Almond oil and extracted diosmin as prophylaxis for the endothelial dysfunction in diabetic rats

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ABSTRACT

The present study was designed to evaluate the efficacy of almond oil and diosmin extracted from lemon peels on the endothelial dysfunction in streptozotocin induced diabetic rats. The study was performed on 75 male white albino rats divided into 5 groups. Healthy rats served as control group, diabetic group, almond oil treated diabetic group, dimethylsulfoxide (DMSO) treated diabetic group, and diosmin treated diabetic group. Experimental diabetes was induced by single subcutaneous injection of 50mg/kg body weight streptozotocin. After two months blood samples were collected for assessment of serum triglycerides(TG), total, high density lipoprotein(HDL), and low density lipoprotein(LDL) cholesterol, fasting blood sugar (FBS), serum insulin, intercellular adhesion molecule-1 (ICAM-1), nitrite and nitrate (NOx), plasma hydrogen peroxide (H₂O₂), glutathione peroxidase activity (GPX) and percent of DNA damage. Mean levels of total cholesterol, TG, LDL-cholesterol, FBS, percent of DNA damage and ICAM-1 were significantly low and HDL-cholesterol, insulin were significantly high in almond oil treated diabetic group compared to diosmin treated diabetic group. On the other hand no significant difference was observed between the two groups regarding NOx, GPX and H₂O₂. Supplementation with almond oil and diosmin exerted an anti-atherogenic effect with amelioration of glycemic status along with decreased DNA damage in STZ induced diabetic rats, these effects are more pronounced in diabetic group treated almond oil. So it is recommended to use almond oil as a prophylactic measure for endothelial dysfunction in diabetics.

Key words: Diabetes, endothelial dysfunction, almond oil, diosmin.

INTRODUCTION

The endothelium is the thin layer of cells that lines the interior surface of blood vessels and lymphatic vessels, the heart, and body cavities. It acts as a blood container; in addition, it actively regulates the passage of nutrients, hormones, and macromolecules into the surrounding tissue [1]. It can detect chemical substances within the blood and physical forces imparted to blood vessel walls (i.e. shear stress and distention) and initiate responses to these chemical and/or physical signals by releasing substances that modulate vascular tone and/or blood vessel structure. Endothelial dysfunction can be defined as the partial or complete loss of balance between vasoconstrictors and vasodilators, growth promoting and inhibiting factors, proatherogenic and anti-atherogenic factors, and pro-coagulant and anti-coagulant factors [2].

Hyperglycemia is a characteristic feature of diabetes and plays a pivotal role in diabetes associated microvascular and macrovascular complications [3]. Endothelial dysfunction comprises a number of functional alterations in the vascular endothelium that are associated with diabetes and cardiovascular disease, including impairment of nitric oxide (NO) production and activity, enhanced generation of reactive oxygen species (ROS), elevated expression and
plasma levels of different vasoconstrictors, increased adhesion molecule expression, and associated enhanced adhesion of vascular cells (e.g. platelets and monocytes) to the endothelium [3]. Increase of glucose oxidation leading to enhanced intracellular production of free radicals (e.g. superoxide and hydrogen peroxide) which has been linked to diabetes-induced which endothelial dysfunction [4], the augmented production of ROS can also promote the inactivation of antioxidant proteins and therefore reduce the antioxidant defense mechanisms [5].

Furthermore, endothelial cells in patients with diabetes are not able to produce sufficient amount of NO and therefore fail to vasodilate in response to vasodilators (e.g. acetylcholine, bradykinin, shear stress) [6].

The key components of diabetic dyslipidemia are elevated plasma low density lipoproteins (LDL), very low-density lipoproteins (VLDL), triglycerides (TG), circulating free fatty acids (FFAs) and lowered high density lipoprotein – cholesterol (HDL-C) [7]. Excess dense LDL is a strong risk factor for cardiovascular disease, it accumulates in the extracellular sub endothelial space of arteries causes toxicity to vascular cells thereby leading to atherosclerosis, hypertension, obesity and functional depression in some organs [8].

