Research Article ISSN 2834-7854

Journal of Nutrition Food Science and Technology

Toxicological Effects of Ethanolic Seed Extract of Picralima nitida on Behavioural, Haematological and Histopathological Responses of Clarias gariepinus Juveniles

Idowu, A. A[1]., Towolawi, A. T[2]., Adesanya, E. O[1*]., Odukoya, A. E.[3], Enilari, S. O[1]., Okesanjo, O. A[1]., Omidele, E. O[1]., Oduntan, A. D[1]., Oladeyi, T. F[1]., Bolarinwa, A. A[2]., Olore, M. Y[1]., Adepitan, D. O[1]., Alimi, M. O[1]., and Akande, O. E[1]

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

[2]Department of Environmental Health Science, Fountain University, Osogbo, Osun State, Nigeria.

[3]Department of Aquaculture and Fisheries, University of Ilorin, Ilorin, Kwara State, Nigeria.

***Corresponding author**

Adesanya, E. O.,
Department of Aquaculture and Fisheries Management, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria.

Submitted: 16 Jul 2026; **Accepted**: 24 Jul 2026; **Published**: 14 Aug 2026

Citation: Idowu et al. (2026). Toxicological Effects of Ethanolic Seed Extract of Picralima nitida on Behavioural, Haematological and Histopathological Responses of Clarias gariepinus Juveniles..*J N food sci tech, 7*(3):1-8. DOI : https://doi.org/10.47485/2834-7854.1071

Abstract

A toxicity test was carried out on 156 juvenile African catfish after two weeks of acclimatisation. Fresh Picralima nitida seed kernels were crushed, extracted using 30% ethanol and 70% distilled water, and concentrated as a stock solution. The definitive static bioassay comprised a control and four extract concentrations (7.5, 10.0, 12.5 and 15.0 ml/10 L), each in triplicate. Behavioural responses, water quality, mortality, haematological, and histopathological alterations in the liver, kidney, and gills were assessed. The extract contained saponins, flavonoids, alkaloids, terpenoids, tannins, and glucosinolates; total flavonoids were 291.58 +/- 0.00 ppm, while saponins were 94.76 +/- 2.27 mg/g. Mortality was 0.00% in the control and 25.93%, 14.81%, 44.44%, and 48.15% at 7.5, 10.0, 12.5, and 15.0 ml/10 L, respectively. Probit analysis gave a 96-h LC50 of 16.66 ml/10 L and an estimated safe concentration of 1.67 ml/10 L. Exposed fish displayed erratic swimming, air gulping, hyperactivity or reduced activity, mucus secretion, spiralling, and loss of equilibrium. Dissolved oxygen declined markedly in treated tanks, especially at 48-72 h. Haematological indices varied across treatments, with lower PCV, Hb, RBC and WBC values at intermediate concentrations and partial recovery at 15.0 ml/10 L, although the analysed parameters were generally not significant at $p > 0.05$. Histopathology showed mild to moderate hepatic and renal lesions, whereas gill tissues showed no observable lesions under the scoring system used. The study indicates that P. nitida seed extract has toxicological potential in C. gariepinus and should be applied in aquaculture only after proper dose standardisation and safety evaluation.

Keywords: *Picralima nitida; Clarias gariepinus; acute toxicity; LC50; haematology; histopathology; dissolved oxygen; plant extract.*

Introduction

Aquaculture increasingly depends on plant-derived products as alternatives to synthetic chemotherapeutics, antibiotics, and chemical disinfectants. This interest is driven by the antimicrobial, antiparasitic, antioxidant, and immunostimulatory activities associated with plant secondary metabolites, as well as their perceived environmental compatibility (Awad & Awaad, 2017; Reverter et al., 2014; Dadras et al., 2023). However, the assumption that botanical products are intrinsically safe is not always valid. Plant extracts contain complex mixtures of alkaloids, saponins, flavonoids, tannins, terpenoids, and other metabolites that may be beneficial at low levels but toxic at higher concentrations or under prolonged exposure (Akinsanya et al., 2016; George et al., 2025).

Picralima nitida, commonly known as the akuamma plant, is a West African Apocynaceae species with established ethnomedicinal relevance. Its seeds are rich in indole alkaloids and other bioactive compounds associated with analgesic, antimicrobial, and antiplasmodial activities (Erharuyi et al., 2014; Kanu et al., 2024). These same constituents may become toxicological stressors when extracts enter aquatic systems through deliberate application, agricultural runoff, poor disposal, or exploratory use in fish health management. Toxicological evaluation is therefore essential before plant extracts are recommended for aquaculture use (Awodele et al., 2019).

Clarias gariepinus is an appropriate model for this assessment because it is economically important, widely cultured in Nigeria and frequently used in ecotoxicological studies. Although the species tolerates variable environmental conditions, it remains responsive to waterborne xenobiotics through behavioural, haematological and tissue-level biomarkers. Behavioural disturbances such as erratic swimming, surface gulping and loss of balance are early warning signs of toxic stress, while blood indices such as packed cell volume, haemoglobin concentration, red and white blood cell counts provide information on oxygen transport, immune condition and physiological disturbance (Iheanacho et al., 2019; Fazio, 2019; Witeska et al., 2023). Histopathological evaluation of liver, kidney and gill tissues further reveals organ-specific injury related to detoxification, excretion, osmoregulation and respiration (Hassan et al., 2023; Bernet et al., 1999).

