Nigerian Journal of Fisheries Vol. 22, No. 1; 2025

POSSIBLE MORTALITY AND NEPHROSIS IN *Clarias gariepinus* (Burchell, 1822) EXPOSED TO ETHANOL LEAF EXTRACTS OF *Senna occidentalis* (L.)

[1]ADESANYA E. O., [1*] A. A. IDOWU, [1] W. O. ALEGBELEYE, [2] M. B. ADEKOLA, [3]A. T. TOWOLAWI, AND [1]F. O. IBRAHIM

[1]*Department of Aquaculture and Fisheries Management, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria*

[2]*Department of Environmental Management and Toxicology, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria*

[3]*Department of Environmental Health Science, Fountain University Osogbo, Osun State, Nigeria*

Corresponding author: 4desanyaoluwatosin@gmail.com, taofiktowolawi@yahoo.com +2348034709759

ABSTRACT

Clarias gariepinus juveniles (250) were stocked; from which 96 fish samples were randomly deployed for range-finding test, while 108 were for the main experiment using 12 plastic tanks of 35 L (4 treatments and 3 replicates), containing 8 fish/tank and 9 fish/tank respectively, filled with 10 L of water. Sub-acute renewal bioassay method was adopted. Range-finding test were done using Senna occidentalis ethanol leaf extracts of 1.00, 1.50, and 2.00 g/ 25 cL of distilled water for 120 hours, followed by the main experiment which had three concentrations of 3.00, 5.00, and 7.00 g/ 25 cL of ethanol leaf extract of S. occidentalis prepared. Daily doses of 5 mL syringes were administered in each treatment for 23 days. The fish were fed twice daily, dead fish in each tank were recorded. The increase in concentrations followed the no-effect observation of the 1.00, 1.50, and 2.00 g/ 25 cL used for the range-finding test. Analysis of variance were used to assess the data. The mortality rate significantly (p < 0.05) varied across the main experiment's treatments and increased with increased concentrations. Treatment A (control) showed no visible lesion, treatment B (3.00 g/ 25 cL) showed vacuolation of the epithelial lining's cytoplasm, treatment C (5.00 g/ 25 cL) showed moderate tubular epithelial necrosis, and treatment D (7.00 g/ 25 cL) showed both glomerular and tubular epithelial necrosis. The toxicity tolerance of C. gariepinus showed that the degree of damage observed increased with an increase in the concentration of S. occidentalis.

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Keywords: Life below water (SDG 14), phytotoxic, shrinkage, physico-chemical, African Catfish.

INTRODUCTION

According to Metian *et al.* (2019), aquaculture appears to be one of the most varied food-producing industries worldwide. Global aquaculture output hit a record of 122.6 million tonnes in 2020, consisting of 35.1 million tonnes of algae valued at USD 16.5 billion and 87.5 million tonnes of aquatic animals valued at USD 264.8 billion. About 54.4 million tonnes were cultivated in controlled waterbodies, compared to 68.1 million tonnes generated by marine and coastal aquaculture. Asia leads the world in aquaculture output, accounting for more than 90% of the overall production via culture. Excluding Africa, every area had steady growth in aquaculture in 2020. The two largest producers, Nigeria and Egypt, saw a decline, whereas the remainder of Africa benefited from a 14.5 per cent rise from 2019. Nigeria, the biggest aquaculture producer in sub-Saharan Africa, has had a downward trend since 2016, which worsened in 2020 with a 9.6 per cent decline. The remainder of Africa had double-digit expansion, rising from 346,400 tonnes in 2019 to 396,700 tonnes in 2020 (FAO, 2022). Like in the economies of other African countries, aquaculture is essential to the Nigerian economy in terms of generating jobs, revenue, food, and food ingredients, among other things (Mulokozi *et al.,* 2020). According to Onyekuru *et al* (2019), Nigeria's fish demand is now 1.4 million metric tons, whereas the country's total annual fish supply from capture and cultural fisheries is less than 0.7 million metric tons. As a result, about 0.7 million metric tons of fish, worth $500 million a year, must be imported to close the gap between supply and demand (Issa *et al.,* 2022).

