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### RESEARCH ARTICLE

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# Assessment of Changes in the Hematological Profile and Behavioural Responses of Juvenile *Clarias gariepinus* Exposed to Raw *Blighia sapida*, Ackee Seed Powder as a Toxicant

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### ABSTRACT

The acute haematological changes of *Clarias gariepinus* juveniles exposed to *Blighia sapida* aqueous extract were investigated in a static bioassay to determine the median lethal concentrations (LC<sub>50</sub>) at 96 hours of exposure. Six graded concentrations (0, 30, 31.7, 33, 35, and 36.7 mg/l) respectively of *B. sapida* seed powder were introduced to the water in 30-liter plastic bowls, containing ten (10) *C. gariepinus* juveniles. The result obtained revealed the 96-hour LC<sub>50</sub> values to be 53.33 mg/l with a 50% confidence interval between the lower and higher limits of 53.33 and 5.3mg/l when multiplied by a constant. Fish exposed to acute toxicity test exhibited several abnormal behaviours, including air gulping, erratic swimming, discolouration, loss of reflex, and molting. Fish were observed motionless at the bottom of the bowl just before death. The oxygen consumption by juveniles decreased from (2.5 to 1.8) with increased concentrations (30–36.7 mg/l) of *B. sapida* seed powder. The result for haematological parameters showed significant decrease in packed cell volume(PCV) that ranged (significantly or not significantly (P>0.05 or P<0.05) from 19.00±1.41 - 16.00±1.41%; the red blood cell counts (RBC) varied (significantly or not significantly (P>0.05 or P<0.05) from 2.95±2.05 - 1.05±0.35 ×10<sup>6</sup>/L; haemoglobin (Hb) (significantly or not significantly (P>0.05 or P<0.05) ranged from 6.33±0.47 - 5.50±1.18g/dl and mean corpuscular haemoglobin concentration (MCHC) was not significantly different when compared with the control. White blood cell counts (WBC) initially decreased in treatment 2 and 3 (3.15 ± 0.35- 2.60 ± 1.13 and 1.50 ± 0.42 (× 10<sup>9</sup>/L)) but significantly increased in Treatment 4 (3.15 ± 0.35- 3.20 ± 0.57 (× 10<sup>9</sup>/L)), where the highest mortality (6 fish) was observed as the concentrations of *B. sapida* seed powder increased. The results showed that *B. sapida* seed powder has negative effects on the haematological values of *C. gariepinus*. Therefore, it was concluded that raw *B. sapida* seed powder causes significant alterations in the haematological profiles of *C. gariepinus* juveniles and should therefore be avoided in proximity to the aquatic environment. Although when processed, it was reported to have potential benefits on the growth and health of *C. gariepinus*.

### Highlights

- African catfish (*Clarias gariepinus*) is a good model for ecotoxicological research.
- Ackee seed (*B. sapida*) contains saponins and hypoglycin A and B, toxic substances that inhibit glyconeogenesis.
- Haematology is of great importance for its active role in monitoring fish health.

### 1. Introduction

The African catfish *Clarias gariepinus* is a significant cultural and commercial species in Nigeria's fishing industry (Tiamiyu *et al.*, 2019). Its great nutritional value has led to its increasing demand in Nigerian

fish markets. The fish has proven to be a good model for ecotoxicological research because of its availability throughout the season and easy acclimatization to laboratory conditions (Adeyemi *et al.*, 2014).

*Blighia sapida* seed has been reported to have potential health benefits, including antioxidant activity (Ahenkorah *et al.*, 2022), anti-inflammatory activity (Kumar and Aderson., 2022), and antimicrobial activity (Adesoyin, 2024). *Blighia sapida* seed, when processed have shown potential benefits on the growth and health of *C. gariepinus*, including enhanced feed utilization, as it has been reported to improve feed conversion ratio and protein efficiency ratio in *C. gariepinus* (Aguemon *et al.*, 2018). It has also been shown to have immunostimulatory effects, increasing the production of antibodies and activating immune cells in fish (Oladimeji *et al.*, 2022) and antiparasitic activity against parasites infecting *C. gariepinus*, such as *Ichthyophthirius multifiliis* (Adebayo *et al.*, 2022). Despite all the aforementioned benefits, the raw seeds contain toxic compounds such as hypoglycin A and B, known toxic substances that can inhibit gluconeogenesis (Bowen – Forbes *et al.*, 2009). If the seeds are not properly processed, they can cause serious health issues in fish, such as reduced growth and organ damage (Surmaitis *et al.*, 2025).

