are central in folklore medicine. In case of *A. obesum* (Lav, Apocynaceae), indigenous people of Oman use it for the treatment of venereal diseases, wounds, skin diseases, headaches, muscle pain as well as catching fish (Akhtar *et al.*, 2016). The plant exhibits anticancer, antiviral, antibacterial, trypanocidal, acaricidal, molluscicidal, antioxidant, and piscicidal activities (Paul *et al.*, 2016).

Fish are an excellent model for determining aquatic ecosystem health (Min & Kang, 2008). The fish *C. gariepinus* is a large, eel-like fish that belongs to the family *Clariidae* (Bruton, 1979). The catfish is widely cultured throughout Africa in both natural and artificial habitats, an omnivore freshwater fish a popular delicacy relished throughout tropical Africa. It is a prominent culture species because of its hardiness and fast growth rate (Ezekiel, 2017). Although acute toxicity effects of ethanol extract of *A. obesum* stem bark on histology of gills, liver and skin of African catfish were evaluated, there is dearth of information on acute toxicity effects of crude extract of *A. obesum* stem bark on gills and liver of catfish juveniles. Therefore, this research aimed at determining the acute effects of crude stem bark extract *of A. obesum* on histology of gills and liver of *C. gariepinus* Juveniles exposed to acute concentrations.

2. 0 Materials and Methods

2.1 Materials

2.1.1 Study Area

The study was carried out in the Hydrobiology and Fisheries Research laboratory of the University of Jos. The University is located, at the northern edge of a pear-shaped upland known as the Jos Plateau. It has a temperate climate averaging 22^0C (72^0F) daily; an average humidity of 60% and average annual rainfall of 1,400mm (56").

2.1.2 Collection and Preparation of Stem Bark of *Adenium obesum*

The experimental material *A. Obesum* was collected from the open fields of Kafin Kawu village in Ganjuwa Local Government of Bauchi State. Nigeria. Between August – October, 2023. The plant was identified by a plant taxonomist as *A. obesum*, in the Department of Biological Sciences, Bauchi State University, Gadau with a voucher number 01260. The stem bark was sheared from the tree plant using an axe and shade-dried over seven days with outdoor relative humidity of 57% and ambient temperature of 28^0C. The dried stem bark was pulverized in the laboratory using mortar and pestle into fine particles, sieved with a meshed utensil (30 µm) and stored in airtight transparent polyethene bag for subsequent use (Audu et al., 2021).

2.3 Experimental Fish Collection and Acclimatization

A total of one hundred and twenty (120) live and apparently healthy, mixed sex Juveniles of *C. gariepinus* of the same brood stock and age were purchased from a Global Integrated Fish Farm in Zarmaganda, Jos, Plateau State. They were transported to Hydrobiology and Fisheries Research laboratory of University of Jos, Nigeria between 0700 and 0900 hours. They were distributed into six clean plastic aquaria of (49 X 23 X 24cm^3) dimension containing dechlorinated tap water with a stocking density of 10 fish/aquaria and were covered with wooden square lid of wire gauze (to stop the fish from jumping out of the aquaria). The fish were allowed to acclimatized to the laboratory condition for a period of two (2) weeks under a static bioassay to ensure that mortality does not exceed 5% and fed daily with 2 mm size pelleted commercial diets (Vital Feed®, Nigeria) equivalent to 3% of their body weight. During this period, the fish were observed closely under a 12 h light: 12 h darkness cycle. Water was changed on daily basis with dechlorinated tap water.

2.2 Acute Toxicity

Acute toxicity bioassays was conducted to determine the 96h LC_{50} value of the crude stem bark powder of *A. obesum* was conducted in semi-static system in a laboratory according to the OECD guideline NO 23 (OECD, 1992). The range-finding test was determined according to the method described by Hoffman et al., 1995. From the range finding tests, six graded concentrations (0.00, 6.50, 8.00, 9.50, 11.00 and 12.5mg/L) of the *A. obesum* extract was used for the definitive test. A complete randomized design was used in the experiment with two aquaria set up for each dose and each aquarium contains ten fish in ten litres of dechlorinated tap water. The performance test lasted for 96 hours. The fish were checked for mortality at different time intervals. The dead fish, if found, were removed immediately to reduce pollution-related effects and were counted for determination of LC_{50}. The toxicant and test water in each aquarium were renewed after 24 hours. The behavior and general conditions of the fish were observed before, during and after each bioassay. The 96 h LC_{50} was determined as a probit analysis using the arithmetic method of percentage mortality data (Thompson & Wilkins, 2003). At the end of 96h, organs (gill and liver) were collected from experimental fishes.

