Journal of Medicinal Plants Research Vol. 5(10), pp. 2029-2034, 18 May, 2011
Available online at http://www.academicjournals.org/JMPR
ISSN 1996-0875 ©2011 Academic Journals

Full Length Research Paper

Effect of supplementation with grape seed *(Vitis vinifera)* extract on antioxidant status and lipid peroxidation in patient with type II diabetes

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Accepted 28 February, 2011

To determine the effects of grape seed extract (GSE) supplementation on antioxidant defense system and lipid peroxidation in persons with type 2 diabetes mellitus (T2D). During a randomized double blind clinical trial adult subjects with type 2 diabetes were supplemented with 200 mg/day of GSE or placebo for two months. Fasting blood samples were obtained at the beginning and the end of study to determine total antioxidant capacity (TAC), superoxide dismutase (SOD) and glutathione peroxidase (GPX) levels in red blood cells and the serum malondialdehyde levels (MDA). After 2 months of grape seed extract supplementation, TAC decreased significantly from 0.71±0.17 to 0.7±0.15 ($p<0.05$) and SOD increased significantly from 1453±210 to 1514±243 ($p<0.05$) with no significant changes in MDA and GPX concentrations. There were no significant differences in the levels of TAC, SOD, GPX and MDA between groups ($P > 0.05$) during study. Although there were significant changes in TAC and SOD levels in GSE group, but there were no significant differences compared to placebo group. Therefore that is better to more investigation performed with upper dose and longer time by attention to special condition of diabetes and the medicines that are consumed.

Key words: Grape seed extract, oxidative stress, antioxidant status, lipid peroxidation, diabetes.

INTRODUCTION

Diabetes mellitus is a chronic metabolic disease which has significant effects on health, quality of life and also on health care system. The worldwide prevalence of diabetes is approximately 3%, of which 90% is type 2 diabetes mellitus (T2D). T2D is characterized by some metabolic abnormalities in carbohydrate and lipid metabolism (Hwang et al., 2009). T2D is regularly associated with increased oxidative stress (Lai, 2008) and oxidative damage due to free radicals (Stephens et al., 2009). There are several potential sources for production of free radicals in diabetes, including: autoxidation of plasma glucose, leucocyte activation, and increased transition metal bioavailability (Lai, 2008) which cause dramatic oxidative damage, illustrated by the high levels of lipid and DNA peroxidation products. Therefore

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Abbreviations: T2D, type 2 diabetes mellitus; **b.w,** body weight; **FBS,** fasting blood sugar; **(HbA1c),** glycated haemoglobin; **TG,** Triglyceride; **(TC),** total cholesterol, **LDL.C,** low-density lipoprotein cholesterol; **(GPX),** glutathione peroxidase; **MDA,** malondialdehyde; **(SOD),** superoxide dismutase; **TAC,** total antioxidant capacity.

the total antioxidant status (TAS) in type 1 or 2 DM is lower than that of age matched controls. All these diabetes-related abnormalities can intensify the endothelial dysfunction, oxidation of LDL and foam cell formation which ultimately lead to the formation of the atheroma plaque (Dierckx et al., 2003). Nowadays there is considerable interest in the potential health benefits of natural remedies such as medicinal plants and their extracts. One of these natural extracts, is grape seed extract (GSE) which has different medicinal properties including anti-inflammatory, anticarcinogenic, platelet aggregation inhibiting, and metal chelating properties, etc (Balu et al., 2005). The antioxidant effects of grape seed extract has been confirmed in different studies (Enginar et al., 2007; Hemmati et al., 2008; Li et al., 2008; Morin et al., 2008; Sharma et al., 2007) which seems to have potentials for improving or treating type 2 diabetes and it's associated metabolic disorders.

Some human clinical trials investigated various effects of GSE (Clifton, 2004; Saada et al., 2009; Sano et al., 2007; Shenoy et al., 2007; Vigna et al., 2003) and there are some experimental studies about it's antidiabetic effects (Hwang et al., 2009; Lee et al., 2008; Lee et al., 2007; Xu et al., 2008). However, to date, there is no study on GSE effects against diabetes in humans. Therefore, the present study investigated the antioxidant effects of GSE on type 2 diabetic patients.

