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Effects of grape consumption on plasma and erythrocyte antioxidant parameters in elderly subjects

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Aim: To investigate the effects of ingesting *Fructus vitis minuta* (black grape) on plasma and erythrocyte antioxidant parameters in elderly subjects.

Materials and methods: Thirteen subjects (mean age 74.67 ± 0.58) participated in the study. They consumed 1 g/kg body weight per day of dried black Maraş grapes for 1 month. Before and after these periods, fasting blood samples were obtained, and oxidant (as MDA) and antioxidant (as SOD) parameters were studied in erythrocytes. Also, plasma samples were obtained from the subjects.

Results: In the erythrocyte hemolysate, MDA (malondialdehydes) levels and SOD (superoxide dismutase) activities were found to be lower ($P < 0.01$) in the second samples compared to the first ones.

Conclusion: We think that because free radical production was decreased SOD activities were not increased. Our results show that reduced peroxidation processes caused by Maraş grape consumption suggest that grape consumption has beneficial effects in elderly subjects.

Key words: Black grape, elderly subjects

Yaşlılarda kurutulmuş siyah üzüm tüketiminin plazma ve eritrosit antioksidan parametreler üzerine etkisi

Amaç: Bu çalışmada yaşlılarda kurutulmuş siyah üzüm tüketiminin plazma ve eritrosit antioksidan parametreler üzerine etkisi incelendi.

Yöntem ve gereç: Çalışmaya 13 kişi (ortalama yaşları 74,67 ± 0,58) katıldı. Katılımcılar 1 ay boyunca 1 g/kg vücut ağırlığı/gün kurutulmuş siyah üzüm tükettiler. Bu periyottan önce ve sonra katılımcıların eritrosit ve plazmalarından açlık kan örnekleri alındı ve oksidan (MDA gibi) ile antioksidan (SOD gibi) belirteçleri çalışıldı.

Bulgular: Eritrosit hemolizatında MDA ve SOD değerleri ikinci örneklerde birincilere göre daha düşük bulundu. Biz serbest radikal üretimi artmadığı için SOD aktivitesinin artmadığını düşünmekteyiz.

Sonuç: Maraş üzümü tüketiminden dolayı peroksidasyon olaylarının azalması, üzüm tüketiminin yaşlılarda yararlı bir etkiye sahip olabileceğini göstermektedir.

Anahtar sözcükler: Siyah üzüm, yaşlılar

Introduction

Aging, a multifactorial process of enormous complexity, is characterized by increased oxidative stress, which can arise from dietary, environmental, and/or genetic sources, and contributes to the decline in cognitive performance.

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Supplementary natural foods that are high in antioxidant potential can compensate for dietary and/or genetic deficiencies that promote increased oxidative stress. Phytochemicals, especially phenolics, in fruits and vegetables are suggested to be the major bioactive compounds for health benefits (1). Among them, black grape is a food with high antioxidant power (2,3) and it deserves special attention in this regard. In previous studies it was established that dried black grape may strengthen the antioxidant potential of the body and thus reduce some oxidant stress-induced health problems (4,5).

Resveratrol, bioactive polyphenol of grape (trans-3,4′,5-trihydroxystilbene), has been reported to exhibit chemopreventive activity against chemical carcinogenesis. It has also been shown to have growth in inhibitory activity toward solid tumors in vivo. Gao et al. studied the antileukemic activity of resveratrol in vitro and in vivo using a mouse myeloid leukemia cell line (32Dp210). They showed that treatment of 32Dp210 leukemia cells with resveratrol at micro molar concentrations (25-50 μmol/L) significantly and irreversibly inhibited their clonal growth in vitro (6).

The present study aimed to investigate the possible effects of black grape consumption on blood lipid profile and oxidant/antioxidant status in elderly subjects.

