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Water Quality Parameters and Histopathological Changes in Gills and Liver of *Clarias gariepinus* Juveniles Exposed to Ethanol Leaf Extract of *Senna occidentalis*

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The leaf of coffee senna or septic weed, *Senna occidentalis,* is among the medicinal and toxic plants whose toxicity on fish has not thoroughly been assessed. The study used 120 *Clarias gariepinus* juveniles with an average body weight of 16 ± 2 g and length of 8.5 ± 0.5 cm, randomly distributed in 12 plastic tanks of 35 L capacity filled up to 10 L volumes of water, acclimatised for 14-day, and fed twice daily with 1.8 mm skretting feed. Various ethanol leaf extracts of *Senna occidentalis* (0, 3000, 5000 and 7000 mg L^{-1}) were added with a 5 mL syringe across the 12 plastic tanks, adopting a renewal bioassay, following the range-finding test of 500, 1000, and 1500 mg L^{-1}, which had no visible impacts on the tested fish samples over 96-h. Five *in-situ* parameters (Dissolved Oxygen, Temperature, Conductivity, and Total Dissolved Solids) were monitored daily for water quality over 96 hours. Analysis of Variance analysed the data with the Duncan Multiple Range Test separating the mean. There were no significant ($p > 0.05$) differences across the monitored water parameters. The control fish had normal histopathology (gills and liver), while the gill of the exposed fish had severe lamellar hyperplasia with complete occlusion and congestion of pillar capillaries. Moderate hepatic necrosis, mild sinusoidal congestion and atrophy of hepatocytes were observed on the liver of the test *C. gariepinus.* The study concluded that levels of the *Senna occidentalis* used in the main experiment exhibited phytotoxicity, thereby necessitating adherence to the levels used in the range-finding test.

Key words: Lamellar hyperplasia, Necrosis, Renewal bioassay, Water quality, SDG 14

According to Oladimeji *et al*. (2016), Nigeria is classified as a typical coastal nation with an abundance of vegetation and water resources, both quantitatively and qualitatively sufficient to support a large fish population. The country has 87 km^3 of groundwater and approximately 214 billion m^3 of surface water, both of which are suitable for artisanal fishing and aquaculture.

Plant phytochemical extracts have biological properties that include illness prevention and nutrition production. For this reason, the tradition of utilising plants in the form of crude extracts to treat common infections and long-term conditions in fish species as a means of controlling parasites and pests as well as dietary nutrients has been adopted in Nigeria and other countries worldwide (Meskin *et al.,* 2002).

According to Haruna (2006), fish is a preferred food because it is readily available as a major source of protein for Nigerians and its prices have been fair and quite affordable. Fish has emerged as one of Nigerians' main sources of protein in their diets over the past few decades. Notably, FAO (2014) states that increasing fish production and lowering reliance on fish imports are two of aquaculture's top priorities. Sall *et al*. (2020) examined global food consumption patterns of fish and its byproducts along with other seafood varieties. Moreover, researchers in fields including microbiology, pathology, toxicology, nutrition, and pharmacology have embraced the use of fish in their fields of study.

Over a billion people worldwide, according to FAO (2014), rely primarily on animal proteins found in foods like fish. Commercial aquaculture also contributes to greater food security by creating jobs and revenue for nearby communities. Fish provides high-quality protein, accounting for up to 16% of all animal protein consumed globally. The production of fish is also advancing globally, outpacing other livestock by roughly 10% annually.

Clarias gariepinus is a popular aquaculture fish species raised for human consumption among Africans; its marketability and acceptability boost its monetary value among Nigerian marketers. Aquaculture production needs to be increased and intensified to attain maximum or sustainable production and guarantee that fisheries do not decline over time (Delgado *et al.,* 2003).

