

Protective effects of purslane seed (*Portulaca Oleracea L.*) on plasma levels of Cystatin C, Cathepsin S, and Creatinine in women with type 2 Diabetes

Parvin Farzanegi¹ Maryam Shaerani² Mohammad Ali Azarbayjani³

Associate Professor Department of Exercise Physiology¹, MSc of Exercise Physiology², Islamic Azad University, Sari Branch, Sari, Iran. Professor Department of Exercise Physiology³, Islamic Azad University, Central Tehran Branch, Tehran, Iran.

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Original Article

Abstract

Introduction: Diabetes is a chronic metabolic disease which is associated with the inflammation of cardiovascular system and kidney. Studies have shown that medicinal plants could be effective in reducing inflammation; however, the effectiveness of purslane (*Portulaca oleracea*) on inflammation is not well defined. Thus, this study attempted to investigate the effect of *Portulaca oleracea* seed consumption on plasma levels of cystatin C, cathepsin S, and creatinine in women with type 2 diabetes.

Methods: In this quasi-experimental study, 14 women with type 2 diabetes were randomly divided into two equal groups of intervention and control (n=7). The subjects received *Portulaca oleracea* seed 2.5 g at lunch and 5 g at dinner (totally 7.5 g) per day for 8 weeks. Blood was collected before and 48 hours after the last intervention. Data were analyzed with paired and independent t-tests, and P < 0.05 was considered significant.

Results: Levels of cystatin C, cathepsin S, creatinine, and lipid profile decreased significantly in the intervention group after 8 weeks (P < 0.05). There was also a significant difference between the intervention and control groups in levels of cystatin C and cathepsin S.

Conclusion: Changes in biochemical markers showed that *Portulaca oleracea* seed could improve the levels of cardiovascular and kidney damage biomarkers and lipid profile in diabetic patients. However, further research is needed for more accurate conclusions.

Correspondence:
Parvin Farzanegi, PhD.
Physiology Department, Islamic
Azad University,
Sari, Iran
Tel: +98 11 33173853
Email:
parvin.farzanegi@gmail.com

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Introduction:

Diabetes mellitus is a chronic metabolic disease. Its global prevalence in adults was 6.4% in 2010 and is predicted to rise to about 7.7% by 2030 (1). The most common side effects of the disease are obesity, hyperlipidemia, hypertension, heart failure,

and kidney damage which are associated with the risk of cardiovascular disease and mortality (2).

Recent studies suggest that high concentrations of cystatin C, age, gender, and body mass index (BMI) are non-independently related to cardiovascular risk factors and play an important

role in its development (3). Cystatin C is a low molecular weight (13 KD), non-glycosylated protein which is produced by nucleated cells. Given the free filtration of cystatin C in glomerulus, its complete reabsorption and catabolism in proximal tubule, and no secretion in tubules, cystatin C concentration is thought to almost entirely depend on glomerular filtration rate (GFR) (4), turning cystatin C to a better marker of kidney function than serum creatinine, particularly in the elderly and patients with mild to moderate renal impairment (5, 6). Increased levels of cystatin C represent primary failure in GFR (7). In addition, high titers of cystatin C is associated with increased risk of cardiovascular disease (CAD) even in the absence of kidney disease, and is known as a strong prognostic factor for cardiovascular disease and mortality (8,9). Increased expression of cathepsins S and L in atherosclerotic lesions show that people with atherosclerosis have higher serum levels of cathepsins S and L than normal. Therefore, they can be used as biomarkers or risk factors of this life-threatening disease in human (10,11). On the other hand, lower expression of cystatin C in the atherosclerotic lesions, as the main inhibitor of cysteine and cathepsins and with the highest inhibitory activity in cathepsins S and L, points out the importance of imbalance between cysteine proteases and their most abundant inhibitor, *i.e.* cystatin C (12).

Special attention has been paid recently to natural products and alternatives by diabetes specialists to minimize problems and complications of diabetes (13). On the other hand, studies have shown that natural antioxidants reduce the risk of chronic diseases (such as diabetes, fatty liver, *etc.*) and promote health through prevention of oxidative stress (14,15). Today, medicinal herbs are considered as the most important natural and functional foods. Some herbs have the highest levels of antioxidants such as catalase, superoxide dismutase, vitamin E, vitamin C, glutathione, polyphenols, carotenoids, selenium, alpha-linoleic acid, omega fatty acids, and unsaturated fatty acids, *etc.* In addition, a significant amount of minerals, group B vitamins, essential amino acids, *etc.* and are found in some of them (16,17).

