

Histopathological Alterations in Gills and Liver of African Catfish (*Clarias gariepinus*) Juveniles Exposed to Acute Concentrations of Aqueous Leaf Extract of Ordeal Tree (*Erythrophleum suaveolens*)

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Abstract: One of the many biomarkers for determining the effects of pollutants on fish is changes in organ histomorphology. This study sought to determine the effect of graded acute concentrations (0.30, 0.40, 0.45 and 0.50 g/L) of *Erythrophleum suaveolens* on the histology of gills and liver of mixed sex of *Clarias gariepinus* juveniles. A total of 120 *C. gariepinus* juveniles were exposed to the acute concentrations of the plant extract. Paraffin wax method and haematoxylin-eosin staining techniques of tissue processing were adopted for the examination of the gills and liver. Dose-dependent histopathological changes were observed in the two organs (gills and liver), i.e. histopathological alterations increased with an increase in the concentration of the plant extract. Gills showed lamellae fusion, haemorrhage, severe necrosis and desquamation of secondary lamellae and severe necrosis of primary lamellae, while the liver indicated necrosis of hepatocytes, congestion and swelling of the intratubular spaces. Structural alterations were evident in the gills and liver of *C. gairepinus* juveniles exposed to the concentrations of aqueous crude leaf extract of *E. suaveolens*.

Keywords: Histopathology; Gill; Liver; Ordeal Tree; African catfish.

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Introduction

The experimental plant, *Erythrophleum suaveolens* is a perennial tree reaching approximately 30 meters in height, featuring a slightly buttressed trunk, with a tendency to be low-branching and forming a dense, spreading crown (Burkill, 1985). Indigenous communities use various names for this tree, such as obo and erun in Yoruba, inyi in Igbo, gwaska in Hausa, aba in Akan-Asante (Ghana), digpende in Bassari (Togo), teli in Koranko (Sierra Leone), and in English, it is commonly known as sassy, sasswood, red

water tree, and ordeal tree (Burkill, 1985). Research has indicated that *Erythrophleum* species pose a significant toxicity risk to livestock, particularly sheep and cows, and because of this, herders in Savannah regions exercise great caution to prevent their animals from grazing in areas where these specific tree species are known to thrive (Akinpelu, Dare, Adebisin, Iwalewa & Oyedapo, 2012).

Numerous authors have documented the impact of plant extracts on the histopathology of gills and liver of different fish species. Akinsanya, Utoh, & Ukwa (2016) reported tissue proliferation, blood vessel congestion, lamellae

fusion, and epithelial lifting in gills of *C. gariepinus* exposed to extracts of *Piper guineense*, *Aframomum melegueta*, *Moringa oleifera*, *Gongronema latifolium*, *Azadirachta indica*, *Garcinia kola* and *Xylopiya aethiopica*

Nasiruddin et al. (2012) examined histopathological alterations such as disintegration of gill filaments and lamellae in gills as well as degeneration of hepatocytes and blood vessels in the liver of *Heteropneustes fossilis* exposed to 50% ethyl alcohol extract of three dry seed extracts of *Lagerstroemia speciosa*, *Dipterocarpus turbinatus*, and *Heveabra siliensis*. Oluwatoyin (2011) documented necrosis, cellular degeneration, malignancy, and inflammation in the gills following the exposure of *Oreochromis niloticus* to acute concentrations of *Ipomoea aquatica* for 96 hours.

Audu, Omirinde, Gosomji, & Wazhi (2017) documented hepatocellular degeneration, central and sinusoidal congestions in the liver of *C. gariepinus* exposed to concentrations of *Vernonia amygdalina*. Abalaka, Fatihu, Ibrahim & Kazeem (2010) documented hyperemia and pronounced oedema with lamellae fusion in the gills of *Clarias gariepinus* exposed to the ethanol extract of *Adenium obesum* stem bark.

In north-central Nigeria, local fishermen use part of *E. suaveolens* to collect fish in water bodies. Leaves of the plant are crushed and thrown into stagnant water bodies, and the stupefied fish are collected. The effects of leaf plant extracts on the fish are, however, not without attendant health effects on fish organs. In consideration of the piscicidal attributes of *E. suaveolens*, this study seeks to investigate the acute toxicity effect of the leaf extract of *E. suaveolens* on the histopathology of gills and liver of *C. gariepinus* juveniles.

Materials and Methods

Collection and Preparation of *Erythrophleum suaveolens* Leaves

The foliage of the *E. suaveolens* plant was gathered in Gindiri, located in the Mangu Local Government Area of Plateau State, North-Central Nigeria. The State is situated to the North-East of Bauchi, to the North-West of Kaduna, to the South-West of Nasarawa, and to the South-East of Taraba, sharing borders with these Nigerian states (Naija Times, 2023).

