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Phytochemical Constituents and Histopathological Effect of Fluted Pumpkin on the Gills and Livers of *Clarias gariepinus* (Burchell, 1822)

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Abstract

A good number of plants have been investigated worldwide to evaluate their pesticidal and piscicidal activities. This study evaluated the phytochemical constituents and acute toxicity of root of Fluted Pumpkin (*Telfaira occidentalis*) on *Clarias gariepinus* juvenile under laboratory conditions. Phytochemical constituents of the root of *T. occidentalis* were screened. Juveniles of *C. gariepinus* were exposed to extract water with varying concentrations of *T. occidentalis* root extract of 25 mg L^{-1}, 50 mg L^{-1}, 75 mg L^{-1} and 0 mg L^{-1} (control). The acute concentration levels of the toxicant caused changes in the histology of the gill and liver of the exposed fish. In conclusion, the aqueous root extract of *T. occidentalis* is capable of interfering with the histological parameters of *C. gariepinus* juveniles. It is suggested that the culturing of *T. occidentalis* along water banks with presence of *C. gariepinus* should be monitored.

1. Introduction

Plant extracts are considered promising agents because of their eco-friendliness, high efficiency, ease of availability, rapid biodegradability and reduced toxicity to non-targeted animals [1, 2]. To date, a large variety of plants have been studied around the world to determine their pesticidal and piscicidal properties [1]. Plant extracts are referred to piscicides if they exert toxicological effects on fishes and cause death to these aquatic animals [3]. Plant piscicides are obtained from a variety of plants belonging to different families and species that may vary considerably not only for their taxonomic variations but also for the plant parts used (leaves, barks, fruits, roots and seeds), mode of use, mode of extraction and species of target fishes [4]. Fluted pumpkin (*Telfaira occidentalis*), a vegetable leaf commonly used in traditional medicine has many benefits [5]. Reports have also shown that leaves of *T. occidentalis* have chemo suppressive properties [6]. The bioactive substance of *T. occidentalis* has been validated by cell culture studies and clinical trial of its immense pharmacological potentials [7]. *T. occidentalis* is popularly used in ethnobotany as antidiabetic, antihypertensive, antitumuoric, antioxidant, immunodulator, antibacterial, antihypercholesterolemic, antiparasitic, anti-inflamatory, and in the treatment of central nervous system-related disorders including convulsion [6]. Aqueous extract of *T.*

occidentalis leaves have hepatoprotection against garlic induced oxidative stress [8], while its aqueous and ethanolic extracts have hypoglycemic properties both in normoglycemic and alloxan-induced diabetic rats [9]. Relative to most vegetable, its protein is very high [10]. Leaves of *T. occcidentalis* are rich in minerals, anti-oxidants and vitamins such as thiamine, riboflavin, nicotinamide and ascorbic acid [11]. Its young leaves are rich in magnesium and iron and can be used for treating anemia due to its heamatinic properties [12]. *T. occidentalis* roots have however been reported to be toxic despite its vast usefulness both in traditional parlance and from scientific report [13].

In Nigeria *T. occidentalis* is mostly cultivated on water banks of most catfish ponds [12], where *Clarias gariepinus* is an important contributor to both inland fisheries and aquaculture [14]. The aquatic environs, as well as the plants along the banks, make up the fish environment, therefore the ability of *C. gariepinus* to grow is reliant on the conditions of its surroundings [7]. In order to satisfy the high demand of the leaf and vegetable seed of *T. occidentalis*, it is cultivated along water banks especially those for commercial production of *C. gariepinus* [7]. The pond supplies the needed water for its growth through irrigation, especially in the dry season [15]. In this system, the excretory product of the fish is broken down by micro-organisms and the resultant product inputted into the hydroponic system for plant growth [16]. As the plant utilizes these metabolites, it purifies the water which in turn is used in the aquaculture system for fish production. However, the phytochemicals from the roots of *T occidentalis* especially those from older and dried up plants cultivated for three to five years may be washed into the body of the water with the rains through the irrigation channels and surface run-offs [17]. Phytochemicals are often sources of bioactive compounds essential in drug discoveries [18]. Flavonoids have been reported to have antioxidant and vasodilatory activity which is beneficial in cardiovascular disorder such as hypertension [19]. Terpenoid compounds (including the monoterrpenoids and diterpenoids), have been shown to produce beneficial effects on the cardiovascular system [20].

Histopathological investigations have long been recognized to be reliable biomarkers of stress in fish for several reasons [21]. The gill surface is more than half of the entire body surface area. In fish, the internal environment is separated from the external environment by only a few microns of delicate gill epithelium and thus, the bronchial function is very sensitive to environmental contamination. Gills are the first organs which come in contact with environmental pollutant, highly vulnerable to toxic chemicals because firstly, their large surface area facilitates greater toxicant interaction and absorption and secondly, their detoxification system is not as robust as that of liver [22]. Gills have been frequently used in the assessment of impact of aquatic pollutants in marine as well as in fresh water habitats [23]. The liver was examined because it plays a primary role in the metabolism and excretion of xenobiotic compounds with morphological alterations occurring in some toxic conditions [24]. This study was therefore aimed at evaluating the phytochemical constituents and histopathological effect of aqueous root extract of *T occidentalis* on gills and liver of *C. gariepinus* under laboratory conditions

2. Methodology

2.1 Sample collection and preparation

Fluted pumpkin (*T. occidentalis*) roots were collected from Akinkugbe Farm in Ondo state, Nigeria. The plant was authenticated by a plant taxonomist at Herbarium Unit of the Department of Plant Biology, Faculty of Life Sciences, University of Ilorin, Ilorin, Nigeria and was assigned a voucher number UIAQF/PB/1111. The root of the *T. occidentalis* was air-dried under room temperature. The air-dried root was pounded using a mortar. 50g of *T. occidentalis* was then weighed using a sensitive scale into three glass bottles which was prepared by cold maceration in 500ml of ethyl-acetate added to each bottle and kept for 72 hours. The mixture was decanted and filtered; the filtrate was concentrated in a distiller

to get the crude extract and was stored at room temperature. The experimental protocol and procedures used in this study were approved by the University of Ilorin, Ilorin, Nigeria; Ethical Review Committee and conform with the Guide to the care and use of Animals in Research and Teaching (Ethical Principles for Medical Research: Declaration of Helsinki).

