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Acute Toxicity Assessment of Ethanolic Extract of Unripe Plantain (*Musa paradisiaca*) Peel on Juveniles of *Clarias gariepinus* (Burchell, 1822): Implications for Its Use as a Feed Additive in Aquaculture Diets

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Abstract

The increasing search for cost-effective and sustainable feed additives in aquaculture has stimulated interest in the utilisation of agricultural wastes such as unripe plantain (*Musa paradisiaca*) peels. This study evaluated the acute toxicity of ethanolic extract of unripe plantain peels on juveniles of *Clarias gariepinus* to determine its safety for potential application in aquaculture feed formulation. Ten juveniles were stocked in each plastic aquarium containing two litres of water and exposed to 0, 20, 25, 30 and 35 mg/L of the extract in duplicate batches (A and B) under static bioassay conditions for 96 hours. Mortality, behavioural responses, water-quality parameters and histopathological alterations of the gills and liver were assessed following standard procedures. No mortality was recorded in the control and 20 mg/L treatments, while 10% mortality was observed at 25 mg/L. Mortality increased to 20-30% and 40-50% at 30 and 35 mg/L, respectively. Behavioural abnormalities, including air gulping, erratic swimming, respiratory distress and vertical erection, increased with concentration and exposure duration. Significant variations ($p < 0.05$) were observed in pH, dissolved oxygen, conductivity and temperature across treatments and exposure periods. Histological examination of the gills revealed no pathological alterations in any treatment group, indicating preservation of gill structural integrity. Similarly, liver tissues from the control and 25 mg/L groups showed normal architecture, whereas moderate to severe hepatic distortions, characterised by tissue degeneration and structural disorganisation, were observed at 20, 30 and 35 mg/L. The findings indicate that lower concentrations of ethanolic extract of unripe plantain peels are relatively safe for *C. gariepinus* juveniles, while higher concentrations may induce toxicological effects. Therefore, careful dosage consideration is necessary before incorporation into aquaculture feed formulations.

Keywords: Clarias gariepinus; Musa paradisiaca peel; ethanolic extract; acute toxicity; LC50; fish behaviour; liver histopathology; gill histopathology; water quality; aquafeed additive.

1. Introduction

Aquaculture has emerged as one of the fastest-growing food-production sectors globally and contributes significantly to food security (Otoh et al., 2024a, 2024b), nutrition (Otoh et al., 2024c), employment (Otoh et al., 2024d) and economic development. The increasing demand for fish protein has intensified the need for sustainable and cost-effective aquaculture practices, particularly in developing countries where feed constitutes the largest operational cost, accounting for approximately 60-70% of total production expenses (Inyang-Etoh et al., 2022; Otoh et al., 2022, 2023; FAO, 2024; Otoh et al., 2025). Consequently, the search for alternative feed ingredients and feed additives derived from agricultural wastes has become a major focus of aquaculture research.

Among the various agricultural by-products generated in tropical regions, plantain (*Musa paradisiaca*) peels are produced in substantial quantities and are often discarded as waste, contributing to environmental pollution. Plantain belongs to the family Musaceae and is widely cultivated across Africa, Asia and Latin America. Nigeria is one of the world's leading producers of plantain, generating large volumes of peel waste annually (Adeniji et al., 2007). These peels have attracted scientific interest because of their rich nutritional composition, including carbohydrates, dietary fibre, proteins, minerals, vitamins and numerous bioactive phytochemicals such as flavonoids, tannins, alkaloids, saponins, terpenoids and phenolic compounds (Emaga et al., 2007; Someya et al., 2002).

Recent studies have demonstrated that plant-derived feed additives can improve growth performance, feed utilisation, immune responses, disease resistance and overall fish health (Reverter et al., 2014). Plantain peels, particularly in their unripe state, contain significant concentrations of antioxidants and antimicrobial compounds that may serve as natural alternatives to synthetic growth promoters and antibiotics in aquaculture feeds (Adeniji et al., 2007). The utilisation of plantain peel waste as a feed additive would not only reduce feed costs but also promote environmental sustainability through waste recycling and circular bioeconomy practices. Recent reviews further emphasise that functional aquafeed additives, including phytogenics, require species-specific dose-response and safety evaluation before practical dietary use (Kalaiselvan et al., 2024; Okon et al., 2025).

Phytotherapy-based aquaculture applications are increasingly evaluated for disease control and health promotion, but recent reviews also stress that dosage, administration route and possible toxicological effects must be

considered (Ng et al., 2024). Despite the nutritional and pharmacological potential of unripe plantain peels, concerns remain regarding the presence of phytochemicals that may exert toxic effects when consumed at high concentrations. Compounds such as tannins, oxalates, phytates and saponins are known to possess antinutritional properties capable of affecting nutrient digestibility, growth performance, physiological functions and tissue integrity in aquatic organisms (Francis et al., 2001). Therefore, before incorporating plantain peel-derived products into aquaculture diets, it is essential to evaluate their safety and determine concentration levels that do not adversely affect fish health.

Toxicity studies provide valuable information regarding the potential hazards of natural products and feed additives to aquatic organisms. Acute toxicity bioassays are widely employed to determine the effects of chemical substances on fish survival, behaviour, physiology and tissue structure over a specified exposure period. Such assessments are important in establishing safe dosage levels for feed ingredients and additives intended for aquaculture applications (Rand, 1995). Histopathological examinations of vital organs such as the gills and liver are particularly useful biomarkers for detecting sublethal toxic effects because these organs are directly involved in respiration, detoxification, metabolism and osmoregulation (Ojutiku et al., 2014; Ogbeide et al., 2019; Musa & Imam, 2022; George et al., 2023a, 2023b; George et al., 2025a, 2025b). Comparable plant-extract bioassays in *C. gariepinus* have reported concentration-related behavioural, oxidative, genotoxic or histopathological responses, supporting the need to screen plant-derived materials before wider aquaculture use (Ashade et al., 2022; Kachi et al., 2024).

