ORIGINAL ARTICLE

HISTOLOGIC ALTERATIONS IN THE KIDNEY OF *CLARIAS GARIEPINUS* EXPOSED TO ETHANOLIC EXTRACT OF *DENNETTIA TRIPETALA*

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ABSTRACT

Background: The description and assessment of histological changes in different organs represent a very sensitive and crucial parameters in determining the impact of toxicants on the ecosystem. To understand the impact of toxicants in aquatic ecosystems, it is necessary to study the histopathological effects of poisons on different fish organs. Patent on kidney toxicity relates the term 'kidney toxicity' to any damage or impairment of the kidney which results in an impaired kidney function, in particular, impaired tubular or glomerular function. **Objective**: This research was aimed at assessing the ichthyotoxic potential of *Dennettia tripetala* fruit extract by investigating its effects on the histology of the kidney of *Clarias gariepinus* (Catfish). **Methods**: Fingerlings of *Clarias gariepinus* (size and body weight ranged from 3.9 – 7.6cm and 0.08 – 3.75g respectively) were exposed to sublethal dose of 0.7g/l (96hrs LC_{50}) and a lethal dose of 1.2g/l of ethanolic extract of *D. tripetala* using static bioassay. **Results**: Kidney tissue treated with sublethal dose (0.7g/l) of D. tripetala revealed condensed renal corpuscle filled with glomerular inflammation while kidney tissue treated with 1.2g/l of the toxicant revealed tubular necrosis and cellular degeneration. These findings revealed the deleterious potency of *D. tripetala* on fish kidney. No fish survived the lethal concentration of 1.2g/l of the toxicant. **Conclusions**: The lesions in the kidney tissue of the fish are attributable to the ichthyotoxic nature of the botanical. Lethal concentrations of *Dennettia tripetala* fruit extract could serve as an environmentally friendly alternative to synthetic piscicides used in fish pond to eliminate unwanted fish prior to stocking with desirable fish species.

Keywords: *Ichthyotoxic, Pesticide, Dennettia tripetala, Clarias gariepinus, lesion, kidney*.

1. INTRODUCTION

The description of histological changes in different organs represents a very sensitive and crucial parameter in determining the impact of toxicants on the ecosystem. To understand the impact of toxicant in aquatic ecosystems, it is necessary to study the histopathological effects of poisons on different fish organs [1]. Patent on kidney toxicity relates the term "kidney toxicity" to any damage or impairment of the kidney which results in an impaired kidney function, in particular, impaired tubular or glomerular function. Affected by kidney toxicity are the excretion related functions of the kidney. Kidney toxicity is induced by or is a result of the administration of a chemical compound or drug [2]. Although plant-derived pesticides are cheaper, safer, and less hazardous to aquatic life than their synthetic counterparts, many, however, are known to have ichthyotoxic properties [3, 4]. *Dennettia tripetala* (Pepper fruit) is of the family Annonaceae. It is found in the tropical rainforest region of Nigeria and also occur in the Savanna areas [5]. It is said to have ethno-medicinal uses. *D. tripetala* fruits are used as carminative (relieving discomfort of gas in the digestive tract) and antiseptic. It is also used as a masticator. The root serves as a valued spice in the preparation of dog meat dishes in South East Nigeria [6].

The fruit is known to be a good source of vitamins. The leaf, fruit, bark and root of the plants possess strong pepperish and pungent spicy taste with a characteristic aroma and fragrance. The fruits are chewed in different forms (fresh green, fresh ripened red, black dry fruit and dry seed). *Dennettia tripetala* fruit serve as mild stimulant to the consumer. It also serves as seasoning which is added to prepared foods such as meat, soup, sausage and in some special local dishes and vegetables [7]. Phytochemical assessment shows it contains saponins, flavonoids, tannins and cyanogenic glycosides [8]. Dennettia oil had been reported to have the ability to protect cowpea against storage insect pests [9].

The fruits are sometimes taken with kolanut, garden egg and palm wine in parts of Nigeria, especially in the Southern part, where they serve for cultural entertainment of guests, particularly during coronation, new yam festivals, and marriage ceremonies [10, 11]. They are often applied to the food meant for pregnant women and are important in the diets of postpartum women to aid uterine Contraction [12]. Ikpi and Nku (2008) reported on the effect of ethanolic extract of *Dennettia tripetala* fruit on haematological parameters in Albino wistar rats [13]. The efficacy of pulverized and extracts of *D. tripetala* as a deterrent of fish beetle (*Dermestes maculates*) on stored dried smoked catfish has also been

reported [14]. This study is aimed at evaluating the icthyotoxicity of the fruit of *Dennettia tripetala* by examining its effect on the histology of the kidney of the fingerling of *Clarias gariepinus*.

