A Randomized Clinical Trial of Beetroot Juice Consumption on Inflammatory Markers and Oxidative Stress in Patients With Type 2 Diabetes

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Abstract

Background/Objectives Oxidative stress and chronic inflammation are both major underlying factors for the initiation and progression of diabetes as well as for the development of long-term diabetes-related consequences in patients with type 2 diabetes mellitus (T2DM). This study was designed to evaluate the effects of concentrated beetroot juice (BJ) consumption on oxidative stress and inflammatory markers in patients with T2DM.

Methods In this 12-weeks randomized clinical trial, forty-six patients with T2DM were randomly assigned to either the concentrated BJ or the control group. The BJ group received 12 ml concentrated beetroot juice twice daily, whereas the control group received no intervention. At baseline and the end of week 12, plasma levels of malondialdehyde (MDA), high sensitive C-reactive protein (hs-CRP), interleukin-6 (IL-6), and tumor necrosis factor- α (TNF- α), and nuclear factor- κ B (NF- κ B) were measured.

Results Compared with control group, concentrated BJ reduced IL-6 (-0.1 vs. 0.83 , $p = .001$), TNF- α (-1.28 vs. 5.51 , $p = .001$), and NF- κ B (-0.03 vs. 0.36 , $p = .005$) after 12 weeks intervention; plasma concentration of MDA and hs-CRP did not change between groups after intervention. Intra-group comparison with paired t-test disclosed that daily BJ consumption could not change oxidative stress and inflammatory markers except for TNF- α compared with baseline values. However, the concentrations of IL-6, TNF- α , and NF- κ B increased significantly in the control group at the end of week 12 compared to baseline ($p = .001$, $p = .001$, $p = .021$, respectively).

Conclusions In conclusion, our study indicates that daily consumption of 24 ml concentrated BJ to patients with T2DM reduces the concentrations of some inflammatory markers including IL-6, TNF- α , and NF- κ B which are involved in the pathogenesis of complications of T2DM.

Trial Registration This study was registered at the Iranian Registry of Clinical Trials (IRCT) with the following identification: IRCT20150815023617N5.

Background

Diabetes mellitus has been proven to be under a status of systemic low-grade chronic inflammation, which is closely involved in the pathogenesis of diabetic complications such as dyslipidemia and atherosclerosis (1). Elevated circulating inflammatory markers interfere with insulin action and disrupt metabolic homeostasis (2). High circulating levels of the inflammatory markers C-reactive protein (CRP), interleukin-6 (IL-6), and tumor necrosis factor (TNF- α) have been proposed as prognostic markers for the development of type 2 diabetes mellitus (T2DM) and its microvascular and macrovascular complications (3). These events can worsen quality of life as well as lead to disability and high mortality rates among subjects with T2DM (4). Therefore, early intervention is a valuable approach to reduce the development of diabetes mellitus and related complications (5). Many clinical trials have suggested that lifestyle intervention, including moderate-intensity exercise and diet control, and pharmacologic intervention are

effective in management of T2DM (5). some dietary intervention can provide significant benefits to improve the health status and reduce exacerbations for patients with T2DM.

Beetroot (*Beta vulgaris L.*) is a vegetable grown throughout worldwide, from the Americas to Europe and Asia (6). It is a rich source of multiple biologically active phytochemical compounds including flavonoids, polyphenols, saponins, carotenoids, ascorbic acid, and a group of bioactive pigments known as betalains (e.g., betacyanins and betaxanthins). Recent evidence has demonstrated that betalains have high antioxidant and anti-inflammatory capabilities through free radical scavenging activity, preventing oxidative damage to lipid molecules and DNA, and inhibiting inflammatory responses (7). Beetroot is also considered as a good source of inorganic nitrates (NO_3^-), which are a substrate for the synthesis of nitric oxide. Nitrite and nitric oxide can reduce the oxidative stress by decreased reactive oxygen species production along with increased activity of antioxidant enzymes, which suggest that nitrate may also exhibit antioxidant properties (8). Beetroot is abundant in minerals such as potassium, sodium, iron, copper, magnesium, calcium, phosphorus, and zinc, in addition to vitamins like retinol, ascorbic acid, biotin, niacin, and folate (9).

