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# Valorization of Ripe Plantain Peel Waste in Aquaculture: Acute Toxicity, Behavioural Responses and Tissue Alterations in Juvenile African Catfish (*Clarias gariepinus*) (Burchell, 1822)

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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

This study evaluated the acute toxicity, behavioural responses, water-quality variations, and histopathological alterations in the gills and liver of juvenile *Clarias gariepinus* exposed to an ethanolic extract of ripe plantain (*Musa paradisiaca*) peels. Fish were exposed to extract concentrations of 0, 20, 25, 30, and 35 mg/L for 96 hours under static bioassay conditions. Behavioural responses, mortality, and water-quality parameters were

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monitored throughout the experiment, while histological examinations of the gill and liver tissues were conducted at the end of the exposure period. Exposed fish exhibited concentration-dependent behavioural abnormalities, including erratic swimming, respiratory distress, vertical erection, air gulping, and escape attempts. Mortality increased progressively with increasing extract concentration, with the highest mortality recorded at 35 mg/L. The 96-hour $LC_{50}$ was estimated at 35 mg/L (log concentration = 1.54), indicating moderate toxicity. Significant variations were observed in dissolved oxygen, pH, temperature, and conductivity across the concentrations and exposure periods. Histological examination of the gills revealed predominantly normal architecture, with only mild focal epithelial degeneration and haemorrhage at 20 mg/L. Liver tissues exhibited concentration-dependent alterations, including macrovesicular steatosis, inflammatory-cell infiltration, cytoplasmic vacuolation, vascular congestion, and sinusoidal congestion. The findings indicate that the ethanolic extract induced physiological and histopathological stress in juvenile *C. gariepinus* at higher concentrations, with the liver showing greater responsiveness than the gills. However, the absence of severe tissue necrosis suggests low-to-moderate toxicity within the tested concentration range. These results provide baseline information for evaluating the potential utilisation of ripe plantain peel-derived products in aquaculture.

*Keywords:* *African catfish; acute toxicity; behavioural responses; circular bioeconomy; ethanolic extract; histopathology; $LC_{50}$; plantain peel waste; tissue alterations; water quality.*

## 1. Introduction

According to the Food and Agriculture Organization (FAO, 2024), aquaculture contributes more than half of the fish consumed worldwide, and production is expected to continue increasing to meet the protein demands of a rapidly growing human population. However, the sustainability of aquaculture is increasingly threatened by the rising cost and limited availability of conventional feed ingredients, particularly fishmeal and soybean meal, which account for a substantial proportion of production expenses. Consequently, there is growing interest in identifying alternative, locally available, and environmentally sustainable feed resources capable of reducing production costs while maintaining fish health and growth performance (Adesina et al., 2025; Peres et al., 2026). Recent syntheses indicate that plant-based and other non-conventional feed resources can support more sustainable aquafeed development, but their suitability depends on ingredient characteristics, processing, inclusion level, and the response of the cultured species (Mugwanya et al., 2023; Qian et al., 2024; Siddik et al., 2024).

The concept of agricultural waste valorisation has gained considerable attention in recent years as part of the circular bioeconomy framework (Park, 2025). Agricultural by-products, which were traditionally regarded as waste, are now increasingly recognised as valuable resources that can be converted into livestock feed, biofertilisers, biofuels, pharmaceuticals, and other value-added products (Olasunkanmi et al., 2021). Among these agro-industrial wastes, plantain peels represent a significant yet underutilised biomass resource, especially in tropical countries where plantain production and consumption are extensive. Global plantain and banana production exceeds 150 million metric tonnes annually, generating substantial quantities of peel waste that constitute approximately 30–40% of the fresh fruit weight. Improper disposal of these peels contributes to environmental pollution, greenhouse-gas emissions, and public-sanitation challenges (Park, 2025). Therefore, effective utilisation of plantain peel waste aligns with sustainable waste-management strategies and contributes to environmental conservation. Recent reviews identify fruit and vegetable peels as potentially useful feed resources within circular production systems, while emphasising that their nutritional value and practical suitability depend on the source material and its processing (Haider et al., 2025; Sulaiman et al., 2025).

Within this framework, the recovery of fruit-processing residues and other agri-food by-products for aquafeed applications has been increasingly examined as a strategy that links feed-resource diversification with waste reduction and value recovery (Dawood et al., 2022; Prabakusuma et al., 2023; Zou et al., 2022).

Plantain (*Musa paradisiaca*) is widely cultivated throughout Africa, Asia, Latin America, and the Caribbean and serves as a major source of carbohydrates for millions of people. In Nigeria, plantain production has increased considerably because of its high nutritional value and economic importance (Adeniji et al., 2007). Consequently, large volumes of peels are generated daily by households, restaurants, food-processing industries, and local markets. Recent studies have shown that plantain peels contain appreciable amounts of carbohydrates, crude fibre, protein, essential minerals, vitamins, and bioactive compounds, making them a promising alternative feed ingredient in aquaculture nutrition (Adesina et al., 2025; Peres et al., 2026).

The nutritional composition of ripe plantain peels includes dietary fibre, potassium, calcium, phosphorus, magnesium, iron, and moderate levels of crude protein. Furthermore, plantain peels contain phytochemicals such as flavonoids, tannins, alkaloids, saponins, phenolic compounds, and antioxidants (Happi Emaga et al., 2007; Someya et al., 2002). These bioactive compounds have attracted scientific interest because of their antimicrobial, antioxidant, anti-inflammatory, and growth-promoting properties. The incorporation of plant-derived bioactive substances into aquaculture diets has been reported to improve feed utilisation, stimulate immunity, enhance disease resistance, and reduce dependence on synthetic growth promoters (Falaye & Oloruntuyi, 1998; Sogbesan et al., 2016). Nevertheless, although these phytochemicals may confer beneficial biological effects at low concentrations, excessive exposure can induce physiological stress and toxic responses in fish (George et al., 2024a, 2024b, 2025a, 2025b).

Evidence from banana-derived materials further indicates that their potential value is influenced by the form of the material, the extraction or processing approach, and the concentration at which bioactive constituents are delivered (Avram et al., 2022; Putra et al., 2022; Rifna et al., 2023; Widoyanti et al., 2023).