MATERIALS AND METHODS

Volunteers were adult males and females, 30 to 65 years of age, with type 2 diabetes for at least five years, using drugs to control blood glucose and not using insulin. Key exclusion criteria included pregnancy and lactation, receiving anticoagulant drugs, receiving antioxidant supplements in the previous three months, persons under gastric treatment, acute renal failure, hepatitis, hyper and hypothyroidism, recent surgery, acute infection, celiac and diarrhea. Subjects were enrolled from November to January from the Imam Reza endocrine clinic of Tabriz in Iran. The study received the agreement of the Tabriz University of Medical Sciences. Subjects were informed of the purposes of the study and were free to ask questions throughout the study.

The study design was randomized, double blind and placebo controlled. Subjects, n = 60, were divided randomly into two groups and supplemented daily with either 200 mg of grape seed extract or placebo for two months'. The alcoholic standardized grape seed extract (*Vitis vinifera*) and placebo, capsules were provided by Drug Applied Research center Tabriz University of Medical Sciences. Volunteers received all of the supplements (for 2 months) at the beginning of study and they were asked to return the non-used supply after two month to help measure their compliance. Volunteers were advised to maintain their normal dietary and exercise habits during the study.

Blood samples were obtained after an overnight fast at the beginning of the study and after two months of supplementation. Height and weight were recorded at the beginning of the study and after two months. The dietary data of these subjects were collected by a 24 h dietary recall for three days (one holiday day and two usual days) at the beginning and during the study and were analyzed with nutrition 4 software. 48 of 60 subjects (26 = grape seed extract, 22 = placebo) completed our study.

Biochemical analysis

Total antioxidant capacity (TAC) were measured by method of Ferric reducing antioxidant power (FRAP). Ferric to ferrous ion reduction at low pH (3.6 in acetate buffer) produces a colored ferrous-tripyridyltriazine complex. FRAP values are obtained by reading the absorbance change at 593 nm, which are linear over a wide concentration range (Benzie and Strain, 1996). Activation of red blood cell superoxide dismutase (SOD) and glutathione peroxidase (GPX) was measured by colorimetric method on an autoanalyser (Abbott, model Alcyon 300, USA) with RANSOD and RANSEL kits respectively (RANDOX Laboratory, UK). Serum MDA level, was measured via reaction with thiobarbituric acid (TBA) as a TBA reactive substance (TBARS) to generate a complex of pink color. Next, its fluorescence intensity was measured at 547 nm with excitation at 525 nm by a spectrofluorimeter (Kontron, model) (Del et al., 2003).

Statistical analysis

Results were expressed as means ± SD. Statistical analysis was conducted on SPSS software using unpaired student's t test to compare results between two groups in different times and with paired student's t test to compare differences within groups $P < 0.05$ was considered statistically significant.

RESULTS

As shown in Tables 1 and 2, at the beginning of the study, the two groups were similar based on body weight (b.w), body mass index (BMI), systolic blood pressure (SBP), diastolic blood pressure (DBP), fasting blood sugar (FBS), glycated hemoglobin (HbA1c), triglyceride (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL.C). Following supplementation with grape seed extract as an antioxidant in T2D there were no significant changes in any of these variables between and within two groups.

Effects of supplementation on dietary intake

The intake of energy, carbohydrate, protein, fat, vitamin C and E and selenium were shown in Table 3. The comparison between two groups showed that there were no significant differences at baseline and after 2 months of supplementation on energy, total fat and vitamin E intake but there were significant differences on carbohydrate, protein and selenium at the beginning of the study and also on vitamin C and selenium at the end of study. There were no significant changes in groups after in the initial and after two months of supplementation.

Effects of supplementation on TAC, SOD, GPX and MDA levels

As shown in Table 4, levels of TAC and MDA were not

Table 1. Basic characteristics of subjects during the study.

