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Effect of French maritime pine bark extract supplementation on metabolic status and serum vascular cell adhesion molecule-1 levels in patients with type 2 diabetes and microalbuminuria

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ABSTRACT

Objectives: This study investigated the effect of French maritime pine bark extract (PBE) supplementation on metabolic parameters, vascular cell adhesion molecule 1 (VCAM-1), urinary albumin-to-creatinine ratio (UACR), and anthropometric indexes in patients with type 2 diabetes (T2DM) and microalbuminuria.
Design: This randomized, double-blind, placebo-controlled clinical trial was conducted on 46 patients with T2DM and the evidence of microalbuminuria aged 30–65 years.
Setting: Patients were recruited from the endocrinology clinic of Sina hospital (Tabriz, Iran) from March 2018 to April 2019.
Interventions: The subjects were randomly assigned to receive two capsules/day each containing 50mg of PBE or placebo for eight weeks.
Main outcome measures: Glycemic parameters, serum VCAM-1 and lipid profile, UACR, and anthropometric indexes were measured for all patients at baseline and the end of the study.
Results: PBE supplementation significantly reduced glycosylated hemoglobin, VCAM-1, total cholesterol, UACR, waist circumference, and waist-to-height ratio compared to the placebo group at the end of the study (all $P < 0.05$). Changes in fasting blood glucose, insulin, triglyceride, low-density lipoprotein cholesterol, and high-density lipoprotein cholesterol were not significant between the two groups (all $P > 0.05$).
Conclusions: The study findings demonstrated some favorable effects of PBE supplementation on glycemic control, serum VCAM-1 and total cholesterol levels, and microalbuminuria, as well as abdominal obesity in patients with T2DM.

1. Introduction

Type 2 diabetes mellitus (T2DM) is one of the major public health problems around the world. International Diabetes Federation has estimated that T2DM will affect 642 million people worldwide until the year 2040.[1] It has been shown that diabetes-related abnormalities including hyperglycemia, oxidative stress, advanced glycation end products (AGEs), and cytokine production, as well as inflammation, are associated with endothelial dysfunction (ED).[2] ED in diabetes contributes to macrovascular and microvascular diseases including atherosclerosis, retinopathy, and nephropathy.[3,4] Albuminuria is the first stage of diabetic nephropathy and is already found in 6.5 % of patients with T2DM. Diabetic nephropathy develops in approximately 40 % of patients with progressive albuminuria.[5,6]

Microalbuminuria is defined as excretion of 30−300 mg of albumin on 24-h urine collection or a urinary albumin-to-creatinine ratio (UACR) of ≥30 to ≤300 mg/g on a random urine sample.[7] Microalbuminuria is considered an important risk factor for cardiovascular abnormalities and mortality in patients with T2DM.[3,8]

Cell adhesion molecules (CAMs) are cell-surface proteins involved in

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binding cells together or attaching them to the extracellular matrix.[9] Vascular cell adhesion molecule (VCAM-1), as one of the CAMs, is up-regulated during inflammation, oxidative stress, and ED.[3,4] VCAM-1 promotes attachment of leukocytes to the vascular wall and their penetration into the intima which leads to the vascular complications, specifically microalbuminuria.[9–12] It has been demonstrated that serum levels of VCAM-1 were higher in diabetic patients with microalbuminuria compared to normoalbuminuria and may be used as a predictive marker for the development of diabetic nephropathy.[13–15] An early study showed that circulating VCAM-1 levels were negatively correlated with glomerular filtration rate in patients with T2DM.[16] Studies also verified that there is a positive correlation between serum VCAM-1 and UACR.[2,3,15]

Diet modification, increased physical activity level, obesity management, and drug therapy are common treatments for T2DM.[17,18] However, the use of plant-derived compounds has been recently noticed in the prevention and treatment of T2DM complications.[18] French maritime pine bark extract (PBE) is a standardized herbal supplement that contains water-soluble bioflavonoids derived from French pine bark (Pinus maritima). The main phenolic compounds in PBE are catechin, epi-catechine, taxol, procyanidin, and proanthocyanidin.[19] Previous studies have shown that supplementation with PBE could have several beneficial effects such as antioxidant, anti-inflammatory, hypolipidemic, hypoglycemic, and antitrombogenic, as well as antiobesity activities.[20–26] In vitro studies reported that PBE treatment decreases the expression of CAMs in activated endothelial cells.[27,28] However, limited intervention studies have been carried out previously to investigate the possible effects of PBE supplementation on certain metabolic parameters such as microalbuminuria and VCAM-1 in patients with T2DM. So, considering the recent findings on the potential effects of PBE in controlling diabetes-induced complications and the limited human research in this regard, the present study was designed to evaluate the effect of PBE supplementation on the metabolic factors including serum glycemic indexes and VCAM-1 levels, UACR, lipid profile, and anthropometric indices in patients with T2DM and microalbuminuria.

