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Research Article

Haematotoxicological Effects of *Pentaclethra macrophylla* Seed Powder on Juvenile African Catfish, *Clarias gariepinus*

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Abstract

The haematotoxic effects of nominal concentrations of *Pentaclethra macrophylla* (African oil bean) seed powder suspension were evaluated in juvenile *Clarias gariepinus* under controlled laboratory conditions. Fish were exposed for 96 h to six treatments: 0.00 mg/L (control), 0.40 mg/L, 0.50 mg/L, 0.60 mg/L, 0.70 mg/L and 0.80 mg/L, each in triplicate. Behavioural responses and haematological indices were assessed after exposure. Treated fish exhibited abnormal behavioural responses, including changes in body colouration, air gulping, barbel deformation, erratic swimming and loss of reflex. These responses appeared earlier and persisted longer at higher nominal concentrations. Exposure also produced significant changes ($p < 0.05$) in most haematological variables. Packed cell volume, red blood cell count and haemoglobin concentration were generally higher in exposed fish than in the control, whereas white blood cell, granulocyte and lymphocyte counts were generally reduced, particularly at intermediate concentrations. Mean corpuscular haemoglobin concentration did not differ significantly among treatments ($p > 0.05$). The results indicate that acute exposure to *P. macrophylla* seed powder suspension can alter the haematological profile and behaviour of juvenile *C. gariepinus*. The findings provide baseline laboratory evidence for assessing potential risks associated with the discharge of African oil bean processing residues into aquatic environments. Further studies incorporating measured environmental concentrations, chronic exposure, histopathology and biochemical biomarkers are recommended.

Keywords: Acute Exposure; African Oil Bean; Behavioural Response; *Clarias gariepinus*; Fish Haematology; *Pentaclethra macrophylla*

Introduction

Aquaculture is one of the fastest-growing food-production sectors and contributes substantially to animal-protein supply, particularly in regions where access to affordable animal protein remains limited. The African catfish, *Clarias gariepinus* [1], is among the most widely cultured freshwater fishes in Nigeria because of its rapid growth, high market acceptability, tolerance of variable environmental conditions, and suitability for intensive production systems [2]. Nevertheless, the productivity and health of cultured fish can be compromised by deteriorating water quality and by anthropogenic inputs such as agricultural runoff, industrial effluents and improperly managed organic residues.

Plant-derived wastes are an important but often under-assessed source of aquatic contamination in agricultural communities. *Pentaclethra macrophylla* Benth., commonly known as African oil bean, is a multipurpose leguminous tree native to West and Central Africa. Its seeds are traditionally processed and fermented into the condiment "Ugba" and are valued for their nutritional composition

[3]. However, seed residues and processing effluents may enter nearby streams, ponds and drainage channels where small-scale aquaculture and artisanal fisheries are practised. Such inputs may introduce organic matter and bioactive plant constituents into aquatic systems.

Previous phytochemical reports have identified several secondary metabolites in *P. macrophylla* seeds, including saponins, alkaloids, tannins, terpenoids, anthraquinones and cardiac glycosides [4]. These compounds may affect fish physiology through processes such as gill irritation, altered membrane stability, respiratory stress, impaired osmoregulation and disruption of normal metabolic function. However, the extent of such effects depends on exposure concentration, duration, fish size, environmental conditions and the form in which the plant material enters the water.

Fish haematology provides a useful tool for assessing physiological disturbance following exposure to aquatic contaminants. Blood parameters such as packed cell volume (PCV), red blood cell count (RBC), haemoglobin concentration (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), white blood cell count (WBC) and differential leukocyte counts are widely used as indicators of oxygen-transport capacity, erythropoietic response, immune status and general stress in fish [5]. Changes in these parameters can reflect sub-lethal effects before overt mortality or gross pathological signs become apparent.

