

## TOXICITY EFFECT OF *Kigelia africana* AQUEOUS EXTRACT ON THE HAEMATOLOGY AND HISTOPATHOLOGY OF JUVENILE NILE TILAPIA (*Oreochromis niloticus*)

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### ABSTRACT

*This study examined the toxicity effect of Kigelia africana on Nile tilapia (Oreochromis niloticus) juveniles. The corresponding effects of this plant extract on the health status of the O. niloticus were similarly studied using their haematological and histopathological profiles. The experiment was carried out at the Hatchery Unit of the Federal University of Agriculture, Abeokuta fish farm. Fish were acclimatized for one week and fed twice daily at the rate of 3% body weight. Water in the culture medium was replenished daily. A total of 150 juveniles of O. niloticus were exposed to concentrations of 0.00, 1.75, 2.50, 3.75 and 5.00 g L<sup>-1</sup> aqueous extract of Kigelia africana set up in three replicates. This toxicity study showed that aqueous bark extract of K. africana caused significant behavioural changes in O. niloticus. Recorded values of the water quality parameters showed significant difference (p < 0.05) across the treatments. The haematological indices of the fish were also observed to be affected with increasing extract concentration, compared to the control treatment (0.0 g L<sup>-1</sup>). Similarly, histology of the liver and gills showed variations in distortions and damages to the tissues; with observed severity increasing with increase in extract concentrations. This study suggested that the 96-h LC<sub>50</sub> of K. africana could be greater than 5 g L<sup>-1</sup>. The study concluded that caution must be taken in the disposal of this plant in water bodies as extended exposure time and at higher concentrations could pose adverse effects on the stock of juvenile Oreochromis niloticus.*

**Key words:** toxicity, *Kigelia africana*, *Oreochromis niloticus*, haematology, histopathology

### INTRODUCTION

Nile tilapia (*Oreochromis niloticus* Linnaeus 1757) is native to Africa, ranging from the upper Nile River south to the equator and west to the Atlantic coast (Petersen, 2005). It is one of the aquatic organisms affected by heavy metals and used as metal biological marker in toxicological studies (Fafioye, 2012). The intensive farming of *O. niloticus* is rapidly expanding and is the second most widely farmed fish in the world, after carps (Nelson, 2006). Several species of tilapia are cultured commercially, but *O. niloticus* is the predominant cultured species worldwide (Fonseca *et al.*, 2013). It presents rusticity, good growth rate, and adaptability to confinement, producing a tasty white-colour meat (Oliveira-Filho *et al.*, 2010). The ease of reproduction of the *O. niloticus* encourages farmers to acquire the species and populate their tanks at a low investment cost. Furthermore, the fish is hardy, occurs in a wide range of environmental variations, tolerating extreme limits of temperature and oxygen, as well as the presence of various pollutants (Beyruth *et al.*, 2004).

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs has been isolated from natural sources. Many of these isolations were based on the uses of the agents in traditional medicines (Cordell, 2000). Though some of these plants, such as *Adenium obesum*, *Vernonia amygdalina* and *Carica papaya* could be toxic to aquatic organisms, they also serve as piscicides during pond preparation to control unwanted aquatic animals; such as leeches, crayfish, snails, tadpoles, and frogs. The plants could also be used to significantly eliminate fish species which are known to reduce aquacultural yields by competing with stocked fish for space and feed. Being eco-friendly, extract from plants can be used as piscicides unlike the use of synthetic chemicals.

*Kigelia africana* (Lam) Benth is commonly referred to as sausage or cucumber tree because of its huge sausage or cucumber-like fruit weighing up to 5-10 kg. It belongs to the *Bignoniaceae* family. The tree can grow up to a height of 20 m. It is an exceptional indigenous medicinal plant, native to and widely distributed in Africa where it grows in open woodlands and wet areas including river

banks and flood plains of Nigeria, Cameroon, Kenya, Guinea, and Senegal (Azu, 2013). It is evergreen where rainfall occurs throughout the year, but deciduous where there is a long dry season. Parts of the plant are used for treating a wide range of traditionally based ailments mainly on cultural practices. The fruit is used to treat skin ailments like fungal infections, boils, psoriasis and eczema. Dysentery, ringworm, tapeworm, postpartum haemorrhage, malaria, diabetes and pneumonia are also treated with the fruit. Gill (1992) reported its wide use in Southern Nigeria as an herbal remedy for malaria, diarrhea, rheumatism, retained placenta and dizziness. Also, infertility, poor libido, sexual asthenia and impotence are treated with medicines containing the fruits, roots or leaves of *K. africana* (Owolabi and Omogbai, 2007).

