Toxicological and Histopathological Effects of *Parkia biglobosa* Leaf Powder on *Clarias gariepinus* Juveniles: Implications for Aquatic Ecosystem Health

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ABSTRACT

This study seeks to determine the Toxicological and histopathological Effects of Parkiabiglobosa Leaf Powder on Clariasgariepinus Juveniles and physicochemical parameters of the cultured water. The fish were exposed to five (5) different concentrations of PLP at 0g/l (control), 15g/l, 30g/l, 45g/l and 60g/l with three replicates, with each containing ten (10) fish sample. One way analysis of variance was used to compare the mean of the different treatments, the experiment employed a completely randomized designed. The histological examination of the liver was conducted after 96 hours using standard histological technique. Behavioural changes such as jumping, abnormal vertical orientation, air gulping, loss of buoyancy, loss of reflex and discoloration increased with increased concentration of PLP. They was a decrease in dissolved oxygen (DO) with increased concentration of Parkiabiglobosa leaf powder (PLP). The DO for the control was highest at 6.03±0.01, while the 45g and 60g of PLP has the lowest DO of 2.86±0.09 respectively. There was a decrease in Potential of Hydrogen (pH) of the cultured water with increasing concentration of PLP, the control showed the highest pH value of 7.16±0.01, followed by 15g at 6.90±0.03, while the lowest pH was recorded for 60g at 6.38±0.02. The Total Dissolved Solids (TDS) was highest at the 60g concentration of PLP (413.00±6.43). The mortality rate was higher with increasing concentration of PLP, with 100% mortality within 24 hours at the 45g and 60g. The mean toxicity level was 4.00. The L_C50 for the 96 hours was 27.55g/L. The Biometric parameters of the varying concentration PLP throughout the 96hrs bioassay show no significant difference (p>0.05) for all parameters. Histological result of the liver of the fish exposed to PLP showed severe cellular generation, pyknotic nucleus and cellular necrosis with increased concentration of PLP, normal liver architecture was observed in the control. The PLP has negative effects on water quality, fish liver histology and on survival rate of the fish. Therefore, fish farmers should not use these plant materials as piscicide in fish harvesting in the natural water bodies as it will decrease fish populations and affect water quality which can lead to serious ecological damages.

Keyword: *Parkiabiglobosa, Clariasgarienpinus, Piscicides, Juveniles, Water quality*

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INTRODUCTION

Fish, amphibians, invertebrates, plants, and microorganisms are all part of the complex networks of biodiversity that make up aquatic ecosystems (Qayoom*et al.,* 2024). These systems, which act as food and oxygen sources, habitats, and zones for the cycling of nutrients, are crucial for preserving ecological balance. However, their stability and functionality are seriously threatened by pesticides (Saba *et al*., 2025). Aquatic ecosystem contamination is a major global concern. The aquatic environment and related living things are negatively impacted by both natural and man-made activities. Numerous contaminants, such as pesticides (Ghafarifarsani*et al*., 2023 ;Rohani, 2023), have caused a great deal of complexity in aquatic communities (fauna and flora) and presented a major risk (Ghafarifarsani*et al*., 2024). The sustainability of aquaculture and wild fish populations was endangered by human activities, such as the careless application of plant-based piscicides. Because plant piscicides were more readily available, biodegradable, and environmentally friendly than synthetic chemicals. Artisanal fishermen in rural Africa had long used ichthyotoxic plants for fishing. Bioactive phytochemicals like alkaloids, tannins, saponins, and anthraquinones that disrupted fish respiratory, circulatory, and neurological systems were mainly responsible for these plants' piscicidal action. Adeniumobesum, Anogeissusleiocarpus, and Datura innoxia are among the plant extracts that have been shown to cause fish mortality and sub-lethal physiological disruptions (Arikpo*et al*,2025**;** Sayyed*et al*., 2025). Piscicide causes oxidative stress, neurotoxicity, endocrine disruption, and immune suppression, among other harmful effects on fish. Fish populations eventually experience decreased growth, reproductive failure, and increased mortality as a result of these effects, which are mediated by intricate biochemical pathways. Plant compounds can cause neuromuscular failure and death by inhibiting acetyl cholinesterase and disrupting mitochondrial function. A good piscicide is one that kills only the target at a relatively low dose without harming other organisms, it has no negative effects on fish ponds, quickly nullifies in water and readily available. (Aman*et al*., 2023).*Clariasgariepinus*, also known as African cat fish, is a fish of considerable ecological significance and commercial importance in Nigeria fishing sector, because of its hardness and ease of maintenance in lab settings, sensitivity to rapid changes in aquatic environment, and connection to humans through the food chain, it has proven to be an appropriate bioindocator species for use in ecoxitological studies involving synthentic chemicals and plant extracts. This study will help in the management of fish health in aquaculture and possible ecological risks inland water bodies. (Kachi*et al*., 2024)