The plasma concentrations of a pro-inflammatory cytokine and tumor necrosis factor alpha (TNFα) are increased in diabetes [9]. They mediate opposite actions on the vasculature thus promote the increase of adhesion molecules intercellular adhesion molecule-1 (ICAM-1) one of the most sensitive cell markers for detecting the atherosclerotic plaque formation process in the endothelial cells [10].

Almond, scientifically known as Prunus dulcis, belongs to the family Rosaceae and is also related to stone fruits such as peaches, plums and cherries [11].

A number of cohort studies have shown a negative association between nut consumption and risk of heart disease [12]. Further benefits of almonds may result from their high polyunsaturated: saturated fatty acid ratio, nut protein, plant sterols, fiber, and associated phenolic substances which possess potent free radical scavenging capacities, depress postprandial glycemia and ROS production [13].

Flavonoids are a large group of polyphenolic compounds naturally occurring in several plants and fruits as glycosides or, less frequently, as their glycosides. Diosmin (diosmetin 7-O-rutinoside), a natural flavones glycoside is readily obtained by dehydrogenation of the corresponding flavanone glycoside, hesperidin that is abundant in the pericarp of various citrus fruits [14]. Diosmin possesses blood lipid lowering [15] and anticarcinogenic activities [16]. It enhances venous tone and microcirculation and protects capillaries [17], mainly by reducing systemic oxidative stress [18]. It has been shown to improve factors associated with diabetic complications [19].

The present study was undertaken to determine the ability of the natural dietary supplement of plants origin namely almond oil and diosmin extracted from lemon peels to modulates biochemical changes of endothelial dysfunction in streptozotocin induced diabetic rats.

**EXPERIMENTAL SECTION**

**Materials**

STZ was purchased from sigma chemical co. (St. Louis, Mo, U.S.A).

**Experimental Protocol**

Male white albino rats, with an initial weight 200-210gm, were obtained from the animal house of the National Research Centre, Cairo, Egypt. Animal procedures were performed in accordance with the Ethics Committee of the National Research Centre.

All rats were housed individually in stainless steel cages for two months they were fed standard rodent chow and water was available ad libitum. They were kept in standard conditions of temperature and light. Rats were divided into 5 groups with 15 animals in each group as follow:

Control group: healthy rats injected with 50mg/kg body weight sodium citrate buffer subcutaneously [20].

Diabetic group: diabetic rats, diabetes was induced by single subcutaneous injection of 50mg/kg body weight streptozotocin. The animals were considered diabetic if fasting glucose level was 200mg/dl after 48 hours of the injection [21].
Almond oil treated diabetic group included rats received intragastric almond oil at concentration of 1.5 mL / kg body weight once daily [22].

Dimethylsulfoxide (DMSO) treated Diabetic group: rats received intragastric DMSO1ml/kg body weight [23].

Diosmin treated diabetic group: rats received intragastric diosmin at concentration of 100 mg / kg body weight dissolved in 0.6 % DMSO once daily [23].

**Extraction of almond oil**
The dried powdered almond seeds were extracted with n-hexane in a soxhlet apparatus, the oil was obtained after evaporation of the solvent at 40˚C under reduced pressure on a rotavapour apparatus [24].

**Extraction of diosmin from citrus peel**
The dried, minced citrus lemon peels were extracted using organic solvents with increasing polarities (Ether, Chloroform, Ethyl acetate, and finally 70% methanol) in a soxhlet apparatus [25].

**Biochemical Analysis**
At the end of the experiment (two months) the animals were fasted 14-16 hours. Blood samples were withdrawn from the orbital vein using ether as general anesthetic. Blood samples were divided into three portions, the first portion was collected in plan tube, and serum was separated at 4000 rpm for 15 min. for estimation levels of fasting blood sugar, NOx, total cholesterol, triglycerides, HDL-cholesterol, insulin and ICAM-1. A second portion was collected in heparinized tube for measure glutathione peroxidase, then plasma was separated for determination of hydrogen peroxide. The third portion was collected in tube contained EDTA as anticoagulant to measure percent of DNA damage using the comet assay.