With its array of uses, toxicity effect of *K. africana* extract on *O. niloticus* can be of economic importance as a way of sustaining the biological sanctity of the water body and also the fish species. Therefore, this present study was aimed at examining the toxicity effect of *K. africana* aqueous extract on *O. niloticus* juveniles.

## **MATERIALS AND METHODS**

### **Experimental Site**

The experimental site was at the Fish Hatchery Centre of the Department of Aquaculture and Fishery Management, Federal University of Agriculture, Abeokuta (FUNAAB), Ogun State, Nigeria.

### **Collection of *Kigelia africana* Bark**

The matured bark of *K. africana* was collected at Gbagba Village, Emere Road, Abeokuta, Ogun State, Nigeria and authenticated at the Department of Forestry and Wildlife Management, FUNAAB.

### **Collection of *O. niloticus* Juveniles**

A total of two hundred (200) juveniles of *Oreochromis niloticus* with initial average body weight of  $15 \pm 0.1$  g and standard length of  $8.1 \pm 0.3$  cm were collected from Premium Aquaculture Farm at Oyan Dam, Abeokuta, Ogun State, Nigeria. The fish were transported in an oxygenated polythene bag, containing water from the culture medium of the farm to the Fish Hatchery unit of the FUNAAB.

### **Acclimatization of the *O. niloticus* Juveniles**

The fish were acclimatized for one week during which they were fed twice daily in the morning and evening with 1.2 mm imported Coppens® feed containing 45 % crude protein at the rate of 3 % body weight during the period. Feeding was terminated 48 hours prior to the start of the experiment while uneaten feed and wastes were removed daily with subsequent water replenishment (Oyelese and Faturoti, 1995).

### **Preparation of Plant Extracts**

The harvested *K. africana* bark was cut into 0.5 cm segments as in the method of aqueous extracts using the modified Katz *et al.* (1987) method. Approximately 10 g of barks material was mixed with 100 ml of distilled water and blended with Arkeys mixer blender (model RK 301 BL). The blended materials were centrifuged for 30 min. at 1800 rounds per min.. The supernatant liquid was filtered through four layers of cheese cloth (0.25 µm pore size). The filtrate obtained was considered to be the original undiluted extracts (stock solution). Serial dilutions were made at different concentrations of 0.00, 1.25, 2.5, 3.75 and 5.00 g L<sup>-1</sup> from the stock solution by mixing the original extract with distilled water to attain desired concentrations.

### **Phytochemical Screening**

Active phytochemical screening was carried out on the crude *K. africana* plant to determine the chemical constituents present quantitatively. The screening was done at the Biology Laboratory, FUNAAB. Qualitative and quantitative assessment was carried out to determine the level of inclusion of the plant extract following the standard procedures as described by Evans (2002) for the presence of alkaloids, tannins, saponins, anthraquinones, steroids, flavonoids and glycosides.

### **Behavioural Studies**

The behavioural responses of the fish in the treatment groups and the mortality rate was observed and recorded at the intervals of 12, 24, 48, 72 and 96 h according to the method developed by Trease and Evans (1989). The responses observed were loss of reflex, hyperventilation, erratic swimming suffocation and spiralling.

### **Bioassay Test**

Bioassay test to determine the 96-h acute toxicity of *K. africana* bark extracts on juvenile *O. niloticus* was conducted following static bioassay procedures described by Yadav and Singh (2010). Clean water was used as the control at 0.0 g L<sup>-1</sup>. Five test acute concentrations of 5.00, 3.75, 2.50, 1.75 and 0.00 g L<sup>-1</sup> (control) were prepared and replicated thrice for each treatment in a transparent plastic container. Each of the tanks was stocked with 10 fish at different concentrations of the *K. africana*. Throughout the 96 hour test, the test fish were fed twice in the morning and evening and the mortality in each tank was monitored and recorded every 24 h till the end of the test. Dead fish were immediately removed to avoid contamination.

### **Water quality parameters**

Temperature, Hydrogen ion concentration (pH) and Dissolved oxygen (DO) concentration were taken in all the treatment groups. Temperature reading was taken using the hand-held alcohol-in-glass

thermometer. The hydrogen ion concentration was measured using the calibrated multi parameter Hanna Instrument (Model HI 98129). The dissolved oxygen concentration was measured with the Griffin oxygen meter (Model 40). The reading on the meter was used in calculating the actual dissolved oxygen level of the water.