Plant-based additives may support fish nutrition and health, although their effects are not uniform across ingredients or species and therefore require case-specific evaluation (Kuebutornye et al., 2024).

The utilisation of agro-industrial by-products in aquafeeds requires a comprehensive evaluation of their safety, palatability, digestibility, and potential toxicological effects. Plant-derived materials often contain antinutritional factors capable of impairing nutrient absorption, metabolism, and tissue function when present at elevated concentrations (Adesina et al., 2025; Peres et al., 2026). Plantain peels contain varying levels of tannins, oxalates, phytates, cyanogenic compounds, and other secondary metabolites, whose concentrations may differ according to fruit maturity, processing methods, and environmental conditions. Therefore, toxicity assessments are essential before plantain peel products are recommended for large-scale inclusion in aquaculture feed formulations. Recent evidence from African catfish also indicates that responses to alternative plant-derived feed ingredients may vary according to ingredient type and inclusion level, supporting the need for ingredient-specific assessment before wider application (Igoche et al., 2025; Shaw et al., 2024).

Accordingly, evaluations of plant-derived aquafeed materials should consider antinutritional constraints and possible tissue-level responses alongside growth and feed-utilisation outcomes (Khieokhajonkhet et al., 2024; Punia et al., 2024).

Among cultured fish species, the African catfish, *Clarias gariepinus*, remains one of the most economically important freshwater fish in Africa because of its rapid growth rate (Otoh et al., 2022, 2023), high fecundity (Otoh et al., 2024a, 2024b), tolerance of environmental stress (Otoh et al., 2024c, 2024d), and consumer acceptance (Otoh et al., 2025). The species constitutes a major component of commercial aquaculture production in Nigeria and several other African countries. Because of its hardiness and sensitivity to environmental contaminants, *C. gariepinus* is frequently used as a bioindicator organism in ecotoxicological studies. Evaluating behavioural responses, mortality patterns, and tissue alterations in this species provides valuable information regarding the safety of novel feed ingredients and environmental contaminants.

Recent feeding studies in *C. gariepinus* have likewise incorporated haematological and histopathological endpoints when assessing unconventional feed resources, reinforcing the value of tissue-based safety indicators in feed evaluation (Ukagwu et al., 2025).

Acute toxicity studies represent a fundamental approach to assessing the potential hazards associated with exposure to natural products and plant-derived compounds. Such studies determine the concentration capable of causing mortality within a specified exposure period, typically 96 hours (George et al., 2023a, 2023b), and provide baseline information for establishing safe exposure limits (Essien-Ibok et al., 2024a, 2024b; George et al., 2025c, 2025d). In fish, toxic substances often induce behavioural abnormalities, including erratic swimming, increased opercular movement, loss of equilibrium, mucus hypersecretion, air gulping, reduced feeding activity, and lethargy. These behavioural changes frequently serve as early indicators of physiological stress preceding mortality and tissue damage (George et al., 2024c, 2024d).

Histopathological assessment is widely regarded as one of the most reliable biomarkers in aquatic toxicology because it reveals cellular- and tissue-level alterations resulting from toxic exposure (Ashade et al., 2014; Fagbohun et al., 2020). The gills, liver, and kidneys are particularly important target organs because of their direct involvement in respiration, detoxification, osmoregulation, and excretion (Sayed et al., 2023; Sulem Yong

et al., 2023; George et al., 2023c, 2023d). Exposure of *C. gariepinus* to toxic plant extracts and environmental contaminants has been associated with pathological changes, including epithelial lifting, lamellar fusion, hyperplasia, congestion, vacuolation, haemorrhage, necrosis, and degeneration of hepatic and renal tissues (Ozoagudike & Bawa-Allah, 2020; Raimi et al., 2022). These alterations provide insight into the mechanisms of toxicity and the overall health status of exposed fish.

Although several studies have investigated the nutritional composition and feed-utilisation potential of plantain peels in livestock and aquaculture systems, information regarding the acute toxicity profile of ripe plantain peel extracts and their effects on fish behaviour and tissue integrity remains limited. Understanding these effects is essential for determining safe inclusion levels and ensuring that the valorisation of plantain peel waste does not compromise fish welfare or productivity. Furthermore, the reported piscicidal activity of papaya peel powder in juvenile *C. gariepinus* suggests that the biological effects of peel-derived materials are source-specific and require direct assessment; however, comparable acute evidence for ethanolic ripe plantain peel extract remains limited (Kachi et al., 2024).

Therefore, the present study was designed to evaluate the acute toxicity, behavioural responses, and histopathological alterations induced by ripe plantain peel extract in juvenile African catfish (*Clarias gariepinus*). Specifically, the study aimed to determine the toxicological effects of varying concentrations of ripe plantain peel extract by assessing mortality, behavioural changes, and tissue alterations in the gills and liver. The findings may contribute to the sustainable utilisation of plantain peel waste in aquaculture, support circular-economy initiatives, and provide evidence relevant to evaluating the safe incorporation of plantain-derived products into fish-feed formulations.

## 2. Materials and Methods

### 2.1 Collection of Specimens

Two hundred (200) healthy juveniles of *Clarias gariepinus* were collected from the Akwa Ibom State University Fish Farm, Obio Akpa, Akwa Ibom State, Nigeria, and acclimatised in a recirculating plastic aquarium measuring 25 × 13 × 8.3 cm and containing hatchery water for 24 h in the Fisheries and Aquaculture Laboratory of the fish farm. This acclimation period helped to stabilise the fish and minimise stress resulting from collection and transportation (George et al., 2014, 2015a, 2015b).

### 2.2 Collection of Plant Samples

Fresh samples of ripe plantain (Musa paradisiaca) were collected from Obio Akpa, Oruk Anam Local Government Area, Akwa Ibom State, on 6 April 2024. The collected plant materials were transported to the University of Uyo, Uyo, Akwa Ibom State, for identification and authentication. The identification process was carried out at the Herbarium of the Department of Botany and Ecological Studies, University of Uyo. The collected ripe plantain peels are presented in Fig. 1.



**Fig. 1. Ripe plantain peels (*Musa paradisiaca*) used in the study**
*(Source: Authors Experimental Preparation, 2026)*

## 2.3 Preparation of Plant Material

After identification, the plantain peels were thoroughly washed, cut into smaller pieces, spread on cellophane sheets, and air-dried at room temperature for 72 hours. The dried peels were subsequently pulverised into a fine powder using a wooden pestle and mortar. The resulting dried and powdered plantain peel material is presented in Fig. 2.




