MEDIA KEDOKTERAN HEWAN VOL 36(2)
https://e-journal.unair.ac.id/MKH
ISSN Online: 2775-975X pISSN : 0215-8930

Ameliorative Effects of *Borreria Verticillata* Aqueous Extract on *Adenium obesum* Stem Bark Extract-Induced Histopathological Response of The Gill, Intestine, Liver, and Kidney in *Clarias gariepinus* (Burchell 1822) Juveniles

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Received: September 11th, 2024
Accepted: January 24th, 2025
Published: May 16th, 2025

Abstract

The study was carried out to determine the effect of *Borreria verticillata* aqueous extract on *Adenium obesum* stem bark aqueous extract induced histopathological responses in the gill, intestine, liver, and kidney of *Clarias gariepinus* juveniles. The study consisted of ten (10) groups of fifteen (15) fish each. Groups II - V received 25, 50, 100, and 200 mg/L of the BVAE, respectively. Group VI received 0.838 mg/L of AOAE only (10% of LC_{50}), while groups VII - X received the combinations of the extracts (GII + GVI, GIII + GVI, GIV+GVI, and GV + GVI, respectively). The histopathology observed in Group VI included complete fusion of secondary lamellae and congested blood vessels, hepatic congestion, periportal inflammation, vacuolation, and hyperplasia of bile canaliculi, hypertrophy of the muscular layer and lamina propria and hyperplasia of the goblet cells, congestion of renal blood vessels, tubular degeneration, and swollen glomeruli. The histopathology induced by exposure in GVI in the gills, liver, and kidneys were ameliorated by the administration of *Borreria verticillata* extract in *Clarias gariepinus* juveniles in terms of severity which is more in the gill where there is complete club shaped gill in exposed fish compared to the *Borreria verticillata* extract treated groups.

Keywords

Aquatic, Bioindicator, Herb, Hepatoprotective, Toxicity

DOI: 10.20473/mkh.v36i2.2025.189-202

Introduction

The plant *Adenium obesum* was first discovered and described in Kenya in 1752 by a German Scientist, P. Forsskal, and the name was derived from the Arabic name of the plant, *Oddaejn*, which means Aden, the former name of Yemen, while obesum was derived from the swelling of the basal part of the plant stem (Plaizeier, 1980; McLaughlin and Garofalo, 2002). *Adenium obesum* is known locally as "Kariya" amongst the Hausa ethnic group of northern Nigeria (Dalziel, 1956; Adamu *et al.*, 2005) just as it is also called "Akpalataa" amongst the Igbo ethnic group of south-eastern Nigeria. Several cardiac glycosides have been reported in *A. obesum* (Mettam, 1941). The main cardiac glycoside in the plant is Oleandrigenin β- gentiobiosyl (1→ 4) β-D- thevetoside (Yamauchi and Abe, 1990; Ahmad and Basha, 2007). In addition, Oleandrigenin-β-D-glucosyl(1→4)-β-D- digitalose was also isolated from the chloroform fraction of the plant (Kiyohara *et al*., 2012). Hoffman and Cole (1977) equally reported the presence of other active cardenolides (Somalin, hongheloside A, 16-acetylstrospeside and honghelin) and an active flavonol (3,3-bis[o-methyl] quercetin) from the ethanol extract of *A. Obesum.* However, an inactive triterpene (dihydroifflaionic acid) and an inactive flavonol (3-0-methylkaempferol) were also reported from the ethanol extract of *A. obesum* (Hoffman and Cole, 1977). The methanol extract of *A. obesum* stem bark has been reported to contain some alkaloids, flavonoids, saponins, tanins, glycosides, anthroquinones and steroids (Tijjani *et al.*, 2011a). However, only saponins, tannins, steroids and glycosides were reported from the petroleum spirit extract of *Adenium obesum* stem bark (Tijjani *et al.*, 2011b). Similarly, a triterpenoid named botulin (Lup-20(29)-ene-3,28-diol) was reportedly isolated from the stem bark of the plant (Tijjani *et al.*, 2012). Cardiac glycosides exert their pharmacological or ionotropic effect by inhibiting the membrane-bound Na+ , K+ - ATPase pump thereby increasing intracellular Na+ and extracellular K+ resulting in the activation of the sodium-calcium pump (Na+- Ca + pump) that increases intracellular Ca + leading to increased myocardial contraction force (Novotny, 2005). However, excessive inhibition of the Na+, K+ - ATPase pump causes a variety of severe arrhythmias resulting in blocked cardiac activity, decreased cardiac output and death, which might be observed in *A. obesum* poisoning (Knight and Walter, 1983). *Adenium obesum* extract has been reported to elicit various histopathological responses in exposed *Clarias gariepinus* (Abalaka *et al*., 2015) and as a piscicide globally (Sharma *et al*. 2024). Studies demonstrate how certain herbs, like garlic and turmeric, have immune-stimulating qualities that boost fish immunity and advance health without the side effects of antibiotics (Singh *et al*., 2022). Studies have established that extracts from Borreria and Spermacoce species as well as their isolated compounds possessed diverse biological activities, including analgesic, anti-inflammatory, antineoplastic, antimicrobial, larvicidal, antioxidant, antiulcer, and hepatoprotective e in man and rat, with alkaloids and iridoids as the major active principles (Abdullahi-Gero *et al*., 2014; Murtala *et al*., 2015). This study was conducted to determine the ameliorative effect of *Borreria verticillata* extract on histopathology induced by *Adenium obesum* on the gill, intestine, liver and kidney of *Clarias gariepinus* juveniles.