Fish haematology is becoming increasingly important in fish culture because of its role in monitoring fish health (Fazio *et al.*, 2024). Most fish's haematological properties have been researched to establish a normal value range, and deviation which indicates a problem with the physiological process (Rainza-Paiva *et al.*, 2000). Studies have also indicated changes in haematological indicators of African clariids treated to several toxicants in laboratory conditions (Adedeji 2009; Okomoda *et al.*, 2010). Fish immunological status is linked to haematological indices such as WBC, platelets, and total counts, which are useful tools for assessing physiological, biochemical, and pathological changes in fish. They have been widely utilized for assessing fish health, monitoring stress responses, estimating the systematic relationship, and physiological adaptations of animals (Okey, 2019). Haematological analysis provides valuable insights into the health conditions of the fish species. Fish hematological parameters, including RBC and WBC, Hb concentration, hematocrit, MCH, and MCHC, contribute to the fish physiology, immune system functionality, and their interactions with aquatic environments (Fazio, 2019). There is not much research on the effects of raw *B. sapida* or the effluent on *C. gariepinus* fish if it accidentally finds its way to the aquatic environment, hence the basis for this research.

This study seeks to assess the changes in haematological profiles and behavioural responses of juvenile *C. gariepinus* exposed to varying concentrations of ackee seed powder for 96 hours.

## 2. Materials and Methods

### Experimental Site

The experiment was carried out in the Central Laboratory of Adekunle Ajasin University, Akungba-Akoko, Ondo State, using a plastic tank with a 50-litre capacity (75 cm × 40 cm × 40 cm) filled with 30 litres of unchlorinated water.

### Fish Collection and Acclimatization

Two hundred and fifty (250) healthy *C. gariepinus* were collected alive from Aquatem farm, Ado Ekiti, Ekiti State, Nigeria, and transported to the Central Laboratory of Adekunle Ajasin University, Akungba-Akoko, Ondo State. The health status of selected fish was

assessed based on the presence or absence of physical injuries and other morphological deformation symptoms. The fish were certified healthy by assessment before the commencement of the study.

The juvenile catfish were acclimatized for two weeks (14 days) within the experimental area and fed with commercial feed (Blue Crown) during the period of acclimatization. Feeding was discontinued 24 hours before the fish were distributed into the experimental tanks.

### Source and preparation of Ackee seed powder

The *Blighia sapida* seeds were collected at Ibaka market, Akungba Akoko, Ondo State. The seed coats were removed, the seeds air-dried, then ground into powder form, kept in a plastic bottle, and stored at room temperature until usage.

### Range Finding Test

A range-finding test experiment was carried out to determine the effective concentration of *Blighia sapida* to be used. Ten (10) fish were distributed into a set of (4) rectangular plastic tanks (75 X 45 X 45cm), each filled with 30liters of unchlorinated water. Powdered *Blighia sapida* seed was introduced at different concentrations of 20, 30, 40, and 50 (mg/l). Each of the test solutions was introduced directly into the plastic tanks. The behavior and mortality of the test fish in each tank were monitored and recorded for 24 hours.

### Definitive Test

The results obtained from the range finding test were subjected to a 96-hour definitive test. Batches of ten juvenile *C. gariepinus* were distributed into a set of 18 rectangular plastic tanks (75 × 45 × 45 cm), each filled with 30litres of unchlorinated water. Six test solutions of Ackee seed powder 0.0, 30, 31.7, 33.0, 35.0, and 36.7 (mg/l) determined from the range finding test were introduced directly into the plastic tanks. The test fish were not fed throughout the 96h test. The behavioral pattern and mortality of the test fish in each tank were monitored and recorded for the first 4 hours, 12 hours, 24 hours, 48 hours, 72 hours, and 96 hours, respectively.

### Blood Collection

Blood samples were collected through the vertebral caudal blood vessel of the test fish using a 2 ml disposable hypodermic syringe and needle after 96 hours of exposure to Ackee seed powder, and the blood was emptied into a 5 ml sample bottle.

