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Assessment of fibroblast growth factor 23, antioxidant enzymes activities and heavy metals in *Oreochromis niloticus* and *Clarias gariepinus*

Soha M. Hamdy[1], Fatma Adel Yahia[1] and Abdelrahman Said Talab [2,*]

[1]Chemistry Department, Faculty of Science, Fayoum University, Fayoum, Egypt.
[2]Fish Processing and Technology Laboratory, Fisheries Division, National Institute of Oceanography and Fisheries, Cairo, Egypt.
[*]Corresponding Author: Abdelrahman_saidh@yahoo.com

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ABSTRACT

The aim of this study was to assess the fibroblast growth factor (FGF23) hormone, chemical composition, antioxidant enzymes activities, biochemical parameters and heavy metals concentration in *Oreochromis niloticus* and *Clarias gariepinus* collected from the River Nile, Rosetta branch during 2019. Fifty samples from *O. niloticus* and *C. gariepinus* were collected from (I) at El-Qanater El-Khyria, (II) after El-Rahawy Drain, (III) at Kom Hamada city, and (IV) at Kafr El-Zayat city. Serum levels of FGF23, glucose, total protein, albumin, urea, and creatinine were assessed. Tissue levels of Fe, Zn, Cu, Mn, and Cd, the activities of superoxide dismutase (SOD), catalase (CAT) and glutathione S-transferase (GST), the values of malondialdehyde (MDA), moisture, protein, lipid, and ash were also assessed. The results showed significant difference ($P \leq 0.05$) in the levels of FGF23, glucose, total protein, albumin, urea and creatinine in serum and the activities of superoxide dismutase (SOD), catalase (CAT) glutathione S-transferase (GST), and malondialdehyde (MDA) in the tissue of *O. niloticus and C. gariepinus* from studied stations of Rosetta branch as compared with control site (I). Metal accumulation in different tissues of the studied fish samples was found to be in the following order: Fe > Zn > Mn > Cu > Cd in muscles and gills. The mean values of moisture, protein, lipid, and ash were 80.16, 17.18, 1.23, and 1.14% for *O. niloticus* and 78.66, 18.16, 1.61, and 1.18% for *C. gariepinus*, respectively. All data indicated that the environmental condition seriously affected the fish quality. Thus, the FGF-23 assay can be used in prospective trials to valid its utility as a biomarker of environmental pollution.

INTRODUCTION

The evaluation of toxic effects of heavy metals in terrestrial and aquatic ecosystems is one of the imperative areas of recent research, and there is an emergent concern on the development of technique for detection of toxic effects in aquatic animals **(Velma & Tchounwou, 2010)**. There are 18 mammalian fibroblast growth factors (FGF) which are grouped into 6 subfamilies based on differences in sequence homology and phylogeny **(Tomlinson *et al.*, 2002; Itoh & Ornitz, 2004; Fu *et al.*, 2004)**. FGF

signaling controls liver specification and regulates the metabolism of lipids, cholesterol, and bile acids in mammals **(Tsai *et al.*, 2013)**. FGF signaling pathways play key roles in the growth and survival of progenitor cells during development, tissue regeneration, and carcinogenesis **(Katoh, 2002; Dailey *et al.*, 2005; Eswarakumar *et al.*, 2005)**. FGF23 is a phosphatonin involved in the regulation of phosphate, calcium, parathyroid hormone, and vitamin D metabolism **(Fukumoto, 2007)**. Higher FGF23 levels have been related to the development of cardiovascular disease (CVD) in chronic kidney disease patients (**Jimbo & Shimosawa, 2014)**. Nowadays, FGF23 has gained wide attention in chronic kidney disease associated mineral bone disease (CKDMBD) and appears to be a candidate as missing link between chronic kidney disease and cardiovascular morbidity and mortality. FGF23 levels increase during progression of CKD **(Ix *et al.*, 2010; Isakova *et al.*, 2011)**. The aim of this study was to assess the effect of pollution on the level of FGF23 hormone, biochemical parameter, heavy metals level, antioxidant enzymes activities, and chemical composition values in *Oreochromis niloticus* and *Clarias gariepinus* collected from the River Nile, Rosetta branch during summer 2019.

MATERIALS AND METHODS

A total of 50 fish samples of each fish species from each station of the Nile tilapia (*Oreochromis niloticus*) and catfish (*Clarias gariepinus*) were collected during summer 2019. Specimens of fish samples were collected from four stations in the River Nile, Rosetta branch as follows: (I) at El-Qanater El-Khyria before bifurcation (Latitude 30^{o} 13′12.93″ N and Longitude 30o 58′33.77″ E); (II) after El-Rahawy Drain (Latitude 30°11’58.42” N and Longitude 31°1’15.02”E); (III) at Kom Hamada city (Latitude 30o 42′52.91″ N and Longitude 30o 45′44.28″ E), and (IV) at Kafr El-Zayat city (Latitude 30o 49′22.64″ N and 30o 48′38.93″ E Longitude).

Sampling and preparation of blood serum

Blood samples were collected in Eppendorf tubes from the caudal vein of *O. niloticus* and *C. gariepinus* fishes. The collected samples were centrifuged at 3000 rpm (1008g) for 15 minutes, then the supernatant serum was obtained by using micropipette and stored at 4^{o}C till determination of FGF23, glucose, total protein, albumin, and kidney function (urea and creatinine). The biochemical metabolites parameters of fish in blood serum were assayed using spectrophotometer (model Jenway 6800UV/Vis double beam), and commercially available kits (Spectrum Diagnostics Kits, Bioassay technology laboratory, Cat.No E0232Fi, www.bt-laboratory.com, 1008 Junjiang Inter. Bldg. 228 Ningguo Rd. Yangpu Dist. Shanghai. China).