2. Materials and methods

This randomized, double-blinded, placebo-controlled trial was conducted on patients with T2DM and microalbuminuria. The study protocol was approved by the Ethics Committee of Tabriz University of Medical Sciences (Tabriz, Iran, NO: IR.TBZMED.REC.1396.1079) and registered in the Iranian Registry of Clinical Trials website (ID: IRCT20100408003664N21). All subjects signed the written informed consent form before participating in this trial. The study was also conducted following the guidelines of the declaration of Helsinki principle.

2.1. Participants

Forty-six T2DM patients (male and female), aged 30–65 years with the evidence of microalbuminuria (UACR 30−300 mg/g) were recruited from the endocrinology clinic of Sina hospital (Tabriz, Iran) from March 2018 to April 2019.

Pregnant, lactating, and postmenopausal women as well as smokers and who had an intensive exercise 24 h before the blood sample collection were not included in the study. Subjects with a history of congestive heart failure, urinary tract infection disease, prostate disease, acute fever, uncontrolled blood pressure, glomerulonephritis, polycystic kidney disease, acute hyperglycemia, and hereditary nephropathy, were excluded from the study. Besides, subjects who used insulin therapy and any kind of nutritional supplements within one month before or during the study period were also excluded.

The sample size was determined based on the changes in fasting blood sugar (FBS) levels according to the information obtained from the Zibadi et al.[24] study. Considering a confidence level of 95 % and a power of 80 %, the sample size was estimated to be 20 per group. Assuming a 15 % possible dropout rate, 23 patients in each group were included.

2.2. Source of the PBE

The PBE capsules were purchased from the Aramis Pharmed Company, Tehran, Iran under the tradename of Oligopin from the Les Derives Resiniques Et Terpeniques SAS (DRT), France. Oligopin is obtained from the pine tree Pinus maritima by a specific selective extraction and purification process of French maritime pine bark. The process starts with a solid-liquid (water) extraction to obtain the total polyphenol content, followed by a liquid-liquid extraction to remove the tannins. Oligopin is characterized by a high content of low molecular weight oligomeric procyanidins (67–75 %).[29]

2.3. Study design

The study population was randomized into the PBE and placebo groups by using a block randomization procedure with matching subjects in each block based on age, sex, and body mass index (BMI). The randomization sequences were generated by using STATA 14/2 software with a randomized block procedure of size 4. Investigators, subjects, and statistician were blinded to the treatment allocation. A general questionnaire was completed for each subject. Patients in the intervention group (n = 23) received two capsules per day, each containing 50 mg PBE for eight weeks. The placebo group (n = 23) received two capsules of identical appearance containing starch (maltodextrin) daily for the same period. Subjects were instructed to consume these capsules during the meals. They were asked to bring back the remaining capsules every two weeks for monitoring the compliance rate by counting the number of returned capsules. The patients would be excluded from the study if the remaining capsule counts were more than 10 % of the expected ones. Participants continued their medications during the study, including blood glucose-lowering medications, angiotensin-converting enzyme inhibitors, and angiotensin receptor blockers. They were also asked not to change their routine dietary pattern and physical activity level until the end of the study.

2.4. Blood sampling

Venous blood samples (5 ml) were obtained at baseline and the end of the study after 12-hr overnight fasting. Two ml of whole blood samples were collected into a complete blood count tube containing ethylene-diamine-tetra acetic acid to assess HbA1c. The serum samples were separated by centrifugation at 3000 rpm for 10 min and were immediately frozen at −70 °C until the analyses were performed.

2.5. Primary outcome measurements

Fasting blood sugar (FBS), HbA1c, serum insulin, and VCAM-1 levels, as well as the UACR, were the primary outcomes of the study. The serum concentration of FBS was measured using the standard enzymatic method with a commercial kit (Pars Azmoon, Karaj, Iran). HbA1c was measured in the whole blood by cation exchange chromatography using the Pishtazteb A1C kit (Pishtazteb, Tehran, Iran). Fasting serum insulin level was measured by enzyme-linked immunosorbent assay (ELISA) kit (Monobind Inc, CA 92630. USA). Insulin resistance was determined by homeostatic model assessment for insulin resistance (HOMA-IR) index using the following formula: HOMA-IR = fasting glucose (mg/dl) × fasting insulin (μIU/mL)/405.[30] The serum level of VCAM-1 was measured using the ELISA kit (Biotech. Cat. No. E0264Hu. China) with a sensitivity of 0.05 ng/mL. The urinary albumin excretion (UAE) was detected by the ELISA kit (Randox, MA1567, United Kingdom) and the urinary creatinine concentration was determined by the Jaffe method.[31] UACR was calculated by dividing the urinary albumin (mg) by the creatinine (g).

