MANSOURA JOURNAL OF BIOLOGY
Official Journal of Faculty of Science, Mansoura University, Egypt
E-mail: scimag@mans.edu.eg ISSN: 2974-492X

Use of fennel seeds extract as a protector against high fructose diet-induced cardiac injury in male rats

Hadeer M. El-Nigomy*, Mamdouh R. El-Sawi & Azza M. El-Wakf

Department of Zoology, Faculty of Science, Mansoura University, Egypt

**** Correspondence to:** H. M. Al-Nigomy; hadiralnigomy179@yahoo.com, Tel.: +201555920029*

Received: 17/ 1/2022
Accepted: 1/3/2022

Abstract The relation between high fructose intake and increased incidence of cardiac injury was assessed, with particular focusing on the possible protective role of oral intake of fennel seeds extract (200 mg/kg bw). Rats fed on high fructose (60%) diet for 12 weeks exhibited raised abdominal fat percent, serum glucose, insulin, leptin, HOMA-IR, and lipid profile with decreased QUICKI, reflecting a state of insulin resistance. This goes with increased myocardial lipid deposition, cardiac hypertrophy, hyperuricemia, reduced cardiac NO and hypertension. Significant reduction in cardiac antioxidants (SOD, CAT and GSH) and TAC, with elevation in oxidative stress indices (NADPH oxidase, $O_2^{\cdot-}$, MDA and H_2O_2) and serum cardiac biomarkers (CK-MB, LDH, AST, and cTn-I) were also observed. Further elevation in cardiac NF-κβ, proinflammatory cytokines (TNF-α, IL-1β and IL-6), and adhesion molecules (ICAM-1, VCAM-1) were recorded. However, administration of fennel seeds extract has shown to reduce fructose-induced metabolic alterations, oxidative injury, inflammation and normalize blood pressure. Thus, supporting fennel seeds extract as a promising supplement against fructose- associated cardiac disease.

keywords: Fructose, abdominal obesity, hypertension, cardiac disease, fennel seeds extract.

1.Introduction

Fructose is a natural monosaccharide sugar found in many fruits, and vegetables **[1].** It is commonly used as a sweetener in the medical and food industry **[2]** and is widely added to processed food and beverages .

Fructose can be used in several forms; the most common usage of fructose is high-fructose corn syrup (HFCS) which is used commercially as sweetener in pastries, ice cream, and frozen desserts, while the other type is crystalline fructose which is free of glucose. It is the pure form of fructose and is included in powdered beverages, flavored enhanced water, carbonated sodas drinks, and chocolate flavored milk **[3].**

Consumption of fructose has risen considerably in the last decades, leading to major health problems **[4].** There is growing evidence that high fructose is closely linked to elevated cardiometabolic risk factors, including hyperglycemia, hyperinsulinemia, insulin resistance, and increased blood lipids **[5].** There is an association among hypertension, insulin resistance and dyslipidemia that promote cardiac abnormalities in fructose-fed rats **[6].** Meanwhile, fructose may also result in increased oxidative stress and inflammation, thus playing a key role in mechanisms leading to cardiac disease **[7].**

Medicinal plants have been studied for many years because they are important sources of biologically active natural products. So, they have been identified as a new target for treatment and management of various diseases. Medicinal herbs are a good alternative to chemical drugs due to their efficacy, low side effects, and low cost **[8].**

Foeniculum vulgare (fennel) is one of the widespread biennial medicinal and aromatic plants belonging to the family Apiaceae that is native to Mediterranean region **[9].** It is one of the important spice plants which is used in the food and pharmaceutical industries **[10].** It can be used in treatment of many diseased conditions such as abdominal pains, cough,

constipation, fever, menstrual disorders, and mouth ulcer **[11].**

Fennel seeds extract (FSE) is a rich source of phytochemicals including polyphenols, flavonoids, alkaloids, amino acids, and fatty acids that have useful effects on human health **[12].** In addition, it has been scientifically proved to possess numerous medicinal activities, including anti-diabetic, anti-atherosclerotic, and hypoglycemic effects, besides anti-tumor and hepatoprotective activities **[14].** It is commonly used as preventive and therapeutic medicines against inflammatory disorders, cardiovascular diseases, and male infertility **[13].** Treatment with FSE leads also to the recovery of lipid profile near to the normal levels by reducing TGs, TC, and elevating HDL-C, confirming the hypolipidemic effect of fennel seeds extract **[14].** It has also used to boost the body's antioxidant defenses through scavenging reactive oxygen species (ROS) and restoring the antioxidative defense system **[8].**

In light of the above information, this research was designed to achieve a better understanding of the pathogenic mechanisms by which fructose consumption may increase cardiac complications and weather prolonged administration of FSE could overcome these complications.