Materials and Methods

Plant Collection and Extraction

The stem bark of *Adenium obesum* was collected from Samaru, Zaria, Kaduna State, Nigeria and it was also authenticated at the Herbarium section Department of Biological Sciences, Ahmadu Bello University, Zaria and a Voucher Specimen Number 01386 was given at the Herbarium for future reference. The leaves were picked and dried under shade until a constant weight was obtained and were crushed into coarse powder using a pestle and mortar and stored for the extraction process (Roshana *et al*., 2016).

Plant Extraction

The extraction process of stem bark of *Adenium obesum* and fresh leaves of *Borreria verticillata* was carried out as described by Saravanan *et al*. (2010).

Experimental Animals

The live juvenile African-Sharp-Tooth catfish, C. gariepinus (N = 450, average weight of 21.48 ± 3.32 g and length 11.37 ± 1.23 cm, respectively, were purchased from a commercial catfish farm of reputable standing and authenticated at the Fishery Section, Department of Biological Sciences, A. B.U., Zaria, Nigeria.

Determination of the median lethal concentration (LC_{50}) of *Adenium obesum* aqueous extract and *Borreria verticillata* aqueous extract

The median lethal dose of *Adenium obesum* extract of 8.3 mg/l used for this study was determined and reported by Muyiwa *et al.* (2019a). The sublethal doses of *Borreria verticillata* used for this study were based on the non-toxic nature of the extract to *Clarias gariepinus* in acute exposure, as reported by Muyiwa *et al*. (2019b).

The experimental design is based on ten groupings (as shown in Table 1) as follows: GI represents the control group where 0.5 liters of unchlorinated water are taken and replaced with 10 ml of distillation water on a daily basis: the water is changed every three days; GII, GIII, GIV, and GV were the groups. 25 mg/l, 50 mg/l, 100 mg/l and 200 mg/l sub lethal doses of BVE, respectively, were introduced into the tank after effectively dissolving the dried BVE in 10 ml of water taken from the group tank in order to maintain a constant concentration of the extract in the tank; GVI, this group contained 10% of LC_{50} AOAE, which represents the sublethal concentration of AOAE is 0.838 mg/l. This was introduced into the tank after effectively dissolving the AOAE in 10 ml of water taken from the group tank in order to maintain a constant concentration of the extract in the tank. 10% LC_{50} AOAE in addition to 25 mg/l, 50 mg/l, 100 mg/l, and 200mg/l sublethal doses of BVAE were introduced into the tanks of Groups VII, VIII, IX, and X, respectively, after effectively dissolving the dried BVAE in 10 ml of water taken from the group tank in order to maintain a constant concentration of the extract in the tank. Fish were fed twice daily (9am and 4pm), extracts were administered into the fish tank after morning feeding daily for 28 days, and water was refreshed every three days. Samples were collected on days 0, 7, 14, 21, and 28.

Table 1. The experimental design 28 days sub-lethal treatment

Treatments	GI	GII	GIII	GIV	GV	GVI	GVII	GVIII	GIX	GX
Control	+	–	–	–	–	–	–	–	–	–
25mg/l BVE	–	+	–	–	–	–	+	–	–	–
50mg/l BVE	–	–	+	–	–	–	–	+	–	–
100mg/l BVE	–	–	–	+	–	–	–	–	+	–
200mg/l BVE	–	–	–	–	+	–	–	–	–	+
0.838 mg/l AOE	–	–	–	–	–	+	+	+	+	+

Note: BVE = Aqueous Extract *Borreria verticillata* Aerial Part, AOE = Aqueous Extract *Adenium obesum* stem bark, + = Administered, - = Not administered

Tissue Collection

The fish were euthanized with 40% ethyl alcohol as described by Doerr and Stoskopf (2019). The gill, liver, intestine, and kidney, from each fish were harvested, fixed into 10% neutral buffered formalin, paraffin embedded, sectioned at 5 µm, and stained with hematoxylin and eosin as described by Zheng *et al*. (2024), for histopathological examination.

