Histopathological alterations in gills, kidney and liver of Nile Tilapia (*Oreochromis niloticus*) fingerlings exposed to aqueous leaf extract of Desert Date (*Balanites aegyptiaca*)

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Abstract

One of the many biomarkers for determining the effects of pollutants on fish is changes in organ histopathology. Leaves of *Balanites aegyptiaca* have been reported to have phytochemicals with fish anaesthetic property. This study sought to determine the effect of graded acute concentrations (200.00, 250.00, 300.00.350.00 and 400.00 mg/L) of *B. aegyptiaca* on histopathology of gills, kidney and liver of mixed sex of *Oreochromis niloticus* fingerlings. A total of 120 *O. niloticus* fingerlings (mean weight 23±0.03 g and mean total length 12.50±0.39 cm) were exposed to the plant extract. Paraffin wax method and haematoxylin-eosin staining techniques of tissue processing were adopted for the examination of the gills, kidney and liver. Dose-dependent histopathological changes were observed in the three organs (gills, kidney and liver) i.e. histopathological alterations increase with increase in concentration of the plant extract. Gills showed lamellae fusion, haemorrhage, desquamation, atrophy and secondary lamellae erosion while kidney and liver indicated atrophy, necrosis, haemorrhage, hyperplasia and hypertrophy. Structural alterations were evident in the gills, kidney and liver of *O. niloticus* fingerlings exposed to the concentrations of aqueous crude leaf extract of *B. aegyptiaca* therefore it should be used with caution during fish anaesthesia.

Keywords: Histopathology; Gill; Kidney; Liver; *Balanites aegyptiaca; Oreochromis niloticus.*

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Introduction

Balanites aegyptiaca, commonly called Desert date, Soapberry tree, Egyptian balsam or thorn tree (Chothani and Vaghasiya, 2011), is a member of the family Zygophyllaceae. It is a multipurpose tree (food, medicines, cosmetics, fodder, and fuel wood) yet is used as pesticides (Hyelda, 2017) and piscicides (Neuwinger, 1994) owing to the presence of saponins (Wakawa *et al* 2018). Different piscicides (chemical) are used to increase aquaculture production (Nasiruddin *et al* 2012) by eliminating undesirable fauna but the use of less expensive, biodegradable and environmentally safer plant piscicides are encouraged in commercial pisciculture ponds (Nasiruddin *et al* 2012). Plant extracts are not only used as piscicides but are also used for poison fishing in lentic and lotic waters (Power *et al* 2008). Parts of *B. aegyptiaca* have been reported as fish poison (Nkunya *et al* 1990) thus used for fishing.

Many authors have reported the effect of plant extracts on histopathology of organs of fish. Akinsanya *et al* (2016) examined the effect of seven rich plant extracts (*Piper guineense, Aframomum melegueta, Moringa oleifera, Gongronema latifolium, Azadirachta indica, Garcinia kola and Xylopia aethiopica*) on histopathology of gills of *C. gariepinus* and reported proliferation of tissue, congestion of blood vessel, complete fusion of lamellae and lifting of epithelia after exposing fish to the extracts for 21 days. In their work, Audu *et al* (2017) reported histo-architectural distortions such as hepatocellular degeneration; central and sinusoidal congestions in the liver of *C. gariepinus* exposed to concentrations of *Vernonia amygdalina* and linked the liver damages to high presence of alkanoids in the extract. Abalaka *et al* (2010) earlier reported hyperaemia and severe oedema with fusion of lamellae in the gills of *Clarias gariepinus* during their experiment on histopathological changes in gill and skin of *Clarias gariepinus* exposed to ethanol extract of *Adenium obesum* stem bark. The authors' statistical analysis showed significant difference (p<0.05) in histopathological changes between the treatment means compared with the control. Nasiruddin *et al.* (2012) studied histopathological changes in gills, liver and intestine of *Heteropneustes fossilis* after exposure to 50% ethyl alcohol extract of three dry seed extracts (*Lagerstroemia speciosa, Dipterocarpus turbinatus,* and *Hevea brasiliensis*) and reported damages such as disintegration

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of gill filaments and lamellae, degeneration of hepatocytes and blood vessels and necrosis of the intestinal epithelia. Oluwatoyin (2011) earlier reported necrosis, cellular degeneration, malignancy and inflammation on the gills after exposure of *O. niloticus* to acute concentrations of *Ipomoea aquatica* for 96 hr.