So far, various interventional studies have addressed the implications of beetroot in cardiovascular diseases, metabolic syndrome, hypertension, dyslipidemia, dementia, cognitive disorders, and diabetes mellitus (10–15). However, few clinical trials have been carried out regarding the anti-inflammatory effects of beetroot and its derivatives. Asgari et al., indicated that short-term supplementation with red beet juice could reduce the levels of inflammatory markers such as TNF- α , high sensitive- C reactive protein (hs-CRP), IL-6, and E-selectin in adults subjects (16).

Although beetroot juice (BJ) has anti-inflammatory and free radical scavenging properties, clinical evidence regarding its activities is rare. To the best of our knowledge, no clinical trial has been designed to show the effects of beetroot juice supplementation on oxidative stress and inflammatory markers in patients with type 2 diabetes mellitus. Therefore, the present study investigated the hypothesis that concentrated BJ consumption would effectively improve oxidative stress and inflammatory markers in patients with T2DM in the setting of randomized clinical trial.

Materials And Methods

Subjects and ethical aspects

This study was a simply randomized, parallel-group, controlled, open-label clinical trial. The trial was carried out between October 2020 and March 2021 and was in adherence with the Declaration of Helsinki and Consolidated Standards of Reporting Trials (CONSORT) statement for randomized trials (17). The trial protocol was approved by Ethics Committee of the National Nutrition and Food Technology Research Institute of Iran (IR.SBMU.nnftri.Rec.1399.035). This study was registered at the Iranian Registry of Clinical Trials (IRCT) with the following identification: IRCT20150815023617N5. Moreover, the author declare that all participants gave written informed consent before initiation of the study protocol.

Forty-six participants with clinically diagnosed T2DM (1–10 years, based on medical history and diabetes medication) according to Standards of Medical Care in Diabetes Guidelines (18) were recruited using simple random sampling from the Shahid Ghasem Clinic, Sari, Iran. The participants were informed about study protocol and signed the informed consent form. They were 35-70 years old with body mass index (BMI) higher than 18.5 kg/m² who treated with oral hypoglycemic agents. Patients were excluded in the initial assessment if they were suffering from inflammatory diseases especially Covid-19 as the major criteria, hepatic and renal disorders, rheumatoid arthritis, having a history of micro- and macro-vascular diseases (such as the history of angioplasty and foot vascular surgery), being pregnant or lactating, being on a weight loss diet within the last 6 months, taking multivitamin and mineral supplements or herbal medicines, poor glycemic control (HbA1c $\geq$ 11), as well as glucocorticoids, nonsteroidal anti-inflammatory drugs and nitrate-containing drugs such as nitroglycerin, isosorbide, and nitropress during last 3 months and during the study.

Protocol

Subjects, after stratification based on sex and age, were randomly and equally allocated to one of the following treatment groups: 1) intervention group with 24 ml concentrated beetroot juice each day between main meals in two doses, and 2) control group with no intervention. For this stratified block randomization, we chose a block size of 4 written in SAS® (Cary, NC) and possible balanced combinations with 2 B (Beetroot) and 2 P (Placebo). Participants were randomly assigned as six blocks (BBPP, PPBB, BPBP, PBPB, BPPB, and PBBP) based on a simple random sampling method in chronological order. The subjects and principal investigators were blinded to the treatment assignment until the final statistical analysis was finished. The participants began taking daily doses of beetroot juice (12 ml twice-daily) immediately after first visit in intervention group and were requested to follow the intervention for 12 weeks. They were requested to consume their juice at the same time each day (between main meals). Patients were advised to keep their routine dietary intake, physical activity habits, and usual medications intake during the study, otherwise they were excluded from the study. Concentrated beetroot juice was prepared from fresh beetroot (*Beta vulgaris* var. *esculenta*, *chenopodiaceae* family) commercially (Takdaneh, Inc., Marand, Iran) without any additives and contained 180 mg nitrate and 0.1 mg nitrite per 24 ml. The quality of beetroot juice was assessed by the Food and Drug Administration, Mazandaran University of Medical Sciences, Sari, Iran at weeks 0, 6, and 12 of the study. Details of the beetroot juice are shown in Table 1. Based on previous studies daily supplementation with 25 ml concentrated beetroot juice does not increase the blood glucose levels in patients with T2DM (19).