2. Materials and methods

2.1. Animals

The present study was performed on thirty male Wister albino rats weighting (160-170g), purchased from animal unit of Helwan Farm-VACSERA, Egypt. Rats were housed in stainless steel cages under controlled environmental conditions (23±2 ^{0}C, relative humidity of 55±5%, and 12-h light/dark cycle). The animals were allowed free access to standard rat chow and tap water *ad libitum*. Experiments were performed with approval of the Experimental Animal Ethics Committee of Mansoura University, Egypt.

2.2. Chemicals and reagents

D-Fructose as white crystals and absolute ethanol were purchased from El-Gomhouria Co. for trading drugs, chemicals, and medical supplies (Mansoura, Egypt). Folin-Ciocalteu reagent was obtained from Fluka Biochemical Inc. (Bucharest, Romania). Gallic acid was purchased from Biomedical Inc. (Orange City, FL, USA), while aluminum chloride from Andenex-Chemie (Hamburg, Germany). Catechin, vanillin, hydrochloric acid, ascorbic acid, and 2.2 diphenyl-1-picryl hydrazyl (DPPH) were purchased from Sigma-Aldrich (St. Louis, USA). Ammonium hydroxide solution was purchased from Alpha Chemika (Panvel, Maharashta, India).

2.3. Preparation of fennel seeds extract

Dried fennel (*Foeniculum vulgare*) seeds were obtained from a local herb market at Mansoura, Egypt and were authenticated by a taxonomist at Botany Department, Faculty of Sciences, Mansoura University, Egypt. The Seeds were washed under running tap water, shade-dried and grinded into fine powder. 500g of powdered seeds was extracted using a mixture of ethanol: water (80:20, v/v) at room temperature for 48 h. The total extract was filtrated and concentrated to dryness under reduced pressure at 45°C using a rotary evaporator. Dried extract was collected and kept at 4°C until further use, where it was dissolved in distilled water and administered to rats at a dose of 200 mg/ kg bw **[15].**

2.4. Experimental design and animal grouping

After acclimatization period of one week, the animals were randomly divided into five groups (n=6): The first group served as normal control where rats were given standard rat chow without treatment. Rats in the second group were given distilled water (0.2 ml/100g bw) by oral gavage as a vehicle and those in the third group were given FSE orally at dose of 200 mg/ kg bw. In the fourth group, rats were fed high fructose diet (60%) daily **[16]**, while in the fifth group, rats were fed high fructose diet (60%) and received FSE as mentioned in the above groups. All treatments were continued consecutively for 12 weeks, and body weights were recorded at the beginning and end of the experiments to determine weight changes in each group.

2.5. Blood pressure measuring

Systolic blood pressure (SBP) was measured at the end of the experiment by using tail cuff method in conscious rats with LE 5001-pressure meter (Panlab Technology, Inc.,

Spain) at Physiology Department, Faculty of Medicine, Mansoura University, Egypt. The cuff was placed at the base of the tail to stop blood flow. The first pulse was referred to SBP **[17].**

2.6. Collection of blood samples and tissue samples

Forty-eight hours following the last treatment, rats were overnight fasted, then sacrificed under light ether anesthesia. Blood samples were collected from jugular vein in clean non-heparinized test tubes, centrifuged at 3000 rpm for 15 min and the separated sera were stored at -20 ºC for later biochemical analysis. Animals were then dissected and the abdominal fat (retroperitoneal and epididymal fat) and heart from each rat were separated, rinsed with ice-cold saline, derided with filter paper, weighed and homogenized for various biochemical assays.

2.7. Abdominal fat percent and myocardial hypertrophy index

Abdominal fat percent was determined by estimating the ratio of abdominal fat weight (AFW) to body weight (BW) as follows: AFW/BW %= AFW (g)/ BW (g) x 100 **[18].** Besides, myocardial hypertrophy index was expressed as heart weight/body weight ratio (HW/BW) % and was calculated using the same equation **[19].**

