

The Effect of Portulacaoleracea L Consumption and Regular Exercise on Levels of Cathepsin S, Cystatin C and C-Reactive Protein in Diabetic Women

Marjan Vahedi (MSc)

Department of Exercise Physiology, Sari Branch, Islamic Azad University, Sari, Iran

Parvin Farzanegi (PhD)

Department of Exercise Physiology, Sari Branch, Islamic Azad University, Sari, Iran

Corresponding Author: Parvin Farzanegi

Email: parvin.farzanegi@gmail.com

Tel: +989112230233

Address: Islamic Azad University of Sari, Sari, Iran

Received: 28 Apr 2014

Revised: 07 Jun 2014

Accepted: 29 Jun 2014

ABSTRACT

Background and Objectives: Diabetes induced oxidative stress plays an important role in pathological damage to the heart and liver by increased production of extracellular matrix. It is thought that the use of medicinal plants, particularly *Portulaca oleracea*. L and regular exercise are effective. The aim of this study was to evaluate the effects of *Portulaca oleracea*. L consumption along with resistance training on cathepsin S, cystatin C and C-reactive protein (CRP) levels on type 2 diabetes patients.

Methods: In this semi-experimental study, 28 female type 2 diabetes patients with a mean age of 52 were randomly divided into 4 groups of control, exercise, supplement and supplement-exercise. *Portulaca oleracea*. L supplement was consumed 7.5 g per day. Resistance training program was performed with a rubber band for 8 weeks, 3 days a week for 60 minutes with 40-50% intensity, up to a maximum repetition. Blood samples were taken before and 48 hours after the last intervention.

Results: After eight weeks, cathepsin S, cystatin C and CRP levels in the supplementation and supplementaion-exercise group were significantly reduced ($P < 0.05$). There were also significant differences between the groups.

Conclusion: *Portulaca oleracea*. L consumption and resistance training have each separate positive impacts on the cathepsin S, cystatin C and CRP levels, but the simultaneous effect of *Portulaca oleracea*. L seed consumption and physical activity can lead to a better efficiency.

Keywords: *Portulaca oleracea*, Resistance training, Cathepsin S, Cystatin C, C-reactive protein, Type 2 diabetes mellitus.

INTRODUCTION

Epidemiological evidences indicate that diabetes is an important risk factor for atherosclerosis, which is the leading cause of death in 80% of diabetic patients (2). The etiological studies have considered obesity, decreased daily physical activity, atherogenic diet, increased serum lipoprotein, increased homocysteine, cathepsin S and C reactive protein (CRP) as newer risk factors of coronary artery atherosclerosis (3, 4). Cathepsin S, an essential lysosomal cysteine protease produced by primary culture of microglia cells and regulated by P2X7 receptor, is associated with Matrix Metalloproteinase (MMPs) and Serine Protease (5). It also has important physiological functions in the extracellular environment including the destruction of Extracellular Matrix (ECM), regulation of growth factors, vascular proliferation, cell migration, proliferation and cell death (6) Sukhova et al. demonstrated the increased expression of cathepsin S in atherosclerotic sites (7). In addition, some other studies have shown that cystatin C is important in the formation and development of atherosclerosis (8,9). Cystatin C is a low molecular weight protein in blood (13000 kDa) which acts as a cysteine proteases inhibitor, effective against both endogenous proteases such as lysosomal cathepsin (B, F, H, K, L) and parasitic and microorganisms proteases. Liu et al. showed that the serum level of cathepsin S in patients with minimal atherosclerotic stenosis of at least one coronary vessel had increased compared to patients without stenosis (11) while the level of serum cystatin C remained unchanged. In a recent study, high serum levels of cystatin C were associated with causes of death, cardiovascular events and congestive heart failure incidents in outpatients with coronary heart disease (13). Researchers have focused their efforts to reduce the associated factors with diabetes and believe that, lifestyle modifications such as appropriate diet and aerobic exercise may prevent or delay the complications of diabetes (15). Previous studies have shown that muscle strength is associated with the prevalence of metabolic disorders while demonstrating that resistance training by increasing muscle mass may reduce the risk factors of cardiovascular diseases (17). Although most studies are on the effect of aerobic exercise on cystatin C and CRP, it seems that resistance trainings may also lead to a decrease in inflammatory markers (18). In this regard,