The African catfish, *Clarias gariepinus*, is one of the most important aquaculture species in Africa because of its rapid growth rate, high tolerance to environmental stress, efficient feed conversion, disease resistance and high market demand (Otoh et al., 2024a, 2024b). Its widespread use in aquaculture and toxicological research makes it an ideal model species for evaluating the safety of novel feed additives. Juvenile stages are especially sensitive to toxic substances and are therefore commonly employed in toxicity studies to assess potential adverse effects on fish health and survival.

Ethanol extraction is one of the most effective methods for recovering bioactive compounds from plant materials because ethanol has excellent solvent properties for extracting phenolics, flavonoids, alkaloids and other phytochemicals (George et al., 2023c). However, concentrated ethanolic extracts may contain elevated levels of biologically active substances capable of eliciting toxic responses in fish if administered beyond safe limits (Azmir et al., 2013). Therefore, evaluating the acute toxicity of ethanolic extracts of unripe plantain peels is a crucial step towards determining their suitability as feed additives in aquaculture nutrition. Information remains limited on the acute concentration-dependent responses of juvenile *C. gariepinus* to ethanolic extract of unripe plantain peel, particularly with respect to mortality, behavioural changes, water-quality variation and gill and liver histoarchitecture under controlled laboratory exposure.

The present study was therefore designed to assess the acute toxicity of ethanolic extract of unripe plantain (*Musa paradisiaca*) peel on juveniles of *Clarias gariepinus*. Specifically, the study aimed to determine mortality patterns, behavioural responses and histopathological alterations in exposed fish. Findings from this investigation will provide baseline information on the safety profile of unripe plantain peel extracts and establish whether the extract can be safely incorporated into aquaculture feed formulations as a natural feed additive.

2. Materials and Methods

2.1 Collection of Specimen

Two hundred (200) healthy juveniles of *Clarias gariepinus* were collected from Akwa Ibom State University fish farm, Obio Akpa, Akwa Ibom State, Nigeria, and acclimated in a recirculatory plastic aquarium measuring 25 x 13 x 8.3 cm^3 containing hatchery water for 24 hours in the Fisheries and Aquaculture Laboratory of Akwa Ibom State fish farm. This acclimation period helped to stabilise the fish and minimise stress resulting from collection and transportation (George et al., 2015a, 2015b).

2.2 Collection of Plant Sample

Fresh plantain (*Musa paradisiaca*) was collected from Obio Akpa in Oruk Anam Local Government Area, Akwa Ibom State. The collection date was 6 April 2024. The plant material was transported to the University of Uyo,

Uyo, Akwa Ibom State, for identification and authentication. This was done at the Herbarium in the Department of Botany and Ecological Studies, University of Uyo, Uyo. The processing of the unripe plantain peels is shown in Fig. 1.



Fig. 1. Processing unripe plantain peels (*Musa paradisiaca*) used in the study
(Source: Authors experimental preparation, 2026)

2.3 Preparation of Plant Material

After identification, the plantain peel was washed, shredded, spread on cellophane and allowed to dry for 72 hours at room temperature. The peel was then pulverised into a fine powder using a wooden pestle and mortar. The dried and powdered peels are shown in Fig. 2.



Fig. 2. Dried unripe plantain peels and Powdered plantain peels (*Musa paradisiaca*) used in the study
(Source: Authors Experimental Preparation, 2026)

2.4 Preparation of Ethanolic Extract (Maceration and Extraction)

The cold extraction method (maceration) was employed in this study following the procedure described by Hidayat and Wulandari (2021). In this process, 240 g of plant material was macerated in 1000 mL of 99% concentrated ethanol in an airtight container and kept at room temperature for 72 hours (3 days). The ethanolic suspension was filtered using a filter net and filter paper, and the extract was evaporated in a water bath at 40 °C for 48 hours and stored in a beaker covered with aluminium foil for bioassay immediately after evaporation was complete.

2.5 Monitoring of Water Quality

Water-quality parameters were monitored before the commencement of the experiment and periodically thereafter, following the standard method (APHA, 2005). The parameters investigated included dissolved

oxygen (DO), pH and temperature (°C). Temperature and pH were measured using a portable pH/EC/TDS/temperature HANNA H1 991301 model instrument, while dissolved oxygen was measured using a digital portable analyser (JPB-607A, Search Tech Instrument).

2.6 Acute Toxicity Test

A preliminary range-finding test was conducted to determine the toxicity level of ethanolic extract of unripe plantain peel following OECD Test Guideline No. 203 (OECD, 2019) and ISO 7346-2:1996 (International Organization for Standardization [ISO], 1996). Five test concentrations were used for this investigation: one control and four test solutions of unripe plantain peel ethanolic extract, in duplicate. Ten (10) *C. gariepinus* juveniles were batch-weighed with a top-loading Mettler balance (Mettler Toledo (K)) and distributed randomly in duplicate per treatment. The plastic aquaria were covered with mosquito nets to prevent fish from jumping out; there was no aeration, water change or feeding throughout the test duration. The toxicant was introduced at concentrations of 5, 10, 15 and 20 mg/L, with a control of 0 mg/L for the range-finding test.

