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Acute Toxicity of Tuber Peels of *Tacca leontopetaloides* on the Biochemistry and Histology of Gills and Liver of *Clarias gariepinus* Juveniles

Bala S. Audu^{1*}, Jamiu O. Omirinde², Audu I. Wakawa³, Ibrahim A. Dalhatu¹, Jacinta B. Sale¹

¹Department of Zoology, Faculty of Natural Sciences, University of Jos, Nigeria

²Department of Anatomy, Faculty of Veterinary Medicine, University of Jos, Nigeria

³Department of Biology, Umar Suleiman College of Education, Gashua, Yobe State

*Correspondence should be addressed to Audu B. Sambo: audusambo@yahoo.com

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Abstract

Background: Increased rate of water pollution has detrimental effects on the health of fish and other aquatic organisms. This invariably affects humans by causing food insecurity, hunger and poverty. In an effort to curb this menace, this study investigated the effects of crude tuber peel extract of *Tacca leontopetaloides* on the biochemical and histological parameters of *Clarias gariepinus*.

Methods: Acute toxicity (96 hr. LC₅₀) test was conducted after a range finding test from which definitive test concentrations of 5.00, 4.00, 3.00, 2.00, and 1.00 g/L were obtained with 0.00 g/L as control. One hundred and twenty (120) mixed sex *C. gariepinus* fingerlings (mean weight, 12.5 g ± 0.5; length, 13.2 cm ± 0.5) were administered definitive concentrations of crude tuber peel extract in duplicate replication. Water quality parameters (pH, dissolved oxygen, free carbon dioxide, alkalinity), tissue enzymes (Alkaline phosphatase (ALP), Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST) and histopathological assessments were analysed using standard procedures.

Results: Physico-chemical parameters (pH, dissolved oxygen, free carbon dioxide and alkalinity) of test tanks containing graded concentrations of *T. leontopetaloides* were significantly altered (p<0.05) compared to the control tank. Similarly, the profile of hepatic enzymes (ALP, AST and ALT) displayed marked progressive dose-dependent increase levels relative to the control. Histopathological examination revealed graded deterioration of gills and liver architecture with increase in the strength of the extract *T. leontopetaloides*.

Conclusion: The crude tuber peel extract of *T. leontopetaloides* is toxic to fish, thus, washing, processing and discard of *T. leontopetaloides* in and around riparian systems should be discouraged.

Keywords: Acute toxicity, Polynesian arrowroot, organs, enzymes and tissue pathology, African catfish

1.0 INTRODUCTION

Water pollution is the contamination of water bodies such as lakes, rivers, oceans and groundwater by anthropological activities either directly or indirectly [1]. Agrawal *et al.*, [1] further stated that all forms of water pollution affect organisms that inhabit such water bodies by damaging not only the individual species and populations but also the natural biological communities. These deleterious effects occur when pollutants are discharged directly or indirectly into water bodies without adequate treatment to remove harmful constituents. Water pollutants (plant extracts, heavy metals, and industrial effluents) have two poisoning mechanisms on fish, one occurring at high concentrations and provoking a rapid suffocation by destruction of the gill epithelium and the other prevailing at low concentrations which inhibits the main metabolic pathways such as gills, kidney and liver [2].

Tacca leontopetaloides commonly known as Polynesian arrowroot [3] is a species of flowering plants of the family Dioscoreaceae [4]. It is naturally distributed across West Africa, Southeast Asia and northern Australia. In Nigeria, the plant is mainly found in the middle belt [5] and in the south western States [3]. *T. leontopetaloides* has been reported as an important food and medicine to Polynesians and Hawaiians [4]. Polynesians, according to Ukpabi *et al.*, [4] apply the starchy content of the plant to wound to stop bleeding and also treat dysentery when mixed with red clay. The authors further reported that Hawaiians eat the raw tubers to treat stomach ailments such as diarrhoea and stop internal haemorrhage in the stomach and colon. Ukpabi *et al.*, [4] further reported that the starchy part of the tuber is used to feed infants. Similarly, Wardah *et al.*, [6] reported that *T. leontopetaloides* was utilized to overcome conditions of famine in South Garut, Indonesia.