Senna occidentalis, also known as Coffee Senna or Septic Weed, is one of the many important wild plant species that are spreading faster across Nigeria's savanna region (Fig. 1). It was brought to Africa from tropical Asia (Umar and Ahmad, 2014). The plants go by a number of common names, including Mogdad coffee, Negro coffee, Stephanie coffee, Stinking coffee, Styptic weed, Coffee senna, and Coffee weed. Local names for the plant include Sanga-sanga or Rai dore (Hausa), Akidi agbara (Ibo), and Abo rere (Yoruba) in Nigeria (Isah *et al*., 2018); Bana Chakunda in Odisha, India (Jain and Jain, 2017).

Studies have shown that *S. occidentalis* is toxic to a variety of animal species, like cattle (André *et al*., 2017), pigs (André *et al*., 2017), horses (André *et al*., 2017), rabbits (Tasaka *et al*., 2000), and poultry (André *et al*., 2017).

Consumers of fish products and food have become more aware of related health issues and are curious about the safety of the fish they eat (Zhou *et al.,* 2020; Hassan *et al.,* 2021; van Bussel *et al.,* 2022). Thus, it is necessary to investigate fish toxicants found in the environment, such as botanical plants which exhibit piscicidal characteristics (Simeon *et al*., 2011).

It has also been established that *S. occidentalis* is both toxic and medicinal (that is, it possesses dual properties). However, *Senna occidentalis* leaves are among the toxic and medicinal plants whose toxicity has not been thoroughly assessed, particularly on fish. Thus, the current study aims to advance our understanding of water quality parameters and histopathological changes in the exposed *C. gariepinus* juveniles' gills and liver to ethanolic leaf extract of *S. occidentalis.*

Histopathology of Fish

When evaluating the health of fish, histology and histopathology, which are used interchangeably, are helpful instruments because they provide important information about the extent of injuries, tissue damage, and organ functionality (Raibeemol *et al.,* 2020). The literature had examples of using histology to ascertain the dietary and medical state of *Clarias gariepinus* (Temitope *et al*., 2020), other fish (Harkrishan *et al.,* 2020), and farm animals like chickens (Hussein *et al.,* 2020). The fish gill is the primary organ through which pollutants enter the body and subsequently travel to other organs; this is because of its respiratory system and direct contact with water. According to Erdoğan *et al*. (2022), fish are susceptible to aquatic toxicants because their gills are respiratory organs that often come into contact with the outside world. The delicate gills are in charge of preserving the ideal balance between acid and base in bodily fluids as well as osmoregulation. Because of their vast surface area and contact with external bodies, the gills are the organs that many pollutants in water target. As a result, they react quickly to even minute alterations in the chemical, biological, or physical environment (Sula *et al*., 2020).

The liver is a crucial organ that participates in the proteins carbohydrates, and fats metabolism. Hepatocyte alterations serve as the primary marker of a toxic environment, according to Hinton and Lauran (1990), who also claim that the liver serves as the body's primary detoxification centre and that this function is carried out by the liver cells (hepatocytes). Furthermore, the liver is the primary metabolic organ where detoxification occurs, making it vulnerable to harm from toxicants (Odigie *et al.,* 2023). Although the liver has an important function in the glucose metabolism to glycogen for storage, lipid regulation, and amino acid deamination (Abubakar *et al*., 2023), it is impossible to overstate how vulnerable it is to any harm from toxicants. Toxins may also target it as an organ (Velkova-Jordanoska and Trajcevski, 2020).

MATERIALS AND METHODS

Experimental Site

The study was carried out at the Fish Hatchery Complex, Department of Aquaculture and Fisheries Management, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria (FUNAAB).

Collection of juveniles of *Clarias gariepinus*

A total of one hundred and twenty (120) juveniles of *Clarias gariepinus* with an initial average body weight of 16 ± 2 grams and length of 8.5 ± 0.5 cm were obtained from Path Farm, Sagamu, Remo, Ogun State, Nigeria, and transported in a 50 L keg to Fish Hatchery, FUNAAB.

