Protective Effects of Bitter Almond Kernel Extract on Liver and Kidney Tissues in Streptozotocin-Induced Diabetic Rats

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Abstract

The bitter almond has been used for a long time by diabetes patients due to some important anti-diabetic effects. In this study, we aimed to determine protective effects of bitter almond extract on the lipid-soluble vitamins, cholesterol, glutathione (GSH), total protein, malondialdehyde (MDA), fatty acid levels of liver and kidney tissues of streptozotocin-induced diabetic rats. According to our findings, in kidney tissue, GSH and total protein were decreased in the Diabetes (D) and Diabetes + Bitter Almond (D+A) groups. Again MDA was increased in the D group, but not changed in the D+A group. Our results indicated that bitter almond extract might have a positively effect on postprandial blood glucose levels, as well as, on the GSH and MDA levels in the kidney tissue. The decrease of postprandial glucose level and these positive results can be attributed to bitter almond extract in the kidney tissue. The bitter almond and streptozotocin (STZ) administrations affected amount of some important fatty acids in the liver and kidney tissues, which substrates in fatty acid metabolism on duty enzymes.

Keywords: Diabetes, bitter almond, malondialdehyde, glutathione, cholesterol, fatty acids.

Streptozotosin-Kaynaklı Diyabetik Sıçanların Karaciğer ve Böbrek Dokularında Acı Badem Çekirdek Ekstresinin Koruyucu Etkileri

Özet

Acı badem bazı önemli anti-diyabetik etkileri nedeniyle diyabet hastaları tarafından uzun bir süredir kullanılmaktadır. Bu çalışmada acı badem ekstresinin streptozotosin kaynaklı diyabetik sıçanların karaciğer ve böbrek dokularında yağda çözünen vitaminler, kolesterol, glutatyon (GSH), total protein, malondialdehit (MDA) ve yağ asitleri seviyeleri üzerinde koruyucu etkilerini saptamayı amaçladık. Bulgularımıza göre böbrek dokusunda, GSH ve total protein miktarı Diyabet (D) ve Diyabet + Acı Badem (D+A) gruplarında azalmıştır. Yine MDA seviyesi D grubunda artmış, D+A grubunda ise değişmemiştir. Sonuçlarımız göstermiştir ki acı badem ekstresi postprandial kan glukoz seviyesi yanında böbrek dokusunda GSH ve MDA seviyeleri üzerinde pozitif etkilere sahip olabilir. Postprandial kan glukoz seviyesindeki azalma ve böbrek dokusundaki bu pozitif etkiler acı badem ekstresine atfedilebilir. Ayrıca acı badem ve streptozotosin uygulamaları karaciğer ve böbrek dokularında yağ asidi metabolizmasında görevli enzimlerin substratı olan bazı önemli yağ asitlerinin miktarını etkilemiştir.

Anahtar Sözcükler: Diyabet, acı badem, malondialdehit, glutatyon, kolesterol, yağ asitleri.

1. Introduction

Diabetes mellitus and its complications are among the major health problems in the world and diabetes comprises a chronic disorder by hyperglycemia or diminished insulin secretion, or both. The diabetes easily affects to some important organs and the quality of live significantly reduces for diabetes patient. Diabetes covers high levels of blood glucose, which contributes to an increase in free radical production [1,2]. Glutathione (GSH) is very important intracellular tripeptide and it plays a key role in maintaining cellular homeostasis, as well as protecting the cell against oxidative stress. GSH is widely distributed in living cells

and involved in their many biological reactions. A measurement of GSH concentration in tissues or cells is a sensitive indicator of their redox status [3]. The kidneys play a critical role in maintaining overall health. These twin organs filter waste products and excess water from the blood and also produce hormones that are essential for the optimal maintenance of bodily functions. Hyperglycemia damages the small blood vessels in the kidney causing diabetic nephropathy. If this occurs, kidney cannot filter the blood properly and this causes the body to retain more water and salt than it should and high levels of protein in the blood occurs [4].