Toxicity of *T. leontopetaloides* in mammals has been widely reported [7, 8, 9]. Agbonna *et al.*, [3] also reported *T. leontopetaloides* to contain anti-nutrients which could be toxic to humans. Similarly, Liu *et al.*, [9] reported tuber peels of *T. leontopetaloides* as highly toxic to other animals. Available literature has shown that there is paucity of data on toxicity effects of the crude tuber peel extract (CTPE) of *T. leontopetaloides* on fish. This study was therefore aimed at investigating the acute

toxicity effects of CTPE of *T. leontopetaloides* on biochemistry and histology of gills (the first organ to come in contact with contaminants) and liver (organ responsible for detoxification, biotransformation and excretion of xenobiotics) of *Clarias gariepinus* in a static non-renewable bioassay. *C. gariepinus* was chosen for this study because of its abundance in freshwater system and resilience to aquatic pollution.

2.0 METHODOLOGY

2.1 Experimental Fish (*Clarias gariepinus*)

A total of 120 mixed sex juveniles of *C. gariepinus* (mean weight and length: 12.5 ± 0.5 g and 13.2 ± 0.5 cm) were purchased from Catfish Experts Global Ventures Farm, Zarmaganda, Jos, Plateau State, Nigeria. The juveniles were acclimatized to laboratory condition for 7 days. Prior to the commencement of the experiment, fish were fed with a commercial feed (Multi feeds[®] ; 2 mm) at 3% body weight twice daily. After acclimatization, experimental fishes were transferred into twelve (12) circular plastic tanks with ten (10) fish per tank and fed twice daily (0800 and 1800 hrs). Photoperiod was natural (12 light: 12 dark) and water from all the tanks were changed daily with fresh dechlorinated municipal water.

2.2 Experimental Plant (*T. leontopetaloides*)

The plant material was obtained from Bakin Ciyawa of Quaapan Local Government Area of Plateau State, Nigeria. The plant floral and tuber parts were taken to the Herbarium section of Plant Science and Technology Department, Faculty of Natural Sciences, University of Jos, Nigeria where it was positively identified as *T. leontopetaloides* (L.) Kuntze, with voucher number: JUHN20000322. The plant tubers were peeled with a sterilized knife, air-dried for 7 days to a constant weight. The dried peel was pounded with ceramic mortar and pestle into powder and sieved using a 0.2 mm mesh size sieve to obtain fine particles desired to enhance dissolution water.

2.3 Determination of Water Quality Parameters

Water quality parameters such as temperature, hydrogen ion concentration (pH), free carbon (iv) oxide (CO₂), total alkalinity (TA), and dissolved oxygen (DO) were monitored according to the conventional method of American Public Health Association (APHA) [10].

Temperature was measured every day while other parameters were monitored at the beginning and end of the bioassay.

2.4 Experimental Design

The 96 hr. acute toxicity experimental protocol involved a static non-renewable bioassay as earlier described by Ogueji and Auta [11]. Six (6) rectangular plastic tanks (45x30x25cm) containing sixty (60) mixed sex *C. gariepinus* juveniles (mean weight and length: 12.5±0.5 g and 13.2± 0.5 cm respectively) with each tank housing 10 fish were filled with 10 L of dechlorinated municipal tap water per tank. Five (5) of the tanks were inoculated with various concentrations (5.00, 4.00, 3.00, 2.00, 1.00 g/L) of crude tuber peel extract (CTPE) of *T. leontopetaloides* while the sixth while the sixth tank, the control, was not inoculated but stocked with ten (10) mixed sex juveniles of *C. gariepinus* [12]. The setup was arranged in a randomized block design [13]. Fish were maintained in a natural light regime (12 light:12 dark) and were not fed throughout the 96 hr bioassay. The setup was replicated.

2.5 Preparation of Gills and Liver of *C. gariepinus* juveniles for Biochemical Analyses

Gills and liver were collected and rinsed in distilled water to remove traces of blood [15]. The organs were macerated in normal saline, crushed with laboratory pestle and mortar [15] then centrifuged for 5 minutes at 1000 rpm [16] and the obtained supernatants were transferred into non-heparinised tubes before storage in a refrigerator (0 °C) for biochemical analysis

2.6 Biochemical Analysis

The activities of various enzymes; alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) in the liver and gills of *C.*

gariepinus juveniles were determined using colorimetric procedure earlier described by Reitman and Frankel [14].

