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# Toxicological Influence of Aqueous Tephrosia bracteolata Leaf Extract on Haematological and Biochemical Parameters in Clarias gariepinus Juveniles

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**Abstract**

Fish farmers traditionally explore plants to catch fish from the wild without knowing the exact dose that would not pose a threat to the fish. The study investigated impacts of varied concentrations (8.00, 4.00, 2.00 and 1.00ml/L and 0.00 (the control) in replicates, using completely randomized design (CRD) and injection method, for a duration of 96hrs) of aqueous leaf extract of Tephrosia bracteolata impacts on haematology, blood serum biochemistry and mortality rate of Clarias gariepinus using 200 juveniles. The data were analysed for mean, standard deviation, and Pearson's correlation using IBM SPSS version 23. Results indicated determination of Alkaloids 52%, Saponin 31%, Flavonoids 29%, Phenol 27%, Glucosides 18% and anthraquinone 12%; and the treatments' $LD_{50}$ was 2 mL (log concentration of 0.301). Unlike in control, levels of the PCV, Hb, RBC, and WBC decreased as the treatments increased, with a strong and direct correlation among the four haematological indices; the glucose levels increased while cholesterol and protein decreased as the treatments increased, with the glucose levels having a very strong but indirect correlation with protein and cholesterol levels. The protein level was strongly correlated ($r = 0.922$, $p < 0.01$) with cholesterol. The study concluded that the plant induced dose-dependent physiological stress on the experimental fish. There was no further observation post-treatment, and cautious usage of the plant around the fish habitat and for fish harvesting by the fish artisans is recommended.

**Keywords:** One health, aquatic toxicity, phytochemicals, post-treatment, plant

## Introduction

Fish production has increased due to its affordability as a protein source compared to animal-based alternatives. The production of aquatic animals in a controlled aquatic environment for human consumption, renewal of fish abundance, and other uses is widely practised among farmers (Robertson and Smith-Vaniz, 2008). One of the fish in the Nigerian aquaculture sector is the catfish (*Clarias gariepinus*), which belongs to the family Clariidae, i.e., the air-breathing catfish possessing large accessory breathing organs composed of modified gill arches (Froese and Pauly, 2014). It can be stocked in a low-oxygen aquatic or polluted habitat, giving it the potential to thrive in water us-

ing the accessory breathing organ.

organic compounds belonging to different classes have been isolated from the plant leaf sample; some have been found useful for their pharmacological properties, while others are still unknown for their effects (Samuel et al., 2019). The study by Dahiya and Jain (2000) encountered aquatic and fish poisoning from the plants, containing highly toxic chemicals, in the target organ. Yet, the study of Okokon et al. (2005) reported leaf alkaloids as an active fraction against microorganisms. It is explored by farmers to improve soil fertility and control pests against storage crops and livestock as a pesticide. Its use in fish harvesting is pronounced among the fish farmers, just like most plants which contain chemicals and are traditionally used to harvest fish across the developing and low-income nations (Yadav and Singh, 2001). Preparation of the leaf extract to catch fish in the wild is quite easy, and the cost implication is affordable, leading to indiscriminate use by some farmers, especially in developing countries. WHO (2007) typified the determination of high plant rotenone posing toxicological threats to fish gills, trachea, skin, or gastrointestinal tract from its lipophilic characteristics. Agbon et al. (2004) discovered that different plant species, including *Tephrosia*, contain phytochemicals with possible threats to aquatic life, including fish.

