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Physiological and Biochemical Studies on Albino Rats Administered Contaminated Water and Fish Supplemented With Fennel

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Abstract: *Male albino rats (Rattus rattus) were divided into four groups of fifteen rats in each group. Two of the studied groups (Group I & II) including the control group (Group I) administered tap water and uncontaminated dried fish collected from a branch of the river Nile, Bahr Yousef at El-Fayoum Governorate. Other studied groups (III & IV) administered highly heavy metals contaminated water and dried fish collected from El-Bats drainage canal, where agricultural and waste municipal of Fayoum Governorate discharged. Moreover, rats in the aforementioned groups administered uncontaminated and contaminated water and dried fish, for eight weeks, supplemented with 300 mg fennel /kg body weight/ day (Second and Fourth groups).The present study revealed highly bioaccumulated heavy metals (copper, lead. Cadmium and zinc) in liver and kidney of rats administered contaminated water and dried fish. Moreover, rats exhibited the lowest growth rate and progressive fall in RBCs count, Hb content, haematocrite value, and HDL and serum total antioxidant. These effects were concomitant with significant increase in the WBCs count, serum glucose, total protein, albumin, globulin, AST, ALT, creatinine, urea, total cholesterol, triglycerides, LDL and malonaldhyde in comparison with those of control group rats before and after supplementation with the studied antioxidants. However, rats administered contaminated water and dried fish supplemented with 300 mg fennel /kg body weight/day for eight weeks showed slight improvement in the growth rate and most of the other studied physiological and biochemical parameters.*

Keywords: *Albino Rats, Heavy Metals, Contaminated fish diet, fennel.*

I. Introduction

Contamination with heavy metals is a serious threat to humans because of their toxicity, bioaccumulation and biomagnifications in the food chain **(1).** Heavy metals act as environmental stressors and may affect the physiological status of aquatic as well as terrestrial organisms **(2, 3).** Due to the regulation rules for use of water resources in El- Fayoum Governorate, fish farms are allowed only to use water from drainage canals mainly El Bats drain, that ramble the most reservoir for agricultural wastewater **(4, 5).** Water quality of El Bats drainage canal varies significantly during the year due to seasonal changes in evaporation rates and water components itself because of the agricultural seasons. Trace metals are of particular concerns as pollutants in aquatic ecosystems as natural processes as most of organic pollutants **(6, 7)** do not rapidly remove them**. (8).** During the past decades, there has been a considerable interest in the use of herbal medicines in the world as people strive to stay healthy in condition of chronic stress, pollution environment and other similar situations. Among the medicinal plants, *Foeniculum vulgare* Mill.(Fennel). Fennel plant has extraordinary antioxidative and other health promoting properties **(9, 10). So, the aim of the present study is to** follow up the extent of contaminated water and fish administration on the experimental rats growth, physiological status as well as biochemical changes. Moreover, elaborate the protective effect of supplemental fennel (*Foeniculum vulgare)* against administration of the contaminated water and fish.

II. Material And Methods

1- Experimental animal:

Adult male albino rats *Rattus rattus* of about 180 + 5 g. in weight and nearly of the same age were used in the present study. They were obtained from the National Research Center, Giza, Egypt and maintained in the laboratory at room temperature, with normal photoperiods. During acclimation period, rats were supplied with tap water and fed *ad libitum* with standard pellet diet formulated in accordance with the composition authorized by the Association of Official Analytical Chemists **(11)** which consists of about 78.5% carbohydrates (inclusive of 50% crude cellulose fibers), 15.2% protein, 3.2% lipids, 2.1% salt mixture and 1.0% multivitamins.

2- Experimental Design

Sixty acclimated adult male albino rats, *Rattus* rattus (180 + 5 g.) were classified into four studied groups, fifteen rats each in a triplicate manner as follows:

Group I: Control, rats administered tap water and uncontaminated dried fish collected from a branch of the river Nile, Bahr Yousef.
Group II: Rats administered tap water and uncontaminated dried fish collected from a branch of the river Nile, Bahr Yousef supplemented with 300 mg fennel/kg body weight daily.
Group III: Rats administered contaminated water and dried fish from El-bats drainage canal at Fayoum Governorate.
Group IV: Rats administered contaminated water and dried fish from El-bats drainage canal at Fayoum Governorate supplemented with 300 mg fennel /kg body weight daily