In north-eastern Nigeria local fishermen use parts of *B. aegyptiaca* to collect fish. Parts of the plant are crushed and thrown into stagnant water bodies and the stupefied fish are collected. The effects of the plant extracts on the fish are, however, not without attendant health effects on fish organs. In consideration of the poisonous and piscicidal attributes of *B. aegyptiaca*, this study seeks to investigate the acute toxicity effect of the leaf extract of *B. aegyptiaca* on histopathology of gills, kidney and liver of *O. niloticus* fingerlings.

Materials and methods

Collection and preparation of B. aegyptiaca leaves

Fresh leaves from different wild *B. aegyptiaca* plants were collected in Gashua, Bade Local Government Area of Yobe State, Nigeria, and identified in the Department of Plant Science and Technology, Faculty of Natural Sciences, University of Jos, Nigeria with voucher no JUHN2000323 . The leaves were washed with clean water several times to remove soil, dust or dirt. A quantity of 3 kg was shade dried to prevent the loss of active components. The dried samples were pulverized with pestle and mortar and sieved (0.5 mm sieve) into fine powder before storing in airtight plastic containers.

Acclimation of the experimental fish (O. niloticus)

A total of 120 mixed sex *O. niloticus* fingerlings were purchased from Alpaks Fish Farm, Rantia, Jos, Plateau State, Nigeria and conveyed in two oxygenated polythene bags (60 fingerlings per bag) to Aquaculture Laboratory of Hydrobiology and Fisheries Unit of University of Jos, Jos, Nigeria. Two weeks prior to the experiment fish were acclimated in six 35 L capacity round plastic containers (20 fingerlings per container) filled with 20 L of water. The water of the holding tanks was changed once every day at 8.00 hours. The fish were fed to satiation artificial diet (Coppens®) twice daily at 10.00 and 17.00 hours, local time.

Experimental design

The experiment consists of six rectangular glass tanks (40x25x23 cm) and sixty mixed sex *O. niloticus* fingerlings, mean weight 23.13±2.43 g and mean total length 12.50±0.39 cm arranged in a randomized block design with six treatments and two replicates each (Rezende, *et al* 2017). Each of the six glass tanks was filled with 10 L of dechlorinated municipal tap water, five of the filled tanks were inoculated with various concentrations of aqueous crude leaf extract of *B. aegyptiaca* and 10 *O. niloticus* fingerlings were introduced into each tank. The other tank which served as the control was also stocked with 10 of the fingerlings but was not inoculated with the test material.

Exactly 50 mixed sex *O. niloticus* fingerlings were randomly introduced into 5 tanks (10 fingerlings per test tank) containing 200.00, 250.00, 300.00, 350.00, and 400.00 mg/L (determined through series of experimental trials) concentrations of aqueous crude leaf extract of *B. aegyptiaca*. The 6th tank (control) was not inoculated with the plant material but 10 fingerlings of *O. niloticus* were introduced into it and setup replicated. The fingerlings (starved 24 hours prior to the experiment) were exposed to the treatment and the control (0.00 mg/L) for 96 hr. There was no renewal of test water or aeration throughout the period of the experiment.

Monitoring water quality parameters

Water quality parameters such as temperature, dissolved oxygen (DO), free carbon dioxide (CO_2), total alkalinity (TA), and hydrogen ion concentration (pH) of the experimental tanks were monitored using the standard methods of American Public health association (APHA) (1985) every 24 hr for four consecutive days.

Excision of organs of O. niloticus fingerlings

After exposure of *O. niloticus* fingerlings to acute concentrations of aqueous crude leaf extract of *B. aegyptiaca*, one fingerling each from treatment and control tanks was removed and dissected to extract the gills, liver and kidney. Each removed organ was rinsed with distilled water to wash off traces of blood. Each organ was preserved in 10 ml specimen bottle containing 5 ml formal saline and transported to National Veterinary Research Institute (NVRI), Vom; Plateau State, Nigeria for histopathological examination of gills, kidney and liver.