This manuscript combines the haematological and histopathological components of the study to provide a more complete toxicological interpretation of ethanolic *P. nitida* seed extract in juvenile *C. gariepinus*. The specific objectives were to characterise the phytochemical profile of the extract, determine mortality and 96-h LC50, assess water quality and behavioural responses, evaluate haematological changes and describe histopathological alterations in target organs. This study examine the toxicity of *C. gariepinus*, using haematological and histopathological components to measure the effect of ethanolic seed extract of *P. nitida*.

Materials and Methods

Study Site and Experimental Fish

The experiment was conducted at the Federal University of Agriculture, Abeokuta (FUNAAB) Fish Farm, DUFARMS, Ogun State, Nigeria. Laboratory analyses were carried out in the Department of Aquaculture and Fisheries Management and the Veterinary Pathology Laboratory, FUNAAB. A total of 156 juvenile African catfish, C. gariepinus, with an average length of 12.2 cm, were sourced from a reputable hatchery and acclimated for two weeks under hatchery conditions. Fish were fed commercial feed during acclimation; water was changed routinely. Feeding was suspended 24 h before exposure to reduce metabolic interference.

Plant Collection and Extraction

Fresh fruits of *P. nitida* were obtained from Odeda market in Abeokuta, Ogun State, Nigeria. Seed kernels were removed, washed with distilled water, and crushed using a mortar and pestle. A 200 g portion of crushed kernels was divided into two 100 g fractions, and each fraction was soaked in 1 L of solvent mixture containing 30% absolute ethanol and 70% distilled water. The mixtures were covered with aluminium foil and left under laboratory conditions for four days, filtered through Muslin cloth and Whatman filter paper, and concentrated using a rotary evaporator. The crude extract was reconstituted in 500 mL distilled water to form the stock solution.

Experimental Design and Exposure

A preliminary range-finding test was conducted for 96 hr before the main experiment. A completely randomised design was adopted, having twelve 35L transparent plastic tanks filled with 10 L dechlorinated water, 4 fish were stocked in each plastic tank. Extracts of 5.0, 7.5, and 10.0 ml/10 L from the stock solution were infused daily into each plastic tank, except for the control tanks. Behavioral response of the fish was observed at 3 hr interval until the 96 hr of the test was completed. Any dead fish were removed immediately to prevent the water from getting contaminated.

The definitive static acute bioassay consisted of a control and four treatment concentrations, using a completely randomised design. Except for the control tanks, extracts of 7.5, 10.0, 12.5 and 15.0 ml/10 L of distilled water were prepared daily and diluted into the treatment tanks; 7 fish were stocked in each plastic tank. Each treatment was in triplicate. Fish were observed for mortality and behavioural responses, including swimming pattern, surface gulping, opercular movement, mucus secretion, spiralling, lethargy and loss of equilibrium. Dead fish were removed immediately to reduce water quality deterioration.

Water Quality, Haematology and Histopathology

Water temperature, electrical conductivity, total dissolved solids, pH and dissolved oxygen were measured at 24-h intervals using a Hanna Instruments HI98129 meter. At the end of exposure, blood was collected from anaesthetised fish through the caudal vein using heparinised syringes. Packed cell volume, haemoglobin concentration, red blood cell count, white blood cell count, erythrocyte indices and differential leukocyte counts were analysed using standard haematological procedures for fish health assessment (Fazio, 2019; Witeska et al., 2023).

For histopathology, liver, kidney and gill tissues were excised, rinsed in saline, fixed in 10% buffered formalin, processed through graded alcohols, cleared in xylene, embedded in paraffin wax, sectioned at approximately 4 microns and stained with haematoxylin and eosin. Tissue alterations were examined under a light microscope and scored using a semi-quantitative lesion system adapted from (Bernet et al., 1999): 0 = no observable lesion, 1 = mild lesion, 2 = moderate lesion and 3 = severe lesion.

Data Analysis

Data were expressed as mean +/- standard deviation where applicable. Treatment means were analysed using analysis of variance and Duncan multiple range test at $p < 0.05$. Mortality data were subjected to probit analysis, and log10 concentration was regressed against probit mortality to estimate the 96-h LC50. The estimated safe concentration was calculated as one-tenth of the LC50, consistent with conventional acute-to-safe concentration extrapolation in aquatic toxicity screening (Brodeur et al., 2018; Newman, 2020).

Results

Phytochemical Profile of Picralima Nitida Seed Extract

The extract contained saponins, flavonoids, alkaloids, terpenoids, tannins, and glucosinolates. Saponin concentration was 94.76 +/- 2.27 mg/g, while total flavonoids recorded 291.58 +/- 0.00 ppm, indicating a high phytochemical burden likely to contribute to biological activity. Alkaloids and terpenoids occurred at moderate levels, while tannins and glucosinolates were detected at lower levels.

Table 1: Qualitative and quantitative phytochemical screening of *Picralima nitida* seed extract.

Phytochemical	Qualitative abundance	Quantitative value
Saponin	+++	94.76 +/- 2.27 (mg/g)
Flavonoids (Total)	+++++	291.58 +/- 0.00 (ppm)
Alkaloids	+	2.70 +/- 0.07 (%)
Terpenoids	+	1.05 +/- 0.03 (%)
Tannin	+	1.61 +/- 0.04 (mg/g)
Glucosinolate (Sinigrin)	+	1.39 +/- 0.03 (mg/g)

High-performance liquid chromatography showed that phloretin was the dominant flavonoid, with a concentration of 260.67 ppm. Naringin was the next most abundant detected compound at 19.52 ppm, while catechin, myricetin, baicalin, and mangiferin occurred at lower concentrations.