*Tephrosia* is a genus of flowering plants in the pea family, Fabaceae, and is found in tropical and warm temperate regions. Hoary pea is a common name for plants in this genus, whose many species are poisonous, particularly to fish. The black seeds of *Tephrosia* species have been used as fish toxins. The genus has three subgenera, including *Marconyx* (e.g., *T. tenuis*), *Brissonia* (e.g., *T. candida*), and *Reineria* (e.g., the rest of the species of *Tephrosia*) (Lakshmi et al., 2008). *Tephrosia bracteolata* is native to tropical Africa, growing best in a nearly 27 °C moderate temperature environment, and is adaptable to many places, including roadsides (Ngegba et al., 2007). *Tephrosia bracteolata* is a flowering plant consisting of more than 300 species distributed across the tropical and subtropical regions of the world (Alghamdi 2013). *T. bracteolata* manifests different biological activities such as anti-diabetic, anti-fungal, antidiarrheal, wound healing, insecticidal, antiviral, anti-ulcer, anti-protozoal, anti-plasmodial, anti-inflammatory, and many others (Chen et al., 2014).

The presence of poisonous plants around and in the aquatic environment poses serious risks to aquaculture, thereby obscuring the cause of fish mortality. The indiscriminate use of leaves and their extracts as piscicides in water bodies has also led to fish loss and mortality of important aquatic life. Thus, plant-derived toxins serve as natural piscicides and are effectively used instead of manufactured pesticides. Given the widespread use of *Tephrosia bracteolata* in artisanal fishing, this study aims to assess the toxicological effects of the aqueous extract of *T. bracteolata* leaves on the haematological and biochemical responses of *Clarias gariepinus* juveniles.

## MATERIALS AND METHODS

### Collection and Acclimatisation of Clarias gariepinus Juveniles

Two hundred (200) juveniles of *Clarias gariepinus* were procured from the Fatola Farm, Camp Junction, Abeokuta, Ogun State, Nigeria. The fish were transported in a 25 L plastic keg (half-filled with water) to the Federal University of Agriculture, Abeokuta (FUNAAB) hatchery section in the morning (around 8:00 AM).

The juveniles of *Clarias gariepinus* were acclimatised under normal conditions for 21 days (three weeks) and fed with 2 mm Aller-aqua feed. Feeding was stopped 24 hours before the start of the experiment. The fish were placed into ten separate rectangular plastic tanks with dimensions of 12 cm by 24 cm, with a water depth of 20 cm.

The cultured water was replenished every morning (i.e., a renewal bioassay was adopted). The test fish were fed throughout the 96-hour period of the experiment. Dead fish were immediately removed to prevent

pollution.

## Collection and Identification of Plant Sample

Mature leaves of Tephrosia bracteolata were harvested from the VC's lodge at FUNAAB. The leaf samples were identified and authenticated at the Forestry Department at FUNAAB.

## Phytochemical Analysis of Plant Sample

The phytochemical composition was analysed at the Animal Science Laboratory of the University of Ibadan (UI), Ibadan, Nigeria, to determine the percentage of phenols, alkaloids, tannins, saponins, rotenone, flavonoids, steroids, glycosides, and anthraquinone in the leaf sample.

## Preparation of Tephrosia bracteolata Leaf Extract

The leaf samples were air-dried for five (5) days at room temperature. The dried leaf samples were then weighed to give a total weight of 50 grams. The air-dried leaf samples were ground using an electric blender to increase their surface area, and then sieved to easily infuse with solvent for extraction. The blended leaf sample was soaked in distilled water (500mL), thoroughly shaken, and left for 24hours for effective extraction. The mixture was filtered into a bowl using a muslin cloth, and the filtration process was repeated twice to ensure the clarity of the stock solution. The filtrate was used as the stock solution.

## Determination of Physico-Chemical Parameters

Using the calibrated multi-parameter Hanna Instrument (Model HI 98129) and DO meter (PCE-DOM Series oxygen meter), measurements of temperature, hydrogen ion concentration (pH), and dissolved oxygen (DO) concentration were made for each treatment group.

## Bioassay test

The bioassay test to determine the 96h acute toxicity of aqueous extracts of Tephrosia bracteolata leaves on juvenile Clarias gariepinus was conducted following the bioassay procedures of Panuganti (2015). Dilutions were made from the stock solution at the concentrations of 8.00, 4.00, 2.00, and 1.00mL treatments, and assumed 0.00mL as the control for comparison. 5ml of each concentration was infused into the plastic tanks during the 96 hr of the experiment. Each treatment was replicated in triplicate, with observations recorded at 24 hour intervals. The solution was stored at room temperature owing to the limited period before the commencement of the experiment.