Tanindi et al observed an increase in cystatin C levels during exercise stress testing of patients with metabolic disorders, especially those with ischemia (19). Hosseini Kakhak et al. reported no significant change in the concentration of cystatin C and CRP in obese girls, after eight weeks of resistance training, four sessions a week with an intensity of 60-70% of up to maximum one repetition (20). *Portulaca oleracea* L. is a traditional medicinal plant, capable of significantly reducing blood glucose and insulin resistance due to containing unsaturated fatty acids, flavonoids and conventional, organic and acidic polysaccharides, which may act as an adjuvant therapy for diabetic patients (22). Researches suggest that consumption of *Portulaca oleracea* L. along with a diet, in addition to reducing glucose levels, can significantly reduce inflammation, oxidation and ultimately vascular injury in type 2 diabetic patients by reducing the amount of cholesterol and triglycerides in the blood, lowering LDL, increasing HDL and preventing the deposition of cholesterol in the blood vessels (23). This study was aimed to examine the effects of a course of *Portulaca Oleracea* L. consumption along with resistance training on the levels of cathepsin S, cystatin C and CRP in diabetic women.

MATERIAL AND METHODS

In this semi-experimental study, 28 diabetic women were selected from the Diabetic Association of Sari with an average age of 52 years. The subject of this study was first brought to the notice of this group of people. Then, the individuals declared their readiness to participate in this research through completion of a questionnaire that was prepared for this purpose. The participants became acquainted with a series of activities that must be performed during the study period in the briefing and became informed with to do steps and procedures, duration of the physical activities and food intake, orally and in writings. Finally, the subjects agreed to the consent form, which allowed them to withdraw their participation in training sessions at any time. In order to relatively control the nutrition of subjects, the 4-weeks questionnaire of food record, nutritional guidelines and recommendations were used daily under a physician's supervision. All patients were then randomly divided into four groups of 7: exercise, supplement, supplement-exercise (experimental groups) and a control group (Table 1).

Table 1 - Characteristics of subjects in all four experimental groups

(Cm) Height	(Kg) Weight	(Years) Age	Index Group
159/28 ± 5/08	76/29 ± 4/39	53/28 ± 1/7	Training
159/17 ± 6/65	73/5 ± 4/89	52/3 ± 4/08	Supplement
159/57 ± 5/25	75/71 ± 5/71	52/57 ± 2/7	Supplement- Training
160/67 ± 6/54	75/67 ± 9/44	50/17 ± 5/34	Control

Table 2 - Characteristics of variables in all four experimental groups

Variables	Groups	Pre test	Post test	P*	P**
Cathepsin S (ng/ml)	Training	34.70	24.51	0.000*	
	Supplement	35.56	26.55	0.000*	
	Supplement- Training	33.41	20.54	0.000*	0.000**
	Control	34.46	34.36	0.885	
Cystatin C (mg/dl)	Training	0.79	0.65	0.000*	
	Supplement	0.77	0.65	0.000*	
	Supplement- Training	0.77	0.28	0.000*	0.000**
	Control	0.78	0.77	0.535	
CRP (ng/ml)	Training	7996.57	6500	0.000*	
	Supplement	7954.43	6821.71	0.000*	
	Supplement- Training	7892.14	4995.57	0.000*	0.000**
	Control	7918.43	7835.14	0.178	