The leaves were air-dried under a shed, pounded with a ceramic mortar and pestle, then sieved into powder (0.5mm) mesh size. From the range fining test (RFT), five definitive concentrations of 0.50, 0.45, 0.40, 0.35 and 0.30 g/L of crude leaves extract of *E. suaveolens* were obtained. The above grams (×10 each) of the powdered crude leaves were weighed and macerated in ten litres (10L) of water for 24 hours; the mixture was filtered through a funnel choked with

non-absorbent cotton wool (Audu et al., 2014). The filtrate obtained was used as stock solution (Olaifa & Lewis, 2003).

Collection and Acclimation of *Clarias gariepinus* Juveniles

A batch of 120 *C. gariepinus* juveniles was purchased from Aquaculture Laboratory, Hydrobiology and Fisheries Unit, University of Jos, Plateau State, Nigeria. The research was conducted in the same Lab. Before the commencement of each experiment, a total of 120 *C. gariepinus* juveniles were acclimated for two weeks. The acclimation took place in twelve round plastic containers, each with a capacity of 35L and accommodating 20 juveniles. The containers were filled with 20L of water each. Throughout the acclimation period, the water in the holding tanks was changed daily at 8:00 hours. The fish were fed a commercial diet (Coppens®) twice daily at 10:00 and 17:00 hours, following Nigerian time.

Experimental Design

Six rectangular glass tanks were measuring 40x25x23cm, and they were arranged in a randomised block design. Each tank housed ten *C. gariepinus* juveniles. The experimental design was replicated. Each of the six glass tanks was filled with 10L of dechlorinated municipal tap water. Among these tanks, five were inoculated with varying concentrations of the aqueous crude leaf extract of *E. suaveolens*, and 10 mixed-sex *C. gariepinus* juveniles were introduced into each of them. The remaining tank, serving as the control, was stocked with 10 juveniles but was free of the test material. The fish were starved 24 hours before the experiment. This entire setup for each experiment was replicated. There was no renewal of test water or aeration throughout the period of the experiment.

Excision of Organs of *Clarias gariepinus* Juveniles

After exposure of *C. gariepinus* juveniles to acute concentrations of aqueous crude leaf extract of *E. suaveolens*, one juvenile each from treatment and control tanks was removed and dissected to extract the gills and liver. Each removed organ was rinsed with distilled water to wash off traces of blood. Each organ was preserved in a 10 ml specimen bottle containing 5 ml formal saline and transported to National Veterinary Research Institute (NVRI), Vom, Plateau State, Nigeria for histopathological examination of gills and liver.

Histopathological Examination of Gills and Liver of *Clarias gariepinus* Juveniles

Paraffin wax method and haematoxylin-eosin staining techniques of tissue processing described by Drury and Wallington (1967) and Avwioro (2011) were adopted for the examination of the gills and liver of *C. gariepinus* juveniles exposed to aqueous crude leaf extract of *E. suaveolens*

Results

Histopathological alterations in organs of *C. gariepinus* juveniles exposed to acute concentrations of aqueous crude leaf extract of *E. suaveolens*.

Gills

Gill of *Clarias gariepinus* juveniles in the control tank (0.00g/L) showed normal secondary lamellae and primary (lamellae plate 1A). Concentrated grades (0.30, 0.35, 0.40, 0.45 and 0.50 g/L) of plant extract induced mild fusion of secondary lamellae, mild haemorrhage of primary Lamellae. Light necrosis, enlargement of primary lamellae, fusion and desquamation of secondary lamellae, necrosis of primary

lamellae, necrosis and fusion of secondary lamellae, mild haemorrhage of primary lamellae, severe necrosis, desquamation of secondary lamellae and severe necrosis of primary lamellae (Plate 1B-F).

Liver

No pathology was observed in the liver of *Clarias gariepinus* from the control (0.00g/L). *Clarias gariepinus* Juveniles Exposed to Acute Concentrations of Aqueous Crude Leaves Extract of *Erythrophleum suaveolens* precipitated dose dependent slight necrosis of hepatocytes and slight intratubular vein to severe necrosis of the hepatocytes and intratubular vein (2B-F).

