J. Endocrinol. Invest. 33: 118-124, 2010
DOI: 10.3275/6564

The effects of polyphenol-containing antioxidants on oxidative stress and lipid peroxidation in Type 2 diabetes mellitus without complications

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ABSTRACT. ***Background:*** **The hyperglycemia-induced oxidative stress in diabetes mellitus (DM) is the major factor in the pathogenesis of cardiovascular complications. The phenolic compounds are potent antioxidants that can reverse the factors leading to cardiovascular complications in DM. The aim of this study was to determine the antagonizing effects of a polyphenol-rich antioxidant supplement containing pomegranate extract, green tea extract, and ascorbic acid, on oxidative stress in Type 2 diabetic patients.** ***Materials and methods:*** **A total of 114 male and female non-smokers (56 study, 58 placebo) with Type 2 DM and without any complications were recruited. The blood levels of fasting blood glucose, glycated hemoglobin, LDL, HDL, triglycerides, plasma malondialdehyde (MDA), total glutathione (GSH), hydrogen peroxide, and antioxidant capacity (AOC) were determined at the beginning and at the end of the 3-month trial. The differences of the data changes between the groups were statistically analyzed by Mann-Whitney U test.** ***Results:*** **The study group showed a decrease in LDL and an increase in HDL and the comparison with the difference in placebo group was statistically significant ($p<0.001$ for LDL and $p<0.001$ for HDL). Accordingly, as a by-product of lipid peroxidation, plasma MDA was decreased in the study group compared to the placebo group ($p<0.001$). As an indicator of increased antioxidant defense, total plasma GSH and AOC increased more in the study group compared to control group ($p<0.001$).** ***Conclusions:*** **These observations indicated that the polyphenol-rich antioxidant supplement containing pomegranate extract, green tea extract, and ascorbic acid has important antagonizing effects on oxidative stress and lipid peroxidation in patients with Type 2 DM and might be beneficial in preventing cardiovascular complications.**
(J. Endocrinol. Invest. 33: 118-124, 2010)

INTRODUCTION

Diabetes mellitus (DM) is a heterogeneous metabolic disorder characterized by hyperglycemia and cardiovascular complications (1-3). It has been shown that diabetic complications may be caused indirectly by increased oxidative stress and impaired antioxidant defense system induced by prolonged hyperglycemia (2-5). Production of reactive oxygen species (ROS) such as hydroxyl radical, hydrogen peroxide (H_2O_2), and superoxide anion, in quantities that overwhelm the endogenous antioxidant defense system is referred to as oxidative stress (6). Hyperglycemia can also directly affect the cardiovascular system as demonstrated both in *in vivo* and *in vitro* studies (7-9).

Increased oxidative stress in DM is shown to be the result of higher rate of glycosylation of proteins (3, 4). It has also been demonstrated that incubation of lipids with glycosylated proteins *in vitro* produces elevated lipid peroxidation (10) and *in vivo* studies proved that elevated levels of by-products of lipid peroxidation such as malondialdehyde (MDA), play an important role in atherosclerosis and late complications of Type 2 diabetic patients (11, 12).

Glutathione (GSH) is an antioxidant compound found in living tissues. It takes up and gives off hydrogen and is important in cellular respiration. Also, GSH participates directly in the neutralization of free radicals, reactive oxygen compounds, and maintains exogenous antioxidants such as vitamins C and E in their reduced (active) forms (3, 13).

Pomegranate (*Punica granatum L.*) fruits are popularly consumed in beverage forms such as pomegranate juice (PJ). Basic and applied research in animals and humans indicates that PJ has potent antioxidant activity and prevents lipid peroxidation by its diverse phenolic compounds (14-17). Ignarro et al. (16) found that PJ, as a strong antioxidant, enhances the biological actions of NO by protecting the NO against oxidative destruction by ROS.

Tea is a part of dietary habits in many countries around the world and there have been claims on their positive influence on human health. The antioxidant capacity (AOC) of phenolic compounds in green tea has been found to be superior to other beverages as well (18-19). In addition to the polyphenol-containing antioxidants, ascorbic acid has long been known as a water soluble, chain-breaking antioxidant which traps the total peroxyl radicals in human plasma (13, 20).

In the present study, we aimed to determine the antagonizing effects of a polyphenol-rich antioxidant supplement

Key-words: Diabetes, glutathione, malondialdehyde, oxidative stress, polyphenol-containing antioxidants.

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Accepted August 31, 2009.