2.3 Water Quality Parameters for Acute (96hr-LC_{50}) Toxicity Test

The water quality parameters, including pH, Free carbondioxide (Free CO_2), dissolved oxygen content (DO), alkalinity and temperature of the experimental media, were determined using standard procedures. Physicochemical parameters of the water were monitored every 24 h using the methods described by Federation (2012).

2.4 Histopathological Examination of *C. gariepinus* Juveniles Exposed to Aqueous Stem Bark Extracts of *Adenium obesum*

At the end of the exposure period, the fish were randomly selected from each concentration and placed on a dissecting board and dissected using a dissecting scissors to remove the gills and liver. The gills and liver excised from the fish were placed in a 30ml Bijou bottles and 10% formaldehyde was added. The samples were taken to the histology laboratory for routine histology using optical microscope under the magnification X400: Haematoxylin-Eosin. (Martins *et al.*, 2018).

2.7 Statistical Analysis

Statistical analyses were performed using IBM SPSS (version 23) software. Data were analysed by One-Way Analysis of Variance (ANOVA). Treatment means were separated using Turkey's multiple comparisons test. The level of significance was determined at a p=0.05 level of probability and a p<0.05 value was considered statistically significant. Data were presented as Mean± SE (standard error).

The gills histo-architecture in the control *C. gariepinus* is characterized by normal parenchyma is evidenced by both intact primary and secondary lamellae, respectively, as well as normal interlamellar space (Plate 2A). The gills structure in the *C. gariepinus* exposed to (6.50 & 8.00 mg/L) seemed to share similar normal architecture with the control. (Plate 2B) mild hyperplastic secondary lamellar epithelium, the gill parenchyma seems normal with patent interlamellar space (star) and (Plate 2C), the gill parenchyma has no visible lesion as evidenced by both intact primary and secondary lamellae (black and red arrows respectively) as well as normal interlamellar space (star). However, *C. gariepinus* exposed to (9.50-12.50 mg/L) of acute concentrations of *A. obesum* displayed moderate to severe gill histopathology including hyperplasia of the apical epithelial cells of the secondary lamellar, interlamellar space occlusion, focal depletion of lamellar cells and cartilaginous matrix (Plate 2D-F). The higher concentration (12.5 mg/L) of acute concentration of *A. obesum* extract, the gill parenchyma was typified by intact interlamellar space but with the presence of focal depletion of lamellar cells (red arrow) and cartilaginous matrix (black arrow) (Plate 2F).

3.0 Results and Discussion

3.1 Water Quality Parameters of Tanks with *C. gariepinus* to Acute Concentrations of *A. obesum* and Control

The water quality parameters evaluated during the acute toxicity phase of the experiment are presented in table 3.1. The temperature, carbon dioxide and pH parameters were not significantly different (P<0.05) in the water fish tanks exposed to different concentrations of *A. obesum* extract and the control. However, the dissolved oxygen content values significantly decreased (3.85mg/L) in the water containing graded of the extract when compared to the control (0.00mg/L).

Table 3.1: -Mean Water Quality Parameters of Tanks with *Clarias gariepinus* Exposed to Acute Concentrations of *A. obesum* during the 96hrs

Parameters	Concentrated Grades of *Adenium obesum Extract* (mg/L)						
	0.00	6.50	8.00	9.50	11.00	12.50	P-values
Temperature (C^0)	23.50± 0.50^a	23.00± 0.00^a	24.00±0 .00^a	23.50± 0.50^a	23.50± 0.50^a	23.50± 0.50^a	0.05
Dissolved (mg/L)	8.40± 0.10^a	6.05± 0.05^{ab}	4.05± 0.05^b	3.90± 0.00^b	3.90± 0.00^b	3.85± 0.05^b	0.05
Carbon dioxide (mg/L)	3.75± 0.05^a	3.95± 0.05^a	4.10± 0.10^a	4.45± 0.05^a	4.65± 0.05^a	4.95± 0.05^a	0.05
Alkalinity (mg/L)	24.25± 0.05^a	19.10± 0.10^b	18.30± 0.10^b	17.40± 0.10^b	16.20± 0.10^b	14.15± 0.05^c	0.05
Ph	6.80± 0.10^a	6.45± 0.05^a	6.10± 0.10^a	5.90 0.00^a	±5.55± 0.05^a	5.25± 0.05^a	0.05

Values in the same row with different superscripts (a, b & c) are significantly different.