Results and Discussion

Histopathological response of gills to Sub-lethal treatment

Sections of gills (Figure 1) from juvenile *Clarias gariepinus* in Group I (control) showed no alterations in the primary and secondary lamellae (Plate A) on days 0 to 28. Similarly, there were no microscopic changes on the gills of Group V during the period (Plate B). On the other hand, fusion of secondary lamellae, atrophied primary lamellae, and congested blood vessels was observed in gills from Group VI on day 14 (Plate C). The sections of gills obtained from group VI on day 28 had fusion of secondary lamella, club-shaped primary lamella, complete fusion of the secondary lamella, and congested blood vessels (Plate D). The gills in Group X had one-sided fusion, curled secondary lamellae, and telangiectasia in primary lamellae as at day 14 (Plate E) and cellular infiltration on day 28 (Plate F) of the study.

Figure 2 (A-G) shows histopathology revealed normal liver in Group I (Plate A) and Group V (Plate B) during the period. The liver sections in Group VI had congestion of blood vessels surrounded by infiltrated inflammatory cells: there was also vacuolation and hyperplasia of bile canaculi (Plate C). The vacuolation was more severe on day 28 (Plate D). These changes were also seen in liver samples obtained on days 14 (Plate E) and 28 (Plate F) in Group X, but appeared to be milder.

Sections of posterior segments of the intestines (Figure 3) showed normal histology in Groups I (Plate A) and V (Plate B and Plate C) during the study. The intestinal sections in Group VI were characterized by hypertrophied lamina propria and hyperplasia of goblet cells (Plate D and Plate E), similar to those in Group X (Plate F and Plate G) on days 14 and 28, respectively.

Figure 4 shows normal kidney structures in Groups I (Plate A) and V (Plate B and Plate C) throughout the four weeks of this study. Tubular generation, congestion of blood

vessels, and swelling of glomeruli were observed in Group VI (Plate D and Plate E) from days 21 to 28. Most of the kidneys in Group X similarly showed near-normal architecture and swollen glomeruli (Plate F and Plate G).