First published online October 15, 2009.

containing pomegranate extract (500 mg), green tea extract (300 mg), and vitamin C (60 mg) (Com Ilac), on oxidative stress in Type 2 diabetic patients who were regularly exercising and on a low carbohydrate diet. We further investigated its contribution to the prevention of diabetic complications, mainly cardiovascular complications. We aimed to demonstrate the improving effects and the antioxidant mechanism of the phenolic compounds in addition to all other measures (diet, exercise) used to control oxidative stress in diabetes and its complications in Type 2 diabetic patients.

MATERIALS AND METHODS

One hundred and fourteen male and female patients with new onset Type 2 DM at Sisli Etfal Education and Research Hospital, Istanbul, Turkey, who were between 40 and 65 yrs of age and who were on oral antidiabetics, were included in our study. All volunteers completed a medical, drug, and supplementation history questionnaire to determine eligibility. Subjects' age, gender, smoking and alcohol habits, exercise and diet regimens were also revealed from this questionnaire. All study protocols were approved by the institutional Ethics Committees of the Yeditepe University Hospital and Sisli Etfal Education and Research Hospital. The study was registered to and approved by National Institute of Health through www.clinicaltrials.gov prior to the commencement of the trial. After explanation of all experimental procedures, written informed consent was obtained from each subject before entry into the study. Patients were excluded from the study if they were unable to sign informed consent; were smokers; were pregnant or lactating; had a known allergy to any drug; were on any nutritional supplements containing substances with antioxidant effect or on any medication with antioxidant effect and had any known cardiovascular, renal or liver disease.

Before the study had begun, a detailed medical evaluation was performed at baseline, including neurological and ophthalmological examination, paying particular attention to underlying diabetic complications, such as autonomic neuropathy, proliferative retinopathy, and cardiovascular complications. Those who were found to have diabetic complications were excluded from the study. One hundred and fourteen subjects who were found to be eligible for the study were using appropriate oral antidiabetic treatment and were started a 1500 kilocalories standard diet rich in vegetables, 3 servings of fruits, maximum 3 slices of bread a day. In addition to these, they were given an aerobic exercise regimen of 150 min a week conducted after breakfast. The aim of these diet and exercise regimens was to build standardization of oxidative stress among the 114 diabetic subjects before the study has begun. After the placebo and the food supplement were given, subjects were called for control physical examination at regular intervals and the examination was always done by the same investigator (T.S.). At each control exam, subjects' blood pressure, weight, and body mass index (BMI) were taken and a full physical examination was done to confirm their wellness.

All of our subjects were new onset Type 2 diabetic patients without any diabetic complication. Therefore, they were not put on any medication like statins and/or antiaggregants which are used to prevent cardiovascular complications but also are known to affect AOC. Also, all of our subjects were only put on oral antidiabetics mainly metformin and/or acarbose. Sulfonylureas and thiazolidinediones were not started to our subjects as some drugs of these groups are known to affect AOC.

There were 2 groups: the study group who received the polyphenol containing antioxidant capsules containing pomegranate extract (500 mg), green tea extract (300 mg), and vitamin C (60 mg) (Com Ilac) and the control group who received the placebo capsules. The initial levels of fasting glucose (mg/dl), glycated hemoglobin (HbA_{1c}) (%), LDL (mg/dl), HDL (mg/dl), triglycerides (TG) (mg/dl), plasma MDA (μmol/l), total GSH (μmol/l), H_2O_2 (mmol/l), and AOC (mmol/l) were determined in both of these groups, 2 weeks after the exercise and diet regimens were started at their first visit. All of these parameters were measured in fasting blood after at least 8 h of fasting. After their blood samples were obtained for these parameters, subjects were randomly divided into 2 groups in a double-blind manner. Fifty-six subjects were enrolled into the study group and received polyphenol-containing antioxidant capsules for 3 months in addition to diet, exercise and their antidiabetic treatment. They took 1 capsule a day. Fifty-eight subjects were enrolled into the control group and received their placebo capsules for 3 months in addition to diet, exercise, and their antidiabetic treatment. The placebo capsules contained 5% polyvinylpyrrolidone as binder, 3% sodium starch glycolate as disintegrant, 1% magnesium stearate as lubricant, and 91% microcrystalline cellulose as diluent. The antioxidant capsule contained 500-mg pomegranate extract which involves a diverse group of polyphenols including ellagitannins, gallotannins, ellagic acid, and flavonoids, such as anthocyanins (14-16). The capsule also contained 300-mg green tea which is known to be rich in polyphenols like catechins, flavanols, flavanol glycosides, flavandiols, phenolic acids, and depsides (18, 19).