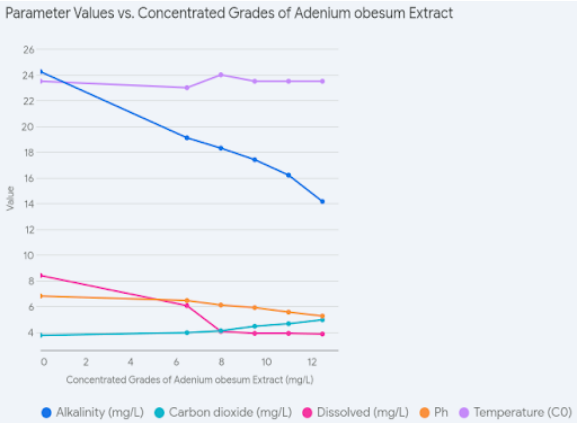
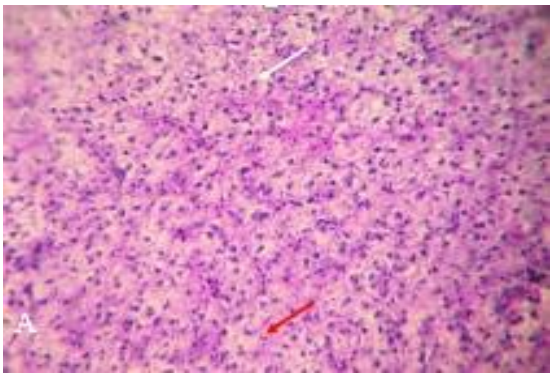
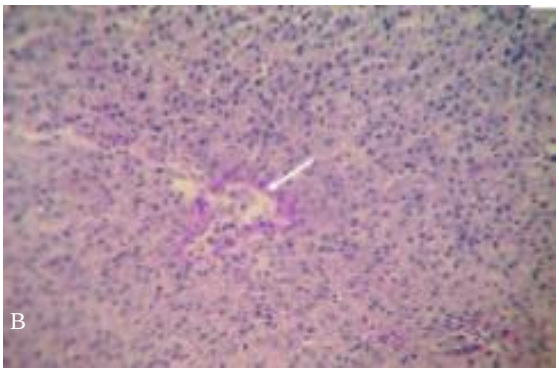


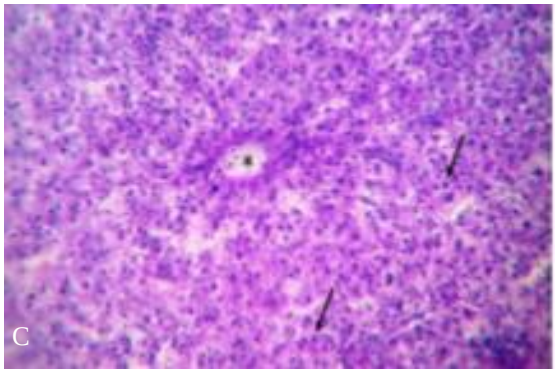
Fig 1: Line Plot showing the trends of each Water Quality Parameters with Increasing Concentrations of *Adenium obesum* Extract

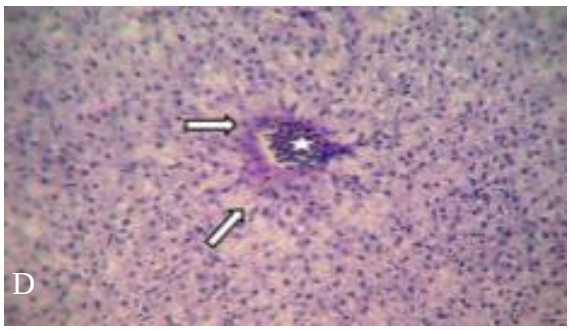
3.2 Histopathological Parameters of *C. gariepinus* Exposed to Acute Concentrations and Control.