2.8. Biochemical analyses

Serum glucose, lipids [triglycerides (TGs), total cholesterol (TC), HDL-C], cardiac hydrogen peroxide (H_2O_2), malondialdehyde (MDA), glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), total antioxidant capacity (TAC), serum uric acid (UA), and cardiac nitric oxide (NO) were determined using commercial kits from Bio-diagnostic Co. (Dokki, Giza, Egypt). Serum leptin and insulin were estimated using ELISA kits provided by DRG instruments GmbH Co. (Germany) and Boehirnger Co. (Mannheim, Germany). Cardiac NADPH oxidase, superoxide anion ($O_2^{\cdot-}$), tumor necrosis factor-α (TNF-α), and interleukin -1β (IL-1β), were determined by ELISA kits from MyBioSource (San Diego, California, USA). Cardiac interleukin-6 (IL-6) and intercellular adhesion molecule-1 (ICAM-1) were measured using ELISA kits provided by BioVision Co. (Minneapolis, MN, USA). Vascular cell adhesion molecule-1 (VCAM-1) was assessed using ELISA kit obtained from Kamiya Biomedical Co. (Seattle, WA, USA). Serum cardiac troponin-I (cTn-I) and nuclear factor-kappa β (NF-κβ) were measured using ELISA kits from CUSABIO (Houston, USA), while enzymes, creatine kinase-MB (CK-MB), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) were assessed using kits from Horiba ABX Co. (France). Low-density lipoprotein cholesterol (LDL-C) was calculated as: TC (mg/dl)- HDL-C (mg/dl)- (TGs (mg/dl)/ 5) **[20].** Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) was calculated as: I_O (μIU/ml)× G_O (mg/dL)/ 405, while Quantitative Insulin Sensitivity Check Index (QUICKI) was calculated as: 1/ [(log I_O (μIU/ml)+ log G_O (mg/dL)] **[21],** where I_O is the fasting insulin and G_O is the fasting glucose.

2.9. Phytochemical investigation of fennel seeds extract

The ethanolic extract of fennel seeds was screened for identifying major phytochemical constituents using standard analytical procedures. The total phenolic content was measured using Folin-Ciocalteu reagent method as described by Bano **[22].** Gallic acid (GA) was used as a standard and total phenolic content was calculated by interpolation of standard curve of GA, where it was expressed as milligram of GA equivalent per gram dry weight (DW) (mg GA/g DW). The amount of total flavonoids present in the extract was determined using the aluminum chloride colorimetric assay, as described by Patel **[23].** Catechin (CE) was used as a standard and the flavonoid content was expressed as CE equivalent (mg CE/g DW). The total tannins were measured using vanillin hydrochloride method as described by Sivananth **[24]** and expressed as tannic acid (TA) equivalent (mg TA/g DW), whereas the total content of alkaloids was determined by using ammonium hydroxide solution for precipitating the alkaloids according to Abifarin **[25].** The percentage of alkaloids was then calculated using the formula: Alkaloid (mg %) = weight of precipitate/ weight of sample taken x100.

2.10. Determination of antioxidative capacity of fennel seeds extract

The antioxidant activity of FSE was measured using the stable 2,2-diphenyl-1-picrylhydrazylhydrate (DPPH) free radical in comparison with the standard antioxidant ascorbic acid as described by Ohikhena **[26].** Samples were added to the methanol solution of DPPH and the radical scavenging activities were measured based on the bleaching of the purple color of DPPH. After an incubation period of 30 min at 37 ^{0}C, absorbance of each test sample (A sample) was measured against the blank (A blank) using spectrophotometer at 517nm. The free radical scavenging activity was measured as percentage inhibition of DPPH that was calculated by using the following equation: Percentage inhibition = (A blank- A sample)/ A blank x 100, where the results were expressed as IC_{50} value which is the concentration required for scavenging 50% of the DPPH.

2.11. Statistical analysis:

All statistical analysis was performed using GraphPad prism 5 software. One way analysis of variance (ANOVA) was adopted to evaluate significance between the individual groups using SPSS statistical package, version 19.00. Values of $P \leq 0.05$ were considered to be significant and the results were expressed as means ± SE (n=6).

3. Results

3.1. Serum glucose, insulin, leptin, insulin resistance, and insulin sensitivity index

Fructose fed rats showed significant elevation in serum levels of glucose, insulin, leptin, and HOMA-IR, with significant reduction in QUICKI as compared with normal control group. Indeed, the oral administration of FSE to fructose fed rats significantly reduced all these changes, however no significant influence was noticed when fennel was given to normal untreated rats **(Table 1)**

3.2. Serum lipid profile

Serum lipids; TGs, TC and LDL-C were found to be significantly increased, while HDL-C was decreased in fructose fed rats compared to control group. Administration of FSE to fructose group attained a significant reduction in values of all lipid parameters except HDL-C which showed significant elevation compared to non-treated fructose group. No marked changes were detected in the normal rats that received FSE **(Table 2).**

3.3. Body weight, abdominal fat percent, cardiac lipids, and hypertrophy index

Fructose fed rats exhibited significant decrease in the body weight, despite an increase in the abdominal fat percent was recorded compared to rats fed on normal diet, indicating establishment of abdominal obesity. Fructose feeding was also associated with significant increase in the cardiac lipids (TGs, TC) concomitantly with cardiac hypertrophy, as confirmed by significant elevation in the relative heart weight. Marked improvement was observed in the studied parameters after administration of FSE to the rats fed on fructose, however no significant changes were recorded when FSE was given orally to the normal untreated rats **(Table 3).**

