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Cadmium Toxicity-Induced Oxidative Stress and Genotoxic Effects on Nile tilapia (*Oreochromis niloticus* L.) Fish: The Protective Role of Fenugreek (*Trigonella foenum-graecum*) Seeds

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ABSTRACT

The present work was designed to study the alleviation influence of fenugreek (*Trigonella foenum-graecum*) on Nile tilapia (*Oreochromis niloticus* L.) experimentally exposed to Cd toxicity. A total of 400 fish were randomly divided into 10 groups (G1-G10) and fish was exposed to sub-lethal dose of Cd and fed on fenugreek seeds (crude or extract), individually or in combination for 45 days. The results showed that Cd exposure increased significantly the serum total protein, albumin, globulin, alanine aminotransferase (ALT), glucose and cholesterol, whereas aspartate aminotransferase (AST) and uric acid were significantly decreased. Also, hepatic protein content, catalase (CAT), superoxidase dismutase (SOD) and glutathione-S-transferase (GST) increased significantly due to Cd exposure. Additionally, Cd induced significantly metallothionein-gene (*MT*) expression and increased Cd concentrations in different fish tissues. Feeding on fenugreek extract 1% and 3% has reduced some Cd-induced biochemical and antioxidant alterations; however, it decreased ALT, AST and uric acid in serum and also reduced CAT and SOD activities. Fenugreek extract induced *MT* up-regulation and decreased Cd accumulation in some fish tissues. Although, feeding on fenugreek extract with Cd exposure somewhat reduced the histopathological changes compared to Cd-exposed group, some pathological changes were observed in fenugreek-fed groups especially in high concentrations. It could be concluded that fenugreek seeds extract at level of 1% could be used as feed supplement to Nile tilapia to alleviate some toxic effects of Cd and reduction of Cd accumulation in fish tissues (especially muscle) in such a way to help fish in facing Cd toxicity and reducing the public health hazard.

INTRODUCTION

Aquaculture had been known in Egypt since the old Egyptian eras (Shaalan *et al.*, 2018). The present aquaculture is depended mainly on the water from agricultural drainage channels where aquaculture projects are not allowed to use irrigation or Nile water (Soliman, 2017). Drainage water was reported as contaminated with agrochemicals, industrial effluents, and domestic sewage (Khallaf *et al.*, 2003). Accordingly, this pollution shows deleteriously effects on farmed fish quality (FAO, 2014) and may have negative effects on the health of human who consume the

polluted fish (Mur, 2014). Drainage water may contains high concentrations of runoff-derived fertilizers, pesticides, heavy metals, and so on (Authman *et al.*, 2012). Among most of heavy metal contaminants released into the aquatic environment, cadmium (Cd) which has been documented as one of the greatest toxic heavy metal that causes adverse effects on aquatic organisms (Shah *et al.*, 2017). Major sources of Cd are industrial and agrochemical wastes (e.g., nickel cadmium batteries manufacturing plants, lead mining and processing units, sewage sludge and fertilizers (i.e. phosphate) that contain high-level of Cd, pigments, ceramics, plastics, glasses, vehicle tires and other synthetics) which discharge into surface water bodies and then be taken by aquatic organisms (Zang and Bolger, 2014; El-Kady and Abdel-Wahhab, 2018). Cadmium has been found to be accumulated in high concentrations in various organs such as intestine, kidney, liver, gills, muscles, and reproductive organs of Nile tilapia, *O. niloticus* (Otludİl *et al.*, 2017). It causes biochemical, physiological, and oxidative stress alterations, anemia, serious histopathological changes and so on (Abdel-Tawwab and Wafeek, 2014; Al-Asgah *et al.*, 2015; Abdel-Tawwab and Wafeek, 2017; Otludİl *et al.*, 2017) and had carcinogenic and endocrine-damaging properties (Bobocea *et al.*, 2008). Cadmium has also adverse effects on osmoregulation, survival, growth and reproduction (Kim *et al.*, 2004; Szczerbik *et al.*, 2006), damage fish health and aquatic ecosystems (Zhou *et al.*, 2017). So, there is an urgent need to develop low-cost, sustainable and effective methods to mitigate its toxic effect on fish. Therefore, there is a need for unusual methods to managing heavy metals toxicity that are appropriate to many toxicants and many species (Sayed *et al.*, 2015).

Recently, many studies have reported using either plants or plant extracts in aquaculture not only as feed additives but also as chemotherapeutics (Wang *et al.*, 2015; Abdel-Tawwab, 2016), as they contains a wide variety of chemical compounds and nutrients (Chang, 2000). It is worth to mention that plants can also induced antioxidant enzymes such as catalase, superoxide dismutase, glutathione S-transferase, and peroxidases, which play an important role in detoxification and removal of toxicity-induced reactive oxygen species (Guardiola *et al.*, 2017; Abdel-Tawwab *et al.*, 2018). In this regard, an interest in using fenugreek (*Trigonella foenum graecum* L.) [FN], in fish diets has gained attention in recent years (Mostafa *et al.*, 2009; Mehboob *et al.*, 2017; Roohi *et al.*, 2017). The leaves of FN are used in diets as green leafy vegetables, which is rich in vitamins and minerals (Srinivasan, 2006; Roohi *et al.*, 2017) and its seeds are used for medication in different countries for aphrodisiac effects, tonic and carminative (Xue *et al.*, 2007). Fenugreek has been shown to have many biological activities as anti-inflammatory, anti-carcinogenic potential, potential allergens, antibacterial, anti-diabetic and antioxidant activity (Belguith-Hadriche *et al.*, 2013; Mehboob *et al.*, 2017).

In Egypt and worldwide, Nile tilapia, *Oreochromis niloticus* is the main farmed fish owing to its high delicacy by a huge number of people and high market value (El-Sayed, 2006). Moreover, Nile tilapia, *O. niloticus* has been used in major laboratory; field and toxicological researches (Authman *et al.*, 2012). Several studies were conducted on the use of FN as a natural feed additive to improve growth performance, enhance disease resistance and immunity of Nile tilapia (Mostafa *et al.*, 2009; Mehboob *et al.*, 2017; Roohi *et al.*, 2017) but there are no studies reporting the role of FN in ameliorating the toxic effects and chelating of Cd in fish. So, the purpose of the present work was: 1) to investigate the FN chelating ability of Cd accumulated in fish tissues, and 2) to determine the role of FN in the amelioration of the biochemical, histopathological and antioxidant alterations induced in *O. niloticus*

by cadmium exposure. This study may give the feasible solution to overcome pollution problems in fish farming.

MATERIALS AND METHODS

Fish

Apparently healthy all-male Nile tilapia, *O. niloticus* (30-40 g body weight; *n* = 400) were obtained from a commercial fish farm at Kafr El-Sheik governorate, Egypt, and were then transported to the Hydrobiology Department, National Research Centre, Dokki. Fish were kept in 120 L glass aquaria under laboratory conditions for 2 weeks for acclimatization, during which fish were fed on a commercial 5-mm floating dry diet pellets (Skretting Egypt, Hendrix) [35% total crude protein, 5.8% fat (ether extract), 3.5% crude fibers and 4100 Kcal digestible energy] at feeding level of 2% of body weight per day. To reduce impurities from metabolic wastes, the water was changed twice a week.

Fenugreek (FN) seeds

FN (*T. foenum graecum*) seeds were obtained from a commercial market (Egypt). Crude crushed seeds and alcoholic extract of FN seeds were prepared. For crude crushed seeds: FN seeds were firstly washed, dried and crushed, whereas for alcoholic extract of FN seeds: 1 kg of crushed FN was soaked in double volume of absolute ethanol in a stoppered container for five days, shaked more than one time per each day, filtered and evaporated by a rotary evaporator, then left for complete dryness and weighed (Azwanida, 2015).