Streptozotocin (STZ) has been widely used to induce diabetes mellitus in experimental animals due to its toxic effects on islet beta cells. The diabetogenic action of STZ is the direct result of irreversible damage to the pancreatic beta cells resulting in degranulation and loss of activity to secretion of insulin. The cytotoxic action of STZ is mediated by reactive oxygen species (ROS) [5]. The increase in ROS and oxidative stress in various tissues are involved in the development of diabetic complications and diabetes mellitus is characterized by hyperglycemia and long-term complications affecting the eyes, kidneys, aorta, nerves, and blood vessels is the important metabolic disease [6].

Over the past decade there is an increased interest in oxidative stress and its role in the development of complications of diabetes. Apart from the traditional antidiabetic treatment, antioxidant, herbal and/or folk medicine therapy, may benefit in diabetes [7]. Many plants and plant-derived compounds were used for the treatment of diabetes, because some of these plants contain some important biological compounds having hypoglycemic activity [8]. Bitter almond has been used for a long time as a part of alternative medicine by diabetic patients [9-13]

The aim of this study was to determine the possible protective effects of bitter almond kernel extract on the fatty acid composition, lipid soluble vitamin contents, reduced glutathione (GSH), total protein and malondialdehyde (MDA) levels of liver and kidney tissue of STZ-induced diabetic rats.

2. Material and Methods

2.1. Chemicals

All the chemicals and reagents were obtained from Sigma Chemical Co. (St. Louis Mo, USA).

2.2. Animals

All the experimental protocols approved by the Ethical Committee of Firat University (Elazig, Turkey). Thirty healthy male Wistar albino rats, aged 8-9 weeks and weight in the range of 200-225 g, were obtained from Firat University Experimental Research Centre (Elazig, Turkey). The animals were housed in polycarbonate cages in a room with a 12 h day-night cycle, at the temperature of 24 ± 3 °C and humidity of 45–65%. During the whole experimental period, animals were fed with a balanced commercial diet and water *ad libitum*.

2.3. Experimental Design

The thirty rats were randomly divided into three groups each containing ten rats.

Group C (Control): Rats were injected with 0.5 mL dimethyl sulfoxide (DMSO) every next day, the rats received tab water and were fed with standard pellet diet as *ad libitum*.

Group D (Diabetes): Rats were injected intraperitoneally with a single dose of 60 mg/kg streptozotocin dissolved in 0.1 mol/L citrate buffer and were fed with standard pellet diet as *ad libitum*.

Group D+A (Diabetes+Almond): Rats were injected with a single dose of 60 mg/kg streptozotocin intraperitoneally, received 1 mg/kg almond bitter extract dissolved in 0.5 mL DMSO intraperitoneally every next day and were fed with standard pellet diet as *ad libitum*.

All treatments were continued for 60 days. Each experimental rat was decapitated after one week of the last injection of DMSO and the last intake of the bitter almond extract. At the end of the experimental period, animals were fasted overnight and sacrificed by cervical decapitation. Liver and kidney tissues were dissected out washed in ice-cold saline to remove blood. The tissues were homogenized in Tris-HCl and ethylenediaminetetraacetic acid (EDTA) buffer at pH = 7.4 and then centrifuged at 9000 rpm for 20 min. The supernatants were used for lipid

peroxidation, and total protein and pellet were used for fatty acids, vitamin and sterol analysis.

2.4. Induction of Diabetes

Overnight fasting animals were made diabetic by a single intraperitoneal injection of freshly prepared STZ (60 mg/kg) dissolved in 0.1 mol/L citrate buffer (pH=4.5) [14]. Control rats were injected with the same volume of isotonic saline. Plasma glucose was determined at the end of 72 h, and the rats with glucose levels greater than 250 mg/dL were used in the present study. The blood glucose levels were measured by using reagent strips (Contour, Bayer, Leverkusen, Germany).