Table 2: HPLC profile of flavonoid compounds detected in *Picralima nitida* seed extract.

Flavonoid compound	Retention time (min)	Concentration (ppm)
Catechin	3.142	2.70
Mangiferin	3.999	0.29
Naringin	4.371	19.52
Myricetin	5.949	5.34
Baicalin	6.569	3.06
Phloretin	13.472	260.67

Mortality response, LC50 and behavioural observations

No mortality occurred in the control group. Mortality among exposed fish was 25.93%, 14.81%, 44.44% and 48.15% at 7.5, 10.0, 12.5 and 15.0 ml/10 L, respectively. Although the 10.0 ml/10 L group showed a non-linear response, overall mortality was higher at the two highest concentrations. The 96-h LC50 was estimated as 16.66 ml/10 L, corresponding to a log10 concentration of 1.2216 and an estimated safe concentration of 1.67 ml/10 L.

Table 3: Mortality and probit response of juvenile *Clarias gariepinus* exposed to ethanolic seed extract of *Picralima nitida* over 96 h.

Treatment	Conc. (ml/10 L)	Log10 C	Stocked	Mortality	Mortality (%)	Probit
Control	0	-	27	0	0.00	-
T1	7.5	0.875	27	7	25.93	4.37
T2	10.0	1.000	27	4	14.81	3.99
T3	12.5	1.097	27	12	44.44	4.86
T4	15.0	1.176	27	13	48.15	4.96

Figure 1: Probit regression line showing the relationship between log10 concentration of *Picralima nitida* seed extract and probit mortality in juvenile *Clarias gariepinus*.

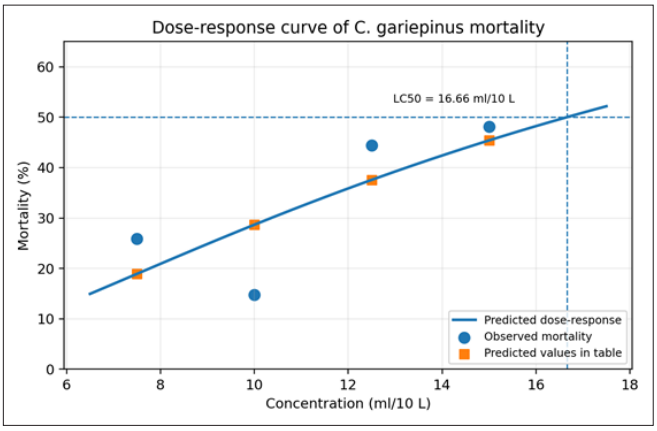

Figure 2: Dose-response curve showing observed and predicted percentage mortality of juvenile *Clarias gariepinus* exposed to ethanolic seed extract of *Picralima nitida*

Behavioural signs recorded in exposed fish included erratic swimming, hyperactivity, restlessness, surface air gulping, mucus secretion, reduced activity, spiralling and loss of equilibrium. These responses were more evident in the treated groups than in the control and became pronounced during periods when dissolved oxygen declined.

Water Quality Dynamics

Temperature remained within a relatively narrow range across exposure time, apart from a transient increase at 48 h. Dissolved oxygen was consistently lower in treated tanks than in the control, particularly at 48 and 72 h. At 72 h, mean DO values were 5.20 mg/L in the control, 2.57 mg/L at 7.5 ml/10 L, 2.53 mg/L at 10.0 ml/10 L, 2.90 mg/L at 12.5 ml/10 L and 2.53 mg/L at 15.0 ml/10 L. Electrical conductivity and total dissolved solids generally increased in treated media over time, while pH tended to decline at the higher concentrations, especially at 12.5 and 15.0 ml/10 L.

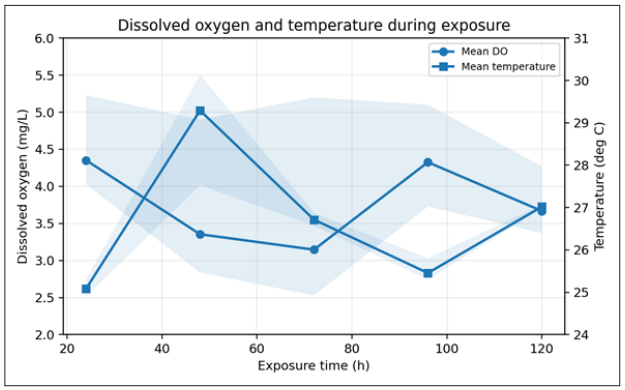

Figure 3: Mean dissolved oxygen and temperature trends across the 96-h exposure period. Shaded bands indicate the observed range across control and treatment groups at each time point.

Haematological Responses

Haematological parameters varied across treatment groups but were generally not statistically significant at $p < 0.05$. PCV, Hb, RBC and WBC values were lower at intermediate concentrations compared with the control, while the 15.0 ml/10 L group showed values close to or above control values for some indices. MCHC showed superscript differences in the descriptive table, but the reported p value remained above 0.05; therefore, it should be interpreted cautiously as a non-significant trend rather than a confirmed treatment effect.

Table 4: Haematological parameters of Clarias gariepinus after exposure to ethanolic seed extract of Picralima nitida.