At the end of 3 months, the measurement of fasting glucose, HbA_{1c}, LDL, HDL, TG, plasma MDA, total GSH, H_2O_2, and AOC levels were repeated. The measurement of these parameters also was done in fasting blood. The statistical comparison of these measures before and after the 3 months period of trial was first done within each group itself (Fig. 1 and 2). Then, the differences of data changes from the beginning until the end of the trial of 3 months duration, was statistically compared between the study and control groups (Table 1).

Determination of antioxidant parameters

Subjects' blood was taken by venipuncture after 8-10 h fasting, at the beginning and at the end of the 3 months period of trial. Plasma MDA, H_2O_2, and AOC were determined from blood collected *via* vacutainer and immediately centrifuged at 3000 rpm for 15 min at 4 C in a Beckman (J2–21) centrifuge (Fullerton, CA). The plasma was then stored in microtubes at –80 C until analyzed. Additionally, 1 ml of blood was collected into EDTA containing tubes for analysis of total GSH.

Determination of plasma MDA (μmol/l) was accomplished using high performance liquid chromatography (HPLC) with a fluorescent detector. The blood sample was reacted with the derivatization reagent and incubated for 60 min at 95 C. The solution was cooled down and centrifuged for 5 min, the supernatant was diluted with the buffer solution and 20 μl of the mixture was injected to the column (Reversed phase C18-column-Bischoff Prontosil Euroband C18 5 μm, 125×4 mm). Standards were run for each determination.

Determination of total GSH (μmol/l) was accomplished using HPLC with a fluorescent detector as well. Initially a reduction solution was added to the sample, the resulting solution was incubated with a derivatization solution for 20 min. A precipita-

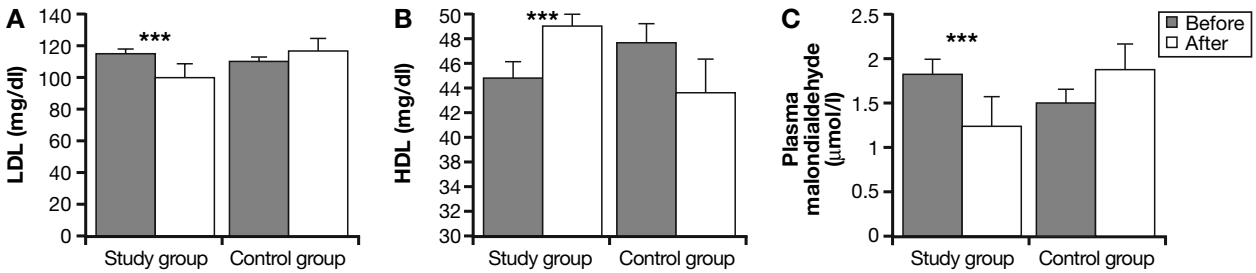

Fig. 1 - *Lipid parameters. A) The effect of polyphenol-containing antioxidant on LDL levels in Type 2 diabetic patients and its comparison with the placebo group. The statistical comparison of the differences of data changes in 3 months after the polyphenol-containing antioxidant and the placebo were given, reveals that LDL decrease in the study group* vs *the LDL increase in the control group was significant (p<0.001) (Table 1). ***p<0.001 comparing data before and after 3 months of the trial in the study group. Data are shown as means±SD. B) The effect of polyphenol-containing antioxidant on HDL levels in Type 2 diabetic patients and its comparison with the placebo group. The statistical comparison of the differences of data changes in 3 months after the polyphenol-containing antioxidant and the placebo were given, reveals that HDL increase in the study group* vs *the HDL decrease in the control group was significant (p<0.001) (Table 1). ***p<0.001 comparing data before and after 3 months of the trial in the study group. Data are shown as means±SD. C) The effect of polyphenol-containing antioxidant on plasma malondialdehyde (MDA) levels in Type 2 diabetic patients and its comparison with the placebo group. The statistical comparison of the differences of data changes in 3 months after the polyphenol-containing antioxidant and the placebo were given, reveals that plasma MDA decrease in the study group* vs *the plasma MDA increase in the control group was significant (p<0.001) (Table 1). ***p<0.001 comparing data before and after 3 months of the trial in the study group. Data are shown as means±SD.*

tion solution was added to separate higher molecular substances and 20 µl of the resulting fluorescent product was injected to the reversed phase C_{18}-column. Internal standards were run during each determination.

For H_2O_2 determinations (mmol/l), blood was taken from patient's finger in a glass pipette and mixed with a special solution and transferred to a tube. The mixture was centrifuged for 1 min and placed in a free radical measuring device (Callegari 1930 Form OX) where measurement lasted for 6 min (250 fort units and less were considered normal, 250-350 fort units medium level oxidative stress and 350-600 units were considered high).