The definitive test was conducted using duplicate concentrations of 20, 25, 30 and 35 mg/L of unripe plantain peel ethanolic extract, as determined from the range-finding test, with a control of 0 mg/L. The behaviour and mortality of the test fish in each tank were monitored and recorded every 24 hours for 96 hours. The inability of fish to respond to external stimuli was used as an index of death. Apart from monitoring and recording fish mortality, fish behaviour, such as erratic swimming, air gulping, loss of reflex, discoloration and moulting, was monitored. The 96-hour LC50 (the concentration of unripe plantain peel ethanolic extract estimated to be lethal to 50% of test organisms after 96 hours of exposure) was determined graphically using probit transformation (USEPA, 2000; George et al., 2013a, 2013b, 2014).

2.7 Histopathological Examinations

The gill and liver tissues were isolated from the test animals and fixed in formalin-saline for 48 hours. The fixed tissues were processed manually through graded ethanol, cleared in xylene, impregnated and embedded in paraffin wax. Sections of the tissue samples were cut with a rotary microtome and stained using the haematoxylin and eosin technique. Prepared tissues were finally observed for pathological changes using a microscope at x100 and x400 magnification.

2.8 Data Analysis

The results of the respective concentration effects of the ethanolic extract of unripe plantain (*Musa paradisiaca*) peels were presented in tables. One-way analysis of variance (ANOVA) was used to test for significant differences between the varying concentrations in both batches (batch A and batch B) at a probability level of $p < 0.05$. Probit analysis was performed using SPSS version 20.0.

3. Results

3.1 Acute Toxicity of *C. gariepinus* Juveniles at Different Concentrations of the Ethanolic Extract of Unripe *Musa paradisiaca* Peels at the End of the Experiment (96 Hours).

Mortality patterns of catfish *C. gariepinus* juveniles over 24, 48, 72 and 96 hours are shown in Table 1 for the two batches of the experiment. In the 0 mg/L concentration of the extract, no mortality was recorded throughout the test period in both batches A and B of the peel extract. In the 20 and 25 mg/L concentrations of the ethanolic extract of unripe plantain peels, no mortality was recorded in batch A. A similar observation was made in the 20 mg/L extract in batch B, except for the 25 mg/L concentration of the extract, where 10% mortality was observed.

A marked decrease in the number of survivors was observed in the 30 mg/L concentration of the ethanolic extract of unripe *Musa paradisiaca* peels at the end of the 96-hour bioassay. Mortalities of 30% and 20% were recorded in batches A and B, respectively, for the unripe *Musa paradisiaca* peel ethanolic extract, leaving 70% and 80% survivors. Mortalities of 50% and 40% were recorded in batches A and B, respectively, at the 35 mg/L concentration of the extract, leaving 50% and 60% survivors in the two batches.

Table 1. Summary of the Percentage Mortality and survivors of *C. gariepinus* Juveniles in the different concentrations of the ethanoic extract of unripe peels of *Musa paradisiaca* at the end of the experiment (96 hours)

Conc. of extract (mg/l)	Batch A				Batch B			
	Mortality (M)	% M	Survivors (S)	% S	Mortality (M)	% M	Survivors (S)	% S
0 mg/l	0	0	10	100	0	0	10	100
20 mg/l	0	0	10	100	0	0	10	100
25 mg/l	0	0	10	100	1	10	9	90
30 mg/l	3	30	7	70	2	20	8	80
35 mg/l	5	50	5	50	4	40	6	60

3.2 96-Hour LC50 Determination for Unripe Peels of *Musa paradisiaca*

The 96-hour LC50 for *C. gariepinus* juveniles exposed to the different concentrations of the ethanolic extract of unripe *Musa paradisiaca* was determined using probit analysis. The concentrations were first transformed into log values for probit analysis (Table 2). The 96-hour LC50 for unripe *Musa paradisiaca* peel extract was given as 35 mg/L, representing a log-transformed concentration of 1.54 mg/L, at which 50% of the test organisms would be killed by the end of the experiment.

Table 2. LC_{50} determination for *C. gariepinus* Juveniles at the end of the 96-hours bioassay for unripe Plantain Peels *Musa paradisiaca*

Concentration (mg/l)	Log Transformation	Mortality (M)	% Mortality	Survivors (S)	% Survivor
0 mg/l	0	0	0	10	100
20 mg/l	1.30	0	0	10	100
25 mg/l	1.39	0	0	10	100
30 mg/l	1.47	3	30	7	70
35 mg/l	1.54	5	50	5	50

3.3 Behavioural Responses of *C. gariepinus* Juveniles to the Toxic Effects of Ethanolic Extract of Unripe *Musa paradisiaca* Peels During the 96-Hour Bioassay

Behavioural responses of *C. gariepinus* juveniles exposed to concentrations of ethanolic extract of unripe *Musa paradisiaca* peels between 24 and 96 hours of exposure were analysed using a semi-quantitative measurement (Table 3). After 24 hours of exposure, the treated groups exposed to 20-35 mg/L concentrations of the peel extract displayed slight to moderate behavioural changes, such as air gulping, erratic swimming, respiratory distress and vertical erection, while the control (0 mg/L) concentration exhibited none of these behavioural changes. Further exposure (48-96 hours) to the peel extract promoted moderate to severe behavioural abnormalities in the treated groups, in a concentration-dependent pattern, except for the 20 and 25 mg/L treatments.