Parameter	Control	T1 7.5 ml	T2 10 ml	T3 12.5 ml	T4 15 ml	p value
PCV (%)	37.33 +/- 3.51a	34.00 +/- 3.00a	30.67 +/- 2.52a	31.00 +/- 8.54a	39.67 +/- 7.51a	0.2799
Hb (g/dl)	12.67 +/- 1.16a	11.34 +/- 1.11a	10.10 +/- 0.89a	10.40 +/- 2.72a	13.30 +/- 2.65a	0.2401
RBC (x10^12/L)	3.03 +/- 0.29a	2.77 +/- 0.23a	2.60 +/- 0.20a	2.67 +/- 0.59a	3.17 +/- 0.59a	0.4386
WBC (x10^9/L)	16.10 +/- 1.45a	14.67 +/- 0.80a	13.60 +/- 0.68a	13.03 +/- 2.54a	16.20 +/- 2.10a	0.1468
MCV (fl)	123.23 +/- 6.67a	122.94 +/- 5.26a	118.10 +/- 8.10a	115.38 +/- 9.03a	125.40 +/- 6.84a	0.4730
MCH (pg)	41.82 +/- 2.40a	41.07 +/- 1.57a	38.87 +/- 2.35a	38.78 +/- 2.45a	41.00 +/- 2.41a	0.2863
MCHC (g/dl)	33.93 +/- 0.17b	33.41 +/- 0.47ab	32.95 +/- 0.55a	33.64 +/- 0.51ab	33.49 +/- 0.35ab	0.1471

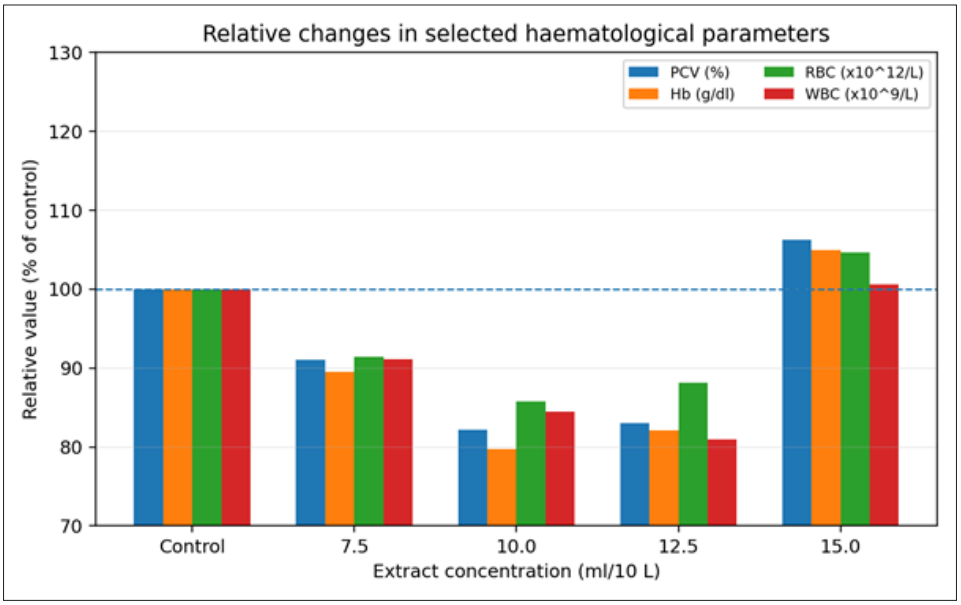

Figure 4: Relative changes in selected haematological parameters of *Clarias gariepinus* exposed to ethanolic seed extract of *Picralima nitida*. Values are expressed as a percentage of the control mean.

Histopathological Alterations

Semi-quantitative histopathological assessment indicated mild-to-moderate organ-specific alterations. Liver lesion scores increased from 0.67 in the control to 1.33 at 15.0 ml/10 L, suggesting a mild concentration-related hepatic response. Kidney scores were variable and did not show a strict concentration-dependent pattern; however, renal lesions were recorded across groups. Gill scores remained 0.00 in all groups, indicating no observable gill lesion under the scoring system and exposure duration used.

Table 5: Semi-quantitative histopathological lesion scores in liver, kidney and gill tissues of Clarias gariepinus exposed to Picralima nitida seed extract.

Treatment	Liver score	Kidney score	Gill score
Control (0 ml)	0.67 +/- 1.15	1.67 +/- 0.58	0.00 +/- 0.00
T1 (7.5 ml)	0.67 +/- 1.15	0.33 +/- 0.58	0.00 +/- 0.00
T2 (10.0 ml)	0.67 +/- 0.58	0.33 +/- 0.58	0.00 +/- 0.00
T3 (12.5 ml)	1.00 +/- 1.00	1.00 +/- 0.00	0.00 +/- 0.00
T4 (15.0 ml)	1.33 +/- 1.15	0.67 +/- 0.58	0.00 +/- 0.00