Analytical methods

Fish FGF23 hormone determination

The concentration of FGF23 hormone was measured by quantitative Sandwich ELISA kit using ELIZA TECAN infinite f50 at Ansary Laboratories, Bab Ellouk, Cairo, Egypt.

Analysis of biochemical parameter

The concentration of glucose was measured using GOD-PAP enzymatic colorimetric method following the description of **Tietz (1995)**. Total serum protein was measured by colorimetric method (Biuret reagent) according to **Tietz (1994)**. Serum albumin was determined by modified bromocresol green colorimetric method according to **Tietz *et al.* (1990)**. Urea levels were determined by urease-colorimetric method according to **Tietz *et al.* (1990)**. Creatinine levels were determined by buffered Kinetic jaffé reaction without deproteinization method according to **Tietz (1986)**.

Heavy metals determination

Fish specimen's muscles and gills were transferred to a beaker and placed in a drying oven thermostatically regulated at 105ºC overnight. A representative sample of 1.0 g dry weight of muscle and gills was taken from the fish specimens. The samples were digested according to the method described by **Goldberg *et al.* (1963)** in which concentrated HNO_3 and $HClO_4$ (65%) with ratio of 5 mL + 5 mL were used in Teflon beakers on hot plate at 50ºC for about 5 hours till complete decomposition of the organic matter. The digested solution was cooled to room temperature, filtered, and diluted to a final volume of 25 mL using deionized water. The concentrations of iron, lead, copper, manganese, and zinc were determined by using atomic absorption spectrophotometer, Hitachi model 170-30 with graphite atomizer (G.A.Z). The results were expressed in mg/kg dry weight.

Analysis of antioxidant enzymes and Malondialdehyde (MDA) levels

The activity of catalase (CAT) was determined spectrophotometrically at wave length of 510 nm according to the method of **Aebi (1984)**. Glutathione S-transferase (GST) activity was determined by the Biodiagnostic Assay Kit (Giza, Egypt) according to the method of **Habig *et al.* (1974)**. The activity of superoxide dismutase (SOD) was determined by colorimetric method using Bio-diagnostic kits according to the method of **Nishikimi *et al.*, (1972)**. Malondialdehyde (MDA) level was determined using Biodiagnostic kit according to the methods of **Ohkawa *et al.* (1979)**.

Proximate Chemical Composition: Moisture, crude protein, fat, and ash content were determined using the conventional methods of **AOAC (2012)**.

Statistical analysis: All data were expressed as mean ± standard deviation. The data were statistically analyzed by one way ANOVA. Results with p value < 0.05 were considered significant. The statistical analysis was carried out by Bonferroni and confirmed by LSD with respect to the reference site (El-Kanater El-Khairia city 30°11'1"N and Longitude 31° 8'20 "E).

RESULTS AND DISCUSSION

FGF23

Phosphatidic hormone FGF23 was cloned in 2000 and has been established as a hormone regulating phosphate and vitamin D metabolism. In addition, several diseases seem to be caused by aberrant functions of FGF23 thereby making a new classification of disorders of phosphate metabolism possible **(Fukumoto, 2013)**.

FGF23 hormone (ng/ml) of *O. niloticus* and *C. gariepinus* collected from different sites of Rosetta branch, the River Nile during summer 2019 are illustrated in Table (1). The highest levels of FGF23 were recorded in catfish serum, while the lowest levels were recorded in the Nile tilapia fish. On the other hand, the fourth station recorded higher amounts of FGF23 in both two fish species samples and this may be due to the increase in pollutants in this site.

Table 1. Mean ±SD of fibroblast growth factor 23 hormone level (ng/ml) of *Oreochromis niloticus* and *Clarias gariepinus* collected from 4 different stations of Rosetta branch, the River Nile during summer 2019

Station		fish species	
		O. niloticus	*C. gariepinus*
I	Mean	102.00	232.54
	± SD	±1.71	±1.19
II	Mean	141.33	247.72
	± SD	±0.95	±1.40
	P value	<0.001	0.001
III	Mean	155.79	251.41
	± SD	±0.89	±2.14
	P value	<0.001	0.002
IV	Mean	226.33	254.02
	± SD	±1.05	±1.36
	P value	<0.001	0.001

[1]*P*≤0.05 significant differences, *P*>0.05 non-significant differences, p<0.001 highly significant differences

Biochemical parameters of *O. niloticus* and *C. gariepinus*

Biochemical parameters level of *O. niloticus and C. gariepinus* collected from four different stations of Rosetta branch were illustrated in Table (2). The studied stations showed significant differences ($P \leq 0.05$) in the values of glucose, total protein, albumin, urea, and creatinine, when compared to reference site I (El-Qanater). Normal value of serum glucose concentration in fish is 56.75 ± 5.87 mg/dL **(Sabae & Mohamed, 2015)**. In the present study, the increase in value of glucose (hyperglycemia) observed in *O. niloticus* and *C. gariepinus* from studied stations of Rosetta branch indicated that fish created more glucose to provide the energy required to combat the stress caused by the environmental pollution. Elevation in glucose level may have been caused by the increase in gluconeogenesis and glycogenolysis, as well as inhibition of glycolysis and

glycogenesis during stress **(Yekeen & Fawole, 2011)**. The current results corroborate previous evidence of elevated plasma glucose levels following exposure to heavy metals and pesticides (**Sabae *et al*., 2008; Ahmad, 2011)**.

Total serum proteins are important in the metabolism and the control of water balance and they can help with disease diagnosis in fish **(Yang & Chen, 2003)**. Serum total protein concentration in fish is normally 4.08±0.61 g/dL, and serum albumin concentration in fish is normally 1.41±0.22g/dL **(Sabae & Mohamed, 2015)**. In the present study, there was elevation in serum total protein (hyperproteinemia) and albumin in *O. niloticus and C. gariepinus* from stations II, III & IV. This may be due to the activation of metabolic processes as a result of exposure to contaminants; the cellular material in the liver is degraded, and several pathological conditions such as liver and kidney damage and relative changes in blood protein mobilization; loss of water in the plasma, and/or stimulation of protein synthesis in liver **(Woo & Tong, 1982; Zaki *et al*., 2008)**.