3- Preparation of fennel seeds

Fennel seeds ***(foeniculum vulgare)****:* Fennel seeds were obtained from Haraz market for herbs and medicinal plants market, Cairo, Egypt. Dried fennel seeds (shamar) were washed with tap water to remove possible potential dust. Afterwards, it was dried by cotton cloth to remove the excess liquid prior to drying. Drying was achieved at room temperature for 48 hr. Then a grinder mill and sieves were used to obtain a powder particle size of less than 0.2mm. **(12**). It was supplied to the third and sixth studied rat groups as a single dose orally (300 mg/kg body weight) according to **(13) Kamal & Mohamed (2009) and (14) Shö ne *et al.* (2006).**

Studied Parameters:

I) Growth rates: Body weight gains were recorded to the nearest gram at the end of the experimental period (8 weeks).

II) Residual heavy metals in liver and kidney tissues: Residual heavy metals (copper, lead, cadmium and zinc) were determined in the liver and kidneys of Albino Rats according to **(15) American Public Health Association. APHA (1995)** then measured using atomic absorption spectrophotometry, Shimadzu AA-7000.

III) Blood sampling and examination:

Blood samples were withdrawn from the retro-orbital venous plexus according to (**16) Schermer (1967).** Heparinized blood samples were examined immediately for the following:

i) Red and white blood cells count:- Total number of erythrocytes (RBCs) and leukocytes (WBCs) were counted using improved Neubauer Haemocytometer.

ii) Haemoglobin content:- Haemoglobin content was estimated using cyanmethoglobin method described by **(17) Van Kamper and Zijlstra (1961).**

iii) Haematocite value (PCV):- Packed cell volume (PCV) was carried out in small haematocrite tubes using haematocrite centrifuge at 3000 r.p.m for 15min.

IV) Biochemical analysis

Kits from Stanbio Laboratory Inc. (Texas, USA) were used in estimating the level of Serum Glucose according to **(18) Trinder (1969)**, Serum total Cholesterol ,Serum Triglycerides , Serum HDL-Cholesterol , Serum LDL-Cholesterol**,** Blood urea nitrogen (BUN)**,** Serum creatinine.

Also kits from Quimica Clinica Aplicada S.A. (QCA Amposta, Spain) were used for determination of the activities of Alanine amino transferase (ALT) and Aspartate amino transferase (AST).

The levels of the above parameters were measured spectrophotometrically by a quantitative enzymatic colorimetric method.

Serum malondialdehyde (MDA) level was determined by colorimetric method using kit purchased from Bio-diagnostic Co., Egypt, according to the method described by **(19) Ohkawa *et al.*, (1979).**

Serum total antioxidant capacity was assayed by colorimetric method using kit purchased from Bio-diagnostic Co., Egypt, according to the method described by **(20) Koracevic *et al.*, (2001)**.

1.1. Statistical analysis:

The results were statistically analyzed using analysis of variance (F-test) followed by Duncan's multiple range test to determine differences in means using Statistical Analysis Systems, Version 6.2 **(21)(SAS, 2000).**

III. Results

Male albino rats (*Rattus rattus*) were divided into four groups of fifteen rats in each group. Two of the studied groups (Group I & II) including the control group (Group I) administered tap water and uncontaminated dried fish collected from a branch of the river Nile, Bahr Yousef at El-Fayoum Governorate. Other studied groups (III & IV) administered highly heavy metals contaminated water and dried fish collected from El-Bats drainage canal, where agricultural and waste municipal of Fayoum Governorate discharged. Moreover, rats in the aforementioned groups administered uncontaminated and contaminated water and dried fish, for eight weeks, supplemented with 300 mg fennel /kg body weight/ day (Second and fourth groups).

Growth rates: Growth indices of an organism reflect its physiological status as well as general health. Data of the present study revealed highly significant decrease in body weight gain (-12.7 ± 1.08 g) of rats administered highly heavy metals contaminated water and dried fish **(Fig. 1).** However, rats administered uncontaminated and

contaminated water and dried fish supplemented with 300 mg fennel/kg body weight daily showed more or less body weight gain values as those of control group rats (0.6 ± 0.04 g and – 0.6 ± 0.34 g respectively).