2.6. Secondary outcome measurements

Serum lipid profile and anthropometric parameters, the secondary outcomes of the study, were determined as follows: serum concentration of triglycerides (TG), Total cholesterol (TC), and high-density lipoprotein cholesterol (HDL-C) were measured using the standard enzymatic method with commercial kits (Pars Azmoon, Karaj, Iran). Low-density lipoprotein cholesterol (LDL-C) was calculated using Friedewald formula: [LDL-C (mg/dl) = TC (mg/dl)-(HDL-C (mg/dl) +TG/5)]. Anthropometric parameters were measured for all patients at baseline and the end of the study. Bodyweight was measured with a light cloth and without shoes by a scale (Seca, Hamburg, Germany) to the nearest 0.1 kg. Height was measured in a standing position and without shoes by measuring tape with a precision of 0.1 cm. BMI was calculated by dividing body weight (kg) by the height squared (m). The waist circumference (WC) was obtained by measuring the area between the last rib and iliac crest and the hip circumference (HC) in the largest area of the buttocks using the irreversible tape. The waist-to-hip ratio (WHR) was calculated by dividing the WC by the hip circumference and the waist-to-height ratio (WHtR) was calculated by dividing the WC by the height.

2.7. Dietary intake and physical activity level assessment

Daily dietary intakes during three days (including two weekdays and one weekend) were estimated using the 24-hr dietary recall method in the week just before the study and during weeks 4 and 8. The average daily energy and macronutrient intakes were analyzed using Nutritionist 4 software (First Databank Inc, Hearst Corp, San Bruno, California, United States). The physical activity level was assessed using the International Physical Activity Questionnaire[32] at the beginning and the end of the study.

2.8. Statistical analysis

The data were analyzed by SPSS version 17 (SPSS Inc., Chicago, IL, USA). The Kolmogorov-Smirnov test was used to check the normality of the distribution of the variables. Non-normal variables were analyzed after logarithmic transformation. The results of the quantitative data with normal distribution were reported as mean ± SD and non-normal distributed data were reported using geometric mean (minimum-maximum). The baseline values of the variables in the two groups were compared using independent samples *t*-test and chi-square test for quantitative and qualitative variables, respectively. A paired samples *t*-test was used for within-group comparisons. Analysis of variance for repeated measurements was used to compare within-group changes in dietary intakes. Analysis of covariance (ANCOVA) was applied to determine any differences between the two groups at the end of the study, adjusting for confounders. The significance level was set at $P<0.05$.

3. Results

Forty-six patients participated in the study and all of the subjects (23 in the PBE group and 23 in the placebo group) completed the 8-week intervention period (Fig. 1). The compliance rate was high and more than 92 % of the supplements consumed in a prescribed manner during the study period. Subjects did not report any adverse effects or symptoms with the supplement consumption throughout the study.

3.1. Characteristics of the participants

General characteristics of the patients are reported in Table 1. There were no significant differences in age, sex, diabetes duration, medications, and physical activity level between the two groups at the baseline. No significant changes were observed in taking medications and physical activity level during the study in any of the study groups ($P > 0.05$).

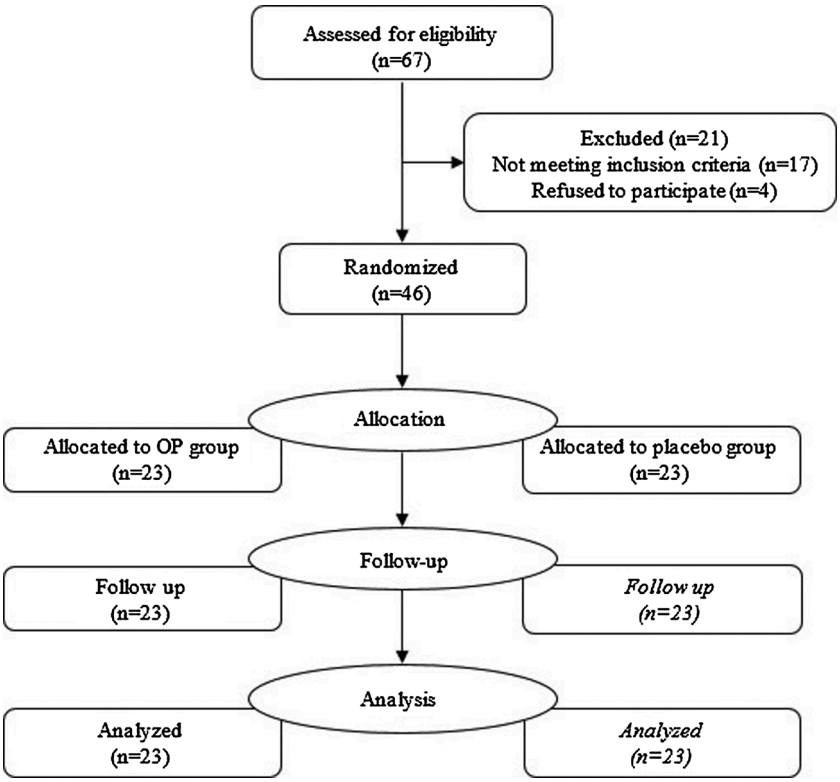

Fig. 1. Study flow diagram.

Table 1
Baseline characteristics of the study patients.