Across the photomicrograph plate, panels A-E represent liver sections, panels F-J represent kidney sections, and panels K-O represent gill sections. The panels are arranged from left to right by concentration: A/F/K = control (0 mL/10 L), B/G/L = 7.5 mL/10 L, C/H/M = 10.0 mL/10 L, D/I/N = 12.5 mL/10 L, and E/J/O = 15.0 mL/10 L. In the liver row, the control section (A) showed no observable lesion in the representative field, while the 7.5 mL/10 L and 10.0 mL/10 L treatments (B and C) showed mild to moderate hepatic degeneration in selected replicates. At 12.5 mL/10 L (D), centrilobular hepatic degeneration with hepatocyte vacuolation around the central vein was evident, and at 15.0 mL/10 L (E), moderate hepatic degeneration persisted. This pattern supports the lesion-score trend, in which hepatic scores increased at the two highest concentrations. In the kidney row, tubular degeneration was evident in the control representative field (F), indicating background renal change that should not be attributed solely to the extract. The 7.5 mL/10 L and 10.0 mL/10 L groups (G and H) showed mild focal tubular degeneration, while the 12.5 mL/10 L group (I) showed the most consistent renal involvement across replicates. The 15.0 mL/10 L group (J) showed mild/localised tubular degeneration, confirming renal involvement but without a strict dose-response pattern. In the gill row, all panels (K-O) retained normal lamellar organisation, with no observable lamellar fusion, epithelial lifting, hyperplasia, oedema, aneurysm or inflammatory infiltration. The gill findings indicate that the observed air gulping and respiratory stress were more likely functional responses to reduced dissolved oxygen than consequences of structural gill injury within the exposure period.

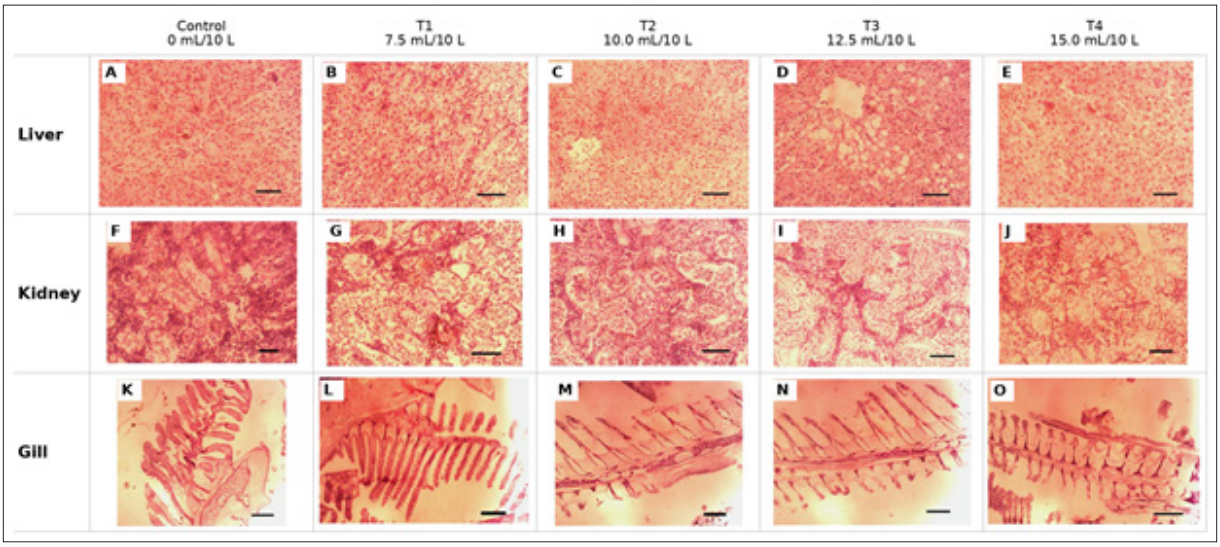

Figure 6: Representative photomicrograph plate of liver (A-E), kidney (F-J) and gill (K-O) sections of Clarias gariepinus exposed to Picralima nitida seed extract for 96 h. A/F/K = control; B/G/L = 7.5 mL/10 L; C/H/M = 10.0 mL/10 L; D/I/N = 12.5 mL/10 L; E/J/O = 15.0 mL/10 L. H&E stain; liver and kidney scale bars = 20 µm; gill scale bars = 100 µm.

Discussion

The present findings show that ethanolic seed extract of *P. nitida* produced behavioural, mortality, haematological and histopathological responses in juvenile *C. gariepinus*. The phytochemical profile provides a plausible basis for these effects. Saponins, flavonoids and alkaloids were prominent in the extract, and similar bioactive compounds have been associated with both therapeutic and toxic effects in fish depending on concentration, exposure duration and delivery route (Reverter et al., 2014; Dadras et al., 2023). The dominance of phloretin within the flavonoid fraction indicates that the extract was chemically uneven, with one major flavonoid accounting for most of the measured flavonoid content.

The observed behavioural abnormalities are consistent with reports that behavioural endpoints are sensitive early-warning indicators of toxic stress in fish (Adesanya et al., 2026). Erratic swimming, surface air gulping, reduced activity and loss of equilibrium may reflect irritation, neurophysiological disturbance and respiratory stress caused by waterborne phytochemicals (Iheanacho et al., 2019; Ismail et al., 2023). Similar behavioural disturbances were reported in *C.*

gariepinus exposed to *Annona muricata ethanolic* leaf extract, where mucus secretion, air gulping, respiratory distress and erratic swimming accompanied mortality and tissue injury (George et al., 2025).

The mortality results indicate acute toxic potential, although the response was not fully monotonic because the 10.0 ml/10 L treatment recorded lower mortality than 7.5 ml/10 L. Non-monotonicity may arise from biological variability, stress tolerance differences among fish, handling effects, or small sample variation. Nevertheless, the two highest concentrations produced the highest mortality, and the probit-derived LC50 of 16.66 ml/10 L suggests that the extract can become hazardous as exposure approaches and exceeds the tested range. This aligns with the work of Brodeur et al. (2018) and Newman (2020), who further mentioned that dose-response modelling is central in aquatic toxicology because it links exposure concentration to biological effect and supports estimation of hazard thresholds.