Histopathological examination of gills, kidney and liver of O. niloticus fingerlings

Paraffin wax method and haematoxylin-eosin staining techniques of tissue processing described by Drury and Wallington (1967) and Avwioro (2011) were adopted for the examination of the gills, kidney and liver of *O. niloticus* fingerlings exposed to aqueous crude leaf extract of *B. aegyptiaca*.

Statistical analysis

Statistical analysis was performed using IBM SPSS (version 20) software. Data were analyzed by one-way of variance analysis (ANOVA). Treatment means were separated using Tukey's multiple comparisons test. Level of significance was determined at p=0.05 level of probability.

Results

Water quality parameters of experimental tanks during acute toxicity test of O. niloticus fingerlings with aqueous crude leaf extract of B. aegyptiaca

Mean water quality parameters during 96 hr acute toxicity test are presented in Table 1. Mean DO and pH of the

experimental tanks inversely decreased with concentrations of aqueous crude leaf extract of *B. aegyptiaca* while mean Free CO-$_2$ and TA directly increased with concentrations of the extract. All concentration tanks, however, maintained constant temperature. Statistical analyses showed no significant difference (p>0.05) between means of all the variables (Temperature, DO, pH, TA and Free CO_2) in the treatment tanks compared with control.

Table 1: Mean water quality parameters of experimental tanks during acute toxicity test of *O. niloticus* fingerlings exposed to concentrations of aqueous crude leaf extract of *B. aegyptiaca.*

Water quality parameter	Concentration (mg/L)						*p*-value
	0.00	200.00	250.00	300.00	350.00	400.00	
DO (mg/L)	5.60±0.41	3.80±1.02	3.00±3001	3.20±1.21	3.10±1.04	3.00±1.49	0.600
Temp (^oC)	24.50±0.28	24.50±0.28	24.50±0.28	24.50±0.28	24.50±0.28	24.50±0.28	1.000
pH	7.20±0.22	7.00±0.16	6.80±0.17	6.90±0.17	6.90±0.19	6.80±0.24	0.863
Free CO_2 (mg/L)	17.00±0.23	16.00±0.30	18.00±0.15	18.00±0.11	18.00±0.02	19.00±0.13	0.888
TA (mg/L)	38.50±8.12	48.30±7.21	49.00±6.41	45.30±7.18	48.30±5.45	50.30±8.27	0.849

Histopathology of gills of O. niloticus *fingerlings exposed to acute concentrations of aqueous crude leaf extract of* B. aegyptiaca *for 96 hr*

The micrographs of gills of *O. niloticus* exposed to acute concentrations of aqueous crude leaf extract of *B. aegyptiaca* are shown in Plate 1. The gill in the control tank fish is characterized by radiation of secondary lamellae (yellow arrow) from primary lamellae (white arrow) with successive gabs maintained between secondary lamellae. There was progressive dose dependent increase in histopathological lesions in gills of *O. niloticus* exposed to grades of *B. aegyptiaca.* The architecture of gills exposed to concentrations (200, 250, 300, 350 and 400 mg/L) of aqueous crude leaf extract of *B. aegyptiaca* showed more lesions ranging from epical fusion of secondary lamellae, haemorrhage, atrophy of primary lamellae, desquamation and complete erosion of secondary lamellae.