## Haematological examination

The blood sample between 1.00 and 1.50 mL was drawn from the caudal peduncle of the fish treated with the aqueous extracts of Tephrosia bracteolate leaf. The blood samples were collected into 10 mL EDTA-treated tubes to estimate PCV and erythrocyte counts, and serum biochemistry tests.

## Erythrocyte count (red blood cell)

The most common diluent used for the red blood cell (RBC) count is formol-citrate. The drawn blood sample was diluted by washing 20.00 $\mu$L of the blood sample into a shellback pipette with 4.00mL of formol-citrate diluent to give a dilution of 1 in 20 $\mu$L. The diluted sample was then mixed thoroughly and loaded into the counting chamber.

## Leucocyte count (white blood cell)

A 2% acetic acid solution with gentian violet was used as the diluent for the white blood cell (WBC) count. The collected blood sample was diluted by washing 50.00 $\mu$L of it into a shellback pipette containing 950.00 $\mu$L of the diluent, to give a final dilution of 1 in 20 $\mu$L. The diluted sample was thoroughly mixed and then loaded into the counting chamber.

### Packed cell volume

Blood was poured into a simple capillary tube until it was about ¾ full. Plasticine was used to seal the tube's open end. The sealed tube was centrifuged at exactly 12,000 revolutions per minute for five minutes in a Hawksley micro-hematocrit centrifuge. The packed cell volume value was determined and expressed as a percentage for each tube after placing it in a micro hematocrit reader.

### Haemoglobin

Haemoglobin concentration was measured spectrophotometrically using the Randox kit. The reagent contained potassium ferricyanide (0.61 mmol/L), potassium cyanide (0.77 mmol/L), potassium phosphate (1.03 mmol/L), and 0.1% v/v surfactant. **Procedure:**
The test tubes were labelled as blank, standard, and test. A 20 $\mu$L sample of whole blood was added to the respective tubes. In addition, 5 mL of the reagent was added to each tube and incubated for 3 minutes at room temperature. The absorbance of all tubes was then read at 540 nm against the reagent blank.
Haemoglobin concentration:

$$HC = Absorbance of sample \times 36.77$$

### Blood Serum Biochemistry

The effect of the leaf extract on glucose, protein, and cholesterol levels was determined using a multichannel analyser (SYNCHRON, Los Angeles, CA), following the method described by Christopher et al. (2016).

### Statistical Analysis

Descriptive statistics were performed to obtain the mean and standard deviation of the data. The mean values were separated using inferential statistics, specifically one-way ANOVA and Duncan's Multiple Range Test, at a significance level of $p < 0.05$, with IBM SPSS Statistics 22. Pearson's correlation was computed to determine possible associations among the assessed parameters. Furthermore, Probit Analysis was applied to analyse the relationship between the aqueous leaf extracts and the quantal response of the experimental fish, thus determining the $LD_{50}$.

## Results

### Phytochemical screening

Qualitative analysis of the aqueous extract of *Tephrosia bracteolata* leaves revealed the presence of various bioactive compounds, with relative concentrations suggesting potential toxicity. Alkaloids were the most abundant, constituting 53.00% of the total phytochemical content (Table 1).

### In situ Water Quality Parameters

Results of the in situ water quality measurements indicated that the temperature remained relatively constant across all treatments. Dissolved oxygen (DO) levels, however, were considerably lower than the typical requirements for fish culture. A clear trend was observed, whereby DO increased with a decrease in the concentration of the aqueous leaf extract. This reduction in DO may be attributed to the interference of the treatments, which likely acted as pollutants and reduced overall water quality. The decrease in DO was statistically significant ($p < 0.05$) across treatments (Tables 2 and 3).