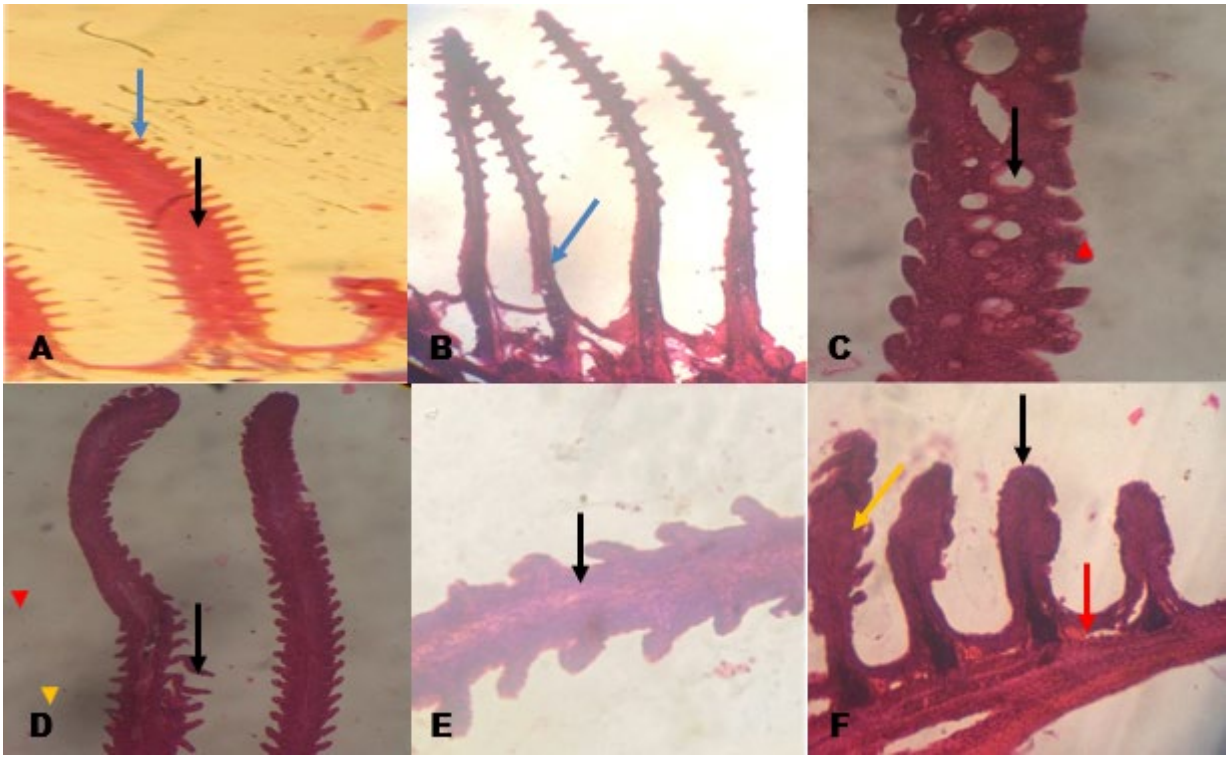

Figure 1. (Plate A) Photomicrograph of the gill in G1 (CONTROL) on D14, showing a normal primary lamella (black arrow) and a normal secondary lamella (red arrow) (H & E, X200); (Plate B) Photomicrograph of the gill in G5 on day 28, showing denuded, fused and atrophied secondary lamellae (blue arrow) (H & E, X400); (Plate C) Photomicrograph of the gill in G6 on day 14, showing vacuolated primary lamella (black arrow), fusion of secondary lamella (red arrow) (H & E, X400); (Plate D) Photomicrograph of the gill in G10 on day 14,showing a one-sided fusion of the secondary lamella (red arrow), curled secondary lamella (black arrow), Telangiectasia within the primary lamella (blue arrow), aneurysm (yellow arrow) (H&E, X100); (Plate E) Photomicrograh of the gill in G10 on day 28; showing an epithelium infiltration within the primary lamella (black arrow) (H & E, X100); (Plate F) Photomicrograph of the gill in G6 on day 28, showing fusion of secondary lamella (Yellow arrow),clubbed-shaped primary lamella with complete fusion of the secondary lamella (Black arrow) and congested blood vessel (Red arrow) (H&E, X600).

