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Research Article

Gill and Liver Histology of *Clarias gariepinus* Exposed to Acute Treatments of Parkia *biglobosa* Husk Extract

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Abstract

Certain plants contain chemicals and have been used traditionally to harvest fish in many parts of the world. There is high tendency to abuse such plants for quick kills. A notable example of such plant piscicide is *Parkia biglobosa*. This research was carried out to investigate the toxic effects of *Parkia. Biglobosa* husk extract on *Clarias gariepinus* juveniles. These effects were measured in terms of acute toxicity (LC_{50}) and the pathological alterations to the liver and gills of *C. gariepinus* juveniles. When the extract was applied in a bioassay test the fish exhibited erratic swimming movements, rapid opercula movement, air surfacing and loss of reflex, especially at the higher concentrations and consequently mortality. The mortality was in direct proportion to the level of the effluent. LC_{50} values obtained from the fish exposed to *P. biglobosa* husk effluent within 96h was found to be 90.02 mg/l. The impact of the *P. biglobosa* extract on the liver and gills of the fish showed pathological alterations when compared to the control. Gills of *C. gariepinus* exposed to different levels of effluent showed various degrees of damage. All fish exposed to the effluent showed separation of respiratory epithelium from underlying supportive tissue. The toxicity of *P. biglobosa* husk extract to *C. gariepinus* was established.

Keywords: Organic Toxicant; Gill; Liver; Histology; *Parkia biglobosa;* Husk Extract

Introduction

Parkia biglobosa is a perennial tree. Its natural ecology is grassland area and is common in such belts within West Africa [1]. It is reported to provide socioeconomic benefits to many communities, for instance, many female dominated trade groups in Burkina Faso depend on it and its products as items of trade as it engaged more than half of the respondents to a 2004 survey [2]. In many parts of Africa this tree has provided economic benefits to women who are mostly in charge of its commerce in the form of dawadawa (fermented seeds). Another research found that the seeds, leaves as well as fruit-pulp are both used to directly feed animals or incorporated into their feed. Trado-medical practitioners have found medicinal qualities in the leaves, stem and bark *P. biglobosa* [3]. Kpodar., *et al.* [4] found that in Togo it is used to treat liver diseases and hypertension. Traditional healers in Guinea and South west Nigeria used it to treat malaria and open-wounds respectively [5-7]. It has been used to treat bacterial infections [8]. Oyeyemi., *et al.* [7] compared its antibacterial properties with that of Streptomycin, an established antibacterial. In the Northern region of Nigeria, some Hausa communities have reported experiencing relief from malaria, general body pains, diarrhoea, inflammations and diabetes mellitus when they used different concoctions *P. biglobosa*. Among these active constituent compounds are certain biochemicals which have pisicidal properties [5]. These chemicals have been used locally to harvest fish in many rural parts of the world. The toxic parts of the plant known to contain piscicidial properties include the bark, pods, fresh seeds and the pulp. These properties

need to be investigated thoroughly because the aquaculture sector is a major contributor towards world fish supply. The risk of diseases and immuno-compromise is heightened by stressors which result from increased production capacities. The African Catfish *Clarias gariepinus*, has been reported to be highly stress tolerant, surviving and thriving in sub optimal conditions. This species is a local favourite in many Nigerian dishes and has dominated the local culture, as many fish farmers prefer its culture over other species. Scientifically, many claims about the medicinal value of *P. biglobosa* have been verified, however information about its toxicities are still sketchy. There is paucity of information on the histopathological changes in the gills and liver of *C. gariepinus* fish acutely exposed to graded concentrations of *P. biglobosa* husk extract.

The aim of this experiment is to determine the 96-hour LC_{50} of aqueous *P. biglobosa* husk extract on *C. gariepinus* juveniles and its histopathological effects on the liver and gills of same fish species.

Materials and Methods

Experimental facility

The Department of Fisheries and Aquaculture toxicology laboratory in Kogi State University Anyigba Nigeria was the research location for this study.

