Antiatherogenic Effect of Almond Oil in Streptozotocin Induced Diabetic Rats

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ABSTRACT

The present study was conducted to evaluate the antiatherogenic effect of almond oil in diabetic rats. Forty five male white albino rats were divided into 3 groups: Control group, diabetic group and diabetic almond oil treated 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 triglycerides, total, HDL, and LDL cholesterol, insulin, intercellular adhesion molecule-1 (ICAM-1), nitrite and nitrate (NOx), hydrogen peroxide (H2O2), glutathione peroxidase activity (GPX) and DNA damage. Results showed that mean 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. Mean concentrations of insulin, NOx , GPX activity were significantly high, and mean levels of H_2O_2 , ICAM-1, percent of DNA damage were significantly low in diabetic group received almond oil compared to diabetic group . The data confirmed property of almond oil as an antioxidant that ameliorates oxidative stress and revealed that it is efficiently improves endothelial function and protects against the development of atherosclerosis in STZ induced diabetic rats.

INTRODUCTION

Endothelial dysfunction is a systemic disorder and a critical element in the pathogenesis of atherosclerotic diseases and its complications (Chhabra, 2009).   In diabetic patients, endothelial dysfunction appears to be a consistent finding; indeed, there is general agreement that hyperglycemia and diabetes lead to an impairment of nitric oxide (NO) production and activity (Bakker *et al.,* 2009). The main components of diabetes include hyperglycemia, oxidative stress, chronic mild inflammation, and dyslipidemia; and they all are associated with increased risk of cardiovascular disease (Li *et al.,* 2011).

The key components of diabetic dyslipidemia are elevated plasma low density lipoproteins , very low-density lipoprotein, triglycerides , circulating free fatty acids   and lowered high density lipoprotein - cholesterol (Ansar *et al.,* 2011).

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Hypercholesterolemia decreases nitric-oxide production in human endothelial cells in vitro. Small dense LDL is a strong risk factor for cardiovascular disease, which is highly atherogenic and serves as a marker for atherosclerosis where the oxidation of LDL leads to its increased penetration of arterial walls (Arcari *et al.,* 2011). Excess LDL in the blood is accumulated in the extracellular sub-endothelial space of arteries and is highly atherogenic and toxic to vascular cells thereby leading to atherosclerosis (Schneider *et al.,* 2011). Conversely, HDL-cholesterol provides an atheroprotective effect by inhibiting cytokine-induced endothelial cell adhesion molecule expression and by enhancing agonist-induced vasodilation in coronary arteries (Brown and Hu 2001).

Atherosclerosis in diabetic patients has been linked to increased oxidative stress. Hyperglycemia directly produces a reactive oxygen species which induce endothelial injury, oxidative modification of LDL, and induction of redox-sensitive genes, including monocyte chemoattractant protein -1 and adhesion molecules such as vascular cell adhesion molecules (Creager *et al.,* 2003). In addition, oxidative stress activates protein kinase C and increases   the   production of advanced glycation end products.

On the other hand hyperglycemia affects activity of glutathione peroxidase which plays an important role in the antioxidant defense of the vascular wall, it transforms hydrogen peroxide into water and lipid peroxides into their respective alcohols. These molecular mechanisms seem to be mainly implicated in the development of atherosclerosis (Hamanishi *et al.,* 2004). ICAM-1 is one of the most important intercellular adhesion molecules involved in atherogenesis. The expression of endothelial ICAM-1 leads to attachment and migration of leukocytes into the vessel wall, and this process is generally considered to be an initial event in the pathogenesis of the early lesion of atherogenesis (Haubner *et al.,* 2007). The release of ICAM-1 from these cells has been found to be inducible by several inflammatory mediators and other factors including, the nuclear transcription factor-kappa B (NF- κ B), IL-1, TNF-α, angiotensin, IL8 , and oxidized LDL(Witkowska , 2005). Epidemiological studies have consistently demonstrated that frequent nut consumption is associated with reduced risk of developing coronary heart disease (CHD), and diabetes (López-Uriarte *et al.,* 2010). Almond, scientifically known as Prunus dulcis, belongs to the family Rosaceae and is also related to stone fruits such as peaches, plums and cherries(Jahanban *et al.,* 2009). Almonds contain high levels of fiber, arginine, magnesium, polyphenolic compounds, vitamin E, and monounsaturated fatty acids (MUFA), specifically oleic acid. These findings have generated proposed mechanisms for these associations including improved insulin sensitivity, increased antioxidant activity, and reduced concentrations of total cholesterol and low-density lipoprotein cholesterol (Wien *et al.,* 2010).