Water quality trends likely intensified the toxic stress response. Dissolved oxygen declined sharply in exposed tanks at 48-72 h, and this period corresponded with behavioural signs such as surface gulping and reduced activity. The oxygen decline may be related to extract-induced changes in water chemistry, increased microbial oxygen demand, or surfactant activity associated with saponins. However, temperature remained generally stable, suggesting that the behavioural and mortality responses were not primarily caused by thermal stress. Similar water quality shifts during exposure to plant extracts have been linked with stress amplification in fish toxicology studies by George et al. (2025).

Haematological changes were evident but mostly non-significant. The decreases in PCV, Hb and RBC at intermediate concentrations may suggest transient impairment of oxygen-carrying capacity or erythrocyte stability, while changes in WBC may reflect immune modulation or stress-mediated leukocyte redistribution. Previous studies by Amaeze et al. (2020) have shown that toxicant exposure can reduce haemoglobin, RBC and PCV in *C. gariepinus* and alter leukocyte profiles as part of systemic stress responses. Because the present haematological trends did not meet the $p < 0.05$ threshold, they should be discussed as biologically relevant patterns requiring confirmation through larger sample size, longer exposure or complementary biochemical biomarkers (Abdel Rahman et al., 2023; Witeska et al., 2023).

Histopathological findings showed mild to moderate hepatic and renal alterations, while the gills showed no observable lesions. The liver sections revealed that the control plate had mostly normal hepatic architecture, whereas the treated plates showed varying degrees of hepatic degeneration. At 7.5 mL/10 L, moderate liver degeneration was noted in one replicate, indicating that at the lowest concentration tested, liver cells were subjected to some degree of stress in some fish. The hepatic response was not completely linear at this concentration since the lesions were milder at 10.0 mL/10 L. This is in contrast with the submission of Adesanya et al. (2025), who reported an absolute linear impact of ethanolic leaf extract of Senna occidentalis on the liver of African catfish. At the same time, the liver exhibited a more prominent centrilobular hepatic degeneration with vacuolated hepatocytes around the central vein in the 12.5 mL/10 L group, whereas the 15.0 mL/10 L group exhibited moderate hepatic degeneration. This general trend of increase in liver lesion score from the control to the maximum concentration implies that the liver was the most responsive organ to the exposure of *P. nitida*.

The vacuolation and hepatic degeneration seen in treated fish could be due to the metabolic stress caused by the phytochemicals (alkaloids, saponins, flavonoids, and tannins) present in the extract. In fishes, the liver is the principal organ involved in xenobiotic metabolism, and hepatocellular swelling, vacuolation, degeneration, sinusoidal changes, and necrosis frequently occur as a result of exposure to toxicants when the detoxification capacity of the liver tissue is exceeded. Plant-derived toxicants cause comparable alterations in the liver of Clarias gariepinus. Hepatic vacuolation, degeneration, and tissue disorganization were reported in catfish exposed to Melaleuca cajuputi extract (Hassan et al., 2023) and liver damage in *C. gariepinus* juveniles exposed to an acute concentration of Anogeissus leiocarpus (Audu et al., 2021).

George et al. (2025) also noted hepatic tissue injury in *C. gariepinus* exposed to ethanolic leaf extract of Annona muricata. These results are similar to the present study, which indicates that high concentrations of medicinal plant extract may be detrimental to the liver of fish, although it may be beneficial at controlled low concentrations.

The greater liver responses at 12.5 and 15.0 mL/10 L also confirm the generally accepted principle of increasing tissue damage with increasing toxicant concentration in fish. The lesion scores and general trend of the plate scores show that the higher the concentration, the more stressful to the liver, although this was relatively mild in this treatment group of 10.0 mL/10 L. The non-linear response at 10.0 mL/10 L could be a result of inter-fish variation, physiological tolerance, or a non-uniform distribution of lesions in tissue sections or adaptive detoxification responses during the brief period of exposure. More complex, non-linear responses of biological systems have been seen in toxicity testing of fish, where the concentration-response relationship is not exact because of variation in sampling and stress tolerance and differences in fish metabolism.

Tubular degeneration was also seen in the kidney sections in some treatment groups; however, the response was not as concentration-dependent as that seen in the liver. Kidney lesion score in the control group was relatively high, suggesting that some kidney changes could have resulted from background stress, handling stress, artefacts from fixation rather than from exposure to the extract itself, or from individual fish variation. The tubular degeneration observed in the treated groups, however, indicated involvement of the kidneys in the toxic

response. Mild tubular degeneration was seen in one replicate each at the 7.5 and 10.0 mL/10 L treatments and in all three at the 12.5 mL/10 L treatment. The lesion was still mild and local at 15.0 mL/10 L. This suggests that the kidney reacted to the extract, albeit in a variable manner, not necessarily in a dose-dependent manner. The physiological imbalances during toxicant exposure may be caused by the damage to the renal tubules. The renal changes observed during this study were similar to those found by Hassan et al. (2023), who found lesions in the kidney of *C. gariepinus* when exposed to Melaleuca cajuputi extract. In the same way, Abdel Rahman et al. (2023) observed the histopathological changes in *C. gariepinus* exposed to imidacloprid, indicating the kidney as a sensitive target organ during toxic stress in African catfish. The similarity in the renal response suggests that kidney tissue is susceptible to both synthetic and natural xenobiotics when exposure levels are above the physiological tolerance of the fish.

