Use of hematological and biochemical parameters and histological changes to assess the toxicity of drumstick tree (*Moringa oleifera*) seeds extract on Tilapia (*Oreochromis niloticus*) fish

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ABSTRACT

Nowadays *Moringa oleifera* (*M. oleifera*), family Moringaceae, is considered an important food source for human in many parts of the world, and as antiviral, antibacterial, sex reversal in tilapia fish and immunostimulant agent in fishes. This study was conducted to investigate the effect of acute and chronic exposure of *Moringa oleifera* seeds extract on some hematological, biochemical variables and histopathological changes of tilapia fish species *Oreochromis niloticus*. The 96 h LC_{50} value of *M. oleifera* seeds extract to the studied fish was 138 mg/L. For acute study, the same concentration of 96 h LC_{50} (138 mg/L) was used while for chronic exposure, 1/10 of 96 h LC_{50} value (13.8 mg/L) was taken. At the end of acute and chronic exposure, hematological parameters like red blood cell count (RBCs), hemoglobin (Hb), hematocrit (Hct), and mean corpuscular hemoglobin concentration (MCHC) were significantly ($P<0.05$) decreased in fish exposed to seeds extract. However a significant ($P<0.05$) increase in white blood cell count (WBCs), mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) values were observed in the exposed fish during above treatment period when compared to that of the control groups. Biochemical parameters such as glucose showed a significant decrease in acute exposure while increased significantly in chronic exposure ($P<0.05$). The obtained results showed a significant decrease in total protein and plasma cholesterol levels in fish exposed to acute and chronic concentrations of seeds extract while a significant ($P<0.05$) increase in aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine, uric acid and alkaline phosphatase (ALP) activity was observed. Chemical muscle composition in fishes exposed to chronic exposure showed a significant increase in muscle water content and ash content while showing a significant decrease in muscle total lipids and total protein. The studied growth indices showed a significant decrease in body weight gain (BWG), specific growth rate (SGR) and hepatosomatic index (HSI). Histopathlogical sections showed pathological changing in gills, liver, kidney and spleen and the obtained results were discussed. This study could provide valuable information about the toxicity of *M. oleifera* seeds extract to *Oreochromis niloticus*. especially with the wide spread of using in the field of aquaculture and to establish safer limit in water purification.

Keywords: *Moringa oleifera*, *Oreochromis niloticus*, hematology, biochemical parameters, histopathology, growth.

INTRODUCTION

The role of aquaculture in food production, economic development and food security is now well recognized. As the fastest growing food production sector, aquaculture holds promise to help provide a growing human population with food as

many of the world's capture fisheries have reached their biological limits of production or have been depleted through over-fishing and habitat degradation (FAO, 2004). Less well recognized is aquaculture's role in conservation and the recovery of threatened and endangered species. In fact, aquaculture has often been implicated in contributing to the endangerment of aquatic biodiversity.

The aquaculture sector has made significant advances in increased production and environmental protection. However, the sector is now being criticized for degrading the aquatic habitat through release of effluents that include uneaten food, waste products, and pharmaceuticals, and through the escape of farmed fish. There is potential to improve the production, efficiency and environmental sustainability of the sector and the effective management of aquatic genetic resources can assist in addressing all of the above issues (Ferreira *et al.* 2007).

Moringa oleifera and *Carica papaya* are bioactive phytochemical plants, rich with saponine (Ayotunde and Ofem, 2008) and nowadays aquaculturists are using many types of extractions from these plants (seeds, leaves and pods) in sex reversal of tilapia fish with different concentrations to avoid using of 17 α-Methyletestosterone hormone (Oluduro and Aderiye, 2009; Abbas and Abbas, 2011).

Such botanical products when used extensively may enter aquatic systems such as streams, river, and lakes, which may have an effect on non-target organisms in a due course of time (Dongameza *et al.*, 2006; Tiwari and Singh, 2006; Winkaler *et al.*, 2007; Gabriel *et al.*, 2009).

Moringa oleifera is the most widely cultivated species of the genus *Moringa*, which is the only genus in the family Moringaceae. In developing countries, *Moringa* has potential to improve nutrition, boost food security, foster rural development, and support sustainable landcare. Many parts of the *Moringa* are edible. Regional uses of the *Moringa* as food vary widely, and include the immature seed pods, called "drumsticks", popular in Asia and Africa, leaves, particularly in the Cambodia, Philippines, South India, Sri Lanka and Africa, mature seeds and oil pressed from the mature seeds (Murakami *et al.* 2007). *Moringa* has numerous applications in cooking throughout its regional distribution. It may be preserved by canning and exported. *Moringa* is undergoing preliminary research to investigate the potential properties of its nutrients and phytochemicals, some of which include antibacterial effects in vitro, improved glucose tolerance in a rat model of diabetes, inhibition of Epstein-Barr virus activity *in vitro* and reduction of skin papillomas in mice. *Moringa* seed powder is being assessed for its potential to make river water potable. Research showed that filtering with seed powder may diminish water pollution and bacterial counts (Afuang *et al.*, 2003; Ayotunde *et al.*, 2011a &b; Compaore *et al.*, 2011 and Melesse *et al.*, 2013) where the powder joins with the solids in the water and sinks to the bottom and this treatment also removes 90-99% of bacteria contained in water. Using *Moringa* to purify water replaces chemicals such as aluminum sulphate, which are dangerous to people and the environment, and are expensive (Muyibi and Evison, 1995; Fahey, 2005).

Moringa has been used in folk medicine, including Siddha medicine and Ayurvedic traditional medicines and in the Philippines. In Ayurvedic traditional medicine, the leaves are believed to affect blood pressure and glucose levels. In Africa, Indonesia and Philippines *Moringa* leaves are given to nursing mothers in the belief that they increase lactation (Peixoto *et al.* 2011). Also, it is also used to reduce swelling, increase sex drive (as an aphrodisiac), prevent pregnancy, boost the immune system, and some people use it as a nutritional supplement or tonic. *Moringa* is sometimes applied directly to the skin as a germ-killer or drying agent (astringent). It

is also used topically for treating pockets of infection (abscesses), athlete's foot, dandruff, gum disease (gingivitis), snakebites, warts, and wounds (HDRA, 2003). *Moringa* also is used for "tired blood" (anemia); arthritis and other joint pain (rheumatism); asthma; cancer; constipation; diabetes; diarrhea; epilepsy; stomach pain; stomach and intestinal ulcers; intestinal spasms; headache; heart problems; high blood pressure; kidney stones; fluid retention; thyroid disorders; and bacterial, fungal, viral, and parasitic infections (Rao *et al.*, 1990; Ferreira, 2004).