3.4. Serum uric acid, cardiac nitric oxide, and systolic blood pressure

Results showed marked elevation in serum UA and SBP, with decrease in cardiac NO in fructose fed rats compared to normal group. These changes tended to be significantly improved when rats were received high fructose diet plus FSE, however no significant alterations were observed when fennel extract was given to the normal rats **(Table 4).**

3.5. Cardiac oxidative stress biomarkers

Results showed significantly elevated levels of NADPH oxidase, $O_2^{\cdot-}$, H_2O_2, and MDA, accompanied by marked reduction of antioxidant defense system; GSH, SOD, CAT, and TAC in the cardiac tissue of fructose fed rats compared to the control animals; which in all were found to be ameliorated when rats were administered with FSE. Indeed, no significant alterations were recorded after administration of fennel to normal untreated rats **(Table 5).**

3.6. Serum cardiac enzymes and cardiac troponin- I

There was elevation in activities of CK-MB, LDH, AST and serum level of cTn-I in fructose fed rats compared to the control group. Indeed, these changes appeared to be improved following oral administration of FSE to rats fed on fructose, where significant reduction in cardiac biomarkers was recorded. No

significant changes were noticed in normal rats received fennel compared to control animals **(Table 6).**

3.7. Cardiac inflammatory markers and adhesion molecules

Cardiac inflammatory markers (NF-κβ, TNF-α, IL-1β and IL-6) and adhesion molecules (ICAM-1, VCAM-1) were found to be significantly increased in fructose fed rats group. Marked reduction was observed in all these parameters following administration of FSE to rats fed on fructose. However, no marked changes were detected when normal untreated rats received fennel **(Table 7).**

3.8. Quantification of phytochemical constituents

Quantification of phytochemical constituents of FSE were illustrated in **Table 8.** Results showed high concentration of total phenolic content, total flavonoids, and tannins, varied between 2762.22 mg GA/100g DW, 897.42 mg CA/100g DW and 731.14 mg TA/100g DW, respectively. Determination of the alkaloid content also revealed high alkaloid percentage (746.38 mg %).

3.9. DPPH radical scavenging assay

Free radical scavenging activity of FSE was assessed in terms of DPPH method. As observed, fennel extract exhibited strong radical scavenging activity with IC_{50} value of 0.002 mg/ml **(Table 8).** Notably, the antioxidant effect is proportional to disappearance of DPPH in test samples, thus the lower the IC_{50} value is the greater the antioxidant power

Table 1. Serum glucose, insulin, leptin, insulin resistance, and insulin sensitivity index in control and treated rat groups

	NCO	VEC	FSE	HFR	HFR+FSE
Glucose (mg/dl)	82.67 ±4.26	82.33 ±6.23	79.67 ±4.67	177.80^{a} ±6.01	119.70^{ab} ±2.73
Insulin (µlU/mL)	58.37 ±1.13	59.10 ±1.07	57.33 ±0.88	74.97^{a} ±2.28	64.33^{b} ±1.45
Leptin (ng/mL)	24.00 ±2.65	24.47 ±2.05	23.33 ±2.03	39.40^{a} ±1.82	28.43^{b} ±1.07
HOMA-IR(µIU×mg/dl)	11.89 ±0.37	11.89 ±0.72	11.26 ±0.49	31.57^{a} ±0.76	18.99^{ab} ±0.17
QUICKI (µIU×mg/dL)	0.27 ±0.001	0.27 ±0.003	0.27 ±0.003	0.24^{a} ±0.002	0.26^{ab} ±0.000

Results are presented as means ±SE for 6 rats. NCO: normal control, VEC: vehicle, FSE: fennel seeds extract, HFR: high fructose. a: significant as compared to normal control group, b: significant as compared to high fructose group.

Table 2. Serum lipid profile in control and treated rat groups

	NCO	VEC	FSE	HFR	HFR+FSE
TGs (mg/dl)	45.32 ±2.20	45.65 ±1.50	43.62 ±1.80	81.63^{a} ±1.48	49.07^{b} ±3.72
TC (mg/dl)	55.00 ±4.65	54.16 ±5.32	53.18 ±4.86	171.70^{a} ±3.48	109.50^{ab} ±3.28
LDL-C(mg/dl)	24.10 ±1.13	23.10 ±1.07	23.00 ±0.58	132.10^{a} ±5.81	68.19^{ab} ±3.00
HDL-C (mg/dl)	37.67 ±2.03	39.33 ±2.33	42.33 ±1.45	23.32^{a} ±1.20	35.00^{b} ±3.06

Results are presented as means ±SE for 6 rats. NCO: normal control, VEC: vehicle, FSE: fennel seeds extract, HFR: high fructose. a: significant as compared to normal control group, b: significant as compared to high fructose group.