### Blood Cell Counts

Haemocytometer was used in the blood cell Counts. The blood diluting fluid was prepared as described by Svobodova *et al.* (1991). The blood cells were counted on the counting chamber of a Haemocytometer with the aid of a compound microscope.

$$RBC = \text{No. of cells counted} \times 3 \times 10 \times 200 \text{ (160 mm}^3\text{)} \text{ - - - - -}$$

Equation 1

$WBC = \text{No. of cells counted} \times 0 \times 25 \times 10 \times 20 (104 \text{ mm}^3) \dots$   
Equation 2

### Hemoglobin Estimation

A haemoglobinometer was used for hemoglobin estimation based on the acid haematin method.

Hemoglobin was expressed as the value obtained  $17.2 \text{ mg}/100 \text{ ml}$  100 packed cell volume - - - - Equation 3

The Packed cell volume was measured by placing a scaled microhaematocrit tube in a centrifuge at 10,500 rpm using a microhaematocrit reader and expressed as a percentage.

### Statistical analysis

All data obtained were subjected to analysis of variance (ANOVA) using Statistical Package for Social Sciences (SPSS) version 20 to generate the means and standard errors. Means generated were compared by Duncan's New Multiple Range Test (DNMRT).

## 3. Results

### Behavioural responses of *C. gariepinus* exposed to *Blighia sapida* seed powder

In the control group (0.0), the fish behaved normally. However, fish exposed to different concentrations of the seed powder showed changes like being motionless, deformed barbels, loss of reflexes, gulping air, producing excess mucus, erratic swimming, and surfacing frequently. These behavioural responses worsened at higher concentrations. The fish also lost their balance, swimming backward and becoming less active, eventually remaining still and leading to death (Table 1).

Behavior Exposure	24 hours	48 hours	72 hours	96 hours
Concentrations (mg/L)	0 30 31.7 33 35 36.7	0 30 31.7 33 35 36.7	0 30 31.7 33 35 36.7	0 30 31.7 33 35 36.7
Coloration	- - - - - -	- + + + + +	- + + + + +	- - + + + +
Air gulping	- - - - - -	- + + + + +	- + + + + +	- - + + + +
Erratic swimming	- - - - - -	- + + + + +	- + + + + +	- + + + + +
Barbell deformation	- - - - - -	- + + + + +	- + + + + +	- + + + + +
Loss of reflex	- - - - - -	- - + + + +	- + + + + +	- + + + + +

**Table 1: Behavioral response of juvenile *C. gariepinus* exposed to *Blighia sapida* seed powder for 96 hours**

**Key:** Positive (+) signifies reaction, Negative (-) signifies no reaction

### Haematological Parameters of *C. gariepinus* exposed to *Blighia sapida* seed powder for 96hours

The highest PCV was observed when the concentration of toxicant was highest at T6 ( $18.00 \pm 1.41\%$ ), while the lowest was at T4 ( $16.00 \pm 1.41\%$ ). There was a significant increase in PCV values with increases in concentrations when compared with the control ( $p < 0.05$ ). The highest RBC was in T6 ( $1.05 \pm 0.35 \times 10^6/\text{L}$ ), and the lowest was in T2 ( $2.75 \pm 0.92 \times 10^5/\text{L}$ ). There was no significant difference ( $p > 0.05$ ) in MCHC after 96 hours of exposure. MCV was highest in T4 ( $297.22 \pm 286.77(\text{fL})$ ) and lowest in T2 ( $59.80 \pm 9.71(\text{fL})$ ). Hb Level was highest in T6 ( $6.00 \pm 0.47 \text{ g/dl}$ ) and lowest in T3 ( $5.33 \pm 0.47 \text{ g/dl}$ ). WBC was highest in T4 ( $3.20 \pm 0.57 \times 10^9/\text{L}$ ) and lowest in T3 ( $1.50 \pm 0.42 \times 10^9/\text{L}$ ). There was a significant difference ( $p < 0.05$ ) in all treatments compared to the control. The lymphocyte count was highest in T1 ( $2.21 \pm 1.49$ ) and lowest in T3 ( $0.74 \pm 0.74$ ). Significant differences were observed at  $p < 0.05$  in all treatments when compared with the control (Table 2).