Research has shown that purslane (*Portulaca oleracea*) is a helpful plant in prevention of

development of diabetes, hyperlipidemia, obesity, fatty liver, *etc.* through preventing oxidative stress and chronic inflammation, improvement of fat metabolism, increasing expression of genes involved in insulin signaling pathway, increasing glucose uptake, adjusting insulin resistance, decreasing triglycerides, LDL, and total cholesterol, regulating the levels of liver enzymes (transaminases), *etc.* (18,19). Although the relationship between cystatin C and cathepsin S has been investigated in some diseases, no conclusive evidence regarding this relationship has been demonstrated in patients with diabetes. In addition, most studies about the effect of purslane in people with type 2 diabetes was performed on blood glucose and lipid profile, and no study investigated the impact of purslane on biomarkers of heart and kidney damage. Thus, this clinical trial aimed at determining the effects of purslane seed consumption on plasma levels of cystatin C, cathepsin S, creatinine, and lipid profile in patients with type 2 diabetes.

Methods:

This clinical trial was conducted in 2012 at Imam Khomeini Hospital of Sari. After initial evaluation, 20 volunteer women with type 2 diabetes who were living in Mazandaran province and referred to the Endocrinology Ward of the hospital were enrolled in the study. A written consent was obtained from the participants before initiating the trial.

Inclusion criteria were type 2 diabetes (as fasting plasma glucose levels of equal or greater than 126 mg/dL in two consecutive measurements) and absence of other diseases. All subjects were under a regular diet prescribed by their physicians. Exclusion criteria were failure to follow the recommended diet and physical activity, and significant change in blood pressure. People with chronic cardiovascular disease or chronic inflammation (diabetic foot ulcer, hepatitis), and those taking vitamin supplements or smoking were also excluded from the study. Accordingly, 6 subjects were excluded because of lack of cooperation, taking vitamin C, and change in life pattern and finally 14 qualified patients completed the study. The subjects were randomly divided into

two groups of intervention and control after matching in terms of age, body mass index, and disease duration. It should be noted that all subjects had an equal chance to be enrolled in each group.

At the baseline, general characteristics of the subjects, including age, height, weight, duration of disease, etc. were assessed using a self-reported questionnaire and physical examination. The subjects were weighted with minimal clothing and barefoot by a digital scale to the nearest 0.1 kg. A tape measure was used to assess the height in a standard position and barefoot. Blood pressure was measured twice in a sitting position using a mercury manometer and the average was reported.

Baseline blood sample (7 mL) was collected after 12-14 h of fasting in sitting position from the brachial vein at 8-10 AM. Then, the intervention group received purslane (as the independent variable) for 8 weeks. At the end of the study, blood samples were collected under the same conditions. Blood samples were collected in EDTA-containing test tubes which were centrifuged at 3000 rpm for 10 min at 4 °C to separate plasma. Plasma obtained was frozen at -80°C until measurement of cystatin C, cathepsin S, creatinine, and lipids.

Subjects in the intervention group consumed 7.5 g purslane seed daily (2.5 g at dinner and 5 g at lunch) for eight weeks (19). The recommended diet had 2500-3000 kcal energy containing 50-55% carbohydrates, 25-30% fat, and 10-15% protein (12).

Plasma lipids were measured by enzymatic method with Acylon 300 autoanalyzer using commercial kits of Pars Azmoon (Tehran, Iran) and creatinine by the Jaffe method. Cystatin C and

cathepsin S were measured using commercial kits of Diazyme Company (USA) (with a sensitivity of 0.19 mg/L and range of 0.62-1.16 mg/L) and Cusabio Company (China) (with a sensitivity of 0.31 ng/mL and range of 1.25-80 ng/mL), respectively, according to the kits instructions.

The results were presented as mean \pm standard deviation. Kolmogorov-Smirnov test was used to determine the normal distribution of the data and t statistical model to evaluate the intra-group changes for dependent groups. The regression homogeneity of the groups was also examined. Analysis of covariance (ANCOVA) was applied in case of regression homogeneity and t statistical model to evaluate for dependent groups in the absence of homogeneity. The significance level for all calculations was $P < 0.05$.