MATERIALS AND METHODS

Plant Collection and Identification

The leaf of *Parkiabiglobosa* were obtained around Ankuri Hospital Phase 2, Lokongoma ($7^0$47'58' N $6^0$42'30'E), Lokoja, Kogi State. The leaves were carefully collected manually using sickle and cutlass respectively. The collected samples were placed in a sterile polythene bag and transported to the Herbarium Unit of the Department of Biological Sciences at Federal University Lokoja, where the leaves of *Parkiabiglobosa were* identified by botanists.

Preparation of Samples

The leaf of *Parkiabiglobosa* was rinsed with running water to remove any particles or debris. They were then air-dried at room temperature for two weeks. After drying, the samples were blended into fine powder using an electronic blender. The powder was placed in an oven to reduce the moisture content, which was then stored in a desiccator until needed for the experiment (10).

Fish Collection

A total number of Seven Hundred (700) healthy juveniles of African catfish (*Clariasgariepinus)* with a mean length of (10:00 ± 1.5cm) and mean weight of (9.5:00 ±1.84g) were purchased from Evergreen Agro Hub, located beside Moremi Junction, Lokoja, Kogi State, Nigeria. They were transported to the Biological Garden of Federal University Lokoja, Adankolo Campus and placed in holding tank, where they were acclimatized for two weeks in a plastic tank with a capacity of 5000 liters of water.

Range Finding Test

A Range Finding Test was conducted to determine the dose concentration for the acute toxicity test, prior to the main experiment. This preliminary test involved concentrations of 0g/10L, 20g/10L, 40g/10L, 60g/10L, 80g/10L and 100g/10L of the leaf powder of *Parkiabiglobosa* with each concentrate tested in triplicate (11). Each concentration contained ten (10) juveniles of African catfish *(Clariasgariepinus*) placed in a 10-litres capacity translucent rectangular aquarium. The container was covered with a net to prevent the fish from jumping out.

Experimental Design

The experimental design was completely Randomized Design. One Hundred and Fifty (150) juveniles of African catfish *Clariasgariepinus*from the same fwere used. The set-up was made up of five treatments of three replicates

Table 1: Behavioral Changes of Fish Exposed to Different Concentrations of *Parkiabiglobosa*Leaves Powder

Exposure Time	Conc. (g/10L)	Erratic Swimming	Jumping	Abnormal Vertical Orientation	Air Gulping	Loss of Buoyancy	Loss of Reflex	Discoloration
24 hr	0	–	–	–	–	–	–	–
	15	+	+	+	+	+	–	+
	30	++	++	++	++	++	+	+
	45	+++	+++	+++	+++	+++	+	+
	60	+++	+++	+++	+++	+++	++	+
48 hr	0	–	–	–	–	–	–	–
	15	–	–	–	–	–	–	–
	30	+	+	+	+	+	+	–
72 hr	0	–	–	–	–	–	–	–
	15	–	–	–	–	–	–	–
	30	+	+	+	+	+	+	–
96 hr	0	–	–	–	–	–	–	–
	15	–	–	–	–	–	–	–
	30	–	–	–	–	–	–	–

Key: – (no significant), + (mild), ++ (severe), +++ (very severe)

each. Five different concentrations of 0g/l (control), 15g/l, 30mg/l, 45g/l and 60 g/l were prepared in triplicate with each concentration containing ten (10) fish samples for the bioassay. The experiment was conducted static–renewal conditions. Fish were fed two times with commercial food I was stopped 24 hours before the commencement of the experiment .

Behavioral Changes

The behavioral response of *Clariasgariepinus*such as discoloration, loss of buoyancy control, unusual surface behavioral, abnormal vertical orientation, loss of reflex, gulping of air and erratic swimming of the fish during the acute toxicity test were monitored over a period of 24 hours to 96 hours following exposure to different concentrations of the leaves powder.

