Damanhour Journal of Veterinary Sciences 3 (2), (2020) 6-11

Damanhour Journal of Veterinary Sciences

Journal homepage: https://djvs.journals.ekb.eg/

Biochemical alterations in serum biomarkers of Nile tilapia Oreochromis Niloticus exposed to sodium fluoride and Moringa oleifera

Nirmen Mekky[1*], Kadry Sadek[1], Magdy Soliman[2], Reyad Khalil[3]

1 Department of Biochemistry, Faculty of Veterinary Medicine, Damanhour University, Damanhour, 22511, Egypt
2 Department of Poultry and Fish diseases, Faculty of Veterinary Medicine, Damanhour University, Egypt
3 Department of Poultry and Fish diseases, Faculty of Veterinary Medicine, Alexandria University, Egypt

ABSTRACT

Fish are extremely sensitive to many water-borne toxicants, due to theirprolonged, constant and direct contact with the aquatic environment where chemical exposure occurs over the entire body surface and ecological significance in any natural systems. This study investigated the effects of sodium fluoride on O. niloticus tissues and protection effect of Moringa oleifera to these tissues by exposed of O. niloticus to 1/10 dose of sodium fluoride 96hr-LC50 (6.1 mg/L) and study the changes in serum biomarkers of liver and kidney functions, total antioxidant capacity (TAC) and lipid peroxidation (LPO). Two hundred and sixty O. niloticus were used for determination of LC50 and chronic toxicity the fish divided into four groups of fifty fishes each. Control group, received no any treatment; 1/10 dose of sodium fluoride LC50 (6.1 mg/L), sodium fluoride plus Moringa oleifera powder and Moringa oleifera 1% of diet. The results showed that, sodium fluoride at dose of 6.1 mg/L significantly decreased serum total protein, albumin, globulin and TAC of O. niloticus. The ALT and AST activities and LPO contents of sodium fluoride exposed group was found higher than the M. oleifera supplemented groups. On the basis of present findings it could be concluded that increased sodium fluoride content in water causes adverse effect on fish blood biochemistry. The changes of plasma biomarkers were the physiological responses of O. niloticus to the stress of sodium fluoride exposure. Moringa oleifera can be grown to produce more natural products and materials against heavy metals toxicity in aquatic ecosystem.

Keywords: *Moringa oleifera*; Sodium fluoride; O. niloticus; Malondialdehyde; Antioxidant

1. Introduction

Fish are usually considered as organism of choice for assessing the effects of environmental pollution on aquatic ecosystem (Olushola et al., 2014). Nile tilapias are considered the most popular widely distributed cheapest and intensively cultured fishes in Egypt (El Deen et al., 2009).

Heavy metals known as staid contaminants of the aquatic environments. At high concentrations, the metals can cause serious impairments in the physiological systems of the fish (Lauer et al., 2006). Interest; in the heavy metals which are required for metabolic activities in organisms, lies in the narrow range between their essentiality and toxicity (Jabeen, 2012). Fluoride pollution characterized by high levels of fluorides in environment, and has significant potential for producing biological and ecological hazards to aquatic life. Sub lethal concentrations may have adverse effects on fish growth, behavior and reproduction (Warrington 1990).

*Corresponding author:
E-mail address: Nirmenfathi@gmail.com
Biochemistry, Faculty of Veterinary Medicine, Damanhour University, Damanhour, 22511, Egypt
P ISSN: 2636-3003 EISSN: 2636-2996
Received: February 20, 2020; Received in revised form: March 7, 2020; accepted: March 17, 2020.

The toxicity of aqueous and sediment-associated fluoride to some species of freshwater organisms was determined by (Metcalfe-Smith et al., 2003). On the other hand Moringa oleifera tree also known as drumstick tree is a rapid growing deciduous shrub or small tree of about 13m tall and 35 cm in diameter with an umbrella-shaped open cap (Anjorin et al., 2010). It has also been reported (Hsu et al., 2006) that, Moringa oleifera oil and micronutrients contain antitumor, antiepileptic, antidiuretic, anti-inflammatory and venomous bite characters.

This study investigated the protective effects of Moringa Oleifera against toxicity of sodium fluoride by determination of 96hr-LC50 for sodium fluoride on O. niloticus. Chronic toxicity by exposed of O. niloticus to 1/10 dose of sodium fluoride 96hr-LC50 values, Changes in antioxidant enzyme activities of O. niloticus gills as activities of superoxide dismutase (SOD) and catalase (CAT) were studied, In addition to changes in enzyme activities of serum as aspartate amino transferase and alanine amino transferase were studied, Amelioration of sodium fluoride toxicity occurred by addition of Moringa oleifera to the ration of exposed O. niloticus by the rate of 1%.