Results:

The mean and standard deviation of the body composition indices of the study subjects are presented in Table 1. The results indicated significant differences between these variables in the study groups; this represents homogeneity of the groups at baseline ($P < 0.05$). Eight weeks consumption of purslane seed resulted in a significant decrease in levels of cathepsin S, cystatin C, creatinine, lipid profile (except HDL), and BMI in the intervention group ($P < 0.05$). In addition, a significant difference was observed between the groups regarding changes in the levels of cathepsin S and cystatin C ($P < 0.05$); however, no significant difference existed in creatinine level, lipid profile, and BMI between the two groups (Figure 1).

Table 1. Characteristics of the study groups in terms of central and distributional indices

| Variable | Intervention group | Control group | P-value |
|---------------------------------|--------------------|-------------------|---------|
| Age (years) | 52.86 \pm 2.41 | 53.50 \pm 2.59 | 0.455 |
| Weight (kg) | 73.14 \pm 2.8 | 75.66 \pm 9.43 | 0.742 |
| Height (cm) | 159.14 \pm 4.56 | 160.66 \pm 6.44 | 0.677 |
| BMI (kg/m ²) | 29.29 \pm 2.33 | 29.31 \pm 2.89 | 0.582 |
| Systolic blood pressure (mmHg) | 151.86 \pm 5.58 | 152.37 \pm 8.91 | 0.463 |
| Diastolic blood pressure (mmHg) | 91.71 \pm 5.02 | 91.42 \pm 9.93 | 0.633 |