Haematological changes in *C. gariepinus* have been reported after exposure to several plant-derived and anthropogenic toxicants, including Jatropha curcas seed powder, cassava mill effluent and pesticide formulations [1,2,6]. Despite the extensive processing and use of *P. macrophylla* in Nigeria, information on its haematological effects in freshwater fish remains limited. This study therefore evaluated the acute haematological and behavioural responses of juvenile *C. gariepinus* exposed to nominal concentrations of *P. macrophylla* seed powder suspension for 96 h.

Materials and Methods

Experimental site

The experiment was conducted in the Central Laboratory of the Department of Fisheries and Aquaculture, Faculty of Agriculture, Adekunle Ajasin University, Akungba Akoko, Ondo State, Nigeria, under ambient laboratory conditions.

Experimental fish and acclimatisation

A total of 180 apparently healthy juvenile *C. gariepinus* were obtained from a commercial fish farm in Akure, Ondo State, Nigeria, and transported to the laboratory in oxygenated containers. The fish were acclimatised for 14 days in 50-L plastic tanks containing 30 L of dechlorinated tap water. During acclimatisation, fish were fed commercial catfish pellets twice daily at 3% of body weight. Uneaten feed and faecal matter were removed by siphoning, and 50% of the water was renewed daily to maintain water quality. Fish showing visible injury, deformity or signs of disease were excluded from the experiment.

Preparation of plant material

Fruits of *P. macrophylla* were collected from mature trees in Akungba Akoko, Ondo State, Nigeria. Seeds were removed from the pods, washed with distilled water and air-dried for seven days on a clean platform. The dried seeds were ground into a fine powder using an electric blender and stored in airtight containers at room temperature until use. For the bioassay, weighed quantities of the seed powder were dispersed directly in test water to obtain the required nominal concentrations. Because the material was used as a powdered plant suspension, the concentrations reported in this study represent nominal mass of seed powder per unit volume of water rather than measured concentrations of dissolved active constituents.

Range-finding test

A preliminary 24-h range-finding test was conducted to identify a suitable concentration range for the definitive exposure. Groups of ten fish were exposed to selected concentrations of *P. macrophylla* seed powder suspension, and mortality and overt behavioural responses were recorded. Concentrations that produced no mortality and a marked toxic response during the preliminary test were used to guide the selection of concentrations for the definitive 96-h exposure.

Definitive 96-h exposure

The definitive bioassay comprised six nominal concentrations: 0.00 mg/L (control), 0.40 mg/L, 0.50 mg/L, 0.60 mg/L, 0.70 mg/L and 0.80 mg/L. Each treatment was replicated three times in rectangular plastic tanks containing 30 L of dechlorinated water. Ten fish were stocked per tank. The exposure was conducted under static conditions for 96 h without feeding or mechanical aeration, consistent with common acute-toxicity bioassay practice.

Behavioural responses and mortality were monitored at 4, 12, 24, 48, 72 and 96 h. Dead fish, if observed, were promptly removed to reduce deterioration in water quality.

Behavioural observations

Behavioural responses were assessed qualitatively at each observation interval. The recorded responses were body-colour change, gulping air at the water surface, barbel deformation, erratic swimming and loss of reflex. Each response was scored as present (+) or absent (-). The control group was maintained concurrently as the reference condition.

Water-quality monitoring

Water-quality variables were monitored at the beginning and end of the 96-h exposure. Dissolved oxygen, temperature, electrical conductivity and pH were measured using calibrated instruments. Monitoring was conducted to confirm that major physicochemical changes did not confound interpretation of the haematological and behavioural responses.

Blood collection and haematological analysis

At the end of the 96-h exposure, surviving fish were sampled for haematological analysis. Fish were anaesthetised before blood collection, and approximately 3 mL of blood was collected from the caudal blood vessel using sterile disposable syringes. Blood samples were transferred immediately into EDTA-treated bottles and kept at 4 °C until analysis.