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

## 2.4 Preparation of Ethanolic Extract (Maceration and Extraction)

A cold extraction (maceration) technique was adopted in this study following the method described by Hidayat and Wulandari (2021). Briefly, 240 g of the plant material was macerated in 1,000 mL of 99% ethanol within an airtight container and maintained at room temperature for 72 hours (3 days). The resulting ethanolic extract was subsequently filtered using a filter net followed by filter paper to remove residual plant materials. The filtrate was concentrated by evaporation in a water bath maintained at 40 °C for 48 hours. The resulting extract was stored in a beaker covered with aluminium foil and used immediately for the bioassay after complete evaporation of the solvent.

## 2.5 Monitoring of Water Quality

Water-quality parameters were assessed prior to the initiation of the experiment and monitored periodically throughout the exposure period in accordance with the standard procedures outlined by the American Public Health Association (APHA, 2005). The evaluated parameters included dissolved oxygen (DO), pH, and temperature (°C). Temperature and pH measurements were obtained using a portable HANNA HI 991301 pH/EC/TDS/temperature meter, whereas dissolved oxygen concentrations were determined using a JPB-607A digital portable analyser (Search Tech Instrument).

## 2.6 Acute Toxicity Test

A preliminary range-finding assay was performed to establish the toxicity threshold of the ethanolic extract derived from ripe plantain peels, in accordance with OECD (2000) Guideline No. 203 and ISO 7346-2:1996. Five experimental concentrations were evaluated in duplicate, comprising a control treatment and four test solutions containing the ethanolic ripe plantain peel extract. Ten juvenile Clarias gariepinus specimens were batch-weighed using a top-loading Mettler balance (Mettler Toledo, K) and randomly allocated to the treatment groups in duplicate. The plastic aquaria were covered with mosquito netting to prevent fish escape, while aeration, water renewal, and feeding were withheld throughout the experimental period. In the range-finding assay, the extract was administered at concentrations of 5, 10, 15, and 20 mg/L, with the control treatment maintained at 0 mg/L.

Based on the outcomes of the range-finding assay, a definitive toxicity test was subsequently conducted in duplicate using ethanolic ripe plantain peel extract concentrations of 20, 25, 30, and 35 mg/L, alongside a control treatment of 0 mg/L. The behavioural responses and mortality of the test organisms in each aquarium

were observed and documented at 24-hour intervals over a 96-hour exposure period. Mortality was confirmed by the absence of response to external stimulation. In addition to survival rates, specific behavioural alterations, including abnormal swimming patterns, air gulping, loss of equilibrium or reflex response, body discolouration, and moulting, were systematically recorded. The 96-hour median lethal concentration ($LC_{50}$), defined as the concentration of ethanolic ripe plantain peel extract required to induce mortality in 50% of the exposed organisms within 96 hours, was estimated graphically using probit analysis following the methodology described by USEPA (2002).

## 2.7 Histopathological Examinations

The gill and liver tissues were excised from the experimental fish and preserved in formalin-saline solution for 48 hours. Following fixation, the tissues were processed manually through a series of graded ethanol concentrations for dehydration, cleared in xylene, impregnated, and embedded in paraffin wax. The embedded tissue blocks were sectioned using a rotary microtome, and the resulting sections were stained with haematoxylin and eosin. The prepared histological slides were subsequently examined under a light microscope at ×100 and ×400 magnifications to assess the presence of pathological alterations.

## 2.8 Data Analysis

The effects of varying concentrations of the ethanolic extract of ripe plantain (Musa paradisiaca) peels were presented in tabular form. One-way analysis of variance (ANOVA) was employed to determine the significance of differences among the tested concentrations in both experimental batches (Batches A and B), with statistical significance established at a probability level of $p < 0.05$. Probit analysis was conducted using SPSS software version 20.0 to estimate the toxicity response parameters.

# 3. Results

## 3.1 Behavioural Responses of Juvenile *Clarias gariepinus* to the Toxic Effects of Ethanolic Ripe *Musa paradisiaca* Peel Extract during a 96-Hour Bioassay

The behavioural responses of juvenile C. gariepinus exposed to varying concentrations of ethanolic ripe Musa paradisiaca peel extract over exposure durations of 24–96 hours were evaluated using a semi-quantitative assessment approach (Table 1). Following 24 hours of exposure, fish subjected to extract concentrations ranging from 20 to 35 mg/L exhibited mild to moderate behavioural alterations, including air gulping, erratic swimming, respiratory distress, and vertical erection. In contrast, no observable behavioural abnormalities were recorded in the control group (0 mg/L). Prolonged exposure for 48–96 hours resulted in moderate to severe expressions of these behavioural disturbances among the treated groups. The severity of responses generally increased in a concentration-dependent manner, although this trend was not consistently observed at the 20 and 25 mg/L exposure concentrations.

**Table 1. Effect behavioral responses of *Clarias gariepinus* juveniles to the toxic effects of ethanolic extract of peels of ripe *Musa paradisiaca* for 96 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*

### 3.2 Acute Toxicity of Juvenile *C. gariepinus* at Different Concentrations of Ethanolic Ripe *Musa paradisiaca* Peel Extract after 96 Hours

The mortality results for juvenile *C. gariepinus* at 24, 48, 72, and 96 hours are presented in Table 2 for both experimental batches. At the control concentration (0 mg/L), no mortality was recorded throughout the test period in either Batch A or Batch B. At 20 mg/L, 10% mortality was recorded in both batches. At 25 mg/L, mortality differed between the batches, with 10% recorded in Batch A and 20% in Batch B, leaving 90% and 80% survivors, respectively.

A marked decrease in the number of survivors was observed at 30 mg/L of the ripe *Musa paradisiaca* peel extract after the 96-hour bioassay. Mortality of 30% was recorded in both Batches A and B, leaving 70% survivors in each batch. At 35 mg/L, mortalities of 40% and 50% were recorded in Batches A and B, respectively, leaving 60% and 50% survivors.