2.2 Qualitative phytochemical screening

The qualitative analyses of the plant constituents were carried out according to the method of Sofowora [25]. The presence of flavonoids, alkaloids, tannins, saponins and terpenoids were tested. To determine flavonoids (Sodium hydroxide test), 100 mg of sample was homogenized by 2ml of methanol and centrifuged for 10 minutes at 10,000 rpm and the supernatant was collected. 200µl of the plant extracts were made up to 1.5 ml using distilled water and added 75µl of 5% $NaNO_2$. The reaction mixture was kept to stand for 5 min and 150 µl of 10% $AlCl_3$ was added to it. The mixture was mixed well and allowed to stand for 5 minutes at room temperature. Thereafter, 0.5ml of 1M NaOH was added and the OD observed against to reagent blank at 510nm. The appearance of a yellow colour indicated the presence of flavonoids.

For the determination of Alkaloids, 5g of sample was mixed with 25 ml of 10% acetic acid in ethanol. The mixture was enclosed and kept for 2 h. Mixture was filtered and filtrate placed on water bath to a quarter of its original volume. Concentrated NH_4OH was added in drops to the extract when precipitation was finished. The solution was allowed to stand, washed with diluted NH4OH, filtered, and the collected dehydrated residue was weighed [26].

The appearance of an orange-yellow precipitate indicated the presence of alkaloid. Alkaloid content was determined by:

$$\% \text{ Alkaloid} = \frac{\text{Weight of precipitate}}{\text{Weight of original sample}} \times 100$$

For the determination of tannins ($FeCl_3$ test), 100 mg of sample was homogenized by 2ml of methanol. Centrifuged for 10 minutes at 10,000 rpm and collected the supernatant. To 1ml of supernatant mixed with 0.5 ml Folin's phenol reagent and 35% Na_2CO_3 of 5ml added and the mixture was kept at room temperature for 5 minutes. The blue color of reaction mixture was observed at 640 nm by UV/visible spectrophotometer. Content of tannin was calculated by calibration curve equation ($y=1.501x+0.102$; $R^2=0.996$) of gallic acid and the results expressed as (mg/g) [27].

For the determination of saponins (Frothing test), an aqueous solution of the extract was shaken and a froth that persisted on warming indicated the presence of saponins. Determination of terpenoids involved the homogenization of 100 mg of sample by 2ml of methanol. Centrifuged for 10 minutes at 10,000 rpm, collected the supernatant. In 100µl of supernatant, added 3ml of the chloroform. Added 200µl of the concentrated sulphuric acid and solution kept at room temperature for 1.5-2hour in dark, for the duration of incubation a reddish-brown color precipitate was formed. Supernatant was decanted without disturbing the precipitate. 3ml of the 95% methanol added and vortex thoroughly until all the precipitate completely mix in methanol. The absorbance was observed at 538 nm against blank, i.e. 95% methanol. Linalool was used as the standard for estimation. Terpenoid content was calculated by the calibration curve equation ($y=1.018x+0.047$; $R2=0.997$) of linalool and results expressed in mg/g [28].

2.3 Fish collection and acclimatization

One hundred and twenty (120) juveniles *C. gariepinus* with mean weight of 13.13±2.27 g were purchased from Mars Fish Farm, Ilorin, Kwara State, Nigeria. Fish were not fed for six hours after transportation after which they were fed using 1.8 mm skretting catfish food (40% crude protein) twice

(morning and evening) in the subsequent days. The tank was half filled with tap water, which had been allowed to stand for 24hours for dechlorination. The water in the tank was replenished with tap water and uneaten food and fecal matter were siphoned out. Dead fish were removed to minimize contamination of water. Feeding of fish was stopped 24hours prior to exposure of acute toxicity of *T. occidentalis* root extract.

2.4 Experimental design

The following concentrations were prepared based on the range finding test [29]:
1 litre =1000g;
1ml = 1mg;
250ml of the toxicant in 10 liters of water = 25mgL^{-1}
500ml of the toxicant in 10 liters of water = 50mgL^{-1}
750ml of the toxicant in 10 liters of water =75mgL^{-1}

Pre-test was conducted to determine the concentration range to be used for the acute toxicity test. One fish was put in a tank at a time and was observed to determine the time mortality occurred. The concentrations of serial dilutions used were arrived at after the fish survived beyond 2 hours on exposure to the toxicant.

Completely randomized design was used, 12 plastic tanks of 35 liters capacity measuring 45.8cm×32.3cm×23.7cm containing 10 liters of water each was used. Three tanks served as control while the remaining nine tanks served as treatment for the test fish. Ten (10) juveniles *C. gariepinus* were randomly distributed to each of the plastic tank and impounded with 10 liters of tap water. Before the commencement of the experiment, the tap water sample was made to stand for two days to reduce the concentration of chlorine. Water quality parameters such as temperature, dissolved oxygen, pH, nitrate (ppm), and nitrite was monitored daily throughout the 96hour exposure using thermometer, digital DO and pH meters.

2.5 Histopathological examination

Fish from each of the test concentrations (75, 50 and 25 mg L^{-1}) were sacrificed and dissected to excise gills and liver. Excised organs were carefully washed of blood stains and kept in specimen bottles containing 0.005 L formal saline [30]. Histopathological examinations were conducted at the Central Research Laboratory, University of Ilorin, Kwara State, Nigeria. Routine paraffin wax method and haematoxylin-eosin staining technique of tissue processing [31] were adopted for the examinations of the excised organs (gills and liver) of *C. gariepinus* exposed to aqueous root extract of *T. occidentalis*. Transverse sections of 2-5µm thick were dried at 60^{o}C and stained with toluidine blue then mounted with DPX [29]. Microphotographs were taken using binocular light microscope equipped with AIPTER-AHDZ600 camera (at x400 magnification).

2.6 Statistical analysis

The data obtained for physico-chemical parameters were subjected to descriptive statistics and one-way analysis of variance. The significantly different means were further separated using Duncan Multiple Range Test using SPSS version 20.