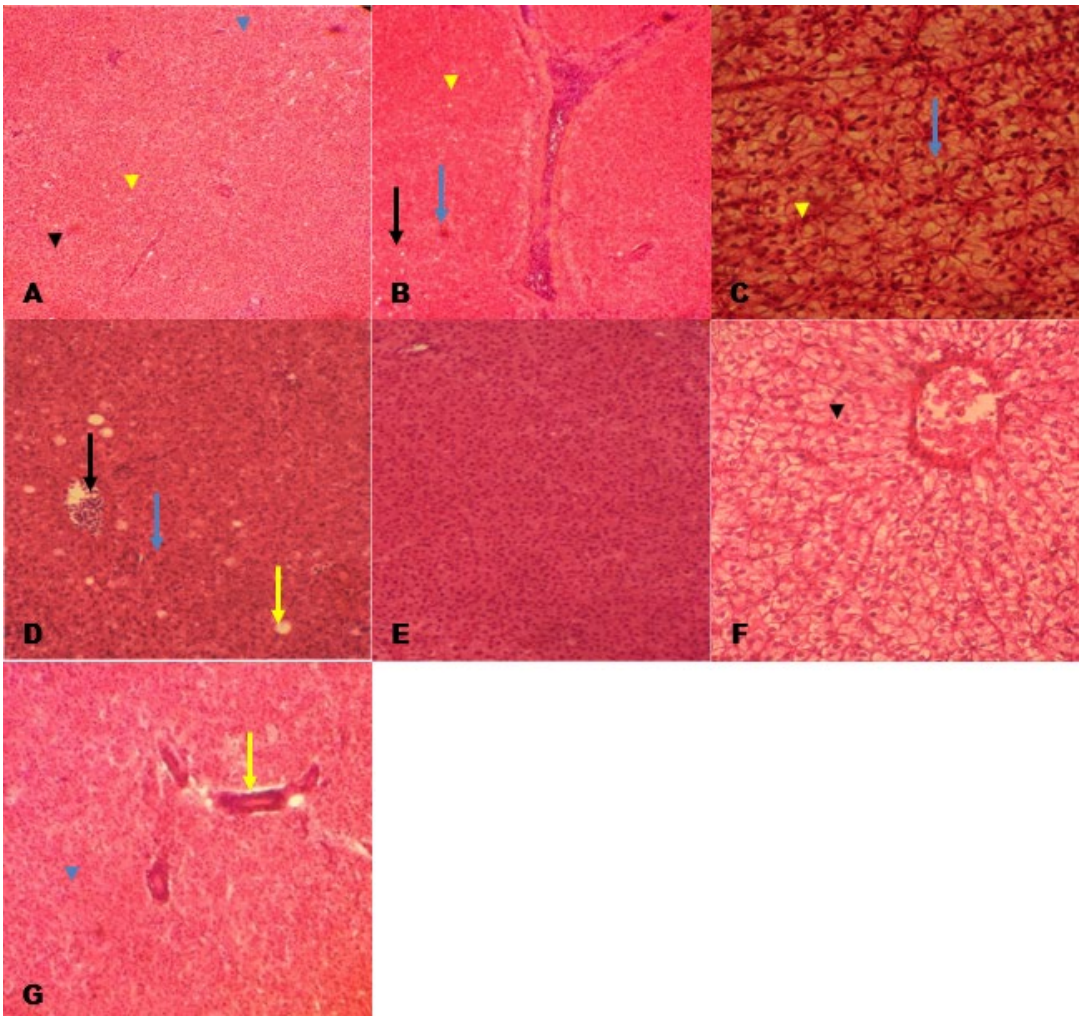

Figure 2. (Plate A) photomicrograph of the liver G1; showing a normal parenchyma of the liver tissue, bile duct (Black arrow),central vein (yellow arrow),melanomacrophage centre (blue arrow) (H & E, X400); (Plate B) Photomicrograph of the liver in G5 on day 14, hepatopancrease (yellow arrow), melanomacrophage (blue arrow), focal areas of vacuolation (black arrow) (H & E, X400); (Plate C) Photomicrograph of the liver in G6 on day 14, showing generalized vacuolation (blue arrow) and hyperplasia of bile canaculi (yellow arrow) (H & E, X400); (Plate D) Photomicrograph of the liver in G10 on day 14, showing a central vein with red blood cells (black arrow),melanomacrophage (blue arrow), areas of vacuolation (red arrow) (H & E, X1360); (Plate E) Photomicrograph of the liver in G5 on day 28; showing normal liver parenchyma (H & E, X40); (Plate F) Photomicrograph of the liver in G6 on day 28; showing congested central vein (black star),diffuse areas of vacuolation (H & E, X2400); (Plate G) Photomicrograph of the liver G10 on day 28; atrophied hepatopancrease (yellow arrow),blood vessel (blue arrow) (H & E, X200).