Significantly, no lesions could be seen on the gills in any of the treatment groups. No lamellar fusion, epithelial lifting, hyperplasia, aneurysm, oedema or inflammatory cell infiltration were observed in the gill sections in the control group 7.5, 10.0, 12.5 and 15.0 mL/10 L. This indicates that the extract was not causing a discernible change in the structure of gill tissue during the exposure period. This is contrary to several studies that show plant extracts caused gill lesions in fish. For instance, gill alteration was reported in African catfish exposed to Melaleuca cajuputi extract by Hassan et al. (2023), while gill damage was reported in *C. gariepinus* exposed to Anogeissus leiocarpus extract by Audu et al. (2021). George et al. (2025) also found gill and liver injuries in African catfish treated with Annona muricata ethanolic leaf extract.

The absence of gill lesions in the present study can be attributed to the adaptive capacity of *C. gariepinus*. African catfish have accessory structures for breathing air and can withstand episodic respiratory stress longer than a number of other freshwater fish species. Thus, behavioural indicators, like surface gulping and air breathing, may have been indicative of functional respiratory strain, not necessarily the gill-visible damage. Although the gill tissue was structurally normal, a reduction of dissolved oxygen that was recorded in the treated tanks may account for the surface gulping and restlessness that was observed during the exposure periods. The exposed fish may have suffered respiratory distress primarily due to water quality stress and physiological oxygen demand instead of direct histolysis of the gill lamellae.

The discrepancy of the present study with the previous reports can also be attributed to differences in the plant species, parts of the plant used, solvents, concentration of phytochemicals, and exposure design. Awodele et al. (2019) reported the toxicological potential of *P. nitida* in experimental animals, which confirms that the bioactive components of *P. nitida* have the potential to affect internal organs when absorbed in the body. The current results lend further support to this apprehension, since it is observed that exposure to *P. nitida* causes a response in the liver and kidney of *C. gariepinus*.

Conclusion

In general, the histopathology findings indicate that ethanolic seed extract of *P. nitida* causes mild to moderate stress on various organs of *C. gariepinus*, with the liver being the most conspicuous and evident, followed by the kidney showing variable tubular degeneration and gills, which exhibited no detectable histopathology lesions. This suggests a systemic toxic effect of the extract over the time of exposure rather than branchial. The results are in accordance with other studies that demonstrated that treatment of medicinal plant extracts at high or uncontrolled concentrations caused histopathological changes in fish. While it is considered that *P. nitida* can be a source for drugs, its application in aquaculture should be done carefully and with careful consideration of the dose, the duration of application, and careful monitoring of the water quality.

More so, the integrated haematological and histopathological profile supports a precautionary interpretation: *P. nitida* seed extract has useful bioactive potential but can pose toxicological risks to *C. gariepinus* under uncontrolled exposure. Its possible aquaculture use should therefore be limited to scientifically validated concentrations, with attention to extraction method, active compound standardisation, water quality monitoring and chronic safety testing.

Ethanolic seed extract of *P. nitida* produced measurable toxicological effects in juvenile *C. gariepinus*. The extract contained high levels of flavonoids and saponins and induced behavioural abnormalities, mortality, reduced dissolved oxygen in exposed media, haematological variation and mild to moderate hepatic and renal lesions. The 96-h LC50 was 16.66 ml/10 L, and the estimated safe concentration was 1.67 ml/10 L. Although haematological changes were mostly not statistically significant, the combined mortality, behavioural and histopathological evidence indicates that the extract should not be introduced indiscriminately into aquaculture systems.

Recommendations

- The use of *P. nitida* seed extract in fish culture should be carefully regulated and kept well below experimentally determined toxic thresholds.
- Further chronic and sub-chronic studies should be conducted to evaluate long-term effects on growth, immunity, reproduction, oxidative stress and organ function.
- Future studies should isolate and quantify the active alkaloids, saponins and flavonoids responsible for toxicity and compare aqueous, ethanolic and hydro-ethanolic extraction systems.
- Routine water quality monitoring, especially dissolved oxygen, should accompany any experimental or practical exposure of fish to botanical extracts.
- Larger sample sizes and additional biomarkers such as antioxidant enzymes, liver enzymes and serum biochemical indices are recommended to strengthen interpretation of haematological trends.