Physicochemical Parameters of the Test Water

The physicochemical parameters of the different test water were monitored during the experiment. These parameters include potential of hydrogen (pH), dissolved oxygen, total dissolved solids and temperature.

Collection of Liver and Skin for Histopathology

The histology examination was conducted with the remaining fish after 96 hours using a standard histology technique (Copper *et al*., 2018). The juveniles *Clariasgariepinus*were randomly selected, with each per replicate representing concentration of 0g, 15g and 30g. The fish samples were euthanized through cervical sections.

The livers were carefully dissected and were placed in small plastic bottles filled with 10% neutral buffered formalin to preserve the quality of the liver sample until they were transported to the laboratory. The fixed tissues were subjected to dehydration in ascending alcohol concentrations and were subsequently embedded in paraffin and sectioned at 15µm thickness. The section of liver was then stained with hematoxylin and eosin (HE), thereafter; the slides were viewed under light microscope (x 40).

Statistical Analysis

Data generated from the physicochemical parameters, condition factors and hepatosomatic indexed were subjected to normality and homogeneity test, data was analyzed using Analysis of Variance (one way ANOVA) to determine the significant difference, Duncan multiple range test was used to separate the means. The analysis was performed using SPSS version 22 software.

RESULTS

Behavioral Changes of Fish Exposed to Different Concentrations of *Parkiabiglobosa*Leaves Powder

The behavioral changes of the fishes exposed to leaf powder concentrates are presented in (Table1). The significant changes that were observed in their behavior include erratic swimming, gulping of air, abnormal vertical orientation, jumping, loss of reflex, loss of buoyancy and discoloration. These reactions increase with increased concentration but decreased with exposure period.

Physicochemical Parameters

The results of the physicochemical parameters test of the different concentrations of the leaf powder of *Parkia*

Table 2: Mean of Physicochemical Parameters of the Different Concentrations of *Parkiabiglobosa* Leaf Powder during the 96 hours Acute Toxicity of Bioassay

Concentration (g/10L)	Nitrite (mg/L)	Phosphate (mg/L)	pH	Temperature (°C)	Conductivity (µs/cm)	Total Dissolved Solids (ppm)	Dissolved Oxygen (mg/L)
0	0.13 ±0.00^{c}	0.06 ± 0.01	7.16 ±0.01^{a}	22.03 ± 0.03^{b}	566.00 ± 11.37^{a}	357.33 ± 3.71^{bc}	6.03 ± 0.01^{a}
15	0.03 ± 0.02^{c}	0.06 ± 0.01	6.90 ±0.03^{b}	21.96 ± 0.09^{b}	675.67 ± 1.33^{b}	332.67 ± 3.76^{b}	6.03 ± 0.01^{a}
30	0.14 ± 0.02^{b}	0.10 ± 0.05	6.76 ±0.17^{c}	22.13 ± 0.13^{b}	725.33 ± 1.76^{c}	360.00 ± 29.51^{bc}	4.90 ± 0.06^{b}
45	0.18 ± 0.01^{ab}	0.12 ± 0.16	6.43 ±0.04^{d}	22.03 ± 0.03^{b}	818.00 ± 5.03^{d}	360.00 ± 1.53^{ab}	2.86 ± 0.09^{c}
60	0.22 ± 0.00^{a}	0.05 ± 0.01	6.38 ±0.02^{d}	22.80 ± 0.06^{a}	777.00 ± 4.73^{e}	413.00 ± 6.43^{a}	2.86 ± 0.15^{d}
P-Value	**0.003**	**0.604**	**0.000**	**0.000**	**0.000**	**0.015**	**0.000**

*Means with the same alphabet along the vertical axis are not different significantly at p<0.05

Table 3: Cumulative Mortality of *Clarias gariepinus* Exposed to various Concentration of Leaf Powder of *Parkiabiglobosa*

Concentration (g/L)	Total Fish Sample	24 Hours MRT	48 Hours MRT	72 Hours MRT	96 Hours MRT	Total MRT	MRT (%)	Survival (%)
0	30	0	0	0	0	0	0	100
15	30	0	0	0	0	0	0	100
30	30	17	2	2	0	21	70	30
45	30	30	0	0	0	30	100	0
60	30	30	0	0	0	30	100	0