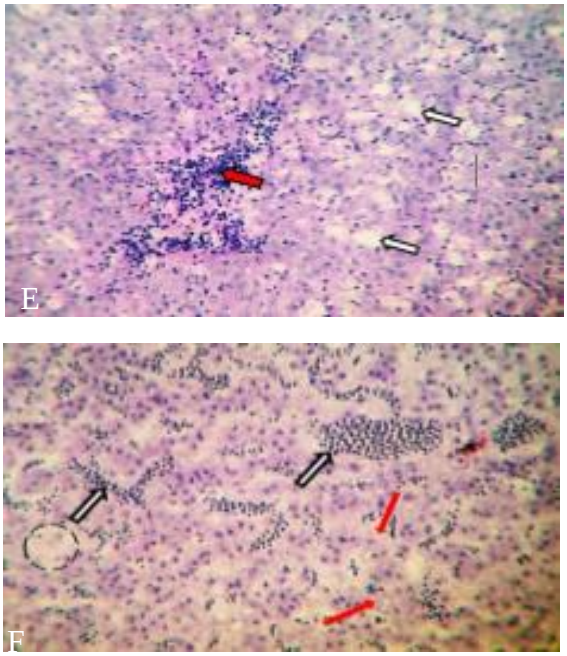
The liver and gills histopathological results of *C. gariepinus* exposed to acute concentrated grades of *A. obesum* stem bark extract are shown in plates (1&2 A-F) respectively. The liver of the control fish displayed normal hepatic histological organization characterized by normal hepatocytes, intact nuclei and substantial cytoplasm. The liver parenchyma also bears normal hepatic sinusoids (Plate 1A). The liver of *C. gariepinus* exposed to 6.50 mg/L of *A. obesum* extract did not differ from those of the control (Plate 1B). However, the *C. gariepinus* exposed to higher concentrated grades of *A. obesum* (8.00 -12.50 mg/L) extracts showed moderate to severe hepatic histopathological lesions comprising of hepatocellular degeneration, central veinous congestions and cellular infiltration of the hepatic parenchyma (Plate 1C-F). *C. gariepinus* exposed to higher concentrated grades of *A. obesum* (8.00 mg/L) with the exception of mild vascular fibrin deposit (white), the hepatocytes architecture appeared to be devoid of visible lesion (Plate 1C). While the liver exposed to (9.50 mg/L) acute concentration of *A. obesum* there is moderate perivascular hepatocellular degeneration (white arrow) and central veinous congestions (star) (Plate 1D). (Plate 1E) the liver of *C. gariepinus* juveniles exposed to (11.0 mg/L) acute concentration of *A. obesum* stem bark extract. There is marked localized perivascular cellular infiltration of the hepatic parenchyma (red arrow) and severe hepatocyte cytoplasmic vacuolation (white arrow). The liver exposed to highest concentration (12.50 mg/L) there is severe cellular infiltration of the hepatic parenchyma (white arrow), marked hepatocellular degeneration (oval outline) with evidence of pronounced hepatocyte cytoplasmic vacuolation (red arrow) (Plate 1F). The trend clearly shows severity of histopathological lesions was concentration-dependent.