Aim of the work

The eventual objective of the present study was to evaluate the ability of the natural supplement of plant origin; almond oil to improve biochemical marker of endothelial dysfunction and oxidative stress in streptozotocin-nicotinamide-induced diabetic rats.

MATERIAL AND METHODS

Materials: STZ was purchased from sigma chemical co. (St. Louis, Mo, U.S.A)., male white albino rats, with an initial weight 200-210gm, were obtained from the animal house of the National Research Center, Giza, Egypt.

Experimental Protocol

All rats were housed individually in stainless steel cages for two months were fed standard rodent chow. They were kept in standard conditions of temperature and light.

Rats were divided into 3 groups with 15 animals in each group as follow:

- Control group included healthy rats injected with 50mg/kg body weight sodium citrate buffer subcutaneously (Fukuharu *et al.,* 2000).

- Diabetic group included 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 (Kohli *et al.,* 2004).

Diabetic almond oil treated group included rats received intragastric almond oil at concentration of 1.5 mL / kg body weight once daily (Zhao *et al.,* 2007).

Extraction of almond oil

The dried powdered almond seeds were extracted with n-hexane in a soxhlet apparatus, the oil was btained after evaporation of the solvent at 40˚C under reduced pressure on a rotavapour apparatus (The united states pharmacopeial conventions, 2012).

Blood Sample Collection

At the end of the experiment the animals were fasted 14-16 h. 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 15min 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 by the comet assay.

Biochemical Analysis

Determination of serum Glucose: This was performed according to the method of Passing and Bablok, 1983, using commercially kit supplied by Biocon Diagnostic, Germany.

Determination of serum insulin: The method was performed according to Judzewitsch *et al.,* (1982). Kit provided from DRG, USA.

Measurement of lipid profile: cholesterol (Allain *et al.,* 1974), triglycerides (Glick *et al.,* 1986), HDL-cholesterol (Lopez-Virella *et al.,* 1977) were determined using commercially kits supplied by Biocon Diagnostic, Germany. LDL-cholesterol was calculated according to equation developed by Friedewald (1972).

$$LDL = \text{total cholesterol} - \frac{(\text{HDL-cholesterol} + \text{triglycerides})}{5}$$

Quantitative determination of glutathione peroxidase activity in whole blood: This was assessed according to the method of Kraus and Ganthen, (1980), using kit supplied by Biodiagnostic .

Quantitative determination of serum hydrogen peroxide: This was assessed according to the method of Davies (1999) using kit supplied by Biodiagnostic.

Quantitative determination of serum nitrite/nitrate (NOx) as index of Nitric oxide: This was measured by the modified Griess method according to Tatsch *et al.,* 2011 using ELISA microplate readers.

Quantitative determination of serum ICAM-1 (Intercellular Adhesion Molecule-1): This was determined by ELISA using Kit provided by RayBio® Rat ICAM-1 (Springer, 1994).

Assessment of DNA Damage by Comet Assay

Comet assay has been developed as to detect cellular DNA damage. It was performed according to Singh and colleagues [Singh *et al.,* 1988] with modifications according to Blasiak and colleagues [Blasiak *et al.,* 2003].

Methodology

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. 10µl 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. To each slide, 20 µl ethidium bromide (10 µg/ml) was added.

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 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

Effects of almond oil on blood glucose and insulin:

Mean concentration of fasting blood sugar of diabetic group received almond oil was found to be significantly low compared to the diabetic group. The recorded values of serum insulin were significantly high in diabetic group received almond oil compared to the diabetic.