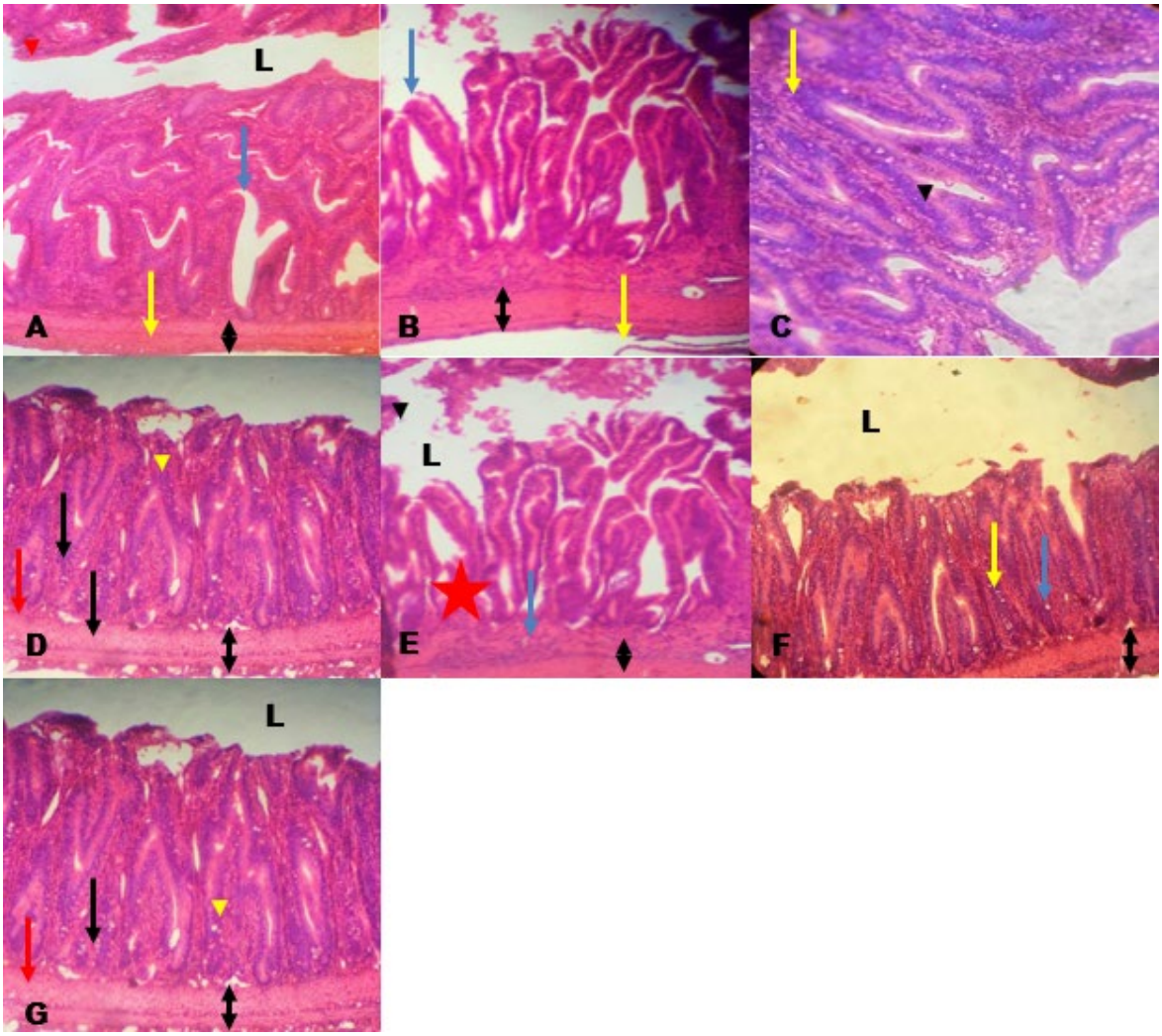

Figure 3. (Plate A) Photomicrograph of the posterior intestine G1;showing,intestinal lumen (L), intestinal villi (red arrow),lamina propria(blue arrow),muscularis mucosa (double arrow),serosa (Yellow arrow) (H & E, X1440); (Plate B) Photomicrograph of the posterioir intestine G5 on day 14; showing intestinal lumen (L), Lamina propria (green arrow), muscularis mucosa (double arrow), serosa (yellow arrow) (H & E, X1440); (Plate C) Photomicrograph of the proximal intestine in G6 of day 14; showing hypertrophied lamina propia (yellow arrow), hyperplasia of goblet cells (black arrow), lumen (L) (H & E, X400); (Plate D) Photomicrograph of the posterior intestine G10 on day 14; showing goblet cells (yellow arrow), hypertrophied lamina propia (black arrow), and muscularis mucusa (double arrow) (H & E, X1280); (Plate E) Photomicrograph of the sagittal section of the posterior intestine G5 day 28; showing villus (black arrow), lamina propria (red star), submucosa(blue arrow), muscularis mucosa (double arrow) (H & E, X1280); (Plate F) Photomicrograph of the sagittal section of the posterior intestine in G6 on day 28; showing, intestinal lumen (L), hyperplasia of the goblet cells (yellow arrow), lamina propia (blue arrow),muscularis mucosa (double arrow) (H & E, X1280); (Plate G) Photomicrograph of the sagittal section of the posterior intestine in G10 on day 28; showing, serosa (black arrow), muscularis mucosa (double arrow), sub mucosa (red arrow), goblet cells (black arrow), lamina propia (yellow arrow) (H & E, X1280).