All of the parts of the tree can be used in a variety of ways. It provides lots of leafy material that is useful when using as human food, animal food, fertilizers, natural pesticides, domestic cleaning agent, fuel wood and other uses (HDRA, 2003; Astuti *et al.*, 2007).

The seeds of *Moringa* are often referred to as peas and can be used from the time they appear until they turn yellow and their shells begin to harden. The seeds contain 35% oil and this is used for cooking purposes. The oil does not turn rancid and also burns without smoke. Oil from *Moringa* seeds is used in foods, perfume, and hair care products, and as a machine lubricant (Richter *et al.*, 2003). The seeds are roasted, pounded, mixed with coconut oil and used for their antibiotic and anti-inflammatory properties to treat arthritis, rheumatism, gout, cramp, sexually transmitted diseases and boils. Roasted seeds and oil can encourage urination and can also be used as a relaxant for epilepsy (Carceres *et al.* 1992).

Biological monitoring techniques like hematological, biochemical variables and histological changes have become attractive and useful for monitoring environmental quality, water pollution and the health condition of aquatic organisms (Olufayo, 2009). The entry of toxicants into aquatic media may affect the water quality parameter which in turn leads to changes in the hematological variables of fish, due to its close association with the external environment (Carvalho and Fernandes, 2006 and Kavitha *et al.*, 2010). Biochemical biomarkers like glucose, protein, creatinine, uric acid and enzymes are frequently used as bioindicators of the general state of health and early warning of stress in fish under stressful conditions (Abou El-Naga *et al.*, 2005; Osman *et al.*, 2010).

MATERIALS AND METHODS

Extraction of *M. oleifera*

The seeds of *M. oleifera* were collected from the plant protection area, agriculture Research Centre, Dokki, Giza, Egypt, and seed powder was prepared according to the method described by Price (2000). Seed powder (25 g) was soaked in 250 ml of distilled water for 15 h. Then, the suspension was shaken thoroughly and filtered through Whatman filter paper No. 1 to collect crude extract (stock solution).

Determination of LC_{50}

The lethal concentration (LC_{50}) of *M. oleifera* was obtained by exposing *Oreochromis niloticus* to different concentrations of *Moringa* in static system. The concentration response relationship (LC_{50}) was determined according to the method of Litchfield and Wilcoxon (1949).

Animals and experimental design

Healthy fishes of *Oreocromis niloticus* (35.2±4.8 g) were collected from Hager Abo-Daghar Farm, Luxor governorate, Egypt and stocked and acclimated in glass aquaria. During the period of acclimatization the fish were fed by 25% protein pelleted diet twice daily. The feeding was withheld for one week before starting the experiment to keep the experimental animals more or less in the same metabolic state.

Aeration was provided throughout the study. Physicochemical parameters of studied water are measured according to APHA (1998) and presented in Table (1). The physicochemical parameters of tested water were maintained throughout the study period. Before the start of the experiment, suitable numbers of fish were transferred into glass aquaria which were continuously aerated.

Two separated experiments were conducted. For acute toxicity test, ten aquaria (80X60X50cm) were prepared and each aquarium filled with 200 L of water. The concentration of *M. oleifera* seed extract (138.0 mg/L) was added after removal of same quantity of water to five aquaria. Then 10 fish were introduced into each aquarium. Simultaneously a control setup without the addition of extract was also maintained in the other five aquaria. After 96 h, the live fish from treatment and control groups were sacrificed for growth indices, chemical muscle composition and the hematological, biochemical assay and histopathological changes.

For chronic toxicity test, a total of 100 fingerlings from the stock were introduced into the same size glass aquaria 200 L (80X60X50 cm) capacity with dechlorinated water. The sublethal concentration (1/10 of LC_{50} which was 13.8 mg/L) of *M. oleifera* seed extract was added to the aquaria after removal of same quantity of water. A control group was maintained in other separated glass aquaria. Chronic exposure was carried out for a period of 42 days (6 weeks) and sampled at the end of exposure.

Sampling

Fish were randomly selected from control and experimental aquaria and blood samples were withdrawn from the *arteria caudalis* using a syringe containing sodium citrate as an anticoagulant. The whole blood was used for hematological assay. Total number of erythrocytes (RBCs) and leukocytes (WBCs) were counted using improved Neubauer Haemocytometer. Hemoglobin (Hb) content was estimated using cyanmethemoglobin method described by Van Kampen and Zijlstra (1961). Hematocrit (Hct) was carried out in small capillary hematocrit tubes using microhaematocrit centrifuge at 3000 rpm for 15 minutes.

Blood indices namely Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH) and Mean corpuscular hemoglobin concentration (MCHC) were calculated according to Gupta (1977). The blood was then centrifuged for 15 min at 3000 rpm to separate plasma which was used for the estimation of glucose, total protein, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) activities, creatinine, uric acid and cholesterol.

Growth indices in chronic exposure

Body weights were recorded to the nearest gram and total body lengths were measured to the nearest 0.l cm to adjust the artificial feed rate every two weeks (Initial weights ranged from 3o-40 g for all the experimental studies at the beginning of the experiment. Then the following growth indices were determined:

-Body weight gain [BWG (g)] = (Final body weight)-(initial body weight).

- Specific growth rate [SGR]: Specific growth rate (growth rate/day) was determined as a percentage of body weight gain/day according to the equation postulated by Allen and Wooton (1982):

$$S.G.R. = \frac{Ln\ W_f - ln\ W_0}{T_f - T_0} x100$$

Where: W_f: The final weight of fish in g., W_0: The initial weight of fish in g.

(T_f - T_o): Time between the final and initial weight in days.

Ln : Logarithm to base e.