Determination of AOC (mmol/l) was accomplished by an automated analyzer. In the assay, antioxidants present in blood caused a reduction in absorption of the colored product proportional to their concentration. The total AOC value of the samples expressed as an equivalent of the mmol concentration of 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid solution. The final results are expressed as mmol 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid equivalent/l.

Statistical analysis

The mean values of the descriptive and metabolic parameters with dispersion measure (SD) prior to the trial were measured separately in the study and control groups and the statistical analysis was done with the student's t-test (Table 2). The comparison of the parameters before and after the trial was done separately in the study and control groups by paired t-test and

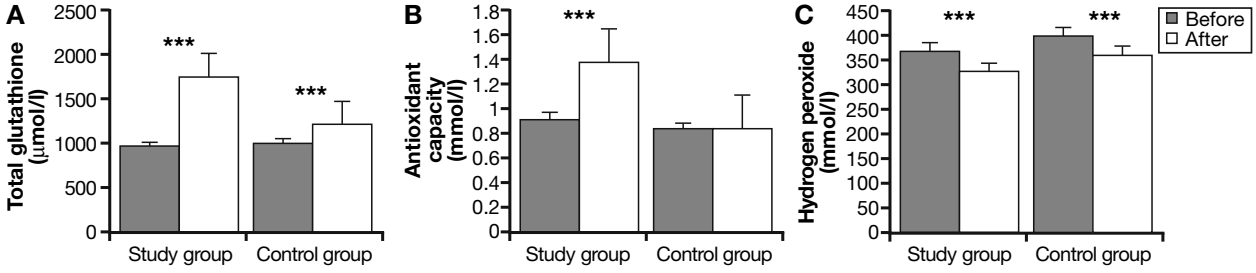

Fig. 2 - *Parameters of oxidative stress. A) The effect of polyphenol-containing antioxidant on total glutathione (GSH) in Type 2 diabetic patients and its comparison with the placebo group. The statistical comparison of the differences of data changes in 3 months after the polyphenol-containing antioxidant and the placebo were given, reveals that total GSH increased in both of the groups but it increased more in the study group. The comparison of the change in groups was significant (p<0.001) (Table 1). ***p<0.001 comparing data before and after 3 months of the trial in the study group and the control group. Data are shown as means±SD. B) The effect of polyphenol-containing antioxidant on antioxidant capacity (AOC) in Type 2 diabetic patients and its comparison with the placebo group. The statistical comparison of the differences of data changes in 3 months after the polyphenol-containing antioxidant and the placebo were given, reveals that AOC increase in the study group* vs *the AOC decrease in the control group was significant (p<0.001) (Table 1). ***p<0.001 comparing data before and after 3 months of the trial in the study group. Data are shown as means±SD. C) The effect of polyphenol-containing antioxidant on total hydrogen peroxide (H_2O_2) in Type 2 diabetic patients and its comparison with the placebo group. The statistical comparison of the differences of data changes in 3 months after the polyphenol-containing antioxidant and the placebo were given, reveals that H_2O_2 decreased in both of the groups but it decreased more in the study group. However, the comparison of the change in groups was not significant (Table 1). ***p<0.001 comparing data before and after 3 months of the trial in the study group and the control group. Data are shown as means±SD.*

Table 1 - *The statistical comparison of the differences of data changes in 3 months after the polyphenol containing antioxidant and the placebo were given.*

Parameters	Study group (no.=56) Mean±SD	Control group (no.=58) Mean±SD	*p*
Blood glucose	–9.87±28.82	–13.75±54.50	ns
HbA_{1c}	–0.32±0.98	–0.61±2.13	ns
LDL	–15.74±29.76	5.93±34.93	<0.001
HDL	4.51±9.16	–4.31±11.30	<0.001
TG	–15.69±37.07	8.39±75.28	<0.05
H_2O_2	–42.21±21.36	–38.71±57.82	ns
AOC	0.45±0.62	–0.09±0.28	<0.001
Total GSH	761.86±652.71	202.11±390.76	<0.001
Plasma MDA	–0.57±0.55	0.37±0.48	<0.001

HbA_{1c}: glycated hemoglobin; TG: triglyceride; H_2O_2: hydrogen peroxide; AOC: antioxidant capacity; GSH: glutathione; MDA: malondialdehyde.

Wilcoxon rank test (Fig. 1 and 2). Then, for the comparisons of the differences of the data changes in the study and control groups before and after the trial, the statistical significance was determined by Mann-Whitney U test (Table 1). Relationships between individual variables in the study and control groups were examined using Spearman correlation coefficients (Tables 3 and 4). *p*-values <0.05 were considered to be statistically significant. The statistical analysis were performed using the SPSS software, version 16.0 for Windows.