Variable		Placebo group (n = 23)	OP group (n = 23)	P-value
Age (years)		51.30 ± 5.84	54.65 ± 7.39	0.09*
Sex	Female	11 (47.8)	11 (47.8)	1.00†
	Male	12 (52.2)	12 (52.2)	
Duration of diabetes (years)		8.65 ± 5.58	6.91 ± 4.88	0.26*
Antidiabetic medication	Metformin (500 mg)	23 (100.0)	22 (95.7)	0.52†
	Gliclazid (80 mg)	17 (73.9)	15 (65.2)	0.43†
	Pioglitazon (30 mg)	2 (8.7)	2 (8.7)	1.00†
Antihypertensive medication	Losartan (25 mg)	18 (78.2)	16 (69.5)	0.66†
	Enalapril (5 mg)	3 (13.0)	4 (17.4)	0.74†
Physical activity level	Light	15 (65.2)	18 (78.3)	0.51§
	Moderate	8 (34.8)	5 (21.7)	
	Vigorous	0 (0.0)	0 (0.0)	

Data are presented as mean ± sd or n (%).
T2DM: type 2 diabetes mellitus; OP: oligopin.
* Independent sample *t*-test.
† Chi-Square test.
§ Fisher's exact test.

3.2. Daily dietary intakes and anthropometric characteristics

Daily dietary intakes of energy and macronutrients of the study groups are presented in Table 2. No significant differences were seen in energy, carbohydrate, protein, and total fat intakes between the two groups at baseline. There were no significant changes in daily intakes of energy and macronutrients throughout the intervention period, neither within nor between the two groups ($P > 0.05$).

The anthropometric characteristics of participants are shown in Table 3. There were no significant differences in weight, BMI, WC, WHR, and WHtR between the two groups at baseline. Following PBE supplementation, significant reductions were observed in WC (by 1.77 %, $P = 0.04$) and WHtR (by 1.51 %, $P = 0.02$) compared to the baseline values. Based on ANCOVA adjusted for baseline values and mean changes in daily energy intake, significant differences were seen in WC ($P = 0.02$) and WHtR ($P = 0.01$) in the intervention group compared to the placebo group at the end of the study.

3.3. Biochemical parameters

The biochemical characteristics of the study groups at baseline and after the intervention are presented in Table 4. No significant differences were found in these values between the two groups at baseline, except for the TG levels ($P<0.001$). Significant decreases in HbA1clevels (by 10.65 %, $P<0.001$), serum levels of TC (by 14.56 %, $P = 0.005$) and VCAM-1 (by 26.79 %, $P<0.001$), and UACR (by 34.24 %, $P<0.001$) were observed in PBE group compared with the baseline values. FBS decreased by 15.22 % in the intervention group in comparison with the baseline, but this reduction was not statistically significant. Serum levels of insulin, TG, HDL-C, and LDL-C and the HOMA-IR remained unchanged within the PBE group. Serum levels of VCAM-1 increased in the placebo group at the end of the study compared to its baseline concentrations (by 10.87 %, $P = 0.02$). Changes in other biochemical variables were not significant within the placebo group.

According to the ANCOVA adjusted for the baseline values and the mean changes in BMI and daily energy intake, significant differences were observed in HbA1c ($P<0.001$), serum levels of TC ($P = 0.03$), VCAM-1 ($P<0.001$), and UACR ($P<0.001$) between the two groups at the end of the intervention. Differences in FBS, insulin, HOMA-IR, TG, LDL-C, and HDL-C were not significant between the two groups at the end.

Table 2
Daily dietary intakes of the study patients at baseline and 4 and 8 weeks after the intervention.

variable	Measurement period	Placebo group (n = 23)	OP group (n = 23)	P-value
Energy (kcal/day)	Before	2277.2 ± 499.6	2118.1 ± 421.0	0.24*
	4th week	2285.3 ± 474.2	2051.6 ± 370.9	0.07†
	after	2289.8 ± 449.6	2084.8 ± 372.7	0.15†
	P-value **	0.91	0.37	
Carbohydrate (g/day)	Before	308.3 ± 64.9	293.9 ± 64.2	0.45*
	4th week	310.2 ± 67.4	284.6 ± 60.0	0.11†
	After	307.5 ± 64.2	289.1 ± 59.3	0.47†
	P-value **	0.82	0.32	
Protein (g/day)	Before	73.2 ± 19.7	64.4 ± 15.6	0.10*
	4th week	76.8 ± 17.9	66.2 ± 14.4	0.15†
	After	75.3 ± 16.9	64.3 ± 14.2	0.11†
	P-value **	0.21	0.71	
Total fat (g/day)	Before	83.0 ± 23.1	77.2 ± 19.1	0.36*
	4th week	83.1 ± 17.4	74.9 ± 13.0	0.09†
	After	85.5 ± 20.7	75.1 ± 15.9	0.053†
	P-value **	0.59	0.46	

T2DM: type 2 diabetes mellitus; OP: oligopin.
Data are presented as mean ± sd.
* P-value is reported based on the analysis of the independent sample *t*-test.
** P-value is reported based on the repeated measures analysis of variance.
† P-Value is reported based on the analysis of covariance (adjusted for baseline values).