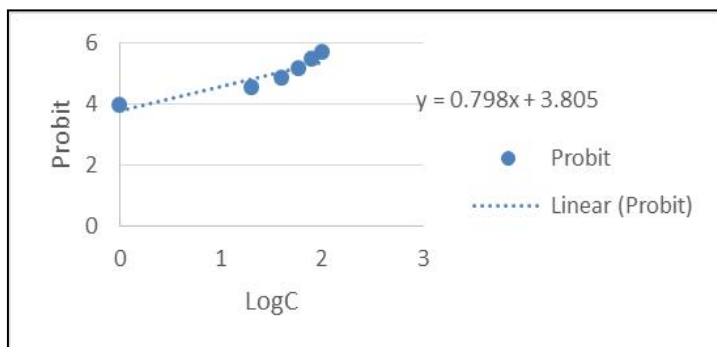
### Log concentration of *Blighia sapida* seed powder and its probit value for juvenile *C. gariepinus* on exposure for 96 hours

The Log of concentration of *B. sapida* seed powder and its probit value for juvenile *C. gariepinus* is presented in Figure 1. Lethal concentration of acute toxicity of *B. sapida* seed to African catfish, *C. gariepinus* is  $53.33 \text{ mg/l}$ , with a permissible concentration range of  $0.5333\text{-} 5.333 \text{ mg/L}$ . This was calculated by multiplying the 96-hour  $\text{LC}_{50}$  by a constant of 0.01-0.1 (Koesoemadinata, 2000). The  $\text{LC}_{50}$  represents the concentration at which 50% of a fish population is killed when exposed to toxins.

**Table 2:** Haematological parameters of *C. gariepinus* exposed to *Blighia sapida* seed powder for 96 hours

Treatments (mg/l) / Parameters	T1 (0.0)	T2 (30.0)	T3 (31.7)	T4 (33.0)	T5 (35.0)	T6 (36.7)
PCV (%)	19.00+1.41 <sup>a</sup>	16.00+2.83 <sup>a</sup>	16.00+1.41 <sup>a</sup>	16.00+1.41 <sup>a</sup>	16.50+3.54 <sup>a</sup>	18.00+1.41 <sup>a</sup>
RBC ( $\times 10^6/L$ )	2.95+2.05 <sup>a</sup>	2.75+0.92 <sup>a</sup>	1.25+0.78 <sup>a</sup>	1.05+1.06 <sup>a</sup>	1.45+0.50 <sup>a</sup>	1.05+0.35 <sup>a</sup>
MCHC (g/dl)	33.42+0.12 <sup>a</sup>	33.45+0.17 <sup>a</sup>	33.43+0.13 <sup>a</sup>	33.43+0.13 <sup>a</sup>	33.36+0.30 <sup>a</sup>	33.34+0.26 <sup>a</sup>
MCV (fL)	87.12+65.35 <sup>a</sup>	59.80+9.71 <sup>a</sup>	154.37+84.74 <sup>a</sup>	297.22+286.77 <sup>a</sup>	116.41+15.36 <sup>a</sup>	179.33+46.91 <sup>a</sup>
MCH (pg/cell)	29.04+21.78 <sup>a</sup>	19.93+3.24 <sup>a</sup>	51.46+28.25 <sup>a</sup>	99.07+95.59 <sup>a</sup>	38.80+5.12 <sup>a</sup>	59.78+15.64 <sup>a</sup>
Haemoglobin (g/dl)	6.33+0.47 <sup>a</sup>	5.33+0.94 <sup>a</sup>	5.33+0.47 <sup>a</sup>	5.33+0.47 <sup>a</sup>	5.50+1.18 <sup>a</sup>	6.00+0.47 <sup>a</sup>
WBC ( $\times 10^9/L$ )	3.15+0.35 <sup>a</sup>	2.60+1.13 <sup>ab</sup>	1.50+0.42 <sup>b</sup>	3.20+0.57 <sup>a</sup>	2.40+0.28 <sup>ab</sup>	2.40+0.57 <sup>ab</sup>
Granulocytes ( $\times 10^9/L$ )	0.74+0.58 <sup>a</sup>	0.52+0.14 <sup>a</sup>	0.36+0.19 <sup>a</sup>	0.31+0.33 <sup>a</sup>	0.39+0.32 <sup>a</sup>	0.27+0.13 <sup>a</sup>
GRA (%)	24.00+2.83 <sup>a</sup>	19.00+1.41 <sup>a</sup>	29.50+3.54 <sup>a</sup>	27.00+4.24 <sup>a</sup>	24.50+13.44 <sup>a</sup>	25.00+4.24 <sup>a</sup>
Lymphocytes ( $\times 10^9/L$ )	2.21+1.49 <sup>a</sup>	2.16+0.74 <sup>a</sup>	0.89+0.58 <sup>a</sup>	0.74+0.74 <sup>a</sup>	1.06+0.18 <sup>a</sup>	0.77+0.20 <sup>a</sup>
LYMPH (%)	75.50+2.12 <sup>a</sup>	78.50+0.71 <sup>a</sup>	70.00+2.83 <sup>a</sup>	71.00+1.41 <sup>a</sup>	75.50+13.44 <sup>a</sup>	74.00+5.66 <sup>a</sup>
Monocytes ( $\times 10^9/L$ )	0.02+0.02 <sup>a</sup>	0.07+0.04 <sup>a</sup>	0.01+0.01 <sup>a</sup>	0.04+0.05 <sup>a</sup>	0.00+0.00 <sup>a</sup>	0.00+0.00 <sup>a</sup>
MON %	1.00+1.41 <sup>a</sup>	2.50+0.71 <sup>a</sup>	0.50+0.71 <sup>a</sup>	2.00+2.83 <sup>a</sup>	0.00+0.00 <sup>a</sup>	1.00+1.41 <sup>a</sup>