Haematological parameters were determined using standard procedures described by Dacie and Lewis [7]. Packed cell volume was determined by the microhaematocrit method; haemoglobin concentration was determined by the cyanmethemoglobin method; and RBC and WBC counts were determined using an improved Neubauer haemocytometer. Differential leukocyte counts were determined from Giemsa-stained blood smears. Erythrocyte indices were calculated as follows: MCV (fL) = (PCV/RBC) x 10; MCH (pg) = (Hb/RBC) x 10; and MCHC (g/dL) = (Hb/PCV) x 100.

Statistical analysis

Data are presented as mean ± standard deviation. One-way analysis of variance (ANOVA) was used to test for differences among treatment means, followed by Duncan's Multiple Range Test for mean separation where significant differences occurred. Statistical significance was accepted at $p < 0.05$. Analyses were conducted using SPSS version 23.0 (IBM Corp., Armonk, NY, USA). Superscript letters in the tables indicate significant differences among treatments within the same row.

Results

Behavioural responses

Behavioural responses of juvenile *C. gariepinus* exposed to *P. macrophylla* seed powder suspension are presented in Table 1. Fish in the control group maintained normal swimming activity, and no abnormal behavioural signs were observed during the 96-h exposure period.

Period	Behaviour	T1 0.00	T2 0.40	T3 0.50	T4 0.60	T5 0.70	T6 0.80
24 h	Colouration change	-	-	+	+	+	+
	Air gulping	-	+	+	+	+	+
	Barbel deformation	-	-	-	-	+	+
	Erratic swimming	-	-	-	+	+	+
	Loss of reflex	-	-	-	+	+	+
48 h	Colouration change	-	+	+	+	+	+
	Air gulping	-	+	+	+	+	+
	Barbel deformation	-	+	+	+	+	+
	Erratic swimming	-	+	+	+	+	+
	Loss of reflex	-	+	+	+	+	+
72 h	Colouration change	-	+	+	+	+	+
	Air gulping	-	+	+	+	+	+
	Barbel deformation	-	+	+	+	+	+

	Erratic swimming	-	+	+	+	+	+
	Loss of reflex	-	+	+	+	+	+
96 h	Colouration change	-	+	+	+	+	+
	Air gulping	-	+	+	+	+	+
	Barbel deformation	-	+	+	+	+	+
	Erratic swimming	-	+	+	+	+	+
	Loss of reflex	-	+	+	+	+	+

Table 1: Behavioural responses of juvenile *Clarias gariepinus* exposed to nominal concentrations of *Pentaclethra macrophylla* seed powder suspension for 96 h.

Values are nominal concentrations in mg/L. + = response present; - = response absent.

At 24 h, body-colour change was observed in fish exposed to 0.50 mg/L or higher concentrations, while air gulping was recorded in all exposed groups at or above 0.40 mg/L. Barbel deformation was observed only at 0.70 and 0.80 mg/L at 24 h, whereas erratic swimming and loss of reflex occurred from 0.60 mg/L upward. By 48 h, all five behavioural responses were recorded in all exposed groups, and these responses persisted through 72 and 96 h. The behavioural observations indicate that exposure to *P. macrophylla* seed powder suspension caused early and persistent signs of stress in juvenile *C. gariepinus*.

Haematological parameters

Haematological parameters after 96 h of exposure are shown in Table 2. Most haematological variables differed significantly among treatments ($p < 0.05$), except MCHC, which remained statistically similar across all treatments.