2. MATERIALS AND METHODS

2.1 Collection And Acclimatization Of *Clarias gariepinus*

The choice of *Clarias gariepinus* was based on its ability to withstand stress and its high commercial value in Nigeria. It is highly fecund, resistant to diseases and can be intensively cultured in poor quality water [14]. The fingerlings of *C. gariepinus* used were obtained from Safe Food farms in Uyo, Akwa Ibom State. Their weight ranged from 0.08 to 3.75g, total length from 3.9 to 7.6cm. They were transported to the laboratory in a polythene bag, containing aerated water and kept in the laboratory for acclimatization in transparent plastic tanks of length 22.6cm and width of 18.5cm. The experimental fish were adapted to laboratory conditions for two weeks before being used in the experiment. They were fed twice a day during the period of acclimatization at 5% of the body weight with formulated feed containing 45% protein, 12% of fat, 2.2g 0f calcium (Ca), 12% of Phosphorus (P) and 8.5% of ash. The water was changed twice a day and at the same time for the two weeks period of acclimatization, in order to remove faecal materials and unconsumed feeds. The tanks were covered with netting material to prevent the fish from jumping out from the plastic containers and to protect them from potential predators. Feeding was discontinued during test period.

2.2 Preparation Of Ethanolic Extract Of *Dennettia tripetala*

Some quantities of *Dennettia tripetala* fruits were air dried for one week and then pulverized by grinding using pestle and mortar. The powdered fruit was stored in an air- tight bottle until used. Thereafter 300g of the homogenized sample was extracted with ethanol. The extract was evaporated to dryness in a rotary evaporator at 60^0C and preserved in the refrigerator until needed.

2.3 Bioassay Procedure

Ten randomly selected fish were distributed in batches and placed in three aquaria containing extract solution and a control tank containing extract free water only. Each set of experiment with the control was replicated twice. The fishes were exposed to a predetermined sub- lethal dose of 0.7g/L (96hr LC_{50}) and a lethal dose of1.2g/l of extract solution for 96 hours. Temperature and pH were determined at the start of the experiment and maintained at optimal levels. Within the duration of the experiment, water in the tank used was replaced after every 48hr with fresh extract solution.

2.4 Preservation of Fish Organs for Histopathology Analysis

After the period of exposure, fish from the experimental and control tanks were dissected. The kidney tissues were collected and fixed in Bouin's fluid embedded in paraffin and sectioned (7 Microns thickness) for staining with haematoxylin/eosin stain. Histological alterations due to treatment with the different doses of ethanolic extract of *D. tripetala* were noted and photomicrographs taken. Available patent pertains to the field of diagnostics for kidney toxicity and toxicological assessment for risk stratification of chemical compounds, specifically, it relates to a method for diagnosing kidney toxicity. It also relates to a method for determining whether a compound is capable of inducing such kidney toxicity in a subject [15].

3. RESULTS

3.1 General Observations

Fish treated with *Dennettia tripetala* exhibited restlessness, gulping of air at the water surface, erratic swimming and frequent attempts at jumping out of the tank. Fish in the control group did not display any abnormal behavior. Exposure of *Clarias gariepinus* to 0.7g/L of ethanolic extract of Dennettia tripetala produced distinct histological alterations in the kidney tissues examined. Plate A shows kidney tissue without treatment and revealed normal cellular pattern with area of distal and proximal convoluted tubules and renal corpuscle. No cellular abnormality was seen. However, the kidney tissue treated with 0.7g/l (Plate B) revealed condensed renal corpuscle with glomerular inflammation while kidney tissues treated with 1.2g/l (plate C) revealed tubular necrosis and cellular degeneration.

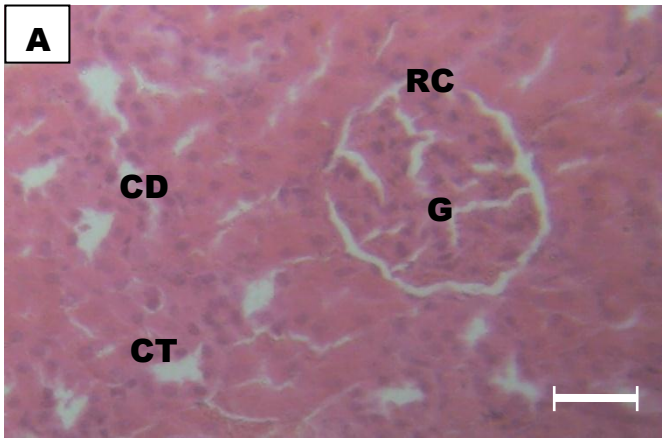

Plate A: Histologic Photomicrograh showing section through the kidney without treatment (control)

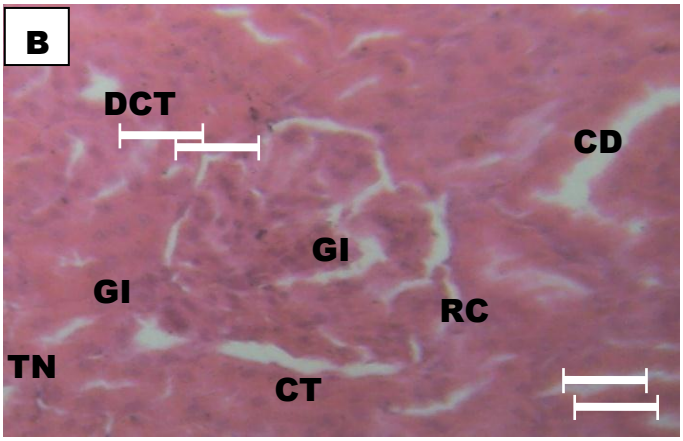

Plate B: Histologic Photomicrograph showing section through the kidney treated with 0.7g/l *Dennettia tripetala*.