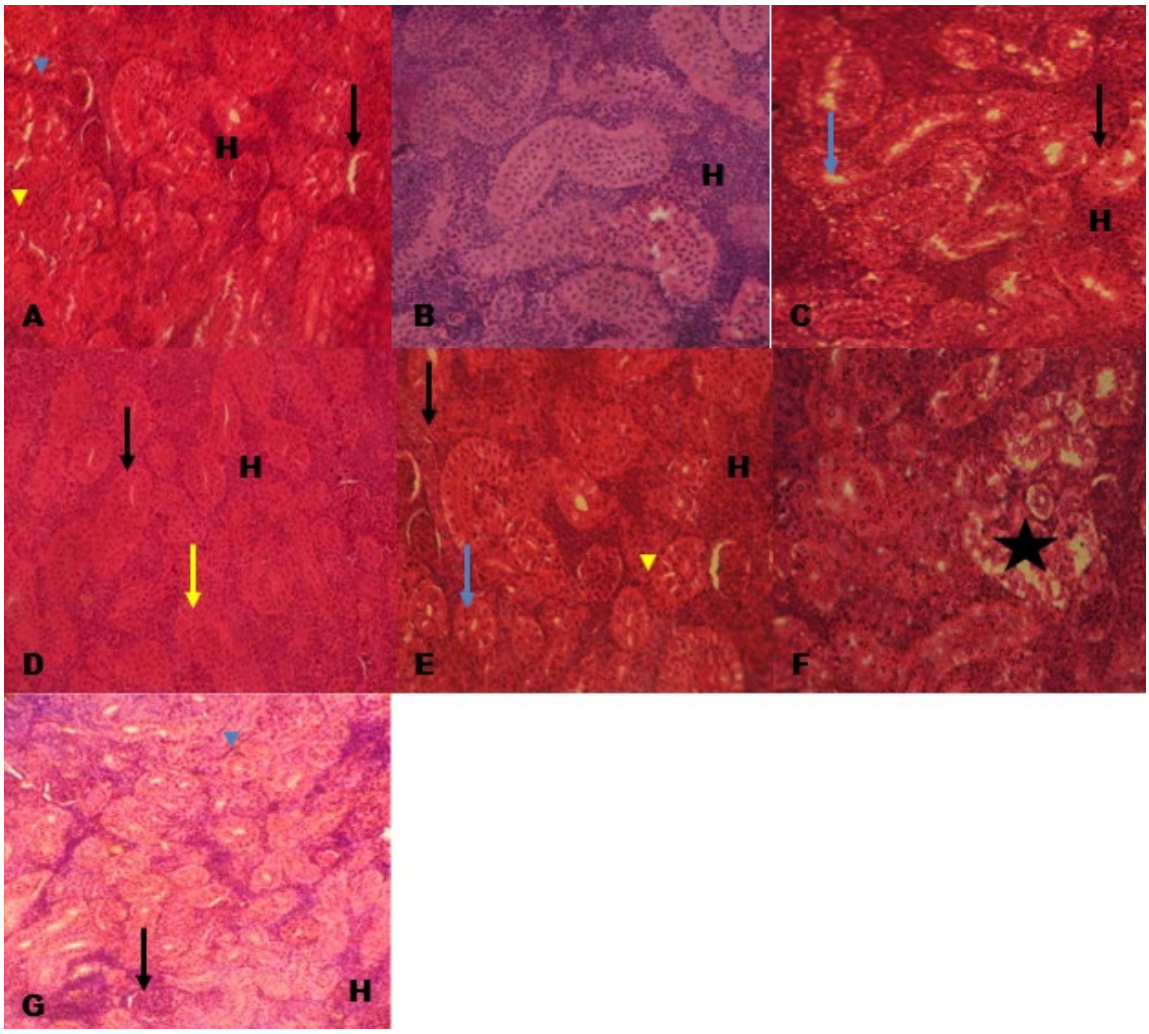

Figure 4. (Plate A) Photomicrograph of the transverse section of the kidney G1, showing; distal convoluted tubule (yellow arrow), proximal convoluted tubule (blue arrow), hematopoietic tissue (H), glomerulus (black arrow) (H & E, X1280); (Plate B) Photomicrograph of the transverse section of the kidney in G5 day 14; showing hematopoietic tissue (H), proximal convoluted tubule (red arrow), (H & E, X1680); (Plate C) Photomicrograph of the transverse of the kidney in G6 on day 14; showing, depleted hematopoietic tissue (H), tubular degeneration (black arrow), distal convoluted tubules (blue arrow) (H & E, X1680); (Plate D) Photomicrograph of the transverse section of the kidney in G10 on day 14, showing proximal convoluted tubule (yellow arrow), hematopoietic tissue (H), distal convoluted tubule (black arrow) (H & E, X1120); (Plate E) Photomicrograph of the transverse section of the kidney in G5 on day 28; showing, hematopoietic tissue (H), glomerulus (yellow arrow), proximal convoluted tubule (black arrow), distal convoluted tubule (blue arrow). (H & E, X1680); (Plate F) Photomicrograph of the transverse section of the kidney in G6 on day28; showing, generalized tubular degeneration (Black star) (H & E, X1680); (Plate G) Photomicrograph of the transverse section of the kidney in G10 on day 28; showing, dilated proximal convoluted tubule (blue arrow), distal convoluted tubule (yellow arrow), hematopoietic tissue (H), hypercellularity of the glomerulus (black arrow) (H & E, X1120).

Examining tissue changes in fish through histopathology enables one to understand how contaminants like pesticides affect organs like the liver and gills. As an illustration of the harmful effects of chemical exposure, notable histopathological alterations were seen in carp subjected to chlorpyrifos (Zaheen and Momand, 2024). It has been helpful to evaluate the effects of both legacy and emerging contaminants by using caged fish for active

monitoring; this approach enables a thorough assessment of environmental health when paired with a variety of biomarkers. In a number of environments, including the contaminated Sepetiba Bay, where fish lesions were observed, suggesting unfavorable environmental conditions, histological abnormalities in fish gills have been successfully employed as bioindicators (de Brito Carvalho *et al.*, 2020). According to Matadamas-Guzman *et al*. (2019), histopathological techniques have been developed to grade the degree of tissue alterations, offering a health indicator for tracking the impacts of pollution in aquatic settings. The gill, liver, intestine, and kidney tissue of exposed C*larias gariepinus* juveniles showed various pathological changes in this study.

Hyperplasia and hypertrophy of the epithelial cells, besides partial fusion of some secondary lamellae observed in AOAE-exposed fish in this study, might have been defense mechanisms since, in general, these result in the increase of the distance between the external environment and the blood and thus serve as a barrier to the entrance of contaminants in this study (Azadbakht *et al*., 2019). Coutinho and Gokhale (2000) found epithelial lifting in the gills of carps (*Cyprinus carpio*) and tilapias (*Oreochromis mossambicus*) exposed to the effluents of wastewater treatment plants. As a consequence of the increased distance between water and blood due to epithelial lifting, the oxygen uptake is impaired. However, fish have the capacity to increase their ventilation rate to compensate for low oxygen uptake (Azadbakht *et al*., 2019). Most of the gill lesions caused by sub-lethal exposures affects lamellar epithelium (Azadbakht *et al*., 2019).