4. Discussion

This study investigated whether PBE supplementation could have beneficial metabolic effects in T2DM patients with microalbuminuria. To our knowledge, this trial is the first to evaluate PBE effects in these patients.

The present study results indicated that an eight-week supplementation with 100 mg/day of PBE, significantly reduced serum HbA1c, TC, and VCAM-1 levels, UACR, and WC compared to the placebo group. Serum FBS levels also decreased in the PBE group, however, this reduction was not significant. Serum levels of insulin, TG, LDL-C, and HDL-C, as well as HOMA-IR, did not change significantly following PBE intervention. PBE has been previously researched and used in several clinical conditions.[21] There is a growing body of evidence that suggests PBE supplementation has some potentially beneficial metabolic properties such as anti-diabetic and hypoglycemic effects.[20,22–26,33–36]

PBE supplementation in the current study induced a significant reduction in serum HbA1c levels and a considerable decrease in FBS levels (by 15.22 %) which could be clinically important. These findings are in agreement in part with the results of some experimental and clinical studies.[20,22–26,34,35] Aydin et al.[34] indicated that treatment of

Table 3
Anthropometric measurements of the study patients at baseline and after eight weeks intervention.

Variable	Measurement period	Placebo group (n = 23)	OP group (n = 23)	MD (95 % CI)	P-value
Weight (kg)	Baseline	77.39 ± 13.30	80.55 ± 13.12	3.15 (−4.69 to 11.01)	0.42*
	After Intervention	77.75 ± 13.59	80.73 ± 13.09	−0.23 (−1.06 to 0.58)	0.56†
	MD (95 %CI)	0.36 (−0.01 to 0.74)	0.18 (−0.55 to 0.91)		
	P-value	0.06 §	0.61§		
BMI (kg/m^2)	Baseline	29.34 ± 5.43	30.28 ± 3.89	0.94 (−1.86 to 3.75)	0.50*
	After Intervention	29.47 ± 5.54	30.36 ± 3.89	−0.08 (−0.40 to 0.23)	0.59†
	MD (95 %CI)	0.13 (−0.01 to 0.28)	0.07 (−0.21 to 0.36)		
	P-value	0.07§	0.59§		
WC (cm)	Baseline	101.17 ± 12.63	105.52 ± 8.38	4.34 (-2.02 to 10.72)	0.17*
	After Intervention	101.65 ± 13.70	103.65 ± 7.98	−2.39 (−4.43 to −0.34)	0.02†
	MD (95 %CI)	0.47 (−0.45 to 1.40)	−1.86 (−3.65 to −0.08)		
	P-value	0.29§	0.04§		
WHR	Baseline	0.94 ± 0.03	0.95 ± 0.03	0.00 (−0.01 to 0.03)	0.48*
	After Intervention	0.95 ± 0.03	0.94 ± 0.05	−0.01 (−0.03 to 0.008)	0.23†
	MD (95 %CI)	0.00 (−0.00 to 0.00)	−0.00 (−0.02 to 0.01)		
	P-value	0.30§	0.53§		
WHtR	Baseline	0.65 ± 0.09	0.66 ± 0.09	0.03 (−0.01 to 0.09)	0.17*
	After Intervention	0.62 ± 0.10	0.65 ± 0.08	−0.01 (−0.02 to −0.003)	0.01†
	MD (95 %CI)	0.00 (−0.00 to 0.00)	−0.01 (−0.02 to −0.00)		
	P-value	0.30§	0.02§		

T2DM: type 2 diabetes mellitus; OP: oligopin; CI: confidence interval; MD: mean difference; BMI: body mass index; WC: waist circumference; WHR: waist to hip ratio; WHtR: waist to height ratio.
Data are presented as mean ± sd.
* P-value is reported based on the analysis of independent sample *t*-test.
§ P-value is reported based on the analysis of paired sample *t*-test.
† P-value is reported based on the analysis of covariance, adjusted for baseline values and mean changes of daily energy intake.

diabetic mice with 50 mg/kg/day of PBE decreased FBS levels at the 14th, 21th, and 28th days of treatment. Jankyova et al.[35] reported improved FBS and postprandial glucose concentrations with PBE treatment in streptozotocin-induced diabetic rats. Several clinical trials also found improvements in glycemia with PBE supplementation.[20,22–26] In the study by Liu et al.[23] PBE consumption decreased HbA1c, FBS, and postprandial glucose in a dose-dependent manner in patients with T2DM during 12 weeks, but it did not affect insulin levels at any dosage of administration. Another study showed that supplementation with 100 mg/day PBE for 12 weeks reduced HbA1c and FBS concentrations;

Table 4
Biochemical parameters of the study patients at baseline and after eight weeks intervention.