2.5. Preparation of Bitter Almond Extract

Bitter almond was collected from Elazig in Turkey. Voucher specimen number is Turkoglu 4908. The bitter almond kernel was dried at room temperature. As-dried kernels were powdered in an electrical grinder and stored at 5 °C. For extraction, 25 g of bitter almond was mixed with 100 mL hexane–isopropyl alcohol (3:2, v/v). The Soxhlet extraction continued until the extraction solvents became colorless. Extracts were centrifuged at 9000 rpm for 10 min and filtered, and the solvent was removed on a rotary evaporator at 40 °C under reduced pressure. The remaining residue dissolved in DMSO was used [15].

2.6. Determination of MDA Level

MDA level in tissue homogenate was estimated using thiobarbituric acid reactive substances by the method of Ohkawa et al. [16]. To 1.0 mL tissue homogenate, 0.5 mL of 8.1% sodium dodecyl sulfate (SDS), 1.0 mL of (20% acetic acid / NaOH, pH = 3.5), 1.0 mL of 10% trichloroacetic acid (TCA), 50 μL of 2% butylated hydroxytoluene (BHT) and 1.0 mL of 0.8% thiobarbituric acid (TBA) were added. The mixture was heated in a water bath at 95 °C for 60 min. After cooling, 4.0 mL of n-butanol/pyridine mixture were added and shaken vigorously. After centrifugation at 4250 rpm for 15 min, the organic layer was taken and its absorbance at 532 nm was measured. 1,1,3,3-

tetramethoxypropane was used as standard. The results were calculated as nmol MDA/g tissue.

2.7. Determination of Reduced Glutathione (GSH)

Reduced glutathione (GSH) was determined by the method of Ellman [17]. Briefly, the tissue homogenate (1.0 mL) was treated with 1.0 mL of 10% TCA. The mixtures were centrifuged in 5000 rpm and the supernatants were taken. After deproteinization, the supernatants were allowed to react with 1.0 mL of Ellman's reagent (30 mM, 5,5'-dithiobisnitro benzoic acid (DTNB) in 100 mL of 0.1% sodium citrate). The absorbance of the yellow product was read at 412 nm in a spectrophotometer. Pure GSH was used as the standard for establishing the calibration curve [18].

2.8. Lipid Extraction

Lipids of tissue samples were extracted with hexane–isopropanol (3:2, v/v) according to the method of Hara and Radin [19]. Tissue sample (1 g) was homogenized with 10 mL hexane-isopropanol mixture. Fatty acids in the lipid extracts were converted into methyl esters including 2% sulfuric acid (v/v) in methanol [20]. The fatty acid methyl esters were extracted with 5 mL n-hexane. Analysis of fatty acid methyl ester was performed in a Shimadzu GC-17A instrument gas chromatography equipped with a flame ionization detector (FID) and a 25 m, 0.25 mm Permabond fused-silica capillary column (Machery–Nagel, Germany). The oven temperature was programmed between 145 and 215 °C, at the heating rate of 4 °C/min. Injector and FID temperatures were 240 and 280 °C, respectively. The nitrogen carrier gas flow was 1 mL/min. The methyl esters of fatty acids were identified by comparison with authentic external standard mixtures analyzed under the same conditions. Class GC 10 software version 2.01 was used to process the data. The results were expressed as percent amount/tissue.

2.9. Saponification and Extraction

Lipid-soluble vitamins and phytosterols were extracted from the lipid fraction according to the method of Sanchez-Machado et al. [21] with minor modifications. 5.0 mL of n-

hexane/isopropyl alcohol mixture treated with 5.0 mL of KOH solution (0.5 M in methanol) were added and immediately vortexed for 20 s. The tubes were placed in a water bath at 80 °C for 15 min, and cooled in iced water. 1.0 mL of distilled water and 5.0 mL of hexane was added, and the mixture was rapidly vortexed for 1 min, then centrifuged at 5000 rpm for 5 min. The supernatant phases were transferred to another test tube and dried under nitrogen. The residue was redissolved in 1.0 mL of the mobile phase (68:28:4, v/v/v, methanol/acetonitrile/water). Finally, an aliquot of 20 μL was injected into the HPLC column. Before injection, the extracts were maintained at −20 °C away from light.