References

1. Awad, E., & Awaad, A. (2017). Role of medicinal plants on growth performance and immune status in fish. *Fish and Shellfish Immunology,* 67, 40-54. DOI: https://doi.org/10.1016/j.fsi.2017.05.034
2. Reverter, M., Bontemps, N., Lecchini, D., Banaigs, B., & Sasal, P. (2014). Use of plant extracts in fish aquaculture as an alternative to chemotherapy. *Aquaculture*, 433, 50-61. DOI: https://doi.org/10.1016/j.aquaculture.2014.05.048
3. Dadras, F., Velisek, J., & Zuskova, E. (2023). An update about beneficial effects of medicinal plants in aquaculture: A review. *Veterinarni Medicina, 68*(12), 96-108. DOI: https://doi.org/10.17221/96/2023-VETMED
4. Akinsanya, B., Utoh, O. U., & Ukwa, U. D. (2016). Toxicological, phytochemical and anthelminthic properties of rich plant extracts on Clarias gariepinus. *Journal of Basic and Applied Zoology,* 74, 75-86. DOI: https://doi.org/10.1016/j.jobaz.2016.09.003
5. George, U. U., Udoeyop, F. A., Abiaobo, N. O., & Eteng, A. O. (2025). Impact of Annona muricata ethanolic leaves extract on behavioural responses and histopathology of the gills and liver of African catfish (Claria gariepinus, Burchell 1822) fingerlings. *International Journal of Biochemistry Research & Review, 34*(5), 199-214. DOI: https://doi.org/10.9734/ijbcrr/2025/v34i51050
6. Erharuyi, O., Falodun, A., & Langer, P. (2014). Medicinal uses, phytochemistry and pharmacology of Picralima nitida in tropical diseases: A review. *Asian Pacific Journal of Tropical Medicine, 7*(1), 1-8. DOI: https://doi.org/10.1016/S1995-7645(13)60182-0
7. Kanu, S. N., Okoriowu, L. I., Ikaraoha, C. I., & Nnodim, J. (2024). Evaluation of the qualitative phytochemical composition of Picralima nitida seeds, leaves and bark. *International Journal of Case Reports and Case Research*.
8. Awodele, O., Coulidiaty, A. G. V., Afolayan, G. O., Agagu, S., Omoseyindemi, B., & Busia, K. (2019). Toxicological evaluation of Picralima nitida in rodents. *Journal of Ethnopharmacology*, 236, 205-219. DOI: https://doi.org/10.1016/j.jep.2019.03.008
9. Iheanacho, S. C., Okoro, N., Nwakpa, J., and Eze, K. (2019). Effects of Chromolaena odorata leaf extract on behaviour and haematology of Clarias gariepinus juveniles. *African Journal of Aquatic Science, 44*(4), 421-427. DOI: https://doi.org/10.2989/16085914.2019.1661823
10. Fazio, F. (2019). Fish hematology analysis as an important tool of aquaculture: A review. *Aquaculture*, 500, 237-242. DOI: https://doi.org/10.1016/j.aquaculture.2018.10.030
11. Witeska, M., Kondera, E., & Bojarski, B. (2023). Hematological and hematopoietic analysis in fish toxicology - A review. *Animals, 13*(16), 2625. DOI: https://doi.org/10.3390/ani13162625
12. Hassan, M., Melad, A. A. N., Zakariah, M. I., and Yusoff, N. A. H. (2023). Histopathological alterations in gills, liver and kidney of African catfish (Clarias gariepinus, Burchell 1822) exposed to Melaleuca cajuputi extract. *Tropical Life Sciences Research, 34*(2), 177-196. DOI: https://doi.org/10.21315/tlsr2023.34.2.9
13. Bernet, D., Schmidt, H., Meier, W., Burkhardt-Holm, P., & Wahli, T. (1999). Histopathology in fish: Proposal for a protocol to assess aquatic pollution. *Journal of Fish Diseases, 22*(1), 25-34. DOI: https://doi.org/10.1046/j.1365-2761.1999.00134.x
14. Brodeur, J. C., Di Giulio, R. T., & Bell, R. A. (2018). Dose-response relationships in aquatic toxicology: Principles and applications. *Aquatic Toxicology*, 199, 1-10.
15. Newman, M. C. (2020). Fundamentals of Ecotoxicology: The Science of Pollution Effects on Organisms in Ecosystems (5th edition.). CRC Press.
16. Adesanya, O. E., Idowu, A. A., Adekola, M. B., Towolawi, A. T., & Odukoya, A. E. (2026). Haematological parameters and behavioural responses of Clarias gariepinus exposed to sub-acute Senna occidentalis ethanol leaf extract. *International Journal of Aquaculture, 16*(3), 149-155. DOI: https://doi.org/10.5376/ija.2026.16.0012
17. Ismail, T. A., Adeyemi, A. O., & Musa, K. (2023). Behavioural biomarkers in fish exposed to plant-based toxicants. *Aquatic Toxicology,* 258, 106526.
18. Amaeze, N. H., Komolafe, B. O., Salako, A. F., Akagha, K. K., Briggs, T. M. D., Olatinwo, O. O., & Femi, M. A. (2020). Comparative assessment of the acute toxicity, haematological and genotoxic effects of ten commonly used pesticides on the African catfish, Clarias gariepinus Burchell 1822. *Heliyon, 6*(8), e04768. DOI: https://doi.org/10.1016/j.heliyon.2020.e04768
19. Abdel Rahman, A. N., Abdel Mageed, M. A., Assayed, M. E. M., Gharib, H. S. A.-R., Nasr, M. A., Elshopakey, G. E., Abdel Moniem, H., Shahin, S. E., EL Husseiny, E., and Ahmed, S. A. A. (2023). Imidacloprid-induced growth, hematological, neuro-behavioral, antioxidant, economic, genetic, and histopathological alterations in Clarias gariepinus: Alleviative role of dietary Hyphaene thebaica. Aquaculture, 564,739058. DOI: https://doi.org/10.1016/j.aquaculture.2022.739058
20. Adesanya, E. O., Idowu, A. A., Alegbeleye, W. O., Adekola, M. B., Towolawi, A. T., & Ibrahim F. O. (2025). Possible mortality and nephrosis in Clarias gariepinus (Burchell, 1822) exposed to ethanol leaf extracts of Senna occidentalis (L.). *Nigerian Journal of Fisheries, 22*(1), https://fisonjournal.org.ng/index.php/njf/article/view/361/354
21. Audu, B. S., Onyia, L. U., & Shunom, S. (2021). Histopathological changes in gills and liver of Clarias gariepinus fingerlings exposed to acute concentrations of dry cell battery. *Sokoto Journal of Veterinary Sciences, 19*(1), 26-33. DOI: https://doi.org/10.4314/sokjvs.v19i1.4