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Pistachio Consumption has Beneficial Effects in Diabetic Rat Model

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In recent years, attention has been developed to the protective biochemical function of natural antioxidants contained in dietary plants. Plant medicines are generally perceived as safe products and the toxicity of these plants has not been reported. Nuts are high in natural antioxidants and other nutrients that may improve glucose homeostasis and support the antioxidant system. In diabetes, the diet must provide the right amount of nutrients and calories in order for the individual to reach and maintain the ideal weight, stabilize the blood glucose levels close to the norm. The objective of this study was to elucidate the hypoglycemic and antioxidative activities of pistachio nuts which are consumed widely in Anatolia, Turkey. Diabetes was induced in rats by intraperitoneal injection of a single dose of 45 mg/kg STZ freshly dissolved in citrate buffer. Plasma and serum samples were used to determine total antioxidant activity (TAA), superoxide dismutase (SOD) activity, reduced glutathione, protein sulfhydryls, malondialdehyde (MDA) and sialic acid levels. According to our results, pistachio consumption caused a significant reduction in MDA levels ($P<0.001$) and an increase in TAA ($P<0.01$) in pistachio treated diabetic rats. Furthermore, in pistachio treated control group, we found an insignificant decrease ($P>0.05$) in glucose levels. The results indicate that pistachio consumption can provide antioxidant protection to membrane lipids, support the antioxidant system and inclusion of pistachios in a healthy diet beneficially affects diabetic risk factors in a dose-dependent manner.

Key Words: *Pistachio, experimental diabetes, antioxidant activity, sialic acid, blood glucose.*

Antep Fıstığı Tüketimi Diyabetik Sıçan Modeli Üzerinde Faydalıdır

Son yıllarda, bitkilerde bulunan doğal antioksidanların koruyucu biyokimyasal işlevine dair ilgi artmıştır. Bitkisel ilaçlar genellikle güvenilirdir ve bitkilerin toksisitesine dair bildirim olmamıştır. Fıstık, glikoz homeostazını destekleyecek ve antioksidan sistemini geliştirecek doğal antioksidan ve diğer besinler yönünden zengindir. Şeker hastalığında perhiz, bireyin ideal kilosunu koruyacak ve kan glikozu seviyelerini norma yakın stabilize edecek şekilde gereken oranda gıda ve kalori temin etmelidir. Bu çalışmanın amacı, Türkiye'de ve özellikle Anadolu'da yaygın olarak tüketilen Antep fıstığının hipoglisemik ve antioksidatif faaliyetlerine açıklık getirmektir. Sıçanlar, tek dozluk (45 mg/kg) STZ ile intraperitoneal enjeksiyon yoluyla diyabetik hale getirildiler. Plazma ve serum örnekleri total antioksidan aktivitesini (TAA), süperoksit dismutaz (SOD) aktivitesini, redüklenmiş glutatyonu, protein sülfhidril miktarlarını, malondialdehit (MDA) ve siyalik asit seviyelerini belirlemek için kullanıldı. Sonuçlarımıza göre, Antep fıstığı tüketimi Antep fıstığı verilen sıçanlarda MDA seviyelerinde anlamlı düzeyde bir azalmaya ($P<0.001$), TAA ($P<0.01$) seviyelerinde ise anlamlı düzeyde bir artışa sebep olmuştur. Bu sonuçlar, Antep fıstığının antioksidan sistemi desteklediğini ve membran lipitlerine antioksidan koruma sağladığına işaret etmektedir ki Antep fıstığının sağlıklı bir perhize dahil edilmesi diyabetik risk faktörlerini doza bağlı olarak faydalı yönde etkileyebilecektir.

***Anahtar Kelimeler*:** *Antep fıstığı, deneysel diyabet, antioksidan aktivite, siyalik asit, kan glukozu.*

Introduction

Diabetes affects more than 135 million people worldwide; the number of people with diabetes will reach an estimated 300 million worldwide by 2025. Because management of diabetes and its complications such as cardiovascular disease, amputation, blindness, and renal failure imposes enormous medical and economic burdens, primary prevention has become a public health imperative (1). In the treatment of diabetes the diet has an important role complementary to the pharmaceutical treatment. The diet must provide the right amount of nutrients and calories in order for the individual to reach and maintain the ideal weight, stabilize the blood glucose levels close to normal (2). In recent years, attention has been developed to the protective biochemical function of natural antioxidants contained in dietary plants, that are candidates for prevention or protection of oxidative damage caused by free radical species.

In human body, a number of biochemical reactions involve the generation of reactive oxygen species (ROS). Excessive ROS can attack lipids, carbohydrates, proteins, DNA, and result in oxidative stress. Under normal conditions, the balance between the generation and diminution of ROS is controlled by the antioxidant defense system (3). The determination of total antioxidant status (TAS) has been used for scientific

purposes to examine the medical importance of free oxygen radicals and antioxidative defense (4, 5).