2.10. Chromatographic Conditions

Chromatographic analysis was performed using an analytical scale (15 cm × 0.45 cm) Supelco LC 18 DB column with a particle size 5 μm (Sigma, USA). HPLC conditions are given as follows: mobile phase 60:38:2 (v/v/v): acetonitrile/methanol/water; a flow rate of 1 mL/min; column temperature 30 °C. The detection was operated using two channels of a diode-array spectrophotometer, and cholesterol and lipid-soluble vitamins were identified by retention and spectral data [22,23].

2.11. Determination of Total Protein

Total protein was estimated by the method of Lowry et al. [24]. Tissue homogenate (10 μL) was added to a 4.0 ml of alkaline copper reagent, and the mix was kept at room temperature. Then 0.5 mL of Folin's-Ciocalteau reagent was added to the mixture and the developing color was read after 30 min at 750 nm. A standard curve was obtained using bovine serum albumin. The level of protein was expressed as mg albumin/g of tissue.

2.12. Statistical Analysis

The experimental results were reported as mean ± S.D. All the data were statistically evaluated with SPSS/15.0 software. The statistical significance of the data was determined using one-way analysis of variance (ANOVA), and the group means were compared by Duncan's multiple range test (DMRT). P values of less than 0.05 were considered to indicate statistical significance.

3. Results

Table 1 shows the postprandial blood glucose levels of Wistar rats at the starting and ending of the study. After the injection of STZ, the postprandial blood glucose levels were significantly increased in the D and D+A groups (p<0.001) when compared to the C group. At the ending of the study, the postprandial blood glucose level was significantly decreased in the D+A group (p<0.001) compared to the D group.

Table 2 shows the lipid-soluble vitamins, GSH, total protein and MDA levels of liver tissue. Compared to the C group, retinol, α-tocopherol, vitamin K_1, vitamin K_2, cholesterol, total protein and MDA levels were increased in the D and D+A groups (p<0.001). Vitamin D_2 level was decreased in the D group, but its level was increased in the D+A group (p<0.001). GSH level was decreased in the D and D+A groups (p<0.001) in comparison to the C group.

Table 3 shows the lipid-soluble vitamins, GSH, total protein and MDA levels of kidney tissue. Retinol, α-tocopherol, δ-tocopherol, vitamin D_2, vitamin D_3 levels were increased in the D and D+A groups compared to the C group (p<0.001). Vitamin K_2, GSH and total protein levels were decreased in the D and D+A groups (p<0.001). Cholesterol level did not differ among all the groups (p>0.05). Vitamin K_1 level was increased in the D group (p<0.001); but its level was decreased in the D+A group (p<0.001) compared to the C group. MDA level was increased in the D group (p<0.001); but its level was protected in the D+A group in comparison to the C group.

Table 4 shows the fatty acid composition of liver tissue. Compared to the C group, 16:1, 18:2, 20:4, PUFA and USFA fatty acid levels were decreased in the D and D+A groups (p<0.001). 18:0, 18:1, 22:6, SFA and MUFA fatty acid levels were increased in the same groups (p<0.001). 16:0 level did not differ among all the groups (p>0.05) in comparison to the C group.

Table 5 shows the fatty acid composition of kidney tissue. 16:0, 16:1, 18:1, 18:2, MUFA and USFA fatty acid levels were decreased in the D and D+A groups (p<0.001) in comparison to the

C group. 18:0, 20:4, 22:6 and PUFA fatty acid levels were increased in the same groups (p<0.001). SFA level did not differ among all the groups (p>0.05).