-Condition factor (k): k factor was calculated for individual fish from the formula recommended by Schreck and Moyle (1990):

$$K = \frac{W}{L^3} \times 100$$

Where: W: is the wet weight in g and L: is the total length in cm.

-Hepatosomatic index (HSI) = (Liver weight (g)/Body weight (g)) x 100

Biochemical analyses

A- Plasma analyses

Blood samples were centrifuged at 3000 rpm to get plasma for the following analyses:

a-The level of plasma glucose was measured using Boehringer Mannheim kits according to Trinder (1969).

b- Total protein content was determined by Biuret test (King and Wootton, 1959).

c- Plasma aspartate aminotransferase (AST, E.N. 2.6.1.1) and alanine aminotransferase (ALT, E.N. 2.6.1.2) activities were determined colorimetrically using transaminases kits according to the method described by Reitman and Frankel (1957).

d- Creatinine was measured using colorimetric method described by Henry (1974).

e- Uric acid content was measured using the enzymatic reaction adopted by Barham and Trinder (1972).

f- Plasma alkaline phosphatase activity was determined according to the method described in Bergemyer (1974).

g- Plasma cholesterol was measured colorimetrically according to Watson (1960).

B- Muscle quality: Muscle quality was determined using the following parameters:

a- Muscle water content was determined according to Sidwell *et al*. (1970).

b- Muscle total protein was determined using the semi-microkjeldahl method as reported by Josyln (1950)

c- Muscle total lipids were determined by the standard method reported in AOAC (1970).

d- Muscle ash was determined by burning samples in a muffle furnace for 16 hours at 550°C (Sidwell *et al*., 1970)

Histological studies:

Tissue specimens from gills, liver, kidneys and spleen were taken from tilapias that were exposed to *M. oleifera* by the end of chronic exposure. The specimens were fixed in 10% formalin. They were processed by conventional method, sectioned at 5 µm and stained with Hematoxylin and Eosin (Bancroft *et al.,* 1996).

Statistical analysis:

The results were statistically analyzed using Duncan's multiple range tests to determine difference in means using Statistical Analyses System (SAS, 2000) and Software Program of Statistical Analysis (SPSS, 2008). One way ANOVA test (Analysis of variance) comparing the treated and untreated control groups in all months. Differences in all the studied parameters were assessed by one way ANOVA.

RESULTS

Water quality characteristics of water used in the experiment are presented in Table (1) and all data of the examined parameters were within the permissible levels of growing fishes (Boyd, 1990).

Table 1: Physicochemical characteristics of water used in the experiment.

Parameters	Mean	Parameters	Mean
pH	6.7	Bicarbonate (HCO_3^-)	118.7 mg L^{-1}
Temperature	27 ^oC	Carbonate (CO_3^-)	0.02 mg L^{-1}
Dissolved Oxygen	5.6 mg L^{-1}	Sulphate (SO_4^-)	86.3 mg L^{-1}
Alkalinity ($CaCO_3$)	145.8 mg L^{-1}	Chloride(Cl^{-1})	14.9 mg L^{-1}
Total Hardness ($CaCO_3$)	128.7 mg L^{-1}	Calcium (Ca^{++})	29.6 mg L^{-1}
Ammonia (NH_3)	0.3 mg L^{-1}	Magnesium (Mg^{++})	6.4 mg L^{-1}
Ammonium (NH_4^+)	0.6 mg L^{-1}	Potassium (K^+)	4.9 mg L^{-1}
Nitrite (NO_2^-)	0.0 mg L^{-1}	Sodium (Na^+)	35. 3 mg L^{-1}
Nitrate (NO_3^-)	0.8 mg L^{-1}	Electric conductivity	0.2 Mmohs cm^{-1}
Total soluble salts	264 mg L^{-1}		

Investigations of blood indices have been suggested to be a valuable tool in assessing the health condition of *Oreochromis niloticus* (Ates *et al.*, 2008).

The effect of acute and chronic exposure of *M. oleifera* seed extract on hematological parameters of *Oreochromis niloticus* was presented in Table (2). Hematological examination showed a significant (P<0.05) decrease in Hb, Hct, RBCs, and MCHC values in fish treated with *M. oleifera* seed extract. In contrast there was a significant increase in WBCs, MCV and MCH value when compared with their control groups (P<0.05) at the end of the experimental periods in both exposures.

Table 2: Acute and chronic effect of *M. oleifera* seeds extract on some hematological parameters of Tilapia fish (*Oreochromis niloticus*).

Parameters	Acute (138 mg/L)		Chronic (13.8 mg/L)	
	Control	Treated	Control	Treated
RBC_S (X 10^6 mm^{-3})	1.27±0.12 ^{a}	1.07±0.11 ^{b}	1.27±0.12 ^{a}	1.34±0.08 ^{b}
WBC_S (X 10^3 mm^{-3})	25.12±1.18 ^{a}	34.17±1.88 ^{b}	25.12±1.18 ^{a}	31.37±2.03 ^{b}
Hb (g/dl)	7.14±0.27^a	6.01±0.19 ^{b}	7.14±0.27 ^{a}	6.95±0.41 ^{b}
HCT (%)	20.88±1.16 ^{a}	15.37±0.94 ^{b}	20.88±1.16 ^{a}	17.84±1.03 ^{b}
MCV (fl)	11.22±1.6 ^{a}	13.87±1.13 ^{b}	11.22±1.6 ^{a}	14.06±1.11 ^{b}
MCH (pg)	38.21±2.71 ^{a}	41.71±2.35 ^{b}	38.21±2.71 ^{a}	44.16±3.12 ^{b}
MCHC (g/dl)	29.84±2.29 ^{a}	27.95±1.89 ^{b}	29.84±2.29 ^{a}	25.23±2.14 ^{b}

The acute exposure of *M. oleifera* seed extract on biochemical parameters of *Oreochromis niloticus*, showed a significant decrease (P<0.05) in plasma glucose, total protein and cholesterol level while showed a significant increase (P<0.05) in plasma creatinine and uric acid values and AST, ALT and ALP activities at the end of exposure time when compared to control.