Table 2. Mean ± SD of serum biochemical parameters level of *Oreochromis niloticus* and *Clarias gariepinus* collected from Rosetta branch, the River Nile during summer 2019

Station		fish species	Glucose (mg/dL)	Total protein (g/dL)	Albumin (g/dL)	Urea (mg/d)	Creatinine (mg/dL)
I	Mean ± SD	*O. niloticus*	153.5 ±1.13	3.82 ±0.04	1.42 ±0.04	8.29 ±0.47	1.6 ±0.13
		C. gariepinus	181.0 ±0.86	5.7 ±0.63	2.0 ±0.007	11.7 ±0.099	2.5 ±0.042
II	Mean ± SD	*O. niloticus*	252.35 ±2.19	5.16 ±0.05	1. 72 ±0.03	20.1 ±0.23	5.42 ±0.60
	P value		0.008	0.015	0.053	0.013	0.04
		C. gariepinus	276.5 ±0.304	7.9 ±0.56	2.3 ±0.08	27.0 ±0.16	7.8 ±0.28
			0.001	0.007	0.053	0.004	0.01
III	Mean ± SD	*O. niloticus*	263.4 ±0.71	6.42 ±0.18	2.11 ±0.03	25.3 ±0.10	7.97 ±1.34
	P value		0.001	0.019	0.023	0.005	0.05
		C. gariepinus	299.2 ±0.47	11.2 ±0.38	2.9 ±0.18	31.8 ±0.42	11.9 ±0.39
			0.001	0.041	0.043	0.006	0.008
IV	Mean ± SD	*O. niloticus*	270.2 ±1.56	7.23 ±0.15	3.21 ±0.02	29.2 ±0.06	10.33 ±1.44
	P value		0.001	0.013	0.008	0.004	0.04
		C. gariepinus	322.3 ±0.170	14.0 ±0.792	5.5 ±0.23	37.0 ±1.31	14.6 ±0.59
			0.001	0.004	0.015	0.011	0.012

[2] ***P*≤0.05 significant differences, *P*> 0.05 non-significant differences**

Serum urea concentration in fish is normally 8.92±0.70 mg/dL and serum creatinine concentration in fish is normally 2.08±0.38 mg/dL **(Sabae & Mohamed, 2015)**. In the present study, serum urea showed high increase in *Oreochromis niloticus* from stations II, III, and IV, and also it showed high increase in *Clarias gariepinus* from studied stations of Rosetta branch. The increase in urea and creatinine level in blood is associated with a decrease in urea and creatinine excretion. As a result, high serum urea and creatinine indicated to necrosis, renal insufficient, impaired nitrogen metabolism, as well as muscle tissue damage **(Murray *et al.*, 1990)**. This result supported that the environmental pollution exerts harmful effects on the kidney tissues causing kidney dysfunction. It is well known that the renal insufficiency or failure is associated with a reduction of urea and creatinine excretion leading to its rise in plasma **(Chang *et al.*, 1996)**. Kidney damage can lead to decreased renal blood flow and a lower glomerular filtration rate, resulting in azotemia, which is marked by a rise in blood urea nitrogen and creatinine levels. In fish, the liver produces urea, which is mainly excreted through the gills rather than the kidney. Thus, in the present study, the elevation of urea level may be attributed to gill dysfunction **(Stoskoph, 1993)**.

Heavy metal concentrations of *O. niloticus* and *C. gariepinus*

Heavy metal concentrations of *O. niloticus* and *C. gariepinus* collected from different sites of Rosetta branch, the River Nile during summer 2019 are shown in Tables (3 &4).

Table 3. Mean ± SD of heavy metal concentrations in flesh of *Oreochromis niloticus* and *Clarias gariepinus* at different stations of Rosetta branch, the River Nile during summer 2019

Station		Fish species	Fe (mg/kg dry wt)	Zn (mg/kg dry wt)	Cu (mg/kg dry wt)	Mn (mg/kg dry wt)	Cd (mg/kg dry wt)
I	Mean±SD	*O. niloticus*	7.58±0.46	3.9±0.28	0.05±0.004	0.26±0.028	0.01±0.001
		C. gariepinus	16.17±1.19	7.6±0.54	0.17±0.36	0.60 ±0.042	0.04±0.004
II	Mean±SD	*O. niloticus*	33.26±0.15	5.80±0.42	0.094±0.002	1.12±0.04	0.084±0.002
	P value		0.003	0.017	0.031	0.004	0.002
		C. gariepinus	54.63±1.58	9.21±0.276	0.47±0.014	3.10±0.170	0.57±0.035
			0.002	0.037	0.038	0.019	0.017
III	Mean±SD	*O. niloticus*	42.91±0.29	7.1±0.28	0.113±0.003	1.62±0.04	0.107±0.008
	P value		0.001	0.04	0.005	0.011	0.023
		C. gariepinus	56.74±0.59	11.80±0.339	0.53±0.049	3.62±0.56	0.61±0.05
			0.01	0.011	0.054	0.038	0.018
IV	Mean±SD	*O. niloticus*	47.3±0.28	9.2±0.71	0.172±0.02	2.52±0.15	0.135±0.02
	P value		0.001	0.042	0.037	0.012	0.041
		C. gariepinus	65.9±0.61	12.78±0.62	1.37±0.127	6.55±1.21	0.64±0.113
			0.003	0.003	0.017	0.047	0.041

[3] ***P*≤0.05 significant differences, *P*> 0.05 non-significant differences**

The obtained results indicated that there was a significant differences in the heavy metals concentrations of *O. niloticus* and *C. gariepinus* between the study sites and the

fourth station (Kafr El Zayat city) that recorded the highest values, while the first station at El-Qanater El-Khiria city recorded the lowest heavy metals concentration. On the other hand, there was significant differences in heavy metal concentrations between *O. niloticus* and *C. gariepinus* and it was found that *C. gariepinus* had the highest concentrations of metals compared with *O. niloticus.* Metal accumulation in different tissues of the studied fish samples was found to be in the following order: Fe > Zn > Mn > Cu > Cd in muscles and gills.