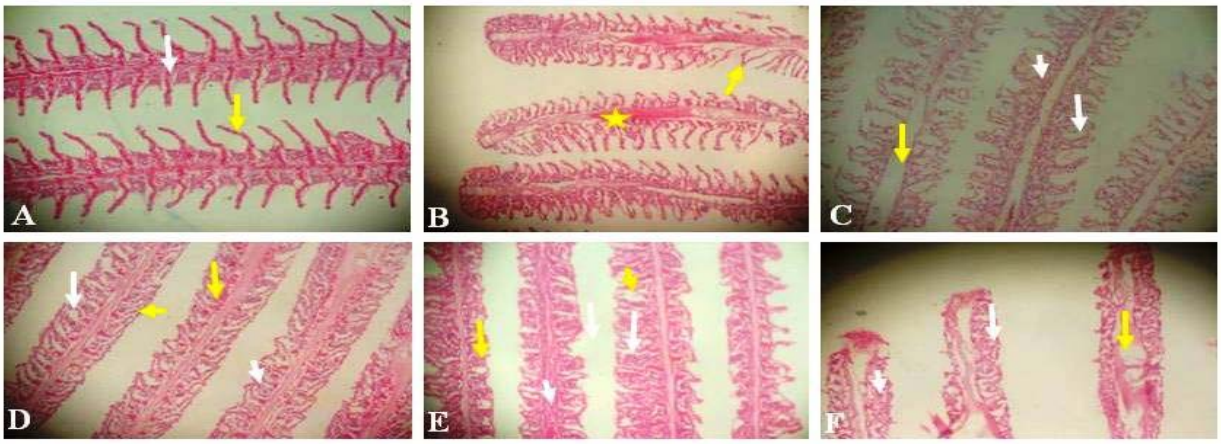

Plate 1: Gills of *O. niloticus* exposed to acute concentrations of aqueous crude leaf extract of *B. aegyptiaca* for 96 hr. **A. Control (0.00 mg/L):** normal gill histology as evidenced by the presence of primary lamellae (white arrow) from which secondary lamellae (yellow arrow) conspicuously radiate out. **B. 200.00 mg/L:** haemorrhage in primary lamellae (yellow star) and epical fusion of secondary lamellae (yellow arrow). **C. 250.00 mg/L:** atrophy of primary lamellae (yellow arrow) evidenced by increased interstitial spaces as well as desquamation of secondary lamellae (white arrowhead) shown by peeling off or flaking of tissues of the primary lamellae while white arrow shows fusion of secondary lamellae. **D. 300.00 mg/L:** haemorrhage in primary lamellae (yellow arrow), fusion of primary lamellae (yellow arrowhead), and desquamation of secondary lamellae (white arrowhead). **E. 350.00 mg/L:** fusion of secondary lamellae (yellow arrow), haemorrhage in primary lamellae (white arrowhead), desquamation of secondary lamellae (white arrow), and atrophy (yellow arrowhead). **F. 400.00 mg/L:** erosion of secondary lamellae (white arrow), atrophy of primary lamellae (yellow arrow) and desquamation (white arrowhead). HandE: x100.

Histopathology of kidney of O. niloticus *fingerlings exposed to acute concentrations of aqueous crude leaf extract of* B. aegyptiaca *for 96 hr*

Plate 2 shows the kidneys' photomicrograph of *O. niloticus* fingerlings exposed to acute concentrations of aqueous crude leaf extract of *B. aegyptiaca* for 96 hr. The control tank (0.00 mg/L) the kidney parenchyma appears normal with intact glomerula substance (star) within the Bowman's capsule separated by capsular spaces (arrow). Concentration dependent damages in the kidney histo-architecture were similar to those observed in the gills with the highest concentration (400.00 mg/L) of the leaf extract having the most severe damage.