Effects of almond oil on lipid profile

The results showed that mean concentrations of serum cholesterol, serum triglyceride, serum LDL-cholesterol were significantly high and serum HDL-cholesterol was significantly low in diabetic treated almond oil group compared to diabetic group.

Effects of almond oil on GPX activity and H_2O_2

GPX activity was significantly high and mean concentration of plasma H_2O_2 activity was significantly low in diabetic treated almond oil group compared to diabetic group.

Effects of almond oil on NOx

Mean concentration of serum NOx was significantly low in diabetic group compared to control group. The recorded values of serum NOx in diabetic treated almond oil group was significantly high compared to diabetic group.

Table. 1: Mean ± SE of the investigated parameters in the different studied groups.

Groups	Control group (n=15)	Diabetic group (n=15)	Diabetic treated almond oil group (n=15)
Parameters			
F.B.S mg/dl	95.7 ±4.56	275 ±8.8	177.8 ±7.2
P value	$P_1 < .00^*$		$P_2 < .00^*$
Serum insulin µIU/ml	145 ±7.7	40.9 ±3.7	97.8 ±2.1
P value	$P_1 < .00^*$		$P_2 < .00^*$
Serum Cholesterol mg/dl	122 ±2.9	188 ±2.3	141.5 ±2.5
P value	$P_1 < .00^*$		$P_2 < .00^*$
Serum Triglycerides mg/dl	98.6 ±2.2	162.9 ±3.2	127.5 ±2.0
P value	$P_1 < .00^*$		$P_2 < .00^*$
Serum HDL-cholesterol mg/dl	49.8 ±1.3	37.4 ±0.90	45.3 ±0.5
P value	$P_1 < .00^*$		$P_2 < .00^*$
Serum LDL-cholesterol mg/dl	54.8 ±0.81	119 ±0.84	71 ±0.78
P value	$P_1 < .00^*$		$P_2 < .00^*$
Plasma H_2O_2 µM / L	565 ±4.2	784 ±10.7	599 ±16.3
P value	$P_1 < .00^*$		$P_2 < .00^*$
GPX activity mU / mL	765 ±15.5	346.5 ±10.9	660 ±11.8
P value	$P_1 < .00^*$		$P_2 < .00^*$
Percent of DNA damage %	4.1 ±0.37	67.5 ±1.7	35.5±1.6
P value	$P_1 < .00^*$		$P_2 < .00^*$
NOx µmole/ml	144± 4.8	79 ± 2.8	118 ± 3.6
P value	$P_1 < .00^*$		$P_2 < .00^*$
Serum ICAM-1 pg/ml	94.4 ±2.7	94.4 ±2.7	137.9 ±1.6
P value	$P_1 < .00^*$		$P_2 < .00^*$

Data are presented as mean ± SE. *P < 0.05 =significant. Significant difference was demonstrated between the following group: P_1 = control group versus diabetic group, P_2 = diabetic group versus group diabetic treated almond oil group.

Effects of almond oil on ICAM-1

Mean of ICAM-1 concentration was significantly elevated in diabetic group compared to control group. It was significantly low in diabetic treated almond oil group compared to diabetic group.

Effects of almond oil 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 group exhibited a distorted appearance .As regard diabetic treated almond oil group percent of DNA damage was significantly low compared to diabetic groups (fig. 1,2,3).
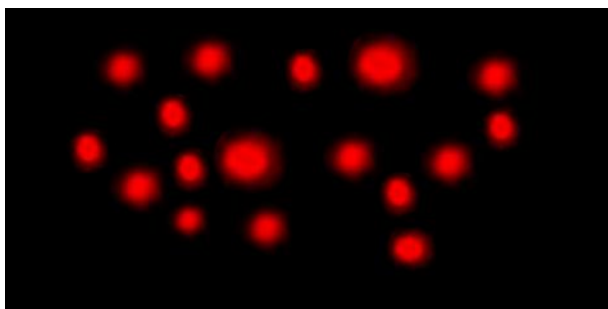