Variable	Measurement period	Placebo group (n = 23)	OP group (n = 23)	MD (95 % CI)	P-value
FBS (mg/dl)	Baseline	155.13 ± 49.67	177.30 ± 71.78	22.17 (−14.50 to 58.85)	0.23*
	After Intervention	165.91 ± 67.01	150.30 ± 48.52	−23.20 (−57.11 to 10.71)	0.17†
	MD (95 % CI)	10.78 (−13.89 to 35.45)	−27.00 (−59.79 to 5.79)		
	P-value§	0.37	0.10		
HbA1c (%)	Baseline	9.50 ± 1.77	9.10 ± 2.04	−0.39 (−1.52 to 0.74)	0.48*
	After Intervention	9.53 ± 1.81	8.13 ± 1.36	−1.17 (−1.75 to −0.59)	<0.001†
	MD (95 % CI)	0.03 (−0.50 to 0.56)	−0.97 (−1.38 to 0.55)		
	P-value§	0.90	<0.001		
Insulin (μU/mL) ‡	Baseline	1.40 (0.15 to 11.38)	1.24 (0.23 to 7.09)	−0.05 (−0.34 to 0.23)	0.71*
	After Intervention	1.16 (0.24 to 7.02)	1.38 (0.21 to 8.48)	0.06 (−0.19 to 0.32)	0.62†
	MD (95 % CI)	−0.08 (−0.28 to 0.12)	0.045 (−0.24 to 0.33)		
	P-value§	0.43	0.75		
HOMA-IR‡	Baseline	0.51 (0.07 to 2.80)	0.50 (0.08 to 2.56)	0.00 (−0.28 to 0.27)	0.98*
	After Intervention	0.44 (0.05 to 2.67)	0.47 (0.05 to 2.68)	0.00 (−0.28 to 0.28)	0.99†
	MD (95 % CI)	−0.061 (−0.27 to 0.14)	−0.03 (−0.32 to 0.26)		
	P-value§	0.55	0.83		
TC (mg/dl)	Baseline	158.08 ± 48.45	162.95 ± 46.72	4.86 (−23.41 to 33.15)	0.73*
	After Intervention	155.21 ± 45.12	139.21 ± 31.31	−20.46 (−39.34 to −1.57)	0.03†
	MD (95 % CI)	−2.86 (−21.45 to 15.71)	−23.37 (−39.59 to −7.88)		
	P-value§	0.75	0.005		
TG (mg/dl)	Baseline	130.47 ± 52.91	218.56 ± 91.92	88.08 (43.51 to 132.66)	<0.001*
	After Intervention	123.08 ± 56.97	195.21 ± 110.40	−15.74 (−68.28 to 36.80)	0.54†
	MD (95 % CI)	−7.39 (−22.11 to 7.33)	−23.34 (−68.59 to 21.89)		
	P-value§	0.30	0.29		
LDL-C (mg/dl)	Baseline	76.60 ± 30.94	87.08 ± 36.09	10.47 (−9.50 to 30.45)	0.29*
	After Intervention	82.95 ± 29.94	84.86 ± 30.88	−3.44 (−19.52 to 12.62)	0.66†
	MD (95 % CI)	6.34 (−6.96 to 19.66)	−2.21 (−15.26 to 10.82)		
	P-value§	0.33	0.72		
	Baseline				0.78*

(continued on next page)

Table 4 (*continued*)

Variable	Measurement period	Placebo group (n = 23)	OP group (n = 23)	MD (95 % CI)	P-value
		39.69 ± 9.92	40.47 ± 9.01	0.78 (−4.85 to 6.41)	
HDL-C (mg/dl)	After Intervention	38.39 ± 9.04	39.82 ± 9.83	1.13 (−3.14 to 5.41)	0.59†
	MD (95 % CI)	−1.30 (−4.80 to 2.19)	−0.65 (−3.65 to 2.35)		
	P-value§	0.44	0.65		
	Baseline	24.00 ± 7.10	26.57 ± 6.76	2.56 (−1.55 to 6.68)	0.21*
VCAM-1 (ng/mL)	After Intervention	26.61 ± 7.35	19.45 ± 7.24	−9.16 (−12.50 to −5.83)	<0.001†
	MD (95 % CI)	2.61 (0.39 to 4.82)	−7.11 (−9.80 to −4.24)		
	P-value§	0.02	<0.001		
	Baseline	46.98 ± 12.76	45.52 ± 9.57	−1.45 (−8.16 to 5.25)	0.66*
UACR (mg/g)	After Intervention	49.23 ± 20.75	29.93 ± 10.39	−16.35 (−22.81 to −9.89)	<0.001†
	MD (95 % CI)	2.24 (−2.56 to 7.05)	−15.59 (−19.95 to −11.23)		
	P-value§	0.34	<0.001		

T2DM: type2 diabetes mellitus; OP: oligopin; CI: confidence interval; MD: means difference; HbA1c: glycosylated hemoglobin; HOMA-IR: homesrtatic model assessment for insulin resistance; TG: Triglyceride; LDL-C: Low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; VCAM-1: vascular cell adhesion molecule-1; UACR: urinary albumin to creatinine ratio. Data are presented as mean ± sd or geometric mean (min, max).

* P-value is reported based on the analysis of independent sample *t*-test.