Senna occidentalis, commonly referred to as coffee senna, is a small, medicinal shrub that is as tall as 3 feet and is a member of the Fabaceae family. It used to be identified as *Cassia occidentalis* (Isah and Mujib, 2013). It has been known for centuries that *S. occidentalis* is used for malaria treatment, and its efficacy has kept malaria from wreaking havoc across Africa (Idowu *et al.,* 2010). According to Odeja (2015), *Senna occidentalis* leaf extracts have desirable pharmacological compounds that have strong antimicrobial and radical scavenging properties. As a result, they may be employed in conventional medicine to cure a variety of infections and conditions. Shittu *et al.* (2014), reported that traditional uses of *S. occidentalis* have included the use of its seeds, roots, leaves, and stems for treating a variety of infections and other conditions. Furthermore, studies on toxicology indicate that *S. occidentalis* is probably safe to use in various circumstances (Silva *et al.,* 2011). For example, *S. occidentalis* extract was found to have antimicrobial activity (Mohammed *et al.,* 2012), larvicidal and suicidal activity (Ibrahim *et al.,* 2010), antioxidant and hepatoprotective activity (Gowrisri *et al.,* 2012), anti-inflammatory actions (Yadav and Singh, 2010), antimalarial activity (Gwarzo *et al.,* 2014), anti-anxiety and anti-depressant activity (Shafeen *et al.,* 2012), analgesic activity (Silva *et al.,* 2011), and anti-diabetic

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activity (Onakpa and Ajagbonna, 2012). The understanding of pathological (and toxicological) studies, as well as changes related to endogenous and exogenous xenobiotics, is aided by the histology of fish kidneys (Van-Dyk *et al.,* 2009). Kidneys help to preserve homeostasis, ensure appropriate production and excretion of metabolic wastes, and aid in the reabsorption of necessary metabolites (Imo *et al.,* 2021). One of the studies that consistently monitors the physiological condition and health status of these species includes hemato-biochemical analyses (Fazio, 2018). Alterations in physiological functions resulting from stressors will lead to disease (Adedeji *et al.,* 2017), which is now a major concern in the production of aquaculture (Idowu *et al.,* 2017). The toxicity trial conducted by Fafioye (2005), on catfish revealed 100% mortalities between 4 and 12 hours of exposure to 120 ppm conc. of *Achyrathes aspera*, 100% of *C. gariepinus* mortalities were recorded within 4 hours of introduction by *T. teraptera* and *S. occidentale* (bark). Fish toxicants affected the fish, as evidenced by their erratic behaviour before death, according to Fafioye (2005). Research on *S. occidentalis* revealed that the nature and quantity of phytochemicals differ based on the season, region, and type of soil; more so, there is ongoing work and an agenda to investigate Nigeria's medicinal plants. Therefore, it is necessary to implement aquaculture management practices that are not only environmentally acceptable but also biotechnologically feasible, socioeconomically viable, cost-effective, and free of water contaminants to avoid health risks for consumers of food fish from aquaculture (Adesina *et al.,* 2013). As a result, research and experimental work on this unique genus remain under-explored, particularly on fish species (Yadav *et al.,* 2009).

The use of medicinal plants has gained interest in aquaculture due to their availability and curative potentials (Nair *et al.,* 2016). However, the safety of these plant extracts to animals, especially fish, should be further studied before its wider applications. Fish toxicologists have concerns about assessing the dose-effect of piscicidal plants on fish. Because, these plants contain compounds including emodin and saponin, which are toxic to fish and have been used in traditional fishing practices for centuries, as well as for medicinal purposes. Hence, this study assessed the toxicity-tolerance of *Clarias gariepinus* (commonly called African catfish) juveniles and their physiological response against different concentrations ethanol leaf extract of *Senna occidentalis* (commonly called coffee senna).