### Haematological profile of the experimental fish

The levels of PCV, Hb, RBC, and WBC significantly ($p < 0.05$) decreased as the aqueous extract concentration of *Tephrosia bracteolata* leaf increased (Table 4). This implies that increasing concentrations of the aqueous extract induced a reduction in the haematological parameters (packed cell volume, haemoglobin, red blood cell, and white blood cell) of the experimental fish. The trend suggests a dose-dependent haematological suppression, which is likely indica-

tive of physiological stress. These reactions were further supported by Pearson's correlation analysis (Table 5), which showed very strong and positive relationships among the haematological indices.

## Blood serum biochemistry

Table 6 showed that the levels of glucose significantly ($p < 0.05$) increased with an increase in the aqueous extract of Tephrosia bracteolate leaf concentrations. Conversely, levels of cholesterol and protein significantly ($p < 0.05$) decreased with an increase in the treatments. Fish exposed to higher concentrations showed elevated glucose levels, possibly as a physiological response to stress, which in turn triggered a physiological response in the fish, prompting them to attempt to adapt by upregulating protein synthesis and lipid metabolism to repair damaged tissues. To clarify the observation, Pearson's correlation buttressed that the glucose level had a very strong and indirect relationship with both cholesterol and protein contents at a 0.01 significance level. The relationship between both protein and cholesterol contents was very strong and direct.

## Mortality observation of the experimental fish and the mean lethal concentration

The experimental plastic indicated the highest mortality (four) in the highest treatment (8.00 mg/ L), and the trend reduced over the study period. However, the 2.00 mg/ L treatment had an irregular mortality rate of the experimental fish (Figure 1). The lethal dose (LD50) of the aqueous extract of Tephrosia bracteolate leaf at which 50.00% of the experimental fish died was 2.00 mg/ L (log concentration of 0.301). The line of best fit for its equation is at Y = 0.40 x + 0.80 ($R^2$ = 0.80) (Figure 2).

Table 1: Phytochemical analysis of Tephrosia bracteolata

| Phytochemical | Quantity (%) | Observation | Remarks |
|---|---|---|---|
| Alkaloids | 52.00 | 5 | A |
| Tannin | 0.87 | 1 | S |
| Saponin | 31.00 | 4 | A |
| Phenol | 27.00 | 3 | H |
| Glycosides | 18.00 | 2 | M |
| Steroids | 0.65 | 1 | S |
| Anthraquinone | 12.00 | 2 | M |
| Flavonoids | 29.00 | 3 | H |
| Rotenone | 0.92 | 1 | S |

S: Slight (1), M: Moderate (2), H: High (3), A: Abundant (4–5).

Table 2: Results of the cultured water in-situ parameters during 96 h of the experiment

| **Conc. (ml/L)** | **pH** | **Dissolved oxygen (mg/L)** | **Temperature (°C)** |
|---|---|---|---|
| 8.00 | $7.10\pm0.00^{a}$ | $1.33\pm0.15^{a}$ | $25.40\pm0.10^{a}$ |
| 4.00 | $7.23\pm0.05^{b}$ | $2.30\pm0.00^{b}$ | $25.23\pm0.05^{ab}$ |
| 2.00 | $7.36\pm0.05^{c}$ | $2.50\pm0.10^{c}$ | $25.13\pm0.05^{ab}$ |
| 1.00 | $7.50\pm0.00^{d}$ | $3.13\pm0.05^{d}$ | $25.30\pm0.17^{b}$ |
| Control (0.00) | $7.43\pm0.05^{d}$ | $3.23\pm0.15^{d}$ | $25.40\pm0.10^{b}$ |

*Note: Mean ± standard deviation. Values with the same superscript down the column were not significantly ($p > 0.05$) different. In other words, $a$, $b$, $ab$, $c$, and $d$ indicate significant differences.*

Table 3: Pearson's correlation among the cultured water in-situ parameters

| | **pH** | **Dissolved oxygen** | **Temperature** |
|---|---|---|---|
| pH | 1 | | |
| Dissolved oxygen | 0.926** | 1 | |
| Temperature | -0.092 | -0.036 | 1 |