Table 3. Effect behavioral responses of *Clarias gariepins* juveniles to the toxic effects of ethanolic extract of peels of unripe *Musa paradisiaca* during 96th hours bioassay at different time intervals

Time in hours	Conc. (mg/l)	Gulping of air	Vertical erection	Respiratory distress	Erratic swimming
24	Control	-	-	-	-
	20	+	-	-	-
	25	+	+	+	+
	30	++	++	++	++
	35	++	++	++	++
48	Control	-	-	-	-
	20	+	-	-	-
	25	+	+	+	+
	30	++	++	++	++
	35	++	++	++	++

Time in hours	Conc. (mg/l)	Gulping of air	Vertical erection	Respiratory distress	Erratic swimming
72	Control	-	-	-	-
	20	-	-	-	-
	25	+	+	+	+
	30	++	++	++	++
	35	+++	+++	+++	+++
96	Control	-	-	-	-
	20	-	-	-	-
	25	+	+	+	+
	30	+++	+++	+++	+++
	35	+++	+++	+++	+++

Where; - = Normal + = Mild ++ = Moderate +++ = Severe

3.4 Initial Water Quality Parameters

The initial water-quality parameters before the commencement of the toxicity studies are shown in Table 4. The values of dissolved oxygen (3.9 mg/L), temperature (28.0 °C), pH (5.34) and conductivity (0.11 µS) observed were within the acceptable range for aquaculture operations.

Table 4. Initial Physico-chemical parameters of the test water prior to stocking of test organism

Fish Species	DO (mg/l)	Temp (°C)	pH	Conductivity (µS)
Clarias gariepinus (Juveniles)	3.9 mg/l	28.0 ^{0}C	5.34	0.11 µS

3.5 Variation in Physicochemical Parameters of the Test Media with *C. gariepinus* Juveniles as the Test Organism during the Experimental Period (96 Hours)

Variation in the water-quality parameters (DO, temperature, pH and conductivity) of the test media at the different concentrations and exposure times is presented in Figs. 3-6.

3.5.1 Dissolved Oxygen (mg/l)

Dissolved oxygen was observed to be generally low across all concentrations of the extract during the 96-hour test. The DO level in the 0 mg/L (control) treatment was observed to be high throughout the test duration. The variation in DO at different time intervals and concentrations is shown in Fig. 3. Significant variation ($p < 0.05$) was observed across the various concentrations of the extract.

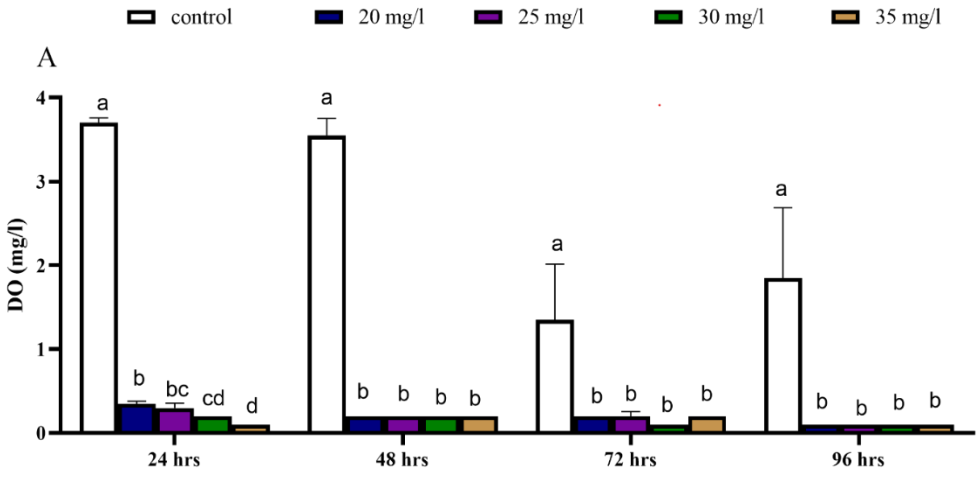


Fig. 3. Dissolved oxygen variations during the 96th hours of test

3.5.2 Temperature (°C)

Significant variation ($p < 0.05$) was observed for temperature across the different concentrations and time intervals, showing the effects of the extract (Fig. 4).

3.5.3 pH

The pH value was observed to vary during the experimental period of 96 hours of exposure to the extract. Significant variations ($p < 0.05$) were observed across all concentrations with respect to the different time intervals (Fig. 5).

3.5.4 Conductivity

Conductivity values showed an interesting pattern, with high values recorded in the control tank across the different time intervals. However, conductivity values in the treated tanks were generally low, as observed in Fig. 6, throughout the study duration. Significant variations ($p < 0.05$) were observed in all concentrations across the different time intervals (Fig. 6).