MATERIALS AND METHODS

Study Area and Experimental Fish

The study was done at the Fish Hatchery Complex, Aquaculture and Fisheries Management Department, Federal University of Agriculture (FUNAAB), Abeokuta, Ogun State, Nigeria. A total of two hundred and fifty (250) juveniles of *Clarias gariepinus* (average body weight 16 ± 2 g and length 8.5 ± 0.5 cm) were obtained from a reputable farm in Sagamu, Ogun State, Nigeria, and transported to the laboratory. Fish were acclimatized for two weeks, during which they were fed 1.8 mm commercial feed (Skretting®) twice daily. Feeding was stopped 48 hours before the beginning of the experiment, wastes were taken out every day along with replenishing water. The leaves of *Senna occidentalis* were obtained at Old Bola Ahmed Tinubu Road, off Iju road, Ifako-Ijaiye LGA, Lagos State, Nigeria. They were authenticated at the Forestry and Wildlife Management Department, Federal University of Agriculture, Abeokuta.

Preparation of *Senna occidentalis* leaf extracts and experimental design

Fresh leaves of *S. occidentalis* were air-dried for two weeks and ground using Binatone BLS-360 1.5 L electronic blender, then 295 g of the powdered leaves were obtained and soaked in 1400 mL of ethanol and 600 mL of distilled water (70% ethanol); the solution was stirred continuously at intervals for 72 hr (Jun *et al.* 2012). The solution was filtered and was made to pass through a rotary evaporator (50 °C), which evaporates the solvent, after which it was oven-dried for 24 hr at 40 ℃ using a low temperature oven drier to achieve a more concentrated extract at the Lagos University Teaching Hospital. The extract was later stored in a refrigerator using an air-tight container. Out of the acclimatized fish, ninety-six were used for the range finding test, while one hundred and eight juvenile catfish were randomly distributed into twelve plastic tanks of 35 L capacity, filled with 10 L of water for the main experiment. The range finding test and the main experiment had four treatments and three replicates, each with 8 fish and 9 fish per treatment tank respectively. Treatment A represent the control tank while Treatment B-D are the experimental tanks.

Range Finding Test

For the range finding test, 12 transparent plastic aquaria each with 35 L capacity, containing 10 L of water were randomly stocked with eight fish. Three stock solutions made of *Senna occidentalis* ethanol leaf extracts at 1.0 g, 1.5 g and 2.0 g per 25 cl of distilled water were prepared, and 5 mL of each concentration were infused into the plastic tanks at 24 hr intervals using a syringe. Throughout the 120 hours of the test, the fish's behavioural response was closely monitored at every three hours. Any dead fish were removed immediately to prevent the water from getting contaminated.

Definitive Test

Sub-acute renewal bioassay method was adopted for the primary experiment, following the steps by Edet and Ikpi, (2008). The increase in concentrations for the main experiment followed the no-effect observation in the range finding test. Clean water was used as the control at 0.00 g/ 25 cL (Treatment A). Three sub-acute concentrations of 3.00 g/ 25 cL (Treatment B), 5.00 g/ 25 cL (Treatment C), and 7.00 g/ 25 cL (Treatment D) were prepared and triplicated for each treatment in a transparent plastic container. A 5 mL of each concentration was infused daily into the plastic tanks housing 9 fish. Throughout the 21 days, the fish were fed twice a day, and the mortality in each tank was monitored and recorded every 24 hours of the experiment. The dead fish were immediately picked out to avoid contamination.

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Histological Test

C. gariepinus were anesthetized after the experiment using benzocaine (0.1 g/l) and then sacrificed by cervical section. The kidney tissues were dissected out, rinsed in physiological saline and fixed in 10% formalin, dehydrated in graded ethanol, cleared with toluene, infiltrated with molten paraffin wax. The microtome sections (approx. 5mm) of kidney were stained with hematoxylin and eosin (H and E) technique and structures were examined under electron microscope. The histopathological changes were evaluated according to Bello *et al.* 2017.