In the chronic toxicity of *M. oleifera* seed extract on biochemical parameters of *Oreochromis niloticus* (Table 3), the plasma glucose, creatinine, uric acid, AST, ALT and ALP levels showed a significant increase (P<0.05) in seed extract treated fish at the end of the experimental period while there was a significant decrease (P<0.05) in plasma protein and cholesterol level.

Table 3: Acute and chronic effect of *M. oleifera* seeds extract on some biochemical parameters of Tilapia fish (*Oreochromis niloticus*).

Parameters	Acute (138 mg/L)		Chronic (13.8 mg/L)	
	Control	Treated	Control	Treated
Glucose (mg/100ml)	102.16±6.14 [a]	88.24±5.52 [b]	102.16±6.14 [a]	167.44±6.33 [b]
Total Protein (µg/ml)	3.79±0.63 [a]	2.46±0.43 [b]	3.79±0.63 [a]	1.91±0.28 [b]
Creatinine (mg/100ml)	1.053±0.17 [a]	1.839±0.33 [b]	1.053±0.17 [a]	1.372±0.29 [b]
Uric acid (mg/100 ml)	18.39±1.92 [a]	23.55±2.06 [b]	18.39±1.92 [a]	21.93±1.88 [b]
Aspartate aminotransferase (IU/L)	29.12±3.15 [a]	38.17±4.22 [b]	29.12±3.15 [a]	34.87±2.94 [b]
Alanine aminotransferase (IU/L)	20.71±2.09 [a]	33.95±3.11 [b]	20.71±2.09 [a]	28.77±2.66 [b]
Alkaline phsphatase (IU/L)	17.34±1.96 [a]	28.66±2.26 [b]	17.34±1.96 [a]	23.05±1.77 [b]
Plasma Cholestrol (mg/dl)	179.61±8.67 [a]	139.27±7.44 [b]	179.61±8.67 [a]	153.12±14.45 [b]

Concerning the chemical muscle composition, there were significant changes in water content, total protein, total lipids and ash composition in fish exposed to chronic exposure of *M. oleifera* seed extract (13.8 mg L^{-1}) at the end of the experiment (42 days) compared to control value and (Table 4). A significant increase occurred in the water content and ash of muscle; whereas total protein and total lipids were significantly decreased compared with the control group.

Table 4: Chronic effect of *M. oleifera* seeds extract on chemical muscle composition of Tilapia fish (*Oreochromis niloticus)*.

Parameters	Chemical muscle composition (13.8 mg/L of *M. oleifera*)	
	Control	Treated
Water content (%)	74.78±1.2[a]	76.3±1.3[b]
Crude protein (% of dry weight)	14.6±0.79[a]	13.3±0.91[b]
Total Lipids (% of dry weight)	5.21±0.38[a]	4.87±0.22[b]
Ash (% of dry weight)	5.64±0.36[a]	5.92±0.31[b]

Regarding the effect of *M. oleifera* seed extract (13.8 mg L^{-1}) on tilapia fish in the chronic exposure (42 days), the obtained results showed a decrease in body weight gain; specific growth rate, hepatosomatic index (HIS) and condition factor (K) compared to the control values (Table 5).

Table 5: Chronic effect of *M. oleifera* seeds extract on some growth indices of Tilapia fish (*Oreochromis niloticus*).

Parameter	Control	Treated
Initial body weight (g)	35.2±4.8	36.3±4.2
Final body weight (g)	48.8±3.4	45.9±3.9
Body weight gain (g)	13.6±4.2	9.6±4.1
Specific growth rate (g/day)	2.6±0.4	1.97±0.3
Hepatosomatic index	1.8±0.3	1.4±0.4
Condition factor (K)	1.1±0.05	0.86±0.07

Histopathological investigations:

The above biochemical investigations were confirmed by the histological sections. The histological alterations found in the gill, liver, kidney and spleen tissues of O. niloticus fish are detailed in Figures (1-2). In the present study, the histopathological examination showed marked deterioration in the gill, liver, kidney and spleen of fishes exposed to chronic exposure of *M. oleifera* than in the controls.

Control gill tissue is shown in Figure (1A). The microscopic examination of gill sections revealed that there was hyperplasia and lamellar fusion between the secondary lamellae which leading to decrease or blocking the interlamellar spaces

Figure (1B), congestion in the lamellar and branchial blood vessels also detected. Degenerative changes in the form of vacuolar degeneration and necrosis also detected in the epithelial lining the secondary lamellae Figure (1C).

Control liver tissue is shown in Figure (1D) and regarding the histopathological changes in the liver of fishes exposed to chronic exposure of *M. oleifera* revealed slight degenerative changes in some of the hepatocytes such as cloudy swelling where the heptatocytes were slightly smaller and elongated, with finely granular easinophilic cytoplasm and apparently normal nuclei Figure (1E & 1F).

Control kidney tissue is shown in Figure (2G) and the main pathological changes in the kidney of examined cases were degenerative changes and necrosis were not restricted to convoluted tubules but extended to involve the collecting tubules where the tubular epithelium showed hydropic degeneration and necrosis. Also the interstitial haemopaeitic renal tissue showed marked necrosis and infiltration of heterophils in the necrotic areas of renal parenchyma Figure (1H & 1I).

Control spleen tissue is shown in Figure (2J) and the spleen of treated fish showed necrosis and depletion of lymphocytic elements associated with increase the number and extension of melanomacrophage centers Figure (1K). Also haemosiderin has been observed to increase considerably within melanomacropahge centers Figure (1L).

DISCUSSION

In the present study, the 96 h LC_{50} value of *M. oleifera* seed extract for the freshwater fish *Oreochromis niloticus* was found to be 138 mg/L which indicates that *M. oleifera* seed extract is toxic to tilapia fish at higher concentrations. Kumar *et al.* (2010) reported that the 24, 48, 72 and 96 h LC_{50} values of aqueous extract of *Euphorbia tirucalli* latex to the fish *Heteropneustes fossilis* was found to be 3.450µl/L, 2.516µl/L, 1.623µl/L and 1.315µl/L respectively. Tiwari and Singh (2003) observed the toxicity of *Nerium indicum* leaf extract to the fish *Channa punctatus* and indicate that the toxicity depends on the solvent used for extraction; the LC_{50} value of diethyl ether, acetone, chloroform and methanol extract of *N. indicum* leaf extract were found to be 17.34 mg/L, 40.01 mg/L, 40.61 mg/L and 106.37 mg/L, respectively. The 24 h LC_{50} value of neem leaf extract for *Prochilodus lineatus* was found to be 4.8 g L^{-1} (Winkaler *et al.*, 2007). The differences in the LC_{50} value of various parts of plant species to fish depend on the chemicals present in the plants, fish size, age, species and also the sensitivity of the fish used for the experiment. Behavior responses of fish were used to examine the toxic nature of pollutants in the aquatic environment. In the present work, copious mucous secretion, loss of equilibrium, restlessness, jerky movements, rapid opercula beat were noted especially during acute treatment.