Acclimatisation of the *C. gariepinus* Juveniles

The *C. gariepinus* juveniles were acclimatised for two weeks; during this period, they were fed 1.8 mm imported Skretting feed, which contained 45% crude protein, two times a day, in the early hours of the morning and the late hours. Feeding was stopped 48-h to the experiment's inception, and wastes and wasted feed were taken out every day along with replenishing water.

Collection of *Senna occidentalis* leaves

The leaves of *Senna occidentalis* were obtained at Old Bola Ahmed Tinubu road, off Iju road, Ifako-Ijaiye LGA, Lagos State, Nigeria, and were validated at the Forestry and Wildlife Management Department, FUNAAB.

Experimental Design and Procedure

The experiment had four treatments and three replicates, each with 10 fish per treatment tank. From the acclimatised fish, one hundred and twenty juvenile catfish were randomly distributed into twelve plastic tanks of 35 L capacity volume, filled with 10 L of water for the main experiment.

Determination of Physico-Chemical Parameters

Using the calibrated multi-parameter Hanna Instrument (Model HI 98129) and DO meter (PCE-DOM Series oxygen meter), measurements of temperature, hydrogen ion concentration (pH), total dissolved solids (TDS), electrical conductivity (EC), and dissolved oxygen (DO) concentration were made in each treatment group (Table 1).

Statistical Analysis

Analysis of variance was used to assess the data, and the distinction between treatment groups was found using the least significance difference (LSD) at the 0.05 significance level. The toxicity indices were obtained using SPSS (version 20.0), a computer statistical program.

RESULTS

Water quality parameters

The study's analysis of the physical and chemical parameters of water quality is highly pertinent to the level of water quality that *C. gariepinus* can tolerate. The outcome of the water parameters is shown in the table below.

Histopathology of the gill

Fish that were exposed to varying amounts of *S. occidentalis* showed altered gill structures, which are shown in Fig. 2 A–D. The findings indicated that Fig. 2 A had no visible lesion, Fig. 2 B had no visible lesion, Fig. 2 C had pillar capillary congestion and Fig. 2 D had significant lamellar hyperplasia with total blockage of the inter-lamellar space.

Results of the effect of *S. occidentalis* ethanolic leaf extract on *C. gariepinus'* gills are shown in plate 2. A = control (0.00 mg/ 25 cL), B = Treatment B (3000 mg/ 25 cL), C = Treatment C (5000 mg/ 25 cL) and D = Treatment D (7000 mg/ 25 cL).

Histopathology of the liver

The observed changes in the liver of *C. gariepinus* exposed to ethanolic leaf extract of *S. occidentalis* revealed no observable lesion in Fig. 3 E, Fig. 3 F showed moderate hepatic necrosis, Fig. 3 G displayed mild sinusoidal congestion, and there was atrophy of hepatocytes in Fig. 3 H.

Results of the effect of ethanolic leaf extract of *S. occidentalis* on the liver of *C. gariepinus* are shown in Fig. 3. E = control (0.00 mg/ 25 cL), F = Treatment B (3000 mg/ 25 cL), G = Treatment C (5000 mg/ 25 cL) and H = Treatment D (7000 mg/ 25 cL).

Figure 1. Picture of *Senna occidentalis*

Table 1. Physico-chemical parameters of the test water

Water Parameters	Unit	Range	Mean
Temperature	°C	25.37-28.43	26.90 ± 1.53
Dissolved oxygen	mg/l	5.34-6.99	6.17 ± 0.83
pH	-	6.83-8.40	7.62 ± 0.79
TDS	mg/l	0.20-0.40	0.30 ± 0.10
Conductivity	µs/cm	0.30-0.40	0.35 ± 0.05