3. Results and Discussion

3.1 Phytochemical analysis

Phytochemical analysis of the aqueous root extract of *T. occidentalis* revealed the presence of flavonoids, alkaloids, tannins, saponins and terpenoids (Table 1). This result corroborated the findings of other authors where these phytochemical compounds exhibited antimicrobials activities [32]. The presence of flavonoids indicates the natural occurring phenolic compound, with beneficial effects in diet as antioxidants and neutralizing free radicals [33]. Alkaloids are used in medicines for reducing headache and fever. Tannins are group of polymeric phenolic compound, which could cause local tumors. Saponins have the properties of precipitating and coagulating red blood cells, anti-inflammatory [34]. Terpenoids were detected in *Moringa pterygosperma* which were reported to be active against antibacterial activity [35].

Table 1. Phytochemical composition of aqueous root extract of fluted pumpkin

Phytochemical constituents	Present or absent
Flavonoids (NaOH test)	+
Alkaloids	+
Tannins ($FeCl_3$ test)	+
Saponins (Frothing test)	+
Terpenoids	+

Key: + present; - absent

3.2 Effect of fluted pumpkin extracts on physicochemical parameters of water

The mean values recorded for the various concentrations compared with those of the control are presented in Table 2. Values of dissolved oxygen with the same superscript for 0 concentration and 25 mg L^{-1} are not significantly different ($P > 0.05$) but vary significantly ($P < 0.05$) from values obtained from concentration of 50 and 75 mg L^{-1}. Values of dissolved oxygen with same superscript for concentrations 50 and 75 mg L^{-1} are not significantly different. Values of pH for concentrations 0 mg L^{-1}, 25 mg L^{-1}, 50 mg L^{-1} and 75 mg L^{-1} are not significantly different at ($P > 0.05$). The values of the water temperature for concentrations of 0 mg L^{-1}, 25 mg L^{-1}, 50 mg L^{-1} and 75 mg L^{-1} are not significantly different. The values of nitrate for concentrations 25 mg L^{-1} and 50 mg L^{-1} are not significant but vary significantly from concentrations 0 mg L^{-1} and 75 mg L^{-1}. The values of nitrate for concentrations of 0 mg L^{-1} and 75 mg L^{-1} are significantly different. The values of nitrite at concentrations of 0mg/L, 25 mg L^{-1}, 50 mg L^{-1} and 75 mg L^{-1} are not significantly different. Statistical analysis showed that the values of temperature, pH and nitrite parameters were within the acceptable range for the fish survival and may not be responsible for the observed disorders [36]. Gaunder [37] recommended pH range of 6.5-9.0. Similarly, Claude [38] recommended a temperature range of 25º C-32 ºC for culture fish species.

Results of the phytochemical analysis show that the root extracts contain toxicants such as an alkaloid which have stimulating effects on the gills thus simulating increased opercula beat which impaired respiration and osmo-regulation [39]. Annune et al. [40] reported that rotenone was extremely toxicant to fish in low concentration. The presence of saponin in the extract impaired the oxygen intake of the exposed fish due to its haemolytic nature[41, 42], lowering the surface tension of the restricted extract and presence of colloidal substances within them [43]. Tannins were reported to be hepatoxic and nephrotoxic due to their ability to precipitate exogeneous and endogenous proteins [25]. This is in disagreement with Sun et al. [44] observation that tannins are beneficial for the remedy of cancer and ulcerated tissues. Islary et al. [45] opined that alkaloid is a capable remedial bioactive substance in plants. Consumption of high alkaloids containing food may cause paralysis and rapid heartbeat which is not

good for health. Gemede and Ratta [46] stated that intake of a high dose of alkaloids will lead to the damage of blood vessels, muscles and other soft tissues and death.

Table 2. Physico-chemical parameters of water

Concentration (mgL^{-1})	Dissolved oxygen	pH	Temperature (°C)	Nitrate	Nitrite
0	5.62 ± 0.25^{a}	7.59 ± 0.08^{a}	25.17 ± 0.15^{a}	33.33 ± 2.89^{c}	1.00 ± 0.00^{a}
25	5.54 ± 0.93^{a}	7.62 ± 0.15^{a}	25.07 ± 0.12^{a}	45.00 ± 5.00^{b}	53.33 ± 10.4^{a}
50	5.32 ± 0.57^{b}	7.82 ± 1.03^{a}	26.26 ± 0.27^{a}	48.33 ± 2.89^{b}	1.02 ± 0.04^{a}
75	4.43 ± 0.93^{b}	7.84 ± 0.22^{a}	25.73 ± 1.02^{a}	53.33 ± 10.41^{a}	1.10 ± 0.21^{a}

Key: Means along the column having the same superscripts are not significantly different at ($p>0.05$)

3.3 Histopathological effects

The effects of toxicants that cannot be quantitatively measured may be described. For instance, the general effects of toxicants on the tissues of organisms can be described by carrying out histopathological analysis of the suspected tissues of a particular organ. In this study, the effects of each of the aqueous concentration on histopathological analysis of *C. gariepinus* were described. Several histopathological changes were observed in the gills and livers of *C. gariepinus* (Fig. 1-8). The degree and levels of lesions were found to be more in fish treated with higher concentrations of aqueous root extracts of *T. occidentalis* because changes were dose-dependent. Fig. 1 shows the photomicrograph of the gill filament of a control fish with normal appearance of gill without observable lesions. Fig. 2 is the photomicrograph of the gill filament of the fish exposed to 25 mg L^{-1} *T. occidentalis* showing moderate diffused lamellae hyperplasia. Fig. 3 presents the photomicrograph section of gill of the fish exposed to 50 mg L^{-1} *T. occidentalis* showing mild lamellae hyperplasia. Fig. 4 is the photomicrograph section of gill of the fish exposed to 75 mg L^{-1} *T. occidentalis* showing marked lamellae and hyperplasia. Fig. 5 shows photomicrograph section of liver of the fish exposed to 25 mg L^{-1} *T. occidentalis* with vacuolation and patchy necrosis of hepatocytes. Fig. 6 presents the photomicrograph section of liver from a control fish showing normal liver appearance with no observable lesion. Fig. 7 is the photomicrograph section of liver of the fish exposed to 50 mg L^{-1} *T. occidentalis* showing diffuse hepatocellular degeneration and necrosis. Fig. 8 shows the photomicrograph section of liver of the fish exposed to 75 mg L^{-1} *T. occidentalis*, with centrilobular vacuolation, patchy necrosis of hepatocytes, mononuclear cellular infiltrates, multifocal degeneration and necrosis of hepatocytes, diffuse and individualization of swelling of hepatocytes necrosis of cryptal and surface enterocyte.