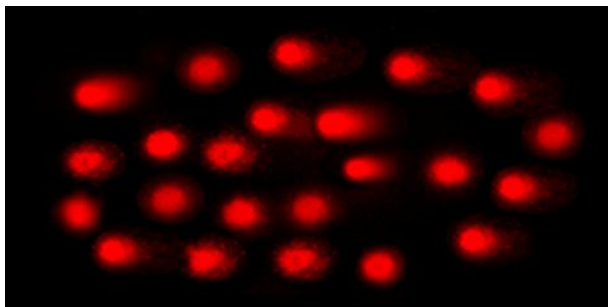**Fig. 1:** Percent of DNA damage in control group.

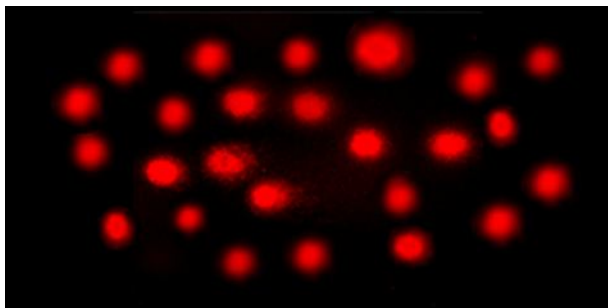**Fig. 2:** Percent of DNA damage in diabetic group.

Fig. 3: Percent of DNA damage in diabetic treated almond oil group.

DISCUSSION

The abnormal metabolic state that accompanies diabetes cause arterial dysfunction. Relevant abnormalities include chronic hyperglycemia, dyslipidemia, and insulin resistant. These factors render arteries susceptible to atherosclerosis. Diabetes alters function of multiple cell types, including endothelium, smooth muscle cell, and platelets, indicating the extent of vascular array in

this disease (Beckman *et al.,* 2002). Emerging evidence suggests an important role of dietary factors in modulating endothelial function. Nuts are fatty foods rich in unsaturated fatty acids. Epidemiological studies have shown that frequent nut consumption decreases the risk of coronary artery disease (Ros *et al.,* 2004). Almond is a rich source of antioxidants and α-linolenic acid, a plant n-3 fatty acid. Further benefits of almonds may result from their high plant sterols, fiber, and associated phenolic substances (Jenkins *et al.,* 2002).

The present study showed that the mean serum glucose level was significantly low and a serum insulin level was significantly high in diabetic group received almond oil compared with diabetic group. These results are in accordance with Wien *et al.,* (2010) who reported that intake almond was significantly associated with a reduction in fasting blood glucose, decreased glycosylated hemoglobin values, 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(GLP-1).

The management of diabetic dyslipidaemia, a well recognized and modifiable risk factor, is a key element in the multifactorial approach to prevent atherosclerosis disease in individuals with diabetes (Hazizi *et al.,* 2009). In the present study level of cholesterol, triglyceride, LDL-cholesterol was significantly high and HDL-cholesterol was significantly low in diabetic group compared to controls groups. These observed are in agreement with finding of Otamere et al (2011) who reported that the most common lipid disorder associated with diabetes is increased level of cholesterol, triglyceride-rich lipoproteins, low levels of HDL, and the presence of small dense as a result, more atherogenic LDL particles where insulin deficiency leads to a variety of derangements in metabolic and regulatory processes, which in turn leads to accumulation of lipids in hepatic tissue (Shepherd, 2005).

As regard diabetic group received almond oil level of cholesterol, triglyceride, LDL-cholesterol was significantly decreased and HDL-cholesterol was significantly increase compared to diabetic groups. These results are in agreement with Li *et al.,* (2011) who reported that Almond consumption is associated with improvements lipid profile. Also Berryman *et al.,* 2011 reported that the cholesterol 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, namely acyl-CoA: cholesterol acyltransferase (ACAT) and 3- hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase, the rate limiting enzyme 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. The present study showed that mean GPx activity was significantly low and mean plasma H_2O_2 levels was

significantly high in diabetic group compared to control group. This result in accordance with Dinçer *et al.,* (2002). This may be due to decrease reduced glutathione (GSH) content found 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.