Residual heavy metals:

Heavy metals (copper, lead, cadmium and zinc) bioaccumulation in rats vital organs liver and kidneys of albino rats before and after antioxidants (300 mg fennel/kg body weight daily) supplementation was carried out are declared in tables 1 & 2. The studied heavy metals bioaccumulation in liver and kidneys of rats administered highly heavy metals contaminated water and dried fish collected from the main drainage canal (El-Bats drain) at Fayoum Governorate where direct agricultural and waste municipal effluents discharged without prior treatment , are much higher in comparison with that of control group rats, administered tap water and uncontaminated dried fish. On the other hand, rats administered highly heavy metals contaminated water and dried fish supplemented with fennel (300 mg/kg body weight daily) , restore liver and kidneys copper content to values more or less similar to that of control group rats.

Haematological analysis:

Data representing blood parameters of albino rats (*Rattus rattus*) administered highly heavy metals contaminated water and dried fish, Table 3 revealed a significant decrease in RBCs count, haemoglobin content and haematocrite values but significant increase in WBCs count in comparison with those of the studied control rat groups before and after supplementation with 300 mg/kg body weight/day for eight weeks. On the other hand, the studied blood parameters of rats fed the highly heavy metals contaminated water and dried fish supplemented with 300 mg fennel/kg body weight daily for eight weeks exhibit more or less similar blood derivatives values as those of rats administered tap water and uncontaminated dried fish before and after supplementation.

Biochemical studies:

(A) Serum protein profile:

Rats administered highly heavy metals contaminated water and dried fish exhibited a significant increase in serum total protein, albumin, and globulin but non-significant differences in A/G ratio in comparison with those of control group rats before and after supplementation with the studied antioxidants (Table, 4).

(B) Serum glucose, liver and kidney functions:

In the present study, table (5) revealed highly significant increase in serum glucose level, disturbances in liver functions with a significant increase in aspartate amino transferase (AST) and alanine amino transferase (ALT) and kidneys dysfunction accompanied by a significant increase in serum creatinine and urea in comparison with those of the control group rats administered tap water and uncontaminated dried fish before and after supplementation.

(C) Serum lipid profile:

Regarding serum lipid profile of the studied albino rats (Table, 6), the present study revealed a significant increase in serum total cholesterol, triglycerides and LDL but significant decrease in HDL of rats administered highly heavy metals contaminated water and dried fish in comparison with those of control group rats before and after supplementation with the studied antioxidants.

(D) Serum antioxidants:

Results in table (7) of the present study revealed that, rats administered highly heavy metals contaminated water and dried fish exhibited the lowest serum total antioxidant but highest malonaldhyde in comparison with those of control group rats before and after supplementation with the studied antioxidants. However, rats administered contaminated water and dried fish supplemented with 300 mg fennel/kg body weight/day for eight weeks restore studied serum constituents to some extent to values more or less similar to those of control group rats.

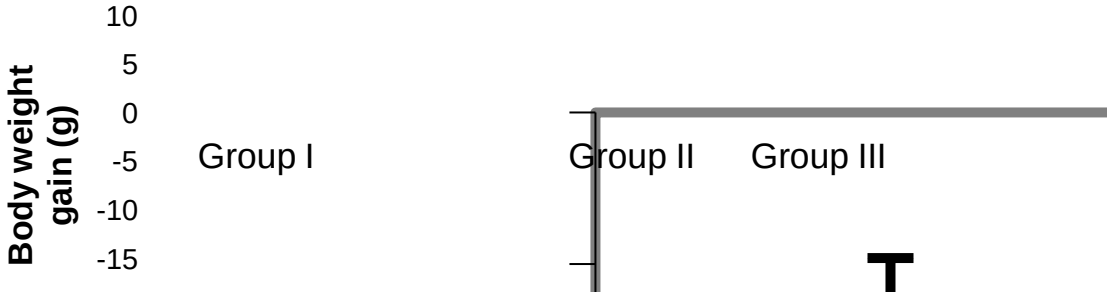

Fig. (1): Body weight gain (g) of albino rats administered contaminated water and fish from El-bats drain at Fayoum Governorate before and after supplementation with antioxidants for 8 weeks.

Table (1): Residual heavy metals in liver (mg/kg dry weight) of albino rats administered contaminated water and fish from El-bats drain at Fayoum Governorate before and after supplementation with antioxidants for 8 weeks.