Table 4. Mean ±SD of heavy metal concentrations in gills of *Oreochromis niloticus* and *Clarias gariepinus* at different stations of Rosetta branch, River Nile during summer 2019.

Stations		Fish species	Fe (mg/kg dry wt)	Zn (mg/kg dry wt)	Cu (mg/kg dry wt)	Mn (mg/kg dry wt)	Cd (mg/kg dry wt)
I	Mean	*O. niloticus*	12.32	7.60	0.09	0.29	0.09
	± SD		±0.064	±0.042	±0.006	±0.006	±0.001
		C. gariepinus	26.72	10.88	0.25	0.83	0.046
			±0.83	±0.61	±0.021	±0.057	±0.006
II	Mean	*O. niloticus*	41.25	12.32	0.112	2.21	0.21
	± SD		±0.68	±0.021	±0.008	±0.085	±0.021
	P		0.006	0.001	0.029	0.011	0.039
	value	*C. gariepinus*	72.59	11.72	0.67	5.29	0.67
			±0.240	±0.73	±0.028	±0.1	±0.028
			0.005	0.032	0.004	0.008	0.013
III	Mean	*O. niloticus*	60.52	13.64	0.213	2.45	0.22
	± SD		±0.530	±0.113	±0.004	±0.057	±0.028
	P		0.003	0.006	0.017	0.007	0.051
	value	*C. gariepinus*	81.28	15.74	0.80	5.62	0.76
			±8.54	±0.93	±0.057	±0.39	±0.11
			0.03	0.02	0.03	0.02	0.03
IV	Mean	*O. niloticus*	72.33	20.53	0.291	3.92	0.23
	± SD		±0.20	±0.15	±0.01	±0.10	±0.03
	P		0.001	0.002	0.014	0.006	0.043
	value	*C. gariepinus*	90.81	17.11	1.81	7.57	0.82
			±3.3	±0.18	±0.042	±0.47	±0.042
			0.009	0.028	0.01	0.018	0.014

[4] ***P*≤0.05 significant differences, *P*> 0.05 non-significant differences**

The lowest value of Fe (7.58 mg/kg dw) was recorded in the flesh of the tilapia at station (I), while the highest value (90.81 mg/kg dw) was recorded in the gills of catfish at station (IV). The lowest value of Zn (3.90 mg/kg dw) was recorded in the flesh of the tilapia at station (I), while the highest value (17.11 mg/kg dw) was recorded in the gills of the tilapia at station (IV). The lowest value of Cu (0.047 mg/kg dw) was recorded in the flesh of the tilapia at station (I), while the highest value (1.81 mg/kg dw) was recorded in

the gills of catfish at station (IV). The lowest value of Mn (0.26 mg/kg dw) was recorded in the flesh of the tilapia at station (I), while the highest value (7.57 mg/kg dw) was recorded in the gills of the catfish at station (IV). The lowest value of Cd (0.011 mg/kg dw) was recorded in the flesh of the tilapia at station (I), while the highest value (0.82 mg/kg dw) was recorded in the gills of the catfish at station (IV). The current results agree with those reported by **Ghannam *et al*. (2014)**, **Talab *et al*. (2014)**, **Ghannam *et al*. (2015a, b)** and **Talab *et al*. (2016)**.

Antioxidant enzyme activities and malondialdehyde (MDA) level of *O. niloticus* and *C. gariepinus*

Antioxidant enzyme activities and biochemical parameters of *O. niloticus* and *C. gariepinus* collected from different sites of Rosetta branch in the River Nile during summer 2019 were illustrated in Tables (5 & 6). In the present study, it was observed that, SOD, CAT, and GST activities in the flesh and the liver of *Oreochromis niloticus* and *Clarias gariepinus* from studied stations of Rosetta branch showed significant differences ($P \leq 0.05$) as compared to the control site (I).

Table 5. Mean ±SD of antioxidant enzyme activities and malondialdehyde (MDA) level as oxidative stress marker in flesh of *Oreochromis niloticus* and *Clarias gariepinus* collected from different stations of Rosetta branch , the River Nile during summer 2019

Station		Fish species	SOD (U/g)	CAT (U/g)	GST (U/g)	MDA (nmol/g)
I	Mean + SD	*O. niloticus*	87.59 ±0.6٠	92.59 ±1.13	0.93 ±0.06	3.49 ±0.03
		C. gariepinus	111.4 ±2.8	102.9 ±0.61	1.32 ±0.092	4.9 ±0.35
II	Mean + SD	*O. niloticus*	79.85 ±0.65	90.08 ±0.43	0.72 ±0.02	3.99 ±0.01
	P value		0.001	0.054	0.045	0.02
		C. gariepinus	107.9 ±1.17	98.5 ±0.416	1.18 ±0.078	16.1 ±0.48
			0.035	0.007	0.021	0.001
III	Mean + SD	*O. niloticus*	71.77 ±0.87	83.18 ±0.44	0.58 ±0.01	8.93 ±0.42
	P value		0.001	0.003	0.045	0.018
		C. gariepinus	88.6 ±0.512	95.5 ±1.00	0.82 ±0.007	17.4 ±0.854
			0.002	0.001	0.04	0.001
IV	Mean + SD	*O. niloticus*	53.47 ±1.45	60.17 ±0.76	0.3٠ ±0.014	14.95 ±0.184
	P value		0.001	0.00١	0.028	0.003
		C. gariepinus	70.27 ±0.998	82.82 ±0.851	0.47 ±0.064	25.44 ±0.87
			0.001	0.001	0.007	0.001