The observed changes on gill sections, such as club-shaped and atrophy of primary lamellae, vacuolations and fusion of secondary lamellae, as well as congestion of blood vessels in the gill arches of Group VI, appeared to be milder in Groups VII-X and absent in Groups I-V. Similar alterations have been reported in fish exposed to permethrin and other toxicants (Abalaka *et al*., 2015). In an earlier study, hyperemia, epithelial proliferation, epithelial detachment, lamellar edema, mucous cell hypertrophy, partial lamellar fusion, epithelial cellular infiltration, hemorrhage, complete lamellar fusion, epithelial degeneration, and epithelial necrosis were recorded in *Clarias gariepinus* exposed to an ethanol extract of *Adenium obesum* (Abalaka *et al.*, 2015). Aqueous extract of *Vernonia amygdalina* (Audu *et al*., 2017). The lamellar fusion were defensive responses to reduce brachial superficial area in contact with the toxicant (Abalaka *et al.*, 2015). The erosion and adhesion or fusions of the secondary lamellae herein reported are in accordance with the findings of earlier investigators (Figueirodo-Fernandes *et al.*, 2007).

The presence of pathology in gills from Groups VII–X in this study may be due to the continuous exposure of these groups to the toxicant without a recovery period to allow for complete healing. However, the fact that the changes appeared to be milder than the group treated with AOAE alone may suggest that the extract of *B. verticillata* contains constituents that have some restorative or protective effects on the gills.

The liver pathology observed in this study was indicative of the effect of *A. obesum* extract. Hepatocytes of *Clarias gariepinus* exposed to *Khaya senegalensis* have shown such changes (Matouke and Aburi, 2015). The liver is

the organ most associated with the detoxification and biotransformation process, and due to its function, position, and blood supply (Cunningham and Porat-Shliom, 2021).

Vacuoles observed in the cytoplasm of hepatocytes in exposed groups in this study may be lipids or glycogen, which are related to the normal metabolic function of the liver, especially when the animals are stressed (Han *et al*., 2015; Wei and Han, 2024). The glycogen acts as a reserve of glucose to supply the energy demand occurring in such situations (Han *et al.*, 2015). Pacheco and Santos (2002) described increased vacuolation of the hepatocytes as a signal of exposure to contaminated water.

The present study showed hypertrophy of lamina propria and hyperplasia of goblet cells in Groups VI and VII–X on day 14. Between days 21 and 28, there was also hypertrophy of the muscular layer of the intestine in these groups. The intestinal pathology here being reported lends support to earlier observations (Suchismita and Abhik, 2013).

However, the ameliorative studies to understand the effects of plant extracts on the fish digestive tract pathology were not undertaken by these previous researchers as was done here. Such pathology observed in the intestines of Group VII–X juvenile *C. gariepinus* exposed to AOAE and treated with BVAE in concurrent administrations gives no strong evidence in favor of using the later extract to ameliorate the pathology seen in fish exposed to AOAE alone; this may be likely due to continuous exposure of the fish to the toxicant without a recovery time to allow for possible tissue healing.

The microscopic changes in fish kidneys from Group VI were tubular degeneration, swollen glomeruli, and congestion of blood vessels. In Groups VII–IX. There was dilation of both distal and proximal convoluted tubules and glomerular hypercellularity. It is apparent that the kidneys were adversely affected by toxic constituents of AOAE, since Groups I–V showed no pathology.

It has been established that the teleostean kidney is one of the major target organs to be affected by contaminants in water (de Brito Carvalho *et al.,* 2020), and it also appears to be particularly sensitive to a variety of toxins due to the high volume of renal blood inflow and its ability to concentrate substances and biotransform some parent compounds to their toxic metabolites. A large quantity of diluted urine may be produced, and many of the enzymatic reactions occurring in the liver have been known to also occur in the kidneys (Mohamed, 2009). The kidneys receive the bulk of the postbrancheal blood flow and are important in the detoxification and elimination of aquatic contaminants in fish (Takvam *et al*., 2023). In fish, detoxification of toxic chemicals depends on the kidneys' function in controlling acid-base balance and eliminating ammonia. Takvam *et al*. (2023) state that they employ a range of transporters and enzymes to efficiently oversee these procedures. These explain why damage to kidneys has been observed following fish exposure to toxicants (Tunna *et al*., 2016).

Conclusion

The pathology induced by exposure to AOAE in the gill, liver, intestine and kidney was ameliorated by the administration of BVAE in *Clarias gariepinus* juveniles in terms of severity which is more evident in the gill tissue where club shaped primary lamellae, which is an irreversible alteration seen in exposed

Clarias gariepinus, was not seen in the groups treated with *Borreria verticillata* aqueous extract.

Approval of Ethical Commission

Ethical clearance approval was given by the Ahmadu Bello University Committee on Animal Use and Care (ABUCAUC) with approval number ABUCAUC/2017/014.

Acknowledgement

Professor Alpha Raj Mekapogu of the Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science, S.V. Veterinary University, Proddatur, Indian, assisted in the acute bioassay guide for the experimental design for the *Adenium obesum* extract.

Author's Contribution

BM: Conceptualization; Methodology; Formal analysis, Writing - Original Draft. JSS: Investigation; Supervision, Data Curation, Validation. AAA: Writing - Review & Editing. UAA: Visualization.

Conflict of Interest

There's no conflict of interest between the authors with regard to this research.

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