Table 3. Body weight, abdominal fat percent, cardiac lipid parameters, and hypertrophy index in control and treated rat groups

	NCO	VEC	FSE	HFR	HFR+FSE
Body weight (g)	315.70 ±9.94	311.30 ±13.69	312.00 ±13.01	187.70^{a} ±4.91	205.00^{ab} ±1.16
abdominalfatpercent	2.78 ±0.35	2.77 ±0.24	2.78 ±0.20	4.69^{a} ±0.17	3.04^{b} ±0.22
Hypertrophy index	0.25 ±0.02	0.25 ±0.01	0.24 ±0.01	0.32^{a} ±0.01	0.26^{b} ±0.01
TGs (mg/g)	35.99 ±2.39	33.14 ±2.31	32.05 ±1.32	136.60^{a} ±5.36	43.61^{b} ±3.21
TC (mg/g)	18.35 ±1.45	14.27 ±1.14	15.70 ±1.68	60.65^{a} ±2.20	27.17^{b} ±1.70

Results are presented as means ±SE for 6 rats. NCO: normal control, VEC: vehicle, FSE: fennel seeds extract, HFR: high fructose. a: significant as compared to normal control group, b: significant as compared to high fructose group.

Table 4. Serum uric acid, cardiac nitric oxide, and systolic blood pressure in control and treated rat groups

	NCO	VEC	FSE	HFR	HFR+FSE
UA (mg/dl)	3.33 ±0.20	3.34 ±0.17	3.55 ±0.18	11.16^{a} ±0.36	4.67^{b} ±0.46
NO (nmol/mg)	16.71 ±0.79	15.72 ±0.63	15.05 ±0.72	10.49^{a} ±0.64	15.68^{b} ±0.69
SBP (mm/Hg)	125.50 ±0.58	124.30 ±0.82	123.00 ±0.21	180.50^{a} ±1.24	147.30^{ab} ±3.71

Results are presented as means ±SE for 6 rats. NCO: normal control, VEC: vehicle, FSE: fennel seeds extract, HFR: high fructose. a: significant as compared to normal control group, b: significant as compared to high fructose group.

Table 5. Cardiac oxidative stress and antioxidant biomarkers in control and treated rat groups

	NCO	VEC	FSE	HFR	HFR+FSE
NADPHoxidae(Pg/mg)	101.30±13.04	102.70 ±9.33	98.33 ±6.64	426.20^{a} ±18.39	230.70^{ab}±12.41
$O_2^{\cdot-}$ (U/mg)	80.20 ±5.80	75.30 ±7.47	73.30 ±6.09	277.40^{a} ±7.25	122.00^{ab} ±5.64
H_2O_2 (Mm/g)	3.71 ±0.61	3.32 ±0.32	3.23 ±0.35	18.34^{a} ±0.95	6.43^{b} ±1.52
MDA (nmol/mg)	787.00 ±20.55	788.30 ±18.48	782.70 ±19.36	1337.00^{a} ±23.52	846.80^{b} ±13.42
GSH (mg/g)	4.45 ±0.12	4.42 ±0.07	4.57 ±0.17	1.62^{a} ±0.06	4.32^{b} ±0.07
SOD (U/g)	280.30 ±8.69	279.30 ±9.68	284.20 ±6.64	142.50^{a} ±11.61	253.50^{b} ±5.33
CAT (U/g)	175.30 ±6.68	175.90 ±6.94	177.20 ±7.96	106.10^{a} ±4.02	130.00^{ab} ±7.66
TAC (Mm/g)	1.42 ±0.01	1.45 ±0.03	1.48 ±0.03	0.81^{a} ±0.05	1.23^{ab} ±0.02

Results are presented as means ±SE for 6 rats. NCO: normal control, VEC: vehicle, FSE: fennel seeds extract, HFR: high fructose. a: significant as compared to normal control group, b: significant as compared to high fructose group.

Table 6. Serum cardiac enzymes and cardiac troponin- I in control and treated rat groups**.**

	NCO	VEC	FSE	HFR	HFR+FSE
CK-MB (U/L)	662.20 ±24.22	661.30 ±21.61	660.30 ±23.26	1370.00^{a} ±28.48	810.00^{b} ±29.47
LDH (U/L)	493.30 ±5.99	492.00 ±6.69	490.3 ±7.83	1295.00^{a} ±17.78	891.20^{ab} ±14.06
AST (U/L)	97.00 ±2.31	96.14 ±5.49	96.57 ±4.03	225.40^{a} ±9.44	110.70^{b} ±7.30
cTn-I (Pg/ml)	58.00 ±1.16	57.23 ±1.13	55.57 ±0.95	181.70^{a} ±10.20	125.30^{ab} ±8.40

Results are presented as means ±SE for 6 rats. NCO: normal control, VEC: vehicle, FSE: fennel seeds extract, HFR: high fructose. a: significant as compared to normal control group, b: significant as compared to high fructose group.