2. Material and methods

2.1. Moringa leaves preparation

Moringa leaves were prepared by personal communication and mixed with the ration by 1% in ration. The Moringa leaves were harvested and air-dried under a shed until they were crispy to touch while retaining their greenish coloration. The leaves were milled to obtain a powder.

2.2. Experimental design

2.2.1. Fish for experimental work

A total of 260 apparently healthy O.niloticus were acclimated in full glass aquaria measuring (40×30×40 cm) and maintained in aerated marine water at 25 ± 2 C for 14 days. They seemed healthy and had a uniform size and weight with average body weight 40 ± 3 grams

2.3. Preparation and experimental design of LC50 of Sodium fluoride in O. niloticus

To obtain a leathal dose 50%, a storage solution was prepared by dissolving sodium fluoride (NaF) in filtered freshwater at the nominal concentration of 1 mg/ml. This solution was subsequently diluted in freshwater to obtain the working solutions of 10, 50 and 250 jig/ml (Ballarin et al., 2014). Freshwater was used in controls. A total number of 60 apparently health O. niloticus, weighting 30 ± 2 grams were selected after the period of acclimation about two weeks and then divided into six equal groups; each group contained of 10 fish. The first five groups were consistently exposed to 0, 20; 40; 60; 80 and 100 mg L-1 of sodium fluoride while the control group (group 6) was act as a control group and the determine the LC50 were carried out according to (Klassen, 1991). The dead fish were removed immediately. Behavioral changes, clinical toxic signs and postmortem lesions of tested fish were closely followed up and recorded daily. The lethal concentration of sodium fluoride after 96 hour (96-h LC50) of exposure was calculated according to (Behrens and Karber, 1953).

2.4. Experimental design of chronic experiment

Four aquaria were used for experimented O. niloticus with an average body weight of 40 ± 3 g and divided to four equal groups (50 fish per each). Fifty fish were served as a control negative group. The groups were arranged as the following; G (2) exposed to sodium fluoride (1/10 LC50, 6.0 mg. /L.); G (3) sodium fluoride (6.0 mg. /L.) plus M. oleifera 1% in ration and G (4) supplemented by M. oleifera 1% in ration only. The experiment was extended to 8 weeks where fish samples were taken every 14 days from all aquaria for analyses (Table 1). Settled fish wastes were cleaned daily by siphoned with three quarters of the aquarium's water, which was replaced by aerated water from the water storage aquaria. Water temperature was kept at 25 ± 1 ℃.

2.5. Fish diets

Fish were fed on a commercial fish diet containing 25% crude protein (table, 2). The diet was daily provided at a fixed feeding ratio of 3 % of body weight of fish as described by (Eurell et al., 1978).

2.6. Sample collection and preparation

At 2nd, 4th, 6th and 8th weeks during the experimental period, blood samples were collected from different groups via the caudal vessels from 3 fish using disposable syringe (Hawak et al., 1965). At same time of blood sampling the specimen of gills, liver, kidney and muscular tissues were collected at 2nd, 4th, 6th and 8th weeks during the experimental period for measurement of different antioxidant parameters from different groups.

2.7. Clinico-biochemical analysis

Determination of serum aspartate aminotransferase (S.AST) and serum alanine amino transferase (S.ALT) were estimated according to (Reitman and Frankle, 1957).

2.8. Statistical analysis

All data are expressed as the mean ± standard deviations (SDs), and the levels of significance are cited. SPSS statistical package version 17.0 for Windows (IBM, Armonk, NY, USA) was used for all data analysis. Differences in values were analyzed by one-way analysis of variance (ANOVA), followed by Duncan's multiple range tests. Differences were deemed significant when $p<0.05$.

3. Results

3.1. Results of determination of L C 5 0 sodium fluoride in O. niloticus

LC50 of sodium fluoride in O. niloticus results were summarized in Table (3) and Figure (1). The results registered that the lethal concentration 50 (LC50) of sodium fluoride in O. niloticus was 61 mg/L; so the 1/10 dose of LC50 of sodium fluoride in O. niloticus to induce chronic toxicity was 6.1 mg/L.

3.2. Effect of sodium fluoride and/or M. oleifera onAspartate aminotransferase in serum of O.niloticus

Our results indicated that there were statistically significant increases in the activities of AST in the serum of O. niloticus exposed to sodium fluoride. But levels of the activities of AST in the serum of M. oleifera supplemented group decreased significantly in comparison to the control group. In relation to control group, sodium fluoride significantly ($p<0.05$) increased the serum levels of AST at 6th and 8th weeks of exposure period of 6.1mg /L. of sodium fluoride which indicate hepatotoxicity (Table, 4). M. oleifera alone has no effect on the measured of levels of AST, fortunately it returned the increased levels of AST to their normal values in sodium fluoride + M. oleifera treated fish.