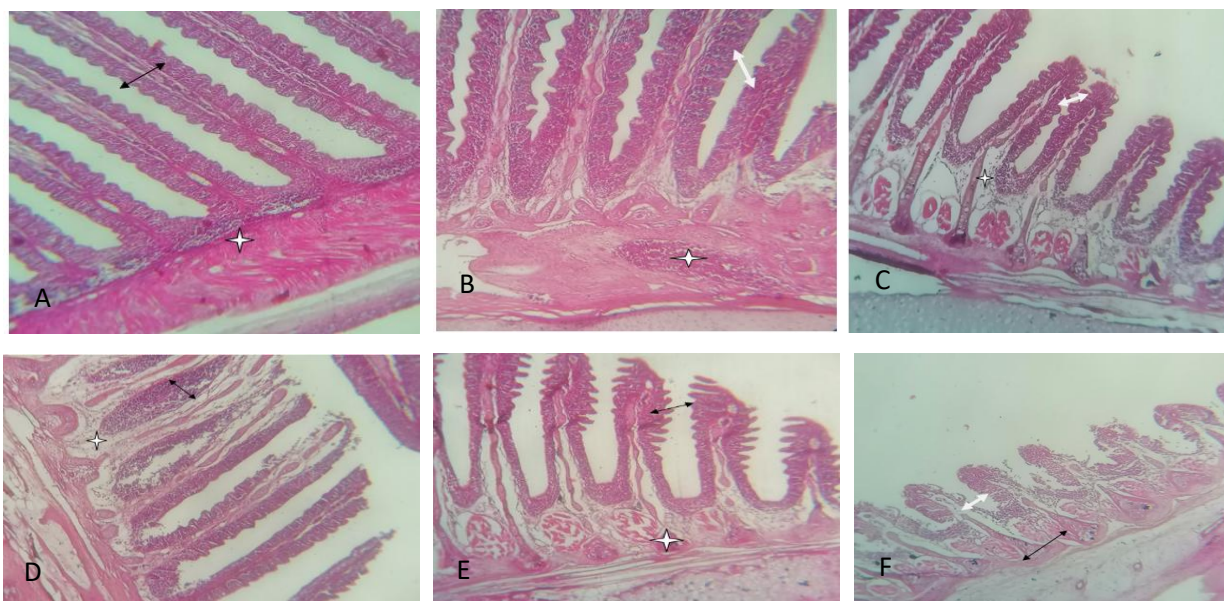
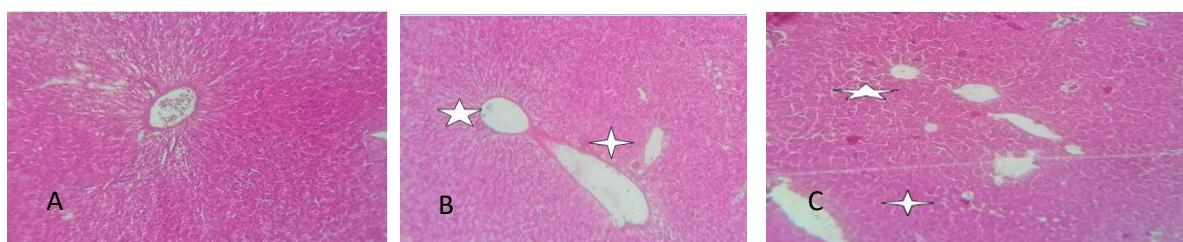


Plate 1. Photomicrographs of the Gill of *Clarias gariepinus* Juveniles Exposed to Acute Concentrations of Aqueous Crude

Leaf Extract of *Erythrophleum suaveolens*. A. (0.00g/L): the gills' histomorphology was intact, as shown by normal secondary lamellae (white star) and primary lamellae (black arrows). B. (0.30g/L): Showing mild fusion of Secondary Lamellae (white-headed arrow) and mild haemorrhage of primary lamellae (White star). C. (0.35g/L): Showing light necrosis and fusion of Secondary Lamellae (White headed arrow) and enlargement of Primary Lamellae (white star).

D. (0.40g/L) Showing fusion and desquamation of secondary lamellae (Black headed arrow) and necrosis of primary lamellae (Whitestar). E. (0.45g/L) showing necrosis and fusion of secondary lamellae (black headed arrow) and mild haemorrhage of primary lamellae (whitestar). F. (0.50g/L) showing severe necrosis and desquamation of secondary lamellae (white headed arrow) and severe necrosis of primary lamellae (Black arrow). H&E: X100