Determination of Water Quality Parameters

Temperature, Hydrogen ion concentration (pH), Total Dissolved Solids (TDS), Electrical Conductivity (EC), and Dissolved oxygen (DO) concentration readings were taken in all the treatment groups using the calibrated multi parameter Hanna Instrument (Model HI 98129) and DO meter (PCE-DOM Series oxygen meter).

Statistical Analysis

Data were analyzed using the Analysis of variance (ANOVA), and the distinction between treatment groups was found using the least significant difference (LSD) at the 0.05 significance level, with the aid of SPSS (version 20.0).

RESULTS

Table 1 demonstrates how *S. occidentalis* ethanol leaf extract affects *C. gariepinus* survival and mortality. The quantity of fish that survived and perished were counted and documented. As observed, the mortality rate of *C. gariepinus* increased with increase in the concentration of *S. occidentalis* leaf extracts. During the 21 days of the experimental exercise, the control tank had 2 mortalities which was the lowest mortality percentage (7%), while treatment B had a 70% mortality rate, treatment C had a 78% mortality rate, and treatment D had 89% mortality rate.

Table 1. Mortality of juveniles of *Clarias gariepinus* exposed to different doses of *S. occidentalis* ethanol leaf extracts

	Treatment A (Control) 0 mg/ 25 cL	Treatment B 3000 mg/ 25 cL	Treatment C 5000 mg/ 25 cL	Treatment D 7000 mg/ 25 cL
No fish exposed	27	27	27	27
Week one	0	4	3	3
Week two	0	4	4	5
Week three	1	6	8	7
Week four	1	5	6	9
Total no (mortality)	**2**	**19**	**21**	**24**
Total no (survived)	**25**	**8**	**6**	**3**
% Survival	**93**	**30**	**22**	**11**
% Mortality	**7**	**70**	**78**	**89**

Water quality parameters

Water quality parameters (Table 2) were measured daily and observed to be at an optimal level. The test water's physicochemical characteristics revealed that its pH ranged from 6.83 to 8.4, dissolved oxygen ranged from 5.34 mg/l to 6.99 mg/l, and temperature fluctuated between 25.37 °C and 28.43 °C. Additionally, conductivity ranged from 0.3 µs/cm to 0.4 µs/cm, while total dissolved solids (TDS) varied between 0.20 mg/l and 0.4 mg/l.

Table 2. Water Quality Parameters

Parameters	Treatment A (Control)	Treatment B	Treatment C	Treatment D
	0 mg/ 25 cL	3000 mg/ 25 cL	5000 mg/ 25 cL	7000 mg/ 25 cL
Temperature (°C)	26.92 ± 2.43 [a]	27.33 ± 1.91 [a]	26.9 ± 3.06 [a]	26.95 ± 3.06 [a]
Dissolved Oxygen (mg/l)	6.06 ± 1.72 [a]	5.99 ± 1.90 [a]	6.02 ± 1.95 [a]	6.01 ± 1.92 [a]
pH	7.63 ± 1.29 [a]	7.56 ± 1.46 [a]	7.48 ± 0.98 [a]	7.74 ± 0.93 [a]
TDS (mg/l)	0.34 ± 0.13 [a]	0.30 ± 0.20 [a]	0.30 ± 0.20 [a]	0.34 ± 0.13 [a]
Conductivity (µs/cm)	0.30 ± 0.20 [a]	0.34 ± 0.13 [a]	0.36 ± 0.22 [a]	0.30 ± 0.20[a]

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Histopathology of the kidney