Hematological investigations have proven valuable for fisheries biologists for quick detection of changes in fish health and these changes may precede changes in fish behavior and visible lesions.

In general the decrease level of RBCs count, Hb and Hct may be due to the destruction of RBCs and erythroblastosis leading to anaemia (Wintrobe, 1978). In the present study the decreased level of RBCs count, Hb and Hct content in fish treated with seed extract might have resulted from hemolysis caused by this extracts. Similar observations were also noted in *Clarias gariepinus* exposed to leaf extracts of tobacco, *Nicotiana tobaccum* and cassava effluents (Omoniyi *et al.*, 2002; Adeyemo, 2005). In contrast Ayotunde *et al.* (2004) noted an increase in RBCs count, Hb and

Hct content in *O. niloticus* exposed to aqueous extracts of *Moringa oleifera* seeds. The decrease in Hb content during stress condition may indicate a decrease in the rate of Hb synthesis which leads to impaired oxygen supply to various tissues resulting decrease in the number of RBCs through haemolysis (Atamanalp and Yanik, 2003). Moreover the lysing of erythrocytes leads to a reduction in hematocrit value (Martinez and Souza, 2002). However the increase in these parameters during sublethal treatment indicates adaptation of the fish to the seed extract toxicity. The observed increase in WBCs during acute and chronic exposure may be due to stimulated lymphopoiesis and/or enhanced release of lymphocytes from lymphomyeloid tissue as a defense mechanism of the fish to tolerate the seed extract toxicity. The increase in leucocyte count indicates the stimulatory effect of the toxicant on immune system and also depends on the toxicant stress (Ates *et al.*, 2008). The MCHC is an indicator of red blood cell swelling and the lowered MCHC during acute treatment might have resulted from release of young erythrocytes containing less hemoglobin into circulation. Whereas the significant increase of MCHC value during chronic exposure may be due to congenital sphaerocytosis as suggested by Sobecka (2001). The significant increase of MCV and MCH during acute and chronic treatment indicates the swelling of red blood cells. Further stress related increase in the erythrocyte volume may be another reason as suggested by Jastrzebska and Protasowickiz (2005).

Measurement of plasma biochemical parameters is mostly used in clinical diagnosis of fish physiology to determine the general status of health (Barnhorn and van Vuren, 2004; Ferreira *et al.*, 2007; Osman *et al.*, 2010). Carbohydrates are the main source of energy in many organisms and their reserve used to meet energy demand in stress condition. The observed increase of plasma glucose level during chronic exposure indicates a stress response triggered by the presence of seed extract in water or might be due to hypoxic condition caused by the seed extract in water. However the decrease in blood glucose during acute exposure can be attributed to high utilization of glucose for oxidation or hypoxic conditions leading to an excess utilization of stored carbohydrates (Kavitha *et al.*, 2012). The decreased level of protein in this study may be due to their degradation and also to their possible utilization for metabolic purposes (Kavitha *et al.*, 2012). Similar observation was also made in *C. punctatus* exposed to latices of *Euphorbia royleana* and *Jatropha gossypifolia* (Singh and Singh, 2002).

Plasma creatinine and uric acid could also be used as a rough index of the glomerular filtration rate (Maita *et al*., 1984). Low values of creatinine and uric acid are not significant but increased values indicated several disturbances in the kidney (Maxine and Benjamin, 1985).

In the present investigation, fish exposed to acute and chronic concentrations of *M. oleifera* showed high level of creatinine and uric acid. It might be attributed to the action of some toxic materials of *M. oleifera* on the glomerular filtration rate which is altered by the pathological changes in the kidney (Ayotunde *et al.*, 2004; Kavitha *et al.*, 2012).

The alterations of enzyme activity under stress condition offer one of the most important biochemical parameter and their level provide information of diagnostic values (Barnhorn and van Vuren, 2004; Adamu, 2009). Transaminase enzymes play an important role in carbohydrate–protein metabolism in fish and determination of transaminases (AST and ALT) can also be used for aquatic biomonitoring (Vutukuru *et al.*, 2007). The enzymes ALT and AST in blood plasma indicate organ dysfunction in aquatic organisms during stress condition (Gabriel and George, 2005). Gabriel *et al.* (2009) noted elevation of both AST and ALT in different organs of catfish hybrid

exposed to aqueous extracts from *Lepidagathis alopecuroides* leaves and suggested that the elevation may be due to disturbances in the Kreb's cycle and the elevation in ALT indicates hepatic damage caused by this plant extracts. The elevation in serum aspartate aminotransferase in Heteroclarias exposed to tobacco leaf dust may be due to the process of either deamination or transamination caused by the plant dust (Adamu, 2009). In the present investigation the significant increase in plasma AST and ALT activity during acute and chronic treatment might have resulted from disturbances in the Kreb's cycle caused by the seed extract. Further structural damages in liver and kidney of the fish due to seed extract toxicity may result leakage of these enzymes into the bloodstream and cause increased activity in plasma. Alkaline phosphatase is an important enzyme of animal metabolism, and plays important role in the transport of metabolites across the membranes (Vorbrodt, 1995). Changes in alkaline phosphatase activity can affect the metabolism of the fish. Increased levels of ALP was noted by Tiwari and Singh (2003) in *C. punctatus* exposed to *N. indicum* leaf extracts and Tiwari and Singh (2006) in *C. punctatus* exposed to aqueous extracts of *E. tirucalli* plant. Gabriel *et al.* (2009) reported an increase in ALP activity in catfish exposed to aqueous extracts from *L. alopecuroides* leaves and suggested that the elevation may be due to damage in the liver and kidney of fish with increased cell membrane permeability. In the present study the elevation of ALP activity may be due to the cellular damage caused by seed extract or a response to overcome toxicity of seed extract (Singh and Singh, 2005).