RESULTS

A total of 114 male and female non-smokers with Type 2 DM were enrolled to the study. Among them, 56 were randomly taken in the study group and given polyphenol-containing capsules, whereas 58 were randomly taken in the control group and given placebo capsules in addition to the exercise and diet regimens and their antidiabetic treatments. There were 22 male and 34 female subjects in the study group whereas 21 male and 37 female subjects in the control group. The difference in gender between the 2 groups did not show any statistical significance (*p*=0.48). There was no statistically significant difference in terms of age, BMI, and systolic and diastolic blood pressures between the study and control groups (Table 2). Also, the initial metabolic parameters like fasting blood glucose (FBG), HbA_{1c}, LDL, HDL, and TG at the beginning of the trial did not show any meaningful difference between the study and the control groups (Table 2).

Table 2 - *Descriptive characteristics and metabolic parameters of the control and study groups before the polyphenol-containing antioxidant and the placebo were given.*

Parameters	Study group (no.=56) Mean±SD	Control group (no.=58) Mean±SD	*p*
Age	53.51±6.82	53.91±7.16	ns
Systolic blood pressure	126.57±14.23	123.33±13.67	ns
Diastolic blood pressure	80.45±9.36	80.09±8.87	ns
BMI	31.37±4.98	30.29±6.28	ns
Blood glucose	162.02±48.11	155.35±58.51	ns
LDL	115.18±31.14	109.95±30.49	ns
HDL	44.05±10.89	47.08±11.40	ns
TG	156.42±68.76	171.93±88.00	ns
HbA_{1c}	7.36±1.78	7.71±2.33	ns

BMI: body mass index; TG: triglyceride; HbA_{1c}: glycated hemoglobin.

Table 3 - *Spearman correlation analysis between variables in the study group.*

	H_2O_2		AOC		Total GSH		Plasma MDA	
	r	*p*	r	*p*	r	*p*	r	*p*
AOC	–0.162	ns						
Total GSH	0.127	ns	0.085	ns				
Plasma MDA	0.209	ns	–0.045	ns	0.083	ns		
BMI	0.151	ns	0.171	ns	–0.087	ns	0.036	ns
Blood glucose	–0.217	ns	0.040	ns	0.147	ns	0.092	ns
HbA_{1c}	–0.052	ns	–0.044	ns	0.101	ns	–0.203	ns
LDL	–0.010	ns	0.179	ns	0.014	ns	0.030	ns

H_2O_2: hydrogen peroxide; AOC: antioxidant capacity; GSH: glutathione; MDA: malondialdehyde; BMI: body mass index; HbA_{1c}: glycated hemoglobin.

Figure 1A shows the comparison of the LDL levels before and 3 months after the subjects started to take the polyphenol-containing antioxidant and the placebo. While in the study group LDL levels showed a statistically significant decrease after the antioxidant supplement was given (*p*<0.001), the LDL levels in the control group showed an increase and the change in LDL levels in the control group was not significant (*p*>0.05).

Figure 1B shows the comparison of the HDL levels before and 3 months after the subjects started to take the polyphenol-containing antioxidant and the placebo. While in the study group HDL levels showed a statistically significant increase after the antioxidant supplement was given (*p*=0.001), the HDL levels in the control group showed a significant decrease (*p*<0.01).

Figure 1C shows the comparison of the plasma MDA levels before and 3 months after the subjects started to take the polyphenol-containing antioxidant and the placebo. While in the study group plasma MDA levels showed a statistically significant decrease after the antioxidant supplement was given (*p*<0.001), the plasma

Table 4 - *Spearman correlation analysis between variables in the control group.*

	H_2O_2		AOC		Total GSH		Plasma MDA	
	r	*p*	r	*p*	r	*p*	r	*p*
AOC	0.117	ns						
Total GSH	0.248	ns	0.131	ns				
Plasma MDA	–0.040	ns	–0.209	ns	–0.163	ns		
BMI	–0.023	ns	0.127	ns	0.045	ns	–0.114	ns
Blood glucose	–0.002	ns	–0.184	ns	–0.049	ns	0.177	ns
HbA_{1c}	–0.178	ns	–0.228	ns	–0.072	ns	–0.014	ns
LDL	–0.207	ns	–0.108	ns	–0.198	ns	–0.120	ns

H_2O_2: hydrogen peroxide; AOC: antioxidant capacity; GSH: glutathione; MDA: malondialdehyde; BMI: body mass index; HbA_{1c}: glycated hemoglobin.