§ P-value is reported based on the analysis of paired sample *t*-test.

‡ Analyzed after log-transformation.

† P-value is reported based on the analysis of covariance, adjusted for baseline values and mean changes of BMI and daily energy intake.

however, the reduction in HbA1c levels was significant only for the first month.[22] Treatment with 150 mg/day of PBE for six months also decreased HbA1c and FBS levels in metabolic syndrome patients.[26]

It has been suggested that the anti-hyperglycemic effect of PBE is related to its oligomeric compounds, especially procyanidins, which inhibit the α-glycosidase enzyme activity and decrease the intestinal glucose absorption.[24,33] A study on diabetic mice showed that PBE has inhibitory effects on both salivary and pancreatic α-amylase enzymes.[36] It was also proposed that PBE decreases glucose uptake in the brush-border membrane through reducing the number of glucose transporter-2 (GLUT-2) by inhibiting the signaling pathways involving phosphatidylinositol-3-kinase (PI3K) and/or p38 mitogen-activated protein kinase (p38-MAPK) pathways.[37] Moreover, Lee et al.[38] showed that procyanidins in PBE may have insulin-like properties which stimulated glucose uptake in the differentiated 3T3-L1 fat cells via GLUT-4, an insulin-regulated glucose transporter in the PI3K/AKT pathway.

In the study by Zibadi et al.,[24] 125 mg/day of PBE supplementation in subjects with T2DM reduced HbA1c levels at the 8th week and FBS at the 12th week of intervention. Thus, it seems that a longer intervention period or a higher dose of supplement might be required to induce significant changes in FBS levels in our study subjects. They also found that the blood glucose lowering effect of PBE is dose-dependent and is not mediated through insulin secretion. Similarly, our results showed no significant changes in insulin levels following PBE supplementation. Such findings were involved in non-significant alterations in HOMA-IR in our study patients.

VCAM-1, a member of the immunoglobulin superfamily of proteins, has been regarded as an inflammatory marker and a risk factor for macrovascular and microvascular complications of diabetes. [4] Earlier in vitro studies demonstrated that isolated endothelial cells from diabetic patients express VCAM-1 by stimulating inflammatory cytokines in presence of high ambient glucose.[2] On the other hand, the expression of VCAM-1 is regulated by nuclear factor-kappa B (NF-κB), a transcription factor that is activated by oxidative stress.[28] So, inflammation and oxidative stress are considered potential targets for the development of therapies to prevent and manage vascular complications of T2DM.[39]

PBE is increasingly known for its anti-inflammatory and antioxidant effects in vitro and in vivo.[39] Based on the results, PBE supplementation remarkably decreased serum VCAM-1 levels compared to the placebo group. To our knowledge, no previous clinical studies have investigated the effect of PBE supplementation on VCAM-1 levels in metabolic diseases. However, a few in vitro studies have examined the effect of PBE on CAMs expression.[27,28] It has been demonstrated that incubation of monocytes with PBE reduced proinflammatory mediators activities and adhesion molecules by inhibiting the NF-κB dependent gene expression and eliminating free radicals.[27] Similarly, a study on TNF-α treated human endothelial cells demonstrated that pretreatment with PBE inhibited the TNF-α induced activation of NF-κB and reduced the expression of CAMs, dose-dependently.[28] PBE also increases plasma antioxidant capacity via stimulating the activity of antioxidant enzymes such as superoxide dismutase and catalase as well as intracellular glutathione level. These antioxidant effects eventually lead to inhibition of the TNF-α mediated NF-κB activation, an essential factor for the gene expression of adhesion molecules.[21,28,39] Our results confirmed the effectiveness of PBE in reducing VCAM-1.[3] However, the magnitude of this reduction remains under debate. The widespread medications used in diabetic patients as well as in our studied subjects are antihypertensive drugs. It has been shown that these medications stimulate nitric oxide production which possesses anti-inflammatory properties and decreases VCAM-1 expression.[40] On the other hand, extra data have indicated the increased expression of adhesion molecules in the kidneys during the progression of kidney disease in T2DM.[41] Additional CAMs may be localized on sites of inflammation, such as microvessels and consequently, in the late stages, their levels could be reduced in the blood.[4] By considering such possible variations in VCAM-1 in the course of disease in patients with diabetes along with taking antihypertensive drugs, the clinical impact of PBE supplementation in patients with diabetes and microalbuminuria needs further studies.

Evaluating urinary albumin excretion (UAE) is one of the methods used for monitoring and early detection of diabetic nephropathy and chronic kidney disease. Studies have shown that there is a close association between early morning void UACR and UAE. Based on a recent guideline, the first-morning void UACR measurement can be used as an alternative to the 24-h UAE levels because collecting the 24-h urine is difficult and error-prone.[42] According to our results, UACR levels decreased significantly by PBE supplementation when compared to the placebo. This finding is in agreement with the study by Zibadi et al.[24] which reported a significant decrease in UAE levels in patients with T2DM with PBE supplementation in eight weeks. Stuard et al.[26] also reported the favorable effect of PBE on UAE reduction in subjects with metabolic syndrome and microalbuminuria. There is no other previous study that investigated the effects of PBE on microalbuminuria indices.