Table 1. The postprandial blood glucose levels of Wistar rats

Blood Glucose Level (mg/dL)	Control	Diabetes	Diabetes + Almond
Before STZ	100.25 ± 0.67^a	96.75 ± 1.23^b	93.23 ± 0.97^c
After STZ	92.25 ± 0.31^c	296.92 ± 1.33^b	326.46 ± 4.61^a
End of Study	107.50 ± 0.89^c	431.75 ± 12.80^a	342.28 ± 6.74^b

Data include mean±SD values for ten rats in each group. There is not any statistically difference among the groups described with same letters. P<0.05

Table 2. The biochemical parameters in liver of Wistar rats

Biochemical Parameters	Control	Diabetes	Diabetes + Almond
Retinol (µmol/g)	2.11 ± 0.02^c	4.32 ± 0.07^a	4.02 ± 0.04^b
α-tocopherol (µg/g)	15.73 ± 0.14^c	108.92 ± 1.73^b	118.74 ± 4.62^a
Vitamin D_2 (µg/g)	0.96 ± 0.03^b	0.71 ± 0.01^c	1.31 ± 0.01^a
Vitamin K_1 (µg/g)	2.36 ± 0.08^c	7.91 ± 0.11^a	5.77 ± 0.14^b
Vitamin K_2 (µg/g)	2.65 ± 0.02^c	5.58 ± 0.05^a	3.91 ± 0.03^b
Cholesterol (µmol/g)	1.79 ± 0.01^c	3.58 ± 0.03^a	3.27 ± 0.02^b
GSH (µmol/g)	10.25 ± 0.06^a	7.11 ± 0.04^b	7.12 ± 0.02^b
Total Protein (µg/g)	151.95 ± 1.38^c	172.34 ± 2.05^b	189.24 ± 1.94^a
MDA (nmol/g)	34.28 ± 0.77^b	44.82 ± 1.44^a	50.32 ± 3.00^a

Data include mean±SD values for ten rats in each group. There is not any statistically difference among the groups described with same letters. P<0.05

Table 3. The biochemical parameters in kidney of Wistar rats

Biochemical Parameters	Control	Diabetes	Diabetes + Almond
Retinol (µmol/g)	3.55 ± 0.04^c	6.82 ± 0.11^a	5.94 ± 0.01^b
α-tocopherol (µg/g)	24.94 ± 0.27^c	95.78 ± 0.29^a	75.81 ± 0.16^b
δ-tocopherol (µg/g)	0.27 ± 0.08^c	0.62 ± 0.01^a	0.43 ± 0.01^b
Vitamin D_2 (µg/g)	0.18 ± 0.02^c	0.23 ± 0.01^b	0.37 ± 0.01^a
Vitamin D_3 (µg/g)	0.20 ± 0.02^c	0.27 ± 0.01^b	0.39 ± 0.01^a
Vitamin K_1 (µg/g)	1.31 ± 0.01^b	2.08 ± 0.03^a	1.19 ± 0.02^c
Vitamin K_2 (µg/g)	14.04 ± 0.03^a	6.22 ± 0.02^b	4.51 ± 0.02^c
Cholesterol (µmol/g)	4.45 ± 0.01	4.28 ± 0.02	4.10 ± 0.01
GSH (µmol/g)	4.03 ± 0.18^a	1.85 ± 0.01^c	3.01 ± 0.03^b
Total Protein (µg/g)	86.94 ± 1.87^a	63.55 ± 0.70^c	67.47 ± 1.16^b
MDA (nmol/g)	22.32 ± 0.24^b	30.19 ± 0.75^a	23.35 ± 0.22^b

Data include mean±SD values for ten rats in each group. There is not any statistically difference among the groups described with same letters. P<0.05

Table 4. The fatty acid composition in liver of Wistar rats (%)