Fish diets

A commercial 5-mm floating dry pellets (35% total crude protein) was firstly crushed, mixed with the appropriate ratios of crude crushed seeds (0.0, 2.5 and 5.0%) or its alcoholic extract (0.0, 1.0 and 3.0%), and wet with 100 ml water to make a paste. The pastes were made again into pellets, and the wet pellets allowed to dry in the open air (protected from bacteria and fungi infection by covering) and then stored at 4^{o}C until use.

Chemicals

Cadmium Chloride [$CdCl_2$; (purity >95% and Mw: 183.32] was obtained from Laboratory Rasayan Fine-Chem LTd. (Haryana, India). A stock solution of Cd was freshly prepared by dissolving $CdCl_2$ in deionized water at a concentration of 1000 mg L^{-1}. Serum total protein, glucose, aspartic aminotransferase (AST), alanine aminotransferase (ALT), albumin, cholesterol, and uric acid were estimated using commercial biochemical kits bought from Spectrum-Diagnostics Co., Egypt. All other chemicals were of analytical grade.

Experimental design

After the fish acclimation, fish were randomly allocated into 10 groups, each group containing 40 fish (20 fish/glass aquarium; two replicates). The 1^{st} group (G1) was the control which received a commercial fish food without any additives. The 2^{nd} group (G2) received a commercial fish food without any additives and subjected to 1/10 LC_{50} of cadmium toxicity (1.5 mg Cd L^{-1} of $CdCl_2$) according to Garcia-Santos *et al.* (2006). The 3^{rd} group (G3) was fed on crushed crude FN seeds-supplemented diet at a concentration of 2.5%. The 4^{th} group (G4) was fed on crushed crude FN seeds-supplemented diet at a concentration of 2.5% and subjected to cadmium toxicity (1.5 mg Cd L^{-1} of $CdCl_2$). The 5^{th} group (G5) was fed on crushed crude FN seeds-supplemented diet at a concentration of 5%. The 6^{th} group (G6) was fed on crushed crude FN seeds-supplemented diet at a concentration of 5% and subjected to cadmium

toxicity (1.5 mg Cd L^{-1} of $CdCl_2$). The 7th group (G7) was fed on alcoholic extract of FN seeds-supplemented diet at a concentration of 1%. The 8th group (G8) was fed on alcoholic extract of FN seeds-supplemented diet at a concentration of 1% and subjected to cadmium toxicity (1.5 mg Cd L^{-1} of $CdCl_2$). The 9th group (G9) was fed on alcoholic extract of FN seeds-supplemented diet at a concentration of 3%. The 10th group (G10) was fed on alcoholic extract of FN seeds-supplemented diet at a concentration of 3% and subjected to cadmium toxicity (1.5 mg Cd L^{-1} of $CdCl_2$). The used ratios of crude and extract FN seeds were chosen based on some previous studies (Zaki *et al.*, 2012; Awad *et al.*, 2015). The duration of the experiment was 45 days. The design of the experiment was represented in table (1).

Table 1: The experimental design.

Groups	Description	Fenugreek seeds (crude or extract) supplemented	Cd concentration
G1	Control	No (0%)	No (0 mg Cd L^{-1})
G2	Cd	No (0%)	Yes (1.5 mg Cd L^{-1})
G3	FN Crude	Yes (2.5%)	No (0 mg Cd L^{-1})
G4	FN Crude Plus Cd	Yes (2.5%)	Yes (1.5 mg Cd L^{-1})
G5	FN Crude	Yes (5%)	No (0 mg Cd L^{-1})
G6	FN Crude Plus Cd	Yes (5%)	Yes (1.5 mg Cd L^{-1})
G7	FN Extract	Yes (1%)	No (0 mg Cd L^{-1})
G8	FN Extract Plus Cd	Yes (1%)	Yes (1.5 mg Cd L^{-1})
G9	FN Extract	Yes (3%)	No (0 mg Cd L^{-1})
G10	FN Extract Plus Cd	Yes (3%)	Yes (1.5 mg Cd L^{-1})

FN = Fenugreek seeds.

Fish of each aquarium were fed the tested diets twice daily at 2% of fish body weight for 45 days. A half of aquarium's water was siphoned off, twice a week, with fish feces and other waste materials and replaced with an equal volume of water maintaining the same Cd concentration.

Parameters of water quality

During the experimental period, the water quality parameters were as follows: temperature (27.17±0.19 °C), dissolved oxygen (7.66±0.26 mg L^{-1}), pH (7.45±0.08), electrical conductivity (751.79 μmhos cm^{-1}), total dissolved solids (488.67±49.64 mg L^{-1}), alkalinity (116.83±7.05 mg L^{-1} as $CaCO_3$), hardness (134.00±11.31 mg L^{-1} as $CaCO_3$), and unionized ammonia (0.56±0.20 mg L^{-1}) with no free chlorine. The water temperature and dissolved oxygen concentrations were measured using SensION6 HACH, HACH Co., USA, whereas pH values were measured using IQ140 pH/Temp meter, IQ Scientific Instruments Inc., USA. Alkalinity, hardness, total dissolved solids, unionized ammonia, and chlorine concentrations were determined according to the standard analytical procedures of APHA (2012). Electrical conductivity (EC) values were calculated from total dissolved solids (TDS) concentrations by using the following equitation: EC= TDS/0.65 (Rusydi, 2018).

Blood samples

At the end of the experiment (after 45 days), 20 fish from each group (10 fish/ replicate) were randomly selected and anesthetized with 0.4 ml L^{-1} of Eugenol (FA-100, Tanabe Seiyaku Co. Ltd, Japan). Blood samples were collected from the caudal vein of fish by a 3-ml sterile syringe and allowed to clot at room temperature in clean, dry centrifuge tubes; centrifuged for 15 min. at 3000 x g to collect the serum, then stored at −80 °C until biochemical analysis. Then fish were killed by overdose of Eugenol, dissected and samples from different tissues were collected for Cd residues, oxidative stress, CYP, MT and histopathological analysis.

Biochemical assays

Serum total protein and albumin concentrations were estimated according to Cannon *et al.* (1974) and Tietz *et al.* (1990), respectively; and then serum globulin concentration was calculated by subtracting albumin from protein concentrations. Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST), uric acid, cholesterol and glucose concentrations were determined colorimetrically (JASCO V-730 spectrophotometer, Tokyo, Japan) according to Reitman and Frankel (1957), Tietz *et al.* (1990), Ellefson and Caraway (1976) and Caraway and Watts (1987), respectively.

Determination of cadmium residues

Cd concentrations, in fish tissues (liver, gills and muscle), were measured, after conc. HNO_3 digestion and extraction of five pooled samples according to Chernoff (1975), using an atomic absorption spectrometry (Perkin-Elmer 3110, USA) with graphite atomizer (HGA-600).

Determination of oxidative stress biomarkers and cytochrome (CYP P450 1A1)

Liver samples were dissected and quickly dropped into liquid nitrogen, then transferred to -80 °C until analyzed. Approximately 0.5 g of each liver sample was homogenized by a high speed glass-Teflon homogenizer in KCl-HEPES buffer; 0.15M KCl, 0.02M HEPES, pH 7.5. The homogenate was centrifuged at 9000 x g for 30 min at 4°C. The supernatant was collected and stored at -80 °C till antioxidant and CYP assays (Parente *et al.*, 2004). Hepatic protein content was determined by the dye binding assay method (Bradford, 1976), using bovine serum albumin (Bio Basic Canada Inc.) as a standard protein. Catalase (CAT), Glutathione-S-transferase (GST), Superoxide dismutase activity (SOD) and Peroxidase (POD) activities were determined in fish liver using Agilent Cary 60 UV-Vis spectrophotometer (USA), according to Aebi (1984), Habig *et al.* (1974), McCord and Fridovich (1969), and Smith (1968), respectively. Cytochrome CYP450 1A1 protein level was determined by a semi-quantitative Enzyme Linked Immuno-Sorbent Assay (ELISA) as developed by Goksøyr (1991) and the optical density values were obtained by the ELISA reader (Gen 5 BioTek, ELx800, USA) at 492 nm.