3.3. Effect of sodium fluoride and/or M. oleifera on Alanine aminotransferase in serum of O.niloticus

The present results indicated that there were statistically significant increases in the activities of ALT in the serum of O. niloticus exposed to sodium fluoride Table (5). But levels of the activities of ALT in the serum of M. oleifera supplemented group decreased significantly in comparison to the control group. In relation to control group, Sodium fluoride significantly ($p<0.05$) increased the serum levels of ALT at 6th and 8th weeks of exposure period of 6.1mg. /L. of sodium fluoride. M. oleifera alone has no effect on the measured of levels of ALT, fortunately it returned the increased levels of ALT to their normal values in sodium fluoride + M. oleifera treated fish.

3.4. Effect of Sodium fluoride and/or M. oleifera on total protein (mg/dL) in serum of O. niloticus

There was a decrease in total protein levels in serum of O. niloticus exposed to 6.1 mg/L sodium fluoride for 8th weeks. We also, observed a strong linear relationship between sodium fluoride periods of exposure and the biochemical parameters in the serum. However, these biochemical endpoints are potential biomarkers for fluoride exposure in O. niloticus (Table, 6). Furthermore, there were statistically significant changes in levels of total protein in serum of groups exposed to sodium fluoride and/or M. oleifera groups ($P>0.05$). In addition to, significant increases in levels of total protein in treated M. oleifera O.niloticus compared with O.niloticus intoxicated and the control group ($P>0.05$).

3.5. Effect of Sodium fluoride and/or M. oleifera on albumin (mg/dL) in serum of O. niloticus

Our resul ts i ndi cat ed that t here were statistically significant increases in the levels of albumin in the serum of Sodium fluoride + M. oleifera group ($P<0.05$) (Table, 7). But levels of albumin in the serum of Sodium fluoride group decreased significantly in comparison to the control group.

3.6. Effect of Sodium fluoride and/or M. oleifera on globulin in serum of O. niloticus

Results have been shown to lead to a decrease in globulin levels in serum of O. niloticus exposed to 6.1 mg/L sodium fluoride for 8th weeks. We also, observed a strong linear relationship between sodium fluoride periods of exposure and the biochemical parameters in the serum. However, these biochemical endpoints are potential biomarkers for fluoride exposure in O. niloticus. Furthermore, there were statistically significant changes in levels of globulin in serum of groups exposed to sodium fluoride and/or M. oleifera groups ($P>0.05$). In addition there were significant increases in levels of globulin in treated M. oleifera O.niloticus compared with O.niloticus intoxicated and the control group ($P>0.05$) (Table, 8).

3.7. Effect of Sodium fluoride and/or M. oleifera on creatinine (mg/dL) in serum of O. niloticus

A significant increase of creatinine in serum of O.niloticus occurred at sodium fluoride exposure of O.niloticus to 6.1mg/L. for 8th weeks (Table, 9). A significant reduction of Creatinine (mg/dL) in serum of O. niloticus was found at M. oleifera supplement group alone. Moreover, an increase in the level of creatinine (mg/dL) in serum of O. niloticus exposed to sodium fluoride+M. oleifera , but it was significantly higher ($P<0.05$) only at sodium fluoride exposure of O.niloticus to 6.1mg/L. for 8th weeks.

3.8. Effect of Sodium fluoride and/or M. oleifera on Lipid peroxidase (MDA) activity in the serum of O. niloticus

There were statistically significant differences between the activities of the groups. The Lipid peroxidase activity at the sodium fluoride exposed group was found higher than at the M. oleifera groups ($p>0.05$). Lipid peroxidase activities at the exposed groups to sodium fluoride in the serum of O.niloticus was found higher than the Moringa oleifera feeding group.The changes in MDA in the serum of O. niloticus exposed to 6.1 mg/L. sublethal concentrations of sodium fluoride were presented in (Table, 10). A slight non-significant increase was observed in MDA activity in the serum of O. niloticus after 2th weeks of exposure to 6.1 mg/L. of sodium fluoride. However after 4th 6th and, 8th weeks of exposure the activity of MDA activity was increased significantly $P<0.05$. The results showed that in MDA activity in the serum of O. niloticus significantly ($p<0.05$) increased in exposed to 6.1 mg./L. sublethal concentrations of sodium fluoride group and returned to normal level in sodium fluoride+M. oleifera supplemented group. However, it was unchanged in M. oleifera group. In serum, MDA level insignificantly ($p>0.05$) increased in sodium fluoride and sodium fluoride+M. oleifera group at 8th weeks of exposure.