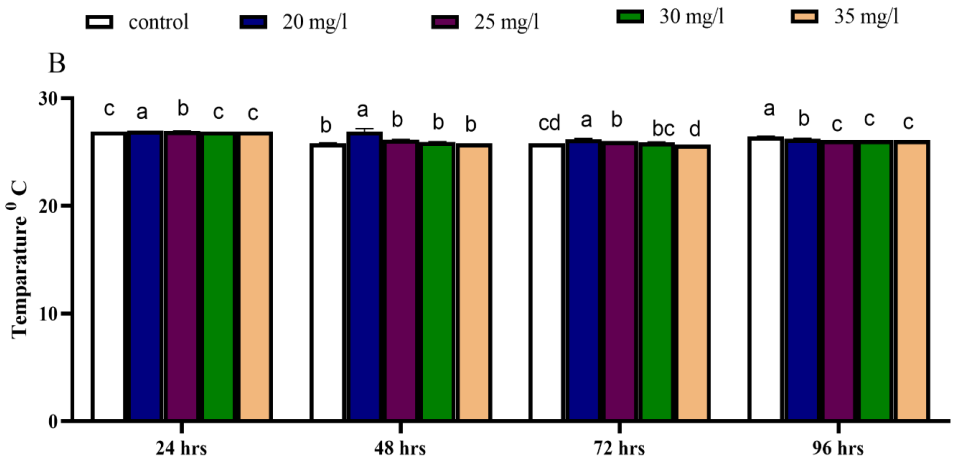


Fig. 4. Temperature variations during the 96 hours of test

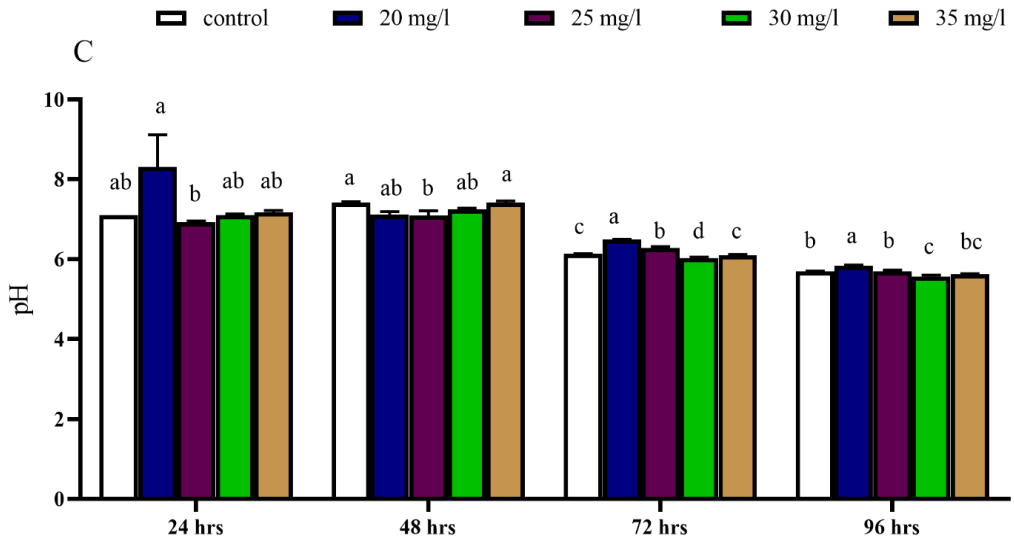


Fig. 5. pH variations during the 96 hours of test

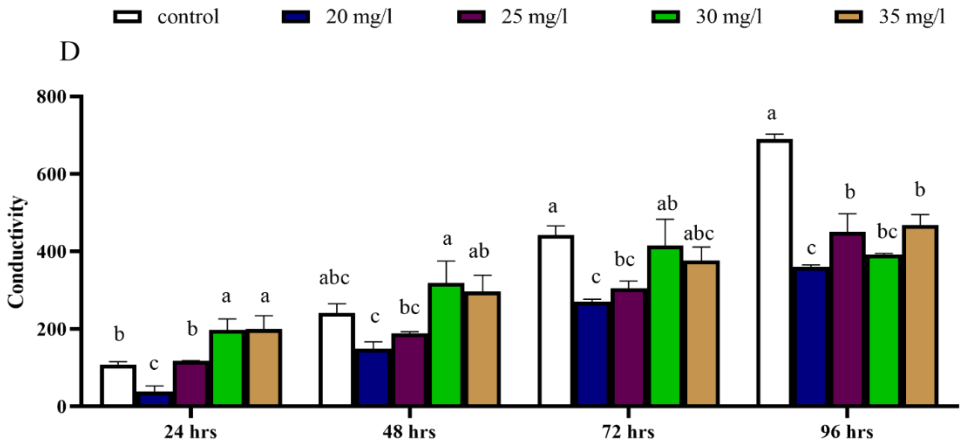


Fig. 6. Conductivity variations during the 96 hours of test

3.6 Histopathology of the Gills and Liver of *C. gariepinus* Juveniles Exposed to Different Concentrations of the Ethanolic Extract of Unripe *Musa paradisiaca* Peels at the End of the Experiment (96 Hours)

3.6.1 Liver Histopathology

Control fish (0 mg/L) displayed normal hepatic architecture with intact hepatocyte cords, well-defined sinusoidal spaces and preserved vascular structures, although diffuse cytoplasmic vacuolation was present. At 20 mg/L, hepatic cords remained largely intact, but focal parenchymal haemorrhage, characterised by erythrocyte extravasation into the hepatic parenchyma and sporadic cytoplasmic vacuolation, was observed. Fish exposed to 25 mg/L showed histological features comparable to controls, with no discernible pathological alterations. These liver sections are shown in Fig. 7.