Table 7. Cardiac inflammatory biomarkers and adhesion molecules in control and treated rat groups

	NCO	VEC	FSE	HFR	HFR+FSE
NF-κβ (ng/mg)	20.33 ±0.88	19.00 ±1.16	19.33 ±1.45	31.00^{a} ±1.16	28.00^{ab} ±1.16
TNF-α (pg/mg)	14.00 ±1.16	12.97 ±1.18	12.60 ±0.96	41.07^{a} ±1.10	19.03^{ab} ±0.55
IL-1β (pg/mg)	73.33 ±1.20	73.03 ±1.70	64.67 ±2.60	273.00^{a} ±12.17	187.30^{ab} ±8.76
IL-6 (pg/mg)	2.50 ±0.23	2.00 ±0.58	1.72 ±0.12	8.00^{a} ±0.58	4.00^{b} ±0.58
ICAM-1 (pg/mg)	100.00 ±1.16	99.60 ±5.04	94.33 ±7.22	420.00^{a} ±15.28	233.30^{ab} ±16.67
VCAM-1 (ng/mg)	14.67 ±0.88	12.67 ±1.76	13.50 ±1.29	102.70^{a} ±12.20	53.33^{ab} ±2.33

Results are presented as means ±SE for 6 rats. NCO: normal control, VEC: vehicle, FSE: fennel seeds extract, HFR: high fructose. a: significant as compared to normal control group, b: significant as compared to high fructose group

Table 8. Phytochemical analysis of ethanolic fennel seeds extrac

Phytochemical constituents	Content
Polyphenols (mg GA/100g DW)	2762.22
Flavonoids (mg CA/100g DW)	897.42
Alkaloids (mg %)	746.38
Tannins (mg TA/100g DW)	731.14
DPPH assay (IC_{50} mg/ml)	0.002

Data represent total phenolic content expressed in mg equivalent gallic acid/100 g dry weight (mg GA/100g DW); total flavonoids content expressed in mg equivalent catechin (mg CA/100g DW); total alkaloids content expressed in mg alkaloid (mg %); tannins content expressed in mg equivalent tannic acid (mg TA/100g DW); antioxidant activity of fennel seeds extract against DPPH radical expressed in IC_{50} value.

4. Discussion

Excessive fructose intake is now emerging as a major risk factor for developing metabolic heart disease **[27].** Fructose unlike glucose which is metabolized in most cells and acts as energy substrate, specifically metabolized in

liver where it is converted into fructose-1-phosphate (F-1-P) by phosphorylating enzyme (phosphofructokinase) that is independent on insulin and stimulated by fructose. Hence, F-1-P is split into two trioses; glyceraldehyde and dihydroxyacetone which could be converted to acetyl-CoA and malonyl-CoA, the key metabolites utilized in de novo synthesis of TGs **[28].** The synthesized TGs are packed into vLDL-C and transported to the peripheral adipose tissue to be stored there and thus causing hyperlipidemia and abdominal fat accumulation, which are core features of fructose associated heart disease. **[27].**

Meanwhile, fructose can alter cholesterol metabolism through the formation of β-hydroxy-β-methylglutaryl-CoA (HMG-CoA) from acetyl-CoA, resulting in hypercholesterolemia **[27],** associated with reduction in HDL-C which is an anti-atherogenic lipoprotein transports circulating cholesterol into liver for clearance. The reduction of plasma HDL-C concentration may accelerate development of atherosclerosis, the most common cause of heart disease **[29].**

In the present study, high fructose feeding led to elevation of serum lipid profile (TGs, TC, LDL-C levels), and abdominal fat percent, while there was significant reduction in HDL-C which seemed in all to be ameliorated by administration of FSE as mentioned in previous study by Samadi-Noshahr *et al.* **[13].** These results indicate that FSE had a hypolipidemic and anti-atherosclerotic effect that correct lipid profile in fructose fed rats. The hypolipidemic effect of fennel may be due to presence of high concentration of polyphenols **[14]** that lead to decrease of cholesterol and FFA synthesis. It also increases HDL-C *via* activating lecithin cholesterol acyltransferase, the key enzyme for lipoprotein and cholesterol metabolism by facilitating the uptake of cholesterol from peripheral tissues into HDL particles, **[30]** promoting HDL-C production **[31].** Modulation of lipid metabolism and elevating level of HDL-C can thus consider as a possible mechanism by which FSE corrects cardiac disease achieved by fructose feeding.