**Table 2. Summary of the percentage mortality and survivors of *C. gariepinus* Juveniles in the different concentrations of the ethanolic extract of ripe 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 | 10 | 10 | 100 |
| 20 mg/l | 1 | 10 | 9 | 90 | 1 | 10 | 9 | 90 |
| 25 mg/l | 1 | 10 | 9 | 90 | 2 | 20 | 8 | 80 |
| 30 mg/l | 3 | 30 | 7 | 70 | 3 | 30 | 7 | 70 |
| 35 mg/l | 4 | 40 | 6 | 60 | 5 | 50 | 5 | 50 |

### 3.3 Determination of the 96-Hour $LC_{50}$ for Ripe *Musa paradisiaca* Peel Extract

The 96-hour $LC_{50}$ for juvenile *C. gariepinus* exposed to different concentrations of ethanolic ripe *Musa paradisiaca* peel extract was determined using probit analysis. The concentrations were first log-transformed for the analysis (Table 3). The estimated 96-hour $LC_{50}$ for the ripe *M. paradisiaca* peel extract was 35 mg/L, corresponding to a log-transformed concentration of 1.54, at which 50% of the test organisms were killed by the end of the experiment.

**Table 3. $LC_{50}$ determination for *C. gariepinus* Juveniles at the end of the 96-hours bioassay for ripe plantain (*Musa paradisiaca)* Peels extract**

| Concentration (mg/l) | Log Transformation | Mortality (M) | % Mortality | Survivors (S) | % Survivor |
|---|---|---|---|---|---|
| 0 mg/l | 0 | 0 | 10 | 10 | 100 |
| 20 mg/l | 1.30 | 1 | 10 | 9 | 90 |
| 25 mg/l | 1.39 | 2 | 20 | 8 | 80 |
| 30 mg/l | 1.47 | 3 | 30 | 7 | 70 |
| 35 mg/l | 1.54 | 5 | 50 | 5 | 50 |

### 3.4 Histopathology of the Gills and Liver of Juvenile *C. gariepinus* Exposed to Different Concentrations of Ethanolic Ripe *Musa paradisiaca* Peel Extract after 96 Hours

#### 3.4.1 Histopathological Changes in the Liver of *Clarias gariepinus* Exposed to Ripe Plantain Peel Extract

Control fish (0 mg/L) displayed normal hepatic architecture, with hepatocytes arranged in distinct cords, average-sized sinusoids, and mild dilation of the hepatic venous system. At 20 mg/L, the hepatic cord arrangement was preserved, but sporadic macrovesicular steatosis was evident, characterised by large cytoplasmic lipid vacuoles with peripheral nuclear displacement. Fish exposed to 25 mg/L showed multifocal mononuclear inflammatory-cell infiltration within the hepatic parenchyma and dilated sinusoids, although the overall architecture remained intact.

At 30 mg/L, moderate cytoplasmic vacuolation of hepatocytes and vascular congestion were observed, while the sinusoidal spaces and general vascular organisation remained preserved. The most pronounced vascular changes occurred at 35 mg/L, comprising diffuse sinusoidal congestion with inflammatory-cell accumulation within dilated sinusoids. Hepatic cords remained visible, but the vascular disturbance was more marked than at any lower concentration. Across the treatment range, hepatic alterations progressed from lipid-based changes (steatosis at 20 mg/L), through inflammatory and vacuolar responses (25–30 mg/L), to predominantly vascular and inflammatory pathology (35 mg/L), suggesting a qualitative shift in the nature of hepatic injury with increasing extract concentration rather than a simple intensification of a single lesion type. The corresponding liver photomicrographs are presented in Fig. 3.

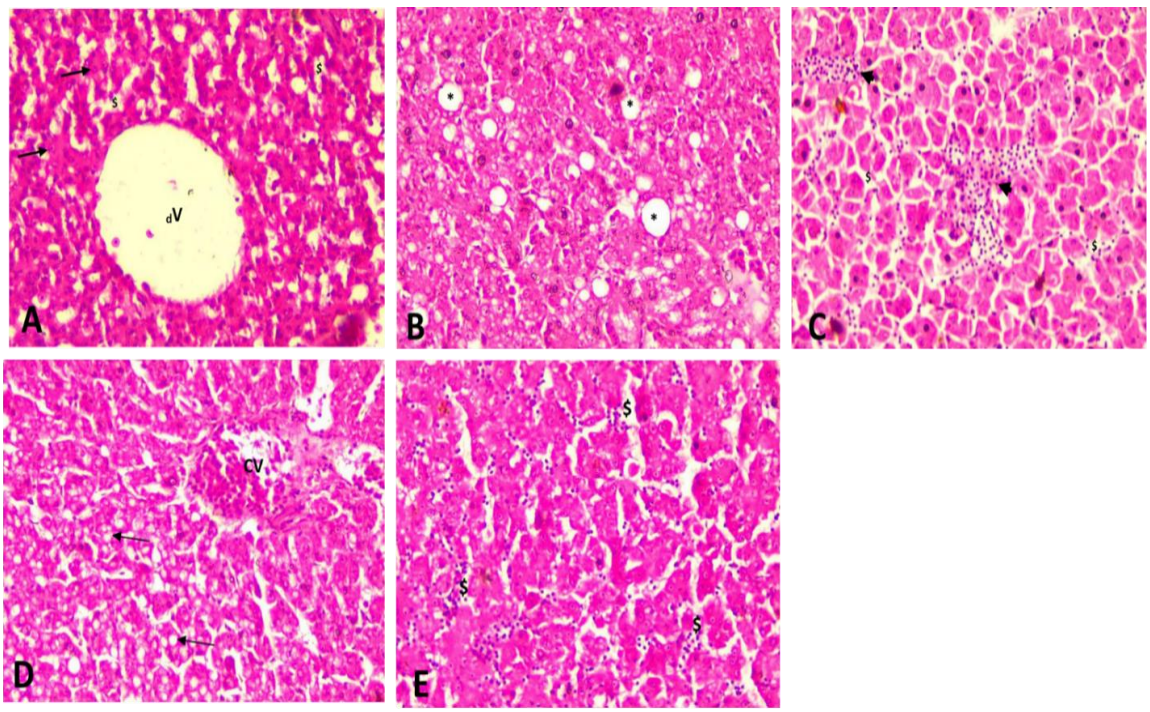



**Fig. 3. Photomicrographs of liver sections of juvenile *Clarias gariepinus* exposed to ripe *Musa paradisiaca* peel extract for 96 h (H&E, ×400)**