Effect of toxicants on the cholesterol level of fishes has been reported by many authors. Munoz *et al*. (1991) studied the stress responses of juvenile rainbow trout; *Salmo gairdneri* exposed to sublethal concentration of copper and found rapid elevation of plasma cholesterol for the first 15 days and then decreased at day 21. However, El-Sabbagh (1996) recorded reduced level of cholesterol in Nile tilapia *Oreochrmis niloticus* after exposure to sublethal concentration of copper sulphate for 3 weeks.

Bentick-Smith *et al*. (1987) mentioned that the serum total cholesterol level in the different species of catfish ranged from 152.0 to 212.0 mg/dl. Kirubagaran and Joy (1992) reported a significant decrease in cholesterol in catfish exposed to 0.04 mg methyl mercuric chloride/l for 90 days. Hilmy *et al*. (1980c) described an increased level of serum total cholesterol in fish exposed to mercury. Santos and Hall (1990) didn't find any changes in the total cholesterol level in lead exposed fish. Holcombe *et al*. (1976) and Kandil (1987) explained the toxic role of the heavy metals on liver concerning the cholesterol synthesis, estrification and excretion.

Nonetheless, saponin from different plant sources could lower the plasma cholesterol levels in a variety of animals as well as in human subjects (Matsuura, 2001). Large mixed micelles formed by the interaction of saponins with bile acids account for their increased excretion when saponin-rich foods are consumed (Oakenfull and Sidhu, 1990). The resulting accelerated metabolism of cholesterol in the liver causes its serum level to go down. In the present study, the hypocholesterolemic response of tilapia exposed to *Moringa* extract could be due to the high Neutral detergent fiber (NDF) content of the *Moringa* seed extract which might have played a major role in decreasing the plasma cholesterol level (Dongmeza *et al.*, 2006).

Quality of meat could be measured by determining its chemical composition (Table 4). Decreased total muscle protein and total lipids of fish exposed to chronic concentration (13.8 mg/L) of *M. oleifera* might be attributed to the action of Moringa

that may critically influence the growth rate and the quality of fish meat (Dongmeza *et al*., 2006).

The increase in muscle water content was explained by Weatherly and Gill (1987) who reported that depletion of body constituents (protein and lipids) results in tissue hydration of inverse dynamic relationships between protein as well as lipids and water content in the muscles. Sakr (2001) and Tawfeek (2004) reached similar results in some freshwater fishes.

Growth in the most general sense reflects significant changes in the metabolic processes occurring in the organism (Glubokov, 1990) and it can be made to approach an optimum in cultured fish by manipulating temperature, light regimes, water quality and amounts and types of nutrients offered (Weatherly and Gill, 1987).

Oreochromis niloticus exposed to chronic concentration of *M. oleifera* revealed a decrease in body weight gain compared to control group (Table 5), this could be due to reduction in food consumption and/or decrease in gross food conversion rate. Inhibition of growth was also reported by Abdelghany (1998).

The condition factor changes (Table 5) may be found to reflect fairly the changes in body protein and lipid content (Weatherly and Gill, 1983) and consequently growth rate (Schreck and Moyle, 1990).

Decreasing of HSI in the present study might be due to the reduced fats absorption and consequently reduced fat retention in the whole body, particularly in the liver. The obtained results showed a lower body lipid content. These finding is in agreement of that of Dongmeza *et al*. (2006).

The values of the condition factor "k" are estimated for comparative purposes to assess the impact of environmental alterations on fish performance (Clark and Fraser, 1983). Therefore, the decrease in factor "k" reflects the health condition of the fish as well as their protein and lipid contents (Weatherly and Gill, 1983).

Decreased factor "k" of fish exposed to chronic exposure of *M. oleifera* might be due to the decreased efficiency of gills, liver and kidney that showed clear hemorrhage and destruction Figures (1-2). However, control fish group exhibited normal parameters.

The above biochemical investigations were confirmed by the histological sections. The histopathological changes in gills may be as a result of *Moringa* extract in water which act as an irritant and accumulated on the gill lamellae because the gills is a site of uptake in freshwater fishes. The obtained results were in agreement with those of Ayotunde *et al.* (2011a & b).

Control gill tissue is shown in Figure (1A) and histopathological changes in the gills after chronic exposure to *M. oleifera* revealed that there was hyperplasia and lamellar fusion between the secondary lamellae Figure (1B) and degeneration changes and necrosis in the epithelial lining the secondary lamellae associated with congestion Figure (1C), similar findings were previously reported by Jiraungkoorskul *et al.* (2003) and Ayotunde (2011b).

Control liver tissue is shown in Figure (1D) and regarding the histopathological changes in the liver of fishes exposed to chronic exposure of *M. oleifera* revealed thinner vascular walls, congestion as well as early fibrosis Figure (1E) and slight degenerative changes in some of the hepatocytes Figure (1F). This result was in agreement with the findings of Oluduro and Aderiye (2009) and Ayotunde *et al.* (2011b).

Control kidney tissue is shown in Figure (2G) and the main pathological changes in the kidney of examined cases were diffuse tubular vascular degeneration and nicrosis Figure (2H) and degenerative changes and necrosis in the tubular

epithelium and the interstitial haemopaeitic tissue associated with infiltration of heterophils in the necrotic areas of renal parenchyma Figure (2I). This result was in the same line with that of Oluduro and Aderiye (2009).

The changes in kidney tissue may be attributed to the renal tissue receive large volumes of blood flaw and serve as a major route of excretion for metabolites of various xenobiotics which may leading to nonspecific histopathological lesions (Ayotunde *et al.*, 2011a).

Control spleen tissue is shown in Figure (2J) and the spleen of treated fish showed necrosis and depletion of lymphocytic elements associated with increase the number of melanomacrophage centers Figure (2K) and accumulation of haemosiderin pigments within the melanomacropahge centers Figure (L). This result was in agreement with the previous findings of Ayotunde (2011a).