Parameter	T1 0.00 mg/L	T2 0.40 mg/L	T3 0.50 mg/L	T4 0.60 mg/L	T5 0.70 mg/L	T6 0.80 mg/L
PCV (%)	19.49 ± 0.00^{a}	22.48 ± 0.01^{b}	21.48 ± 0.01^{ab}	25.49 ± 0.00^{c}	20.49 ± 0.00^{ab}	27.99 ± 0.00^{d}
RBC ($\times 10^{6}$/ mm^{3})	1.00 ± 0.11^{ab}	0.85 ± 0.02^{a}	0.95 ± 0.02^{a}	1.15 ± 0.02^{bc}	1.20 ± 0.00^{c}	1.40 ± 0.05^{d}
MCHC (g/dL)	33.25 ± 0.10^{a}	33.33 ± 0.00^{a}	33.48 ± 0.00^{a}	33.33 ± 0.00^{a}	33.41 ± 0.85^{a}	33.38 ± 0.55^{a}
MCV (fL)	202.09 ± 20.43^{ab}	264.59 ± 1.21^{c}	226.10 ± 2.25^{b}	221.59 ± 1.96^{b}	170.84 ± 2.39^{a}	201.54 ± 12.44^{ab}
Haemoglobin (g/dL)	6.50 ± 0.11^{a}	7.50 ± 0.28^{b}	7.18 ± 0.28^{ab}	8.50 ± 0.28^{c}	6.84 ± 0.86^{ab}	9.34 ± 0.20^{d}
WBC ($\times 10^{3}$/ mm^{3})	1.30 ± 0.23^{c}	0.95 ± 0.14^{abc}	0.60 ± 0.00^{a}	0.65 ± 0.08^{a}	0.85 ± 0.02^{ab}	1.10 ± 0.05^{bc}
Granulocytes ($\times 10^{3}/mm^{3}$)	0.54 ± 0.14^{b}	0.35 ± 0.08^{ab}	0.15 ± 0.02^{a}	0.20 ± 0.05^{a}	0.25 ± 0.02^{a}	0.31 ± 0.05^{ab}
Lymphocytes ($\times 10^{3}/mm^{3}$)	0.75 ± 0.08^{b}	0.59 ± 0.05^{ab}	0.44 ± 0.05^{a}	0.44 ± 0.05^{a}	0.59 ± 0.00^{ab}	0.78 ± 0.20^{b}
MCH (pg)	67.38 ± 6.81^{ab}	88.19 ± 0.40^{c}	75.39 ± 0.75^{b}	73.85 ± 0.66^{b}	56.94 ± 0.77^{a}	67.19 ± 4.15^{ab}

Table 2: Haematological parameters of juvenile *Clarias gariepinus* exposed to nominal concentrations of *Pentaclethra macrophylla* seed powder suspension for 96 h.

Values are mean ± standard deviation. Values in the same row with different superscript letters differ significantly at $p < 0.05$. PCV = packed cell volume; RBC = red blood cell count; MCHC = mean corpuscular haemoglobin concentration; MCV = mean corpuscular volume; WBC = white blood cell count; MCH = mean corpuscular haemoglobin.

PCV and haemoglobin concentration were generally higher in the exposed groups than in the control group, with the highest values recorded at 0.80 mg/L. RBC count showed a non-linear response: it decreased at 0.40 mg/L and 0.50 mg/L compared with the control, then increased at higher nominal concentrations. MCV and MCH also fluctuated among treatments, with the highest values recorded at 0.40 mg/L and lower values at 0.70 mg/L. WBC, granulocyte and lymphocyte counts were generally lower in exposed fish than in the control, although partial increases were observed at the highest concentration.

Discussion

The present study shows that nominal concentrations of *P. macrophylla* seed powder suspension can induce behavioural disturbance and haematological alteration in juvenile *C. gariepinus* during acute laboratory exposure. The absence of abnormal behaviour in the control group and the early occurrence of stress-related responses in exposed fish suggest that the plant material contributed to the observed responses.

Air gulping, erratic swimming and loss of reflexes are common behavioural indicators of physiological stress in fish exposed to aquatic contaminants. Air gulping may reflect impaired respiratory efficiency or increased oxygen demand, while erratic swimming and loss of reflexes may indicate neuromuscular stress or reduced capacity to maintain normal orientation. In the present study, all behavioural indicators in exposed fish were recorded by 48 h, suggesting that the test material induced persistent stress under the exposure conditions. However, because gill histology, oxygen consumption and neurochemical endpoints were not measured, the precise mechanisms underlying these behavioural responses cannot be confirmed from the present data alone.