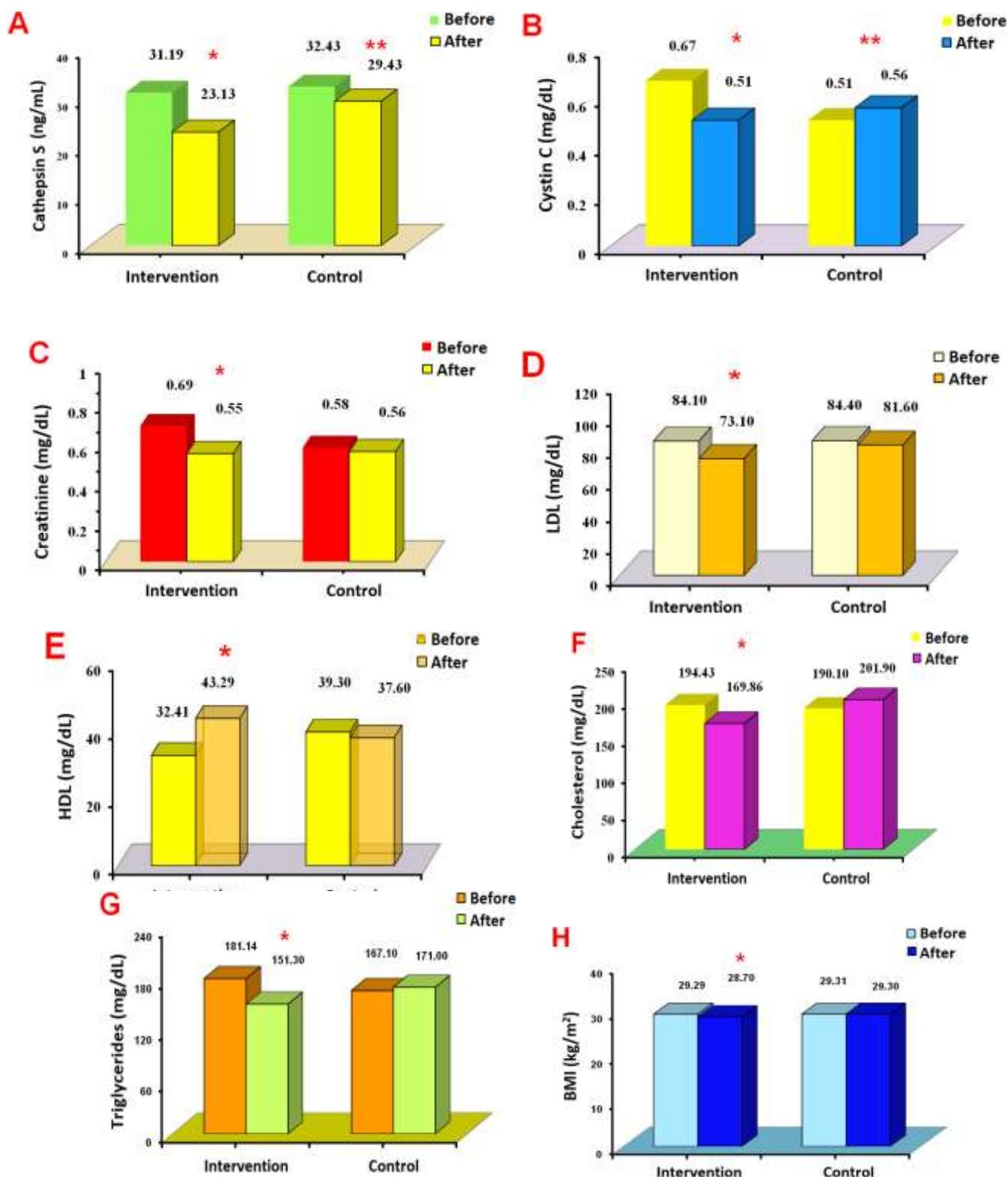


Figure 1. Comparison of the variables before and after intervention in the studied groups; A) Cathepsin S; B) Cystatin C; C) Creatinine; D) LDL; E) HDL; F) Cholesterol; G) Triglycerides; H) BMI.

* P-value: significance of changes between before and after test based on paired t-test

** P-value: significance of changes between the means of groups based on ANCOVA test

Conclusion:

The results showed that treatment with purslane seed significantly reduced cystatin C, cathepsin S, creatinine, triglycerides, cholesterol, LDL, and BMI, and significantly increased HDL in diabetic women.

However, no significant effect of purslane consumption was observed on creatinine, lipid profile, and BMI in the intervention group compared with the controls.

Recent studies showed that diabetes was not merely a hyperglycemic disease; rather, it is an inflammatory disorder (20). In addition, type 2 diabetes is associated with cardiovascular risk factors, obesity, hypertension, increased triglycerides and LDL, decreased HDL, hyperinsulinemia, insulin resistance, and chronic inflammation (21). In all these conditions, inflammatory factors damage the vessels wall and kidney and result in their dysfunction (22). Cardiovascular and kidney dysfunction are associated with increased cathepsin S and cystatin C (23). It has been also shown that patients with type 2 diabetes have an in vivo low-grade inflammatory state (12) and inflammation may lead to insulin resistance and related diseases, such as diabetes, metabolic syndrome, and atherosclerosis. In this regard, Jobs et al. (9) showed that serum cathepsin levels were associated with C reactive protein. In addition, Naour et al. (10) demonstrated that cathepsins may also involve in energy balance in adipose tissue and blood circulation.

These studies suggest that serum cathepsin may incorporate to incidence of diabetes through specific routes including inflammatory reactions, independent of insulin resistance.

According to literature, serum levels of cystatin C and cathepsin S increase with obesity. Taglieri et al. (2012) reported that serum levels of cystatin C significantly increased in overweight and obese compared with normal weight individuals, therefore the results of this study can confirm the relationship between obesity and increased cystatin C in obese individuals (25).

In the present study, even though the subjects in both groups had a high BMI at baseline, it was decreased significantly in the intervention group at the end of the eighth week, although it was insignificant in comparison with the controls.

However, no significant difference was observed between the two groups. A significant decrease in lipid profile (cholesterol, triglycerides, and LDL) and a significant increase in HDL level were also seen after eight weeks compared with baseline in the intervention group. However, the difference between the two groups was not significant. It seems that reduced levels of lipids in the intervention group are arisen from decrease in BMI.

Some studies have demonstrated the role of purslane as a non-medicinal treatment in lowering blood glucose and insulin resistance, which was attributed to its polyunsaturated fatty acids, flavonoids, and polysaccharides (19-24). The mechanism of action of purslane raw polysaccharide is not well known, however it is likely that purslane increase insulin secretion through closing K^+ -ATP channel, membrane depolarization, and stimulation of Ca^{2+} influx (16). Ghatrehsamani et al. (1390) showed that consumption of 50-60 g raw purslane per day for 45 days significantly decreased BMI, total cholesterol, LDL, and OXLDL and increased HDL, paraoxonase activity, and apolipoprotein A-1 in patients with LDL higher than 100 mg/dL. It seems that purslane's fiber binds to cholesterol of the ingested food, prevents its absorption, and hence facilitates its disposal from the GI tract. Furthermore, increased levels of paraoxonase-1 activity increase HDL. This is a calcium-dependent enzyme which activity increases through the positive effect of purslane on intracellular calcium resulting in reduced form of oxidized LDL (OXLDL) (24).

Lee et al. (2012) showed that daily intake of 300 mg/kg purslane extract for 10 weeks significantly reduced serum glucose, creatinine, water consumption, and urine volume in mice with diabetes (17).

