

The Effect of Purslane Seeds on Biomarkers of Oxidative Stress in Diabetic Patients: A Randomized Controlled Cross-over Clinical Trial

Elahe Zakizadeh, Elham Faghihimani¹, Parvane Saneei, Ahmad Esmailzadeh

Food Security Research Center, Department of Community Nutrition, School of Nutrition and Food Science, Isfahan University Medical Sciences, Isfahan, Iran, ¹Endocrine and Metabolism Research Center, Isfahan University Medical Sciences, Isfahan, Iran

Correspondence to:

Dr. Ahmad Esmailzadeh, Food Security Research Center, Department of Community Nutrition, School of Nutrition and Food Science, Isfahan University of Medical Sciences, P.O. Box: 81745-151, Isfahan, Iran. E-mail: Esmailzadeh@hlth.mui.ac.ir

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ABSTRACT

Background: This study was designed to examine the effects of purslane seeds on biomarkers of oxidative stress in type 2 diabetic patients.

Methods: This cross-over randomized controlled clinical trial was conducted on 40 patients with type 2 diabetes. Participants were randomly assigned to receive either 10 g/day purslane seeds with 240 cc low-fat yogurt (intervention group) or only 240 cc low-fat yogurt (as a control group) for 5 weeks. After a 2-week washout period, subjects were moved to the alternate arm for an additional 5 weeks. At baseline and end of each study phase, fasting blood samples were collected to quantify biomarkers of oxidative stress.

Results: We observed a slight decrease in plasma total antioxidant capacity in both groups, however, between-group changes were not significant (-2.67 vs. -2.95 $\mu\text{g/dL}$, $P = 0.94$). Plasma malondialdehyde in purslane group increased slightly, however, we observed no significant effect in the control group (1.62 vs. -0.47 $\mu\text{g/dL}$, $P = 0.58$). Although both groups had a slight reduction in plasma oxidized low-density lipoprotein (ox-LDL), we failed to find any significant effect of purslane on plasma ox-LDL (-1.06 vs. -1.71 $\mu\text{g/dL}$, $P = 0.85$).

Conclusions: This cross-over clinical trial revealed that consumption of purslane seeds for 5 weeks in type 2 diabetic patients did not result in improved oxidative stress.

Keywords: Diabetes, medicinal plant, oxidative stress, purslane

INTRODUCTION

Diabetes is one of the most common endocrine disorders with increasing prevalence worldwide.^[1] It has been estimated that 7.7% of Iranian adult population is affected

by type 2 diabetes.^[2] Diabetic patients are at greater risk of morbidity and mortality from microvascular and macrovascular complications.^[3] It seems that oxidative stress plays a key role in the pathogenesis of diabetes complications.^[4] Patients with diabetes are susceptible to increased oxidative stress due to the excessive production of reactive oxygen species and impaired antioxidant defense mechanism.^[5,6] Hyperglycemia in turn results in increased production of free radicals which cause membrane damage due to peroxidation of membrane lipids and protein glycation.^[7] In order to prevent complications of diabetes, the control of oxidative stress is necessary in diabetic patients.

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Earlier studies have shown that the use of medications, consumption of antioxidants, as well as herbal medicine, might help control the oxidative stress.^[8] The use of medicinal plants for the management of diabetes has been common in Iranian population.^[9] Among others, the use of purslane, *Portulaca oleracea*, has been more prevalent by diabetic patients and has recently attracted great attention by scientists. Purslane is a rich source of antioxidants including Vitamins A, C, and E, and beta-carotene, which might help strengthening body's total antioxidant capacity (TAC).^[10,11] Previous studies have indicated that phenolic alkaloids of purslane, including oleracin A, B, and E increased scavenging of free radicals in rat brains.^[12] Administration of purslane aqueous juice was beneficial for hepatic, renal, and testicular tissue due to its antioxidant properties.^[13] Other studies have also demonstrated that polysaccharides from purslane led to lower exercise-induced oxidative stress.^[14] In diabetic rats, administration of purslane extract has been resulted in reduced lipid peroxidation.^[15]

Despite lots of information about the role of oxidative stress in the pathogenesis of diabetic complications, limited data are available on the application of dietary strategies for alleviating oxidative stress in diabetic patients. The antioxidant properties of purslane in prior studies have been mostly examined in animal models, and we are aware of no study that examined the effects of purslane intake on oxidative stress in humans. Assessing the effect of purslane intake on biomarkers of oxidative stress is particularly relevant for diabetic patients who are at increased risk of oxidative stress. The current study was designed to examine the effects of purslane seeds on biomarkers of oxidative stress in type 2 diabetic patients.