The haematological profile indicates systemic physiological disturbance. PCV and haemoglobin concentration were generally elevated in exposed fish, particularly at the highest nominal concentration. Such increases may reflect haemoconcentration, altered plasma volume or compensatory adjustment in oxygen transport under stress. RBC responses were non-linear, with a decrease at lower-exposure treatments followed by increases at higher-exposure treatments. This pattern may be associated with competing processes such as erythrocyte damage, altered erythrocyte volume, splenic release of erythrocytes or stress-mediated shifts in circulating cells. Because plasma osmolality, erythrocyte fragility and organ histology were not assessed, these explanations should be regarded as plausible interpretations rather than confirmed mechanisms.

MCHC remained statistically unchanged among treatments, indicating that the relative haemoglobin concentration within erythrocytes was not markedly altered, even though PCV, RBC and haemoglobin concentration varied among treatments. In contrast, MCV and MCH fluctuated across treatments, suggesting that erythrocyte size and haemoglobin content per cell may have been affected by exposure. These changes further support the view that *P. macrophylla* seed powder suspension can disturb erythrocyte-related indices in juvenile *C. gariepinus*.

The reduction in WBC, granulocyte and lymphocyte counts in most exposed groups suggests possible leukocyte depletion or stress-related redistribution of immune cells. Such changes may reduce fish's capacity to respond to secondary stressors and infectious agents. The partial increase in WBC and lymphocyte counts at the highest concentration should not be interpreted as full immune recovery, because behavioural signs of stress were also pronounced in exposed fish. Rather, the pattern may reflect an acute stress response or variability in leukocyte mobilisation during exposure.

The toxicological effects observed in this study may be associated with bioactive constituents reported in *P. macrophylla* seeds, including saponins, alkaloids, tannins and cardiac glycosides [4]. Saponins, in particular, are known to interact with biological membranes and may contribute to membrane instability, while other plant secondary metabolites may influence respiratory or metabolic processes. Nevertheless, the present study did not quantify the seed powder's phytochemical profile or measure specific biochemical pathways. Future work should therefore include phytochemical characterisation, gill and liver histopathology, oxidative stress biomarkers, acetylcholinesterase activity and chronic-exposure designs.

From an environmental perspective, the findings suggest that residues from *P. macrophylla* seed processing may pose risks to fish if they enter ponds, streams or drainage systems at biologically active concentrations. However, direct field risk assessment requires measured environmental concentrations, fate-and-transport data, and information on dilution, degradation, and repeated exposure.

The present study should therefore be interpreted as a controlled laboratory assessment that provides baseline evidence for further ecological and aquaculture risk evaluation [8-10].

Conclusion

Acute 96-h exposure to nominal concentrations of *P. macrophylla* seed powder suspension altered the behaviour and haematological profile of juvenile *C. gariepinus* under laboratory conditions. Exposed fish showed stress-related behaviours, including air-gulping, colouration changes, barbel deformation, erratic swimming and loss of reflex. Haematological responses included changes in PCV, RBC, haemoglobin concentration, MCV, MCH and leukocyte counts, while MCHC remained statistically unchanged. These findings indicate that *P. macrophylla* seed powder suspension can produce haematological disturbance in juvenile African catfish. Further studies using measured environmental concentrations, chronic exposure, histopathological endpoints, biochemical biomarkers and phytochemical characterisation are recommended to clarify the ecological relevance and mechanism of toxicity.

Authors' Contributions

All authors contributed to the conception and design of the study. Adene I. C. coordinated the study, supervised the experimental work and contributed to manuscript preparation. Adaba O. E. and Ajagunna O. E. contributed to sample preparation, laboratory analysis, data collation and revision of the manuscript. All authors read and approved the final manuscript.

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Availability of Data and Materials

The datasets generated and analysed during the current study are available from the corresponding author upon reasonable request.

Competing Interests

The authors declare that they have no competing interests.

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