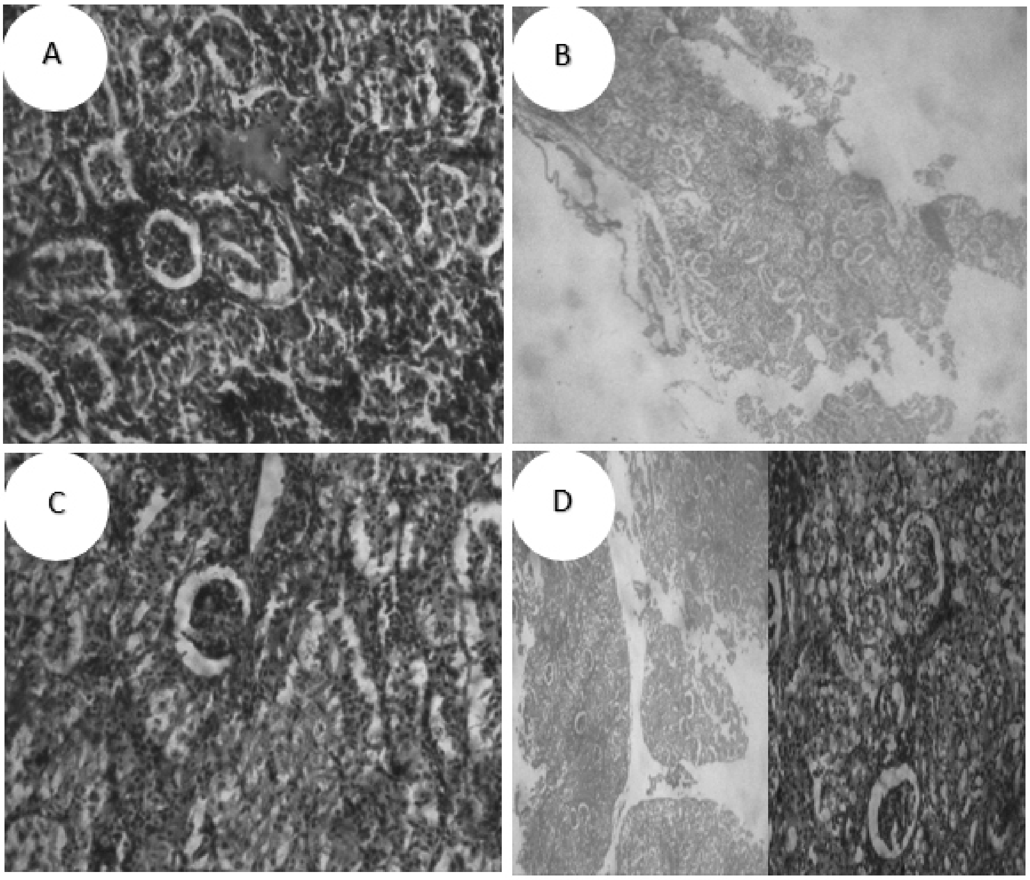

Fig. 1. The histological sections of *C. gariepinus* kidneys subjected to various concentrations of *S. occidentalis* as shown above. A represents the control tanks (0.00 mg/25cl), B represents Treatment A (3000 mg/25cl), while C represents Treatment C (5000 mg/25cl) and D represents Treatment D (7000 mg/25cl). The kidneys of the fish in the control tanks showed no visible lesion, while there was formation of vacuoles in the membranous tissue of the fish in treatment B (vacuolation of the epithelial lining's cytoplasm). There was moderate death of cells caused by toxins in Treatment C (represented by a moderate tubular epithelial necrosis), and Treatment D showed both glomerular and tubular epithelial necrosis, that is, the glomerulus, which is responsible for filtering blood to release urine was affected by toxins caused by leaves of *S. occidentalis*.