### Haematological Test

Haematological parameters such as red blood cell, white blood cell, packed cell volume, haemoglobin, red blood cell indices and white blood cell differential counts were carried out following standard procedures (Erhunmwunse and Ainerua, 2013).

### Histopathological Test

Histopathological analyses of experimental fish were carried out by removing the gill and liver of both the fish exposed to *K. africana* extracts and the control treatment after 96 hours.

### Statistical Analysis

Analysis of variance (ANOVA) was used to analyse the data collected and least significance difference (LSD) at the 0.05 significance level was used to determine the difference among treatment groups. The lethal concentration that caused 50 % mortality (96 h LC<sub>50</sub>) was determined using the probit analysis. The indices of toxicity and their 95 % confidence limits were derived from a computer statistical program, SPSS (version 20.0).

## RESULTS

### Qualitative and Quantitative Analysis of *Kigelia africana* Bark Extract

The qualitative analysis of *K. africana* leaves extract revealed the presence of tannin, saponin, alkaloid, flavonoid, phenol, steroid, oxalate, phytate, glycoside, anthraquinone and quantitative abundance levels of toxicity were also determined as shown in Table 1.

### Behavioural Response of *Oreochromis niloticus* to Aqueous Extract of *K. africana*

The behavioural responses of the fish in the treatment groups are presented in Table 2.

**Table 1:** Qualitative and quantitative analysis of *Kigelia africana* bark extract

Phytochemicals	Qualitative	Quantitative (%)
Tannins	+++	23.18
Saponins	+++	21.26
Alkaloids	++	11.26
Flavonoids	++	9.21
Phytate	+	4.67
Phenols	+	2.86
Oxalate	+++	24.28
Steroids	+	0.50
Glycoside	+	1.62
Anthraquinones	+	1.16

Key: + Low, ++ Mild, +++ High

**Table 2:** Behavioural response of *Oreochromis niloticus* to aqueous extract of *K. africana*

Behavioural response	Concentrations (g L <sup>-1</sup> )				
	0.00	1.25	2.50	3.75	5.00
Loss of reflex	-	-	+	++	+++
Hyperventilation	-	-	-	++	+++
Erratic swimming	-	-	+	++	+++
Suffocation	-	-	+	++	+++
Spiralling	-	-	+	++	+++

Key: - Absent, +Low, ++ Mild, +++ High

**Table 3:** Mean water quality parameters changes of *O. niloticus* exposed to aqueous extract of *K. africana*

Concentrations (g L <sup>-1</sup> )	DO (mg L <sup>-1</sup> )	pH	Temp (°C)
0.00	5.9±0.1 <sup>a</sup>	7.0±0.1 <sup>a</sup>	26.0±0.6 <sup>a</sup>
1.75	5.8±0.2 <sup>a</sup>	7.0±0.1 <sup>a</sup>	26.9±0.2 <sup>b</sup>
2.50	4.1±0.1 <sup>b</sup>	6.8±0.2 <sup>b</sup>	26.8±0.2 <sup>b</sup>
3.75	4.0±0.1 <sup>b</sup>	6.7±0.3 <sup>b</sup>	27.0±0.1 <sup>b</sup>
5.00	3.0±0.1 <sup>c</sup>	6.4±0.1 <sup>c</sup>	7.3±0.2 <sup>ab</sup>

Mean values followed by the same superscript along the columns were not significantly ( $p > 0.05$ ) different

DO - Dissolved oxygen, Temp - Temperature

### Water Quality Parameters

The observed water quality parameters across the treatment groups are presented in Table 3.

### Mortality of *O. niloticus* Exposed to Aqueous Extract of *K. africana* for 96-h Exposure Period

There were no mortalities of *O. niloticus* recorded during the 96-h exposure to *K. africana* aqueous extract across all the treatment groups.

### Haematological Parameters of *O. niloticus* Exposed to Aqueous Extract of *K. africana*

The haematological indices of *O. niloticus* exposed to aqueous extract of *K. africana* are shown (Table 4.)