3.9. Effect of Sodium fluoride and/or M. oleifera on Lipid peroxidase (MDA) activity in the liver and kidney of O. niloticus

There were statistically significant differences between the activities of the groups. The Lipid peroxidase activity at the sodium fluoride exposed group was found higher than at the M. oleifera groups ($p>0.05$). Lipid peroxidase activities at the exposed groups to sodium fluoride in the liver and kidney of O.niloticus were found higher than the Moringa oleifera feeding group. The changes in MDA in the liver and kidney of O. niloticus exposed to 6.1 mg/L. sublethal concentrations of sodium fluoride were presented in (Table, 11&12). A slight non-significant increase was observed in MDA activity in the liver and kidney of O. niloticus after 2th weeks of exposure to 6.1 mg/L. of sodium fluoride. However, after 4th, 6th and 8th weeks of exposure the activity of MDA activity was increased significantly $P<0.05$. The results showed that in MDA activity in the liver and kidney of O. niloticus significantly ($p<0.05$) increased in exposed to 6.1 mg./L. sublethal concentrations of sodium fluoride group and returned to normal level in sodium fluoride+M. oleifera supplemented group. However, it was unchanged in M. oleifera group. In liver and kidney, MDA level insignificantly ($p>0.05$) increased in sodium fluoride and sodium fluoride+M. oleifera group at 8 t h weeks of exposure.

4. Discussion

The toxicological impact of fluoride on a variety of aquatic species is well predictable with its harmful effects on humans, livestock, and plants (Gikunju, 1992). A number of studies on aquatic invertebrates like Daphnia, Artemia, Penaeus, and Hydropsyche reveal that fluoride affects survival, growth, behavior, and reproduction. In our study, the results showed that in MDA activity in the gills, liver, kidney and muscular tissue of O. niloticus significantly increased in exposed to 6.1 mg./L. sublethal concentrations of sodium fluoride group and returned to normal level in sodium fluoride + M . oleifera supplemented group. However, it was unchanged in M. oleifera group. In gills, liver, kidney and muscular tissue, MDA level insignificantly increased in sodium fluoride and sodium fluoride + M. oleifera group at 8th weeks of exposure. These findings are in good agreement with those of (Xiangguo et al., 2011) who recorded a significant increase in MDA level in embryo-larval stages of zebrafish intoxicated with different doses of cypermethrin. Fluoride ions act as enzymatic poisons, disturbing enzyme activity and, finally, interfering metabolic processes such as glycolysis and synthesis of proteins (Camargo, 2003).The elevation of Aspartate amino transferase (AST) and Alanine aminotransferase (ALT) is altered due to fluoride toxicity and utilization of amino acids (Devi and Piska, 2006b). M. oleifera improved the serum (ALT), (AST), creatinine during lead acetate admenstration in fish

Ouedraogo et al. (2013) reported that M. oleifera at doses of 150 and 300 mg/kg body weights prevent gentamicin induced nephrotoxicity in rabbits by significantly decreasing the markers of kidney damage including lipid peroxidation, serum creatinine and urea as well as histological changes. Onah et al. (2016) reported that M. oleifera extracts supplementation was associated with significant decreases in the levels of ALP, ALT, and AST, GGT, bilirubin, urea, creatinine and uric acid. Results showed decrease in total protein levels in serum of O. niloticus exposed to 6.1 mg. /L sodium fluoride for 8th weeks. Furthermore, there were statistically significant changes in levels of total protein in serum of groups exposed to sodium fluoride and/or M. oleifera groups. On the same manner, (Kumar et al., 2007 ; Bajpai and Tripathi, 2010) found that the toxicity of fresh water catfish exposure to two sub-lethal doses of fluoride NaF (35 mg F ion/L and 70 mg F ion/L) for 90 days after their a significant decrease of total protein occurred.

Devi and Piska, (2006a) found that the fluoride decrease of the tissue proteins of fresh water cat fish. The decreased levels of protein in the serum, liver, kidney, muscle, and brain of NaF exposed fish were found by (Bajpai and Tripathi, 2010). Results indicated that there were statistically significant increases in the levels of albumin in the serum of sodium fluoride + M. oleifera group. But levels of albumin in the serum of sodium fluoride group decreased significantly in comparison to the control group. On the same manner, the (Fırat et al., 2011; Jianjie et al., 2013) reported that the elevated sodium fluoride cause decrease of albumin and globulin in fish. A significant increase of creatinine in serum of O. niloticus occurred at sodium fluoride exposure group to 6.1mg. /L. for 8th weeks. Moreover, an increase in the level of creatinine in serum of O. niloticus exposed to sodium fluoride+M. oleifera, but it was significantly higher ($P<0.05$) only at sodium fluoride exposure of O. niloticus to 6.1mg. /L. for 8th weeks. Fluoride ions have been reported to act as enzymatic poisons, inhibiting enzyme activities and ultimately, interrupting metabolic process (Fcdsw, 1984 Onah et al. (2016) reported that M. oleifera extracts supplementation was associated with significant decreases in the levels of bilirubin, urea, creatinine and uric acid.

5. Conclusion

On the basis of present findings it could be concluded that increase the administration of sodium fluoride to the water causes adverse effect on fish especially O. niloticus. Sodium fluoride toxicity induced changes in some liver and kidney function parameters as well as some oxidative markers of these organs and also revealed possible amelioratory effects to these changes after M. oleifera extracts supplementation.