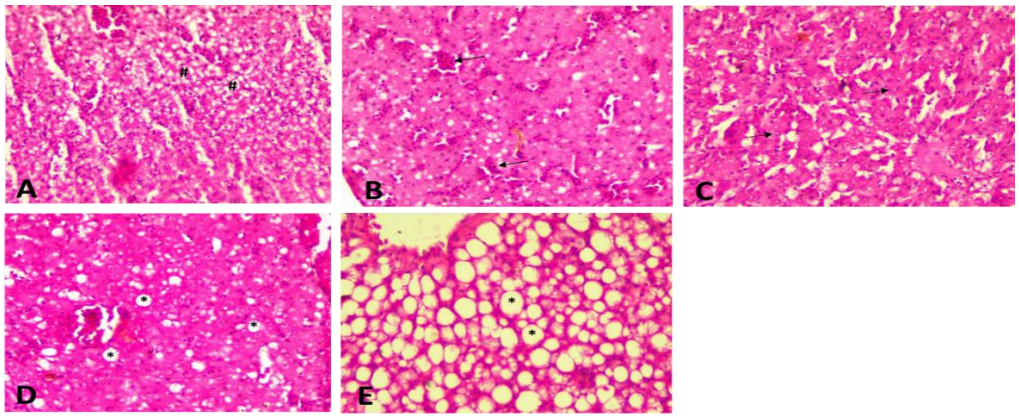


Fig. 7. Photomicrographs of liver sections of juvenile *Clarias gariepinus* exposed to ethanolic extract of unripe *Musa paradisiaca* peel for 96 hours (H&E, ×400). ***(A) Control (0 mg/L): normal hepatic architecture with intact hepatocyte cords (HC), well-defined sinusoidal spaces (S), preserved vascular structures, and diffuse cytoplasmic vacuolation (V). (B) 20 mg/L: largely preserved hepatic cords with focal parenchymal haemorrhage (arrow) characterised by erythrocyte extravasation and sporadic cytoplasmic vacuolation. (C) 25 mg/L: normal hepatic architecture comparable to control, with intact hepatocyte cords, preserved sinusoidal spaces, and no discernible pathological alterations. (D) 30 mg/L: intact hepatic tissue organization with sporadic macrovesicular steatosis (MS) characterised by large lipid vacuoles and peripheral nuclear displacement (arrowhead). (E) 35 mg/L: severe diffuse macrovesicular steatosis with widespread hepatocellular lipid vacuolation and marked peripheral nuclear displacement (arrowheads). Note the progressive increase in lipid accumulation from D to E***

A concentration-dependent increase in hepatic lipid accumulation was evident at higher doses. At 30 mg/L, sporadic macrovesicular steatosis appeared, with large lipid vacuoles and peripheral nuclear displacement within scattered hepatocytes, although overall tissue organisation remained intact. The most pronounced changes occurred at 35 mg/L: severe diffuse macrovesicular steatosis with widespread hepatocellular lipid vacuolation and marked peripheral nuclear displacement throughout the parenchyma. Despite this progressive lipid accumulation, basic hepatic architecture remained discernible at all concentrations, and no necrosis, fibrosis or inflammatory infiltrates were observed in any treatment group.

3.6.2 Gills Histopathology

Gill architecture remained normal across all treatment groups. Control fish (0 mg/L) displayed well-developed primary lamellae with orderly secondary lamellae, intact epithelial lining and preserved vascular structures. Fish exposed to 20, 25, 30 and 35 mg/L showed gill morphology indistinguishable from controls, with no evidence of epithelial degeneration, hyperplasia, lamellar fusion, oedema, haemorrhage or necrosis at any concentration tested. The complete absence of branchial pathology across the full dose range indicates that the ethanolic extract of unripe *M. paradisiaca* peel exerted no detectable toxic effect on gill tissue within the acute exposure period. The gill sections are shown in Fig. 8.

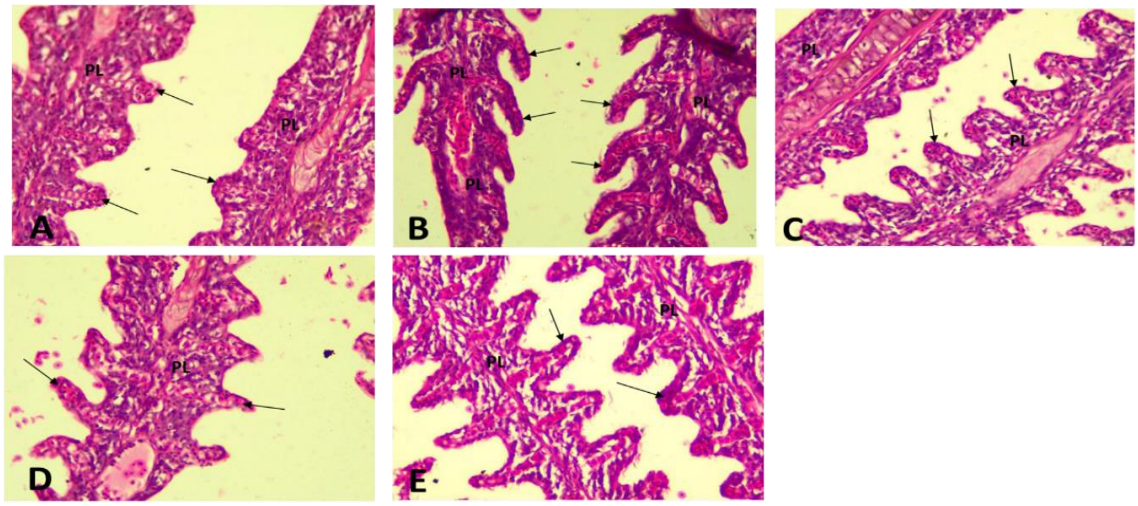


Fig. 8. Photomicrographs of gill sections of juvenile *Clarias gariepinus* exposed to ethanolic extract of unripe *Musa paradisiaca* peel for 96 h (H&E, ×400). *(A) Control (0 mg/L): normal gill architecture with well-developed primary lamellae (PL), orderly secondary lamellae (SL), intact epithelial lining, and preserved vascular structures. (B) 20 mg/L: normal branchial morphology comparable to control, with intact primary and secondary lamellae. (C) 25 mg/L: preserved lamellar organization with no pathological alterations. (D) 30 mg/L: intact primary and secondary lamellae with no evidence of degeneration or structural abnormality. (E) 35 mg/L: normal gill histoarchitecture indistinguishable from control. No concentration-dependent histopathological changes were observed across treatment groups*