**Serum Glucose**
It was performed according to the method of Passing and Bablok [26], using kit supplied by Biocon Diagnostic, Germany.

**Serum insulin**
It was performed according to the method of Judzewitsch et al. [27] using kit provided by DRG, USA.

**Lipid profile**
Total cholesterol [28], triglycerides [29], HDL-cholesterol [30] were determined using kits supplied by Biocon Diagnostic, Germany. LDL-cholesterol was calculated according to equation developed by Friedewald [31].

\[
LDL= \text{total cholesterol} - (\text{HDL-cholesterol} + \text{triglyceride})
\]

**Quantitative determination of GPX activity**
It was assessed according to the method of Kraus and Ganthen [32].

**Quantitative determination of serum H₂O₂**
It was assessed according to the method of Davies [33].

**Quantitative determination of serum NOx**
It was measured by the modified Griess method [34].

**Quantitative determination of serum ICAM-1**
This was determined by ELISA using Kit provided by RayBio [35].

**Comet Assay**
Comet assay has been developed to detect cellular DNA damage. It was performed according to Singh et al.[36] with modifications according to Blasiak et al.[37].

**Methodology of the comet assay**
Lymphocytes were isolated by Ficoll–Hypaque density gradient (Pharmacia LKB Biotechnology, Piscataway, NJ, USA) and washed by phosphate-buffered saline (PBS) at pH 7.4. Ten µl of the cells were suspended in 75 µl of 0.5% low melting agarose for pipetted on microscopic slides with a layer of 1% agarose, spread using a coverslip and maintained on an ice-cold flat tray for 5min to solidify. After removal of the coverslip, the slides were immersed in cold lysis solution at 4 °C for 1 h, followed by electrophoresis at 25 V, 300 mA, for 40 min at steady temperature.
After electrophoresis, the slides were gently removed from the tank and washed three times with neutralising buffer 0.4 M Trisma base at pH 7.5 for 10 min. Twenty μl ethidium bromide (10 μg/ml) was added to each slide.

Visualization and analysis of Comet Slides
The slides were examined at 40× magnification using an inverted fluorescence microscope (IX70; Olympus, Tokyo, Japan) equipped with an excitation filter of 549 nm and a barrier filter of 590 nm, attached to digital camera (Olympus). Damaged cells were visualized by the “comet appearance”, with a brightly fluorescent head and a tail to one side formed by the DNA containing strand breaks that were drawn away during electrophoresis. Samples were analyzed by counting the damaged cell out of 100 cells per slide to calculate the percent of damage.

Statistical Analyses
Data entry and analysis were done using the statistical package for the social sciences (SPSS) program, version 16 and Microsoft Excel 2007. Data are presented means ± standard error (SE). The significance difference between values was estimated using student’s t-test. A p value less than 0.05 was considered to indicate a statistically significant difference.

RESULTS AND DISCUSSION

Effects of almond oil and diosmin on FBS, insulin and lipid profile
Mean concentration of FBS of diabetic groups received almond oil and diosmin was found to be significantly low compared to the diabetic group and diabetic group received DMSO respectively. The mean FBS of diabetic group received almond oil was significantly low compared to diabetic group received diosmin. The recorded values of serum insulin were significantly high in diabetic groups received almond oil and diosmin compared to the diabetic group and diabetic group received DMSO respectively. On the other hand the mean of serum insulin in diabetic group received almond oil was significantly high compared to diabetic group received diosmin. The results of the present study revealed that levels of cholesterol, triglyceride, LDL-cholesterol were significantly low, and HDL-cholesterol was significantly high in diabetic group received almond oil compared to diabetic group received diosmin (Table 1).