Metallothionein gene expression

Total RNA was extracted from fish liver and head kidneys using GeneJET RNA Purification Kit (Fermantas, UK) according to the manufacturer's protocol. RNA concentration and quality were assessed spectrophotometrically. RNA was reverse transcribed with Revert Aid First Strand cDNA Synthesis Kit™ (Fermentas life science Co.) using random hexamers. Reaction was performed using Bio-Rad thermo-cycler machine, USA. cDNA was PCR amplified using corresponding primers for metallothionein gene (*MT*) and 18S rRNA was used as a reference gene. Primers were designed through NCBI web site (Table 2) and purchased from Invitrogen Corporation (Van Allen Way, Carlsbad, Canada). The relative expression level of a specific gene in the immunized fish was compared to that of non-immunized fish (Pfaffl, 2001). Statistical analyses for the mRNA transcription levels were performed with the aid of the SPSS.16 statistical package (SPSS Inc., Microsoft Co., Redmond, USA).

Table 2: Sequences of the 5' and 3' synthetic MT primer used in PCR.

Accession number	Primers (sense and antisense 5'→3'	Annealing Temp.
MT	Sense: 5'- AGAGACAAGAGCAACGCCAG-3'	
XM_003447045.4	Antisense: 5'- ATGCTGCAGACTCCTCACTG	58 ℃
18sRNA	Sense: 5'- GGACACGGAAAGGATTGACAG3´	
JF698683.1	Antisense:5´- GTTCGTTATCGGAATTAACCAGAC3´	58℃

Histopathological examination

Small portions of different tissues (gills, liver and muscles) from each treatment were fixed in 10% formalin, then dehydrated in ascending grades of alcohol and cleared in xylene. The fixed tissues were embedded in paraffin wax, sectioned at 5 µm, and subsequently stained with hematoxylin and eosin (H & E), and examined and photographed by light microscopy (Olympus CX41, Japan) fitted with camera (Olympus Camera Model E-620, Japan).

Statistical analysis

The obtained data were subjected to one-way ANOVA test. Significant differences in mean values between the experimental groups were tested using Duncan test (Duncan, 1955). All statistical analyses were performed using a computer program of SPSS Inc. (version 17.0 for Windows) at the $P<0.05$ level of significance.

Ethical statement

All experiments were conducted in accordance to the Egyptian laws and with the principles and procedures guidelines approved by the Review Committee for the use of Human or Animal Subjects, National Research Centre (NRC), Dokki, Giza, Egypt.

RESULTS

Biochemical parameters

The results of the investigated biochemical parameters revealed that Cd-treated group (G2) showed significant increase ($P<0.05$) in serum total protein, albumin, globulin, ALT, glucose and cholesterol, whereas AST and uric acid values showed significant ($P<0.05$) decrease in relation to the control (G1) (Table 3). Dietary supplementation with FN-only for 45 days caused significant increase ($P<0.05$) in serum protein and globulin in all groups that not subjected to Cd (G3, G5, G7 and G9) compared with G1. The cholesterol level was also increased in all FN-only groups except of G9 that received 3% FN extract. On the other hand, there was a significant decrease ($P<0.05$) in serum glucose in all groups that not subjected to Cd compared with G1, while, only FN extract 1% and 3% fed groups showed the more significant decrease in serum ALT, AST and uric acid values than crude FN groups. Feeding on FN in Cd-exposed groups (G4, G6, G8 and G10) showed more increase in protein levels compared with both G1 and G2. Interestingly, total globulins, showed the same pattern of protein levels in serum of these groups. The fish feeding on 1% and 3% FN extract caused significant decreases ($P<0.05$) in serum ALT and uric acid in Cd-exposed groups compared with G2. While serum AST showed its lowest value in only G8 that feeding on 1% FN extract compared with G2 (91.5 and 102.3 U/l, respectively). Also the lowest serum uric acid value was recorded in G8 group (32.3 mg/l) compared with G2 (43.4 mg/l). Unfortunately, FN caused more significant ($P<0.05$) elevation in glucose and cholesterol levels in Cd-exposed groups than the Cd-only values (G2).

Table 3: Levels of serum biochemical parameters, and oxidative stress biomarkers of Nile tilapia (*O. niloticus*) experimentally exposed to 1.5 mg Cd L^{-1} and feeding on fenugreek (FN) seeds crude or extract-supplemented diets for 45 days.