4. Discussion

The acute toxicity test of ethanolic extract of unripe plantain peels on juveniles of *Clarias gariepinus* demonstrated a concentration-dependent toxic effect, as evidenced by increasing mortality and behavioural abnormalities with increasing extract concentrations. The absence of mortality in the control group confirmed that the experimental conditions were suitable and that the observed mortalities in the treated groups were attributable to the toxic effects of the extract. The absence of mortality in the control group is consistent with earlier research (George et al., 2024b, 2024c). The low mortality observed at 25 mg/L (10%) suggests that the fish were able to tolerate this concentration with minimal physiological stress. However, mortality increased markedly at 30 and 35 mg/L, indicating that higher concentrations exceeded the physiological tolerance limits of the fish.

The progressive increase in mortality with increasing extract concentration may be attributed to the presence of bioactive phytochemicals such as tannins, saponins, flavonoids, alkaloids and phenolic compounds commonly reported in unripe plantain peels (Adeniji et al., 2007). Although these compounds possess antimicrobial and antioxidant properties that may be beneficial when used as feed additives at low levels, excessive concentrations can exert toxic effects on aquatic organisms. Saponins are known to disrupt cell membrane integrity and impair respiratory functions in fish, thereby contributing to increased mortality under acute exposure conditions (Francis et al., 2002). Similar concentration-dependent mortality patterns have been reported in fish exposed to various plant-derived extracts and phytogenic compounds (Ayotunde et al., 2010; Fafioye et al., 2004; George et al., 2024a; George & Essien-Ibok, 2024).

Behavioural responses are widely recognised as sensitive indicators of environmental stress and toxicant exposure in fish. The occurrence of air gulping, erratic swimming, respiratory distress and vertical erection among exposed fish indicates physiological impairment resulting from exposure to the extract. Air gulping and respiratory distress are commonly associated with reduced respiratory efficiency and attempts by fish to compensate for impaired oxygen uptake at the gill surface (Svobodova et al., 1993). Erratic swimming behaviour reflects neurophysiological disturbances caused by toxic substances affecting the nervous system, while vertical erection and loss of equilibrium are indicators of severe stress and disruption of normal locomotor activities.

The observed increase in behavioural abnormalities from 48 to 96 hours, particularly at higher concentrations (30-35 mg/L), suggests cumulative toxic effects of the extract over time. The concentration-dependent nature of these responses indicates that the severity of physiological stress increased with increasing extract concentration. Similar behavioural manifestations have been reported in fish exposed to plant extracts (George et al., 2025c, 2025d), pesticides (Momta et al., 2024; Ezekiel et al., 2025; Bala et al., 2023) and other toxicants (Isyaku & Solomon, 2016; Ivon et al., 2017), where toxic stress alters metabolic processes, oxygen utilisation and nervous system function (Omitoyin et al., 2023; Agbohessi et al., 2023). The relatively mild behavioural changes observed at 20 and 25 mg/L further support the possibility that lower concentrations may be tolerated without causing severe physiological disruption.

Water-quality parameters play a critical role in determining fish health and survival during toxicity studies. The significant variations observed in pH, dissolved oxygen, conductivity and temperature among treatment groups suggest that the ethanolic extract influenced the physicochemical characteristics of the culture medium. Changes in pH may have altered the bioavailability and toxicity of phytochemical constituents present in the extract. Similarly, reductions in dissolved oxygen could have contributed to the respiratory distress and air-gulping behaviour observed in exposed fish. Increased conductivity may reflect the release of dissolved organic and inorganic substances from the plantain peel extract into the water. Although the recorded values remained within acceptable limits for short-term exposure, their significant variation across concentrations indicates that part of the observed stress responses may have resulted from both direct toxic effects of the extract and indirect effects mediated through alterations in water quality. Similar alterations in water quality in toxicity studies have been reported by George et al. (2023d, 2023e) and Essien-Ibok et al. (2024a, 2024b).

The liver is a major organ involved in metabolism, detoxification, nutrient storage and biotransformation of xenobiotics in fish. Consequently, it is one of the most sensitive organs for evaluating the toxic effects of environmental contaminants and plant-derived extracts (Hinton & Laurén, 1990; George et al., 2023d, 2023e). In the present study, the control fish exhibited normal hepatic architecture, indicating that the experimental conditions were suitable and free from confounding stressors.

The liver exhibited the primary histopathological damage, showing a dose-dependent progression from focal parenchymal haemorrhage and sporadic vacuolation at 20 mg/L to severe diffuse macrovesicular steatosis at 35 mg/L. This monotonic trend aligns with a classical dose-response model in which tissue injury increases proportionally with toxicant concentration (Yancheva, 2016). As the principal site of xenobiotic biotransformation in teleosts, the liver concentrates absorbed phytochemicals through phase I and II enzyme systems (Wolf & Wolfe, 2005), rendering hepatocytes particularly susceptible to metabolic disruption even when other organs remain unaffected (Yancheva, 2016). The absence of necrosis, fibrosis or inflammatory infiltration indicates that the extract impairs hepatocellular lipid metabolism, likely by interfering with fatty-acid oxidation or lipoprotein export, rather than causing direct cytotoxic cell death. This pattern is consistent with

nutritional-pathology findings showing that exceeding hepatic fatty-acid oxidation capacity leads to triglyceride accumulation and steatosis without accompanying cellular necrosis (Caballero et al., 2004).