<table>
<thead>
<tr>
<th>Groups</th>
<th>F.B.S (mg/dl)</th>
<th>Insulin (μIU/ml)</th>
<th>Cholesterol (mg/dl)</th>
<th>TG (mg/dl)</th>
<th>LDL-C (mg/dl)</th>
<th>HDL-C (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>95.7 ±4.56</td>
<td>145 ±7.7</td>
<td>122 ±2.9</td>
<td>98.6 ±2.2</td>
<td>54.8 ±0.81</td>
<td>49.8 ±1.3</td>
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<tr>
<td>Diabetic</td>
<td>275 ±8.8</td>
<td>40.9 ±3.7</td>
<td>188 ±2.3</td>
<td>162.9 ±3.2</td>
<td>119 ±0.94</td>
<td>174 ±0.90</td>
</tr>
<tr>
<td>P1 &lt; .00</td>
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<tr>
<td>Almond oil diabetic</td>
<td>177.8 ±7.2</td>
<td>97.8 ±2.1</td>
<td>141 ±2.5</td>
<td>127.5 ±2.0</td>
<td>71 ±0.78</td>
<td>45.3 ±0.5</td>
</tr>
<tr>
<td>DMOSO diabetic</td>
<td>277 ±12.04</td>
<td>41.2 ±3.8</td>
<td>183 ±2.2</td>
<td>162 ±3.2</td>
<td>114 ±1.1</td>
<td>37.9 ±0.45</td>
</tr>
<tr>
<td>P2 &lt; .00</td>
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<td>P2 &lt; .00</td>
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<td>P2 &lt; .00</td>
</tr>
<tr>
<td>Diosmin diabetic</td>
<td>226 ±4.02</td>
<td>83.7 ±4.4</td>
<td>132 ±1.1</td>
<td>139.9 ±1.6</td>
<td>80 ±1.09</td>
<td>42.2 ±0.59</td>
</tr>
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<td>P3 &lt; .00</td>
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</tr>
</tbody>
</table>

F.B.S Fasting blood sugar    TG Triglyceride
HDL-C High density lipoprotein Cholesterol
LDL-C Low density lipoprotein Cholesterol

Effects of almond oil and diosmin on NOx , GPX activity, H2O2 and ICAM-1
The recorded values of NOx in diabetic group received almond oil were significantly high compared to diabetic group. Also it was significantly high in diabetic group received diosmin when compared to diabetic group received DMSO. The result showed that no significant difference in mean of NOx in diabetic group received almond oil and diabetic group received diosmin .

GPX activity was significantly high and mean concentration of plasma H2O2 was significantly low in diabetic group received diosmin and diabetic group received almond oil compared with diabetic group received DMSO and diabetic group respectively. There is no significant difference in activity GPX and concentration of plasma H2O2 between diabetic group received almond oil and diabetic group received diosmin .