METHODS

Participants

Diabetic patients aged 35–65 attending Endocrine Research Center of Isfahan University of Medical Sciences, were included in this randomized cross-over clinical trial. The study was carried out in Isfahan, Iran, during January 2012–July 2012. On the basis of sample size formula suggested for cross-over clinical trials, a sample size of 38 people was needed for the whole trial. Given the high drop-outs in cross-over trials, we enrolled 40 subjects that diagnosed with type 2 diabetes, based on the criteria of American Diabetes Association in this trial.^[16] In addition to being a diabetic patient, subjects were needed to be nonsmokers, nonusers of alcohol, nonpregnant, and nonlactating women. They were recruited in the study if they had not changed the

dosage and type of medications in the last 2 months prior to the study and had no history of cardiovascular, lung, kidney, and liver diseases. A written informed consent was obtained from all participants. The study protocol was approved by the Ethical Committee of Isfahan University of Medical Sciences, Isfahan, Iran.

Study design

Diabetic patients were randomly assigned to the initial arm of the study to receive either 10 g/day purslane seeds powder, with 240 cc low-fat yogurt (intervention group) or only 240 cc low-fat yogurt (as control group) for 5 weeks. The prescribed dose of 10 g/day purslane seeds powder was selected based on a previous study.^[17] After a 2-week washout period, subjects were crossed over to the alternate treatment arm for an additional 5 weeks. Patients were asked not to change their regular diet and routine physical activity levels for the duration of the study. They were also requested not to start insulin injection and not to change the dosage and type of medications throughout the study. We assessed dietary intakes of subjects by the use of dietary records three times during each study phase (2 weeks days and a weekend day). Nutritionist IV software (First Databank) was used to calculate nutrient intakes based on dietary records. Physical activity levels were also assessed through physical activity records during the study once every 2 weeks. Data from physical activity records were expressed as MET-h/day.

Assessment of biochemical measures

Fasting blood samples (10 mL) were taken at baseline and end of each study phase in an early morning after an overnight fast. Blood samples were immediately centrifuged (Hettich D-78532, Tuttlingen, Germany) at 3500 rpm for 10 min to separate serum. Then, the samples were stored at -70°C before analysis at the laboratory. Plasma TAC and plasma oxidized low-density lipoprotein (ox-LDL) was assessed by the use of ELISA kit (Glory Science, USA). Serum malondialdehyde (MDA) levels were quantified using ELISA kit (Glory Science, USA). All inter-and intra-assay coefficients of variation for biochemical indicators were $<5\%$.

Statistical methods

All statistical analyses were done using the Statistical Package for Social Science version 16 (SPSS Inc., Chicago, Illinois, USA). Data on dietary intakes were compared by paired *t*-test. For each dependent variable, we computed the changes from baseline by subtracting the baseline value from the end-of-trial value. Within and between-group changes in dependent variables were compared by the use of paired samples *t*-test. Data are expressed as mean \pm standard deviation, and $P < 0.05$ was considered as statistically significant.

RESULTS

Overall, 40 subjects with type 2 diabetes mellitus who fulfilled the enrollment criteria entered in this study. No adverse events were reported from the subjects throughout the study period. There was no significant difference in dietary intakes of the participants between the two groups [Table 1]. Comparison of physical activity levels in study participants throughout the study revealed no significant differences between participants in the intervention and those in control groups [Figure 1].