*Note: Correlation is significant at the 0.01 level (2-tailed). Correlation strength: 0.00–0.19 very weak, 0.20–0.39 weak, 0.40–0.59 moderate, 0.60–0.79 strong, 0.80–1.0 very strong.*

Table 4: Phytochemical analysis of Tephrosia bracteolata

| **Conc. (ml/L)** | **PCV (%)** | **Hb (g/dL)** | **RBC ($\times10^{12}$/L)** | **WBC ($\times10^{9}$/L)** |
|---|---|---|---|---|
| 8.00 | $20.67 \pm 1.15^{a}$ | $6.80 \pm 0.10^{a}$ | $1.83 \pm 0.11^{a}$ | $7.77 \pm 0.25^{a}$ |
| 4.00 | $25.33 \pm 1.53^{b}$ | $8.70 \pm 0.10^{b}$ | $2.38 \pm 0.36^{b}$ | $8.57 \pm 0.15^{b}$ |
| 2.00 | $31.33 \pm 1.53^{c}$ | $10.30 \pm 0.17^{c}$ | $3.30 \pm 0.18^{c}$ | $9.67 \pm 0.15^{c}$ |
| 1.00 | $38.00 \pm 10.00^{d}$ | $12.30 \pm 0.26^{d}$ | $3.84 \pm 0.03^{c}$ | $11.30 \pm 0.26^{d}$ |
| Control | $35.67 \pm 1.15^{d}$ | $11.33 \pm 0.15^{e}$ | $3.05 \pm 0.02^{d}$ | $10.33 \pm 0.20^{e}$ |

Values are expressed as mean ± standard deviation. Values with the same superscript in a column are not significantly different ($p > 0.05$).

Table 5: Pearson's correlation among the haematological parameters of *Clarias gariepinus*

| | **PCV (%)** | **Hb (g/dL)** | **RBC ($\times10^{12}$/L)** | **WBC ($\times10^{9}$/L)** |
|---|---|---|---|---|
| PCV (%) | 1 | | | |
| Hb (g/dL) | $0.982^{**}$ | 1 | | |
| RBC ($\times10^{12}$/L) | $0.921^{**}$ | $0.936^{**}$ | 1 | |
| WBC ($\times10^{9}$/L) | $0.969^{**}$ | $0.959^{**}$ | $0.852^{**}$ | 1 |

** Correlation is significant at the 0.01 level (2-tailed). Correlation strength: 0.00–0.19 very weak; 0.20–0.39 weak; 0.40–0.59 moderate; 0.60–0.79 strong; 0.80–1.0 very strong.

Table 6: Results of blood serum biochemistry after 96-h exposure

| **Conc. (ml/L)** | **Glucose (mg/dL)** | **Cholesterol (mg/dL)** | **Proteins (g/L)** |
|---|---|---|---|
| 8.00 | $17.60 \pm 0.20^{e}$ | $38.33 \pm 0.21^{a}$ | $27.70 \pm 1.14^{a}$ |
| 4.00 | $15.63 \pm 0.31^{d}$ | $42.60 \pm 0.36^{b}$ | $34.90 \pm 1.51^{b}$ |
| 2.00 | $14.37 \pm 0.35^{c}$ | $50.03 \pm 0.50^{c}$ | $37.17 \pm 0.87^{c}$ |
| 1.00 | $12.50 \pm 0.20^{b}$ | $55.30 \pm 0.10^{d}$ | $40.27 \pm 0.86^{d}$ |
| Control | $6.87 \pm 0.16^{a}$ | $56.33 \pm 0.15^{e}$ | $39.33 \pm 0.42^{d}$ |

Values are expressed as mean $\pm$ standard deviation. Values with the same superscript in a column are not significantly different ($p > 0.05$).