For more interpretation, the increased lipid accumulation and abdominal adiposity by fructose feeding could induce insulin resistance that is defined as a defect in insulin-induced glucose uptake, glycogen synthesis, and glucose oxidation, resulting in hyperglycemia **[32].** Consequently, pancreas tries to compensate by increasing the secretion of insulin, leading to hyperinsulinemia and insulin resistance **[33].**

Leptin is a hormone secreted mainly by adipocytes **[34]** and stimulated by insulin. Both adipose tissue and insulin can elevate the level of leptin. High fructose diet leads to excessive insulin secretion and insulin resistance, thereby stimulating leptin production **[35].** According to previous study, the increased leptin was positively associated with hyperinsulinemia, insulin resistance, and hyperlipidemia which is a key factor for developing cardiac disease and increased myocardial lipid deposition **[36].** As such, the present study showed significant elevation in serum glucose, insulin, leptin, and insulin resistance in fructose fed rats which together may contribute to increased deposition of myocardial lipids, thus predisposes to increased heart weight/ body weight ratio and cardiac hypertrophy **[37].**

Administration of FSE to fructose fed rats significantly decreased hyperglycemia, hyperinsulinemia, hyperleptinemia, and insulin resistance, thereby reduces myocardial lipid accumulation and cardiac hypertrophy. This effect is likely attributed to presence of flavonoids which are known for their health benefits through promoting insulin sensitivity and cellular glucose uptake **[38].** Flavonoids may also play a critical role in maintaining normal lipid metabolism and leptin production **[39].** Thus, consumption of FSE could significantly contribute to ameliorating fructose- induced metabolic disorders and related cardiac disease.

Indeed, fructose can also stimulate muscle wasting and loss of lean body mass, an effect which is likely mediated *via* decrease in muscle insulin sensitivity in response to lipid influx by fructose **[40].** Meanwhile, fructose tends also to promote synthesis of catabolic hepatokines such as, Fetuin A, which is an endogenous inhibitor of insulin receptor tyrosine kinase in muscles, associated with insulin resistance and lowered protein synthesis **[41].** As support, Rashwan *et al.* **[42]** addressed an association

between fructose intake and sarcopenia which probably corresponds to the significant decrease in body weight gain observed by previous studies. Similar pattern of body weight loss has been established by the present data following high fructose feeding which was further attenuated by administration of FSE probably through presence of flavonoids having a capacity for maintaining protein metabolic balance and preventing muscle wasting **[13].**

Beyond this effect, fructose has also shown to stimulate production of UA, which is closely associated with development and progression of hypertension **[43].** This metabolic aspect is specific to fructose due to its fast uncontrolled metabolism, leading to depletion of cellular ATP and accumulation of AMP, followed by acute rise of UA **[44]** Increased UA may contribute to impaired endothelial function through reducing bioavailability of NO, a major vasodilator helps to maintain vascular compliance **[45].** Thus, deriving elevation of SBP and may eventually manifest features of cardiac disease **[46].** UA can also inhibit insulin-induced NO production by endothelial cells, which is a strong predictor for vascular dysfunction and increased arterial pressure **[47].** Similar results were achieved by the present data showing increased serum UA with decreased production of cardiac NO and increased SBP in fructose fed rats.

However, administration of FSE to fructose fed rats was found to be effective in ameliorating elevation of UA and decrease of NO level, leading to reduced blood pressure, indicating strong antihypertensive and cardioprotective effect of FSE. This may be explained by the capacity of the plant polyphenols to activate NO metabolism and reduce vasoconstriction by acting on angiotensin converting enzyme (ACE) and angiotensin receptor activity **[48].** Polyphenols have also reported for their potent vasodilating effect through activating endothelial nitric oxide synthase (eNOS) and hence NO release **[49],** which is a regulating factor of blood pressure and cardiac function **[50].** Thus, it seemed possible that intake of FSE is of great value in improving vasculature and cardiac health under condition of excess fructose feeding.

Although different mechanisms have identified, oxidative stress generally regarded as a major factor in development of cardiac disease by fructose feeding **[51].** According to previous study occurred by Radmanesh *et al.* **[52]**, fructose feeding causes excessive production of mitochondrial ROS, and it is associated with an increase in cardiac oxidative stress. More interpretation may involve the ability of dietary fructose to increase serum glucose levels which in turn activates the pro-oxidant enzyme, NADPH oxidase in cardiac tissue **[28].** Once activated, it will catalyze electron transfer from NADPH to O_2, allowing generation of $O_2^{\cdot-}$ which is the precursor of most ROS and mediator of oxidative reactions. Alternative pathway may include increased lipid accumulation, which is suggested as aggravating factor by fructose, promoting mitochondrial dysfunction and activating the production of ROS **[53].**