*(A) Control (0 mg/L): normal hepatic architecture with hepatocytes arranged in distinct cords (HC), average-sized sinusoids (S), and mild dilation of the hepatic venous system. (B) 20 mg/L: preserved hepatic cord arrangement with sporadic macrovesicular steatosis (MS) characterised by large cytoplasmic lipid vacuoles and peripheral nuclear displacement (arrowhead). (C) 25 mg/L: multifocal mononuclear inflammatory cell infiltration (arrows) within the hepatic parenchyma and dilated sinusoids; overall architecture intact. (D) 30 mg/L: moderate cytoplasmic vacuolation of hepatocytes (V) with congestion of blood vessels (asterisk); sinusoidal spaces preserved. (E) 35 mg/L: diffuse sinusoidal congestion (asterisks) with inflammatory cell accumulation within dilated sinusoids (arrows); hepatic cords remain visible. Note the qualitative progression from lipid-based injury (B) through inflammatory response (C) to vascular pathology (D–E)*

### 3.4.2 Histopathological Changes in the Gills of *Clarias gariepinus* Exposed to Ripe Plantain Peel Extract

Gill architecture was preserved across most treatment groups. Control fish (0 mg/L) displayed intact primary and secondary lamellae, with normal epithelial lining, supporting cartilage, and erythrocytes visible within the lamellar blood channels. Fish exposed to 25, 30, and 35 mg/L showed histological features indistinguishable from those of the controls, with intact lamellar organisation, preserved epithelial and vascular structures, and no pathological lesions.

The sole exception was the 20 mg/L group, which exhibited focal epithelial degeneration and haemorrhage within the primary lamellae. The overall gill architecture remained preserved despite these localised changes. The restriction of branchial pathology to the lowest treatment concentration, with no lesions at higher concentrations, does not support a concentration-dependent toxic effect on gill tissue. The corresponding gill photomicrographs are presented in Fig. 4.

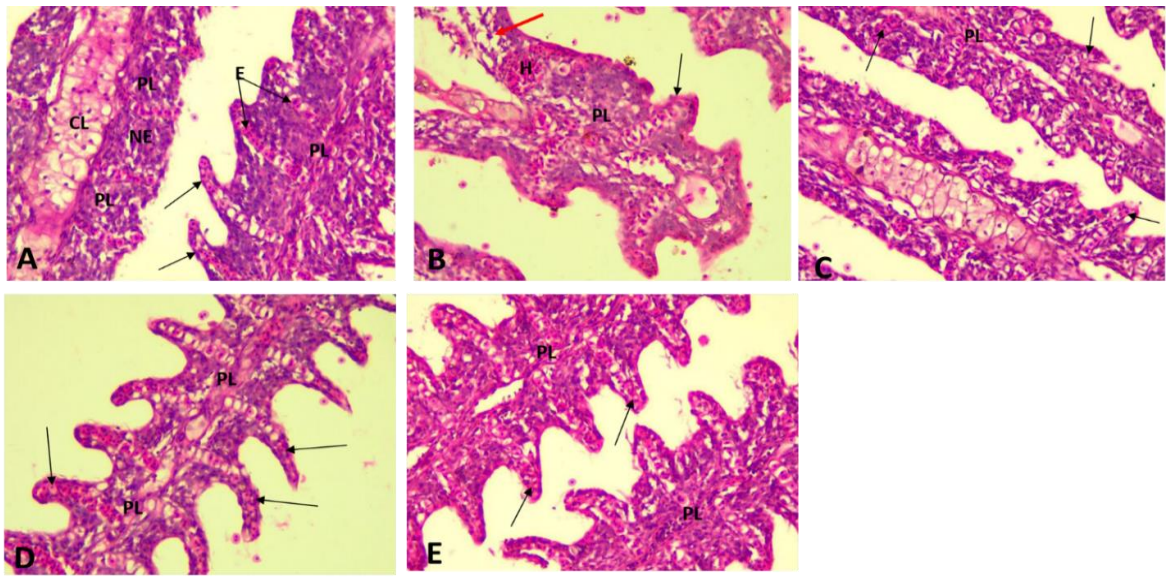



**Fig. 4. Photomicrographs of gill sections of juvenile Clarias gariepinus exposed to ripe Musa paradisiaca peel extract for 96 h (H&E, ×400)**

*(A) Control (0 mg/L): normal gill architecture with intact primary lamellae (PL), orderly secondary lamellae (SL), normal epithelial lining, supporting cartilage, and erythrocytes within lamellar blood channels. (B) 20 mg/L: focal epithelial degeneration (arrow) and haemorrhage (arrowhead) within the primary lamellae; overall gill architecture preserved. (C) 25 mg/L: normal branchial morphology comparable to control, with intact primary and secondary lamellae and preserved vascular structures. (D) 30 mg/L: normal gill histoarchitecture with no pathological alterations. (E) 35 mg/L: intact primary and secondary lamellae indistinguishable from control. Pathological changes were confined to the 20 mg/L group; no concentration-dependent gill damage was observed*

## 3.5 Initial Water Quality Parameters

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

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

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

### 3.6 Variation in the Physicochemical Parameters of the Test Media Containing Juvenile *Clarias gariepinus* during the 96-Hour Experimental Period

Variations in the water-quality parameters (DO, temperature, pH, and conductivity) of the test media at different concentrations and exposure times are presented in Figs. 5–8.

#### 3.6.1 Dissolved Oxygen (mg/L)

The lowest dissolved-oxygen values across all concentrations of the ripe peel extract were observed at 96 hours, whereas the highest DO level was recorded in the control (0 mg/L) at 24 hours. Variations in DO at different time intervals and concentrations are shown in Fig. 5. Significant variation ($p < 0.05$) was observed among the extract concentrations.