2.7 Preparation of Gills and Liver *C. Gariepinus* juveniles for Histopathological Examinations

The routine paraffin wax method and haematoxylin-eosin staining techniques during tissue processing previously described by Drury and Wallington [17] and Avwioro [18] were adopted for the processing and examination of the gills and liver tissues of *C. gariepnus* juveniles exposed to *T. leontopetaloides*

2.8 Data Analysis

Data were analysed using one-way analysis of variance (ANOVA) single classification. Treatment means were separated using Tukey's multiple comparisons test. Level of significance was determined at P=0.05 level of probability

3.0 RESULTS

3.1 Water Quality Parameters of Tanks with *C. gariepinus* Juveniles Exposed to Acute Concentration of CTPE *T. leontopetaloides*

The results of the water quality parameters of experimental tanks are summarized in Table 1. The pH and dissolve oxygen (DO) values in tanks laden with concentrated grades of CTPE significantly decreased ($p<0.05$) with the increasing concentration of the extract. On the other hand, the total alkalinity (TA) levels increased significantly ($p<0.05$) with the rising concentrations of CTPE. There was no marked difference in the free carbodioxide (FCO₂) levels in treated tanks except for significant elevated levels in tanks containing 2 and 3 g/L of the CTPE. With respect to temperature, there was no significant difference ($p=0.59$) in the temperature

Table 1. Water quality parameters of experimental tanks during acute bioassays of *C. gariepinus* juveniles exposed to CTPE of *T. leontopetaloides*

Parameter	Concentration (g/L)					
	0.00	1.00	2.00	3.00	4.00	5.00
pH	6.93±0.04	6.25±0.35	6.43±0.04	6.38±0.11	6.23±0.04	5.83±0.04*
Free CO ₂ (mg/L)	2.63±0.04	2.43±0.04	3.63±0.04*	4.53±0.04*	2.93±0.04	2.03±0.04
TA (mg/L)	39.50±0.71	29.55±0.64	94.00±1.41*	107.50±0.71*	125.50±0.71*	137.00±1.41*
DO (mg/L)	3.53±0.04	3.33±0.04	3.25±0.35	3.42±0.04	2.93±0.04*	1.93±0.04*
Temp (°C)	24.75±0.35	25.55±0.64	25.60±0.57	25.60±0.57	25.60±0.57	25.60±0.57

Values with Asterisks (*) in the row are significantly different compared with the control

of all the tanks laden with concentrated grades of CTPE compared with the control.

3.2 Effect of Acute Toxicity of CTPE of *T. leontopetaloides* on Biochemistry of Gills of *C. gariepinus* Juveniles

The mean values of ALP, AST and ALT of gills of *C. gariepinus* juveniles exposed to *T. leontopetaloides* are presented in Figure 1. Biochemical activities in the gills increased with increase in concentration of the plant extract. The highest mean ALP activity (6587.63 ± 1.94 U/L) was recorded in 4.00 g/L concentration of *T. leontopetaloides* while the lowest value (5957.88 ± 1.94 U/L) was recorded in the control. AST recorded the highest activity (1903.23 ± 1.25 U/L) in treatment concentration 5.00 g/L and progressively decreased to the lowest value (1799.83 ± 2.50 U/L) in concentration 2.00 g/L. Similarly, the highest ALT activity (2011.99 ± 2.50 U/L) was recorded in the highest treatment concentration (5.00 g/L) while the lowest value (1974.86 ± 2.50 U/L) was recorded in treatment concentration 1.00 g/L. There was no significant differences ($p > 0.05$) in the activities of ALP, AST and ALT compared to the control.

Figure 1. Effect of Crude Tuber Peel Extract of *T. leontopetaloides* on Gills Biochemistry of *C. gariepinus* Juveniles

3.3 Effect of Acute Concentrations of CTPE of *T. leontopetaloides* on Liver Biochemistry of *C. gariepinus* Juveniles

The effect of CTPE of *T. leontopetaloides* on liver biochemistry of *C. gariepinus* juveniles is shown in Figure 2. The liver ALP values were significantly reduced ($p < 0.05$) in *C. gariepinus* exposed to 3 and 4 g/L of CTPE of *T. leontopetaloides* compared to other groups. There was no significant difference ($p > 0.05$) in the liver ALP values of the control and cat fish treated with 1 g/L of the extract.