[5] $P \leq 0.05$ significant differences, $P > 0.05$ non-significant differences

Table 6. Mean ±SD of antioxidant enzyme activities and malondialdehyde level as oxidative stress marker in liver of *Oreochromis niloticus* and *Clarias gariepinus* collected from different stations of Rosetta branch , the River Nile during summer 2019

Station		Fish species	SOD (U/g)	CAT (U/g)	GST (U/g)	MDA (nmol/g)
I	Mean	*O. niloticus*	122.04	1٢0.87	1.93	14.34
	+ SD		±0.13	±0.84	±0.08	±0.05
		C. gariepinus	157.6	144.3	2.3	23.95
			±4.04	±0.896	±0.014	±0.23
II	Mean	*O. niloticus*	112.63	112.56	1.35	27.47
	+ SD		±0.55	±0.88	±0.04	±0.50
	P value		0.00١	0.007	0.046	0.01
		C. gariepinus	124.8	119.7	1.50	35.01
			±0.94	±0.475	±0.064	±0.50
			0.004	0.001	0.023	0.001
III	Mean	*O. niloticus*	91.67	102.32	0.92	48.61
	+ SD		±2.36	±0.52	±0.01	±0.40
	P value		0.001	0.001	0.022	0.002
		C. gariepinus	98.4	117.4	1.05	38.9
			±0.55	±1.33	±0.035	±1.2
			0.001	0.001	0.004	0.001
IV	Mean	*O. niloticus*	77.50	95.67	0.65	58.86
	+ SD		±3.50	±0.68	±0.028	±0.219
	P value		0.001	0.00١	0.019	0.001
		C. gariepinus	91.36	103.97	0.80	47.48
			±0.86	±1.70	±0.057	±1.154
			0.001	0.001	0.012	0.001

[6] $P \leq 0.05$ significant differences, $P > 0.05$ non-significant differences

Moreover, a significant elevation of MDA was observed in the flesh and the liver of *Oreochromis niloticus* and and *Clarias gariepinus* as compared to the control site (I). The accumulation of heavy metals in the fish organs may be responsible for the rise in lipid peroxidation. The large increase in lipid oxidation markers could indicate lipid molecules' susceptibility to reactive oxygen species and the degree of oxidative damage they suffered **(Farombi *et al*., 2007)**. The decline in antioxidant enzyme activities may also be contributed to the strong increase in lipid oxidation and its marker. The metal-catalyzed formation of ROS is capable of destroying tissues such as DNA, proteins, and lipids **(Pandey *et al*., 2003)**.

Chemical composition of *O. niloticus* and *C. gariepinus*

The chemical composition of *O. niloticus* and *C. gariepinus* collected from different sites of Rosetta branch in the River Nile during summer 2019 are shown in Table (7). The obtained results indicated that, there is no significant differences in the chemical composition of two fish species collected from four different site in the River Nile, Rosetta branch and the mean values of moisture, protein, lipid, and ash of *O.*

niloticus and *C. gariepinus* were "80.16, 17.18, 1.23, and 1.14%" and "78.66 ,18.16, 1.61, and 1.18 %", respectively.

Table 7. Chemical composition of *O. niloticus* and *C. gariepinus* collected from different sites of Rosetta branch, the River Nile during 2019

Parameter		Fish species	Station				
			I	II	III	IV	Mean
Moisture (%)	Mean	*O. niloticus*	80.21	80.18	80.23	80.03	80.16
	± SD		±0.028	±0.04	±0.03	±0.02	
	P value			0.10	0.35	0.06	
	Mean	*C. gariepinus*	78.15	78.87	78.83	78.77	78.66
	± SD		±0.06	±0.2	±0.8	±0.33	
	P value			0.07	0.23	0.13	
Protein (%)	Mean	*O. niloticus*	17.15	17.15	17.25	17.18	17.18
	± SD		±0.03	±0.04	±0.04	±0.03	
	P value			0.50	0.15	0.30	
	Mean	*C. gariepinus*	18.05	18.11	18.37	18.12	18.16
	± SD		±0.06	±0.01	±0.06	±0.02	
	P value			0.16	0.08	0.13	
Lipid (%)	Mean	*O. niloticus*	1.08	1.21	1.24	1.38	1.23
	± SD		±0.07	±0.01	±0.07	±0.01	
	P value			0.10	0.18	0.06	
	Mean	*C. gariepinus*	1.94	1.73	1.42	1.35	1.61
	± SD		±0.03	±0.09	±0.54	±0.21	
	P value			0.13	0.21	0.07	
Ash (%)	Mean	*O. niloticus*	1.14	1.17	1.08	1.16	1.14
	± SD		±0.04	±0.03	±0.01	±0.01	
	P value			0.33	0.10	0.37	
	Mean	*C. gariepinus*	1.12±0.0	1.16	1.29	1.14	1.18
	± SD		1	±0.03	±0.07	±0.04	
	P value			0.16	0.10	0.25	

The present results are in parallel with those reported by **Talab *et al*. (2016)** who found that, moisture, protein, fat, ash, carbohydrates and calorific values of *O. niloticus* collected from the River Nile Rayahs varied between (78.55–80.77%), (16.10–17.88%), (1.10–1.95%), (0.55–1.50%), (0.10–0.94%) and (78.37–89.73%), respectively. On the other hand, **Ghannam *et al*. (2015a)** reported that, the average proximate composition of *Clarias gariepinus* ranged from 76.91-78.45% moisture, (19.28-20.54%) protein content, (0.95-1.42%) fat content, (1.08-1.19%) ash content (0.04-0.92%), and carbohydrates content on wet weight basis.