	GSE (26)	Placebo (22)
b.w (Kg)		
Initial	75±17	73 ± 11
End	75±18	73 ± 11
BMI (Kg/m^2)		
Initial	31±6	30±4
End	31±6	31±6
SBP (mmHg)		
Initial	13±2	13±2
End	13±2	12±2
DBP (mmHg)		
Initial	8±1	8±1
end	8±1	8±1

(b.w): body weight; (BMI), body mass index; (SBP), systole blood pressure; (DBP), diastol blood pressure.

significantly different at baseline between two groups. Differences were noted, however, comparing the initial and post-treatment levels of TAC and SOD changed after two months of supplementation with grape seed extract. The levels of TAC decreased significantly from 0.71±0.17 to 0.7±0.15 ($p<0.05$) and SOD increased significantly from 1453±210 to 1514±243 ($p<0.05$) in GSE Group. The levels of TAC, SOD, GPX and MDA weren't significantly different ($p<0.05$) in the treatment groups compared to placebo at the end of study.

DISCUSSION

In this clinical trial study, we investigated the effect of supplementation with GSE on antioxidant status and lipid peroxidation of type 2 diabetic patients. There were no significant differences between two group at the initial and end of the study. In the GSE group, there were significant reduction in TAC and significant increase in SOD after treatment with no significant effects on GPX and MDA. It has been shown that grape seed proanthocyanidin extract (GSPE) provides significantly greater protection against free radicals and free radical-induced lipid peroxidation and DNA damage than vitamins C, E and β-carotene using similar doses (Bagchi et al., 1998). Moreover the antioxidant effect of GSE given to animals was reported more effective than vitamin E administered before whole-body irradiation in rats (Enginar et al., 2007). Morin et al. (2008) demonstrated that GSP has significant protective ability against oxidative damage in leukocytes and may be effective in the prevention of oxidative lymphocyte damage by reactive oxygen species *in vitro*. The results of Enginar et al. (2007) indicate that GSE supplementation significantly enhanced the antioxidant status (the levels of GSH, retinol, β -carotene and ceruloplasmin concentration), and decreased the incidence of free radical induced lipid peroxidation (the level of MDA and nitrite concentration) in blood samples of rats exposed to x-radiation (Lee et al., 2008).

Standardized grape seed extract contains 92 to 95% oligomeric proanthocyanidin (Arora and Ansari, 2010) and suggests that oligomeric proanthocyanidins could act as a regulator in inflammatory reactions associated with oxidative stress in type 2 diabetes and purposed that proanthocyanidin administration, especially the oligomeric form, may improve oxidative stress via the regulation of hyperlipidemia than hyperglycemia in type 2 diabetes. (Al-Awwadi et al., 2005) offered polyphenolic extracts enriched in different types of polyphenols possess differential effects on insulin resistance, hypertension, and cardiac hypertrophy, and polyphenols modulate the expression of NAD(P)H oxidase. In the study of saada et al. (2009) pre-irradiation GSE administration, significantly decreased radiation-induced oxidative stress in heart tissues which was substantiated by a significant amelioration of serum lactate dehydrogenase (LDH), creatine phosphokinase (CPK) and aspartateaminotransferase (AST) activities. GSE treatment also attenuated the oxidative stress in pancreas tissues which was associated with a significant improvement in radiation-induced hyperglycemia and hyperinsulinemia. In another study after 12 week administration of tablet containing 0, 200 or 400 mg grape seed extract to healthy subject MDA-LDL level,

Table 2. Laboratory characteristics of subjects during the study.

	GSE (26)	Placebo (22)
FBS (mg/dl)		
Initial	121±39	116±31
End	122±45	110±26
HbA1c (%)		
Initial	6.5±1	6.5±1
End	6.6±1	6.4±1
TG (mg/dl)		
Initial	144±42	162±61
End	136±27	172±73
TC (mg/dl)		
Initial	158±26	146±18
End	159±21	154±19
LDL (mg/dl)		
Initial	79±27	71±19
End	80±35	83±21

Fasting blood sugar, (HbA1c), glycated haemoglobin; (TG), Triglyceride; (TC), total cholesterol; (LDL.C); low-density lipoprotein cholesterol of subject during the study.