Mean of ICAM-1 concentration was significantly low in diabetic group received almond oil and diabetic group received diosmin when compared to diabetic group and diabetic group received DMSO respectively. Our findings showed that mean concentration of ICAM-1 was significantly low in diabetic group received almond oil compared to diabetic group received diosmin (Table2)
Table 2: Mean levels of NOx, GPX activity, H$_2$O$_2$, ICAM-1 and % DNA damage in different studied groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>NOx (µmole/ml)</th>
<th>GPX activity (mU/mL)</th>
<th>H$_2$O$_2$ (µM/L)</th>
<th>ICAM-1 (pg/ml)</th>
<th>% DNA damage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>144 ± 4.8</td>
<td>765 ±15.5</td>
<td>565 ±4.2</td>
<td>94.4 ±2.7</td>
<td>4.1 ±0.37</td>
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<tr>
<td>Diabetic</td>
<td>79 ± 2.8</td>
<td>346.5 ±10.9</td>
<td>784 ±10.7</td>
<td>94.4 ±2.7</td>
<td>67.5 ±1.7</td>
</tr>
<tr>
<td></td>
<td>*P &lt; .00</td>
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<tr>
<td>Almond oil diabetic</td>
<td>118 ± 3.6</td>
<td>660 ±11.8</td>
<td>599 ±16.3</td>
<td>137.9 ±1.6</td>
<td>35.5 ±1.6</td>
</tr>
<tr>
<td></td>
<td>*P &lt; .00</td>
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<tr>
<td>DMSO diabetic</td>
<td>80 ± 4.8</td>
<td>383 ±7.1</td>
<td>760 ±10.9</td>
<td>212 ±4.4</td>
<td>65.5 ±1.16</td>
</tr>
<tr>
<td>Diosmin diabetic</td>
<td>112 ± 3.8</td>
<td>609 ±11</td>
<td>592 ±10.7</td>
<td>147 ±3.09</td>
<td>39 ±0.6</td>
</tr>
<tr>
<td></td>
<td>*P &lt; .00</td>
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<td></td>
<td>*P4 &lt; .3</td>
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<td></td>
<td>*NS</td>
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</table>

Effects of almond oil and diosmin on DNA damage

The comet analysis showed that the control group had the most compact DNA and maintained the circular form of a normal nucleus, with no evidence of comet formation. In contrast, cells from diabetic groups exhibited different degrees of a distorted appearance, diabetic group received almond oil showed the least distorted cells (Table 2).

Person’s correlation coefficients

Results of the present study showed positive correlation between ICAM1 and FBS, H$_2$O$_2$ and percent of DNA damage (Figure 1, 2, 3), in addition negative correlation with NOx and GPX (Figure 4, 5). As regard percent of DNA damage the results revealed positive correlation with FBS and H$_2$O$_2$ (Figure 6, 7), negative correlation was observed with NOx and GPX (Figure 8, 9).

Fig.1: Correlation between serum glucose and ICAM-1

\[ y = 1.376x - 16.38 \]

\[ R^2 = 0.842 \]
Fig. 2: Correlation between Serum ICAM-1 and H₂O₂

$$y = 0.434x - 125.1$$

$$R^2 = 0.802$$

Fig. 3: Correlation between ICAM-1 and DNA

$$y = 0.467x - 33.36$$

$$R^2 = 0.907$$

Fig. 4: Correlation between Serum ICAM-1 and NOx

$$y = -1.442x + 317.7$$

$$R^2 = 0.738$$
Fig. 5: Correlation between ICAM-1 and GPX

\[ y = -0.249x + 300.8 \]
\[ R^2 = 0.805 \]

Fig. 6: Correlation between serum glucose and % DNA damage

\[ y = 2.880x + 84.62 \]
\[ R^2 = 0.888 \]

Fig. 7: Correlation between DNA damage and H\(_2\)O\(_2\)

\[ y = 0.198x - 88.47 \]
\[ R^2 = 0.692 \]
This study investigated the effect of almond oil and extracted diosmin on endothelial dysfunction in diabetic rats. The present findings showed that the mean serum glucose level was significantly low and serum insulin level was significantly high in diabetic group treated almond oil compared to diabetic group. These results are in agreement with Wien et al. [38] who reported that intake almond was significantly associated with a reduction in fasting blood glucose, lower glycosylated hemoglobin value, and enhanced insulin sensitivity, suggested that the high oleic acid content in the almonds may improve beta-cell efficiency through enhanced intestinal secretion of glucagon-like peptide-1. As regard diabetic group received diosmin mean serum glucose levels was significantly low and serum insulin levels was significantly high compared to control diabetic group treated DMSO. These result in agreement with Srinivasan and Pari [23,19] who reported that the administration of diosmin to diabetic rats resulted in a significant restoration of the plasma glucose, insulin, glycosylated hemoglobin, and the activities of carbohydrate metabolic enzymes.