*biglobosa*during the 96hours bioassay are shown in (Table 2). All the parameters evaluated were significantly different (P<0.05) across the different concentrations except for phosphate. Nitrite concentrations were significantly higher at 45g/10L and 60g/10L concentrations compared to the control group 0g/10L. The lowest dose of 15g/10L at lower concentrations of the leaf power from (0—15g) nitrate concentrations did not show any significant difference but at higher concentrations above 30g/10L nitrate concentrations begin to change significantly. The phosphate concentrations showed slight increase with increased in the leaf power concentration from the control (0g/10L, 0.06) up to 45g/10L (0.12), then dropped to 0.05 at the 65g/10 concentration, this observed fluctuations were statistically non-significant (p>0.05). The Potential of Hydrogen (pH) level reduces with increase in concentration of the leaf powder, shifting from neutral (7.10) to 0g/10L towards being acidic (6.38) to the highest concentration of 60g/10L volume of water. The pH at control set-up significantly (p<0.05) differs from those of experimental set- ups. However, the 45g and 60g concentration showed no significant difference (P>0.05).

The temperature changes from 0g/10L to 45g/L were not significantly different (P>0.05) but at 60g/L the temperature increased significantly (<0.05) higher than the other concentrations. The electrical conductivity increases with increase in the concentration of the leaf powder from 566.00±11.37µs/cm at 0g/10L to 777.00±4.73µs/cm at 60g/10l concentration. These progressive increased significantly (p<0.05) different from each other from the control to the highest concentration. The Total Dissolved Solids (TDS) the results shows that concentration of dissolved solids increases significantly (p<0.05) from 0g/10L concentration (357.33±3.71ppm) to the 60g/10L concentration (413.00±6.43ppm). Dissolved oxygen had its highest value of 6.03mg/L at 0g/10L and 15g/10L concentrations. The Dissolved oxygen showed a significant decreased (p<0.05) from 30g/10L (4.90±0.06mg/L) to 60g/10L (2.86±0.15mg/L)

Mortality Rate of *Clariasgariepinus*Exposed to various Concentrations of the Leaf Powder of *Parkiabiglobosa*

Table 3 is the outcome of the mortality rate observed at the different concentrations during the 96 hours exposure. The percentage mortality of *Clariasgariepinus*juveniles exposed to P*arkiabiglobosa* leaf powder for 96hours recorded zero mortality in the control group. The mortality rate increases with increased concentration of the leaf powder. The 15 g of leaf powder, which was the lowest concentration tested, had 0% mortality as in the control throughout the 96hours exposure. The 30g leaf powder exposure had 70% mortality (MRT). However, at 45g and 60g concentration it resulted in the highest mortality rate of 100% at the end of the 96 hours experiment.

Table 4: Acute Toxicity Analysis on *Clarias gariepinus* exposed to different concentration of *Parkiabiglobosa*leaf powder

Concentration (g/L)	Leaf (Mean ± SE)
0	0.00 ± 0.00^{c}
15	0.00 ± 0.00^{c}
30	0.00 ± 0.00^{b}
45	10.00 ± 0.00^{a}
60	10.00 ± 0.00^{a}

*Means with the same alphabets are not different significantly at p<0.

Table 5: Biometric parameters of *Clariasgariepinus* exposed to various concentrations of *Parkiabiglobosa* leaf powder during the 96hour of acute toxicity

Concentration (mg/10L)	Body Weight (Unit)	Condition Factor	Visceral Index	Leptosomatic Index
0	11.46 ± 0.64	0.90 ± 0.03	6.43 ± 2.84	1.01 ± 0.26
15	11.61 ± 0.39	1.03 ± 0.06	7.01 ± 3.03	0.95 ± 0.07
30	10.77 ± 0.22	0.93 ± 0.02	10.65 ± 1.14	3.22 ± 1.38
P-Value	**0.435**	**0.069**	**0.475**	**0.159**