Table (1): Design of the chronic experiment

Group	Treatments	No. of fish	Dose of sodium fluoride and other additives	Reference
G1	Control (without treatment)	50	0	
G2	Sodium fluoride only	50	6.0 mg/L	1/10dose of LC50
G3	Sodium fluoride plus *Moringa oleifera* extract	50	6.0 mg/L+ *Moringa oleifera* 1% in ration	
G4	*Moringa oleifera* extract	50	*Moringa oleifera* 1% in ration	

Table (2): The ingredient composition (%) of the basal diet (without supplementation of Moringa oleifera extract

Ingredients	%
Fish meal (65%)	15
Barley	30
Ash	10.5
Soybean meal	23
Crude fiber (CF)	12.5
Wheat bran	14.5
Ether extract (EE)	2.46
Limestone	1.8
N-free extract (NFE)	56.04
Bone meal	1.0
Digestible energy, Kcal/g (DE)	2.63
Salt	0.2
Calcium	1.10
Premix	0.3
Phosphorus	0.59
DL – methionine	0.2
Lysine	0.97
Methionine	0.57

Each 3 Kg vitamin and mineral mixture provides: Vitamin A: 12000000 IU, Vit.D3: 2200000 IU, Vit. E: 10000 mg, Vit. K, :2000 mg, Vit.B:11000mg, Vit.B2 :4000mg, Vit.B6 :1500mg, Vit.B12 :10mg, Pantothenic Acid : 10000mg, Niacin :20000mg, Biotin :50 mg, Folic Acid : 1000mg, Choline chloride : 500gm, Selenium: 100mg, Manganese : 55000mg, Zinc : 50000mg, Iodine : 1000 mg and carrier CaCo3, to 3000 gm.

Table (3): Results of determination of $_{LC50}$ sodium fluoride in *O. niloticus*

Sodium Flouride Dose (Mg/L)	Number of exposed fish	Overall death 96 hrs	A	B	AB
0	10	0	0	0	0
20	10	0	20	0	0
40	10	2	20	1	20
60	10	5	20	3.5	70
80	10	7	20	6	120
		10	20	8.5	170
					∑ A x B=390

A = differences between the two consecutive doses , B = arithmetic mean of the mortality caused by two consecutive doses. 96 h LC50 = LC100 - □ (A x B)/N = 100 – 390/10 = 61.0 ppm. Or (mg/L)

• lethal concentration 50 (LC50) of Sodium fluoride in O. niloticus = 61 mg/L.

•1/10 dose of LC50 of Sodium fluoride in O. niloticus to induce chronic toxicity was = 6.1 mg/L.

Table (4): Effect of sodium fluoride and / or *M. oleifera* on AST (IU/L) in serum of *O. niloticus*

Groups	Period of exposure			
	2nd week	4th week	6th week	8th week
CTR	48.13 ± 0.24 $^{A b}$	47.01 ± 1.28 $^{A c}$	46.95 ± 0.43 $^{A c}$	46.84 ± 0.78 $^{A c}$
SF	53.84 ± 0.16 $^{D a}$	58.79 ± 0.22 $^{C a}$	68.43 ± 0.71 $^{B a}$	75.29 ± 1.16 $^{A a}$
FM	49.65 ± 0.61 $^{D b}$	54.79 ± 0.81 $^{C b}$	64.94 ± 0.77 $^{B b}$	72.19 ± 1.61 $^{A b}$
ME	41.80 ± 1.00 $^{A c}$	40.70 ± 0.34 $^{A d}$	39.35 ± 0.58 $^{A,B d}$	36.64 ± 0.45 $^{B d}$

Different superscript small letters within the same column indicate significantly different mean values (p< 0.05) between different groups. Different superscript capital letters within the same raw indicate significantly different mean values (p< 0.05) between different periods of exposure. CTR, Control; SF, Sodium fluoride (6.1 mg/L.); FM: Sodium fluoride (6.1 mg/L.) + Moringa oleifera extract (1% in ration); ME, M. oleifera extract (1% in ration).

Table (5): Effect of sodium fluoride and / or *M. oleifera* on ALT (IU/L) in serum of *O. niloticus*

Groups	Period of exposure			
	2nd week	4th week	6th week	8th week
CTR	53.17 ± 0.31 $^{A b}$	52.64 ± 0.94 $^{A b}$	51.10 ±1.11 $^{A c}$	50.75 ± 1.41 $^{A c}$
SF	55.78 ± 0.24 $^{C a}$	58.46 ± 0.49 $^{C a}$	64.47 ± 0.60 $^{B a}$	68.63 ± 0.60 $^{A a}$
FM	53.49 ± 0.36 $^{D a,b}$	56.73 ± 0.34 $^{C a}$	61.22 ± 0.93 $^{B b}$	64.53 ± 0.49 $^{A b}$
ME	48.42 ± 0.65 $^{A c}$	45.99 ± 0.28 $^{A,B c}$	43.54 ± 0.60 $^{B,C d}$	40.87 ± 0.38 $^{C d}$

Different superscript small letters within the same column indicate significantly different mean values (p< 0.05) between different groups. Different superscript capital letters within the same raw indicate significantly different mean values (p< 0.05) between different periods of exposure. CTR, Control; SF, Sodium fluoride (6.1 mg/L.); FM: Sodium fluoride (6.1 mg/L.) + Moringa oleifera extract (1% in ration); ME, M. oleifera extract (1% in ration).