DISCUSSION

The adoption of using ethanol and water for extraction was in agreement with Jun *et al.* (2012), who stated that "extraction of phytochemicals can be more effective when an organic solvent like ethanol is mixed with aqueous solvent". The mortality rate in this research significantly ($P < 0.05$) varied across the treatments and increased with an increase in concentration. The survival rate was lowest in treatment D (11%), where *S. occidentalis* ethanol leaf extract had the highest concentration compared to the control tank, which had 7% mortality. Water quality parameters were observed to be at optimal levels; therefore, the report of death cannot be attributed to contaminated or polluted water quality; this agrees with the report by Jimoh *et al.* (2023) who worked on histological observations on liver and gills of *C. gariepinus* juveniles treated with some prophylactics and stated that "the mortality recorded might not be connected to poor water quality". However, it was frequently noted that the fish responded to environmental changes as soon as the extract was added to the body of water. These responses included morphological and behavioural indicators. 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), as well as a condition wherein living organisms exhibit changes in their bodily systems and manifest symptoms due to impaired physiological functions (Adedeji *et al.,* 2017), such as aggression, loss of balance, and erratic swimming. Tissue histopathological examination provides a reliable means of identifying and observing different kinds of tissue damage (Yang *et al.,* 2014). From the results of this study, it can be deduced that *S. occidentalis* has a significant toxic effect on the kidneys of *Clarias gariepinus* juveniles. This was ratified by the regular trend observed in the mortality rate, which increased with an increase in the concentration of ethanol leaf extract of *S. occidentalis* and this is in line with the

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report from Ogbonne *et al.* (2018) who investigated the toxicity effects of exposure of aqueous extract of *Chromolaena odorata* leave on gill/liver of Juveniles catfish.

According to Agbebi *et al.* (2013), and Adegbesan *et al.* (2018), mild tubular epithelial necrosis in the kidney of fish may be caused by toxins (such as emodin, saponin etc.) and infection; this was observed in treatment C and D, where there was a mild tubular epithelial necrosis. That is, the membranous tissue covering the internal surface of the kidney was affected by toxins, which are capable of causing the death of cells or tissues; this could also result in uncontrolled cell death, as reported by Shahida *et al.* (2021). Treatment D (7000 mg/ 25 cL) showed evidence of this, resulting in tubular and glomerular epithelial necrosis. The glomerulus is responsible for filtering blood in order to release urine; damage to the glomerulus could result in death. Every juvenile of *C. gariepinus*, with the exception of those receiving the control treatment, had kidney damage. The increased vacuolation of the cytoplasm of the epithelial lining seen in treatment B is consistent with the findings of Pacheco & Santos (2002), who reported that this phenomenon indicates significant cell degeneration and direct exposure to toxins, which could lead to damage to cells and tissue.

CONCLUSION

This study showed that *S. occidentalis* ethanol leaf extracts are toxic to the kidneys of *C. gariepinus*; caution should be taken when administering them to fish as phytomedicine. Despite *C. gariepinus'* strong immunity and survival adaptations, the results of this study showed histopathological changes and damage to the kidney. The degree of damage increased with the concentration of *S. occidentalis* ethanol leaf extract; therefore, the mortality rate increased with an increase in the concentration of *S. occidentalis.*

Acknowledgments

The authors acknowledge the Department of Aquaculture and Fisheries Management, FUNAAB. For the release of a section of the FUNAAB hatchery unit. The authors would like to express their gratitude to the anonymous reviewers in advance for their constructive feedback, which will undoubtedly enhance the quality and clarity of the manuscript. The authors would also appreciate one another for working together to ensure the success of this experiment.

We, the authors, had no conflict of interest to declare.

Authors' contributions

All authors were involved in the conception and design of the study as well as the review of the manuscript. AOE experimented, WOA and AAI supervised the main experiment, MBA supervised the procedures and the range finding test, while ATT thoroughly contributed to the manuscript writing and editing, and FOI performed data collection.

Author's statement

This manuscript has been read and approved by all the authors, with the belief that this manuscript represents honest work. Also, the requirement for authorship has been met.

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