The nutrient content of nuts shows a high proportion of mono and polyunsaturated fatty acids. They also have other components, such as protein (arginine), fibre, copper, magnesium, vitamin E, folic acid, plant sterols and phytochemicals. Additionally nuts enhances satiety (1, 6-8). Agents with an antioxidative effect may improve pancreatic β-cell function and contribute to the medical treatment of diabetes (9). During our research, we focused on the improvement of the antioxidant system produced by pistachio nuts.

Streptozotocin (STZ) is frequently used to induce diabetes mellitus in experimental rat models through its toxic effects on pancreatic ß-cells. The cytotoxic action of STZ is associated with the generation of ROS causing oxidative damage (9). Oxidative stress plays an important role in chronic complications of diabetes and is postulated to be associated with increased lipid peroxidation (10). Moreover, diabetes manifested by experimental animal models exhibit high oxidative stress due to persistent and chronic hyperglycemia, which thereby depletes the activity of antioxidative defense system (11).

Human sialic acids (SA) are N-acetylated derivates of neuraminic acid that are abundant terminal monosaccharides of glycoconjugates (12). SA, increases rapidly following the inflammatory and injury process. Therefore the detection of total SA concentrations may be a valuable indicator of tissue damage and tissue proliferation (13).

Accordingly, in this study we aimed to investigate the effects of pistachio consumption on the antioxidant status, oxidative stress and SA levels in STZ induced diabetic rats.

Materials and Methods

All animal protocols were approved by the committee on the use of live animals in teaching and research of The University of Istanbul. Thirty-two Wistar Albino rats aged 8 weeks (initial body weight, 200-250g), were used. Rats were obtained from Istanbul University Animal Laboratory and they were housed in cages in an environment-controlled room (room temperature, 22±2^{0}C; relative humidity, light/dark cycle, 12h/12h) with free access to food and tap water.

Induction of Experimental Diabetes: Diabetes was induced in rats by intraperitoneal injection of a single dose of 45 mg/kg STZ freshly dissolved in citrate buffer (0.05 M, pH 4.5). Controls were injected with citrate buffer. The diabetic state was characterized by the measurement of plasma glucose concentration 2 days after the STZ injection. Animals with serum glucose levels higher than 11mmol/L were included in this study (14).

Experimental Design: In the experimental design, a total of 32 rats were divided into 4 groups, each containing 8 rats. The groups were: Control group, Control+pistachio group, Diabetic group and Diabetic+pistachio group. Each group had equal number of male and female rats. Control group and Diabetic groups were fed on the Standard chow containing: 20% crude protein, 2.85% crude oil, 5.96% cellulose, 8 % crude ash, 0.97 % calcium, 0.5 % phosphorous, 1.03% lysine, 0.33% methionine, 0.65% methionine+cysteine, 0.14 % sodium, 1.13 % linoleic acid; whereas Control+pistachio and Diabetic+pistachio groups were fed on standard chow supplemented with 1.26 % g pistachio for 8 weeks. The diets were prepared once a week in the laboratory and stored at a temperature of + 4^{0}C.

Blood collection: At the end of 12 weeks the rats were sacrificed under urethane anaesthesia (1.25 g/kg) after overnight fasting. To minimize diurnal variations, the rats were routinely sacrificed between 07.00 and 08:00 hours. Blood samples were collected from the heart and transferred into test tubes. Serum samples were separated by centrifugation and used for glutathione analysis on the same day without delay. The remaining serum samples were stored at -20^{0}C until lipid peroxidation SOD, TAA, TT, SA and glucose analysis.

Analytical methods

Assay of total antioxidant activity: Antioxidant activities were determined by the method of Koracevic *et al* (5). A standardized solution of Fe–EDTA complex reacts with hydrogen peroxide (H_2O_2) by a Fenton type reaction, leading to the formation of hydroxyl radicals ($OH^{\cdot}$). These ROS degrade benzoate, resulting in the release of thiobarbituric acid reactive substances (TBARS). Antioxidants from the added sample of human fluid cause suppression of the production of TBARS. Finally absorbance values were measured by a spectrophotometer at 532nm.

Assay of CuZn SOD activity: CuZn SOD activity was determined by the method of Sun *et al.* (15) based on the inhibition of nitroblue tetrazolium reduction. The absorbance of the reduction product was read at 560 nm in a spectrophotometer. One unit of SOD is defined as the amount of protein that inhibits the rate of NBT reduction by 50 %.

Assay of GSH: GSH levels were determined by the method of Beutler *et al*. (16) using metaphosphoric acid for protein precipitation and 5'5'-dithiobis-2-nitro-benzoic acid for colour development.