As regard diabetic group received almond oil GPX activity was significantly increase and H2O2 was significantly decrease compared to diabetic groups . These results are in agreement with Li *et al.,* (2007) 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

The accumulation of the products of the oxidative stress, and reduced antioxidant defenses, such as diminished activity of glutathione peroxidase, catalase and superoxide can cause the damage to biological macromolecules: proteins, lipids and DNA. In consequence, this may lead to oxidative stress-related diseases, including atherosclerosis, the most significant complication in diabetes (Blasiak *et al.,* 2004). The comet assay 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 group exhibited a distorted appearance, indicating substantial DNA damage.

In diabetic group received almond oil percent of DNA damage was significantly low compared to diabetic groups as the antioxidant properties of almond flavonoid were shown to highly biologically available work synergistically with other antioxidants (Vitamins C and E) to protect against LDL oxidation in vitro, as well as enhancing resistance to Cu+1 which participate in generation hydroxyl radical (OH˙) that cause oxidative damage of deoxyribonucleic acid (Yen-Chen *et al.,* 2005). So it can prevent lipid peroxidation, and DNA damage (Cyril *et al.,* 2010).

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 those Suresh and Undurti 2006 who reported that diabetes mellitus is associated with hyperglycaemia, increased oxidative stress and decreased nitric oxide production from endothelial cells, as elevated glucose levels are exacerbated by increased aldose reductase activity leading to depletion of the NADPH required for the generation of nitric oxide from L-arginine by nitric oxide synthase.

As regard diabetic group received almond oil mean serum NOx concentration was significantly increase compared to diabetic groups as it has an important role in improving the bioavailability of nitric oxide as it contains sizeable amounts of L-arginine, the precursor amino acid of the endogenous vasodilator nitric oxide constituent (Sabaté *et al.,* 2003). On the other hand, it is rich in phenolic antioxidants and α-tocopherols which preserves bioavailability of nitric oxide (Ros, 2009).

Atherosclerosis is initiated by the adhesion of monocytes to arterial endothelial cells, followed by their transmigration into the subendothelial space along a chemotactic gradient. One mechanism by which high glucose conditions may enhance this process involves activation of atherogenic cytokines nuclear factor κ-B (NF-κB) , TNF-α and IL-1 which lead to the expression of several inflammatory genes, specially adhesion molecule ICAM-1 that facilitate monocyte adhesion to endothelial cells(Piga *et al.,* 2007).

Our study showed that ICAM-1 concentration was significantly elevated in diabetics compared to controls, this may be due to that diabetes cause TNF-α induction which cause overexpression cytokines IL-1, monocyte chemoattractant protein-1 and IL-6, IL-8 as ICAM-1 receptors are expressed following induction by these cytokines and then expression of ICAM-1 (Lehle *et al.,* 2007& Haubner *et al.,* 2007).

As regard diabetic group received almond oil ICAM-1 was significantly decreased compared to diabetic groups. These results are in line with previous studies of López-Uriarte *et al.,* 2010 & Damasceno *et al.,* 2011& Mandalari *et al.,* 2011 who demonstrated that almond rich in MUFA which had a larger diminution in the RNA expression of TNF-α messenger and rich vitamin E which was found to increase the resistance of LDL against oxidative modification , and inhibit LDL-induced adhesion of monocytes to endothelial cells.

CONCLUSION

It can be concluded from the present study that administration of almond oil improving endothelial function ,exerted an antihyperlipidemic effect together with protection against oxidative damage in STZ induced diabetic rats that could suggest its potent antiatherogenic effect, so it is advisable to incorporate it into daily diet of diabetic patients. Nevertheless, more studies are needed for better understanding the exact cellular mechanisms underlying the protective effects of almond oil.

DISCLOSURE STATEMENT

All authors confirm that no competing financial, personal, political, or academic interests exist in connection with this article.

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