Fatty Acids	Control	Diabetes	Diabetes + Almond
16:0	17.02±0.20	17.18±0.15	17.12±0.21
16:1	1.71±0.01[a]	1.22±0.02[b]	0.89±0.03[c]
18:0	15.85±0.18[c]	19.57±0.15[b]	20.20±0.19[a]
18:1	3.54±0.13[c]	7.68±0.05[b]	8.66±0.15[a]
18:2	20.17±0.24[a]	18.63±0.15[c]	19.28±0.17[b]
20:4	33.11±0.15[a]	23.84±0.21[b]	22.81±0.20[c]
22:6	4.52±0.06[c]	5.45±0.07[b]	5.83±0.09[a]
Others	4.08±0.21	6.43±0.39	5.21±0.19
∑SFA	32.87±0.30[b]	36.75±0.17[a]	37.32±0.26[a]
∑MUFA	5.25±0.36[c]	8.90±0.06[b]	9.55±0.15[a]
∑PUFA	57.80±0.37[a]	47.92±0.31[b]	47.92±0.30[b]
∑USFA	63.05±0.37[a]	56.82±0.30[b]	57.47±0.27[b]

MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; SFA: saturated fatty acids; USFA: unsaturated fatty acids. Data include mean±SD values for ten rats in each group. There is not any statistically difference among the groups described with same letters. $P<0.05$

Table 5. The fatty acid composition in kidney of Wistar rats (%)

Fatty Acids	Control	Diabetes	Diabetes + Almond
16:0	21.71±0.18[a]	16.31±0.19[b]	16.11±0.16[b]
16:1	4.29±0.11[a]	0.92±0.03[b]	0.89±0.02[b]
18:0	11.25±0.25[b]	16.72±0.14[a]	16.17±0.20[a]
18:1	17.96±0.11[a]	10.44±0.07[c]	11.30±0.21[b]
18:2	19.47±0.32[a]	16.89±0.14[c]	17.70±0.10[b]
20:4	21.44±0.34[c]	29.10±0.22[a]	27.64±0.10[b]
22:6	0.94±0.02[c]	1.93±0.03[b]	2.06±0.10[a]
Others	2.94±0.09	7.69±0.85	8.15±0.98
∑SFA	32.96±0.33	33.03±0.23	32.27±0.20
∑MUFA	22.25±0.13[a]	11.36±0.09[c]	12.19±0.21[b]
∑PUFA	41.85±0.32[b]	47.92±0.23[a]	47.39±0.16[a]
∑USFA	64.10±0.41[a]	59.28±0.24[b]	59.59±0.26[b]

MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; SFA: saturated fatty acids; USFA: unsaturated fatty acids. Data include mean±SD values for ten rats in each group. There is not any statistically difference among the groups described with same letters. $P<0.05$

4. Discussion

The reduction of fasting and postprandial blood glucose level is very important for preventing of some specific diabetes complications in the diabetes patients [25]. In previous studies, it has been shown that almond contains the phenolic compounds, such as catechin, epicatechin, cyaniding and procyanidin [26,27]. Also it was suggested that procyanidins blocked the activity of alpha glucosidase enzyme to digest the carbohydrates in the intestine [28]. It was reported that the epicatechin regenerates the beta cells in the pancreas [29], and the catechin and cyanidins inhibit the glucose absorption in the intestine [30-32]. In this study, the postprandial glucose level was significantly decreased in the bitter almond extract given compared to the diabetes group. In the previous studies, it was shown that the consumption or administration of almond was significantly reducing the blood glucose levels in the humans and experimental animals [13,33-36]. The reduction of postprandial glucose level in the diabetic group treated with bitter almond extract can be explained according to above mentioned studies and/or these relationships.