Table 7: Pearson's correlation among blood serum biochemical parameters of *Clarias gariepinus*

| | **Glucose (mg/dL)** | **Cholesterol (mg/dL)** | **Proteins (g/L)** |
|---|---|---|---|
| Glucose (mg/dL) | 1 | | |
| Cholesterol (mg/dL) | -0.865** | 1 | |
| Proteins (g/L) | -0.744** | 0.922** | 1 |

** Correlation is significant at the 0.01 level (2-tailed). Correlation strength: 0.00–0.19 very weak; 0.20–0.39 weak; 0.40–0.59 moderate; 0.60–0.79 strong; 0.80–1.0 very strong.

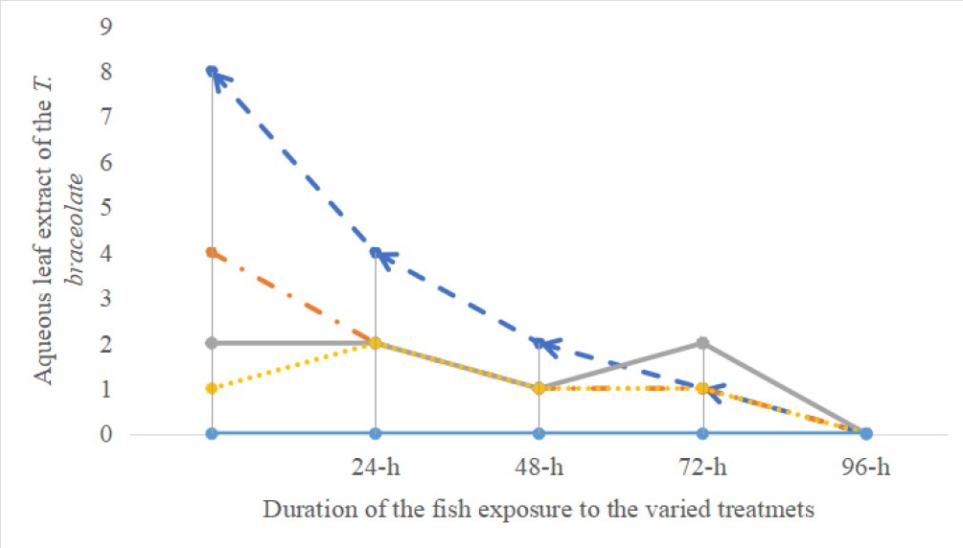


Figure 1: Mean Mortality of the *C. gariepinus* Juvenile

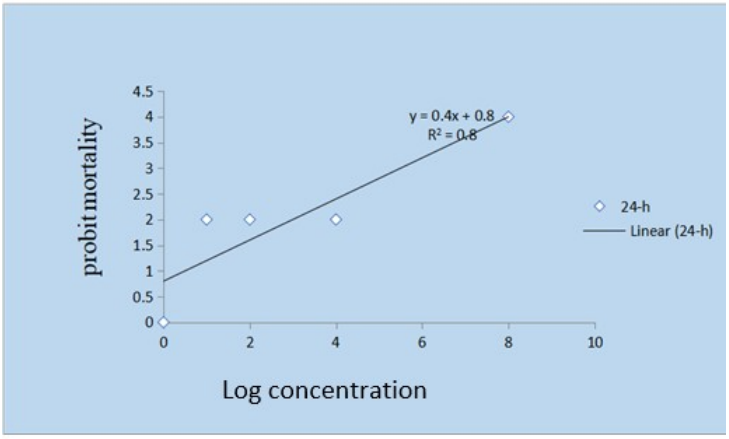


Figure 2: Relationship between probit mortality and log conc. of the aqueous extract of *Tephrosia bracteolate* leaf.