Studied Groups	Copper	Lead	Cadmium	Zinc
Group I (Control group) (Rats administered tap water and uncontaminated fish)	0.927 ± 0.149 B/C	0.218 ± 0.008 B	0.089 ± 0.004 B	1.23 ± 0.049 A
Group II: Rats administered tap water and uncontaminated fish supplemented with 300 mg fennel/kg body weight daily	0.83 ± 0.15 C	0.185 ± 0.018 B/C	0.037 ± 0.016 B	1.05 ± 0.06 A
Group III: Rats administered contaminated water and fish from El-bats drain)	1.26 ± 0.023 A	0.64 ± 0.01 A	0.576 ± 0.12 A	1.16 ± 0.17 A
Group IV: Rats administered contaminated water and fish from El-bats drain supplemented with 300 mg fennel/kg body weight daily)	1.157 ± 0.102 A/B	0.167 ± 0.015 B/C	0.15 ± 0.004 B	0.95 ± 0.23 A

Data are represented as means of six samples ± S.E.
Means within the same column, with the same letter for each parameter are not significantly different, otherwise they do (SAS, 2000). ** Highly significant difference at P ≤ 0.01

Table (2): Residual heavy metals in kidney (mg/kg dry weight) of albino rats administered contaminated water and fish from El-bats drain at Fayoum Governorate before and after supplementation with antioxidants for 8 weeks.

Studied Groups	Copper	Lead	Cadmium	Zinc
Group I : Control group (Rats administered tap water and uncontaminated fish)	0.783 ± 0.076 A/B	0.211 ± 0.032 B	0.056 ± 0.012 B	0.916 ± 0.027 A
Group II: Rats administered tap water and ncontaminated fish supplemented with 300 mg fennel/kg body weight daily	0.75 ± 0.085 B	0.211 ± 0.029 B	0.039 ± 0.015 B	1.067 ± 0.021 A
Group III : Rats administered contaminated water and fish from El-bats drain	1.027 ± 0.056 A	0.454 ± 0.014 A	0.426 ± 0.126 A	1.25 ± 0.12 A
Group IV: Rats administered contaminated water and fish from El-bats drain supplemented with 300 mg fennel/kg body weight daily	0.87 ± 0.059 A/B	0.158 ± 0.015 B	0.020 ± 0.004 B	0.92 ± 0.21 A

Data are represented as means of six samples ± S.E.
Means within the same column, with the same letter for each parameter are not significantly different, otherwise they do (SAS, 2000). ** Highly significant difference at P ≤ 0.01

Table (3): Blood parameters of albino rats administered contaminated water and fish from El-bats drain at Fayoum Governorate before and after supplementation with antioxidants for 8 weeks.

Studied Groups	RBCs (X 10^6/mm^3)	Hb (g/100 ml)	Ht (%)	WBCs (X 10^3/mm^3)
Group I : Control group (Rats administered tap water and uncontaminated fish)	6.08 ± 0.199 A	13.35 ± 0.26 A	36.61 ± 0.36 A	4.9 ± 0.21 B
Group II: Rats administered tap water and uncontaminated fish supplemented with 300 mg fennel/kg body weight daily	5.68 ± 0.16 A/B	12.65 ± 0.34 A	36.43 ± 0.49 A	4.76 ± 0.198 B
Group III: Rats administered contaminated water and fish from El-bats drain	4.23 ± 0.09 D	10.12 ± 0.18 B	29.83 ± 0.54 B	6.98 ± 0.39 A
Group IV: Rats administered contaminated water and fish from El-bats drain supplemented with 300 mg fennel/kg body weight daily	5.28 ± 0.16 C	12.83 ± 0.33 A	35.58 ± 0.52 A	4.83 ± 0.17 B

Data are represented as means of six samples ± S.E.
Means within the same column, with the same letter for each parameter are not significantly different, otherwise they do (SAS, 2000). ** Highly significant difference at P ≤ 0.01

Table (4): Serum protein profile of albino rats administered contaminated water and fish from El-bats drain at Fayoum Governorate before and after supplementation with antioxidants for 8 weeks.