The reactive oxygen species (ROS) generated in the oxidative stress, are toxic for the cells. These affect the lipid bilayer in the cell membrane and cause the formation of the lipid peroxidation [37]. The products of lipid peroxidation are harmful to most cells in the body and are associated with a variety of diseases, such as atherosclerosis and brain damage [38]. Our study and previous studies have shown that MDA level was increased in the liver, kidney and other tissues of diabetic rats [2]. The liver is the most important center of the body detoxification. Therefore, in this tissue, increasing of MDA level can be caused by some substances, which are contained bitter almond. Compared to the C group, the MDA level was significantly decreased in the kidney of the bitter almond extract given D+A group. This decrease may be due to various phytochemical components in the bitter almond extract, such as vitamins, sterols, antioxidant compounds, and phenolic composition with potential healthy biological properties. It was determined in previous studies that these compounds prevented of aging, cardiovascular disease, diabetes, and cancer [39,40].

The liver is the most important center of the body detoxification. Therefore, increasing of MDA level and decreasing of GSH level in liver can be caused by some substances, such as amygdaline. The bitter almonds contain this compound and in the in the presence of water (hydrolysis), amygdaline yields glucose and the chemicals, benzaldehyde and hydrocyanic acid (HCN). HCN, the salt of which is known as cyanide, is poisonous and bitter almonds yield 4-9 mg of hydrogen cyanide per bitter almond [41]. In the present study, the hydrogen cyanide can formed in the diabetes + almond group rats; consequently the MDA level was increased, GSH level was decreased in the liver tissue of bitter almond extract given group.

In this study, the total protein levels and GSH were significantly decreased in the kidney tissue of D and D+A groups. The previous studies have shown that the decreasing of GSH level was inhibited the synthesis of protein, and therefore the total protein levels were significantly decreased in the tissues [42].

In the present study, it was observed that GSH level was decreased in the D and D+A groups of the liver and kidney tissues. This fact can be caused by diabetes mellitus. Because, previous studies have shown that GSH level decreased in some tissues of streptozotocin-induced diabetic rats, such as brain, liver, and kidney [43].

The changes of fatty acid composition were reported in the experimental and clinical diabetes [44,45]. Diabetes can change the fatty acid composition because it inhibits Δ-6 and Δ-5 desaturase enzyme activities due to the decreased amount of metabolic products related with these enzymes [45-49]. Δ-6 desaturase is first and rate-limiting microsomal enzyme and catalyzes the synthesis of the arachidonic acid (20:4 n-6) and docosahexaenoic acid (22:6 n-3) from linoleic acid (18:2 n-6) and linolenic acid (18:3 n-3) [50].

In our study, although the bitter almond generally did not have effects on the fatty acid levels in the diabetic rats, the arachidonic acid level was significantly decreased in the liver of diabetic rats, and the docosahexaenoic acid level was significantly increased in the same tissue. Previous studies have shown that arachidonic

acid levels were decreased in the experimental and clinical diabetes [46,51,52].

The arachidonic acid and docosahexaenoic acid levels were significantly increased in the kidney tissue of the D and D+A groups compared to the control group. These increases may be related to increased activity of the Δ-6 desaturase enzyme in the kidney tissue. These results are explaining the reduction of linoleic acid level. When the Δ-6 and Δ-5 desaturase enzyme activities are suppressed in diabetes, the linoleic acid level can be decreased [46,52-55].

In the present study, while stearic acid and oleic acid levels were increased in the liver tissue of the D and D+A groups; the palmitoleic acid and linoleic acid levels were decreased in the same groups. The decrease of palmitoleic acid level can be attributed to the suppression of Δ-9 desaturase enzyme activity in the liver tissue [56]. Δ-9 desaturase, also known as stearoyl-CoA desaturase (SCD), is a key enzyme, which catalyzes the biosynthesis of monounsaturated fatty acids (MUFA) from saturated fatty acids [46].

The palmitic acid and palmitoleic acid levels were decreased in the kidney tissue of D and D+A groups compared to control group. Previous studies have reported that the palmitic acid level was decreased in the diabetes [44,55]. Additionally, while the strearic acid level was increased in the D and D+A groups, the oleic acid level was decreased in kidney tissue of these groups. This fact can be explained by inhibition of Δ-9 desaturase enzyme activity in the diabetes [46,57].