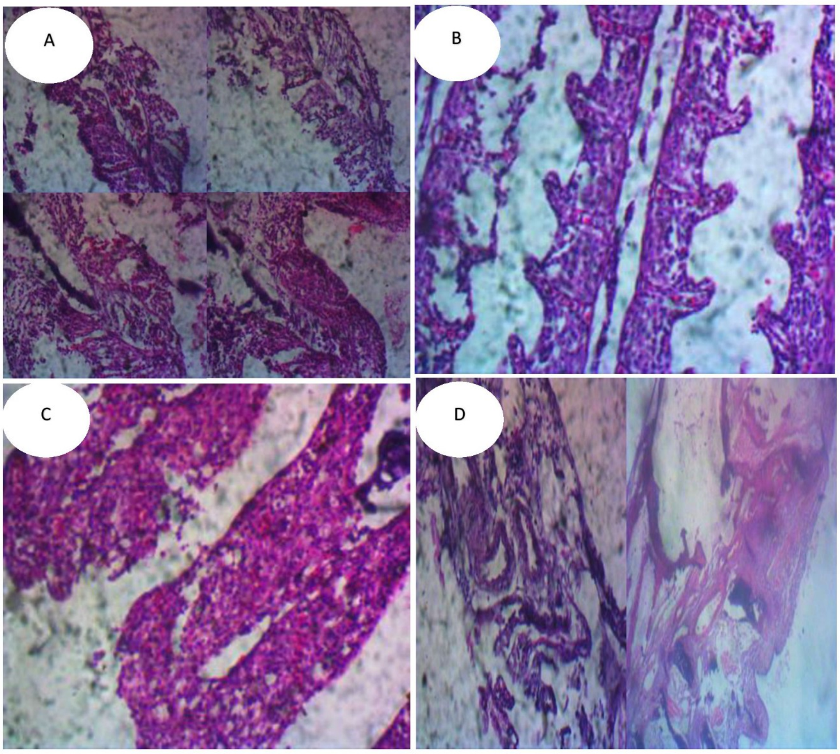

Figure 2. A had no visible lesion; **B** had no visible lesion; **C** had pillar capillary congestion; **D** had significant lamellar hyperplasia with total blockage of the inter-lamellar space.

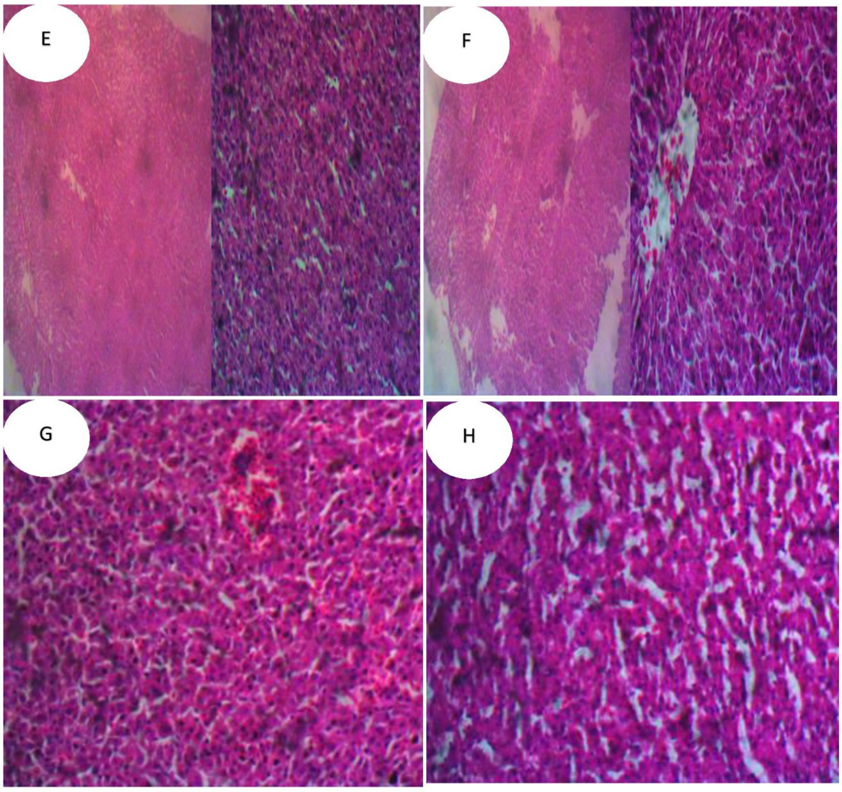

Figure 3. E there is no observable lesion; **F** showed moderate hepatic necrosis; **G** displayed mild sinusoidal congestion; **H** showed atrophy of hepatocytes.