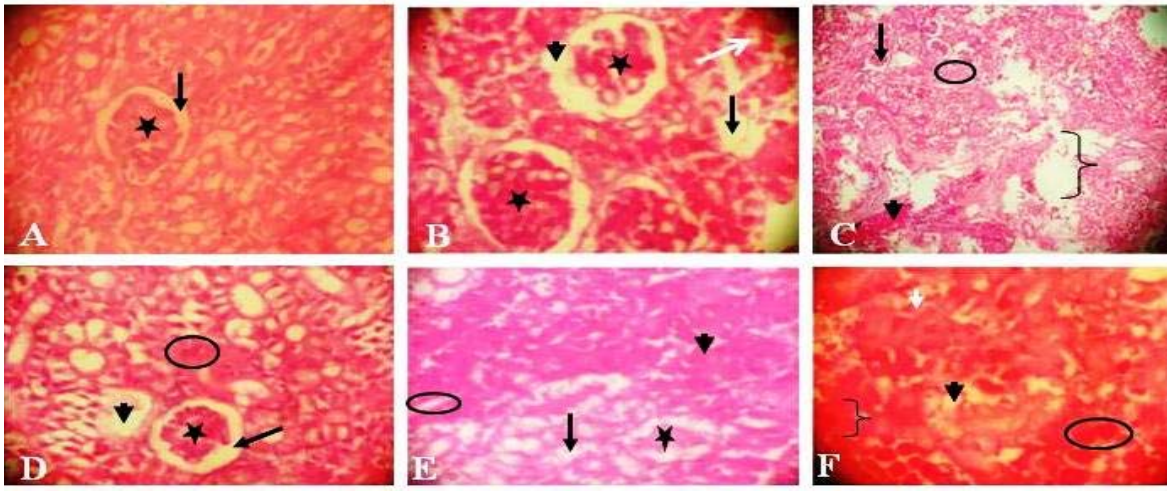

Plate 2: Kidney of *O. niloticus* exposed to acute concentrations of aqueous crude leaf extract of *B. aegyptiaca* for 96 hr. **A. Control (0.00 mg/L):** normal glomeruli (Star) and capsular space (arrow). **B. 200.00 mg/L:** atrophy (black arrow), vacuolation of the tissues (White Arrow), glomeruli (star), and capsular space (arrowhead). **C. 250.00 mg/L:** tissue atrophy (brace), haemorrhage (arrowhead), necrosis (circle), and glomerulus (black Arrow). HandE: x100. **D. 300.00 mg/L:** necrosis (circle), atrophy (arrowhead), glomerulus (star), and capsular space (arrow). **E. 350.00 mg/L:** tissue atrophy (circle), hyperplasia (arrowhead), necrosis (arrow), and glomeruli (star). **F. 400.00 mg/L:** nuclear hypertrophy ((circle), hyperplasia (white arrowhead), atrophy (black arrowhead), and necrosis (brace). HandE: x40.

Histopathology of liver of O. niloticus *fingerlings exposed to acute concentrations of aqueous crude leaf extract of* B. aegyptiaca *for 96 hr*

The histopathological results of liver of *O. niloticus* fingerlings exposed to acute concentrations of aqueous crude leaf extract of *B. aegyptiaca* is presented in Table 3: The observed liver in the control (0.00 mg/L) showed morphologically normal histopathological features with blood vessels (arrowhead) seen in sinusoids (arrow). In contrast, the liver of fingerlings exposed to the various concentrations of the leaf extract showed moderate to severe lesions. The higher concentrations of the leaf extract showed more severe lesions compared to the lower concentrations. The concentration-dependent hepatic histopathological alterations are characterized by hypertrophy, atrophy, haemorrhage, hyperplasia, necrosis and nuclear karyolysis. Alterations in the histopathology of gills, kidney and liver are summarized in Table 2.