The management of diabetic dyslipidaemia, a well recognized and modifiable risk factor, is a key element in the multifactorial approach to prevent atherosclerosis in individuals with diabetes [39]. In the present study level of cholesterol, triglyceride, LDL-cholesterol was significantly high and HDL-cholesterol was significantly low in diabetic groups compared to control group. These are in agreement with finding of Otamere et al. [40] who reported that the most common lipid disorders associated with diabetes are increased level of cholesterol, triglyceride-rich lipoproteins, low levels of HDL and the presence of small dense as a result, more atherogenic LDL particles. After diosmin supplementation, significant decrease in level of cholesterol, triglyceride, LDL-cholesterol and significantly increase HDL-cholesterol in diabetic treated diosmin compared to diabetic group received DMSO. The results of the
present study revealed significant low levels of cholesterol, triglyceride, LDL-cholesterol and significant high HDL-cholesterol level in diabetic group treated diosmin compared to diabetic group treated DMSO. These results are in accordance with Mulero et al. [41] and Srinivasan and Pari [42] who reported that the administration of diosmin to diabetic animals stimulates insulin output that could activate lecithin cholesterol acyl transferase and lipoprotein lipase there by increasing the HDL fraction, lowering the intestinal absorption of cholesterol and enhancing the excretion of ingested cholesterol. As regard diabetic group treated almond oil level of cholesterol, triglyceride, LDL-cholesterol was significantly decreased and HDL-cholesterol was significantly increase compared to other diabetic groups. These results are in agreement with Li et al.[43] who reported that almond consumption is associated with improvements lipid profile. Also Berryman et al.[44] reported that total and LDL-cholesterol reduction associated with almond consumption, has been primarily attributed to the replacement of saturated fat with unsaturated fat where the major fatty acids in almonds are oleic acid and linoleic acid, accounting for 91–94% of its total lipids. It also rich phytosterols which may exert hypocholesterolemic effects via interactions with intracellular enzymes, acyl-CoA: cholesterol acyltransferase and hydroxyl methylglutaryl-CoA reductase, the rate limiting enzymes in cholesterol synthesis as well as viscous fiber in almond decreases LDL-cholesterol by disrupting enterohepatic circulation, thus increasing bile acid and cholesterol excretion and up regulating the LDL-cholesterol receptor [44]. Diabetic group treated almond oil showed a significant hypocholesterolemic effect compared to diabetic group treated diosmin.

The current study showed that mean GPx activity was significantly low and mean plasma H2O2 levels was significantly high in diabetic group compared to control group. This results are in accordance with Dinçer et al.[45] who referred this observation to decrease reduced glutathione (GSH) content reported in diabetic patients, since GSH is a substrate and cofactor for GPx, therefore GPx catalyzes the reduction of hydrogen peroxide and organic hydroperoxides into H2O and molecular oxygen by using GSH. Yasım et al.[46] reported increase in GPx activity by addition of diosmin–hesperidin combination to the diet, indicating its antioxidant properties. In the current study, GPx activity was significantly high and H2O2 was significantly low in diabetic group treated diosmin compared to diabetic groups treated DMSO. These results are in agreement with Srinivasan and Pari [19] who explained that, GPx activity were elevated in diabetic rats treated with diosmin through increase the biosynthesis of GSH and reduction of the oxidative stress as free radical scavenging ability of diosmin could exert a beneficial effect against pathogenic alterations caused by O2•− and OH•. As regard diabetic group treated almond oil GPx activity was significantly high and H2O2 was significantly low compared to diabetic groups. These results are in agreement with Li et al. [47] who reported that phytosterols of almond such as b-sitosterol induce the increase in the GSH/total glutathione ratio, induce the antioxidant enzymes glutathione peroxidase and superoxide dismutase. No significant difference in activity GPx and H2O2 between diabetic group treated almond oil and diabetic group treated diosmin was observed.