	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	*F*-value	*P*-value
Serum biochemical parameters												
Protein (mg/ml)	35.0 ± 0.3^{a}	52.6 ± 0.5^{c}	71.7 ± 0.5^{g}	78.4 ± 0.4^{i}	56.5 ± 0.7^{d}	76.7 ± 0.3^{h}	39.4 ± 0.6^{b}	61.2 ± 0.4^{f}	52.7 ± 0.0^{c}	58.7 ± 0.5^{e}	1009.3	0.000**
Albumin (mg/ml)	4.6 ± 0.2^{c}	9.7 ± 0.1^{e}	3.8 ± 0.1^{b}	2.2 ± 0.1^{a}	8.2 ± 0.3^{d}	14.7 ± 0.5^{g}	4.2 ± 0.3^{bc}	16.3 ± 0.2^{h}	14.7 ± 0.2^{g}	11.4 ± 0.2^{f}	502.7	0.000**
Globulin (mg/ml)	30.5 ± 0.2^{a}	42.9 ± 0.2^{d}	67.9 ± 0.4^{i}	76.1 ± 0.5^{j}	48.3 ± 0.2^{g}	61.9 ± 0.2^{h}	35.2 ± 0.1^{b}	44.9 ± 0.1^{e}	38.0 ± 0.2^{c}	47.3 ± 0.5^{f}	2348.3	0.000**
ALT (Unit/L)	37.1 ± 0.2^{ef}	38.2 ± 0.6^{g}	38.0 ± 0.4^{fg}	39.4 ± 0.6^{g}	35.3 ± 0.2^{cd}	36.0 ± 0.5^{de}	30.2 ± 0.5^{a}	34.5 ± 0.5^{c}	32.5 ± 0.5^{b}	35.9 ± 0.5^{cde}	36.5	0.000**
AST (Unit/L)	119.7 ± 0.2^{e}	102.3 ± 0.5^{c}	111.4 ± 0.6^{d}	139.6 ± 0.3^{h}	123.0 ± 0.7^{g}	121.3 ± 0.2^{f}	103.1 ± 0.6^{c}	91.5 ± 0.3^{a}	98.1 ± 0.6^{b}	110.6 ± 0.4^{d}	927.0	0.000**
Glucose (mg/L)	302.9 ± 0.5^{e}	354.3 ± 0.2^{f}	151.4 ± 0.5^{a}	502.9 ± 0.5^{i}	262.9 ± 0.6^{c}	394.3 ± 0.2^{h}	222.9 ± 0.6^{b}	622.9 ± 0.5^{j}	294.3 ± 0.4^{d}	377.1 ± 0.6^{g}	81477.0	0.000**
Cholesterol (mg/L)	70.2 ± 0.6^{b}	91.2 ± 0.5^{d}	280.7 ± 0.5^{f}	228.1 ± 0.3^{e}	435.1 ± 0.2^{h}	705.3 ± 0.4^{j}	84.2 ± 0.3^{c}	603.5 ± 0.1^{i}	42.1 ± 0.5^{a}	347.4 ± 0.2^{g}	344709.2	0.000**
Uric acid (mg/L)	44.3 ± 0.6^{e}	43.4 ± 0.3^{d}	45.2 ± 0.5^{e}	50.8 ± 0.4^{h}	47.1 ± 0.6^{f}	48.9 ± 0.6^{g}	36.0 ± 0.2^{b}	32.3 ± 0.6^{a}	41.5 ± 0.6^{c}	36.9 ± 0.5^{b}	132.4	0.000**
Oxidative stress biomarkers												
Hepatic protein (mg/ml)	6.6 ± 0.2^{a}	9.8 ± 0.6^{bcde}	9.6 ± 0.5^{bcd}	11.2 ± 0.4^{e}	9.1 ± 0.5^{bc}	9.8 ± 0.2^{bcde}	9.1 ± 0.5^{bc}	10.7 ± 0.5^{de}	8.9 ± 0.3^{b}	10.5 ± 0.5^{cde}	8.1	0.000**
CAT (Unit/ml)	153.3 ± 0.2^{f}	197.4 ± 0.4^{i}	193.1 ± 0.3^{h}	92.6 ± 0.6^{c}	160.2 ± 0.2^{g}	141.4 ± 0.6^{e}	139.2 ± 0.2^{d}	75.8 ± 0.6^{a}	200.6 ± 0.6^{j}	79.5 ± 0.5^{b}	11705.0	0.000**
SOD (Unit/ml)	6.5 ± 0.6^{d}	7.3 ± 0.3^{e}	4.5 ± 0.3^{b}	5.0 ± 0.3^{bc}	6.7 ± 0.6^{d}	2.5 ± 0.4^{a}	6.0 ± 0.4^{cd}	4.5 ± 0.5^{b}	8.6 ± 0.5^{f}	4.7 ± 0.5^{bc}	15.6	0.000**
GST (µmole/min/mg protein)	0.03 ± 0.0^{a}	0.6 ± 0.1^{d}	0.1 ± 0.0^{a}	0.4 ± 0.1^{b}	0.6 ± 0.0^{cd}	0.7 ± 0.1^{d}	0.8 ± 0.0^{d}	0.6 ± 0.1^{bcd}	0.4 ± 0.0^{b}	0.4 ± 0.0^{bc}	13.4	0.000**
POD (Unit/ml)	0.3 ± 0.0^{a}	0.3 ± 0.0^{ab}	0.4 ± 0.1^{bc}	0.4 ± 0.0^{ab}	0.3 ± 0.1^{b}	0.4 ± 0.1^{ab}	0.6 ± 0.0^{d}	0.3 ± 0.0^{ab}	0.3 ± 0.1^{ab}	0.5 ± 0.0^{cd}	7.2	0.000**
CYP P450 1A1 (OD at 492 nm)	0.2 ± 0.0^{a}	0.18 ± 0.0^{a}	0.6 ± 0.0^{c}	0.6 ± 0.0^{c}	0.6 ± 0.0^{c}	0.4 ± 0.1^{b}	0.6 ± 0.0^{c}	0.5 ± 0.0^{c}	0.4 ± 0.0^{b}	0.5 ± 0.0^{c}	30.9	0.000**

G1 = Control, G2 = 1.5 mg Cd L^{-1}, G3 = 2.5% Crude FN; G4 = 2.5% Crude FN + Cd; G5 = 5% Crude FN; G6 = 5% Crude FN + Cd; G7 = 1% Extract FN; G8 = 1% Extract FN + Cd; G9 = 3% Extract FN; G10 = 3% Extract FN + Cd.

Data are expressed as mean ± SE (n= 10). SE = standard error.

Means with the same letter within the same raw are not significantly different ($P>0.05$).

F-value = ANOVA's *F*-test. **Highly significant ($P<0.001$).

Antioxidant biomarkers and Cytochrome P450 1A1 (CYP1A1)

A highly significant (P<0.001) differences in hepatic protein, CAT, SOD, GST, and POD activities were observed within the different treatment groups (Table 3). The Cd treated group (G2) showed significant increase (P<0.05) in hepatic protein, CAT, SOD, and GST values by 1.48, 1.3, 1.1 and 20 folds, respectively, compared with G1. On the other hand, Cd exposure didn't significantly affect POD and CYP values compared with G1. Dietary supplementation with FN-only for 45 days caused increase in hepatic protein content, CAT, GST and CYP over their comparable control values (G1). The highest values of CAT and SOD activities were recorded in fish fed 3% FN extract group (G9), and highest GST and POD activities were recorded in 1% FN extract group (G7). Fortunately, feeding on FN with Cd exposure caused significant decreases (P<0.05) in hepatic CAT and SOD activities compared with the Cd-only group (G2). The lowest CAT value was recorded in fish fed 1% FN extract group (G8) compared with G2 (75.8 and 197.4 U/ml, respectively). Also, the lowest SOD values were recorded in 5% FN crude group followed by 1% FN extract group compared with G2.

Metallothionein (*MT*-gene expression)

The results of MT-gene expression was presented in Fig. 1. There was a significant up-regulation (P<0.05) in *MT*-gene expression in Cd-only group compared to Cd non-exposed groups (Fig. 1A & B). On the other hand, *MT*-gene expression in FN only groups; that not exposed to Cd toxicity showed insignificant (P>0.05) changes (Fig. 1A). While, feeding on FN for 45 days in Cd-exposed groups caused significant (P<0.05) up-regulation in *MT*-gene expression, with the highest value of expression, as fold change, reported with 3% FN extract followed by 1% FN extract, 5% FN crude and finally 2.5% FN crude (Figure 1B).

Cadmium residues

The Cd accumulation in the investigated fish organs was in the following order: liver>gills>muscles and these accumulations increased 9.05, 4.77, and 1.35-fold, respectively, in G2 compared with G1 (Table 4). Cd residues in fish liver were only reduced in G8 group. And Cd residues in fish gills were reduced in G6, G8 and G10. While Cd residues in fish muscles showed their lower values in G6 and G8. So feeding on 1% FN extract with Cd exposure (G8) was the only group that showed significant reduction in Cd residues in fish liver, gills and muscles as compared with G2.

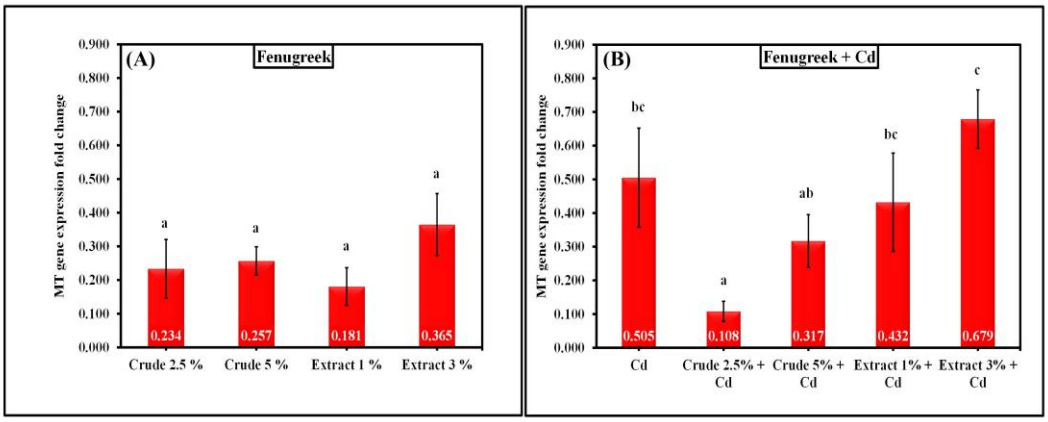

Fig. 1: The fold change of metallothionein (*MT*) gene expression in Nile tilapia, *O. niloticus*: (A) fed with FN-supplemented diet, (B) fed with FN-supplemented diet and exposed to 1.5 mg Cd L^{-1}. Bars assigned by different letters are significantly differed at P<0.05.