*Means with the same alphabets are not different significantly at p<0.05

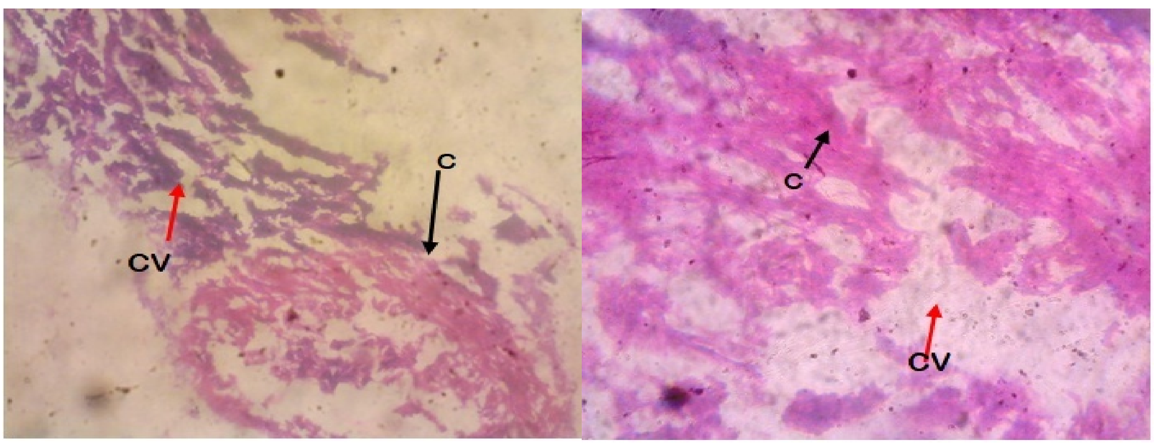

Figure 1: liver histology of *Clarias gariepinus* exposed to 0g concentration of *Parkia biglobosa* leaf powder during the 96 hour of acute toxicity

Acute Toxicity Analysis on *Clariasgariepinus* exposed to different concentration of *Parkiabiglobosa*leaf powder

The acute toxicity of leaf powder on *Clariasgariepinus*was evaluated in Table 4 below. The mean toxicity levels were 4.000 for the leaf, with a standard error of 0.471. The 95% confidence intervals indicated that the leaf group toxicity ranged from 3.017 to 4.983.

The analysis of Biometric parameters

The results of biometric parameters of the different concentrations of *Parkiabiglobosa* leaf (Table 5) powder throughout the 96hours bioassay show the body weight, condition factor, visceral index and hepatosomatic index measured for different concentrations of the leaf power for 96hours showed no significant difference (P>0.05) for all the parameters between 0g to 60g concentrations. This indicates that the administration of *Parkiabiglobosa* leaf and stem bark powders did not produce any noticeable effects on the body weight, condition factors or the visceral and hepatosomatic index, regardless of the concentration used. The study revealed that the leaf powders did not exhibit any acute toxic effects on the measured parameter.

Histological observation of liver in *Clariasgariepinus*exposed to various concentration of *Parkiabiglobosa* leaf powder during the 96hour of acute toxicity

The histological changes observed in the liver of *Clariasgariepinus*exposed to leaf are presented in the Figure 1 above: At 0 g of the leaf powder, the cytomorphology

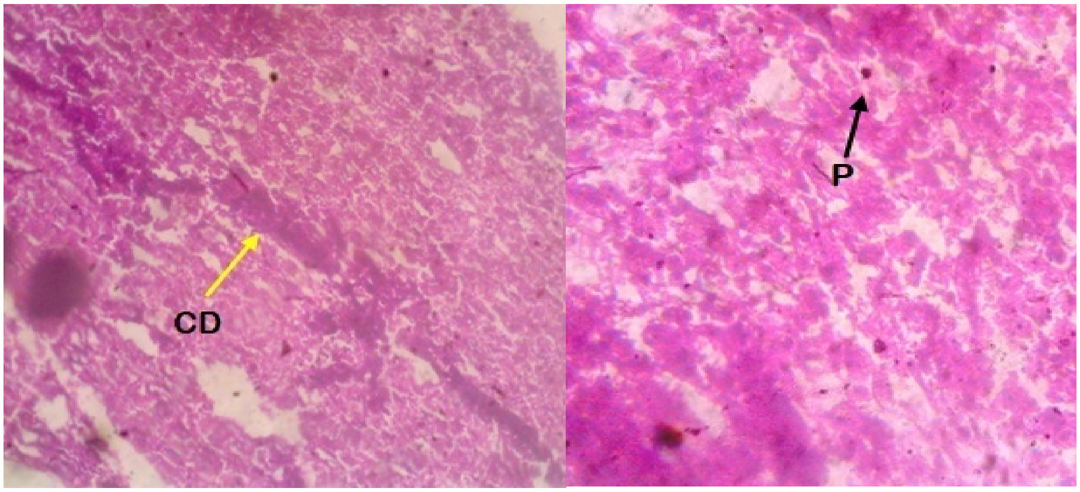

Figure 2: liver histology of *Clarias gariepinus* exposed to 15g concentration of *Parkia biglobosa* leaf powder during the 96 hour of acute toxicity