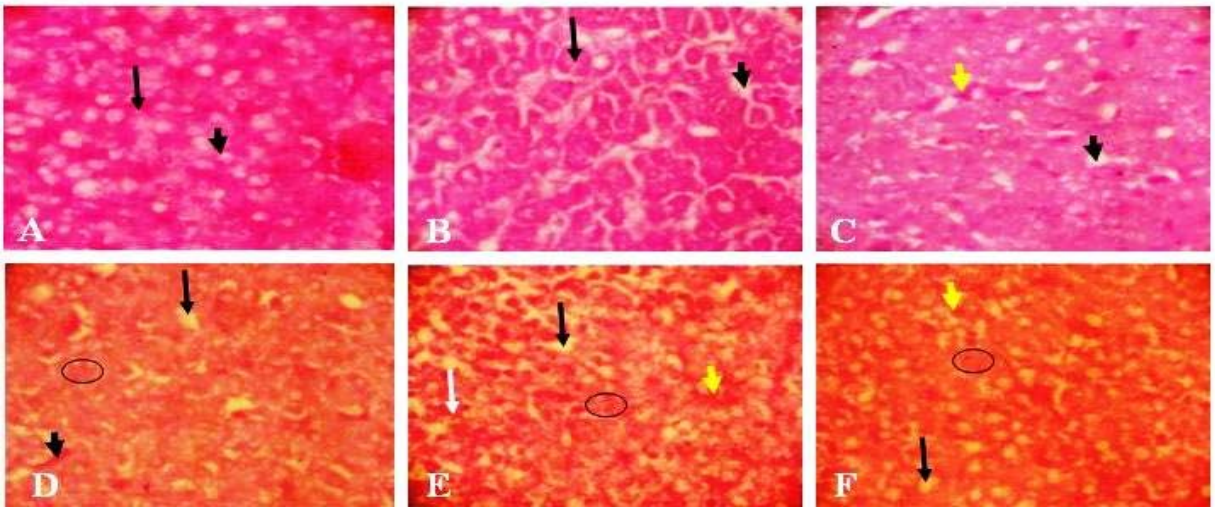

Plate 3: Liver of *O. niloticus* exposed to acute concentrations of aqueous crude leaf extract of *B. aegyptiaca* for 96 hr. **A. Control (0.00 mg/L):** normal hepatic parenchyma evident by normal appearance of cytoplasm and sinusoids (black arrow) with normal blood vessels (black arrowhead). **B. 200.00 mg/L:** mild hypertrophy (black arrow) revealed by presence of enlarged hepatocytes, and mild hepatic atrophy (black arrowhead). **C. 250.00 mg/L:** mild haemorrhage (yellow arrowhead) and moderate hepatic atrophy (black arrowhead). **D. 300.00 mg/L:** moderate hepatic atrophy (black arrow), mild necrosis (circle), and mild haemorrhage (black arrowhead). **E. 350.00 mg/L:** severe hepatic atrophy (black arrow), hyperplasia evident by presence of increased number of cells (white arrow), necrosis (circle), and karyolysis (yellow arrowhead) revealed by increase in cell size and dissolution of nuclei. **F. 400.00 mg/L:** karyolysis (yellow arrowhead), severe necrosis ((circle), and hepatic atrophy (black arrow).

Table 2: Summary of histopathological changes in gills, kidney and Liver of *O. niloticus* fingerlings exposed to 96 hr acute concentrations of aqueous crude leaf extract of *B. aegyptiaca*.

Conc.(mg/L)	Organs	FL	EL	N	A	D	V	H	HR	HP	K
0.00	Gill	-	-	-	-	-	-	-	-	-	-
	Kidney	-	-	-	-	-	-	-	-	-	-
	Liver	-	-	-	-	-	-	-	-	-	-
200.00	Gill	-	-	-	-	-	-	-	-	-	-
	Kidney	-	-	+	+	-	+	-	-	-	-
	Liver	-	-	-	+	-	-	-	-	+	-
250.00	Gill	+	-	-	+	+	-	-	-	-	-
	Kidney	-	-	+	+	-	-	-	+	-	-
	Liver	-	-	+	+	-	-	+	+	-	-
300.00	Gill	+	-	-	+	+	-	-	+	-	-
	Kidney	-	-	+	+	-	-	-	-	-	-
	Liver	-	-	+	+	-	-	-	+	-	-
350.00	Gill	+	-	-	+	+	-	-	+	-	-
	Kidney	-	-	+	+	-	-	+	-	-	-
	Liver	-	-	+	+	-	-	+	-	-	+
400.00	Gill	-	+	-	+	-	-	-	-	-	-
	Kidney	-	-	+	+	-	-	+	-	+	-
	Liver	-	-	+	+	-	-	-	-	-	+

FL = Fusion of Secondary Lamellae; EL = Erosion of Secondary Lamellae; N = Necrosis; A = Atrophy; D = Desquamation; V = Vacuolation; H = Hyperplasia; HR = Haemorrhage; HP = Hypertrophy; K = Karyolysis; + = Presence; - = Complete Absence.

Discussion

Monitored water quality parameters during the 96 hr acute toxicity of aqueous crude leaf extract of *B. aegyptiaca* on *O. niloticus* fingerlings were temperature, DO, TA, pH, and Free CO_2. Mean DO in the present study inversely decreased with plant extract concentrations. Similar findings were recorded by Reboucas *et al* (2015) and Makori *et al*. (2017) in acidic rearing water of juveniles of Nile tilapia and earthen ponds for growing *O. niloticus* respectively. Minimum DO requirement of *O. niloticus* is 3mg/L (Makori *et al* 2017) therefore the DO content of the test water in this study is within tolerable range for *O. niloticus*, hence it could not have contributed to the observed histopathological changes in the gills, liver and kidney of fingerlings of *O. niloticus*. Mean pH in the present study range between 6.80 and 7.20 which is within the optimum pH (5.0-8.0) requirement for rearing Nile tilapia (Nobre *et al* 2014).