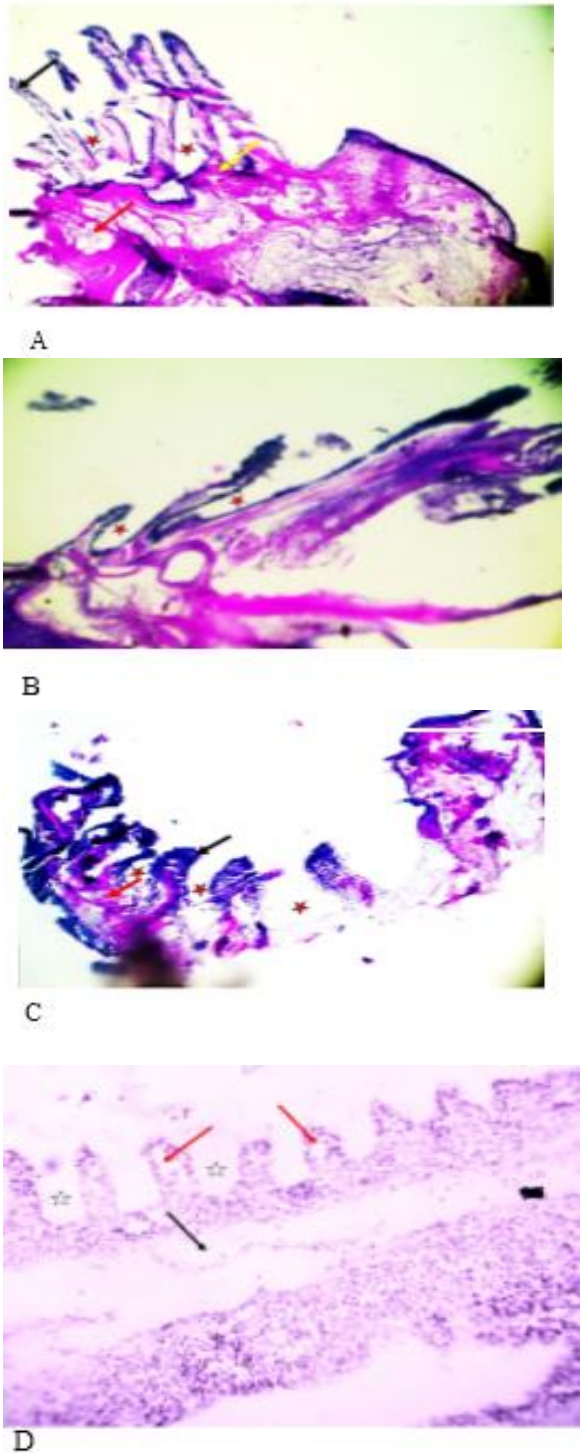


Plate 1: Photomicrograph of the liver of *Clarias gariepinus* juveniles exposed to acute concentrations of aqueous crude stem bark extract of *Adenium obesum* for 96 hours.

The gills histo-architecture in the control *C. gariepinus* is characterized by normal parenchyma is evidenced by both intact primary and secondary lamellae, respectively, as well as normal interlamellar space (Plate 2A). The gills structure in the *C. gariepinus* exposed to (6.50 & 8.00 mg/L) seemed to share similar normal architecture with the control. (Plate 2B) mild hyperplastic secondary lamellar epithelium, the gill parenchyma seems normal with patent interlamellar space (star) and (Plate 2C), the gill parenchyma has no visible lesion as evidenced by both intact primary and secondary lamellae (black and red arrows respectively) as well as normal interlamellar space (star). However, *C. gariepinus* exposed to (9.50-12.50 mg/L) of acute concentrations of *A. obesum* displayed moderate to severe gill histopathology including hyperplasia of the apical epithelial cells of the secondary lamellar, interlamellar space occlusion, focal depletion of lamellar cells and cartilaginous matrix (Plate 2D-F). The higher concentration (12.5 mg/L) of acute concentration of *A. obesum* extract, the gill parenchyma was typified by intact interlamellar space but with the presence of focal depletion of lamellar cells (red arrow) and cartilaginous matrix (black arrow) (Plate 2F).

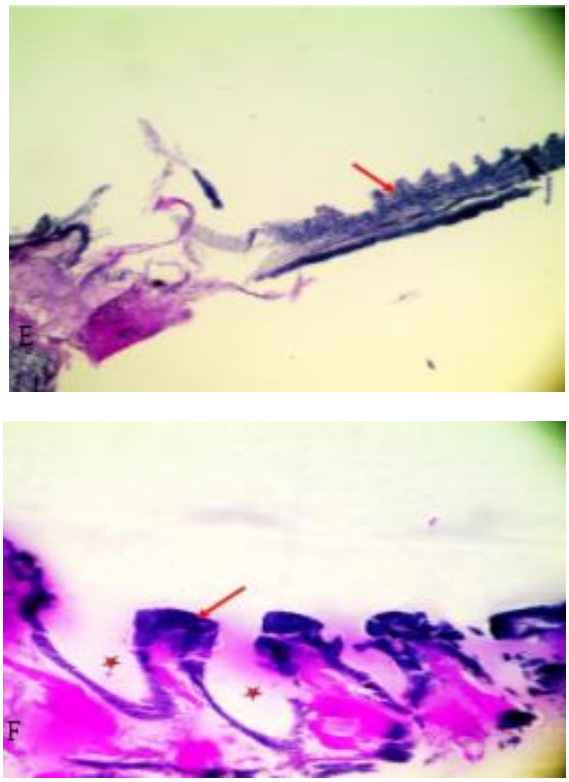


Plate 2: Photomicrograph of the gills of *Clarias gariepinus* juveniles exposed to acute concentrations of aqueous crude stem bark extract of *Adenium obesum* for 96 hours.