MDA levels in the control group showed a significant increase ($p<0.001$).
Figure 2A shows the comparison of the total GSH levels before and 3 months after the subjects started to take the polyphenol-containing antioxidant and the placebo. Total GSH increased in both groups and the increase within each group was statistically significant ($p<0.001$ for both the study and the control groups).
Figure 2B shows the comparison of the AOC levels before and 3 months after the subjects started to take the polyphenol-containing antioxidant and the placebo. While in the study group AOC levels showed a statistically significant increase after the antioxidant supplement was given ($p<0.001$), the AOC levels did not show a significant change in the control group ($p>0.05$).
Figure 2C shows the comparison of the H_2O_2 levels before and 3 months after the subjects started to take the polyphenol-containing antioxidant and the placebo. Hydrogen peroxide decreased in both groups and the decrease within each group was statistically significant ($p<0.001$ for both the study and the control groups).
In Table 1, the statistical comparison of the differences of the data changes in parameters before and after the subjects started to take the polyphenol-containing antioxidant and the placebo is shown. For the comparison of the differences of the data changes, we first determined the data change in 3 months for each group and then we compared the differences of the change in data between the groups. The positive effects of polyphenol-containing antioxidant supplement on lipid peroxidation was shown by the decrease in LDL and increase in HDL in the study group whereas the control group showed an increase in LDL and a decrease in HDL (Fig. 1A and 1C). The comparison of the changes in these parameters between the 2 groups was found to be statistically significant ($p<0.001$ for both LDL and HDL) (Table 1). Accordingly, as a by-product of lipid peroxidation, plasma MDA was decreased by the addition of polyphenol-containing antioxidant supplement in the study group ($p<0.001$) whereas an increase in plasma MDA was seen in the control group ($p<0.001$) (Fig. 1C). The comparison of the changes in plasma MDA between these 2 groups was found to be statistically significant, as well ($p<0.001$) (Table 1). Although the H_2O_2 levels showed statistically significant decreases in both groups ($p<0.001$ for both the study and the control groups) the comparison of the difference in H_2O_2 levels changes before and 3 months after the polyphenol-containing antioxidant and the placebo were given, did not show any significance ($p>0.05$) (Table 1). On the other hand, AOC showed a statistically significant increase in the study group ($p<0.001$) compared to the control group in which there was a minor change in AOC levels ($p>0.05$) (Fig. 2B). The comparison of the changes in AOC between these 2 groups was found to be statistically significant ($p<0.001$) (Table 1). Total plasma GSH showed statistically meaningful changes in both groups ($p<0.001$ for each group) (Fig. 2A) and the comparison of the differences of the data changes revealed that GSH increased more in the study group compared to control group and this was a statistically meaningful difference ($p<0.001$) (Table 1). The FBG levels and HbA_{1c} levels were both decreased in both the study and control groups, but the reduction in their levels within each group were minimal and statistically not significant. The comparison of the changes in these parameters between the 2 groups was not found to be statistically significant (Table 1).
Tables 3 and 4 show the Spearman correlation analysis of the metabolic and oxidative variables in the study and control groups. Neither positive nor negative correlations were found between the metabolic and oxidative parameters. The statistically meaningful changes in the antioxidant and oxidative parameters of the study group were solely related to the effect of polyphenol-containing antioxidant supplement (Table 3). Also, in the control group, there was not any statistically significant correlation between the metabolic parameters and the oxidative parameters (Table 4).

DISCUSSION

The present study indicated that the administration of a polyphenol-rich antioxidant supplement containing pomegranate extract, green tea extract, and vitamin C to Type 2 diabetic patients for 3 months: (a) prevented the decrease in GSH concentration and increased AOC thus increased antioxidant activity; (b) prevented the increase in plasma MDA thus inhibited lipid peroxidation; (c) decreased LDL and increased HDL.
Currently published studies showed that oxidative stress in DM seems to be caused by both an increased production of free radicals and a reduction in antioxidant defenses (1, 3, 4, 21, 22). The increased oxidative stress in DM induces an increase of lipid peroxidation products such as MDA (2, 5, 12, 21) and a decrease of glutathione levels (1, 2, 22). In the present study, the addition of food supplement to the antidiabetic diet and exercise regimens in the study group increased total GSH and AOC in blood more than the control group. It also caused a remarkable decrease in plasma MDA and desirable cholesterol levels in the study group. Bloomer et al. (6), claimed that plasma MDA can be increased by aerobic exercise which causes an oxidative stress to the body, whereas Lazarevic et al. (2) and Coskun et al. (21) demonstrated that regular exercise can decrease LDL and MDA and increase HDL and glutathione in Type 2 diabetics. Bloomer et al. (6) also, concluded that diets rich in polyphenols can cause attenuation in the MDA increase observed with exercise similar to the results of the present study.
Our study is unique in the point that all of our subjects were given an antidiabetic diet and exercise regimens in conjunction to their regular oral antidiabetics, 2 weeks prior to the commencement of the trial and advised to continue those regimens by the end of the trial. So, all of our subjects were brought to the same level in terms of metabolic measures prior to the trial (Table 2).
Among the most popular polyphenol-rich antioxidant beverages including 100% fruit juices, iced tea beverages, and red wine, PJ has the most potent antioxidant capacity followed by red wine and grape juice (14, 16). The Pomegranate fruit extract (PFE), because of its high content of polyphenolic antioxidants, is expected to possess beneficial effects similar to those exerted by PJ (17).