CONCLUSION

Even though seed extract of *M. oleifera* has wide applications in aquaculture operations, the health hazard of these plant extract to aquatic organisms has not been studied in detail. The findings of the present study indicate that seed extract of *M. oleifera* has significant effect on hematological and biochemical parameters and also cause histopathological changes of the fish. Application of *M. oleifera* in aquatic activities need more investigations before widespread in its uses and for safe applied.

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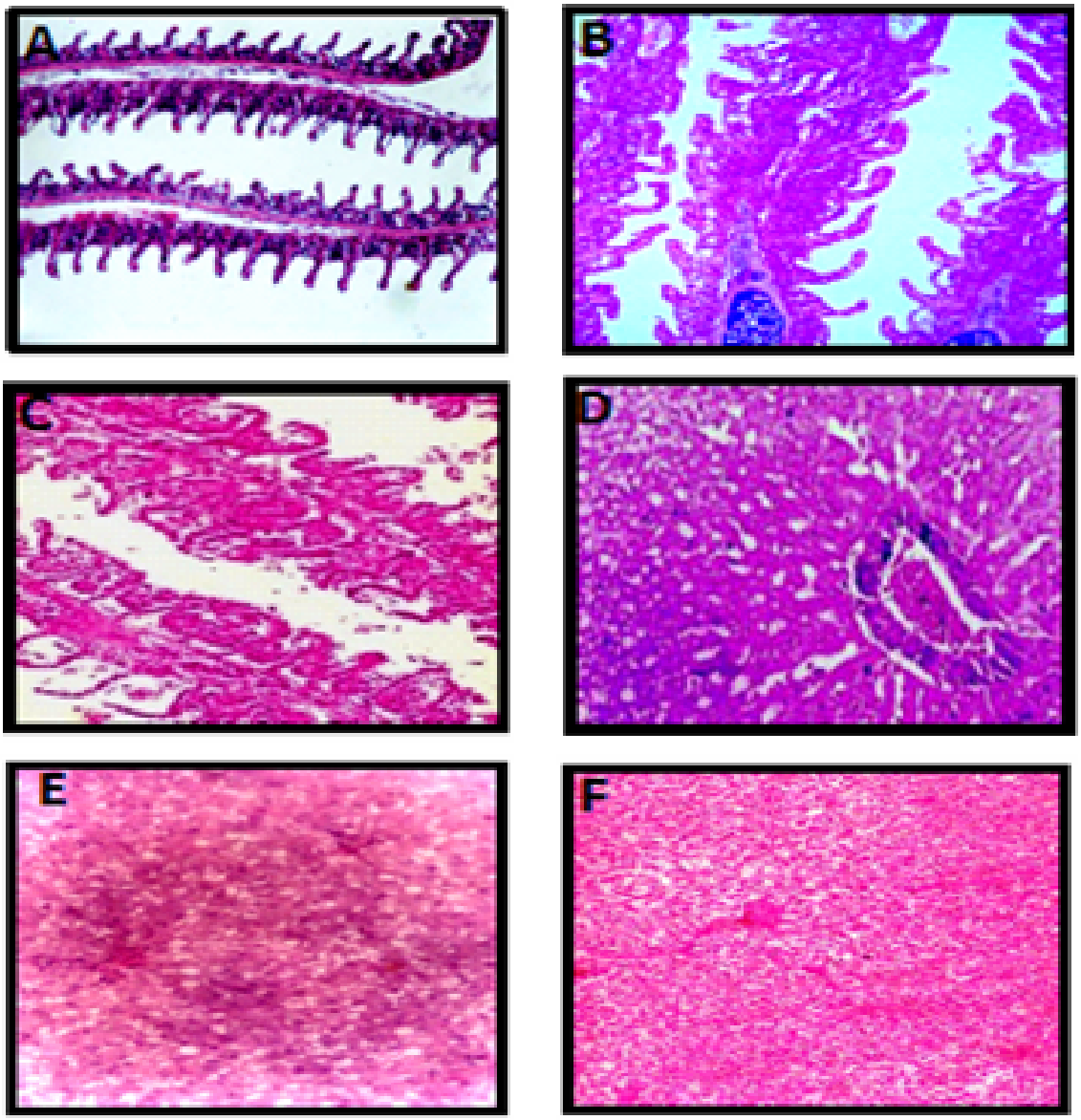

Fig. 1: Photomicrographs of transverse sections of gill and liver tissue of *O. niloticus* (H&E x 400).
A- Control gill tissue.
B- Gill showing hyperplasia and lamellar fusion between the secondary lamellae.
C- Gill showing and degeneration changes and necrosis in the epithelial lining the secondary lamellae associated with congestion.
D- Control liver tissue.
E- Liver showing thinner vascular walls, congestion as well as early fibrosis.
F- Liver showing slight degenerative changes in some of the hepatocytes.