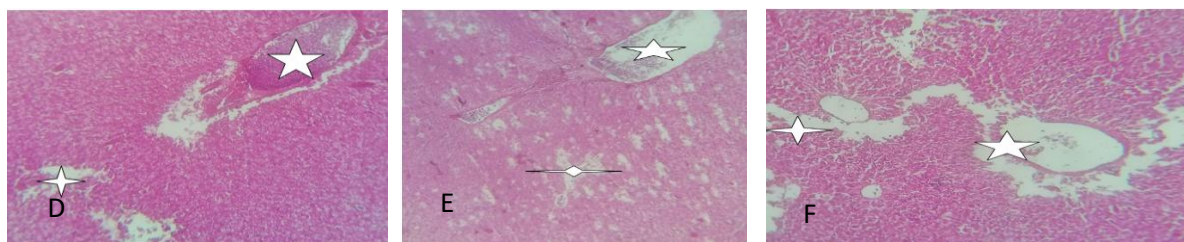


Plate 2. Photomicrographs of the Liver of *Clarias gariepinus* Juveniles Exposed to Acute

Concentrations of Aqueous Crude A. (0.00g/L): no pathology observed. B. (0.30g/L): slight necrosis of the hepatocytes (4-point star) and slight intratubular vein (5-point star). C. (0.35g/L) necrosis of the hepatocytes (4-point star) and mild congestion of the intratubular vein (5-point star). D. (0.40g/L) necrosis of the hepatocytes (4-point star) and swelling of the intratubular vein (5-point star). E. (0.45g/L) slightly severe necrosis of the hepatocytes (4-point star) and intratubular vein (5-point star). F. (0.50g/L) chronic necrosis and desquamation of the hepatocytes (4-point star) and intratubular vein (5-point star). H&E: X100.

Discussion

Histopathological alterations, as noted by Thophon et al. (2003), serve as commonly utilized biomarkers for assessing the impact of pollutants on fish. The gill, crucial for functions like respiration and osmoregulation, is particularly susceptible to environmental changes, which can disrupt its function (Camargo and Martinez, 2007). This study observed pronounced effects on the gill structures, notably the lamellae and blood vessels, with the severity increasing with increasing concentration of aqueous crude leaf extract of *E. suaveolens*. This aligns with findings by Wakawa et al. (2023), who documented escalating histopathological alterations in the gills of *C. gariepinus* juveniles exposed to increasing concentrations of *Thevesia peruviana* leaves extract.

The observed changes in the gills, including tissue atrophy, shortening, desquamation, adhering and erosion of secondary lamellae, along with haemorrhage in interstitial spaces, parallel findings by Shuaibu et al. (2022) after exposing *C. gariepinus* to varied treatments of *Parkia biglobosa* husk extract. Similarly, Adebola and Ayo (2014) reported gill alterations such as lamellae hypertrophy, hyperplasia, and desquamation of the secondary lamellae in *Heterobranchius bidorsalis* exposed to cypermethrin. Nasiruddin et al. (2012) observed similar changes in *H. fossilis* following exposure to 50% ethyl alcohol extract of three dry seeds. Additionally, Camargo and Martinez (2007) noted epithelial lifting, hyperplasia, hypertrophy of epithelial cells, partial fusion of secondary lamellae, and rupture of gill epithelium causing haemorrhage in a Neotropical fish.

Histopathology of organs serves as one of the most reliable biomarkers of stress in fish, with the liver being particularly vulnerable due to its role in detoxification and biotransformation processes (Abuh et al., 2024). Exposure to various concentrations of aqueous crude leaf extract of *E. suaveolens* resulted in tissue atrophy, necrosis, haemorrhage, and cellular degeneration in the liver of *C. gariepinus* juveniles. These changes resemble hepatocyte hypertrophy, nuclear hypertrophy, cytoplasmic vacuolation, and cellular degeneration in *Tilapia zilli* exposed to aluminium (Hadi & Alwan, 2012). Adebola and Ayo (2014) reported similar findings in *H. bidorsalis* exposed to cypermethrin. Additionally, Wakawa et al. (2023) observed hepatic atrophy, necrosis, and Kupffer cell proliferation in *C. gariepinus* juveniles exposed to graded concentrations of *T. peruviana* leaves extract. These liver histological changes in *C. gariepinus* juveniles may be attributed to the increased detoxification workload required to eliminate the toxic leaf extract from the body (Adebola & Ayo, 2014).

Conclusion

Exposure of fish to acute concentrations of aqueous crude leaf extract of *E. suaveolens* for 96 hr could be toxic. It could therefore be concluded that exposure of *C. gariepinus* juveniles to acute concentrations (0.30 - 0.50 g/L) of *E. suaveolens* for 96hr could cause histopathological alterations in their gills and liver.

Recommendation

Indiscriminate dumping of leaves of *E. suaveolens* in lentic water should be avoided or regulated to preserve fish diversity.

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