Similarly, pH could not have affected the histopathology of gills, liver and kidney of the test animal in this study. Mean TA in the present study directly increased with concentration of aqueous crude leaf extract of *B. aegyptiaca*. The minimum TA (38.50±8.12 $CaCO_3$) in this study is within the minimum (20 mg/L $CaCO_3$) is required for acceptable water pH buffering (Reboucas *et al* 2016), therefore, alkalinity could not have caused the alterations in histopathology of gills, liver and kidney of *O. niloticus* fingerlings.

Histopathological changes, according to Thompson's study (as cited in Naeemi, *et al* 2013), have been widely used as biomarkers in determining the effects of pollutants on fish. The gill which is always in contact with the external environment participates in many important functions such as respiration and osmoregulation (Camargo and Martinez, 2007) and these roles predispose it to structural damages which make the fish vulnerable to respiratory or osmoregulatory difficulties (Olusegun and Adedayo, 2014). Any change in the environment due to external factor may adversely affect the function of the gill (Camargo and Martinez, 2007), particularly, the lamella epithelium and blood vessels (Hinton and Lauren, 1990). In this study the structures most affected by the leaf extract of *B. aegyptiaca* are lamellae and blood vessels. The effect of the extract directly increased with concentration of the plant extract. Similar findings were reported by anonymous (n.d) on fish exposed to waste water.

The changes observed in the gills in this study include tissue atrophy, shortening, desquamation, fusion and erosion of secondary lamellae, and haemorrhage in interstitial spaces and hyperplasia. This is similar to the findings of Abalaka *et al* (2010) on gills of *Clarias gariepinus* exposed to ethanol extract of *Adenium obesum* stem bark. This study also corroborates the finding of Nasiruddin *et al* (2012) on *Heteropneustes fossilis* exposed to 50% ethyl alcohol extract of three dry seeds. Present study is also in line with the findings of Camargo and Martinez (2007) on Neotropical fish in urban stream. One of the first fish organs to be affected by toxicants in the water is the kidney in which the tubules (glomeruli and Bowman's capsule) are the most affected (Takashima and Hibiya, 1995). Kidney also receives the largest postbranchial blood therefore; renal lesions could be good indicators of environmental pollution (Naeemi *et al* 2013). In the present study alterations such as tissue necrosis, tissue atrophy, vacuolation in the glomeruli and mild haemorrhage outside the blood vessels were observed. These results are similar to the findings of Naeemi *et al* (2013) on *Caspian kutum* exposed to linear Alkylbenzene

sulfonate and Camargo and Martinez (2007) on Neotropical fish caged in urban stream.

Rodrigues and Fanta's study (as cited in Hadi and Alwan, 2012) and Olusegun and Adedayo (2014) reported that the organ most associated with detoxification and biotransformation process is the liver and due to its function it is one of the organs most affected by contaminants in the water. In this study the liver of *O. niloticus* fingerlings showed tissue atrophy, hypertrophy, necrosis, haemorrhage in sinusoids, hyperplasia, and nuclear degeneration (karyolysis). Similar results were reported by Hadi and Alwan (2012) and Adebola and Ayo (2014) in *Tilapia zilli* exposed to aluminium and *Heterobranchus bidorsalis* exposed to cypermethrin concentrations respectively. The present result is also in line with the report of Al-Zaidan (2017) on Zebra catfish (*Danio rerio*) exposed to un-ionized ammonia. The alterations in liver in the present study are probably due to excess work required by the fingerlings to get rid of the toxicant from their body during the process of detoxification (Adebola and Ayo, 2014).

Conclusion

Exposure of fish to acute concentrations of aqueous crude leaf extract of *B. aegyptiaca* for 96 hr could be toxic. It could therefore be concluded that exposure of *O. niloticus* fingerlings to acute concentrations (200.00-400.00 mg/L) of *B. aegyptiaca* for 96 hr could cause histopathological alterations in their gills, kidney and liver. Indiscriminate dumping of leaves of *B. aegyptiaca* in lentic water should be avoided or regulated to preserve fish diversity.

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