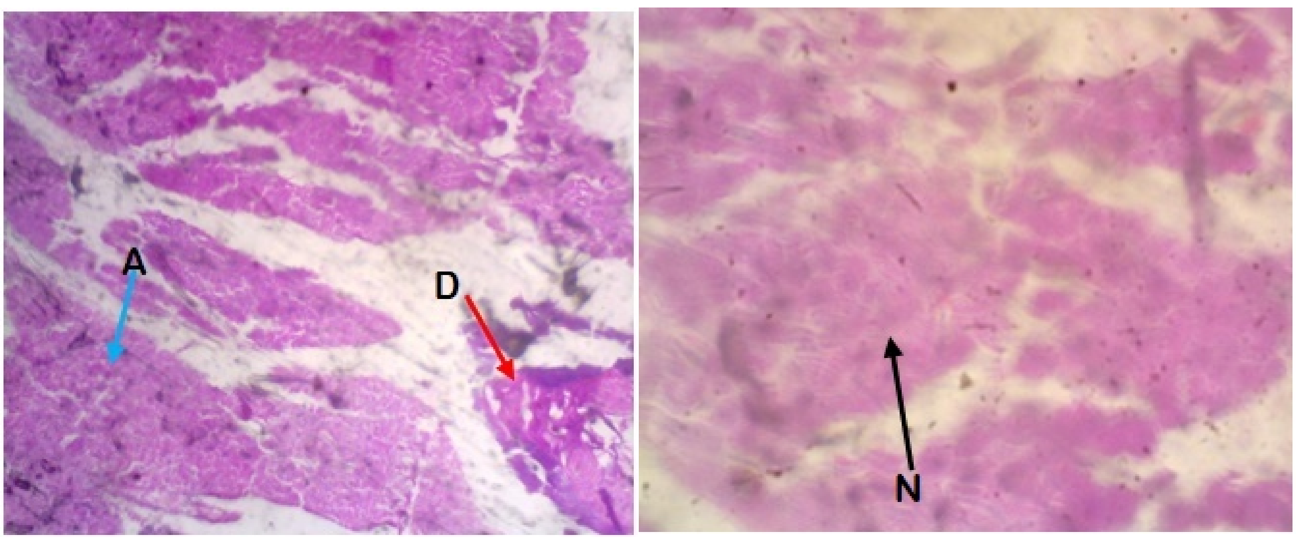

Figure 3: liver histology of *Clarias gariepinus* exposed to 30g concentration of *Parkia biglobosa* leaf powder during the 96 hour of acute toxicity

features were intact. The Hepatic cords (HC), Sinusiod (S), Central vein (CV) and the Blood vessel (BV). Figure 2: At 15 g of the Leaf powder, the cytomorphology features showed severe cellular degeneration and pyknotic nucleus. Figure 3: At 30 g of the Leaf powder, the cytomorphology features showed severe cellular degeneration and cellular necrosis (CN).

The results of histopathological effect of Leaf Powder of different concentrations exposed to the *Clariasgariepinus*

Figure 1: The liver histology of *Clariasgariepinus* exposed to 0 concentration of *Parkiabiglobosa* leaf powder during the 96hour of acute toxicity. The cytomorphology features were intact. The Hepatic cords (HC), Sinusoid (S), Central vein (CV) and the blood vessel (BV) showing mild congestion (black). Stainning uptake: H/E. X100/400MG.

Figure 2: The liver histology of *Clariasgariepinus* exposed to 15g concentration of *Parkiabiglobosa* leaf powder during the 96 hour of acute toxicity, the cytomorphology features showed severe cellular degeneration and pyknotic nuclei. Staining uptake: H/E. X100/400MG.