Increased lipid peroxidation and AGEs have been shown in diabetic nephropathy.[43] Hyperglycemia induces oxidative stress and causes apoptosis in renal tubular cells. Previous studies demonstrated that PBE with antioxidant and anti-inflammatory properties reduces ROS production and AGEs levels, and prevent the activation of NF-κB, an important transcription factor in the pathogenesis of diabetic nephropathy, in renal tubular cells. Besides, PBE inhibits apoptosis in renal tubular cells by up-regulation of anti-apoptotic Blc2 and reducing pro-apoptotic Bax protein levels.[39,44] Such mechanisms might be

involved in inducing favorable effects of PBE supplementation on UACR levels in our study patients with microalbuminuria.

Hypercholesterolemia is a major risk factor for cardiovascular disease especially in patients with T2DM.[45] Based on our results, PBE supplementation declined TC level in the intervened group compared to the placebo group. Reduced LDL-C levels by PBE supplementation was previously reported in subjects with TD2M and hypertension.[24] Devaraj et al.[46] also demonstrated a significant reduction in LDL-C levels and increased HDL-C levels in healthy subjects who received 150 mg/day PBE for six weeks. The beneficial effects of PBE administration with different dosages and durations on the lipids profile were also reported in some other experimental and clinical studies.[20,25,34,47–49] However, Stuard et al.[26] found that supplementation with 150 mg/day of PBE for six months in patients with T2DM did not affect TC and HDL-C levels.

Peroxisomal proliferator-activated receptor α (PPAR-α) is a ligand-activated transcription factor that regulates the expression of different genes involved in lipid metabolism. Exogenous and endogenous activators of PPAR-α induces lipid-lowering properties via regulating the fatty acids β-oxidation in the liver.[50] Shimada et al.[51] indicated that administration of a diet containing 3% or 5% PBE in obese diabetic mice for eight weeks, increased the expression of enzymes involved in fatty acid oxidation such as PPAR-α, dose-dependently. It has been also demonstrated that PBE may up-regulate hepatic LDL-C receptors or increase the sterols excretion.[46] Such mentioned mechanisms might be contributed to decreased TC levels in our intervened group. However, the varying dosages of PBE supplementation together with the differences in supplement formulation, treatment duration, clinical and metabolic characteristics of the patients involved in the studies, as well as experimental designs might influence the results. Further studies are required to evaluate the effects of PBE supplementation on serum lipid profile and the involved mechanisms in subjects with T2DM.

We also found that PBE supplementation decreased WC and WHtR measurements compared to the placebo. Weight and BMI did not show any changes. Similarly, Belcaro et al.[20] reported decreased WC in subjects with metabolic syndrome following PBE supplementation without significant changes in weight and BMI. Sedighian et al.[25] found significant decreases in weight, BMI, WHR, and central obesity in obese women with metabolic syndrome after supplementation with PBE for eight weeks. However, some other studies did not confirm the beneficial effects of PBE on anthropometric parameters.[26,52] It has been concluded that PBE has an anti-obesity effect via suppression of fat accumulation in adipose tissue.[53] Such effect was attributed to the stimulation of lipolysis in adipose tissue by activation of cAMP-dependent protein kinase A, which regulates hormone-sensitive lipase. Furthermore, it has been reported that procyanidin, one of the major components of PBE, suppresses fat accumulation via inducing the fatty acid oxidation enzyme expression, as well as down-regulating the fatty acid synthase enzyme expression.[52] Our findings confirmed the hopeful effects of PBE supplementation on reducing visceral adiposity in T2DM patients.

It should be noted that in the present study, daily dietary intakes and physical activity levels of subjects did not change throughout the study in any of the groups. Therefore, these factors could not be considered as confounding factors in the interpretation of the study findings. This study was conducted as a randomized double-blind placebo-controlled trial to minimize the possible bias and confounding factors. However, our study had some limitations including the short duration of the intervention period and using the low dose of PBE. Thus, our results may not be generalized to other dosages of PBE, other intervention periods, or patients taking other diabetic medications.

5. Conclusion

The present study indicated that daily supplementation with 100 mg of PBE in patients with T2DM and microalbuminuria had favorable effects on glycemic control, serum VCAM-1, and UACR level, as well as TC concentrations and abdominal obesity which could be helpful in the control of diabetes complications.

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CRediT authorship contribution statement

Elham Navval-Esfahlan: Visualization, Investigation, Data curation, Writing - original draft. **Maryam Rafraf:** Supervision, Conceptualization, Project administration, Writing - original draft. **Somayyeh Asghari:** Data curation, Writing - review & editing. **Hossein Imani:** Conceptualization, Visualization. **Mohammad Asghari-Jafarabadi:** Methodology, Software, Formal analysis, Validation. **Sanaz Karimi-Avval:** Resources.

Declaration of Competing Interest

The authors report no declarations of interest.

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