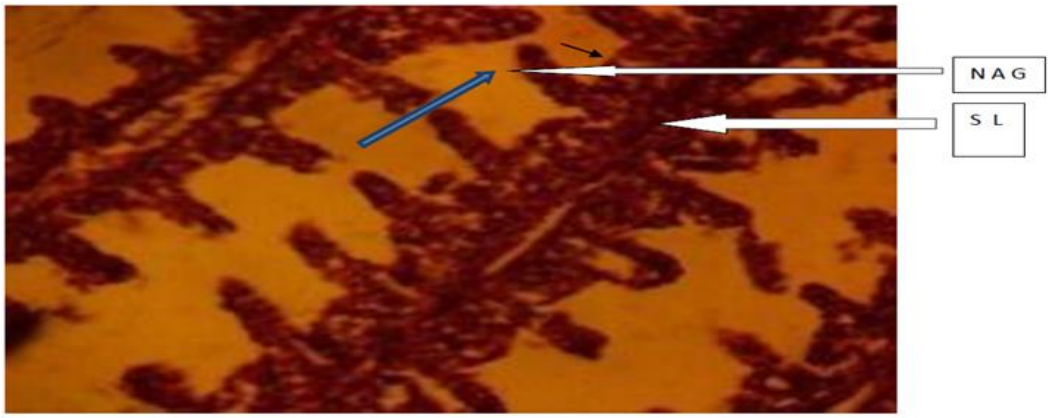

Figure 1. Photomicrograph section of gill of a control *Clarias gariepinus* showing normal appearance of gill with no observable lesion ((Hematoxylin and eosin, x40)

Figure 2. Photomicrograph section of gill of *Clarias gariepinus* exposed to 25 mg L^{-1} *Telfairia occidentalis* showing moderate diffused lamellae hyperplasia (Hematoxylin and eosin, x40)

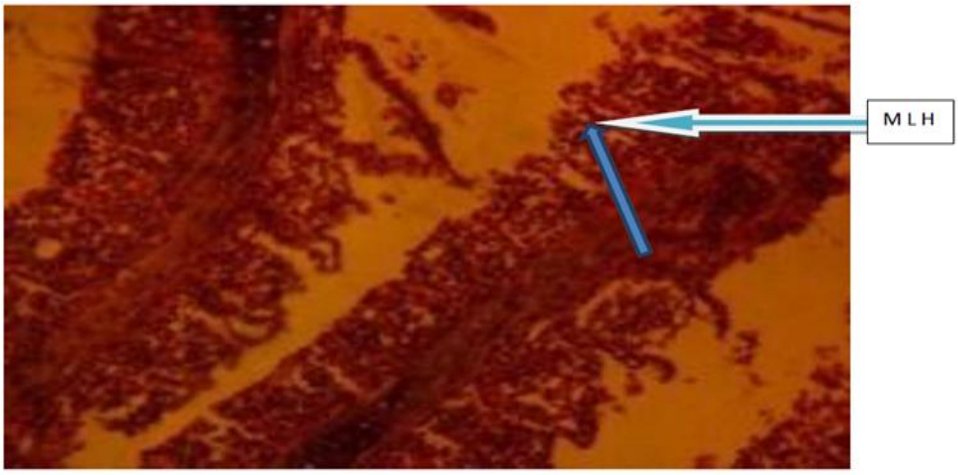

Figure 3. Photomicrograph section of gill of *Clarias gariepinus* exposed to 50 mg L^{-1} *Telfairia occidentalis* showing mild lamellae hyperplasia (Hematoxylin and eosin, x40)

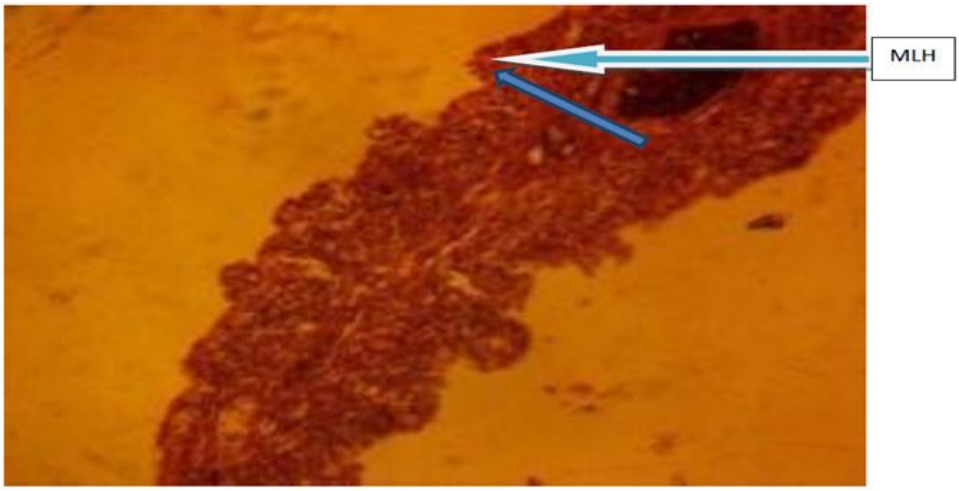

Figure 4. Photomicrograph section of gill of *Clarias gariepinus* exposed to 75 mg L^{-1} *Telfairia occidentalis* showing marked lamellae and hyperplasia (Hematoxylin and eosin, x40)