Table 1
Composition of concentrated beetroot juice

composition	amount
Total polyphenols (mg/100 mL)	190
Total flavonoid (mg/100 mL)	120
TAC (%) DPPH	15
Nitrate (mg/dL)	720
Nitrite (mg/d L)	0. 4
Sugar (g/100 g)	42
NACL(%)	2

Sample size

For estimating the sample size, we used the standard formula suggested for randomized clinical trials based on a power ($1-\beta$) of 80% and type one error (α) of 0.05. According to a previous study (20) and considering the TNF- α as a key variable with a pooled standard deviation of 0.6 pg/mL, the current study needed 19 patients in each group to have 80% study power. Assuming 20% potential loss of samples in each group, we calculated to have 23 participants per group.

Measurements

Anthropometric assessment

At baseline, a general questionnaire was completed during a personal interview for each patient regarding demographic information, medical history, drug history, present illnesses, medication use, and health status. Anthropometric assessment was performed at the beginning and the end of the intervention. Body weight was measured in light clothing without shoes in an overnight fasting status with a precision of 0.1 kg using a Seca beam balance scale (Seca, Hamburg, Germany). Height was measured using a seca stadiometer (Seca, Hamburg, Germany) without shoes and based on standard protocol at week 0. Waist and hip circumferences were also undertaken in the standing position using non-stretchable flexible constant tension measuring tape and according to a standard protocol by a trained investigator at baseline and the end of week 12. Moreover, body mass index (BMI) was calculated as weight in kg divided by height in meters squared (kg/m^2).

Dietary and physical activity assessment

The dietary intake over the study period was assessed by the completion of 3 day 24-h dietary recalls (one weekend and two weekdays) at baseline and at the end of study. First 24-h dietary recall was recorded by an expert dietitian through an in-person interview. The other two intake data were collected by

telephone. The dietary recalls were analyzed using Nutritionist IV software (N Squared Computing, San Bruno, CA) modified for Iranian foods. Physical activity was also evaluated at the baseline and final visit using a validated semi-quantitative questionnaire, based on metabolic equivalent (MET)-h/day values (21).

Biochemical assessment

Following overnight fasting (12-14 h) in an early morning, 10 ml peripheral blood samples were collected from each subject at baseline and the end of the trial. Blood samples were centrifuged at 3700 rpm for 10 min and then serum was separated and frozen at -80°C to quantify serum IL-6, hs-CRP, and malondialdehyde (MDA). The remaining process was related to peripheral blood mononuclear cells (PBMCs) isolation. To isolate PBMCs from whole blood, we transferred the buffy coats to a new falcon tube. Then buffy coats were diluted by Phosphate-buffered saline (PBS) and homogenized completely. In the next step, the entire solution was added to a new falcon tube containing 4 mL ficoll solution gently, and we centrifuged the samples at 2300 rpm for 20 min (22). The PBMCs were isolated and frozen at -80°C until they were used. All biochemical variables were measured in the same laboratory by using standard laboratory methods.