Table 4: Cadmium residues (mg kg^{-1} wet weight) in liver, gills and muscles of Nile tilapia, *O. niloticus*, experimentally exposed to 1.5 mg Cd L^{-1} and feeding on fenugreek (FN) seeds crude or extract-supplemented diet for 45 days.

	G1	G2	G4	G6	G8	G10	*F*-value	*P*-value
Liver	4.8 ± 0.1^{a}	43.7 ± 0.3^{c}	45.0 ± 0.7^{c}	50.3 ± 0.4^{d}	20.1 ± 0.5^{b}	59.0 ± 0.6^{e}	1758.6	0.000**
Gills	1.5 ± 0.1^{a}	6.9 ± 0.6^{c}	12.1 ± 0.6^{d}	5.4 ± 0.4^{b}	4.5 ± 0.7^{b}	5.3 ± 0.2^{b}	55.8	0.000**
Muscles	0.3 ± 0.0^{a}	0.4 ± 0.1^{b}	0.8 ± 0.1^{d}	0.3 ± 0.0^{a}	0.3 ± 0.0^{a}	0.6 ± 0.1^{c}	8.2	0.001**

G1 = Control; G2 = 1.5 mg Cd L^{-1}; G4 = 2.5% Crude FN + Cd; G6 = 5% Crude FN + Cd; G8 = 1% Extract FN + Cd; G10 = 3% Extract FN + Cd.
Data are expressed as mean ± SE. SE = standard error.
Means with the same letter within the same raw are not significantly different ($P>0.05$).
F-value = ANOVA's *F*-test. **Highly significant ($P<0.001$).

Histopathological alterations

The histopathological examination of the control group revealed normal structure of musculature, gills, and liver (Fig. 2A-C). In groups exposed to FN extract, the musculature showed edema and Zenker's necrosis (Fig. 2D). Gills exhibited congestion in the lamellar and branchial blood vessels, and severe hyperplasia in the secondary lamellae, associated with degenerative and necrotic changes in the respiratory epithelial cells lining the secondary lamellae (Fig. 2E). Liver showed severe vacuolar degeneration and necrotic changes in the hepatocytes (Fig. 2F). The histopathological picture became very severe, in fish exposed to FN crude, where the musculature showed high degree of degenerative changes, edema and Zenker's necrosis (Fig. 2G). Gills appeared congested associated with telangiectases (lamellar capillary aneurisms) at the tips of the secondary lamellae, and moderate degree of hyperplasia in the epithelial lining of the secondary lamellae (Fig. 2H). Liver showed severe congestion in the hepatic blood vessels, associated with severe vacuolar degeneration and necrotic changes in the hepatocytes (Fig. 2I). In fish exposed to 1.5 mg L^{-1} of $CdCl_2$, the musculature showed edema, degenerative and necrotic changes (Fig. 3A). Gills showed telangiectases at the tips of the secondary lamellae, severe hyperplasia, degenerative and necrotic changes in the respiratory epithelial lining (primary and secondary lamellae) and congestion were also observed (Figs. 3B1 & 3B2). Liver showed diffuse cloudy swelling, necrotic changes, vacuolar degeneration with infiltration of mononuclear cells in-between the hepatocytes, with activation of melano-macrophage centers (Fig. 3C). The musculature showed mild edema and Zenker's necrosis (Fig. 3D) in the group exposed to 1.5 mg L^{-1} of $CdCl_2$ plus FN extract. Gills showed clubbing at the ends of the primary gill lamellae, moderate hyperplasia at the base of secondary gill lamellae associated with degenerative and necrotic changes in the respiratory epithelial cells lining the secondary lamellae, and congestion (Fig. 3E). Liver showed moderate diffuse vacuolar degeneration and necrotic changes in the hepatocytes with infiltration of mononuclear cells in-between the hepatocytes (Fig. 3F). In the fish treated with 1.5 mg L^{-1} of $CdCl_2$ plus FN crude, the musculature revealed edema, degenerative changes and Zenker's necrosis (Fig. 3G). Gills have congestion in the lamellar and branchial blood vessels, clubbing at the ends of the primary gill lamellae, severe degree of hyperplasia and separation of the epithelial lining of the secondary gill lamellae (Fig. 3H). Liver showed severe congestion in the hepatic blood vessels, severe vacuolar degeneration and necrotic changes in both hepatocytes and pancreatic tissues associated with infiltration of mononuclear cells in-between the hepatocytes and infiltration of melano-macrophage cells nearby the necrotic areas (Fig. 3I). Generally, it was found that there was a reduction in the size of inflammatory cell infiltration and hyperplasia areas and a reduction in the number of necrotic cells in fish exposed to 1.5 mg L^{-1} of $CdCl_2$ plus FN extract compared to FN crude.