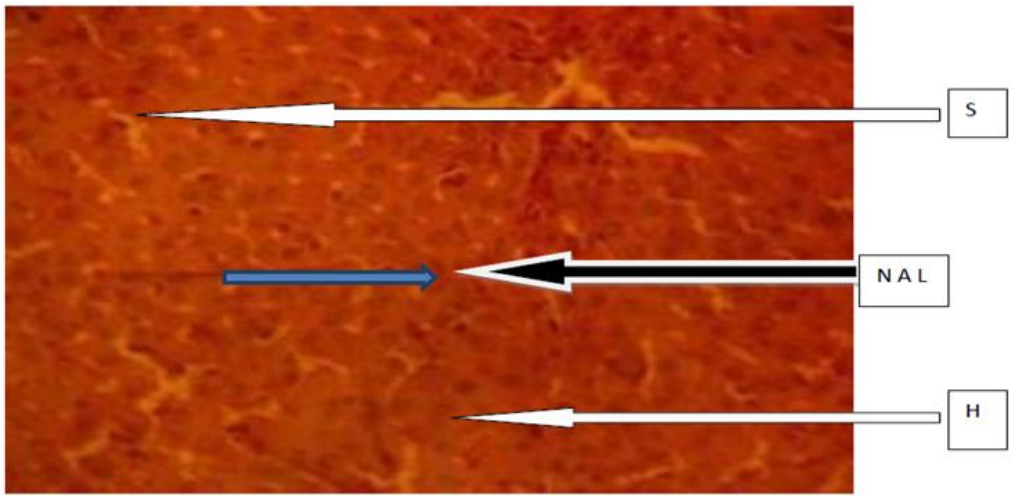

Figure 5. Photomicrograph section of liver of *Clarias gariepinus* exposed to 25 mg L^{-1} *Telfairia occidentalis* showing vacuolation and patchy necrosis of hepatocytes

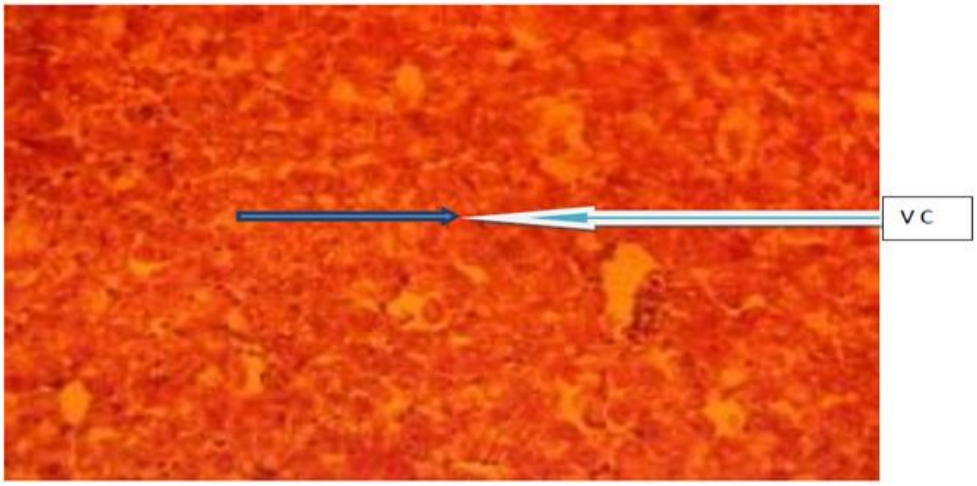

Figure 6. Photomicrograph section of liver from a control of a control *Clarias gariepinus* showing normal liver appearance with no observable lesion (Hematoxylin and eosin x 40)

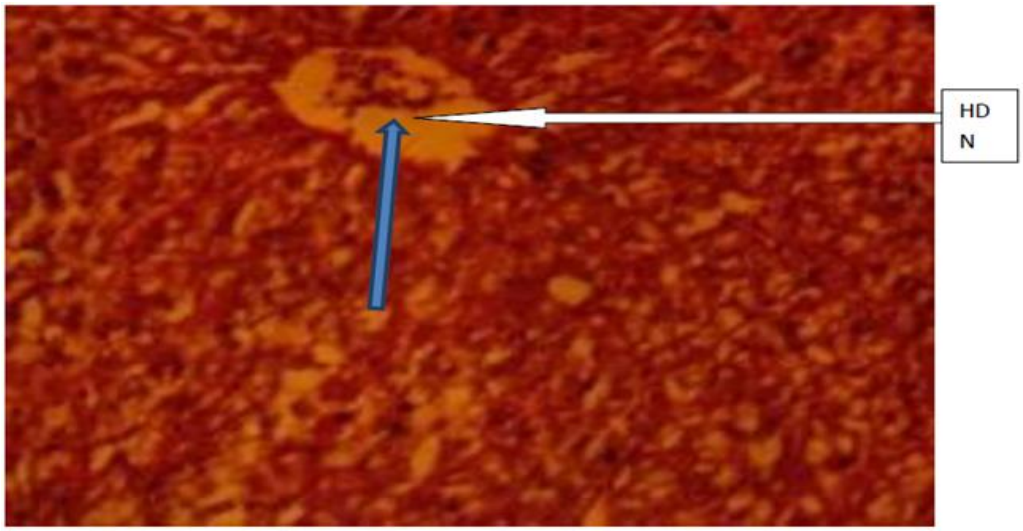

Figure 7. Photomicrograph section of liver of *Clarias gariepinus* exposed to 50 mg L^{-1} *Telfairia occidentalis* showing diffuse hepatocellular degeneration and necrosis (Hematoxylin and eosin, x40)

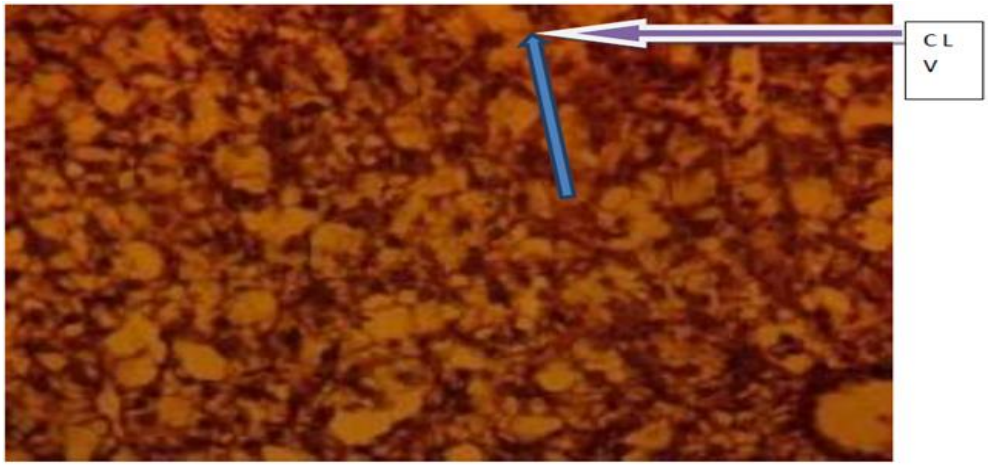

Figure 8. Photomicrograph section of liver of *Clarias gariepinus* exposed to 75 mg L^{-1} *Telfairia occidentalis* showing centrilobular vacuolation, patchy necrosis of hepatocytes, mononuclear cellular infiltrates, multifocal degeneration and necrosis of hepatocytes, diffuse and individualization of swelling of hepatocytes necrosis of cryptal and surface enterocyte (Hematoxylin and eosin, x40)