Exercise program was performed in the gym everyday for 8 weeks and 3 days per week for 60 minutes. According to the procedure of this study, diabetic individuals in the supplement-exercise group completed their first training session with low intensity in order to familiarize them with the exercise and the method of performing activities coordinately. For period of 8 weeks, supplement-exercise and supplement groups consumed 7.5 grams (g) of *Portulaca oleracea*. L seed including 2.5g and 5 grams along with their meals for lunch and dinner respectively (23,25). The amount of consumed *Portulaca oleracea* was calculated based on the average consumption amount of seeds, which is used in some places. In order to measure cathepsin S, cystatin C and CRP levels, blood test was done 48 hours before the start of training, and then the process was repeated after 8 weeks. Cystatin C concentration was measured using Diazyme method, with sensitivity of 0.19mg/l, range of 0.62 -1.16mg/l. Cathepsin S was measured using Cusabio method and finally CRP was evaluated by ELISA. Descriptive and analytical statistics were used to analyze the obtained data. To evaluate interrelated group changes, paired T-test statistical model was used for related groups. The ANOVA test was used to evaluate the differences between different group indices of research and Tukey test was applied to determine the place of differences between groups. All statistical analyzes indicators were used to test the hypothesis at a significance level of $P \leq 0.05$ using SPSS (version 11).

RESULTS

Based on the results, eight weeks of supplementation, exercise and supplements combined with exercise, lead to a reduction in cathepsin S, cystatin C and CRP levels in diabetic women (Table 2). One-way ANOVA test showed a significant difference between the average levels of cathepsin S in study groups after eight weeks of supplementation, supplementation-exercise and exercise ($P=0.000$). Tukey test results showed that the average levels of cathepsin S in the group of supplementation, supplementation-exercise and exercise compared to the control group was lower but no significant difference was observed in the levels of cathepsin S between the supplementation and exercise group ($P= 0.519$)(table2). The one-way analysis of variance showed a significant difference between the mean levels of cystatin C after eight

weeks of intervention between all three study groups. Tukey test results showed that the average levels of cystatin C in supplements, supplement-exercise and exercise group was lower compared with the control group however, these differences were not statistically significant ($p=0.999$). Moreover, based on the results of one-way analysis of variance between the mean CRP levels after eight weeks of intervention, there was a significant difference between all three study groups. Tukey test results showed that the average post-test levels of CRP in the study groups was lower compared with the control group but no significant difference was observed between the said groups ($C=0.540$).

DISCUSSION

Based on the findings of this study, the levels of cathepsin S, cystatin C and CRP decreased after supplementation intervention with *Portulacaoleracea*. L and/or combined with resistance training. The interactive effects of exercise and supplementation led to further changes in the above variables compared with supplementation and exercise alone. This may indicate the strengthening effects of resistance training with *Portulacaoleracea*. L supplementation, in reducing coronary heart damages caused by cathepsin S, cystatin C and CRP and thereby, reducing other inflammatory markers that are induced by them. Previous studies showed that cathepsin S has a key role in the development of inflammation caused by diabetes (7,11). Cathepsin S is released by macrophages and becomes involved in pathophysiological remodeling of extracellular matrix, which leads to adipogenesis or lipohypertrophy (26). Expansion of adipose tissue may cause hypoxia, which ultimately leads to weak local inflammation and eventually result in insulin resistance (27). Also, plasma levels and adipose tissue expression of cystatin C (endogenous cathepsin S inhibitor) in obese individuals increases, independent of decreased glomerular filtration rate that could be reflective of adipose tissue growth control by cathepsin inhibitor (28). Cathepsin S is known as an inflammation inducing factor which through attraction of inflammatory cells and increasing cystatin C and CRP levels lead to the development of cardiovascular disease (29). It has been also demonstrated that patients with