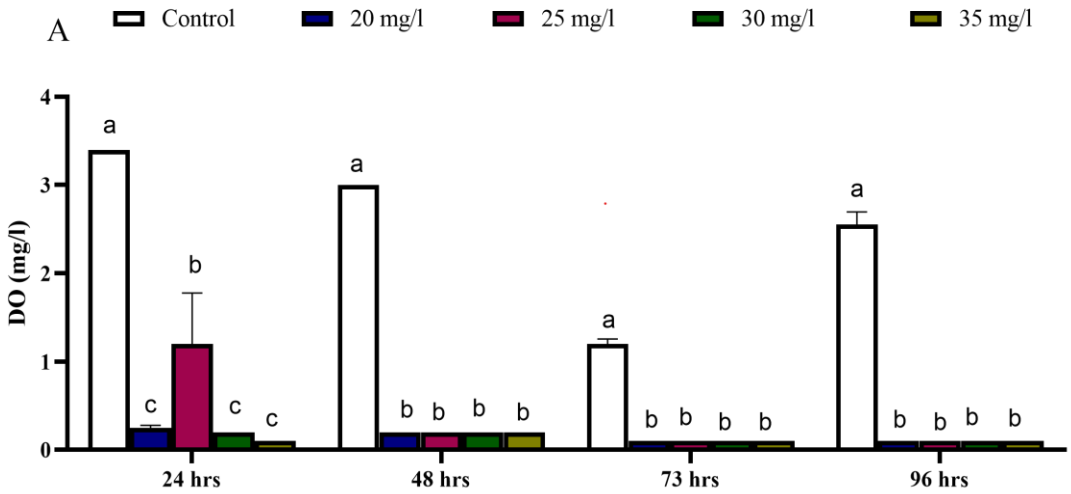



**Fig. 5. Dissolved oxygen variations during the 96 hours of test**

#### 3.6.2 Temperature (°C)

Significant variation in temperature was observed among the different concentrations of ripe plantain peel extract at 24, 48, and 72 hours ($p < 0.05$). No significant difference was observed at 96 hours (Fig. 6).

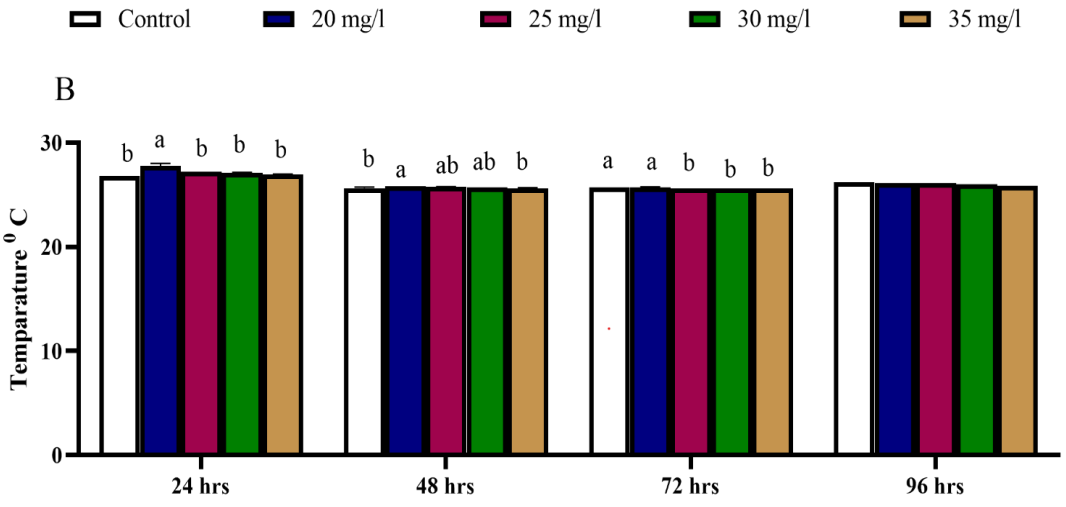



**Fig. 6. Temperature variations during the 96 hours of test**

### 3.6.3 pH

The pH varied during the 96-hour experimental period in the ripe peel extract treatments. Significant differences ($p < 0.05$) were observed among the concentrations at the different time intervals (Fig. 7).

### 3.6.4 Conductivity

Conductivity values showed a distinct pattern, with relatively high values recorded in the treated tanks across the different time intervals. Significant differences ($p < 0.05$) were observed among the concentrations at 24, 48, 72, and 96 hours (Fig. 8).