All phenolic compounds are known to be strong antioxidants which result in a reduction of lipid peroxidation (15). Accordingly, administration of regular PJ and, recently, PFE to animals and humans had a significant protective effect on atherosclerosis or hypertension (16, 17). Ignarro et al. (16) demonstrated in their study that PJ shows its antioxidant activity by enhancing the biological actions of NO which in turn, prevents the oxidation of LDL-cholesterol.

Polychronopoulos et al. (18) demonstrated that tea consumption (either green or black) is associated with reduced levels of FBG among non-obese elderly people in Mediterranean Islands. Conversely, Buyukbalci et al. (19) revealed that some herbals like black and green tea do not inhibit glucose diffusion using *in vitro* model glucose absorption, but they concluded that their phenolic compounds and antioxidant activities may be useful for the prevention of the development of vascular diseases seen in Type 2 DM. In the present study, although significant effects of the phenolic compounds on MDA, total GSH, HDL, and LDL levels were observed, no such effects were measured neither on blood glucose nor HbA_{1c} levels of the patients. The reason for this might be that the effect of the food supplement had been indirectly through its antagonizing action on ROS induced oxidative stress rather than by the direct action of glycosylated proteins on vascular endothelium in our study.

In the present study, H_2O_2 decreased significantly both in the placebo group and the study group and we did not observe any statistical significance when we compared the differences of the 2 groups. Although oxidative stress markers have been studied extensively, a consensus has not been reached yet which of these has been the most reliable one for clinical practice. Since oxidants are highly reactive compounds, their half-life is only of seconds (5). Therefore, after exposing to oxyradicals, the modified products of lipids, nucleic acids and carbohydrates have been accepted as more reliable markers of oxidative stress because of their longer half-life. For this reason, we propose MDA as a marker of oxidative stress and total GSH as a measure of antioxidant defense.

Naturally occurring antioxidants like vitamin E, vitamin C, β-carotene, green tea and, recently, PJ/PFE (13-16, 19, 23) can inhibit the oxidative modification of LDL. This action could positively influence the atherosclerotic process and, as a consequence, the progression of coronary heart disease. Marfella et al. (22) also suggested that acute hyperglycemia increases blood pressure by reducing the availability of endogenous NO and this can be prevented by GSH and reversed by the precursor of NO synthesis, L-arginine.

The evidence presented in this study suggested that the polyphenol-rich food supplement containing PFE, green tea, and vitamin C prevented the oxidation of LDL-cholesterol and increased GSH and antioxidant capacity in Type 2 diabetic patients who were without any complications. Thus, this action of the food supplement can prevent atherosclerosis and cardiac complications in Type 2 diabetic patients.

In conclusion, the polyphenol-rich food supplement containing pomegranate extract, green tea extract, and ascorbic acid has been found to have beneficial effects in patients with Type 2 DM. These polyphenol-rich compounds caused an additional decrease in the levels of MDA and an increase in GSH and AOC. The increase in HDL and the decrease in LDL levels were observed in the study group, indicating the significance of these phenolic compounds on cholesterol transport. Therefore, the present study suggested that the use of the polyphenol-rich food supplement containing pomegranate extract, green tea extract, and ascorbic acid might be beneficial in patients with Type 2 DM for prevention of cardiovascular complications.

ACKNOWLEDGMENTS

The present study was supported by Yeditepe University and Com Ilac Chemistry Industry and Trade Company.