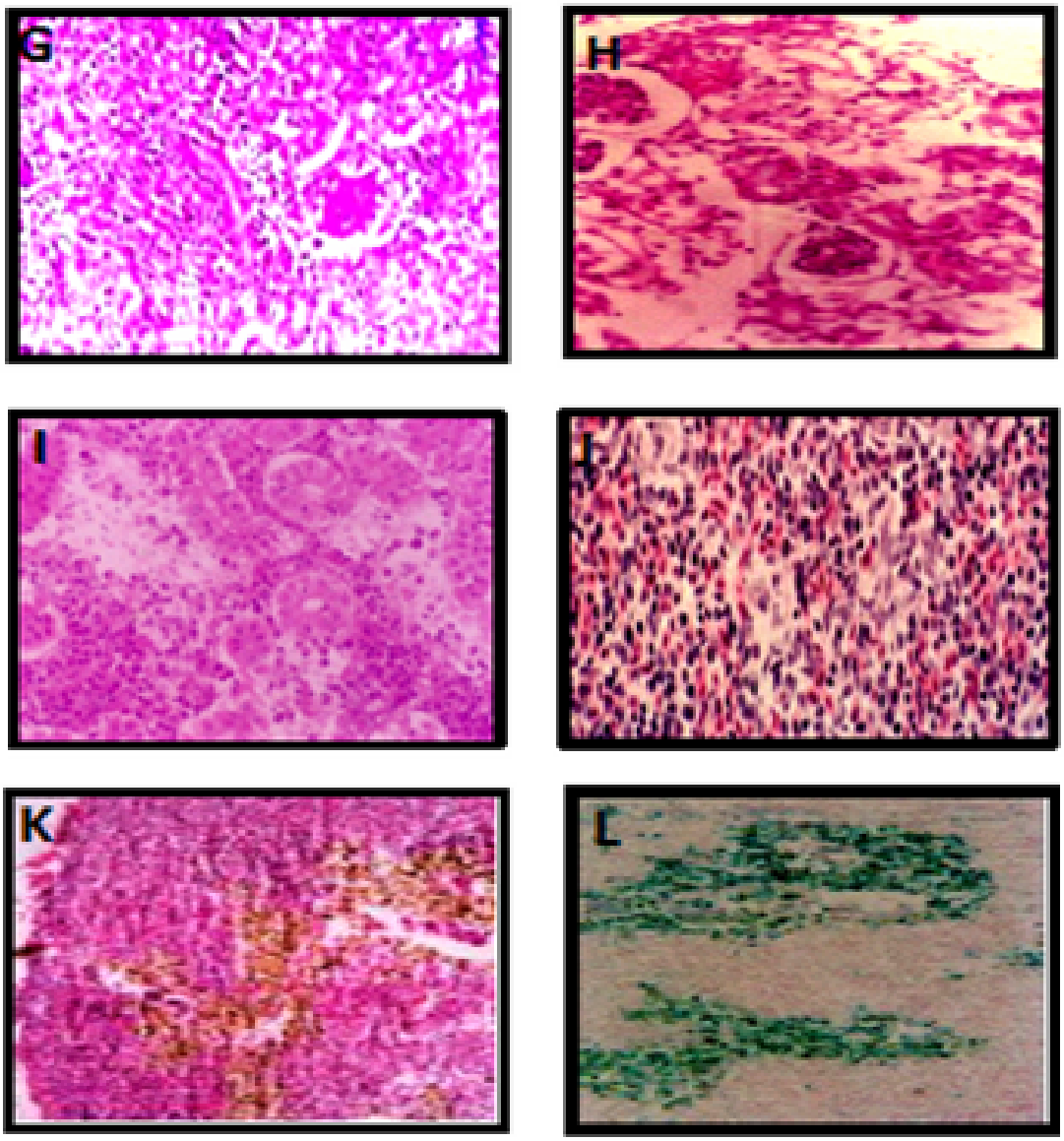

Fig. 2: Photomicrographs of transverse sections of kidney and spleen tissue of *O. niloticus* (H&E x 400).

G- Control kidney tissue.

H- Kidney showing diffuse tubular vascular degeneration and nicrosis.

I- Kidney showing degenerative changes and necrosis in the tubular epithelium and the interstitial haemopaeitic tissue associated with infiltration of heterophils in the necrotic areas of renal parenchyma.

J- Control spleen tissue.

K- Spleen showing showed necrosis and depletion of lymphocytic elements associated with increase the number of melanomacrophage centers.

L- Spleen showing accumulation of haemosiderin pigments within the melanomacropahge centers (Prusion blue stain).

ARABIC SUMMARY

استخدام دلائل الدم والتغيرات الهستولوجية لتقييم سميه مستخلص بذور نبات المورينجا (مورينجا اوليفيرا) على اسماك البلطى النيلى (اريوكرومس نيلوتكس)

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يعتبر نبات المورينجا من اهم المصادر الغذائية للانسان فى عديد من مناطق العالم حيث انها تعتبر مضاد للفيروسات والبكتريا ومحول جنسى لاسماك البلطى النيلى ومقوى للجهاز المناعى لها.

وقد ركزت هذة الدراسة على تاثير الجرعة النصف مميتة (13.8ppm) LC_{50} وجرعة تحت مميتة 1/10 LC_{50} (1.38ppm) لمستخلص بذور نبات المورينجا على التغيرات بدلائل الدم والبيوكيميائية والهستوباثولوجية لاسماك البلطى النيلى.

فقد وجد فى نهاية التعرض للتركيزين معنوى لكلا من عدد كرات الدم الحمراء والهيموجلوبين والهيماتوكريت وبالتالى ارتفاع معنوى لكلا من عدد كرات الدم البيضاء و (MCV) و (MCH)بالمقارنة بالمجموعة الضابطة (الكنترول).

اما التغيرات البيوكيميائية فقد انخفض مستوى الجلوكوز عند التعرض للجرعة النصف مميتة بينما ارتفع عند التعرض للجرعة التحت مميتة.

خلال النتائج التى تم الحصول عليها وجد انخفاض معنوى بمستوى كلا من البروتين الكلى والكولسترول ببلازما الدم للاسماك المعرضة بكلتينما التركيزين ارتفع معنوى لمستوى كلا من اسبارتات امينوتانسفيريز والكرياتنين و حمض اليورك والفوسفاتيز القلوى ببلازما الدم للاسماك.

كما وجد بالتحليل الكيميائى لمكونات العضلات للاسماك المعرضة للجرعة التحت مميتة ارتفاع المحتوى المائى بينما لوحظ انخفاض معنوى فى كلا من محتوى البروتين والدهون الكلية بالعضلات.

كما لوحظ عند دراسة مؤشرات النمو انخفاض معنوى فى قيم كلا من (BWG)معدل النمو (GR) و معامل العلاقة بين الكبد ووزن الجسم (HIS).

وبدراسة القطاعات الهستوباثولوجية لوحظ تغيرات بانسجة كلا من الخياشيم والكبد والكلى والطحال.

وقد امددتنا تلك الدراسة على معلومات توضح التاثير السام لمستخلص بذور نبات المورينجا على اسماك البلطى النيلى وبالاخص عند استعمال العشائي والغير مسبحقول المزارع السمكية يجب تقنين استعمال الاضافات ذات المصادر النباتية بحرص شديد وبعد تجارب علمية متعددة ومتخصصة.