**Table 4:** Haematological indices of *O. niloticus* exposed to aqueous extract of *K. africana*

Haematological parameters	Concentrations (g L <sup>-1</sup> )				
	0.00	1.25	2.50	3.75	5.00
HB (g/dl)	13.85 ± 0.35 <sup>d</sup>	11.78 ± 0.25 <sup>b</sup>	11.30 ± 0.26 <sup>b</sup>	12.00 ± 1.00 <sup>c</sup>	10.33 ± 0.28 <sup>a</sup>
PCV (%)	41.66 ± 0.33 <sup>a</sup>	34.33 ± 0.66 <sup>b</sup>	33.33 ± 1.45 <sup>b</sup>	34.33 ± 1.20 <sup>a</sup>	31.33 ± 0.66 <sup>c</sup>
RBC (× 10 <sup>12</sup> /L)	1.70 ± 1.00 <sup>a</sup>	1.51 ± 0.23 <sup>b</sup>	1.40 ± 0.11 <sup>c</sup>	1.52 ± 0.40 <sup>b</sup>	1.31 ± 0.17 <sup>d</sup>
WBC (× 10 <sup>3</sup> /L)	12.26 ± 0.14 <sup>b</sup>	9.40 ± 0.23 <sup>a</sup>	13.23 ± 0.23 <sup>c</sup>	12.96 ± 0.26 <sup>b</sup>	12.11 ± 0.38 <sup>b</sup>
HET (%)	28.33 ± 1.33 <sup>c</sup>	32.66 ± 1.66 <sup>b</sup>	39.66 ± 0.07 <sup>a</sup>	40.11 ± 0.47 <sup>a</sup>	38.00 ± 0.57 <sup>a</sup>
LYM (%)	65.66 ± 0.16 <sup>a</sup>	62.66 ± 0.33 <sup>b</sup>	57.33 ± 1.52 <sup>c</sup>	54.33 ± 0.66 <sup>d</sup>	57.00 ± 0.66 <sup>c</sup>
EOS (%)	3.00 ± 0.00 <sup>a</sup>	2.00 ± 0.00 <sup>b</sup>	2.00 ± 0.00 <sup>b</sup>	3.00 ± 0.01 <sup>a</sup>	2.00 ± 0.00 <sup>b</sup>
MON (%)	0.00	1.00 ± 0.00 <sup>a</sup>	0.00	0.00	1.00 ± 0.00 <sup>a</sup>
BAS (%)	0.00	0.00	0.00	0.00	0.00

Means with different superscripts along a row were significantly ( $p < 0.05$ ) different.

\*\*Hb: Haemoglobin; PCV: Packed cell volume; RBC: Red blood cell; WBC: White blood cell;

HET: Heterophils; LYM: Lymphocytes; EOS: Eosinophils; MON: Monocytes; BAS: Basophils

**Table 5:** Histopathological changes of *O. niloticus* exposed to aqueous extract of *K. Africana*

Treatment concentration (g L <sup>-1</sup> )	Duration (hours)	Organs	Lesion	Malignancy	Necrosis	Inflammation	Pigment	Cellular degeneration
0.00	96	Gill	-	-	-	-	-	-
		Liver	-	-	-	-	-	-
1.25	96	Gill	+	+	+	+	+	+
		Liver	+	-	+	+	-	-
2.50	96	Gill	+	-	+	+	+	-
		Liver	+	-	+++	+	-	+
3.75	96	Gill	-	++	-	++	++	+
		Liver	++	-	+	++	-	+
5.00	96	Gill	-	+++	-	+++	+++	-
		Liver	+++	-	++	+	+	-

Key: - completely absent, + present, ++ mild, +++ severe

**Histopathological Changes of *O. niloticus* Exposed to Aqueous Extract of *K. africana***

Observed histopathological changes in fish across the treatment groups are presented in Table 5.

**DISCUSSION**

In the present study, the observed water quality parameters did not deviate from regulatory standards. However, the dissolved oxygen concentration decreases with increasing concentration levels of the *K. africana* extract. This could be due to the introduction of the extract to the treatments in increasing concentration, thus distorting the oxygen balance of the water. The quality of water of aquatic systems is a predisposing factor to the biological living of aquatic organisms (including fish) inhabiting it. Thus, any deviation from the natural settings of the water will be observed in agitation of the species, as this portends imbalance in the biological sanctity of the water, which directly affects the species as it is in close relationship with it. Results from the phytochemical screening of the *K. africana* showed consonance with several phytochemical studies that the extracts from many species of *Bignoniaceae* contained secondary metabolites such as saponins, tannins, flavonoids in varying amounts (Choudhury *et al.*, 2011; Gouda *et al.*, 2006). The behavioural changes observed in this study include loss of reflex, hyperventilation, erratic swimming, suffocation and spiralling. From the present study, these behavioural changes were dominant as the concentration of the extract increases. This led to the fish exhibiting haphazard movement and aggressiveness in the medium (Eyo *et al.*, 2013). Generally, when water quality is affected by toxicants, physiological changes will be observed in the values of some of the haematological parameters and swimming activity of the fish (Heath, 1991; Adeyemo, 2005).