Figure 3: The liver histology of *Clariasgariepinus* exposed to 30g concentration of *Parkiabiglobosa* leaf powder during the 96hour of acute toxicity the cytomorphology features showed severe cellular necrosis (black); cellular degeneration (red); cellular aggregation and inflammation (blue arrow). Staining uptake: H/E. X100/ 400MG.

DICUSSION

Ecotoxicology plays a vital role in assessing the impact of natural substances on aquatic organisms (Smith, 2023**)**. In this study, the use of *Parkiabiglobosaleaf and bark* for fish harvesting shows an innovative approach that merits thorough investigation. The behavioral changes in *Clariasgariepinus* such as erratic swimming, gulping of air, vertical orientation and loss of buoyancy with increased concentration of *Parkiabiglobosa* leaf powder, may be as a result of the alteration of the physicochemical parameter of the cultured water as result of the *Parkiabiglobosa* leaf powder. The present study shows reduction in dissolve oxygen concentration as the concentration of the toxicant increased. The behavioral abnormalities such as gulping of air, loss of reflex and abnormal surface area observed prior to death could be an indication of depletion of oxygen. This report is similar to the findings made by Akpa*et al.,*(2023), who noted behavioral changes due to variation in water quality parameters such as erratic swimming and gulping of air *in Clariasgariepinus*when exposed to ethanolic extracts of *Launaeataraxocifolia*. This result is also in line with Orpin*et al.,* (2023), who reported that behavioural changes such as erratic swimming, air gulping and loss of equilibrium in fish exposed to *Calotropisprocera* were attributed to alteration in water quality parameters like oxygen level and pH during the experiment. This behavoural changes were linked to toxic effects from the primary factor observed when they expose *Clariasgariepinus*juveniles to *Datura innoxia* stem extracts and to *Clariasgariepinus*fingerlings T*etrapleuratetraptera* plants extracts respectively. The mortality rate increased progressively with increased concentration*Parkiabiglobosa* leaf powder, with the control having no mortality, the higher rate of mortality recorded in fish with increased concentration of *Parkiabiglobosa* leaf powder. The mortality may be as a result of change in fish physiology, body biochemistry and contamination of the cultured water as a result of the leaf extract. The same finding was also recorded byBabangida et *al.* (2024), when juveniles of *Clariasgariepinus*were exposed to *Carica papaya* leaf, seed and peel powder. (Okownko*et al*., 2024; Ganesh *et al*., 2023). In this research, the biometric responses included bodyweight, condition factors, hepatosomatic index and visceral which showed no significant differences. The body weight showed no significant variation across the concentration in the leaf extracts. The body weight has over all P- value of 0.434indicates that these differences are not statistically significant, suggesting that the concentration of *Parkiabiglobosa* bark power does not significantly impact the body weight. This result contrasts with Ibrahim *et al.*(2023) , all of whom reported significant difference in biometric parameters. The results of Histopathological examination of *Parkiabiglobosa* leaf extract revealed that the liver of *Clariasgariepinus*in the control group shows no alteration. Thecytomorphology features were found to be intact. The examination showed well preserved hepatic cords, clearly defined sinusoid and intact central veins. Also, the blood vessel displayed normal structure and function. At a concentration of 15g and 30g of the extracts, the cytomophology features exhibited significant changes, including the presence of pynotic nuclei, hydropic degeneration as indicated by the vacuolation observed in the tissue sample arrow of the sunosoid and severe cellular degeneration, severe cellular degeneration and severe cellular necrosis, including significant hydropic degeneration (edema). The finding of Nahar*et al*. (2025) revealed similar result on The ethyl alcohol extract of moringaoleifera leaves shows disorganized parenvhyma, vacuoles, hyperplasia in the liver of Heteropneustesfossilis fish. Pyrogallol exposure at sublethal levels in African catfish lead decreased immune parameters increased pro-inflammatory cytokines, and histhopathological damages in the liver and spleen. (Hamed*et al.,* 2023).

Conclusion

Based on the results from this research**,** *Parkiaboglobosa* leaf powder had adverse effects on the liver of *Clariasgariepinus and* the behavior of the fish. The presence of this plant powder in water also had negative impact on the physicochemical parameters of the water, fish farmer should desist from the use of *Parkiabiglobosa* leaf powder as a piscicidal substance in fish harvesting as they are effective at killing fish but can also harm non-target aquatic organisms, disrupt food webs, and reduce biodiversity if used indiscriminately or in large quantities.

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