Means with different superscripts show significant differences at ( $P < 0.05$ ). Packed cell volume (PCV), Haemoglobin (Hb), Red blood cell (RBC), White blood cell (WBC), Mean cell volume (MCV), Mean cell haemoglobin (MCH), and Mean cell haemoglobin concentration (MCHC).



**Fig 1:** Log concentration of *Blighia sapida* seed powder and its probit value for juvenile *C. gariepinus* on exposure for 96 hours

#### 4. Discussion

The haematology of fish serves as an important tool to fishery biologists due to its frontline sensitive indicator of vital physiological and biochemical functions. Several studies have been done on fish using haematology as a biomarker for exposure to different toxicants and to monitor the interaction between the toxicant and the biological system (DaCuna *et al.*, 2011). The major blood function is the regulation of the internal environment of a system (homeostasis) (Ada *et al.*, 2012). Any intense attack on the blood outrightly leads to the death of an organism. The results of haematological indices of juvenile *C. gariepinus* on

exposure to *B. sapida* powder for 96 hours showed a significant decrease in the following: PCV, RBC, Hb, lymphocyte, granulocyte, and monocyte. The reductions are indications of anemic conditions caused by the destruction of erythrocytes (Omoniyi *et al.*, 2002), which were similar to the observations of Okomoda *et al.* (2013) and Aderolu *et al.* (2010) when different freshwater fishes were exposed to pollutants under laboratory conditions. There was an increase in MCH and MCV with an increase in concentration. The WBC decreased with an increase in concentrations of *B. sapida* seed powder but increased in T4, where the highest mortality was observed. This increase may serve as a means by which the fish is fighting against the toxicant in the blood, because WBC is an antigen that fights any unwanted microorganisms in the body. The increases in WBC are in confirmation of the observation of Oladimeji *et al.* (2022) that *B. sapida* seeds have immunostimulatory effects, thus increasing the production of antibodies and activating immune cells. These results of the haematological parameters of this work are in conformity with what was observed by Adene *et al.* (2019) on exposure of *Heterobranchius bidorsalis* to sodium hypochlorite.

The behavioural responses observed in *C. gariepinus* juveniles on exposure to *B. sapida* seed powder demonstrated a sensitive indication of physiological stress in fish, such as skin colouration, air gulping, and loss of reflex, erratic swimming, barbell deformation, motionlessness, and finally death. The behavioural responses showed tolerant variations to *B. sapida* seed powder with increasing concentrations, which are in

conformity with the reports of some researchers (Adene *et al.*, 2019, and Ayotunde *et al.*, 2011) on the toxicities of some plant seeds and chemicals to *C. gariepinus* juvenile freshwater fish.

## 5. Conclusion

The study presented significant risks to fish health with significant/major decreases in most haematological parameters, thus suggesting anemia or impaired erythropoiesis. An increase in white blood cell count (Leucocytosis) indicated possible stress or inflammation in the fish, which may be attributed to toxins present in the seed. Its application as a botanical or piscicidal or as a toxic product to other aquatic organisms, such as toads/frogs, turtles, during pond preparations should be investigated.

## Funding

There is no funding obtained for this research work.

## Competing Interest

There is no competing interest for this research work.

## Data Availability Statement

Available upon request.

## Ethics Approval

This study did not involve the use of human subjects.

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