Assay of plasma protein thiols (TT): Membrane protein sulfhydryl concentration was measured spectrophotometrically using 5,5'-dithiobis-2-nitrobenzoic acid as described by Ellman (17).

Assay of lipid peroxidation: Lipid peroxidation was determined by measuring malondialdehyde (MDA) levels in serum. MDA levels in serum were determined by measuring the levels of TBARS according to the method of Yagi (18).

Assay of sialic acid: SA content was determined by Warren's thiobarbituric acid method (19).

Statistical Methods

Friedman's test was used to compare the differences between the groups. The level of significance was set at P<0.05. The data was analysed using SPSS software (Version 12.0, Chicago, IL).

Results

Prior to inducing diabetes (day 0), the groups were checked for the differences in weight and no significant difference between their weights was found (P >0.05). STZ injection significantly increased serum glucose levels at 48h after injection, and glucose levels of the STZ induced diabetic groups remained significantly higher than that of the control groups during the experimental period. Blood glucose, SA and TBARS levels increased (P<0.01, P<0.01 and P<0.001 respectively) whereas TAA, SOD and GSH levels significantly decreased (P<0.01, P<0.01 and P<0.05 respectively) in the diabetic group when compared with the control group. In pistachio treated control group, we found an insignificant decrease in glucose levels (P>0.05); whereas insignificant increases in SOD and GSH levels were also observed when compared with the control group. Total thiol levels insignificantly decreased in diabetic group compared with the control group (P>0.05). Pistachio consumption in the diabetic group significantly decreased TBARS levels (P<0.05) and significantly increased TAA levels (P<0.05) when compared with the diabetic group. Moreover, pistachio consumption in the diabetic group led to slight increases in GSH and SOD levels and a slight decrease in glucose levels when compared with the diabetic group (Table 1).

Table 1. Effect of pistachio consumption on antioxidant defense system, oxidative stress and glucose levels in control, control+pistachio, diabetic and diabetic+pistachio groups.

	Control (n=8)	Control +Pistachio (n=8)	Diabetic (n=8)	Diabetic +Pistachio (n=8)
TAA (mmol/L)	1.65 ± 0.34	2.14 ± 0.39[b]	1.12 ± 0.20[b]	1.42 ± 0.30[d]
SOD (U/ml)	5.53 ± 2.12	5.93 ± 2.31	3.27 ± 1.95[c]	4.03 ± 1.71
GSH (%mg)	29.61 ± 3.80	31.07 ± 3.15	26.72 ± 4.04[a]	27.01 ± 3.10
TT (µM)	317.85 ± 35.99	321.58 ± 37.62	263.08 ± 56.20	284.15 ± 45.32
TBARS(nmolMDA/mL)	3.84 ± 1.42	3.80 ± 1.65	5.86 ± 1.67[c]	3.99 ± 1.55[d]
SA (mM)	16.56 ± 2.48	16.20 ± 2.21	20.83 ± 4.15[b]	17.65 ± 2.98
Glucose (mmol/L)	5.03 ± 0.48	4.76 ± 0.61	21.94 ± 1.69[b]	19.87 ± 1.33

Values expressed as Mean ± SEM, n=8 in each group.
[a]Significantly different from control (P<0.05),
[b]Significantly different from control group (P<0.01),
[c] Significantly different from control group (P<0.001),
[d] Significantly different from diabetic group (P<0.05).

Discussion

In recent years, attention has been developed to the protective biochemical function of natural antioxidants contained in dietary plants that are candidates for prevention or protection of oxidative damage caused by free radical species. Population studies indicated that in individuals regularly consuming nuts have reduced risk for cardiovascular disease and diabetes. In clinical and experimental trials, nuts appear to have beneficial effects on glucose profile as well (20-22). In our study, pistachio consumption insignificantly reduced blood glucose levels and supported the antioxidant system.

In the present study, we aimed to adapt the dosage of pistachio (10 oz =310 g) to rats, in order to examine the effect of pistachio consumption on glucose, SA levels and oxidant-antioxidant status in a rat model with moderate diabetes. For this purpose, we have calculated the equivalent dose of pistachio for rats as 0.88 g pistachio/week, consuming human weight as 70kg and rat weight as 200g. Accordingly, pistachio added diets that contain 1.26 % pistachio were prepared.

Experimentally, STZ produced a significant increase in serum glucose levels which was reduced insignificantly due to pistachio consumption. Nuts contain 70% to 80% fat, and most fatty acids in nuts are unsaturated (polyunsaturated and monounsaturated), which may be beneficial for glucose and insulin homeostasis. Several studies have shown that a higher intake of monounsaturated and polyunsaturated fatty acids improves insulin sensitivity (21).