DISCUSSION

Gebrelibanos *et al.* (2014) reported that *Senna species* have been known to cause a variety of toxicities despite their many potential medicinal benefits; this has been a major concern in aquaculture production (Idowu *et al.,* 2017). Considering the level of water quality that *C. gariepinus* can tolerate, the physical and chemical water quality parameters examined in this study are of great importance. Table 1 described that there was no significant difference ($p > 0.05$) in the water parameters; the values recorded were within the optimal range, meaning the observed parameters did not stray from regulatory norms; this is consistent with the findings of Jamabo *et al*. (2015); it can therefore be said that the water had no negative impact on *C. gariepinus* juveniles. According to this study, different amounts of *S. occidentalis* ethanolic leaf extracts caused varying degrees of histopathological alterations in *C. gariepinus* liver and gills. Tissue histopathological examination provides a reliable means of identifying and observing different kinds of tissue damage (Yang *et al.,* 2014). The healthy gills of fish are essential for breathing, osmoregulation, and the excretion of nitrogenous waste (Jimoh *et al*., 2023), among other metabolic activities (Herrero *et al*., 2018). The result of histology of the gills revealed that there was no visible lesion in the control treatment. Congestion in the pillar capillaries affected the juveniles in treatment B, which caused a major blockage or obstruction of oxygen flow. The gill of *C. gariepinus* juveniles in treatment D was severely affected; this might be a result of its vast surface area and direct contact with external bodies, as reported by Sula *et al*. (2020); the gills are the organs that many pollutants in water target and it was earlier said that they react quickly to even minute alterations in the chemical, biological, or/and physical environment. According to Foyle *et al*. (2020), an abrupt change in gill activity may be fatal, which was likely the case in this research work. Moreover, Agbebi *et al*. (2013) observed hyperplasia in the gills of *C. gariepinus* fed different dietary treatments; in contrast, the fish in treatment D was typified with severe lamellar hyperplasia with complete occlusion of inter-lamellar distance; meaning, there was an unusual increase in the size of the organ due to an increase in the number of cells which led to an obstruction in the lamellar of the gills.

As earlier reported, the liver is the primary metabolic organ where detoxification takes place. For this reason, the liver becomes vulnerable to harm from toxicants (Odigie *et al.,* 2023) and is also a target for toxins (Velkova-Jordanoska and Trajcevski, 2020). The result of the liver histology revealed that there was no visible lesion in the control treatment. However, the fish's liver displayed moderate hepatic necrosis in treatment B, which was in line with studies of Jimoh *et al.* (2015) and Olukunle (2011), who also noted minor hepatocyte alterations; this could lead to cirrhosis in the long run, as the injury or death of hepatic cells in the organ could result in diseases of the liver caused by damage from toxins. Hepatocyte atrophy was seen in treatment D and mild sinusoidal congestion in treatment C, respectively; this could result in a decrease in the liver's functionality because sinusoidal congestion means a block in the channel through which blood flows in the liver and can also result in cirrhosis; this aligned with the findings of Jia *et al*. (2022), who reported hepatic cell shrinkage, which could cause cirrhosis and impaired portal flow through the liver.

CONCLUSION

Given the values of the water quality parameters recorded, it can be concluded that the water quality did not deviate from optimal value despite the toxins released by *S. occidentalis.* However, the toxicity of *S. occidentalis* was capable of causing intensive damage to the internal organs of *C. gariepinus,* most especially the gills, which have direct contact with external bodies. The study would advise that usage of the test plant should not exceed the concentration of the range-finding test to prevent the phytotoxic effect of the likes of the test fish in this study.

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CONFLICTS OF INTEREST

The authors declare that they have no potential conflicts of interest.

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