Vitamins cannot be synthesized by humans and therefore humans need to take these compounds to prevent metabolic disorders. Vitamin K is a fat-soluble vitamin essential for the functioning of several proteins involved in blood clotting [58]. Yoshida et al. [59] reported the positive effects of vitamin K providing glucose homeostasis for men and women. In contrast to their study, in our study, while the vitamin K_2 level was significantly increased in the liver tissue, its level was significantly decreased in the kidney tissue of the D and D+A groups compared to control group.

Vitamin E (α-tocopherol) is a lipophilic antioxidant and inhibits lipid peroxidation [60]. In the present study, the α-tocopherol level was significantly increased in the liver and kidney tissue of the D and D+A groups in comparison to control group. This increase was probably due to the deterioration of vitamin E binding protein activity. This fact may be caused by STZ. The highest α-tocopherol levels were observed in the bitter almond group. The STZ may have increased the vitamin E level in these tissues due to it has toxic properties.

The two major biologically inert precursors of vitamin D are vitamin D_3 (cholecalciferol) and vitamin D_2 (ergocalciferol). The vitamin D has an important role in the treatment and pathogenesis and/or progression of several disorders including some cancers, cardiovascular disease, hypertension and diabetes [61]. Some researches reported that vitamin D endocrine system has a pivotal role in the regulation of insulin secretion of the pancreatic beta cells [62-64]. In the present study, although vitamin D levels were significantly decreased in the D group of liver tissue; their level was significantly increased in the D+A groups of kidney and liver tissues.

Vitamin A is required for normal growth, vision, and to fight infections [65]. Researches have identified that level of vitamin A (Retinol) was increased in the liver of rats in experimental diabetes. Also, the retinol carrier protein with the transthyretin level is decreased in the liver, kidney and plasma [66]. In this study, the retinol level was significantly increased in the liver and kidney tissues of D and D+A groups. This fact can be explained by decreasing of retinol binding protein and transthyretin levels in these tissues. Basu and Basulado [66] showed that retinol binding protein and transthyretin levels were decreased in the plasma and tissues of experimental diabetes.

Diabetes mellitus is associated with significant abnormalities in lipoprotein metabolism. This relationship is associated with accelerated atherosclerosis and subsequent cardiovascular disease [67,68]. Diabetes causes certain metabolic abnormalities such as high blood cholesterol and triglyceride [69]. In the present study, while the cholesterol level was significantly increased in the liver of D and D+A groups; its level was not changed in the kidney of the same groups compared to control group. Cholesterol is one of the most important

molecules forming cell structure. SREBP-1c and SREBP-2 are sterol regulatory element binding proteins and they control gene expression for acetyl CoA carboxylase, steroyl CoA desaturase 1 and 2 enzymes in the fatty acid synthesis as transcription factors. It was determined that the activity of these factors decreased in the STZ-induced diabetic rats, and the application of insulin recovered this activity after occurring of diabetes [70,71]. In our study, the administration of STZ may affect these factors.

In conclusion, the postprandial blood glucose level was decreased in the diabetic group treated with bitter almond extract compared to the diabetes group. This study has shown that the GSH level was significantly decreased in the diabetes group and diabetic group treated with bitter almond extract, and the MDA level was significantly increased in the liver tissue of the same groups. In kidney tissue, although the GSH level was significantly decreased in the diabetes group, its level was protected in the diabetic group treated with bitter almond extract. Also in the same tissue, MDA level was significantly increased in the diabetes group, its level significantly decreased in the diabetic group treated with bitter almond extract. The bitter almond and STZ administrations affected the amount of some important fatty acids which substrates in fatty acid metabolism on duty enzymes when compared to control group values. Additional improved studies can be performed to understand the reasons of these situations in more detail. The observed positive results (decrease of postprandial blood glucose and MDA levels and protection of GSH level for the kidney tissue) can be attributed to bitter almond kernel, because it contains some important phytochemical compounds.

5. Acknowledgements

6. References

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