Table (6): Effect of Sodium fluoride and / or *M. oleifera* on Total protein (mg/dL) in the serum of *O. niloticus*

Groups	Period of exposure			
	2nd week	4th week	6th week	8th week
CTR	4.34± 0.095 $^{A b}$	4.47± 0.039 $^{A b}$	4.44± 0.076 $^{A c}$	4.29± 0.124 $^{A c}$
SF	3.89± 0.014 $^{A c}$	3.56± 0.040 $^{B c}$	3.34± 0.020 $^{C d}$	3.16± 0.020 $^{C a}$
FM	4.27± 0.034 $^{C b}$	4.62± 0.018 $^{B b}$	4.73± 0.037 $^{A,B b}$	4.90± 0.014 $^{A b}$
ME	4.71± 0.018 $^{C a}$	5.32± 0.020 $^{B a}$	5.76± 0.036 $^{A a}$	5.79± 0.017 $^{A a}$

Different superscript small letters within the same column indicate significantly different mean values (p< 0.05) between different groups. Different superscript capital letters within the same raw indicate significantly different mean values (p< 0.05) between different periods of exposure. CTR, Control; SF, Sodium fluoride (6.1 mg/L.); FM: Sodium fluoride (6.1 mg/L.) + Moringa oleifera extract (1% in ration); ME, M. oleifera extract (1% in ration).

Table (7): Effect of Sodium fluoride and / or *M. oleifera* on albumin (mg/dL) in the serum of *O. niloticus*

Groups	Period of exposure			
	2nd week	4th week	6th week	8th week
CTR	2.37± 0.039 A a,b	2.21± 0.029 A b	2.40± 0.125 A b	2.39± 0.252 A a
SF	2.10± 0.023 A b	1.92± 0.070 A c	1.99± 0.009 A c	1.97± 0.014 A b
FM	2.48± 0.011 A a	2.52± 0.029 A a	2.69± 0.037 A a	2.18± 0.021 B a,b
ME	2.52± 0.029 A a	2.21± 0.011 B b	1.93± 0.001 B,C c	1.90± 0.013 C b

Different superscript small letters within the same column indicate significantly different mean values (p< 0.05) between different groups. Different superscript capital letters within the same raw indicate significantly different mean values (p< 0.05) between different periods of exposure. CTR, Control; SF, Sodium fluoride (6.1 mg/L.); FM: Sodium fluoride (6.1 mg/L.) + Moringa oleifera extract (1% in ration); ME, M. oleifera extract (1% in ration).

Table (8): Effect of Sodium fluoride and / or *M. oleifera* on globulin (mg/dL) in the serum of *O. niloticus*

Groups	Period of exposure			
	2nd week	4th week	6th week	8th week
CTR	1.97± 0.068 B a,b	2.26± 0.071 A b	2.04± 0.138 A,B b	1.90± 0.160 B c
SF	1.79± 0.011 A b	1.61± 0.020 A,B c	1.35± 0.015 B,C c	1.17± 0.026 C d
FM	1.76± 0.033 C b	2.09± 0.047 B b	2.03± 0.068 B b	2.72± 0.026 A b
ME	2.18± 0.047 C a	3.12± 0.020 B a	3.84± 0.020 A a	3.93± 0.017 A a

Different superscript small letters within the same column indicate significantly different mean values (p< 0.05) between different groups. Different superscript capital letters within the same raw indicate significantly different mean values (p< 0.05) between different periods of exposure. CTR, Control; SF, Sodium fluoride (6.1 mg/L.); FM: Sodium fluoride (6.1 mg/L.) + Moringa oleifera extract (1% in ration); ME, M. oleifera extract (1% in ration).