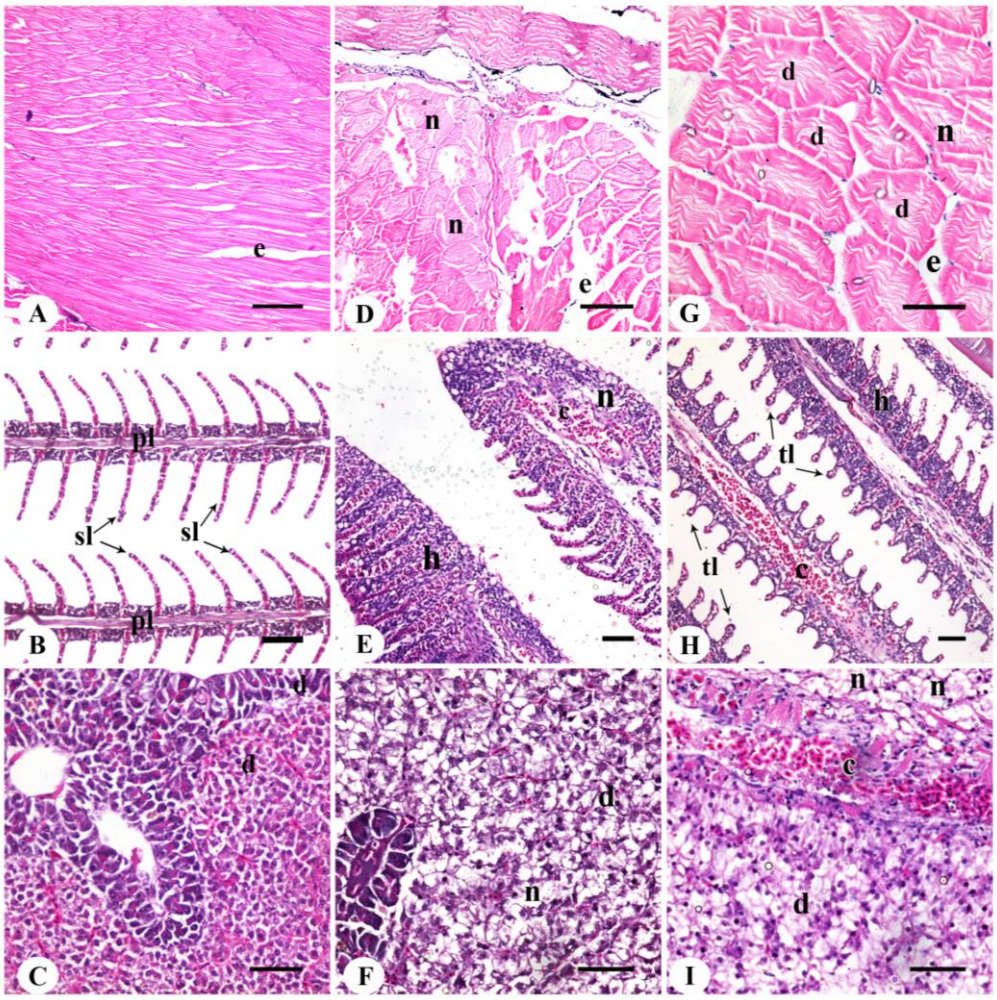

Fig. 2: (A-C) *O. niloticus* control fish group: (A) Musculature showing normal structure with slight edema between the muscular fibers. (B) Gills showing normal lamellar structure (primary and secondary lamellae). (C) Liver (Hepatopancreas) showing normal hepatic and pancreatic structures with mild degenerative changes in the pancreatic acinar cells also in some hepatocytes. (D-F) *O. niloticus* fish treated with FN seeds extract: (D) Musculature showing edema and Zenker's necrosis. (E) Gills showing congestion in the lamellar and branchial blood vessels, severe hyperplasia in the secondary lamellae, associated with degenerative and necrotic changes in the respiratory epithelial cells lining the secondary lamellae. (F) Liver showing severe vacuolar degeneration and necrotic changes in the hepatocytes. (G-I) *O. niloticus* fish treated with crude FN seeds: (G) Musculature showing high degree of degenerative changes, edema and Zenker's necrosis. (H) Gills showing congestion in the lamellar and branchial blood vessels, telaniegctasis and moderate degree of hyperplasia in the epithelial lining of the secondary lamellae. (I) Liver showing severe congestion in the hepatic blood vessels, severe vacuolar degeneration and necrotic changes in the hepatocytes. e = edema, n = necrosis, h = hyperplasia, d = degeneration, c = congestion, pl = primary lamellae, sl = secondary lamellae, tl = telaniegctasis. (H&E, Bar = 50 µm).

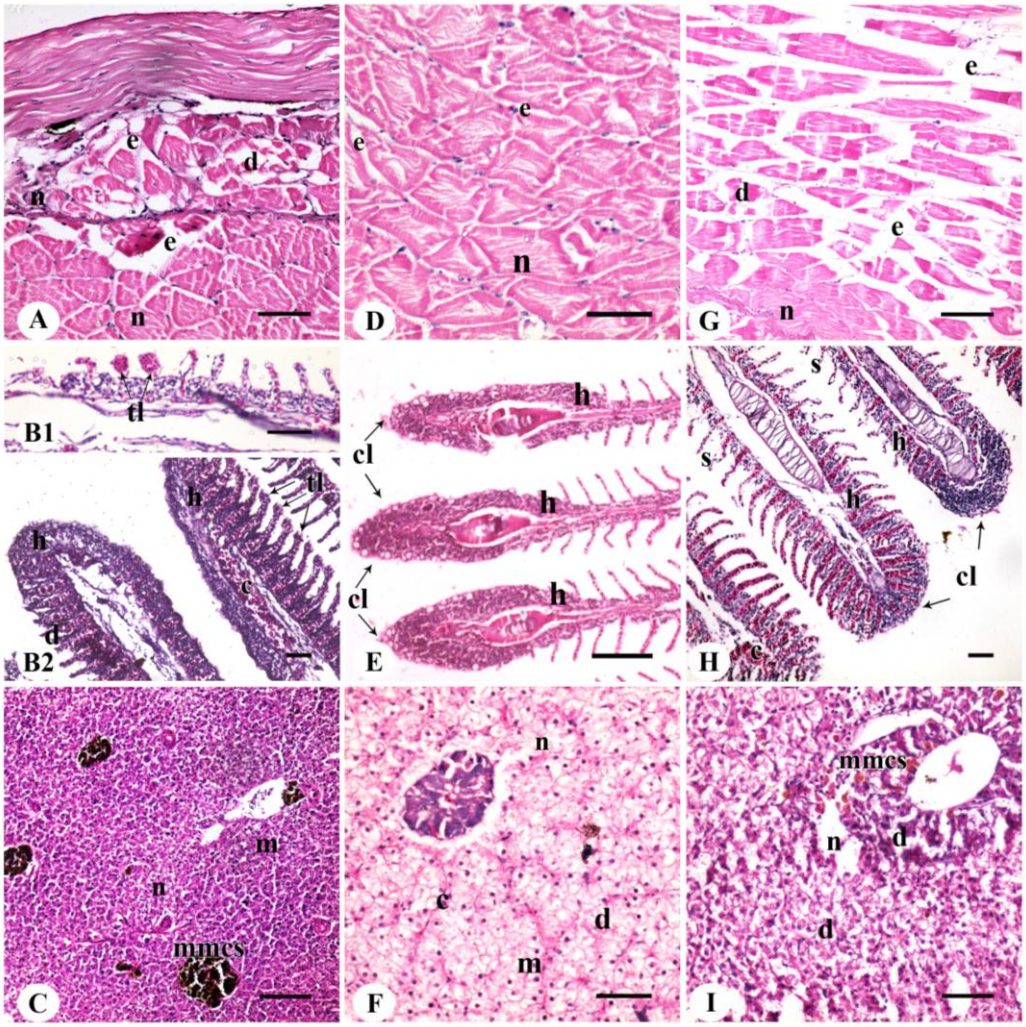

Fig. 3: (A-C) *O. niloticus* fish exposed to 1.5 mg Cd L^{-1}: (A) Musculature showing edema, degenerative and necrotic changes. (B1) Gills showing telangiectasis. (B2) Gills showing telangiectasis, severe hyperplasia, degenerative and necrotic changes in the respiratory epithelial lining (primary and secondary lamellae) and congestion. (C) Liver showing diffuse cloudy swelling, necrotic changes, vacuolar degeneration, and infiltration of mononuclear cells in-between the hepatocytes with activation of melano-macrophage centers. (D-F) *O. niloticus* fish exposed to 1.5 mg Cd L^{-1} and fed with FN seeds extract: (D) Musculature showing edema and Zenker's necrosis. (E) Gills showing clubbing, moderate hyperplasia at the base of secondary gill lamellae, degenerative and necrotic changes in the respiratory epithelial cells lining the secondary lamellae, and congestion. (F) Liver showing moderate diffuse vacuolar degeneration and necrotic changes and infiltration of mononuclear cells in-between the hepatocytes. (G-I) *O. niloticus* fish exposed to 1.5 mg Cd L^{-1} and fed with crude FN seeds: (G) Musculature showing edema, degenerative changes and Zenker's necrosis. (H) Gills showing congestion in the lamellar and branchial blood vessels, clubbing, hyperplasia and separation of the epithelial lining of the secondary gill lamellae. (I) Liver showing severe congestion, severe vacuolar degeneration and necrotic changes in both hepatocytes and pancreatic tissues, infiltration of mononuclear cells in-between the hepatocytes and infiltration of melano-macrophage cells nearby the necrotic areas. e = edema, n = necrosis, h = hyperplasia, d = degeneration, c = congestion, m = mononuclear inflammatory cells, mmcs = melano-macrophage cells/centers, tl = telaniegctasis, cl = clubbing, s = separation. (H&E, Bar = 50 µm).