Table 1: Dietary intakes of the participants throughout the study^a

Variables	Intervention ^b	Control ^c	P ^d
Energy (kcal/day)			
Before	1932.2±479.4	1964.2±524.4	0.39
After	1914.5±489.4	2139.6±147.3	
P ^d	0.8	0.44	
Carbohydrate (g/day)			
Before	295.9±92	293.3±82.4	0.87
After	286.5±78.4	281.7±63.2	
P	0.43	0.27	
Protein (g/day)			
Before	77.05±22.1	81.9±24.6	0.56
After	73.7±23.8	75.4±21.1	
P	0.42	0.08	
Fat (g/day)			
Before	52.7±16.3	54.5±23.2	0.32
After	55.6±23.6	82.6±164.2	
P	0.42	0.36	
Vitamin C (mg)			
Before	166.4±124.9	163.6±95	0.48
After	170.1±146.1	137.5±95	
P	0.89	0.12	
Vitamin E (mg)			
Before	3.48±1.7	3.8±2.6	0.07
After	5.04±7.1	3.2±1.7	
P	0.17	0.29	
Vitamin A (mcg)			
Before	571.5±489.6	544.5±517.6	0.44
After	455.3±429	533.1±436.6	
P	0.24	0.91	
Zinc (mg)			
Before	8.6±2.5	9.01±3.2	0.97
After	8.1±2.6	8.5±2.3	
P	0.44	0.42	
Fiber (mg)			
Before	18.8±8.05	18.2±7.5	0.57
After	16.9±7.8	17.9±5.7	
P	0.31	0.8	

^aReported values are mean±SD, ^bIntervention group received 10 g/d purslane seeds powder with 240 cc low-fat yogurt, ^cControl group received only 240 cc low-fat yogurt, ^dFor comparison of within group differences by paired t test,

^eFor comparison of between-group differences by paired t-test. SD=Standard deviation

The effects of the 5-week intervention on biomarkers of oxidative stress are presented in Table 2. We observed a slight decrease in plasma TAC in both groups, but between-group changes were not significant (-2.67 vs. -2.95 $\mu\text{g/dL}$, $P = 0.94$). Plasma MDA in purslane group increased slightly, but we observed no significant effect in the control group (1.62 vs. -0.47 $\mu\text{g/dL}$, $P = 0.58$). Although both groups had a slight reduction in plasma ox-LDL, we failed to find any significant effect of purslane on plasma ox-LDL (-1.06 vs. -1.71 $\mu\text{g/dL}$, $P = 0.85$).

DISCUSSION

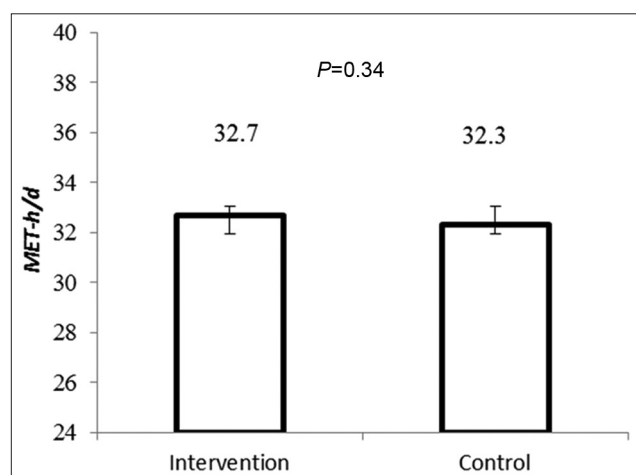
We failed to find any significant effect of purslane on plasma levels of TAC, MDA, and ox-LDL in this randomized cross-over clinical trial. To our knowledge, this study is among the first investigations that examined the effects of purslane seeds on biomarkers of oxidative stress in type 2 diabetic patients.