In literature, The Nurses' Health Study has shown that the consumption of peanuts and peanut butter 5 times a week was associated with a 27% and 21% reduction in risk of type 2 diabetes, respectively (1). In the same study of 86,016 female registered nurses those consuming at least 5 oz (155 g) of nuts/week had a % 35 lowering in nonfatal myocardial infarction compared with those eating less than 1 oz of nuts (23).

The polyphenol resveratrol (RSV) was detected in the aqueous extracts from the edible nut of cultivars of pistachio (24) and resveratrol treatment in STZ induced diabetic rats improved blood glucose levels (25). The

hypoglycemic effects of RSV administration seen in STZ induced rats was explained by a reduced food intake and/or a reduced digestion and absorption of the foods by the gastrointestinal system. Furthermore, RSV is a strong antioxidant and it may improve the functional states of the metabolic machinery of the cells (26). In our study, pistachio consumption insignificantly reduced the glucose levels of the control group. They are energy-dense and good sources of fiber and protein, and contain dietary factors that increase satiety ratings. Moreover people who consume nuts may tend to engage in higher levels of physical activity than non-nut eaters (6). Hu *et al.* (23) reported that nut consumption was associated with greater frequency of vigorous exercise among the Nurses Health Study participants. The Physicians' Health Study also noted that men who ate nuts frequently were more physically active (27).

It was reported that human plasma has a chain breaking antioxidant capacity and ability to trap free radicals (5, 28). Induction of STZ significantly depleted the antioxidant activity in our experimental model. This reduction might be due to the higher production of oxidative agents. Reduced antioxidant levels as a result of increased free radical production in experimental diabetes has previously been reported (29). Biological effects of ROS are controlled in vivo by a wide spectrum of enzymatic and non-enzymatic defense mechanisms, in particular SOD, which catalyzes dismutation of superoxide anions to H_2O_2; and catalase, which then converts H_2O_2 into molecular oxygen and water (5, 30). According to our study, decreased SOD activity in the diabetic group could be due to its depletion or inhibition as a result of the increased production of free radicals. However pistachio consumption insignificantly increased SOD levels in diabetic rats. GSH also protects tissue from damage caused by diabetes. Depletion of tissue GSH is one of the primary factors that permit lipid peroxidation. GSH participates in many cellular functions particularly elimination of H_2O_2, interaction with free radicals and regeneration of vitamin E. Therefore, decreased GSH content in plasma may impair antioxidant defenses and accelerate the oxidative damage (31). Also, GSH significantly decreased in the diabetic group. In literature, serum GSH levels has been reported to be decreased indicating that the reduced activity of the antioxidant defense system was caused by long-term diabetes (10). Administration of pistachio had no effect on GSH levels in diabetic rats.

Free radicals may cause the oxidation of protein thiol groups. TT groups in proteins serve as antioxidants by scavenging free radicals, thus sparing antioxidants and/or cellular constituents from attack. Therefore, the measurement of sulphydryl groups in proteins may be useful (32). Consistent with the literature, although statistically insignificant, TT levels decreased in diabetic group compared with the control group which might result from an increased consumption to detoxify an augmented load of free radicals generated in diabetes. However, treatment with pistachio insignificantly increased total thiol levels in our diabetic group. The decrease in TT levels in diabetic rats were consistent with the findings of other investigators (11, 33).

Hyperglycemia is the primary symptom of diabetes and is blamed for the complications of diabetes because elevated glucose concentration directly injures cells and induces lipid peroxidation (34). In our study, we observed a significant increase in TBARS levels in diabetic rats. However, pistachio consumption decreased TBARS levels. Thus, we propose that pistachio treatment may have a role in scavenging free radicals generated by STZ induction.

Sialic acid is a terminal component of the non-reducing end of carbohydrate chains of glycoproteins and glycolipids. These are essential constituents of hormones, enzymes and tissues. SA is reported to increase in humans and animals during a number of pathological situations where the contributory event is either of tissue damage, tissue proliferation or inflammation (35-37). It has been demonstrated that the activity of sialidase (the enzyme that liberates sialic acid) is increased in human leukocytes from people with type 2 diabetes, in endothelial cells stimulated with advanced glycation endproducts, and in an animal model of diabetes (37, 38). In diabetic rats, we observed a significant increase in SA levels when compared to the control group and consumption of pistachio nuts insignificantly decreased serum SA in the diabetic group.

The results of our study support a positive effect of pistachio consumption on oxidant-antioxidant status. Other than the positive effects of nuts on blood glucose levels; pistachio nuts may be included in diets to lower the oxidative stress induced by diabetes. However, studies with longer period of observation times are needed to prove the reliability of pistachio consumption in the long term.

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