Introduction of the extract to juveniles of *O. niloticus* at increasing concentrations remarkably led to progressive changes in the haematological composition, as well as the histological set up of the gills and liver of the species. It has been widely reported that deviations (in terms of toxicants) from the natural physiological formations has impacts on

the histological functioning of fish exposed to plant extracts; *Adenium obesum* (Abalaka *et al.*, 2015), *Luffa cylindrica* (Odioko *et al.*, 2016) and *Vernonia amygdalina* (Audu *et al.*, 2017) and many more. Akinrotimi *et al.* (2012) affirmed that haematological studies are often used as a tool in determining the health status of fish species and also to detect physiological changes in stressed conditions such as exposure to pollutants and toxicants. The packed cell volume (PCV) of the fish across all treatments showed significant difference ( $p < 0.05$ ). The values recorded deviated in a decreasing manner with respect to the control treatment. This could be due to the effect of the toxic potentials of the aqueous extract of *K. africana* on the blood of the *O. niloticus* as the concentration of the extract increases. This was in agreement with Adakole (2012), who reported similar trend in fish exposed to toxicants. Eriegha *et al.* (2017) also affirmed that PCV value of all fish exposed to the toxicants was lower than those for healthy fish (control treatment). Haemoglobin has been reported to be a major index to the survivability of fish, as it is directly related to the oxygen binding capacity of the blood. From this study, it was observed that there was a significant difference ( $p < 0.05$ ) in the values of the haemoglobin and dissolved oxygen. That is, decrease in haemoglobin corresponded with decrease in dissolved oxygen. This according to Ali *et al.* (2008) is caused by the effect of toxicants on blood. Notably, heterophil function majorly as the first line of defense against infections and response to toxic elements. Thus in the present study, this showed significant difference ( $p < 0.05$ ), as the levels of heterophils observed increased with increasing exposing of the fish to the *K. africana* extracts. Similarly, the white blood cell (WBC) counts showed significant difference, as the observed values increased in counts with increasing extract concentration of the *K. africana*. This could be attributed to alterations in defense mechanism against the action of the toxic potentials of the extract (Zaghloul, 2001; Zaghloul *et al.*, 2005). Histopathological observations from this study revealed damages ranging from mild to severe effects. The damage to the gills and liver of the *O. niloticus* increased with increase in the

concentration levels of the extract of the *K. africana*. Being a sensitive organ of extreme morphological importance, the gills of fish remains an essential organ for biological homeostasis in the aquatic medium. The gills remain vulnerable to unstable water quality due to effects of contaminants or pollutants in the aquatic environment (Reddy and Waskale, 2013). Gills of healthy (control treatment) *O. niloticus* in the present study showed similar orientation to that of other finfishes, with no observed tissue alteration or distortion. However, as concentration of the extract increases, variations were evident in the structural formations of the gill tissues of the fish in the treatments (Audu *et al.*, 2017). This includes observed lesion, malignancy and inflammation of the tissue, necrosis, pigment discoloration and cellular degeneration. Similarly, liver of the control treatment of the *O. niloticus* from the present study showed healthy status, as there was no observed distortion or disorientation to the tissue. However as with the gills, structural deformations across the treatments varied with levels of exposure of the *K. africana* extract. These observations in tissue anomalies could pose biological malfunctioning abilities of the liver. Furthermore, this aligned with results from previous observations of the exposure of different plant extracts to fish, in ascertaining the potency or otherwise of such plants (Sheikhlar *et al.* 2011; Adeogun *et al.* 2012; Abalaka *et al.* 2015).

## CONCLUSION

This study revealed *Kigelia africana* extract had no mortality effect over the 96-h period. Thus, the 96-h LC<sub>50</sub> of *K. africana* could be said to be greater than 5 g L<sup>-1</sup>. However, it could be deduced from this study that the introduction of *Kigelia africana* extract could cause mortalities to *O. niloticus* over longer exposure periods as seen in its effects in the water quality. At higher concentrations of the extracts, haematological and histological indices of the fish species were also affected. Therefore, disposal of parts or whole *Kigelia africana* plant into water bodies should be done with extreme caution as it could threaten the life and existence of fish.

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