The correlation matrix for fibroblast growth factor (FGF23), biochemical parameters, heavy metals, antioxidant enzymes activities and chemical composition in *Oreochromis niloticus* and *Clarias gariepinus* collected from the Rosetta branch during summer 2019 are illustrated in Tables (8 & 9).

Table 8. Correlation matrix of fibroblast growth factor 23, biochemical parameters, heavy metals, antioxidant enzymes activities, and chemical composition in Oreochromis niloticus.

	FGF23	Glucose	Total protein	Albumin	Urea	Creatinine	Fe	Zn	Cu	Mn	Cd	SOD	CAT	GST	MDA	Moisture	Protein	Lipid	Ash
FGF23	1																		
Glucose	0.776	1																	
Total protein	0.934	0.889	1																
Albumin	0.988	0.687	0.912	1															
Urea	0.902	0.959	0.982	0.851	1														
Creatinine	0.945	0.908	0.997	0.914	0.989	1													
Fe	0.857	0.980	0.962	0.795	0.995	0.970	1												
Zn	0.981	0.852	0.985	0.963	0.961	0.989	0.929	1											
Cu	0.993	0.838	0.966	0.972	0.945	0.976	0.910	0.996	1										
Mn	0.982	0.862	0.984	0.959	0.964	0.990	0.934	1.000	0.997	1									
Cd	0.910	0.963	0.975	0.854	0.998	0.986	0.993	0.961	0.951	0.966	1								
SOD	-0.993	-0.746	-0.945	-0.996	-0.894	-0.947	-0.846	-0.983	-0.987	-0.980	-0.896	1							
CAT	-0.968	-0.601	-0.862	-0.994	-0.786	-0.863	-0.721	-0.926	-0.940	-0.921	-0.790	0.979	1						
GST	-0.989	-0.827	-0.976	-0.975	-0.946	-0.980	-0.909	-0.999	-0.997	-0.998	-0.947	0.991	0.944	1					
MDA	0.952	0.649	0.919	0.983	0.837	0.908	0.780	0.949	0.942	0.941	0.830	-0.982	-0.977	-0.959	1				
Moisture	-0.861	-0.424	-0.623	-0.870	-0.573	-0.649	-0.502	-0.747	-0.800	-0.750	-0.600	0.833	0.900	0.778	-0.787	1			
Protein	0.278	0.505	0.586	0.293	0.554	0.537	0.576	0.447	0.361	0.434	0.505	-0.353	-0.226	-0.414	0.427	0.216	1		
Lipid	0.988	0.863	0.960	0.956	0.952	0.974	0.921	0.990	0.997	0.993	0.960	-0.974	-0.920	-0.990	0.917	-0.796	0.334	1	
Ash	0.103	-0.102	-0.212	0.052	-0.155	-0.149	-0.179	-0.063	0.031	-0.044	-0.097	-0.006	-0.093	0.034	-0.118	-0.514	-0.907	0.069	1

Table 9. Correlation matrix of fibroblast growth factor 23, biochemical parameters, heavy metals, antioxidant enzymes activities, and chemical composition in *Clarias gariepinus*

	FGF23	Glucose	Total protein	Albumin	Urea	Creatinine	Fe	Zn	Cu	Mn	Cd	SOD	CAT	GST	MDA	Moisture	Protein	Lipid	Ash
FGF23	1																		
Glucose	0.999	1																	
Total proteir	0.889	0.904	1																
Albumin	0.681	0.713	0.907	1															
Urea	0.993	0.997	0.935	0.763	1														
Creatinine	0.959	0.967	0.981	0.826	0.983	1													
Fe	0.993	0.993	0.855	0.670	0.983	0.932	1												
Zn	0.913	0.921	0.988	0.832	0.945	0.989	0.869	1											
Cu	0.759	0.788	0.916	0.986	0.828	0.862	0.761	0.844	1										
Mn	0.902	0.921	0.960	0.925	0.945	0.953	0.902	0.922	0.966	1									
Cd	0.987	0.981	0.803	0.572	0.962	0.900	0.992	0.835	0.672	0.840	1								
SOD	-0.784	-0.805	-0.981	-0.946	-0.850	-0.926	-0.743	-0.954	-0.926	-0.924	-0.673	1							
CAT	-0.785	-0.812	-0.950	-0.988	-0.853	-0.897	-0.776	-0.890	-0.995	-0.972	-0.692	0.959	1						
GST	-0.836	-0.854	-0.994	-0.936	-0.893	-0.955	-0.799	-0.972	-0.930	-0.948	-0.736	0.996	0.962	1					
MDA	0.956	0.968	0.942	0.852	0.980	0.965	0.959	0.921	0.913	0.987	0.915	-0.876	-0.922	-0.913	1				
Moisture	0.924	0.910	0.647	0.382	0.874	0.779	0.942	0.695	0.505	0.705	0.974	-0.488	-0.519	-0.563	0.809	1			
Protein	0.552	0.524	0.437	0.035	0.504	0.527	0.471	0.571	0.042	0.245	0.545	-0.352	-0.133	-0.383	0.327	0.546	1		
Lipid	-0.927	-0.932	-0.975	-0.790	-0.951	-0.988	-0.882	-0.997	-0.808	-0.903	-0.857	0.929	0.857	0.952	-0.914	-0.729	-0.622	1	
Ash	0.498	0.466	0.333	-0.085	0.436	0.441	0.424	0.475	-0.067	0.152	0.512	-0.234	-0.021	-0.271	0.250	0.544	0.991	-0.533	1