The trend of ALP values appeared to decrease with increasing concentrations of CTPE of *T. leontopetaloides*. There was no significant difference ($p > 0.05$) in the levels of liver AST across the entire exposed groups compared to the control. With respect to ALT levels, there were significant reductions in the hepatic ALT levels in the cat fish exposed to different grades of CTPE of *T. leontopetaloides* compared to the control. However, there was no significant difference ($p > 0.05$) in ALT levels in the liver of *C. gariepinus* treated with the 1 g/L of the extract and the control.

Figure 2. Effect of Crude Tuber Peel Extract of *T. leontopetaloides* on Liver Biochemistry of *C. gariepinus* Juveniles

3.4 Histopathology of Gills of *C. gariepinus* Juveniles Exposed to CTPE of *T. leontopetaloides*

The histopathological outcomes of gills of the test fish exposed to CTPE of *T. leontopetaloides* are presented in Plate 1. There were progressive concentration-dependent increases in histopathological alterations in the gills exposed to various concentrations of *T. leontopetaloides*. The control showed normal gill histo-architecture characterized by patent interlamella space (star), normal primary (arrowhead) and secondary (black arrow) lamellae. Fish exposed to 1.00 and 2.00 g/L of the plant extract showed moderate lamella cell depletion (red arrow) with intact interlamella space (star). There was mild focal lamella cell depletion (red arrow) in gills exposed to 3.00 g/L of the plant extract. Extract concentration (4.00 g/L) was characterized by severe lamellar cell depletion (red arrow), matted water channels (circle outline) and apical lamella clubbing (black arrow) while the highest extract concentration (5.00 g/L) was typified by severe lamella cell depletion and complete histo-architectural distortion (circle outline).

Plate 1: Light micrographs of the gill of *Clarias gariepinus* exposed to acute concentrations of *Tacca leontopetaloides*

A. 0 g/L (Control): Normal gill histo-architecture characterized by patent interlamellar space (star), presence of normal primary (arrowhead) and secondary (black arrow) lamellae and intact ceratobranchial bone (c) **B. 1 g/L, C. 2 g/L:** Mild lamellar cell depletion (red arrow) with intact interlamellar space (star) **D. 3 g/L:** Moderate focal lamellar cell depletion (red arrow) **E. 4 g/L:** Severe lamellar cell depletion (red arrow), matted water channels (circle outline) and apical lamellar clubbing (black arrow) **F. 5 g/L:** Severe lamellar cell depletion and complete histo-architectural distortion (circle outline). Stain: HE

3.5 Histopathology of Liver of *C. gariepinus* Juveniles Exposed to CTPE of *T. leontopetaloides*

The photomicrographs of Liver of *C. gariepinus* juveniles exposed to CTPE of *T. leontopetaloides* are shown in Plate 2. The liver in the control showed normal hepatic histoarchitecture typified by hepatocytes with regular cellular outline, intact cytoplasm and roundish nucleus (arrow). Liver of the juveniles exposed to concentrations of the plant extract also indicated progressive concentration-dependent increase in histopathological alterations. No visible lesions were observed in the liver of *C. gariepinus* juveniles exposed to lower concentrations (1.00 and 2.00 g/L) CTPE of *T. leontopetaloides* whereas concentration the liver exposed to 3.00 g/L of the plant extract showed mild hepatocyte degeneration (circle outline). There was severe sinusoidal congestion (arrow) in 4.00 g/L concentration while the highest concentration (5.00 g/L) was characterized by severe cellular infiltration (arrows) and marked hepatocellular degeneration (circle outline).

Plate 2: Light micrographs of the liver of *C. gariepinus* exposed to acute concentrations of *T. leontopetaloides*.

A. 0 g/L (Control): Normal hepatic histo-architecture typified by hepatocytes with regular cellular outline, intact cytoplasm and roundish nucleus (arrow) **B. 1 g/L, C. 2 g/L:** No visible lesion **D. 3 g/L:** Mild hepatocyte degeneration (circle outline) **E. 4 g/L:** Severe sinusoidal congestion (arrow) **F. 5 g/L:** Severe cellular infiltration (arrows) and marked hepatocellular degeneration (circle outline). Stain: HE.