Photomicrographs of the liver of *C. gariepinus* exposed to varying concentrations of aqueous extract of *T. occidentalis* root extract showed histomorphology distortion of the liver's parenchyma, stoma and sinusoids. The parenchyma is represented by hepatocytes (H), the stoma consisting of connective tissue and vessels while the sinusoids (S) are capillaries traveling between the hepatocytes. Hepatocytes are 80% of the largest polyhedral cells of the liver. The hepatocytes are made up of two-to- four nuclei which are large and spherical.

The acute exposure of *C. gariepinus* juvenile to concentrated grades of *T. occidentalis* induced progressive striking histological alterations in the gill (moderate diffuse lamellae hyperplasia, mild and marked lamellae hyperplasia). Gill of fish plays important function including respiration, osmoregulation and excretion [46] due to its contact with the immediate water environment [47]. This proximity with the external environment predisposes it to histological damages such that the fish becomes vulnerable to respiratory and osmoregulatory difficulties [48] especially when toxicants enter the body and cause damage to gill membranes and affect its physiological functions [49]. Succinctly put, fish exposed to toxicants die when their gill lamella epithelia and blood vessels are adversely affected [50]. Therefore, the observed moderate to severe gill histo-architectural alterations (lamellar vascular congestion, lamellar clubbing, partial to complete inter-lamellar space occlusion and lamellar cell hyperplasia) in this study further established the toxic potential of *T. occidentalis* extract. The gill histopathogical profiles in this study corroborate lesions earlier reported in similar studies [51, 52]. The detoxification and biotransformation gills are the primary corridor for molecular exchange between the internal milieu of fish and their external environment such as gas transfer, acid-base regulation and ionic regulation [14]. The filament of the gills and their secondary lamellae represent two general types of epithelium that contain three cell epithelia, the pavement, the chloride (ionocytes or mitochondria-rich) cells and the mucous cells, which are most prevalent [29].

The control liver had normal internal arrangement components while *T. occidentalis* root extract induced progressive striking histological alterations in the liver of the exposed fish (disorganized hepatocytes, random vacuolation of cytoplasm, diffuse hepatocellular degeneration, necrosis, centrilobular vacuolation, patchy necrosis of hepatocytes, multifocal degeneration, individualization of swelling of hepatocytes, necroses of cryptal and surface enterocytes). The findings from this study are related to previous reports on fish exposure to plant extracts [53], fenvalerate [54], aluminium [55].

The alterations observed in the various plates are indications of its toxic effects. This is in agreement with Omitoyin et al. [17] and Fafioye [56] who exposed *C. gariepinus* and *O. niloticus* to lethal and sublethal concentrations of *Parkia biglobosa* and *Raphia vinifera* respectively.

Conclusion

This study has revealed that aqueous root extract of *T. occidentalis* are toxic to *C. garipinus* at all concentration levels. The histological analysis revealed the occurrence and degree of alterations which were dose dependent to the concentrations of root extract of *T. occidentalis* while organ samples taken from the control groups remained normal throughout the duration of the experiment. The use of *T. occidentalis* root extract in water bodies should be checked to prevent contamination of the aquatic environment as well as the aquatic biotas.

Disclosure statement: *Conflict of Interest:* The authors declare that there are no conflicts of interest.
Compliance with Ethical Standards: This article does not contain any studies involving human subject.

References

[1] B. Akinsanya, O. Utoh and U. D. Ukwa. Toxicological, Phytochemical and Anthelminthic properties of rich plant extract on *Clarias gariepinus*. *Journal of Basic and Applied Zoology,* 74(2016) 75-86.

[2] I.F. Jesuniyi, R. O. Moruf and A. O. Lawal-Are. Immunomodulatory effect of Moringa oleifera Lam. aqueous extract on the burrowing crab, *Cardiosoma guanhumi* (Latreille, 1828). *Nigerian Veterinary Journal,* 41 (3) (2020) 264 – 273.

[3] H. M. Burkill. The useful plants of West Tropical Africa, vol 1-3.Royal Botanic Gardens, Kew (1995).

[4] H. D. Neuwinger. Plants used for poison fishing in tropical Africa. *Toxicon,* 44(2004) 417–430

[5] J. K. Mensah, R. I. Okoli, J. O. Ohaju-Obodo and K. Eifediyi. Phytochemical, nutritional and medical properties of some leafy vegetables consumed by Edo people of Nigeria. *African journal of Biotechnology*, 7(14) (2008) 2304-2309

[6] O. A. Igbeneghu and A. B. Abdu. Multiple antibiotic-resistant bacteria on fluted pumpkin leaves, a herb of therapeutic value. *Journal of Health, Population and Nutrition*, 32(2) (2014) 176–182.

[7] O. A. Eseyin, M. A. Sattar, H. A. Rathore, A. Ahmad, S. Afzal, M. Lazhari and S. Akhtar. Hypoglycemic potential of polysaccharides of the leaf extract of *Telfairia occidentalis*. *Annual Research & Review in Biology*, (2014) 1813-1826.

[8] A. A. Dada, and A. D. Abiodun. Effect of dietary fluted pumpkin (*Telfairia occidentalis*) extract on growth performance, body composition and haematological parameters of Nile tilapia (*Oreochromis niloticus* Linnaeus). *Journal of Fisheries*, 2(3) (2014) 203-208.