ROS such as $O_2^{\cdot-}$, hydroxyl radical (OH^-), and H_2O_2 are continuously generated inside the human body as a consequence of exposure to exogenous chemicals or a number of endogenous processes **[54].** However, increased generation of ROS consequently attacks the cell membrane lipids, leading to increased lipid peroxidation manifested by over accumulation of MDA **[37].** Lipid peroxidation is one of the most important destructive effect of free radicals which destroys cell membrane and causes a loss in membrane integrity and a change in related enzymes **[55].** The increase in MDA suggests enhanced tissue damage and failure of antioxidant defense mechanisms to prevent formation of excessive free radicals **[56].**

The present study showed significantly increased oxidative stress markers including NADPH oxidase, H_2O_2, O_2^-, and MDA with reduction in endogenous antioxidants (SOD, CAT, and GSH) and TAC in the heart of fructose fed rats, thereby generating a state of oxidative stress. One of the harmful consequences is elevation in serum levels of cardiac enzymes (CK-MB, LDH and AST) and cTn-I in the serum, suggesting their leakage into the blood circulation due to cardiomyocytes damage and disruption of cellular membrane integrity **[57].** However, when fructose fed rats were supplemented by

FSE, the elevated levels of NADPH oxidase and related oxidative stress mediators showed significant decline with elevation in the cellular antioxidant capacity, reflecting improved myocardial oxidative status. This seemed to be closely linked with observed reduction of serum cardiac biomarkers (CK-MB, LDH, AST, and cTn-I), in fennel treated rats compared to non-treated animals. These results may contribute to presence of several polyphenolic constituents including tannins and flavonoids which are known to possess strong antioxidant properties and reducing power **[58],** as determined herein by the highest DPPH radical scavenging activity. In accordance, Wu *et al.* **[59]** suggested tannins as potent inhibitors for NADPH oxidase that is responsible for generation of ROS and interfering with cellular detoxifying systems such as SOD and CAT **[60].** Meanwhile, flavonoids have reported to be effective protectors against over production of free radicals and oxidation of cellular lipids **[61].** The present results could thus confirm the beneficial role of fennel to attenuate disrupted redox status and cardiac damage produced by high fructose feeding.

Besides, it has accepted that inflammation can participate directly in fructose pathophysiology and its cardiac complications **[62].** Lately, the involvement of oxidative stress in initiating inflammation has gained wide interest **[63].** On this basis, a possibility existed that increased generation of ROS stimulates multiple signaling pathways through activation of redox-sensitive transcription factors such as NF-κβ **[64].** Activation of NF-κβ might be an important regulator that controls expression of genes encoding pro-inflammatory cytokines **[65]** and endothelial adhesion molecules **[66]** which eventually contribute to pathogenic processes of several cardiac diseases **[67].** As such, the present data proposed a correlation between increased levels of cardiac NF-κβ and progression of heart disease in fructose fed rats through promoting elevation of pro-inflammatory cytokines (TNF-α, IL-1β and IL-6) and adhesive molecules (VCAM-1 and ICAM-1) in cardiac tissue.

However, administration of FSE to fructose fed rats clearly exhibited lowered inflammatory response through reducing levels of NF-κβ and its inflammatory mediators (TNF-α, IL-1β and IL-6). This action is thought to be derived from the plant polyphenols particularly flavonoids which have suggested to exert beneficial effect towards the heart through modulatory signaling pathway like NF-κβ which has be linked to lowered levels of pro-inflammatory cytokines and improved cell survival and function **[68].** Added to this, flavonoids have shown to possess strong anti-inflammatory properties through decreasing the expression of adhesive molecules involving ICAM-1 and VCAM-1 in endothelial cells **[69].** Indeed, results of this study should also be interpreted based on presence of other plant constituents including alkaloids and tannins, which are known for their anti-inflammatory activities **[70].** Hence, it seems possible to establish the anti-inflammatory effect of FSE and its promising protective effect against cardio-pathogenic effect of increased fructose feeding.

5. Conclusion

The present study demonstrated a strong association between high fructose intake and development of metabolic cardiac disease. Proposed mechanisms may involve altered metabolism, insulin resistance, oxidative stress, and inflammation which together are responsible for developing hypertension, and cardiac disease. However, administration of FSE tended to modulate altered metabolic pathways and other cardiac risk factors, thereby eliciting marked anti-hypertensive and cardio-protective action in fructose fed rats. Thus, regular consumption of FSE could be recommended as a complementary alternative therapy for patients with metabolic cardiac diseases. However, further clinical studies are needed to stablish the plant therapeutic effectiveness.

Acknowledgment

The authors are highly grateful to Zoology Department, Faculty of Science, Mansoura University for providing necessary facilities during research work.

6. References

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