The correlation matrix for (FGF23) in the present work was calculated with confidence level of 95% and (P < 0.05) showed that, significant correlations were detected between FGF23 and biochemical parameters, heavy metals, antioxidant enzyme activities and chemical composition in *Oreochromis niloticus* as shown in Table (8) which presents the values as follows: glucose (r=0.776), total protein (r= 0.934), albumin (r= 0.988), urea (r= 0.902), creatinine (r= 0.945), Fe (r= 0.857), Zn (r= 0. 981), Cu (r= 0.993), Mn (r= 0.982), Cd (r= 0.910), MDA (r= 0.952), protein (r= 0.278) , lipid (r= 0.988) and ash (r= 0.103). In contrast, FGF23 is negatively correlated with SOD (r= -0.993), CAT (r= -968), GST (r= -0.989) and moisture (r= -0.861). On the other hand, FGF23 in *Clarias gariepinus* (Table 9) was significantly correlated with glucose (r=0.999), total protein (r= 0.889), albumin (r= 0.681), urea (r= 0.993), creatinine (r= 0.959), Fe (r= 0.993), Zn (r= 0. 913), Cu (r= 0.759), Mn (r= 0.902), Cd (r= 0.987), MDA (r= 0.956), moisture (r= 0.924) ,protein (r= 0.552) and ash (r= 0.498). In contrast, FGF23 is negatively correlated with SOD (r= -0.784), CAT (r= -785), GST (r= -0. 836) and lipid (r= -0.927). The concentrations of the toxic elements in fish are mainly affected by the levels of these metals in the habitats, the food of the fish and the rate of metal detoxification **(Urena *et al*., 2007, Talab *et al*., 2016)**. **Giuseppe *et al*. (2015)** informed that, increased fibroblast growth factor 23 (FGF23); a bone-derived hormone involved in the regulation of phosphate and vitamin D metabolism, has been related to the development of cardiovascular disease (CVD) in chronic kidney disease patients and in the general population. However, what determines higher FGF23 levels is still unclear. Furthermore, little is known about the influence of diet on FGF23.

CONCLUSION

The observations of the current data showed that biochemical parameters are well used to assess fish health. The studied stations showed significant differences ($P \leq 0.05$) in the values of glucose, total protein, albumin, urea and creatinine, when compared to reference site (I El-Kanater) in both *O. niloticus* and *C. gariepinus*. In addition, the activities of antioxidant enzymes SOD, CAT, and GST and MDA level showed significant differences ($P \leq 0.05$) when compared to site (I). A change was observed in the chemical composition of the two fish species that may be related to differences in water quality and environmental conditions. Thus, FGF23 assay can be used in prospective trials to validate its utility as a biomarker of polluted freshwater fish.

REFERENCES

Aebi H. (1984). Catalase. Methods in Enzymology 105:121–126. Bhattacharjee G, Chaudhuri PS. 2002. Cocoon production, morphology, hatching pattern and fecundity in seven tropical species – a laboratory based investigation. Journal of Biosciences 27:283–294.

Ahmad Z. (2011). Acute toxicity and haematological changes in common carp (*Cyprinus carpio*) caused by diazinon exposure. African Journal of Biotechnology, 10(63):13852-13859.

AOAC (2012). Official Method of Analysis (15th ed.) Association of Official Analytical Chemists, Inc., Washington, DC, USA, 478pp.

Chang L.; Magos L. and Suzuki T. (1996). Toxicology of metals. Lewis Publishers, New York.

Dailey L.; Ambrosetti D.; Mansukhani A. and Basilico C. (2005). Mechanisms underlying differential responses to FGF signaling. Cytokine Growth Factor Rev 16(2):233–247.

Eswarakumar V.P.; Lax I. and Schlessinger J. (2005). Cellular signaling by fibroblast growth factor receptors. Cytokine Growth Factor Rev 16(2):139–149

Farombi E. O.; Adelowo, O. A. and Ajimoko Y. R. (2007). Biomarkers of Oxidative Stress and Heavy Metal Levels as Indicators of Environmental Pollution in African Cat Fish (*Clarias gariepinus*) from Nigeria Ogun River. Int. J. Environ. Res. Public Health 4(2):158-16.

Fu L.; John L.M.; Adams S.H.; Yu X.X.; Tomlinson E.; Renz M.; Williams P.M.; Soriano R.; Corpuz R.; Moffat B.; Vandlen R.; Simmons L.; Foster J.; Stephan J.P.; Tsai S.P. and Stewart T.A. (2004). Fibroblast growth factor19 increases metabolic rate and reverses dietary and leptindeficient diabetes. Endocrinology 145: 2594–2603.

Fukumoto S. (2007). Regulation of phosphorus-vitamin D metabolism by FGF23. Nihon Naika Gakkai Zasshi., 96(4):719-24. PMID: 17506310.

Fukumoto S. (2013). Clinical Aspects of Fibroblast Growth Factor 23. Translational Endocrinology of Bone, pp.151–166.

Ghannam H.E.; El Haddad E.S. and Talab A.S. (2015a). Bioaccumulation of heavy metals in tilapia fish organs. Journal of Biodiversity and Environmental Sciences, pp.88-99.

Ghannam H.E.; Talab A.S. and Elewa A.A. (2015b). Impact of seasonal changes on the quality of water and fish from Abu Za'baal Lakes. Journal of Bioscience and Applied Research, 1(4):192-199.

Ghannam H.E.; Talab A.S.; Gaber S.E. and Jahin H.S. (2014). Assessment of heavy metals distribution in some freshwater fish organs using inductively coupled plasma optical emission spectrometry (ICP-OES). Ecology, Environment and Conservation Journal, 20 (2): 63-74.

Giuseppe R.; Kühn T.; Hirche F.; Buijsse B.; Dierkes J.; Fritsche A.; et al. (2015). Potential Predictors of Plasma Fibroblast Growth Factor 23 Concentrations: Cross-Sectional Analysis in the EPIC-Germany Study. PLoS ONE 10(7): e0133580. doi:10.1371/journal.pone.0133580

Goldberg E.D. (1963). Geochronology with lead-210. In: Symposium on radioactive dating, Athens, November, 1962. Vienna, International Atomic Energy Agency. pp. 121-131.