Type 2 diabetes is the most prevalent metabolic disease all over the world. Oxidative stress plays an important role in the pathogenesis of microvascular and macrovascular complications of diabetes.^[15] There are several ways to control the oxidative stress in diabetic patients. Earlier studies have shown that some medicinal plants may protect diabetes against oxidative stress.^[8] Due to the high concentrations of antioxidants in purslane, it has potential antioxidant effects.^[10,11] However, we did not find any beneficial effect of purslane seeds on oxidative stress in the current study. A limited number of studies has been published about the health effects of purslane. In addition, previous studies have reached opposite findings. Administration of aqueous juice of purslane has been resulted in improvement in antioxidant parameters in rats.^[13] Consumption of purslane leaves for 3 weeks led to a significant reduction in TBARS and a tremendous increase in GSH-R in diabetic rats.^[15] In addition, Hao *et al.*^[18] reported that purslane administration resulted in the increased levels of superoxide dismutase and decreased levels of MDA in the brains of mice treated with D-galactosamine. Others have also shown that purslane leaves can reduce some cardiovascular risk factors through decreasing ox-LDL and increasing the activity of the paraoxanase-1 enzyme in hyperlipidemic subjects.^[19] Previous studies have documented that intake of 5 g/day *P. oleracea* seeds in type 2 diabetic subjects could decrease serum levels of triglycerides, total cholesterol, LDL-cholesterol, liver alanine aminotransferase, aspartate aminotransferase and gamma-glutamyl transaminase, total and direct bilirubin, fasting and postprandial blood glucose, insulin, body weight, and body mass index while increased high density lipoprotein cholesterol and albumin.^[17] The discrepancy between our findings and other studies might be explained by the discrepancies in

Table 2: Effect of purslane on stress oxidative biomarkers of diabetic patients^a

	Intervention ^b				Control ^c				
	Before	After	Change ^d	P ^e	Before	After	Change ^d	P ^e	P ^f
TAC	13.2±14.6	10.5±11.0	-2.67±11.5	0.15	15.8±19.6	12.8±17.4	-2.95±23.4	0.43	0.94
MDA	13.4±15.2	15.0±13.2	1.62±21.5	0.63	15.2±16.9	14.8±12.8	-0.47±19.2	0.87	0.58
Ox-LDL	20.6±14.3	19.5±12.4	-1.06±11.8	0.57	22.4±14.6	20.7±13.6	-1.71±18.0	0.55	0.85

^aReported values are mean±SD. ^bIntervention group received 10 g/d purslane seeds powder with 240 cc low-fat yogurt. ^cControl group received only 240 cc low-fat yogurt. ^dCalculated by subtracting values at 5th week from values at baseline. ^eFor comparison of with-in group differences by paired t-test. ^fFor comparison of between-group differences by paired t-test. TAC=Total antioxidant capacity, MDA=Malondialdehyde, Ox-LDL=Oxidized-low density lipoprotein, SD=Standard deviation

**Figure 1: Comparison of physical activity levels in study participants throughout the study intervention periods**

study design, duration of intervention and participants of the study. Furthermore, it must be kept in mind that almost all previous studies have been done on animals. In addition, we used purslane seeds rather than purslane leaves or other preparations in this study.

Several mechanisms might explain the antioxidant effects of purslane. It has been shown that purslane intake results in better glycemic control in diabetic patients.^[17] Purslane mechanism can be in connection with increasing insulin secretion by closing of the channel gate ATP-K⁺, membrane depolarization, and Ca²⁺ entry stimulation as the first key step in insulin secretion.^[20] Improved homeostasis of glucose in diabetic patients might explain the beneficial effects of purslane on biomarkers of oxidative stress. However, as we published previously,^[21] we did not find any significant effect of purslane seeds on plasma glucose levels in these patients. Purslane has been shown to elevate antioxidant enzymes through which it might lead to reduced oxidative stress.^[22]

The strength of the present study was the use of a crossover design which decreased the effect of confounders. Also, the study was among the first investigations that examined the effects of purslane seeds on biomarkers of oxidative stress in patients with type 2 diabetic. Some limitations need to be taken into account in the interpretation of our findings. One of our study limitations is the method of assessment of compliance to purslane seeds intake. We

assessed compliance through phone interviews as well as asking participants to deliver the empty sachets to study personnel. Future studies are better to assess the compliance through the use of an appropriate biomarker. Although finding a suitable biomarker for purslane intake is difficult, measurement of the alpha-linolenic acid content of red blood cells' membranes might provide some information in this regard.^[23,24] The short duration of intervention might prohibit us to reach significant effects of purslane on biomarkers of oxidative stress. In addition, a single measurement of the metabolic profile, which has day-to-day variations, in the current study, might lead to misleading findings.

CONCLUSIONS

This cross-over clinical trial revealed that consumption of purslane seeds for 5 weeks in type 2 diabetic patients did not result in improved oxidative stress. Additional studies with a much longer experimental period and a larger sample size are needed to further explore the effects of purslane on oxidative stress.

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