REFERENCES

1. Miranda M, Muriach M, Almansa I, et al. CR-6 protects glutathione peroxidase activity in experimental diabetes. Free Radic Biol Med 2007, 43: 1494-8.
2. Lazarevic G, Antic S, Cvetkovic T, Vlahovic P, Tasic I, Stefanovic V. A physical activity programme and its effects on insulin resistance and oxidative defense in obese male patients with type 2 diabetes mellitus. Diabetes Metab 2006, 32: 583-90.
3. Colak E, Majkic-Singh N, Stankovic S, et al. Parameters of antioxidative defense in type 2 diabetic patients with cardiovascular complications. Ann Med 2005, 37: 613-20.
4. Ceriello A. New insights on oxidative stress and diabetic complications may lead to a "causal" antioxidant therapy. Diabetes Care 2003, 26: 1589-96.
5. Atli T, Keven K, Avci A, et al. Oxidative stress and antioxidant status in elderly diabetes mellitus and glucose intolerance patients. Arch Gerontol Geriatr 2004, 39: 269-75.
6. Bloomer RJ, Goldfarb AH, and Mckenzie MJ. Oxidative stress response to aerobic exercise: comparison of antioxidant supplements. Med Sci Sports Exerc 2006, 38: 1098-105.
7. Bohlen HG, Lash JM. Topical hyperglycemia rapidly suppresses EDRF-mediated vasodilatation of normal rat arterioles. Am J Physiol 1993, 265: 219-25.
8. Giugliano D, Marfella R, Coppola L, et al. Vascular effects of acute hyperglycemia in humans are reversed by L-arginine. Evidence for reduced availability of nitric oxide during hyperglycemia. Circulation 1997, 95: 1783-90.
9. Kawano H, Motoyama T, Hirashima O, et al. Hyperglycemia rapidly suppresses flow-mediated endothelium- dependent vasodilation of brachial artery. J Am Coll Cardiol 1999, 34: 146-54.
10. Draper HH, Hadley M. A review of recent studies on the metabolism of exogenous and endogenous malondialdehyde. Xenobiotica 1990, 20: 901-7.
11. Ugochukwu NH, Figgers CL. Attenuation of plasma dyslipidemia and oxidative damage by dietary caloric restriction in streptozotocin-induced diabetic rats. Chem Biol Interact 2007, 169: 32-41.
12. Griesmacher A, Kindhauser M, Andert SE, et al. Enhanced serum levels of thiobarbituric-acid-reactive-substances in diabetes mellitus. Am J Med 1995, 98: 469-75.
13. Henning SM, Zhang JZ, McKee RW, Swendseid ME, Jacob RA. Glutathione blood levels and other oxidant defense indices in men fed diets low in vitamin C. J Nutr 1991, 121: 1969-75.
14. Seeram NP, Aviram M, Zhang Y, et al. Comparison of antioxidant potency of commonly consumed polyphenol-rich beverages in the United States. J Agric Food Chem 2008, 56: 1415-22.
15. Kaur R, Arora S, Singh B. Antioxidant activity of the phenol rich fractions of leaves of Chukrasia tabularis A. Juss. Bioresour Technol 2008, 99: 7692-8.
16. Ignarro LJ, Byrns RE, Sumi D, Nigris FD, Napoli C. Pomegranate juice protects nitric oxide against oxidative destruction and en-

hances the biological actions of nitric oxide. Nitric Oxide 2006, 15: 93-102.

17. de Nigris F, Balestrieri ML, Williams-Ignarro S, et al. The influence of pomegranate fruit extract in comparison to regular pomegranate juice and seed oil on nitric oxide and arterial function in obese Zucker rats. Nitric Oxide 2007, 17: 50-5.
18. Polychronopoulos E, Zeimbekis A, Kastorini CM, et al. Effects of black and green tea consumption on blood glucose levels in non-obese elderly men and women from Mediterranean Islands (MEDIS epidemiological study). Eur J Nutr 2008, 47: 10-6.
19. Büyükbalci A, El SN. Determination of in vitro antidiabetic effects, antioxidant activities and phenol contents of some herbal teas. Plant Foods Hum Nutr 2008, 63: 27-33.
20. Wayner DD, Burton GW, Ingold KU. The antioxidant efficiency of vitamin C is concentration-dependent. Biochim Biophys Acta 1986, 884: 119-23.
21. Coskun O, Ocakci A, Bayraktaroglu T, Kanter M. Exercise training prevents and protects streptozotocin-induced oxidative stress and beta-cell damage in rat pancreas. Tohoku J Exp Med 2004, 203: 145-54.
22. Marfella R, Nappo F, Angelis L, Paolisso G, Tagliamonte MR, Giugliano D. Hemodynamic effects of acute hyperglycemia in type 2 diabetic patients. Diabetes Care 2000, 23: 658-63.
23. Marchioli R. Antioxidant vitamins and prevention of cardiovascular disease: laboratory, epidemiological and clinical trial data. Pharmacol Res 1999, 40: 227-38.