The accumulation of oxidative stress products and reduction of antioxidant defences, such as diminished activity of glutathione peroxidase, catalase and superoxide can cause damage to biological macromolecules as proteins, lipids and DNA. In consequence, this may lead to oxidative stress-related diseases, including atherosclerosis [48]. The current results revealed that the control group had the most compact DNA and maintained the circular form of a normal nucleus, with no evidence of comet formation. In contrast, cells from diabetic group exhibited a distorted appearance, indicating substantial DNA damage. Our results showed low percent of DNA comet assay in diabetic group treated diosmin compared to diabetic groups treated DMSO. These results are in agreement with Virginia et al.[49] who reported protective effect of detralex containing diosmin and hesperidin against oxidative DNA damage of lymphocytes of chronic venous insufficiency patients, as both are good chelators of free radicals because of reactivity of their hydroxyl groups. Due to binding metal ions, they inhibit Fenton reaction and thus limit DNA damage. In diabetic group received almond oil percent of DNA damage was significantly low compared to other diabetic groups as the antioxidant properties of almond flavonoid in protecting against LDL oxidation, as well as enhancing resistance to Cu2+ which participate in generation hydroxyl radical (OH•) that cause oxidative damage of DNA so it can prevent lipid peroxidation and restore DNA [50,51]. The percent of oxidative DNA damage was significantly low in diabetic group treated almond oil compared to diabetic group treated diosmin.

According to our results serum NOx concentration was significantly low in diabetic group compared to control group. These results are in agreement with finding of Suresh and Undurti [52] who reported that diabetes mellitus is associated with decreased nitric oxide production from endothelial cells, as high glucose level exacerbates aldose reductase activity leading to depletion of the NADPH required for the generation of nitric oxide from L-arginine by nitric oxide synthase. The diabetic group treated diosmin showed low mean NOx concentration compared to diabetic group received DMSO. This result is in agreement with Silambarasan and Raja [53] who reported that treatment with diosmin elevated plasma nitrite and nitrate levels, indicating its ability to protect nitric oxide from free radicals, thereby increasing its availability. As regard diabetic group treated almond oil mean serum NOx concentration was significantly high compared to other diabetic groups, as it contains sizeable amounts of L-
arginine, the precursor amino acid of the endogenous vasodilator nitric oxide [54]. On the other hand, it is rich in phenolic antioxidants and α-tocopherols which preserve bioavailability of nitric oxide [55]. No significant difference in mean NOx level was observed in diabetic group treated almond oil compared to diabetic group treated diosmin.

Hyperglycemia enhances activation of cytokines, nuclear factor κ-B (NF-κB) , TNF-α and IL-1 which lead to the expression of adhesion molecule ICAM-1, that facilitate monocyte adhesion to endothelial cells [56]. Our study showed that ICAM-1 concentration was significantly high in diabetic group compared to control group. As regard diabetic group treated almond oil, ICAM-1 was significantly low compared to other diabetic groups. These findings coincident with previous studies of López-Uriarte et al. and Damasceno et al.[57,58] as almond is rich in MUFA which causes inhibition in RNA expression of TNF-α messenger and also rich in vitamin E which increases the resistance of LDL against oxidative modification and inhibits LDL-induced adhesion of monocytes to endothelial cells [59]. Katherine and Caroline [60] reported that diosmin significantly reduced plasma level of ICAM-1 in patients with chronic venous insufficiency. This results is in accordance with our findings which demonstrated that ICAM-1 concentration was significantly low in diabetic group treated diosmin compared to control diabetic group received DMSO. In present study mean of ICAM-1 concentration was significantly low in diabetic group treated almond oil compared to diabetic group treated diosmin.

CONCLUSION

Supplementation with almond oil and diosmin exerted have an antihypercholesterolemic effect with amelioration of oxidative stress and glycemic status, along with decreased percent of DNA damage in STZ induced diabetic rats, these effects are more pronounced in almond oil treated group.

So administration of diosmin may be beneficial, but almond oil has more prophylactic effect in diabetic endothelial dysfunction.

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