Materials and methods

Thirteen subjects (mean age 74.67 ± 0.58) participated in the study. They consumed dried black Maraş grapes at a daily dose of 1 g/kg body weight for a month (study period). Before and after this period, fasting blood samples were obtained, and oxidant (MDA and XO) and antioxidant (SOD, GSH-Px, and CAT) parameters were studied in erythrocyte hemolysate, and MDA levels were studied in plasma fraction as described previously (7-11). The CAT and GSH-Px activities were given in international units (IU)/mg protein and SOD activity in U/mg protein. One unit of SOD activity was defined as the amount of protein causing 50% inhibition of the nitroblue tetrazolium salt (NBT) reduction rate. The MDA concentration was determined using the thiobarbituric acid reaction. The CAT activity was determined by measuring the decrease in absorbance of hydrogen peroxide (H_2O_2) at 240 nm. The GSH-Px activity was measured by following changes in NADPH absorbance at 340 nm. Xanthine oxidase activity was determined by measuring the uric acid formation from xanthine at 293 nm. In the activity calculations, extinction coefficients of uric acid, H_2O_2, and NADPH were used for XO, CAT, and GSH_Px enzymes, respectively. Additionally, routine parameters were studied in sera from the subjects. In the statistical analysis, a paired t test was used.

Results

As can be seen from the tables, MDA levels and SOD activities in the erythrocytes were found to be lower ($P < 0.01$) in the second samples compared to the first ones. Xanthine oxidase activity was found to be lower in the second samples, but this decrease was

Table 1. Mean ± SD values of oxidant and antioxidant parameters in erythrocytes from elderly subjects who consumed dried black grapes.

Groups	GSH-Px	CAT	SOD	MDA	XO
Before grape consumption (n = 15)	22.35 ± 1.69	50,727 ± 6668.5	13,576 ± 2676	269.1 ± 42.1	33.5 ± 12.98
After grape consumption (n = 15)	21.95 ± 1.81	50,434 ± 6882.4	9272 ± 2376	220.2 ± 29.9	30.5 ± 9.64
P value	P = 0.552	P = 0.836	P = 0.001	P = 0.001	P = 0.558

Paired- t test
MDA: nmol/mg protein
SOD: U/mg protein
GSH-Px: IU/mg protein
CAT: IU/mg protein
XO: mIU/mg protein

not significant. However, no differences were found between the blood lipid parameters.

Table 2. Mean ± SD values of MDA levels in plasma from elderly subjects who consumed dried black grapes.

Groups	MDA
Before grape consumption (n = 15)	0.42 ± 0.31
After grape consumption (n = 15)	1.42 ± 0.67
P value	P > 0.05

Paired t test

Discussion

The pathogenesis of aging and age-related diseases involves oxidative stress at an early stage in its development (12) as confirmed by a decrease in antioxidant defense systems and an increase in oxidative reactions (13,14). Decreased glutathione (GSH) levels (15,16) and glutathione peroxidase activity (17) are coupled to increased oxidative damage to various molecules such as DNA (18,19), lipids (20,21), and proteins (22,23).

Reactive oxygen species (ROS) cause oxidant stress in tissues of living organisms. The main ROS to be considered is superoxide anion (O_2^-), which is predominantly generated by the mitochondria. Hydrogen peroxide (H_2O_2) is produced from O_2^- by the action of superoxide dismuase (SOD), and peroxynitrite ($ONOO^-$) is generated by the reaction of O_2^- with nitric oxide (NO). These ROS are scavenged by antioxidant enzymes namely SOD, GSH-Px, and CAT. Under certain circumstances, these endogenous antioxidative defenses are likely to be disturbed as a result of overproduction of oxygen radicals and failure to regenerate antioxidants in tissues adequately. Besides the antioxidant enzymes and vitamins, some foods that have antioxidant power may ameliorate oxidant stress.