Studied Groups	Total protein (g/100 ml)	Albumin (g/100 ml)	Globulin (g/100 ml)	A/G ratio
Group I : Control group (Rats administered tap water and ncontaminated fish)	**5.16 ± 0.11 C**	**1.12 ± 0.06 B**	**4.1 ± 0.17 B**	**0.273 ± 0.03 A**
Group II : Rats administered tap water and uncontaminated fish supplemented with 300 mg fennel/kg body weight daily	**5.84 ± 0.02 B**	**1.27 ± 0.15 B**	**4.54 ± 0.06 A**	**0.280 ± 0.02 A**
Group III : Rats administered contaminated water and fish from El-bats drain	**6.27 ± 0.04 A**	**1.49 ± 0.07 A**	**4.74 ± 0.04 A**	**0.314 ± 0.01 A**
Group IV : Rats administered contaminated water and fish from El-bats drain supplemented with 300 mg fennel/kg body weight daily	**6.12 ± 0.07 A/B**	**1.47 ± 0.02 A**	**4.6 ± 0.08 A**	**0.320 ± 0.01 A**

Data are represented as means of six samples ± S.E.
Means within the same column, with the same letter for each parameter are not significantly different, otherwise they do (SAS, 2000).
** Highly significant difference at P ≤ 0.01
* Significantly difference at P < 0.05

Table (5): Serum constituents of albino rats administered contaminated water and fish from El-bats drain at Fayoum Governorate before and after supplementation with antioxidants for 8 weeks.

Studied Groups	Glucose (mg/100 ml)	AST (u/l)	ALT (u/l)	Creatinine (mg/100 ml)	Urea (mg/100 ml)
Group I : Control group (Rats administered tap water and uncontaminated fish)	110.8 ± 2.31 C	50.6 ±0.79 C	25.4 ± 0.6 C	0.596 ± 0.055 C	33.4 ± 0.60 C
Group III: Rats administered tap water and uncontaminated fish supplemented with 300 mg fennel/kg body weight daily)	98.8 ± 2.1 D	49.6 ± 0.65 C	27.2 ± 1.2 C	0.57 ± 0.01 D	32.9 ± 0.6 C
Group IV: Rats administered contaminated water and fish from El-bats drain)	141.2 ± 1.25 A	124.4 ± 2.03 A	44.0 ± 1.58 A	1.18 ± 0.013 A	101.2 ± 0.44 A
Group VI: Rats administered contaminated water and fish from El-bats drain supplemented with 300 mg fennel/kg body weight daily	127.0 ± 1.42 B	91.2 ± 0.85 B	36.4 ± 0.40 B	0.978 ± 0.007 B	69.4 ± 0.52 B

Data are represented as means of ten samples ± S.E.
Means within the same column, with the same letter for each parameter are not significantly different, otherwise they do (SAS, 2000). ** Highly significant difference at P ≤ 0.01

Table (6): Serum lipid profile of albino rats administered contaminated water and fish from El-bats drain at Fayoum Governorate before and after supplementation with antioxidants for 8 weeks.

Studied Groups	Total cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
Group I (Control group) : Rats administered tap water and uncontaminated fish	78.1 ± 1.2 C	70.3 ± 0.85 C	48.7 ± 0.52 A	47.0 ± 0.39 C
Group II: Rats administered tap water and uncontaminated fish supplemented with 300 mg fennel/kg body weight daily	77.4 ± 1.43 C	68.0 ± 0.56 C	49.0 ± 0.15 A	46.7 ± 0.26 C
Group III: Rats administered contaminated water and fish from El-bats drain	128.2 ± 0.81 A	114.9 ± 0.78 A	36.2 ± 0.74 C	64.1 ± 0.60 A
Group IV: Rats administered contaminated water and fish from El-bats drain supplemented300 mg fennel/kg body weight daily	94.2 ± 1.39 B	90.7 ± 1.76 B	40.7 ± 0.42 B	59.6 ± .79 B

Data are represented as means of ten samples ± S.E.
Means within the same column, with the same letter for each parameter are not significantly different, otherwise they do (SAS, 2000). ** Highly significant difference at P ≤ 0.01

Table (7): Serum malonaldhyde and total antioxidant of albino rats administered contaminated water and fish from El-bats drain at Fayoum Governorate before and after supplementation with antioxidants for 8 weeks.