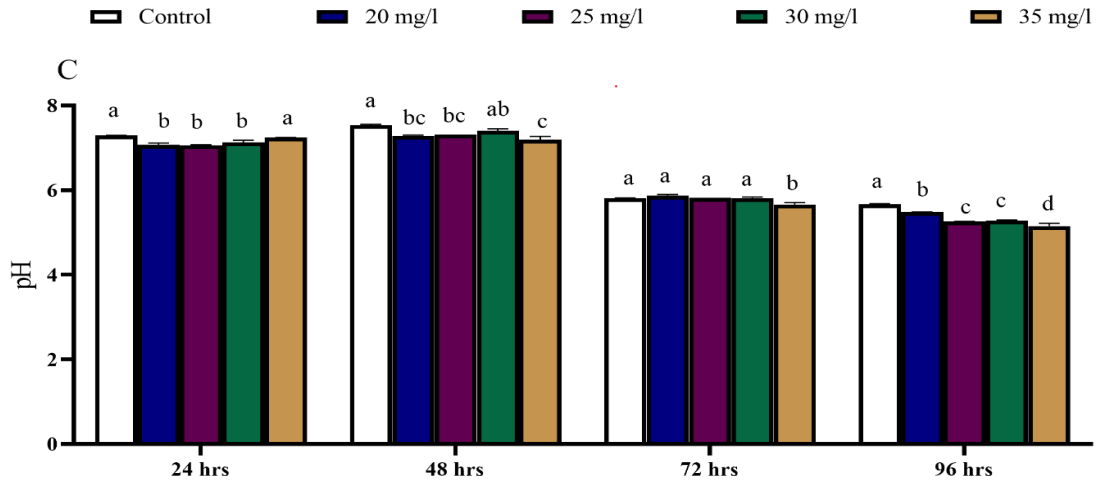



**Fig. 7. pH variations during the 96 hours of test**

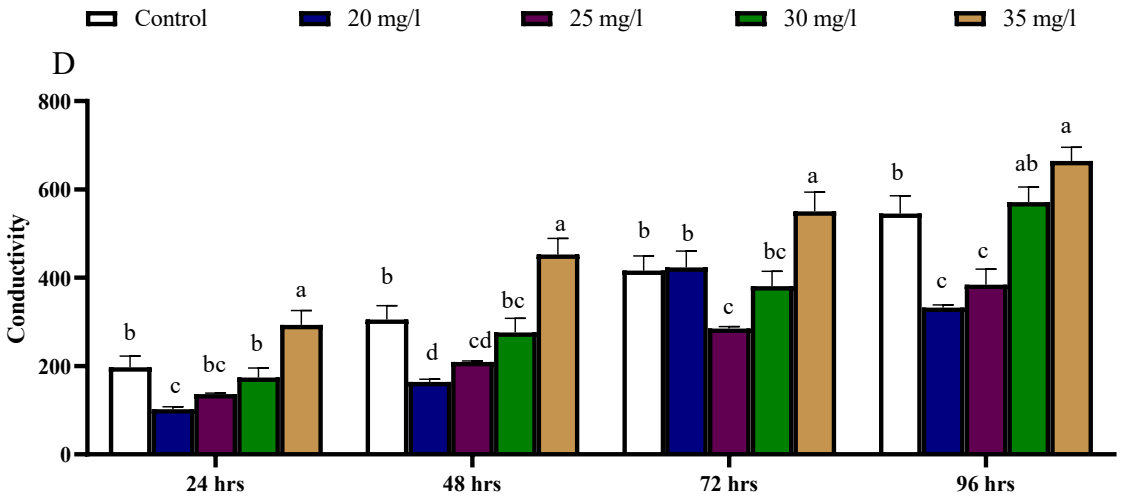



**Fig. 8. Conductivity variations during the 96 hours of test**

## 4. Discussion

The behavioural alterations observed in juvenile *Clarias gariepinus* exposed to the ethanolic extract of ripe plantain peels—including erratic swimming, respiratory distress, vertical erection, air gulping, and attempts to jump out of the test medium—indicate physiological stress induced by the extract. Such behavioural responses are widely recognised as early indicators of toxicant exposure and often precede mortality and tissue damage (Omitoyin et al., 1999). The progressive increase in the severity of these abnormalities with increasing

concentration and exposure duration suggests a concentration-dependent toxic effect of the extract. Similar behavioural disturbances have been reported in *C. gariepinus* exposed to plant-derived toxicants, pesticides, and industrial effluents, in which disruption of respiratory and neurological functions resulted in abnormal swimming patterns and increased surface activity (Ayoola, 2008; Ekpenyong et al., 2022).

The mortality pattern recorded during the bioassay further supports the toxic effects of the extract. Mortality increased with increasing concentration, indicating a dose-dependent response. Such concentration-dependent mortality is characteristic of acute toxicological exposure and has been reported in several studies involving botanical extracts and agricultural wastes tested on fish species (Fafioye et al., 2004; Omitoyin et al., 1999). The relatively low mortality observed at 20 and 25 mg/L suggests that these concentrations may be within the tolerance limits of the species, whereas higher concentrations imposed greater physiological stress and resulted in increased mortality.

The 96-hour $LC_{50}$ value of 35 mg/L was interpreted as indicating moderate toxicity of the ethanolic ripe plantain peel extract to juvenile *C. gariepinus*. According to the toxicity classification criteria proposed by Sprague (1971), substances with $LC_{50}$ values within this range are considered moderately toxic to aquatic organisms. Similar $LC_{50}$ values have been reported for several plant-derived products used in aquaculture and fisheries management, suggesting that, although the extract contains bioactive compounds capable of eliciting toxic effects, its toxicity is not excessively high relative to conventional piscicides and botanical toxicants (Fafioye et al., 2004).

The significant variations observed in dissolved oxygen, pH, temperature, and conductivity across exposure concentrations and durations may have contributed to the behavioural and physiological responses recorded. Water-quality parameters are known to influence fish metabolism, respiration, and toxicant uptake (Boyd, 2015). Reduced dissolved-oxygen levels, in particular, may explain the respiratory distress and air-gulping behaviour observed in the exposed fish. Nevertheless, the relatively stable temperature values at 96 hours suggest that temperature was unlikely to be the primary factor responsible for the observed toxic responses. Similar interactions between water-quality variables and toxicant-induced stress have been documented in acute toxicity studies involving *Clarias gariepinus* and other freshwater fish species (Svobodova et al., 1993).

Hepatic histology showed a clear concentration-dependent response characterised by a qualitative shift in the nature of injury rather than the simple intensification of a single lesion type. At 20 mg/L, the lesions were lipid-based, with sporadic macrovesicular steatosis and peripheral nuclear displacement consistent with triglyceride accumulation when hepatic fatty-acid oxidation capacity is exceeded (Caballero et al., 2004). At 25–30 mg/L, the pattern diversified, with mononuclear inflammatory infiltration, sinusoidal dilation, and moderate cytoplasmic vacuolation indicating concurrent metabolic and early vascular disturbances (Yancheva, 2016). At 35 mg/L, the dominant pathology comprised diffuse sinusoidal congestion and inflammatory-cell accumulation within dilated sinusoids, matching the circulatory-disturbance pattern documented in fish exposed to a wide range of organic and inorganic toxicants, in which dilation of blood sinusoids and cytoplasmic degeneration of hepatocytes are among the most consistently observed hepatic changes (Yancheva, 2016). This triphasic lipid → inflammatory → vascular progression is consistent with the central role of the teleost liver in the uptake, elimination, toxification, and detoxification of xenobiotic compounds (Wolf & Wolfe, 2005) and reflects progressive overloading of metabolic and circulatory processes as the concentration increases.

The pattern aligns with previously documented hepatic responses of *C. gariepinus* to phytogenic and metallic stressors, including concentration- and duration-dependent hepatocellular enlargement, vacuolation with pyknotic nuclei, cytolysis, and focal necrosis under sublethal glyphosate exposure (Olurin et al., 2006), as well as progressive architectural distortion described as dose- and time-dependent under chronic lead exposure (Olojo et al., 2005). This consistency supports the interpretation of a concentration-dependent shift rather than idiosyncratic variation. The finding at 25 mg/L—namely, inflammatory infiltration without overt steatosis or the overt congestion observed at 30–35 mg/L—may represent a threshold zone between metabolic and vascular injury rather than sampling variability. Such intermediate responses highlight the value of quantitative reaction-pattern scoring, which combines lesion extent and pathological importance factors to distinguish reversible from irreversible changes (Bernet et al., 1999). The fatty infiltration without accompanying necrosis observed in this study is consistent with the steatosis pattern that arises when dietary lipid exceeds hepatic oxidation capacity (Caballero et al., 2004), supporting the interpretation that the extract primarily affects hepatocellular lipid metabolism rather than acting through direct cytotoxicity.