Sourcing of the Parkia biglobosa husk and preparation of the experimental sample

Freshly harvested pods were bought from a farmer's market in Dekina. The pod was peeled open and the seeds removed from the pulp by washing in running water. The seeds were confirmed to be that of *P. biglobosa* at the Forestry and Wildlife Department, Kogi State University Anyigba. For 2 hours the seeds were boiled, and then peeled manually. The manual peeling process to remove the husk was aided by the addition of water (room temperature). Distilled running water was used to wash the husks, which were then oven-dried to a constant weight. After drying the husks were ground and sifted with 0.25 mm sieve to ensure uniform fines, and then preserved in a tightly corked bottle.

Experimental design

Juvenile *Clarias gariepinus* were used for this experiment. One hundred and eighty healthy pieces were obtained from a farm well known for quality fish seed in Anyigba, Nigeria. The juveniles were then moved using best practices to the hatchery section, Department of Fisheries and Aquaculture, Kogi State University Anyigba where they were kept for 2 weeks. This was done to allow the fish adjust to the new environment ruling out effect of stress. During this adjustment period, the fish were fed at 5% BW with Coppens® feed. Water quality was monitored and adjusted accordingly.

Preparation of Parkia biglobosa husk extract

A portion (250g) of the dried and powdered sample (*P. biglobosa* husk) was dissolved in 1L of distilled water at a room temperature (25 ± 5°C). It was mixed thoroughly by shaking and allowing it to stand for 24 h and then decanted and filtered using Whatman filter paper (125 mm). The filtrate was stored in an air-tight bottle and used for the bioassay tests.

Phytochemical screening of Parkia biglobosa husk extract

Using the methods described by AOAC [9] the presence and quantities of some phytochemical components of the *P. biglobosa* husk extract were evaluated. This screening was done at the Biochemistry department, Bayero University Kano. The phytochemical components screened for include flavonoids, steroids, alkaloids, hydrocyanic glycosides, terpenoids, tannins and saponins.

Water quality analysis

The quality of the water in the rearing tanks was closely monitored during the bioassay test. Using a Hanna "Combo" easy-to-carry metre (Hi 98129, Hanna Instruments, Inc Mauritius) the electrical conductivity and total dissolved solids were measured. A digital pH metre was used to measure pH, while temperature and dissolved oxygen levels were monitored with a combined digital metre (Model JPB 607).

Preliminary test

Range finding tests were carried out to gauge the tolerance level of the test fish to the toxin after a two-week acclimatization period. The results of this test were used to set reasonable intervals for application of the *P. biglobosa* husk extract.

Acute bioassay test

30 litres of dechlorinated tap water was pumped into each of the 18 ($1m^3$) concrete tanks used for the bioassay test. Aeration was continuously supplied sing battery powered aerators throughout the experimental period. The range of quantities of the toxicants used were staggered around the results of the preliminary test. 108 mg/L, 101 mg/L, 94 mg/L, 87mg/L, 80 mg/L and 0 mg/L (control)

were used in triplicates. These exact quantities of the aqueous *P. Biglobosa* husk extract were introduced into the respective tanks using a micropipette. The concrete tanks were then stocked with 10 randomly selected *C. gariepinus* juveniles (20.43 ± 0.11g) each. Feed was withdrawn from the test fish 24h to the commencement of the experiment. The tanks were covered with plastic nets to prevent the fish from jumping out during the experiment. Monitoring for changes in the way the fish acts was carried out, these alterations (some of which led to mortality) were recorded at 6h, 12h, 24h, 48h, 72h and 96h after introduction of the toxicant. Dead fish were promptly taken out of the tanks to avoid water pollution and their numbers recorded. The ASTM [10] protocols for acute toxicity tests were followed for this test.

A probit analysis, arithmethic percentage mortality data, log concentration, graph and slope function, upper and lower confidence limits were used to find the LC_{50} (the concentration of a toxicant that is capable of killing 50% of the test population) for acute toxicity test.