Studied Groups	Malonaldhyde (nano mol/ml)	Total antioxidant (mol/ml)
Group I : Control group (Rats administered tap water and uncontaminated fish)	1.99 ± 0.054 C	1.930 ± 0.087 A
Group II: Rats administered tap water and uncontaminated fish supplemented with 300 mg fennel/kg body weight daily	1.98 ± 0.046 C	1.85 ± 0.05 A
Group III: Rats administered contaminated water and fish from El-bats drain	4.11 ± 0.149 A	1.27 ± 0.079 C
Group IV: Rats administered contaminated water and fish from El-bats drain supplemented300 mg fennel/kg body weight daily	2.596 ± 0.168 B	1.61 ± 0.018 B

Data are represented as means of six samples ± S.E.
Means within the same column, with the same letter for each parameter are not significantly different, otherwise they do (SAS, 2000). ** Highly significant difference at P ≤ 0.01

IV. Discussion

Dependent of industrial revolution in most industries to heavy metals, led in turn to pollution of aquatic as well as terrestrial habitats and hence become a threat to man. So, the present study focused on the usage of some physiological parameters in evaluating and monitoring heavy metals toxicity in some tissues of albino rats before and after supplementation with 300 mg fennel/kg body weight daily as therapy detoxifications.Heavy metals cause losses of weight and a deceleration of the growth **(22 and 23)**. Heavy metals would have caused a loss of appetite in the rats**.** Indeed, there is a correlation between the losses of body weight and the heavy metals concentration recorded in the studied vital organs in the present study as previously reported by **(22 and 23) Sajjadet *et al,* (2014) and Vandjiguiba *et al.* (2016).**

It is known that the polyunsaturated fatty acids constituting the cellular membrane are highly prone to react with ROS and get peroxidized in completely different chemical entities which perturbs varied key functions of the cell membrane such as membrane permeability and receptors, activities of membrane bound enzymes/trans membrane proteins (**24,; 25, 26)**, ion channels and transport of ions, and exo- and endocytosis as well as signal transduction. Some in vitro and in vivo animal studies have indicated that lead-induced oxidative damage significantly contributes to enhancement of erythrocytes membrane fragility during lead intoxication (**26).**White blood cell count increase is an implication of systemic inflammatory reaction within the body **(27, 28).** It involves the production of more white blood cells such as T cells, B lymphocytes, monocytes and neutrophils. All these cells have their own important role in an in-flammatory reaction **(28, 29).**

Progressive destruction of RBCs due to binding of lead with RBCs, leading to increasing fragility and destruction; could be another reason for decrease in haematological values **(30, 31**). Similarly, significant decrease in Hb and PCV were observed following exposure of rats to lead acetate **(31, 32).** The biochemical parameter makes it possible to evaluate possible toxic effects of an agent on the physiological functions of the organism responsible for many diseases (**23, 33**). The aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are used in animal toxicology like markers of the dysfunction of liver (**23**). The quantification of the activity of these enzymes in the animals is a marker of the exposure to pollutants (**23**). The activities of AST and ALT are cytosolic marker enzymes reflecting hepatocellular necrosis as they are released into the blood after cell membrane damage. Therefore, we used the activities of AST and ALT in the circulation as indicators of hepatic functions **(12)**.

The recorded disturbances in the studied blood parameters and serum constituents of rats administered highly heavy metals contaminated water and fish may be attributed to Oxidative stress, altered physiological and biochemical characteristics (**34)** leading to organ damage (**35, 36)** occur with heavy metal exposures. Heavy metals impart their toxicological effects mainly through molecular interactions with sulfhydryl groups on various molecules, (**36, 37)** generation of reactive oxygen species (ROS) and weakening the antioxidant defense system of cells and altering calcium and Fe^{2+} channels transport (**36)** Antioxidant activity Fennel is known as an excellent source of natural antioxidants. This plant can inhibit free radicals due to the high content of polyphenols and flavonoids. Phenolic compounds in this herb such as, caffeoyl quinic acid ,rosmarinic acid, eriodictyol-7-orutinoside, quercetin -O-galactoside, kaempferol-3-O-glucoside showed antioxidant activity as previously reported by **(38)Chatterjee *et al.* (2012) and (39)Kooti *et al.* (2015**). Other studies revealed that fennel seed has various antioxidant activities such as total antioxidant, free radical scavenging, superoxide anion radical scavenging, hydrogen peroxide scavenging, metal chelating activities, and reducing power **(40).**

V. Conclusion

In conclusion, the present study showed that contamination of the environment with heavy metals may threaten human beings life. Food supplementation with fennel as antioxidant (300mg/kg body weight) has shown some improvement of the observed effects of heavy metals toxicity. This study therefore suggests carrying out a study on another useful preventive agent against the effect of the heavy metals contaminated water and food at least partly due to its antioxidant properties.

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