type 2 diabetes have a low degree of inflammation that may lead to insulin resistance and its associated diseases such as metabolic syndrome and atherosclerosis (28). In this regard, Jobs et al. suggested that there is relationship between the serum levels of cathepsin S and CRP in obese men (14). Furthermore, Naour et al. showed that cathepsin S has a significant impact on energy balance in adipose tissue and blood circulation (30). It seems that cathepsin S, independent of the resistance to insulin, has an important impact on the incidence and development of diabetes by specific routes, including the inflammatory response (31). But in this study, despite a significant reduction in cathepsin S levels after eight weeks of supplementation (25.33%), exercise (29.36%) or along with resistance training (37.34%), compared with the percentage of change in levels of cystatin C (14% reduction in the supplementation group, 17.7% in the exercise group and 62% in exercise-supplementation group) and CRP (14.3% reduction in the supplementation group, 18.7% in the exercise group and 34.7% in exercise-supplementation group) was greater in the study groups. It seems that despite the short duration of supplementation and resistance training intervention, a favorable anti-inflammatory compatibility was developed in diabetic women. Recent studies showed that regular exercise could increase muscle strength through reduction of oxidative stress and inflammation caused by it (32). In this regard, Luo et al. stated that 9 weeks of resistance training in older mice prevented the loss of muscle mass and result in improved strength. Resistance training increases the levels of regulatory proteins such as Beclin 1, Atg5/12, Atg7 and cathepsin L. It seems that resistance training by increasing the autophagy activity and reducing death of muscle cells has beneficial effects on muscular atrophy (33). The results of a study also showed that muscle damage, necrosis and inflammation increase in the early stages of exercise, which result in the accumulation of macrophages and lysosomal damage and subsequent increased release of cathepsin S. Nevertheless, through exercising thrice a week, because of increased endurance, vulnerability and enzymes release become lower (34). It has been shown that level and intensity of stress is important in cell death pathway selection. Milder stress results in limited release of lysosomal contents that can lead to apoptosis or

apoptosis-like cell death while more extreme stress causes complete disruption of lysosomes and rapid cell necrosis (35).

Some studies have reported the lowering properties of *Portulacaoleracea*. L derived polysaccharides on blood sugar levels of laboratory animals (23). This study showed that after eight weeks of *Portulacaoleracea*. L consumption, levels of cathepsin S, cystatin C and CRP were significantly reduced which was even greater among the exercise-supplement group. El-Sayed et al. indicated that consumption of *Portulacaoleracea*. L for four weeks significantly reduces lipid profile, liver enzymes, fasting blood glucose, insulin and albumin in type 2 diabetic patients (23). Gong et al also reported that consumption of *Portulacaoleracea*. L for 28 days delayed the lipid metabolism, decreased triglycerides and fasting blood glucose and a significant increase in HDL and insulin levels (40). *Portulacaoleracea*. L is considered as an excellent source of alpha-tocopherol antioxidant vitamins, ascorbic acid, beta-carotene and glutathione as well as a rich source of essential amino acids such as isoleucine, leucine, lysine, methionine, cysteine, phenylalanine, tyrosine, threonine and valine. This plant plays an important role in biological systems against oxidative stress and liver damage, likely due to increased levels of antioxidants including catalase, glutathione, glutathione reductase and glutathione peroxidase. Possible mechanism involved in the reduction of hyperglycemia and insulin accumulation can be made by blockage of ATPK + channels, membrane depolarization and stimulation of Ca⁺⁺ infiltration which is the first step in insulin secretion (41). Polysaccharides in this plant are capable of clearing anion superoxide, 1,1-diphenyl-2-picrylhydrazyl (DPPH), nitric oxide and hydroxyl radicals, and thus has a protective effect against free radicals (42). It is also possible that the decrease in the variables is due to herbal antioxidant compounds such as phenolic compounds. These flavonoid compounds such as quercetin, found in *Portulacaoleracea*. L have hypoglycemic activities. Flavonoids and polyphenolic compounds can protect cells against reduced glutathione depletion by increasing glutathione antioxidant enzymes, glutathione reductase, glutathione peroxidase and catalase (40).

CONCLUSION

Consumption of *Portulacaoleracea*. L seed

in diabetic patients, due to the positive effect on cathepsin S, cystatin C and CRP levels can be useful. Resistance training also has a positive impact on these factors but the simultaneous effect of Portulacaoleracea. L and physical activity can lead to a better efficiency.

ACKNOWLEDGMENTS

This article is the result of a Master thesis,

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