Two interpretive caveats warrant emphasis. The diffuse cytoplasmic vacuolation observed in control livers mirrors the diffuse hepatic vacuolation documented in *C. gariepinus* fed standard commercial diets (Jimoh, 2020), establishing an elevated baseline against which mild treatment-related vacuolation is difficult to distinguish. Apparent sinusoidal congestion and dilated sinusoids must also be interpreted cautiously because liver congestion can arise from the degree of exsanguination at sacrifice, manual compression of the liver sample during necropsy, or tissue-processing and microtomy artefacts rather than from true in-life circulatory disturbance (De Sales-Ribeiro et al., 2020). Subchronic feeding trials incorporating quantitative histopathological scoring (Bernet et al., 1999), lipid-specific staining of cryosections, and strictly controlled basal diets are therefore needed to determine the reversibility of the steatotic and inflammatory changes and to establish a no-observed-adverse-effect concentration suitable for assessing aquaculture applications of *M. paradisiaca* peel extract (Crane & Newman, 2000). Furthermore, distinguishing physiological glycogen depletion from pathological degeneration remains important because the PAS technique frequently reveals dynamic shifts in carbohydrate storage that can be misconstrued as structural damage (Sayed et al., 2023). Future research should implement semi-quantitative grading systems to measure the degree of cytoplasmic vacuolation objectively and distinguish alterations in lipid or glycogen storage from toxicant-induced degenerative states (Matos et al., 2007).

Branchial architecture was preserved across most treatment groups, with fish exposed to 25, 30, and 35 mg/L showing histology indistinguishable from that of the controls. Only the 20 mg/L group displayed focal epithelial degeneration and primary-lamellar haemorrhage. This near-complete preservation at higher concentrations is notable because the fish gill is regarded as one of the most sensitive histopathological indicators of aquatic exposure, and its structural alterations have been quantitatively catalogued across a wide range of toxicants and irritants (Mallatt, 1985). Comparative plant-extract studies on *C. gariepinus* at sublethal concentrations consistently report concentration-dependent branchial damage: ethanolic *Justicia extensa* leaf extract elicits hyperplasia, secondary-lamellar fusion, desquamation, and cellular degeneration (Ekpenyong et al., 2022), while methanolic *Melaleuca cajuputi* leaf extract produces epithelial lifting, oedema, hydropic degeneration, lamellar disorganisation, and necrosis (Hassan et al., 2023). Concentrations of 20–35 mg/L lie within the range at which similar plant-derived toxicants produce clear dose-dependent lesions; therefore, branchial preservation up to the highest tested concentration indicates that the ethanolic *M. paradisiaca* peel extract did not exert a direct dose-dependent effect on branchial tissue in this species.

The pattern is consistent with mechanism-driven organ selectivity. Dissolved extract constituents absorbed across the gills reach the liver via the circulation, where biotransformation and dose-dependent accumulation in hepatic tissue (Yancheva, 2016) may concentrate reactive metabolites within hepatocytes, while the gill epithelium, which lacks equivalent metabolic capacity and responds to injury through a restricted set of stereotyped defensive responses (Mallatt, 1985), is exposed only transiently to parent compounds. The focal damage confined to 20 mg/L and not reproduced at higher concentrations may reflect transient tissue-handling variation rather than toxicant-driven pathology. Quantitative histopathological scoring that weights lesion reversibility using importance factors (Bernet et al., 1999), alongside subchronic trials establishing a no-observed-adverse-effect concentration for aquaculture application (Crane & Newman, 2000), would strengthen these conclusions and clarify whether the apparent branchial sparing persists under prolonged exposure.
Overall, the histopathological findings indicate that ripe plantain peel extract caused only mild and localised liver and gill alterations, with no evidence of severe or progressive tissue damage. These results suggest a low level of branchial toxicity and indicate the potential use of ripe plantain peel-derived products in aquaculture, although longer-term exposure studies are required to evaluate their safety more fully.

## 5. Conclusions

The ethanolic extract of ripe plantain peels produced measurable toxic effects in juvenile *Clarias gariepinus* during the 96-hour exposure period. Behavioural abnormalities, including air gulping, erratic swimming, respiratory distress, and vertical erection, increased with exposure concentration and duration, indicating physiological stress. Mortality also increased with concentration, and the 96-hour $LC_{50}$ was estimated at 35 mg/L. Changes in water-quality parameters, particularly dissolved oxygen, pH, and conductivity, suggest that the conditions of the test medium may have contributed to the observed responses. Histopathological findings showed that the liver was more responsive to exposure than the gills, with alterations including steatosis, inflammatory infiltration, cytoplasmic vacuolation, vascular congestion, and sinusoidal congestion. In contrast, gill architecture remained largely intact, with only mild localised alterations. Overall, the findings indicate that

ripe plantain peel extract has low-to-moderate acute toxic potential within the tested concentration range. However, studies conducted under controlled water-quality conditions, with longer exposure periods and feeding-based designs, are needed before its safe use in aquaculture can be recommended.

## 6. Limitation

This study was limited to a 96-hour acute exposure under static bioassay conditions without aeration, water renewal, or feeding. These conditions may have affected water quality and contributed to the behavioural and mortality responses. The $LC_{50}$ estimate was based on a limited concentration range and requires confirmation using a larger sample size and appropriate confidence intervals. The study assessed extract exposure rather than dietary inclusion; therefore, the findings cannot directly establish safe feed-inclusion levels. Histopathological observations were descriptive and were not supported by quantitative lesion scoring. Future studies should include chronic exposure, controlled water quality, growth performance, and nutritional-safety assessments.

## 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, and reference formatting. These AI-assisted tools were not used as authors and did not replace the intellectual contributions or scholarly judgement 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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