Habig W.H.; Pabst M.J. and Jacoby W.B. (1974). Glutathione S-transferases: the first enzymatic step in mercapturic acid formation. J. Biol. Chem. 249:7130-7139.

Isakova T.; Wahl P.; Vargas G.S.; Gutierrez O.M.; Scialla J.; Xie H.; Appleby D.; Nessel L.; Bellovich K.; Chen J.; Hamm L.; Gadegbeku C.; Horwitz E.; Townsend R.R.; Anderson C.A.; Lash J.P.; Hsu C.Y.; Leonard M.B. and Wolf M. (2011). Fibroblast growth factor 23 is elevated before parathyroid hormone and phosphate in chronic kidney disease. Kidney Int., 79(12):1370-1378.

Itoh N. and Ornitz D. M. (2004). Evolution of the Fgf and Fgfr gene families. Trends Genet. 20, 563–569.

Ix J.H.; Shlipak M.G.; Wassel C.L. and Whooley M.A. (2010). Fibroblast growth factor-23 and early decrements in kidney function: the Heart and Soul Study. Nephrol Dial Transplant 25(3):993-997.

Jimbo R. and Shimosawa T. (2014). Cardiovascular Risk Factors and Chronic Kidney Disease-FGF23: A Key Molecule in the Cardiovascular Disease. Int J Hypertens. 1-9:381082. doi: 10.1155/2014/381082.

Katoh M. (2002). WNT and FGF gene clusters (review). Int J Oncol 21(6):1269–1273.

Murray R.; Mayes P.; Granner D. and Radwel V. (1990). Harper's Biochemistry? 22nd Edition, Appleton and Lange, London, Toronto.

Nishikimi M.; Rao N.A. and Yagi K. (1972). The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochem. Biophys. Res. Commun., 46: 849-854.

Ohkawa H.; Ohishi N. and Yagi K. (1979). Assay of lipid peroxides in animal tissues by thiobarbituric acid reaction, Annals of Biochemistry. (95):351-358.

Pandey S.; Parvez S.; Sayeed I.; Haque R.; Bin-Hafeez B. and Raisuddin S. (2003). Biomarkers of oxidative stress: a comparative study of river Yamuna fish Wallago attu (Bl. & Schn.). Sci. otal Environ. 309:105-15.

Sabae S. Z. and Mohamed F. A. S. (2015). Effect of environmental pollution on the health of Tilapia spp. from Lake Qarun. Global Veterinaria, 14 (3): 304-328.

Sabae S. Z.; Emam W. M. and Rabeh S. A. (2008). Microbial characteristics of Wadi El-Raiyan Lakes, Egypt. J. Egypt. Acad. Soc. Environ. Develop. (D-Environmental Studies), 9 (2): 17-28.

Stoskoph M. (1993). Fish Medicine. W.B. Saunders company, pp. 116, 128,129.

Talab A. S.; Goher M. E.; Ghannam H. and Abdo M. H. (2016). Chemical compositions and heavy metal contents of *Oreochromis niloticus* from the main irrigated canals (rayahs) of Nile Delta. Egyptian Journal of Aquatic Research, 42: 23-31.

Talab A.S.; Jahin S.H.; Gaber S.E. and Ghannam H.E. (2014). Influence of Modern Cooking Techniques on Heavy Metals Concentrations of Some Freshwater Fish Fillets. Research Journal of Applied Sciences, Engineering and Technology, 8(1): 69-75. 15.

Tietz N.W. (1986). Textbook of Clinical Chemistry. W.B. Saunders Co., Philadelphia, 1271-1281.

Tietz N.W. (1994). Fundamentals of Clinical Chemistry. 2nd Edn., WB Saunders, Philadelphia, pp: 692.

Tietz N.W. (1995). Clinical guide to laboratory tests, 3rd Ed., (Saunders, W.B., Ed.), Philadelphia, USA, pp: 130-273.

Tietz N.W.; Finley P.R. and Pruden E.L. (1990). Clinical Guide to Laboratory Tests. 2nd Edition, W.B. Saunders, Philadelphia, 304-306.

Tomlinson E.; Fu L.; John L.; Hultgren B.; Huang X.; Renz M.; Stephan J.P..; Tsai S.P.; Powell-Braxton L.; French D. and Stewart T.A. (2002). Transgenic mice expressing human fibroblast growth factor-19 display increased metabolic rate and decreased adiposity. Endocrinology 143, 1741–1747 (2002). Initiated interest in FGF19 as a metabolic regulator by detailing the phenotype of FGF19 transgenic mice.

Tsai S.M.; Da-Wei L. and Wen-Pin W. (2013). Fibroblast growth factor (Fgf) signaling pathway regulates liver homeostasis in zebrafish. Transgenic Res (2013) 22:301–314.

Urena R.; Peri S.; Del Ramo J. and Torreblanca A. (2007). Metal and metalloithionein content in tissues from wild and farmed *Anguilla anguilla* at commercial size. Environ. Int. 33, 532-539.

Velma V. and Tchounwou P. B. (2010). Chromium-induced biochemical, genotoxic and histopathologic effects in liver and kidney of goldfish, *carassius auratus*," Mutation Research: Genetic Toxicology and Environmental Mutagenesis, vol. 698, no. 1-2, pp. 43–51, 2010

Woo N. and Tong W. (1982). Salinity adaptation in the snakehead, Ophiocephalus maculatus Lacépéde: Changes in oxygen consumption, branchial Na+ -K+-ATPase, body composition. J. Fish Biol., 20:11-19.

Yang J. L. and Chen H. C. (2003). Serum metabolic enzyme activities and hepatocytes ultra-structure of common carp after gallium exposure. Zoological Studies, 42(3): 455-461.

Yekeen T.A. and Fawole O. (2011). Toxic effects of endosulfan on haematological and biochemical indices of *Clarias gariepinus*. Afri. J. Biotechnol., 10(64):14090-14096.