[9] T. M. Salman, I. A. Alagbonsi, S. A. Biliaminu, O. A. Ayandele, O. K. Oladejo and O. A. Adeosun. Blood glucose-lowering effect of *Telfairia occidentalis*: A preliminary study on the underlying mechanism and responses. Biokemistri, 25(3) (2021) 54-64.

[10] E. M. Aregheore. Voluntary intake, nutrient digestibility and nutritive value of foliage of fluted pumpkin (*Talfairia occidentialis*) haylage mixtures by goats. *Livestock Research for Rural Development*, 19(4) (2007) 71-79.

[11] A. A. Kayode and O. T. Kayode. Some medicinal values of *Telfaira occidentalis*: a review. *Amer. J. Biochem. Molecular Biol*., 1(2011)30-38.

[12] W. A. Ajibade, I. A. Ayodele and S. A. Agbede. Water quality parameters in the major rivers of Kainji Lake National Park, Nigeria. *African Journal of Environmental Science and Technology*, 2(7) (2008). 185-196.

[13] E. A. Ogbonnaya and P. O. Uadia. Effect of sub-acute exposure to *Telfairia occidentalis* root, pod and stem extracts on some liver and renal function parameters in rats. *Br J Pharm Res*, 11(3) (2016) 1-8.

[14] M. I. Abubakar. Toxicity of 2, 3-dichlorovinyl dimethyl phosphate on respiratory dynamics of *Clarias gariepinus* (Burchell, 1822) under laboratory conditions. *FUW Journal of Agriculture and Life Sciences* 3(1) 2019) 101-107.

[15] B. Zhang, C. Xiao, S. Xie, J. Liang, X. Chen and Y. Tang. Iron–nickel nitride nanostructures in situ grown on surface-redox-etching nickel foam: efficient and ultrasustainable electrocatalysts for overall water splitting. *Chemistry of Materials*, 28(19) (2016) 6934-6941.

[16] R. H. Bosma, L. Lacambra, Y. Landstra, C. Perini, J. Poulie, M. J. Schwaner, and Y. Yin. The financial feasibility of producing fish and vegetables through aquaponics. *Aquacultural Engineering*, 78(2017) 146-154.

[17] B. O. Omitoyin, A. O. Ogunsanmi and B. T. Adesina. Studies on acute toxicity of piscidal plant (*Tetrapheura tetraptera*) extracts on Tilapia (*Sarotherodon galilaeus*) fingerlings, *Tropical journal of Animal Science*, 2(2) (1999) 189-197.

[18] H. H. Kashani, E. S. Hoseini, H. Nikzard and M. H. Aarabi. Pharmacological properties of medicinal herbs by focus on secondary metabolites. *Life Science Journal,* 9(1) (2012)34-42.

[19] P. G. Pietta. Flavonoids as antioxidants. *J. Nat. Prod.,* 63(7) (2000) 1035-1042

[20] U. V. Hipolito, J. T. Rocha, N. B. Palazzin, C. J. Rodrigues, C. C. Crestani, Correa, F.M., Bonaventura, D., Ambrosio, S.R., Bendhack, L.M., Resstel, L.B and C. R. Tirapelli. The semi-synthetic kaurane ent-16alpha-methoxykauran-19-oic acid induces vascular relaxation and hypotension in rats. European Journal of Pharmacology. 660(2011) 402-410.

[21] R. Van der Oost, J. Beyer and N. P. Vermeulen. Fish bioaccumulation and biomarkers in environmental risk assessment: a review. *Environmental toxicology and pharmacology*, 13(2) (2003) 57-149.

[22] G. Barbin, M. Mallat and A. Prochiantz. In vitro studies on the maturation of mesencephalic dopaminergic neurons. *Developmental neuroscience*, 7(5-6) (1985) 296-307.

[23] S. Athikesavan, S. Vincent, T. Ambrose and B. Velmurugan. Nickel induced histopathological changes in the different tissues of freshwater fish, *Hypophthalmichthys molitrix*(Valenciennes). *Journal of Environmental Biology*, 37(2) (2006) 391-395.

[24] E. Rocha, R. A. Monteiro, M. H. Oliveira and M. W. Silva. The hepatocytes of the brown trout (*Salmo trutta* f. fario): a quantitative study using design-based stereology. *Histology and histopathology*, 16(2) (2001) 423-437.

[25] A. Sofowora. Medicinal plants and traditional medicine in Africa.2nd edition. Specimen bookslimited. (1993)134-156

[26] B. E. Omoruyi, G. Bradley and A. J. Afolayan. Antioxidant and phytochemical properties of *Carpobrotu sedulis* (L.) bolus leaf used for the management of common infections in HIV/AIDS patients in Eastern Cape Province. BMC Complementary and Alternative Medicine, 12:215.Schanderl SH. 1970. Methods in food analysis. New York: Academic Press(2012) 709.

[27] S. H. Schanderl. Methods in food analysis. New York: Academic Press (1970) 709.

[28] N. Ghorai, S. Chakraborty, S. Gucchait, S. K. Saha and S. Biswas. Estimation of total Terpenoids concentration in plant tissues using a monoterpene, Linalool as standard reagent. *Nat Protocol Exc*, 5(2012) 1038

[29] M. I. Abubakar. Toxicity of 2, 3-dichlorovinyl dimethyl phosphate (Sniper 1000EC) on *Clarias gariepinus* (Burchell, 1822) and *Oreochromis niloticus*(Trewavas, 1983) under laboratory conditions. Unpublished Ph.D Thesis, Department of Aquaculture and Fisheries Management. Federal University of Agriculture, Abeokuta, Nigeria. (2013) 184.

[30] B. S. Audu, J. O. Omirinde, I. J. Gosomji and P. E. Wazhi. Histopathological changes in the gill and liver of *Clarias gariepinus* exposed to acute concentrations of *Vernoniaa mygdalina*. *Ani. Res. Int.*, 14 (2017) 2576-2587.

[31] R. Drury and E. Wallington. Histological technique. 4th edition, Oxford University Press, USA (1967) 279-280.

[32] F. Nikkon, A. Saud, M. H. Rahman and M. E. Haque. In vitro antimicrobial activity of the compound isolated from *Moringa pterygosperma*. *Pakistan Journal of Biological Sciences* 6(22) (2003) 1888-1890

[33] A. Del-Ri, B. G. Obdululio, J. Casfillio, F. G. Marin and A. Ortuno. Uses and properties of Citrus flavonoids. *J. Agric. Food Chem.,* 45 (1997) 4505-4515.