An important finding of this study was the significant reduction (20%) in creatinine concentration in the intervention group after 8 weeks of purslane seed consumption. This reduction was not significant in comparison with the intervention group, which is possibly due to short duration of consumption, dose, and type of purslane consumed by the subjects in comparison with other studies (17,19,22).

Although taking purslane seed was not well effective in reducing blood lipids and creatinine, it had a significant impact on levels of cystatin C and cathepsin S in diabetic women, which may indicate its role in modulation of cardiovascular damage indicators independent of blood lipids and creatinine.

According to some studies, decrease in the levels of cystatin C and cathepsin S occurs after a significant reduction in proinflammatory mediators such as IL-6, interferon gamma, CRP, and TNF- α (7,21). Although the effect of purslane consumption on changes in inflammatory markers was not investigated in this study, which can be considered a limitation, it seems that purslane ingestion was associated with desired effects on reduction of cystatin C and cathepsin S in diabetic women.

Overall, according to the findings of this study, it can be stated that purslane seed consumption improves the levels of biomarkers of heart and kidney damage in diabetic patients, but it could not alter creatinine level and lipid profile. However, more research is needed for more accurate results.

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اثر حفاظتی دانه خرفه (*Portulaca oleracea* L.) بر سطوح پلاسمایی سیستاتین C، کاتپسین S و کراتینین در زنان مبتلا به دیابت نوع ۲

پروین فرزانی^۱، مریم شعرانی^۲، محمد علی آذربایجانی^۳

^۱ دانشیار، گروه فیزیولوژی ورزشی، ^۲ کارشناس ارشد، گروه فیزیولوژی ورزشی، دانشگاه آزاد اسلامی، واحد ساری، ساری، ایران. ^۳ استاد، گروه فیزیولوژی ورزشی، دانشگاه آزاد اسلامی، واحد تهران مرکز، تهران، ایران.

مجله پزشکی هرمزگان سال نوزدهم شماره اول ۹۴ صفحات ۸-۱

چکیده

مقدمه: دیابت بیماری مزمن متابولیکی است که موجب التهاب در دستگاه قلب و عروق و کلیه می‌شود. مطالعات نشان دادند استفاده از گیاهان دارویی می‌تواند در کاهش التهاب مؤثر باشد، ولیکن اثربخشی گیاه خرفه به خوبی مشخص نشده است. بنابراین هدف از مطالعه حاضر، بررسی اثر مصرف دانه خرفه بر سطوح پلاسمایی سیستاتین C، کاتپسین S و کراتینین در زنان میانسال مبتلا به دیابت نوع ۲ می‌باشد.

روش کار: در این مطالعه نیمه تجربی، ۱۴ زن مبتلا به دیابت نوع ۲ به طور تصادفی و مساوی به گروه‌های مداخله و کنترل تقسیم شدند میزان مصرف دانه خرفه ۷/۵ گرم در روز (۲/۵ و ۵ گرم در نهار و شام، به ترتیب) بود. خون‌گیری قبل و ۴۸ ساعت پس از آخرین مداخله انجام شد. تجزیه و تحلیل یافته‌ها با استفاده از آزمون‌های زوجی و مستقل انجام شد ($P < 0.05$).

نتایج: غلظت سیستاتین C، کاتپسین S و کراتینین و پروفایل لیپیدی پس از هشت هفته مصرف دانه خرفه در گروه مداخله کاهش معنی‌داری یافت ($P < 0.05$). همچنین بین دو گروه فقط در سیستاتین C، کاتپسین S تفاوت معنادار مشاهده شد.

نتیجه‌گیری: با توجه به تغییرات نشانگران بیوشیمیایی مداخله مطالعه مشخص شد، مصرف دانه خرفه موجب بهبودی در سطوح بیومارکرهای آسیب قلبی و کلیوی و پروفایل لیپیدی افراد دیابتی می‌شود. البته برای نتیجه‌گیری دقیق‌تر نیاز به تحقیقات بیشتری می‌باشد.

کلیدواژه‌ها: سیستاتین C - کاتپسین S - کراتینین - دیابت نوع ۲

نویسنده مسئول:

دکتر پروین فرزانی

گروه فیزیولوژی ورزشی، دانشکده

علوم انسانی دانشگاه آزاد اسلامی،

واحد ساری

ساری-ایران

تلفن: ۳۳۱۷۳۸۵۲ ۱۱ ۹۸+

پست الکترونیکی:

parvin.farzanegi@gmail.com

نوع مقاله: پژوهشی

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