DISCUSSION

Cadmium pollution is one of the most harmful heavy metal pollutants in the aquatic environments. Even at sub-lethal concentrations, Cd has a cumulative effect and causes serious physiologic disturbances in fish (Abdel-Tawwab and Wafeek, 2014; Abdel-Tawwab and Wafeek, 2017; Khalesi *et al.*, 2017) and humans (Marengo *et al.*, 2018). So, for Cd detoxification, a scientific tool is essential to sustain the health of the economic farmed fish. Natural products, such as plants and their extracts, have received much attention because they could increase appetite, promote growth, enhance immune ability, and have anti-pathogenic properties in fish (Abdel-Tawwab, 2016; Abdel-Tawwab *et al.*, 2018). In recent years, one of these plants that have gained attention is FN (Mostafa *et al.*, 2009; Mehboob *et al.*, 2017; Roohi *et al.*, 2017).

In the current study, the significant increase in the serum total protein (hyperproteinemia), albumin and globulin contents that were observed in G2 indicated that the total protein influential balance was broken and body health of fish, immune response and antioxidant capability were affected (Sayed and Authman, 2018). During stress conditions, fish need more energy to overcome and detoxify pollutants, so proteins in the liver degrade and the serum protein level increase (Authman *et al.*, 2013). In line with our results, El-Serafy *et al.* (2013) observed a very highly significant increase in the plasma total proteins, albumin and globulin content in Nile tilapia, *O. niloticus* fish fed on $CdCl_2$ contaminated diet. Consistent with the results of Mostafa *et al.* (2009) and Roohi *et al.* (2017), FN-only supplementation resulted in a significant increase in the serum protein and globulin compared with the control, because FN contains selenium that may increase production of protein in liver (Abdel-Tawwab *et al.*, 2007). Concerning Cd+FN exposed groups; protein level is higher than the FN groups without Cd exposure that may be returned to the toxic effect of Cd, redox reactions and antioxidant enzymes induction (Asagba *et al.*, 2008). Concerning liver enzymes such as ALT and AST, they are considered important plasma indicators to examine the animal health (Mohiseni *et al.*, 2016) and also used as stress indicators (Al-Asgah *et al.*, 2015). In the present study, Cd-only exposure significantly increased ALT activity and decreased AST activity, compared with the control group. Metals can either decrease or increase activities of hepatic enzymes and can induce histopathological changes in liver, depending on the metal type, its concentration, duration of exposure, fish species and other factors (Figueiredo-Fernandes *et al.*, 2007). During metal stress, to counter the energy demand, enhancement of ALT or AST activities may happen; while sometimes high metal accumulations in the tissues decrease ALT or AST activities (Öner *et al.*, 2009). High levels of ALT or AST in blood may be because of liver cellular damage that represents a usually indicative of necrosis and disease in the animal's liver (Mohiseni *et al.*, 2016). This was matched the histopathological changes noted in current study in liver sections of Cd-exposed fish (Fig. 3C). In accordance, Abbas *et al.* (2007) observed inhibition in AST activities of Nile tilapia, *O. niloticus*, whereas, Al-Asgah *et al.* (2015) mentioned significant increase in ALT activity after *O. niloticus* exposure to Cd. The significant ($P<0.05$) low values of ALT and AST in both Cd-exposed and non-exposed groups, supplemented by 1% and 3% FN extract, than their comparable control, agrees with the findings of Sushma and Devasena (2010) who found reduction in hepatic enzymes (ALT and AST) in rats fed on FN which have protective effect against hepatotoxicity. On the other hand, Mostafa *et al.* (2009) found no significant difference in AST activity and significant increase in ALT

activity in monosex Nile tilapia, *O. niloticus* fingerlings feeding on FN. Urea, uric acid and creatinine are valuable indices for health of the gills and kidneys (Yılmaz *et al.*, 2012). Plasma uric acid can be considered as a glomerular filtration rate rough index (Maita *et al.*, 1984). In the present work, Cd-only exposure displayed a significant decreased level of uric acid compared to the control fish. Abbas *et al.* (2007) confirmed similar data of uric acid in *O. niloticus* exposed to Cd. Yılmaz *et al.* (2012) found that FN as feed additive did not change uric acid levels of sea bass (*Dicentrarchus labrax*). The present study results proved that FN extract had an ameliorative effect against liver injury induced by Cd toxicity. This may be due to its antioxidant activity (Sakr *et al.*, 2009).

Glucose level in blood have long been used as a reliable and sensitive indicator of contaminants causing stress in fish, whereas the increase in blood cholesterol concentration is used as an liver dysfunction indicator; because one of the main liver functions is lipids homeostasis (Osman *et al.*, 2018); or damages to kidney (Roohi *et al.*, 2017). In the current work, the significantly higher glucose value in Cd-only intoxicated fish might be because of the vulnerable stress induced by Cd toxicity lead to hyperglycemia. Levesque *et al.* (2002) concluded that trace metals modulate the carbohydrates metabolism, causing hyperglycemia by stimulating glycogenolysis in fish. Our results agreeing with the data reported by Al-Asgah *et al.* (2015) and Mohiseni *et al.* (2016) who showed blood glucose levels elevation in Nile tilapia and common carp exposed to Cd. Moreover, the blood cholesterol level in Cd-only intoxicated fish was significantly increased (hypercholesteremia) compared to control group. As a response to pollution, the cholesterol level increased because organisms required excess energy reserves to mediate the stress effects. In addition, the failure of liver and kidney causes release of cholesterol into the fish blood (Mohiseni *et al.*, 2016). In Cd+FN exposed groups, the continuous elevations in glucose and cholesterol levels may be returned to the Cd effect and pointed that FN has no effect to modulate glucose and cholesterol levels in Cd-exposed fish. In stress state, the increase in the circulating corticosteroids or catecholamines concentration results in hyperglycemia (Min and Kang, 2008). On the other hand, fish feeding on FN-only (crude and extract) and not subjected to Cd toxicity stress showed decrease in glucose level in the serum, that may be returned to the hypoglycemic effect of FN. Roohi *et al.* (2017) mentioned that, FN has a hypoglycaemic effect on the body because it contains galactomannan (mucilaginous fibers). In the current study, unexpected increase in cholesterol level in *O. niloticus* feeding on crude FN seeds (2.5 and 5%) may be explained by the possible toxic effect of high FN doses on fish. Contradicting to our results, Roohi *et al.* (2017) demonstrated significantly lower cholesterol levels in common carp, *C. carpio* fed supplemented diet by 1% FN seed. However the 1% and 3% FN extracts decreased the cholesterol level. Previous studies indicated that the FN seeds ethanol extract contained saponins, which have hypocholesterolemic effects, where in the digestive tract; it increases the conversion of hepatic cholesterol to bile salts, and it increases the loss of these substances complexes and neutral sterols in the feces (Srinivasan, 2006; Helal *et al.*, 2018).