Where LC_{50} Probit value = 5.00, LC_{84}, probit value = 5.99, LC_{16} probit value = 4.01, D = the log dose concentration values, while Log_{10} D = 1.21.

Where, N = numbers of individuals tested between the range of concentrations corresponding to LC_{16} to LC_{84}, Ƒ = Frequency of individuals that are in the LC_{84} and LC_{16} range. N = 30+30+30+30+30, N = 150, Upper Limit = LC_{50} x Ƒ, Lower Limit = LC_{50} ÷ Ƒ LC_{50} = LC_{50} (Lower limit to Upper limit; 95% Confidence Limit).

Gills and liver histology

The liver and gill histology was conducted at the Department of Pathology Aminu Kano Teaching Hospital, Kano. Histological assessment methods adopted from AOAC [11] were used. Micrographs were taken after observation of the plates at 400X magnification.

Data analysis

The means from the acute bioassay test and physicochemical water analysis were computed and variance analysed (ANOVA) using Microsoft Excel package 2013. The resulting means were separated using Duncan Multiple Range Test (DMRT), and were reported as mean ± SE.

Results

Phytochemical screening

The phytochemical screening of *P. biglobosa* extract revealed the presence and quantity of certain secondary metabolites in the plant extract as shown in table 1. Steroids, cardiac glycoside and terpernoids were not quantitatively detected by the phytochemical screening but were detected at medium and low levels respectively by quantitative analysis.

Chemical constituent	Quantitative Analysis (mg/g)	Qualitative Analysis
Steroid	ND	++
Saponin	4.15 ± 0.03	++
Flavonoid	6.54 ± 0.01	+++
Cardiac Glycoside	ND	++
Tannins	2.03 ± 0.01	+
Alkaloid	3.01 ± 0.01	+
Phenol	2.31 ± 0.01	++
Steriods	ND	++
Carbohydrate	1.43 ± 0.02	+++
Terpernoids	ND	+

Table 1: Phytochemical Analysis of *P. biglobosa.*

Keys

ND = Not Detected

+ = Low

++ = Medium

+++ = Relative.

Water Quality

Water quality parameters monitored during this experiment are presented in table 2, all parameters differed significantly between the treatments. TDS, EC and temperature increased as the *P. biglobosa* extract concentration increased while DO and pH decreased as the concentration of *P. biglobosa* increased.

Probity

The regression equation (Figure 1) of the relationship estimated to be probit y = 15.904x with log conc - 26.1, and R^2 =1. The expression, R^2 value indicates that, mortality rate of fish increased with increase in concentration of *P. biglobosa* pods extract.

Treatment (mg/L)	pH	Temperature (°C)	Electrical Conductivity (µS/cm)	Total Dissolved Solids (mg/L)	Dissolved Oxygen (mg/L)
Control	6.90 ± 0.00^{a}	27.90 ± 0.09^{d}	841.33 ± 0.33^{e}	420.33 ± 0.33^{d}	3.90 ± 0.01^{a}
85.50	6.41 ± 0.01^{b}	28.30 ± 0.15^{c}	870.00 ± 0.68^{d}	434.67 ± 0.33^{d}	3.53 ± 0.01^{b}
90.50	6.35 ± 0.01^{b}	28.50 ± 0.07^{bc}	912.33 ± 0.33^{c}	456.00 ± 0.58^{c}	3.52 ± 0.01^{b}
95.50	6.28 ± 0.02^{b}	28.50 ± 0.12^{bc}	919.67 ± 0.48^{c}	465.33 ± 0.33^{c}	3.39 ± 0.01^{c}
100.50	6.17 ± 0.00^{c}	28.80 ± 0.15^{a}	953.33 ± 1.63^{b}	478.67 ± 1.33^{b}	3.21 ± 0.01^{c}
105.50	6.10 ± 0.01^{c}	28.70 ± 0.15^{a}	991.33 ± 0.67^{a}	496.67 ± 0.33^{a}	3.07 ± 0.01^{d}

Table 2: Water quality parameters during the experiment

Means in the same column with different superscripts differ significantly (P ≤ 0.05).