## DISCUSSIONS

The in-situ water quality parameters revealed dissolved oxygen (DO) levels ranging between 1.33 and 3.23 mg/L, values consistent with the previously reported range of 1.00–3.00 mg/L, and indicative of sub-lethal effects on the test fish. The low DO levels observed in this study could be attributed to the accumulation of the aqueous extract in the water (Anku 2017). Pollutants are known to directly impair fish gill function, thereby reducing oxygen uptake. Breitburg et al., 2001) similarly reported that reduced DO concentrations in aquatic environments increase fish mortality due to air gulping, a response also noted in the present experiment. While other water quality parameters remained suitable for fish growth and survival, the increasing concentration of *Tephrosia bracteolata* extract contributed to the observed stress responses.

Phytochemical analysis confirmed that *T. bracteolata* contained a relatively low level of rotenone (0.92% of total phytochemicals). Although rotenone is only mildly toxic to humans and mammals, it is highly toxic to aquatic organisms. Wynne and Masser (2010) demonstrated that rotenone concentrations of 1.00–3.00 mg/L (0.003%) are lethal to fish, causing up to 50% mortality in cultured species. This supports the present findings, where the aqueous extract of *T. bracteolata* leaves produced an $LD_{50}$ value of 2.00 mg/L, while the highest mortality occurred within the first 24 h at the highest treatment concentration (8.00 mg/L). Thus, the determined rotenone content (0.92%) in the extract likely accounted for the recorded mortality.

Hematological parameters, including PCV, Hb, RBC, and WBC, showed significant reductions with increasing concentrations of the aqueous extract, corroborating the results of Ramesh and Saravanan (2008), who observed similar declines in *Cyprinus carpio* exposed to aqueous plant extracts. The observed mortality in *Clarias gariepinus* juveniles in this study may therefore be attributed to the combined effects of reduced DO levels and the phytochemical-induced suppression of hemoglobin, impairing oxygen transport. Akpa et al. (2010) similarly reported reduced hematological indices and increased mortality in tilapia juveniles exposed to *Tephrosia* leaf extracts.

In terms of blood serum biochemistry, glucose levels in the experimental fish increased significantly with increasing concentrations of *T. bracteolata* extract. Elevated blood glucose is a typical stress response, often mediated by cortisol-induced gluconeogenesis, which reduces glucose uptake by tissues and raises circulating levels (Malini et al., 2018). This response reflects the increased metabolic demand required to fuel osmoregulation and maintain homeostasis under stress. Conversely, both protein and cholesterol levels decreased with rising extract concentrations. The reduction in cholesterol could be attributed to alkaloid interference, consistent with the findings of Kou et al. (2016) and Prasetyastuti et al. (2016), who reported cholesterol depletion in fish exposed

to alkaloid-rich plant extracts.

Behavioral changes were also evident. Within 24 h, the fish exhibited surface-oriented respiration and lethargy—symptoms consistent with hypoxic stress. Such responses were also observed by Akpa et al. (2010) in tilapia exposed to *Tephrosia* extracts. Activity levels progressively declined over the 96-h exposure period, culminating in mortality. However, complete mortality (100%) was not achieved across treatments. The highest recorded mortality was 70% at the 8.00 mg/L concentration.

Overall, the present findings highlight that *T. bracteolata* extract exerts dose-dependent physiological, biochemical, and behavioral impacts on juvenile *Clarias gariepinus*, with rotenone content, hematological suppression, and hypoxia contributing synergistically to fish mortality.

## RECOMMENDATIONS

The study concluded that the aqueous extract of *Tephrosia bracteolata* leaves posed a threat to the experimental fish across treatments. Abnormal physiological behaviors were observed, with severity increasing at higher concentrations. Hematological indices, blood serum biochemistry, and mortality (with $LD_{50}$ at 2.00 mg/L and log concentration of 0.301) affirmed the toxic potential of the extract.

Based on these findings, it is recommended that fish farmers exercise caution regarding both deliberate and accidental exposure of cultured fish to *T. bracteolata* leaf extracts, and carefully monitor pond environments for phytochemical contamination. Furthermore, future studies should investigate the histopathological effects of *T. bracteolata* on fish to better understand organ-level toxicity mechanisms.

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