A noteworthy anomaly was observed at 25 mg/L, where hepatic architecture resembled that of controls despite clear pathology at flanking concentrations. This discontinuity may reflect within-treatment biological variability or a genuine threshold between sublethal adaptation and overt metabolic disruption (Bernet et al., 1999). Additionally, the diffuse cytoplasmic vacuolation in control livers is consistent with dietary lipid loading documented in *C. gariepinus* reared on standard commercial diets (Jimoh, 2020). Subchronic feeding trials using quantitative histopathological scoring (Bernet et al., 1999) and lipid-specific staining are needed to determine whether the steatosis is reversible and to establish a no-observed-adverse-effect concentration suitable for aquaculture dietary application (Crane & Newman, 2000).

The complete preservation of branchial architecture across all concentrations, including the highest dose that produced severe hepatic steatosis, demonstrates a clear organ-selective toxicity pattern. As the primary contact surface with waterborne toxicants, gills possess a geometrically complex multifunctional epithelium widely regarded as one of the most sensitive histopathological indicators of aquatic exposure, with structural gill alterations quantitatively catalogued across more than one hundred toxicological studies (Mallatt, 1985). Plant extracts tested on *C. gariepinus* at comparable sublethal ranges have consistently produced branchial epithelial lifting, lamellar disorganisation, haemorrhage and necrosis; ethanolic *Justicia extensa* leaf extract elicits hyperplasia, erosion, fusion and disorganisation of secondary lamellae, desquamation and cellular degeneration in fingerlings (Ekpenyong et al., 2022), and methanolic *Melaleuca cajuputi* leaf extract produces epithelial lifting, oedema, hydropic degeneration, lamellar disorganisation and necrosis in proportion to concentration (Hassan et al., 2023).

The dissociation between hepatic and branchial responses is consistent with the exposure route: dissolved extract constituents absorbed across the gills reach the liver via portal circulation, where the liver has a high capacity for uptake, elimination, toxification and detoxification of xenobiotic compounds (Wolf & Wolfe, 2005). The gill epithelium, which lacks equivalent phase I and II xenobiotic handling and depends largely on stereotyped physiological reactions to chemical irritants (Mallatt, 1985), therefore remains structurally unaffected despite comparable dose exposure. Taken together, these findings indicate that ethanolic extract of unripe *Musa paradisiaca* peel produces organ-selective, dose-dependent hepatic lipid toxicity in juvenile *C. gariepinus* without branchial compromise, positioning the liver as the critical target organ for safety evaluation.

Overall, the preservation of normal gill morphology in all treatment groups demonstrates that exposure to unripe plantain peel extract up to 35 mg/L did not visibly affect the respiratory tissues of juvenile *Clarias gariepinus* during the acute exposure period. The results support further evaluation of the extract as a potential eco-friendly aquaculture feed additive or bioresource, with attention to concentration-dependent hepatic responses.

The findings of this study suggest that, while unripe plantain peel possesses biologically active compounds with potential application as a feed additive, excessive concentrations can induce toxic effects in juvenile fish. The low mortality and limited behavioural alterations observed at 20 and 25 mg/L indicate that these concentrations may be relatively safe for further nutritional evaluation. However, the pronounced mortality and behavioural disturbances recorded at 30 and 35 mg/L demonstrate that higher concentrations may compromise fish health and welfare. Therefore, careful dose optimisation is necessary before incorporating unripe plantain peel products into aquaculture feed formulations.

5. Conclusions

This study showed that ethanolic extract of unripe *Musa paradisiaca* peel produced concentration-dependent effects in juvenile *Clarias gariepinus* during 96 hours of acute exposure. The control and 20 mg/L treatments recorded no mortality, while low mortality occurred at 25 mg/L in one replicate. Higher concentrations resulted in greater mortality, with 20–30% mortality at 30 mg/L and 40–50% mortality at 35 mg/L. Behavioural abnormalities, including air gulping, erratic swimming, respiratory distress and vertical erection, became more pronounced as extract concentration and exposure time increased. Histological observations indicated that gill architecture remained preserved across all treatments, suggesting no visible branchial damage within the acute exposure period. In contrast, liver tissues showed concentration-related alterations, with macrovesicular steatosis becoming more evident at 30 and 35 mg/L. Overall, the lower concentrations appeared better tolerated,

whereas higher concentrations may pose risks to fish health. Chronic feeding trials, physiological assessment and dose optimisation are necessary before recommending the extract for aquaculture feed formulation.

6. Limitation

This study was limited to a 96-hour acute exposure period under laboratory conditions and did not assess long-term dietary effects, growth performance, feed utilisation or biochemical responses. The experiment used juvenile *Clarias gariepinus* only, so the findings may not directly apply to other fish species or developmental stages. Further chronic feeding trials with wider physiological and nutritional assessments are required to establish a safe inclusion level for aquaculture diets.

Declaration of AI Use

This manuscript was prepared through the combined contributions of all author(s), including contributions to the study design, data, content development, results, interpretation, and related scholarly work. The author(s) acknowledge the use of Grammarly and ChatGPT to assist with grammar checking, language refinement, reference formatting. These AI-assisted tools were not used as authors and did not replace the intellectual contributions or scholarly judgment of the author(s). All AI-assisted outputs, including content, references, and interpretations, were carefully reviewed, revised, verified, and approved by the author(s). The author(s) accept full responsibility for the accuracy, integrity, and final content of the manuscript.

Competing Interests

Authors have declared that no competing interests exist.

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