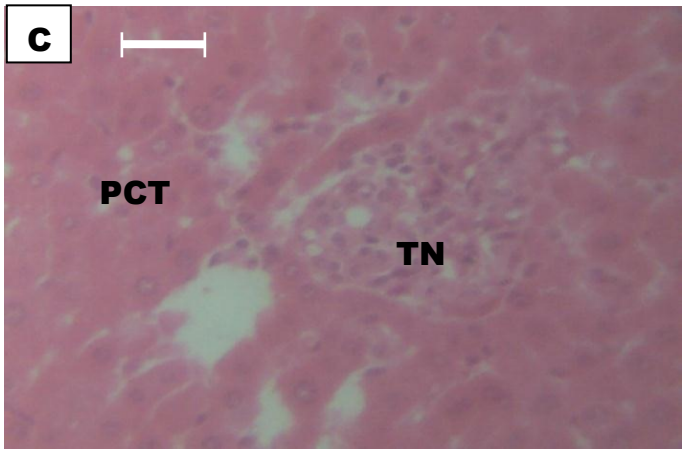

Plate C: Histologic Photomicrograph showing section through the kidney treated with 1.2g/l *D. tripetala*
Scale Bar=50 micron.

KEYS:

CT	–	Connective tissue
RC	–	Renal corpuscle
G	–	Glomerulum
CD	–	Cellular degeneration
DCT	–	Distal convoluted tubule
GI	–	Glomerular inflammation
PCT	–	Proximal convoluted tubule
TN	–	Tubular necrosis

4. DISCUSSION

The lesions observed in the kidney of the fish clearly implicate *D. tripetala* as a toxicant. Similar observations were made when *Clarias gariepinus* was exposed to a sub-lethal dose of *Piper guineense* [16] and when *Heteropneustes fossilis* was exposed to extracts of two plants, *Pongamia pinnata* (L) and *Clerodendrum viscosum* (vent) [patent]. The fish kidney is one of the first organs to be affected by contaminants in water and appears to be particularly sensitive to a variety of toxins due to the high renal blood flow, the ability to concentrate substances, and the biotransformation of the parent compound to a toxic metabolite. The present study confirms the submission by Adeogun *et al.*, (2012) [3], that the most common alterations found in the kidney of fish exposed to water contamination are inter alia, tubular degeneration and changes in the corpuscles. Pathological changes have also been observed in several organs of *Clarias gariepinus* exposed to sub-lethal concentrations of methanolic extracts of *Raphia hookeri*. The plant which is used by artisanal fisher folks in Nigeria to stupefy fish for easy catch caused lesions on the gill, liver, kidney, brain and heart of the fish [3]. Other piscicidal plants used in harvesting fish by local fisher folks in Nigeria include *Cassia alata, Erythrophloem ivorensis, Omphalocarpum elatum, Piptadenastrum africanum, Abizia ferruginea, Albizia adianthifolia, Strychnos aculeatus and Tetrapleura tetraptera* [17]. The utilization of *D. tripetala* in agricultural fields should be done with caution because of it deleterious effects on fish as indicated in the present study. Its ichthyotoxic property can however be utilized in the eradication of unwanted, resident species of fish from aquaculture ponds prior to stocking. According to a patent on fish toxicity, control or eradication of unwanted fish species such is essential to efficient fishery management in many areas. Killing of undesired species of fish in lakes, ponds, streams etc., is often desirable prior to restocking with desired species of fish. Various toxic materials such as antimycin A have previously been used for killing undesirable fish. However, such toxicants have generally suffered from one or more deficiencies such as the requirement of uneconomical amount of toxicant, resistance of specific types of fish to the toxicant, repelling of fish by the toxicant, etc., [15]. The toxicity of biocides wears off after a while due to biodegradation [18], making them an environmentally friendly alternative to synthetic piscicides.

5. CONCLUSION

This study revealed the ichthyotoxic property of *Dennettia tripetala*. The plant extract induced lesions on the fish kidney leading to its malfunctioning and death. The use of this botanical in agricultural field should be done with caution. However, lethal concentrations of the extract could constitute an environmentally friendly alternative to synthetic piscicides and be harnessed to get rid of unwanted fish in aquaculture prior to stocking of pond with desirable fish species.

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