Water quality parameters assessment in fish culture during an experiment is necessary considering the complete reliance of the whole life process of fish on the quality of the immediate surroundings (Bolorunduro and Abdullahi, 1996). Apart from this, water quality is determined to establish its involvement in any role in the alterations observed during toxicity experiment as reduction in water quality influences stress and disease in fish (Devi *et al.*, 2017). Therefore, the non-significant difference in the temperature, carbon dioxide and pH parameters of the water containing experimental fish exposed to acute concentrated grades of *A. obesum* connotes that the parameters were within normal range that is required for fish survival and growth as confirmed by Audu *et al*. (2021) on the effect of acute concentrations of stem bark extract of *Anogeissus leiocarpus* on *C. gariepinus* juveniles..

4.0 Discussion

The dissolved oxygen content showed significant concentration dependent decrease values in the water containing grades of the extract when compared to the control. This impact of this is in the progressive accumulation of *A. obesum* extracts as its concentrated grades increases. It thus poses a serious threat to the dissolve oxygen content in the water through its degrading effects on the oxygen molecules. These findings confirmed the reports of Makori *et al*. (2017) on physico-chemical properties of natural earthen pond for culturing tilapia, Audu *et al*. (2020) on sub-lethal concentration of leaves extract of the desert date on cat fish and Audu *et al*. (2021) on the effect of acute concentrations of stem bark extract of *Anogeissus leiocarpus* on *C. gariepinus* juveniles.

Histo-architectural alterations have been widely used as biomarkers of pollutants in fish (Naeemi *et al.*, 2013). The gills of fish play crucial roles of respiration, osmoregulation and excretion (Camargo & Martinez, 2007; Jalaludeen *et al.*, 2012; Audu *et al.*, 2017) as a result of its proximity with the surrounding water environment (Olojo *et al.*, 2005). This closeness to the external environment predisposes it to histo-architectural disruptions that make fish to become vulnerable to respiratory and osmoregulatory difficulties (Olusegun & Adedayo, 2014), particularly when toxicants diffuse into their body and initiate gill membranes damage and in turn disrupt its physiological functions (Bala & Malachy, 2020). Most fish exposed to toxicants die when their gill lamella epithelia and blood vessels are adversely affected (Hinton & Lauren, 1990).

Hence, the observed moderate to severe gill histo-architectural alterations (hyperplasia of the apical epithelial cells of the secondary lamellar, interlamellar space occlusion, focal depletion of lamellar cells and cartilaginous matrix) precipitated by acute concentrated grades of *A. obesum* extract more particularly the higher concentrations of both toxicity phases further established the toxic potential *A. obesum*.

The gills histopathogical results observed in this study corroborate lesions earlier reported in similar studies conducted by Abalaka *et al.*, (2015) on *A. obesum*, Audu *et al.*, (2020) on *Tacca leontopetaloide*, Audu et al., (2020) on desert dates and Audu *et al.*, (2021) on *A. leiocarpus*, common active ingredients and possible mechanisms may contribute to the gill histological changes observed in fish exposed to these plants.

Two major processes, detoxification and biotransformation are known hepatic functions that have positioned the liver as an important organ to be probed in morpho-physiological disruption perturbed by accumulation of contaminants in the water (Hadi & Alwan, 2012). Owing to these backgrounds, the observed moderate to severe hepatic histopathological lesions comprising of hepatocellular degeneration, central veinous congestions and cellular infiltration of the hepatic parenchyma in catfish exposed to higher concentrated grades of the acute (8.0 -12.5 mg/L) of *A. obesum* stem bark extracts. The histopathological

lesions seen in this study tallies with those reported by Audu *et al*., (2020) on desert dates and Audu *et al*., (2021) on *Anogeissus leiocarpu*, flavonoids and saponins are the common bioactive ingredients in both the plants which may contribute to the observed histological alterations.

5.0 Conclusion

When fish are exposed to high levels of the liquid extract from the stem bark of *A. obesum*, it can be harmful. Therefore, it can be concluded that exposing *C. gariepinus* to varying concentrations of *A. obesum* (ranging from 0.00 to 12.5mg/L) for 96hrs could lead to changes in the liver and gills tissues. To protect fish diversity, it's important to avoid or control the careless disposal of *A. obesum* stem bark into stagnant water bodies.

It is recommended that use of *A. obesum* stem bark extracts for cropping is capable of affecting non-targeted aquatic biota negatively, and as such should be discouraged.

Declarations

Ethics approval and consent to participate

Not Applicable.

Consent for publication

All authors have read and consented to the submission of the manuscript.

Availability of data and material

Not Applicable.

Competing interests

All authors declare no competing interests.

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