Table (9): Effect of Sodium fluoride and / or *M. oleifera* on creatinine (mg/dL) in the serum of *O. niloticus*

Groups	Period of exposure			
	2nd week	4th week	6th week	8th week
CTR	0.20± 0.014 A b	0.21± 0.005 A b	0.21± 0.015 A b	0.21± 0.014 A c
SF	0.24± 0.003 D a	0.34± 0.017 C a	0.46± 0.006 B a	0.78± 0.008 A a
FM	0.23± 0.003 D a,b	0.30± 0.017 C a	0.43± 0.005 B a	0.68± 0.017 A b
ME	0.20± 0.011 A b	0.19± 0.005 A b	0.18± 0.005 A b	0.17± 0.003 A d

Different superscript small letters within the same column indicate significantly different mean values (p< 0.05) between different groups. Different superscript capital letters within the same raw indicate significantly different mean values (p< 0.05) between different periods of exposure. CTR, Control; SF, Sodium fluoride (6.1 mg/L.); FM: Sodium fluoride (6.1 mg/L.) + Moringa oleifera extract (1% in ration); ME, M. oleifera extract (1% in ration).

Table (10): Effect of Sodium fluoride and / or *M. oleifera* on Lipid peroxidase (MDA) activity (nmol (µM MDA/mg protein) /ml.) in serum of *O. niloticus*

Groups	Period of exposure			
	2nd week	4th week	6th week	8th week
CTR	24.92± 0.40 A b,c	24.74± 0.24 A b	24.31± 0.93 A b	25.06± 0.24 A c
SF	26.68± 0.51 D a	28.95± 0.25 C a	32.70± 0.31 B a	35.65± 0.38 A a
FM	26.62± 0.62 C a,b	27.59± 0.58 C a	30.97± 0.47 B a	33.55± 0.70 A b
ME	24.26± 0.22 A c	23.25± 0.06 A,B b	21.81± 0.24 B c	19.63± 0.37 C d

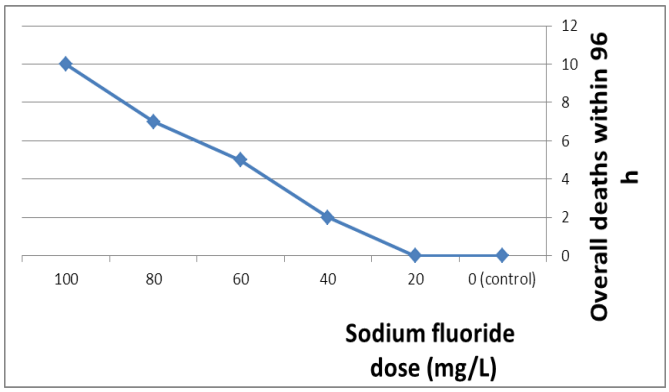
Different superscript small letters within the same column indicate significantly different mean values

Table (11): Effect of Sodium fluoride and / or *M. oleifera* on **Total antioxidant capacity (TAC)** (mML-1/ml.) in serum of *O. niloticus*

Groups	Period of exposure			
	2nd week	4th week	6th week	8th week
CTR	0.85± 0.015 A a	0.84± 0.008 A b	0.85± 0.043 A b	0.89± 0.011 A b
SF	0.83± 0.015 A a	0.80± 0.009 A,B c	0.75± 0.009 B,C c	0.69± 0.012 C c
FM	0.81± 0.017 A,B a	0.82± 0.018 A b,c	0.79± 0.017 A,B b,c	0.76± 0.005 B c
ME	0.86± 0.015 D a	0.094± 0.006 C a	1.02± 0.020 B a	1.14± 0.008 A a

Different superscript small letters within the same column indicate significantly different mean values

Fig. (1): LC50 of Sodium fluoride in O. niloticus



Conflict of interest

The authors declare no conflict of interests, financial or otherwise.

Author contributions

All authors are contributed equally to this work.

References

Anjorin, S.T., Ikokoh, P.S., Okolo, A., 2010. Mineral Composition of Moringa oleifera Leaves Pods and Seeds from Two Regions in Abuja. Nigeria. International Journal of Agriculture and Biology, 12 (3),1560-9.

Bajpai,, S., Tripathi, M., 2010. Effect of fluoride on growth bioindicators in stinging catfish, Heteropneustes fossils (Bloch). Research Report Fluoride, 43, 232-236.

Ballarin,, L., Covre, V., Masiero, L., Casellato, S.N., 2014. Immunotoxic effects of fluoride on the hemocytes of Venerupis philippinarum. Research report, ISJ, 11, 22-29.

Behrens, A. S., Karber, L. 1953. Determination of LC50. Arch. Exp. Path. Pharm., 28: 177.

Camargo, J.A., 2003. Fluoride toxicity to aquatic organisms: A review. Chemosphere. 50(3), 251-64.

Devi, A.J., Piska, R.S., 2006b. Effect of fluoride on aspartate and alanine amino transferase activities in the fresh water fish, Clarias batrachus (Linn.). Bulletin of Pure and Applied Sciences-Zoology, 25A,19-24.

Devi, A.J., Piska, R.S. 2006a. Effect of fluoride on tissue proteins of fresh water cat fish Clarias batrachus (Lin.). J. Aquat. Biol., 21, 184-187.