In the current study, hepatic protein, CAT, SOD, and GST activities were significantly increased in Cd-only exposed *O. niloticus* fish as compared with the control. The increase in total protein levels in the serum of Cd-only exposed fish confirmed the hepatic protein increase. Roohi *et al.* (2017) mentioned that, increased total protein levels in fish serum possibly reflects increased protein synthesis in liver, because of the close relationship between the rate of liver tissue protein synthesis and the serum total protein concentrations. The observed elevated CAT, SOD and GST

levels show the possible shift towards a mechanism of detoxification under long-term Cd exposure. Zirong and Shijun (2007) mentioned that, in fish, Cd exposure causes harmful effects on oxidative stress responses, because it could produce ROS (directly or indirectly). Atli and Canli (2010) reported that Cd (redox-inactive metal) can cause increases in ROS production significantly, followed by oxidative stress situation, leading to DNA, proteins and lipids dysfunctions. Similarly, Abdel-Tawwab and Wafeek (2017), Mushtaq *et al.* (2017) and Li *et al.* (2018) reported increased activities of oxidative system enzymes (i.e. CAT, SOD and GST) in different fish species exposed to Cd and other metals, indicating that the antioxidant system response to metal stress and a possible shift toward a detoxification mechanism. Feeding on FN extract with Cd exposure showed significant decreases in CAT and SOD activities, compared to the control group. This indicates to the protective role of FN since it can modulate the toxic effect of Cd. Also, the variations in other antioxidant enzymes may be returned to Cd toxic effect. The effect of heavy metal exposure on the activities of antioxidant enzymes had been inconclusive, showing no change (Porte *et al.*, 2002), inhibition (Messina *et al.*, 2014), or induction of these enzymes (Abdel-Tawwab *et al.*, 2017). In fish groups feeding on FN-only supplemented diet, FN increased the hepatic protein content, CAT and GST activities over the control group. Present findings agree with Awad *et al.* (2015) who found an up-regulation in liver gene expression; CAT, SOD and GR (glutathion reductase) of *Sparus aurata* (L.) fed 5% and 10% FN-supplemented diets. Adding to that, Bahi *et al.* (2017) mentioned that the increase in the serum peroxidase activity of *Sparus aurata* (L.) feed with diets supplemented with FN seeds and probiotics may have been due solely to FN supplementation. The flavonoid and phenolic compounds contained in FN would help to increase its antioxidant ability in liver (Dixit *et al.*, 2005; Guardiola *et al.*, 2017). The characteristic functions of higher content of flavonoids (such as quercetin, kaempferol and apigenin) and saponins (such as yamogenin and diosgenin) in FN are to protect the oxidative damage and have immunostimulatory properties (Yılmaz *et al.*, 2012). Also, the phenolics antioxidant property is due to their redox properties. They act as hydrogen donors, reducing agents (free radical terminators) and singlet oxygen quenchers (Belguith-Hadriche *et al.*, 2013).

The present study revealed that exposure to Cd-only for 45 days, inhibited CYP 450 activity compared with the control and other treatment groups. Several previous studies have reported similar inhibition of activities of CYP 450 in fish after exposure to Cd which may be due to cell damages (Hassanain *et al.*, 2007; Chen and Chan, 2018). Because of the metals high affinity to SH (sulfhydryl)-residues, they react with cellular ligands, producing complexes with SH-containing molecules, such as the thiol group of the CYP cystein which is linked to the heme iron (Risso-de Faverney *et al.*, 2000). The hastened heme turnover caused by metals may significantly decrease the oxidative role of the cell, including that of the protective microsomal CYP450 monooxygenase system (Hassanain *et al.*, 2007). Many reports are harmonious with the perspective that CYP450 enzymes were depressed because of heme depletion (Xu *et al.*, 2018). In the current work, administrating FN-supplemented diet to fish (without or with Cd exposure) showed significantly up-regulation of CYP450 activity compared to control. The significant increase in CYP450 activity in fish fed on FN may be due to its active compounds such as flavonoids compounds (Guardiola *et al.*, 2017). Many flavonoids have been reported to be potent inducers of various CYPs (Hodek *et al.*, 2002). For example, diosmin, and its aglycone form, diosmetin, quercetin and galangin, increased the CYP1A1 expression (Zhou *et al.*, 2003).

In the current work, the higher *MT* mRNA expression found in Cd-only exposed group may be because of the affinity between Cd and *MT* where *MT* is able to bind Cd (Atli and Canli, 2003; Abdel-Tawwab and Wafeek, 2014). The presence of many thiol groups is the *MT* main characteristic, those are capable to bind to divalent cations. Forming this complex prevents Cd from remaining as a free ion, its most toxic form (Le Croizier *et al.*, 2018). Similarly, Abdel-Tawwab and Wafeek (2014) observed increase in *MT* concentrations in different organs of Nile tilapia exposed to Cd. In the present study, it was found that administration of FN enhanced the expression of *MT* in Cd-exposed groups more than non-exposed groups. Present results show that FN has a role in stimulating *MT* expression for binding to Cd and eliminating its hazardous effects. This effect may be possibly attributed to their antioxidant properties that seemed to be associated with total flavonoids; total phenolic and anthocyanin content (Li *et al.*, 2017).

The present data agreed with Al-Asgah *et al.* (2015) who reported that muscles of *O. niloticus* accumulated the lowest metal concentration during Cd exposure. In Cd-exposed fish groups feeding on FN; it was found that Cd levels were decreased significantly with the 1% FN extract, which is the more effective dose in reducing Cd accumulation in tissues (especially muscle) of Cd-exposed fish groups. So, present results suggested that 1% FN extract supplementation may have a role in reducing the Cd accumulation in fish tissues. The major pharmacologically active ingredients of FN are flavonoids, which appear to be responsible for reducing the Cd accumulation in fish tissues. The probable mechanism of flavonoids influential lowering effect on Cd content in tissues of Nile tilapia may be its metal chelating capacity which sequentially may lessen the tissue Cd load. Agreeing with this suggestion, Li *et al.* (2017) reported that flavonoids can alleviate Cd toxicity by means of three aspects: 1) unabsorbed flavonoids can lessen accumulated Cd in the body by chelating it in the intestine and increasing its excretion; 2) absorbed flavonoids in the circulation play role in toxicity reduction; 3) flavonoids metabolites also have protective effects against damage induced by Cd. Also, phenolics in FN act as metal chelators (Belguith-Hadriche *et al.*, 2013). Abdel-Tawwab (2015) also recorded a reduction of copper in fish tissues after supplementation with American ginseng.

In agree with the present work, Abbas *et al.* (2007) and Al-sawafi *et al.* (2017) observed that Cd exposure resulted in many histopathological alterations in fish gills, liver, and muscles. In the current work, after FN-only (crude or extract) supplementation to fish, the observed histopathological lesions may be due to the presence of saponins in FN. Francis *et al.* (2002) mentioned that, saponins are toxic to fish due to their harmful effect on the respiratory epithelia. Also, mild hepatitis and early liver degeneration were noted in mice at FN extract higher doses (Abdel-Barry and Al-Hakiem, 2000). Besides, in the present study, increasing CYP 450 expression may be responsible for the FN side histopathological effects in fish tissues. Yarru *et al.* (2009) suggested that, the over CYP gene expression produces more ROS, which impelling an oxidative stress that lead to tissues damage. In the present study, fish exposed to Cd and feed on FN-supplemented diet (crude and extract) showed reduction in the damage of gills, liver and muscle histology, with FN extract gave more reduction in Cd histopathological effects than FN crude. These histopathological findings revealed that FN extract-supplemented diets (especially 1% FN extract) can overcome the negative effects of Cd better than the FN crude-supplemented diets. This may be due to the higher content of antioxidants (i.e. phenolics and flavonoids) in FN extract. Dixit *et al.* (2005) reported that the extract of FN had a high flavonoid

and phenolic content being responsible for the radical scavenging and antioxidant activities of the extract.

CONCLUSION

The present study confirmed the toxic effect of Cd on biochemical, antioxidant and histopathological features of Nile tilapia, *O. niloticus*. Also, it revealed the promising role of fenugreek extract to alleviate Cd-induced biochemical and antioxidant changes. Feeding of fish on fenugreek extract with Cd exposure also reduced the histopathological changes compared with that induced by Cd toxicity only. Generally, it was found that FN extracts, especially 1% was more effective in alleviation most of the destructive effects of Cd than FN crudes. In addition 1% FN extract reduced the Cd accumulated in fish muscles, which is very important issue from the public health (human consumer's health) viewpoint.

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