Table 3. Average daily intake of calorie, carbohydrate, protein, fat, vitamin C, E and Se during the study.

Group	Grape seed extract (26)	Placebo (22)
Energy (kcal)		
Initial	2237±77	1932±507
end	2300±730	1951±498
Carbohydrate (g)		
Initial	296±95	236 ±106[a]
End	307±109	264 ±93
Protein (g)		
Initial	78±24	62±15[a]
End	81±25	69±17
Fat (g)		
Initial	90±52	78±24
End	91±42	79±21
Vitamin C (mg)		
Initial	113±86	93±58
End	140±73	104±40[a]
Vitamin E (IU)		
Initial	19±18	15±7
End	18±11	15±6
Selenium		
Initial	0.058±0.025	0.043±0.016433[a]
End	0.056±0.025	0.043±0.15532[a]

Values are the mean ± SD. [a] means significant difference ($p<0.05$) between the placebo group and grape seed extract.

Table 4. Effects of 2 months of grape seed extract supplementation on TAC SOD, GPX and MDA.

Group	Grape seed extract (26)	Placebo (22)
TAC UmM Fe (• •)/L		
Initial	0.722±0.17	0.697±0.15
End	0.688±0.17[b]	0.690±0.11
SOD U/mgHb		
Initial	1453±210	1024±152[a]
End	1514±243[b]	1035±169[a]
GPX U/gHb		
Initial	29.107±3.951	22.950±2.121[a]
End	29.034±4.031	23.087±2.466
MDA nmol/ml		
Initial	3.5±0.67	3.5±0.75
End	3.1±0.88	3.1±0.77

Values are the mean ± SD. [a] means significant ($p<0.05$) difference between the placebo group and the grape seed extract. [b] means significant ($p<0.05$) difference between the initial and end of the study.

was significantly decreased in the 200 mg and in 400 mg groupcompared to the basal level (Sano et al., 2007).

The results of vigna et al. (2003) showed that supplementation of healthy male heavy smokers with a polyphenolic extract of grapes significantly decrease the concentration of thiobarbituric acid reactive substances (TBARS) as a marker of lipid peroxidation compared with placebo and basal values. More over Clifton (2004) demonstrate that 2 g/day of GSE (1 g of polyphenols) alone, or with 1 g/day of added quercetin in yoghurt, favourably alters vascular function, endothelial function, and degree of oxidative damage in comparison to a control (yoghurt). Thus sufficient polyphenols from GSE appear to be absorbed to influence endothelial nitric oxide production (Clifton 2004). Reasons of difference between our finding with other research is: diversity in qualification of tests and subjects, difference in dose of supplementation, study duration, and variation in flavonoid content of grape seed extracts. Variations in the flavonoids content have been correlated with sample origin, variety, degree of ripeness, refrigeration technique, as well as carbohydrate, fat and protein content. Cooking and industrial food-processing practices also decrease or chemically modify total flavan-3-ol content (Clifton, 2004). In our study, diabetic subject were no exclusion criteria on the basis of some medication. Studies on diabetic animals performed whereas no drugs except grape seed extract or other point supplement be used and in the *in vitro* studies major intermediated factor be controlled. Perhaps if our subject had abnormal lipid profiles or HbA1c or we medicated their's with upper dose, would achieved better responses.

Similarly that observed, although grape seed extract and proanthocyanidin are effective antioxidant *in vitro*, there are only a limited number of animal studies and fewer human studies that addressed the efficacy of these compounds as antioxidant. Thus more studies about their effects on human disease and drugs interference (*in vitro* and *in vivo*) would be needed.

ACKNOWLEDGEMENTS

Authors would like to thank Mr. Amir M. Vatankhah for technical assistance throughout the work. This research was financially supported by Drug Applied Research Center, Tabriz University of Medical Sciences, Iran. This work is the results of S. Abedini MSD dissertation.

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