In fact, intake of fruits and vegetables is associated with reduced incidence of many common forms of diseases, especially cardiovascular diseases and some types of cancer (24). Diets rich in vegetables and fruit are also associated with a reduced risk of many chronic diseases of ageing (25-28). Singh et al. reported that grape seed extract possesses in vivo anticancer efficacy against hormone-refractory human prostate cancer (PCA), which is associated with its antiproliferative, proapoptotic, and antiangiogenic activities together with up-regulation of insulin-like growth factor binding protein (IGFBP) (29).

Natella et al. demonstrated that supplementing a meal with grape seed proanthocyanidins can minimize the postprandial oxidative stress by decreasing the oxidants and increasing the antioxidant levels in plasma, and, as a consequence, enhance the resistance to oxidative modification of LDL (30). Sharma et al. suggested a strong possibility of synergistic efficacy of grape seed extract and doxorubicin combination for breast cancer treatment, independent from estrogen receptor status of the cancer cell (31). Singletary and Meline indicated that grape seed proanthocyanidins exert significant inhibition on colon aberrant crypts and breast tumors in a rat dual-organ tumor model, and grape polyphenolics warrant further evaluation as potential colon cancer chemopreventive agents (32). Agarwal et al. suggested that grape seed polyphenols may exert strong anticarcinogenic effect and this effect possibly involves modulation of mitogenic signaling and cell-cycle regulators and induction of G1 arrest, cell-growth inhibition, and apoptotic death (33). In a

Table 3. Blood routine parameters of the subjects before and after dried black grape consumption (mean ± SD values).

Groups	T.CHOL.	LDL	VLDL	HDL	TG
Before	210.3 ± 53.7	126.6 ± 37.9	29.1 ± 13.0	53.8 ± 15.0	146.1 ± 65.2
After	201.2 ± 43.9	125.2 ± 33.0	32.1 ± 15.0	52.5 ± 11.0	158.5 ± 73.1
P value	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05

(T.CHOL: total cholesterol, LDL: low density lipoprotein, HDL: high density lipoprotein, VLDL: very low density lipoprotein, TG: triglyceride)
Paired t test

previous study, it was suggested that chemopreventive properties of trans-resveratrol in grape extracts are associated with inhibition of activation of the IkappaB kinase (34). Tamura and Matsui reported that grape juice exhibited the most potent inhibitory action on the phenol sulfotransferase activity of mouse intestines and human colon carcinoma cells and that the inhibitory activity of grape juice was located mainly in the skin and seeds of grape (35).

Roychowdhury et al. showed that, in addition to its antioxidative property, grape seed proanthocyanidin extract (GSPE) enhances low-level production of intracellular NO in primary rat astroglial cultures. Furthermore, GSPE pretreatment protects the microglial GSH pool during high output NO production and results in an elevation of the H_2O_2 tolerance in astroglial cells (36).

Rho et al. found that grape intake, especially grape pomace with the highest content of flavonoids, beta-carotene, tocopherols and dietary fiber among the 3 parts, showed the prominent antioxidative capacity of inhibiting age-related or Cd-induced increase of lipid peroxidation and DNA damage effectively, promoting liver and red blood cell antioxidant enzyme activities (37).

In another study, Devi et al. similarly showed that proanthocyanidin (PA) intake in a moderately low quantity is effective in up-regulating the antioxidant defense mechanism by attenuating lipid peroxidation (LPO) and protein oxidation (PO). Changes in the cholinergic system, however, indicate an increase in the ACh concentration with a moderate reduction in AChE activity, suggesting further that PA may have a potent role in enhancing cognition in older rats (38).

Shukitt-Hale et al. showed that grape consumption leads to significantly lowered erythrocyte MDA levels, which indicate that consumption of grape decreases oxidation reactions in elderly subjects (39).

Our study showed that MDA levels and SOD activities in the erythrocyte hemolysate were found to be lower ($P < 0.01$) in the second samples compared to the first ones. Xanthine oxidase activity was found to be lower in the second samples, but this decrease was not significant. We suggest that because free radical production was decreased SOD activities were not increased. Our results show that grape consumption leads to significantly lowered oxidation reactions in erythrocytes.

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