4.0 DISCUSSION

In any aquatic toxicology study, water quality parameters are determined due to the important function they play in the healthy existence of fish [19] since the whole life process of aquatic organisms solely depends on the immediate environment [20]. Any alteration in water quality may affect lives of the inhabitants [21] and any addition of toxicant may cause fluctuations in the water quality variables [22]. In the present study the significant decrease in DO in water loaded with high concentrations of *T. leontopetaloides* is suggestive of degrading effects of the plant extract on the oxygen molecules [23] since the DO minimum requirement of *C. gariepinus* is 3-4 mg/L [24]. The increase in CO₂ could be due to the higher rate of oxidation of organic materials than the rate of oxygen replenishment [25] leading to respiratory asphyxiation [26]. Also the decrease in DO and increase in CO₂ could have serious impacts on biochemical and histopathological alterations in the gills and liver of *C. gariepinus* juveniles exposed to CTPE of *T. leontopetaloides*.

These findings on dissolve oxygen and free carbondioxide are similar to the report of Audu et al., [27] on acute concentrations of *B. aegyptiaca* exposed to *Oreochromis ni-*

loticus fingerlings. Water with high TA level is considered productive for fish culture [12]. The test water of the present study could be considered as ideal for fish culture since the TA range between 29.55 and 137.00 mg/L is within the recommended level of alkalinity for fresh water system of 5-500 mg/L [28, 29].

Evaluation of organ biochemical parameters are used to ascertain destructive effects of toxicants [30] in fish organs. Plasma enzymes are good pointers in determining ideal range of phytotoxin concentrations [31] in stressed organisms. Enzymes such as ALT, ALP, AST and LDH are analysed to determine different stress conditions in fish [32, 33]. The increase in ALP, AST and ALT in the serum of gills of *C. gariepinus* juveniles exposed to *T. leontopetaloides* in this study is an indication of tissue impairment caused by stress [34].

This result is similar to the findings of Audu *et al.*, [27] after exposing *C. gariepinus* fingerlings to grades of waste dry cell battery. The irregular pattern of activities of ALP, AST and ALT observed in the liver of *C. gariepinus* juveniles exposed to *T. leontopetaloides* in this study is dissonant with the findings of Mekkiawy *et al.*, [35], Al-Asqah *et al.*, [32] and Audu *et al.*, [27].

Changes in histopathology of organs are used to evaluate the effect of pollutants on fish health [36]. Histopathological alterations in gills in the present study are positively related with the concentrations of *T. leontopetaloides* extract. This is in line with the findings of Kumar *et al.*, [37] after exposing *Heteropneustes fossilis* to varied concentrations of Neem extract, Azadirachtin. Gill lesions (lamella cell depletion and complete histo-architectural distortion) observed in the present study corroborate the observations of [38, 39, 12]. Similar lamella depletion has also been reported by Kumar *et al.*, [37]. The liver of the *C. gariepinus* juveniles exposed to concentrations of *T. leontopetaloides* extract also indicated progressive concentration-dependent increase in histopathological alterations. This trend has been similarly reported by Adeyemo [40], Jalaludeen *et al.*, [41], Hadi and Alwan [42] and Rajkumar *et al.*, [43]. The grades in the severity of hepatic lesions (hepatocellular degeneration, sinusoidal congestion and cellular infiltration) observed in this study have further validated the hepatic enzymes results earlier stated and could reflect a remarkable derangement in the normal functioning of the liver. These observations on hepatic histopathological lesions are in agreement with similar

studies reported by Audu *et al.*, [12, 39].

Conclusively, CTPE of *T. leontopetaloides* was found to cause negative effects on physicochemical parameters of water as well as on biochemical and histopathological alterations in the gills and liver of *C. gariepinus* juveniles. Therefore, adequate precautionary measures must be taken when discarding the peels to avoid deposition into aquatic ecosystem so as to maintain healthy aquatic life.

Conflict of Interest

The authors declare that there is no conflict of interest.

Authors Contribution

BSA conceived and designed the study, performed manuscript writing and revision and contributed to financial obligations; **JOO** Performed morphometric analysis, contributed to photomicrographic plates analysis and financial obligations; **AIW** develop and patterned manuscript to conform with journal style and contributed to financial obligations; **AID** performed data collection, contributed to data analysis tools and actively involved in the bench work; **JBS** contributed to data collection and data analysis tools and actively involved in the bench work

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