[34] J. Shi, K. Arunachalam, D. Yeung, Y. Kakuda, G. Mittal and Y. Jiang. Saponins from edible Legumes, Chemistry, Processing and health benefit. *J. Med. Food,* 7 (2004) 67-78.

[35] D. E. Okwu. Phytochemicals and Vitamins content of indigenous species of Southeastern Nigeria. *J. Sustain. Agric. Environ*., 6(1) (2004) 30-37.

[36] A. J. Makori, O. O. Abuom and R. Kapiyo. Effects of water physico-chemical parameters on Tilapia (*Oreochromis niloticus*) growth in earthen ponds in Teso North Sub-County, Busia County. *Fish Aquatic Sci.,* 20 (2017) 23-30.

[37] M. O. Sunmonu, E. O. Ajala, M. M. Odewole, S. Morrison, A. M. Alabi, Comparative analysis of physico-chemical properties of oil extract from two varieties of fluted pumpkin seeds using different extraction methods, *Kathmandu University Journal of Science Engineering and Technology*, 13, No. II (2017) 48-60;

[38] E. B. Claude. Water Temperature in Aquaculture. (Global Aquaculture Alliance) (2018); https://www.globalseafood.org/advocate/water-temperature-in-aquaculture/

[39] M. I. Abubakar, I. Adeshina, I. Abdulraheem and S. A. Abdulsalami. Histopathology of gills, livers and kidney of *Clarias gariepinus* (Burchell, 1822) exposed to sniper 1000EC under laboratory conditions. *Acta Biologica*, 26(1) (2019) 19-30.

[40] P. A. Annune, S. O. Gbele and A. A. Oladimeji. Acute Toxicity of Zinc to fingerlings of *Clarias lazera. J. Aquatic Sciences,* 3(1991) 1357-1385.

[41] A. P. Ikeyi, G. T. Onah, A. O. Ogbonna, R. N. Udedibo and R. C. Ugwuanyi. Review of the Potentials of some Selected Vegetables in Nigeria-Towards Eradication of Malnutrition and Food Insecurity among Vulnerable Groups. *Idosr Journal of Biochemistry, Biotechnology and Allied Fields,* 5(1) (2020)72-77.

[42] Y. A. Geidam, U. I. Ibrahim, M. M. Bukar, H. I. Gambo and O. Ojo. Quality Assessment of broiler day-old chicks supplied to Maiduguri, North-Eastern Nigeria. *International Journal of Poultry Science*, 6(2) (2007) 107-110.

[43] S. D. Desai, D. Desai, and H. Kaur. Saponins and their biological activities. *Pharma Times*, 41(2009)13-16.

[44] Y. Sun, T. Zhang, B. Wang, H. Li and P. Li. Tannic acid, an inhibitor of poly (ADP ribose) glycohydrolase, sensitizes ovarian carcinoma cells to cisplatin. *Anti-can D*, 23(2012) 979-990.

[45] A. Islary, J. Sarmah and S. Basumatary. Proximate composition, mineral content, phytochemical analysis and in vitro antioxidant activities of a wild edible fruit (*Grewia sapidaRoxb*. ex DC.) found in Assam of North-East India. *American J of PhysBiochem and Pharma,* 5(2016) 21-31.

[46] H. F. Gemede and N. Ratta. Antinutritional factors in plant foods: potential health benefits and adverse effects. *International Journal of Nutrition and Food Science*, 3(2014) 284-289.

[47] E. A. A. Olojo, K. B. Olurin, G. Mbaka and A. D. Oluwemimo. Histopathology of gills and liver tissues of the African Catfish (*Clarias gariepinus*) exposed to Lead. *Afri. J Biotech*.,4 (2005) 117- 122.

[48] A. A. Olusegun and O. O. Adedayo. Haematological responses, serum biochemistry and histology of *Clarias gariepinus* (Burchell, 1822) exposed to sublethal concentrations of coldwater fresh root bark extracts of *Plumbago zeylanica* (leadwort). *J. Aqua. Res. Dev.,* 5(2014) 282-288.

[49] S. A. Bala and N. O. A. Malachy. Metabolic enzyme profile, behavioural changes and morpho physiological parameters of African catfish *Clarias gariepinus* juveniles in response to burnt waste tyres. *Comp ClinPathol*., 29 (2020) 787–797.

[50] D. E Hinton and D. J. Laurén. Liver structural alterations accompanying chronic toxicity in fishes: Potential Biomarkers of Exposure. In: McCarthy JF and Shugart LR (Eds.). Biomarkers of Environmental Contamination, Boca Raton: Lewis Publishers. (1990) 51-65.

[51] M.M.P Camargo and C.B.R. Martinez. Histopathology of gills, kidney and liver of a neotropical fish caged in an urban stream. *NeotropIchthyo.*, 5(2007) 327-336.

[52] M. Nasiruddin, M. A. Azadi and A. Jahan. Histopathological changes in gill, liver and intestine of *Heteropneustes fossilis* (bloch) treated with three dry seed extracts. *J Asia SocBangladash Sci.,* 38(2012) 217-226.

[53] A. O. Adeogun, O. O. Alaka, V. O. Taiwo and S. O. Fagade. Some pathological effects of sub-lethal concentration of the methanolic extract of *Raphiahookerion Clarias gariepinus. Afr. J. Biomed. Res.,* 15 2012 105-115.

[54] B. Velmurugan, M. Selvanayagam, E. Cenyiz and E. Unlu. The effects of fenvalerate on different tissues of freshwater fish *Cirrhinus mrigala*. L *Environ. Sci. Health B.*, 42(2) (2007)157-163.

[55] J. B. Hunter, S. L. Ross and J. Tannahill. Aluminium pollution and fish toxicity. *J. Water Pollut. Control Fed*, 79(1980). 413-420.

[56] O.O. Fafioye. Lethal and sublethal effects of extracts of *Parkia biglobosa* and Raphia *viniferaon* some freshwater fauna. Ph.D. These University of Ibadan, Nigeria, (2001) 216pp.