El Deen, Ahmed I.N., Razin, A.M., 2009. Application of some medicinal Plants to eliminate Trichodina sp. in tilapia (Oreochromis niloticus). Report and Opinion, 12(17), 1.

Eurell,, T.E., Lewis, S.D.H., Grumbles, L.C. 1978. comparison of selected diagnostic tests for detection of motile Aeromonas Septicemia in fish. Am. J. Vol. Res 39(8), 1384 - 1386.

FCDSW.1984. Oral Health Fact Sheet, Flagstaff CitiZens for safe drinking water, USA.

Fırat, O., Cogun, H.Y., Yuzereroğlu, T.A., Geok, G., Fırat, O., Kargin, F., 2011. K€otemen, Y. A comparative study on the effects of a pesticide (cypermethrin) and two metals (copper, lead) to serum biochemistry of Nile tilapia, Oreochromis niloticus. Fish Physiol Biochem, 37, 657– 666.

Gikunju, J.K. 1992. Fluoride concentration in Tilapia fish (Oreochromis lecosticus) from Lake Naivasha, Kenya. Fluoride, 25, 37-43.

Hawak, P.B., Oscaand, B.L., Summerson, W., Hawak, S., 1965. physiological Chemistry.; London Journal; and A.Churchill.L.T.D.14 Th Ed.

Tian-Jian, H., Steve, E., Guza, R. T. 2006. Wave induced sediment transport and onshore sandbar migration. Coastal Engineering, 53, 10, 817-824.

Jabeen, G. 2012. Studies on fish species specific metals bio-accumulation patterns in relation to water, sediments and plankton in the river Ravi. Ph.D. Thesis. Dept of Zoology Fish. Uni. Agri. Faisalabad.

Klassen, C.D. 1991. Principles of toxicology.1991; In: Gilman, A.G., Tall, T.W., Nies, A.S., Taylor, P. (Eds.), Pharmacological Basis of Therapeutics, 8th ed. McGraw Hill, pp. 49–61.

Koroliuk, M.A., Ivanova, L.I., Maiorova, I.G., Tokarev, V.E, 1988. A method of determining catalase activity - Lab. Delo.; 1: 16-19.

Kumar, A., Tripathi, N., Tripathi, M. 2007. Fluoride induced biochemical changes in fresh water catfish (clarias batrachus, linn). Research Report Fluoride, 40, 37-41.

Lauer, T., Preik, M., Rassaf, T., Strauer, B.E., Deussen A., Feelisch, M., Kelm, M. 2006. Plasma nitrite rather than nitrate reflects regional endothelial nitric oxide synthase activity but lacks intrinsic vasodilator action. Proc. Natl. Acad. Sci. 98, 12814-12819.

Metcalfe-Smith, J.L., Holtze, K.E., Sirota, G.R., Reid, J.J., de Solla, S.R., 2003..Toxicity of aqueous and sediment-associated fluoride to freshwater organisms. Environ Toxicol Chem, 22,161–166.

Olushola, S.A., Olalekan, S.F., Folahan, A.A., Leslie, F.P., Ximba, B.J. 2014. Application of nano zinc oxide (nZnO) for the removal of triphenyltin chloride (TPT) from dockyard wastewater. Water SA, 40(4), 659-664.

Onah, C.E., Meludu, S.C., Dioka, C.E., Onuegbu, A.J., Onah, C.F., Ajaghaku, D.L., Nnodim, J. K,. Obi, E. 2016. Amelioratory Effect of Methanolic Leaf Extract of Moringa oleifera on Some Liver and Kidney Function and Oxidative Stress Markers in Lead-intoxicated. EJMP, 12(4), 1-12.

Onah, C.E., Meludu, S.C., Dioka, C.E., Onuegbu, A.J., Onah, C.F.. Ajaghaku, D.L, Nnodim, J.K., Obi, E. 2016. Amelioratory Effect of Methanolic Leaf Extract of Moringa oleifera on Some Liver and Kidney Function and Oxidative Stress Markers in Lead-intoxicated. EJMP, 12(4),1-12.

Ouedraogoa, M., Lamien-Sanoub, A., Ramdeb, N., Ouedraogob, A.S., Zongoa, S, P., Goumbrib, O., Duezc, P. , Guissoua, P.I., 2013. Protective effect of Moringa oleifera leaves against gentamicin-induced nephrotoxicity in rabbits. Exp Toxicol Pathol,, 65, 335-339.

Warrington, P.D. 1990. Ambient Water Quality Criteria for Fluoride.Technical Ap- pendix.British Columbia Ministry of Environment.

Xiangguo, S., Aihua, G., Guixiang, J., Yuan, L., Jing, D., Jing, J., Fan, H., Yan, L., Yan, L., Yankai, X., Chuncheng, L., Ling, S., Wang, X. 2011. Developmental toxicity of cypermethrin in embryo-larval stages of zebrafish. Chemosphere, 85, 1010-1016.