Figure 1: The regression equation of the relationship between probit and Log concentration.

Figure 2a: Gills in the control tank shows normal aspect of the gill filaments (black arrow), the Lamellae (L), epithelial with a pillar cell (white arrow), Primary Lamellae (PL) and Secondary Lamellae (SL).

Histology of Gill and Liver Cells

Analysis showing the effect of *Parkia biglobosa* on liver and gills of *Clarias gariepinus* juveniles at different concentrations of treatment. The histopathological alterations observed in the intestine of *C. gariepinus* juveniles are presented in figure 2a-d is an indication of the toxic effect of *P. biglobosa* husk extracts. The histology micrographs showed moribund specialized enzyme secreting tissue, overcrowding of the sinusoids and fibrosis. The gills of *Clarias gariepinus* exposed to *Parkia biglobosa* effluent showed varying degrees of epithelia hyperplasia compared to the normal gill filament (control).

Figure 2b: Gill Cell of *Clarias gariepinus* juveniles at T3 alterations observed are blood congestion (B), erosion (white Arrow) and blanketing (black arrow).

Figure 2c: Gill Cell of *Clarias gariepinus* juveniles at T4 showing fusion with destruction of Secondary Lamellae hyperplasia (Black Arrow) and Blood Congestion in the Vascular Axis of the primary filament (white arrow) and vacoulation (G).

Figure 2f: Photomicrograph of gill of C. *gariepinus* shows distortion of gill filaments as some are blanketed and eroded (Black Arrow). Epithelium rupture with hemorrhage (*), intense cellular necrosis, blanketing and blood congestion (white arrow).

Figure 2d: Gill Cell of *Clarias gariepinus* Juveniles at T5 showing lamellar disorganization (black arrow) of lamellar epithelium cell (white arrow), deformed secondary lamellae (D).

Figure 2g: Photomicrograph of Gill Cell of C. *gariepinus* at T6 Destruction observed are Epithelial erosion (White Arrows) Hyperplasia of lamellar epithelium (Black Arrows), Necrosis (N) and malformed secondary lamellae (M).

Figure 2e: Gill Cell of C. *gariepinus* 0.0mg/l showing normal hepatic tissue (H), hepatocytes with granular cytoplasm (white arrow) and central round nucleus (black arrow).

Figure 3a: Liver Cells of C. *gariepinus* at 0.0 mg/l showing normal hepatic tissue (H), hepatocytes with granular cytoplasm (white arrow) and central round nucleus (black arrow).

Figure 3b: Liver of juveniles exposed to T3 shows liver with Hepatic tissue showing focal necrosis (black arrow) bile stagnation(white arrows) and cytoplasmic degeneration.

Figure 3c: T5 liver shows fibrosis (black arrows), rapture of hepatocytes (white arrows), haemorrhages (H) and (D) vacoulation of cytoplasm.

Figure 3d: Photomicrograph of Gill Cell of *Clarias gariepinus* juveniles at T6 Destruction observed are Epithelial erosion (White Arrows) Hyperplasia of lamellar epithelium (Black Arrows), Necrosis (N) and malformed secondary lamellae (M).

Figure 3e: T4 liver shows rapture of hepatocytes (white arrows), haemorrhages (H) and vacoulation of cytoplasm (A).

Discussion and Conclusion

Toxicants (organic or inorganic) usually elicit certain physical and biochemical reactions from finned fish. These reactions have been studied in detail and reported by many authors: Ayuba., *et al*. [12] tested the toxic effects of NPK 20:10:10 on *C. gariepinus*, Nwanna., *et al.* [13] used gramozone and detergent on *O. niloticus* fingerlings, Ogah., *et al*. [14] and Fafioye, [1] tested *C. gariepinus* and *O. niloticus* respectively with plant extracts. These authors reported certain common reactions to these inorganic and organic toxicants. These reactions can be classified into two main categories, physical and cellular reactions. The former being the immediate observable response to the later. Quick opening and closing movements of the opercular, uncoordinated swimming and unusual surfacing behaviours are established signs of internal organ stress and attempts to create distance with the toxicant. It was found that the intensity of these actions increased with increased concentration of the pollutants, and this continued until mortality was observed. The concentration at which 50% of the test fish exposed the toxicant *P. biglobosa* husk extract died (LC_{50}) in this experiment was found to be 90.02 mg/l.

In this experiment, the gills and liver were sampled because of the physiological importance of these organs to the absorption and metabolism of chemicals. For instance, fish gills are considered appropriate indicators of water pollution levels and perform respiratory, osmoregulatory, excretory acid base balance functions without which the fish would not survive. Therefore, functional impairment of gills caused by pollutants can significantly damage health of fish, and lead to death [15]. The liver in its role as the

primary organ for detoxification of organ xenobiotics, is a target of a wide variety of pollutants and other toxic by-products. These tend to accumulate in high concentrations within it and the fish consequently suffers harmful effects including death [14,16,17]. Pathological alterations in the liver also serve as useful markers of exposure to environmental stress.

The toxicant (*P. biglobosa* husk extract) in this experiment was observed to impact the test subject *C. gariepinus* negatively at the cellular level. These deleterious effects were observed specifically in the cells of the gills and liver of the fish. The micrographs (Figure 2a-2g and 3a-3e) of the fish treated with *P. biglobosa* husk extract was compared with the non-exposed fish (control) and the result showed varying degrees of epithelial hyperplasia, fusion of lamellae, and separation of the epithelium. When compared with the non-exposed fish (control) the gills of the *C. gariepinus* exposed to *Parkia biglobosa* effluent showed epithelia hyperplasia. Generally, this overgrowth of cells was more obvious towards the extremes of the filament. The surface respiratory area was greatly reduced due to lamella fusion from the 96hr exposure. The gills showed graded levels of pathological changes in response to different concentrations of the toxicant. Experiments carried out in fish toxicity are unanimous in the conclusion that any fish exposed to toxicants are pathologically altered. Some of the major changes include the detachment of respiratory epithelium from the supportive tissues that underly it. This separation severely impacts negatively on the physiology of test fish usually leading to death [18,19]. This 96 hrs LC_{50} is different from the results of other researchers for other species of fish using different toxicants. This could be due to the differing nature of toxicants, active ingredients and mechanism of action, age, size and genetic composition of the test fish [1,13,14,17,20-23].

The liver histology of the test fish showed lesions, fibrosis, necrosis of the parenchyma, crowding of red blood cells which are common signs of organ interaction with toxins. Other observable signs were the reduction and sometimes total stoppage of blood flow (stasis) in parts of the liver. This stasis was associated with obstructive red blood cells congesting the sinusoid and resultant dying tissue. These alterations were similarly observed by Rana and Raizada [24] while carrying out acute short term bioassay on *Labeo rohita* with tannery and textile dye industry effluents. The *L. rohita* used showed severe histological alteration in the liver tissue of fish exposed to the effluents. *C. gariepinus* and *O. niloticus* are the favoured culture species in Nigeria, with fish farming being a major source of protein these much-needed sources of essential amino and fatty acids are at risk if toxicants like *P. biglobosa* are continuously dumped degrading the water quality and causing pathological alterations to the biology of these organisms. As wastewater treatment and water recycling becomes more mainstream, note must be made of the initial pollutants. Organic pollutants like *P. biglobosa* are usually overlooked as culprits of water pollution, as more emphasis is placed on inorganic pollutants like heavy metals. This experiment and others like it serve as a cautionary tale, encouraging the scientific community and general public to minimize indiscriminate dumping of wastes in water bodies as they can cause pathologic alterations to the biota and bioaccumulate endangering the final consumers.

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