Plasma concentrations of hs-CRP and IL-6 were measured by enzyme-linked immunosorbent assay (ELISA) kits (LDN, Nordhorn, Germany for hs-CRP, and Demeditec, Kiel-Wellsee, Germany for IL-6). Plasma level of MDA was determined using colorimetry method by a commercial kit (ZellBio GmbH, Ulm, Germany), according to the manufacturer's instructions. In addition, nuclear factor kappa B (NF- κ B) p65 and TNF- α were detected in PBMC nuclear extracts by using an ELISA kit (ZellBio GmbH, Ulm, Germany, Diaclone, Besancon, France; respectively).

Concentrated beetroot juice characteristics and analytical assays

Concentrated beetroot juice prepared by Takdaneh, Inc., Iran, without any additives (contains 180 mg nitrate and 0.1 mg nitrite per 24 ml). Total Phenolic Content (TPC) was measured by the Folin-Ciocalteu reagent, using Gallic acid as a standard. The absorbance was measured at 765nm using the colorimetric assay. Total flavonoid content was measured by the aluminum chloride and potassium acetate. The absorbance of the reaction mixture was subsequently measured at 415 nm colorimetric assay using quercetin (10 mg/100 ml) as standard (23). Concentrated beetroot juice was diluted at 1:10 (v: v) to measure its total antioxidant capacity (TAC), based on the inhibition percent of DPPH. The working solution (6×10^{-5} M) was prepared, and then the absorbance at 515 nm was recorded using a spectrophotometer. A control with no added extract was also analyzed (24). Nitrate and nitrite content was measured by the colorimetric assay. Nitrate content was measured according to the cadmium reduction method (25). Sugar content was measured by the HPLC method. Concentrated beetroot juice composition is summarized in Table 1.

Compliance

For the ascertainment of patients' compliance, we contacted the study participants by phone every 15 days to assay any problem with the consumption of beetroot concentration and possible side effects. A bottle of concentrated beetroot juice, adequate for 30 days, was given to the intervention group at baseline. The second and the third bottles were given at the follow-up visits every 4 weeks. Every 4 weeks following initiation of the study, the participants were asked to return the unused bottles. Patients who had not consumed at least 90% of the expected BJ were regarded as noncompliance; and were consequently excluded from the study. The degree of compliance for our patients was over 90%. Also, the concentrated beetroot juice was stored at 0 to 1 ° C before distribution, and the intervention group was asked to keep it at refrigerator temperature (less than 5 ° C). In this study, no adverse effects were reported. Moreover, the participants were instructed to maintain their usual lifestyle and dietary habits during the study.

Statistical analysis

Data was analyzed using Statistical Package for the Social Sciences (SPSS, Inc., Chicago, IL) for Windows version 20. Two-sided *P* values < 0.05 were considered as statistically significant. Data are presented as mean ± standard deviation (SD) and controlled for normality by the Kolmogorov–Smirnov test to ensure that the assumptions of the analysis are met. Log transformation was performed before statistical analyses for variables with non-normal distribution. Baseline characteristics and biochemical variables of the study participants were compared using the independent t-test. Comparison of paired parametric variables for each study group was performed separately using paired t test. In order to eliminate the effects of confounding factors, either at the beginning or during the study, analysis of covariance (ANCOVA) was used. All analysis of covariance models were adjusted for baseline covariates. Intention-to-treat principle (ITT) based on expectation maximization clustering algorithm and per-protocol analysis were applied for analyzing the data; the per-protocol analysis results were only displayed because of the same outputs.

Results

Characteristics of the study subjects

A total of 200 volunteer participants were screened for inclusion and exclusion criteria; of which 46 were enrolled in the trial. Among participants who were assigned to the groups, three women and one man (having car accident, changing living area, infection to COVID-19, and death caused by COVID-19) in the BJ group, two women and two men (initiating insulin administration, infection to COVID-19, and quarantine and the risk of attending at blood sampling center) in control group, failed to complete the study. Finally, thirty-eight participants were included in the final analysis, as shown in figure. 1.

As shown in Table 2, at the beginning of the study, there was no statistically significant difference among patients between groups in terms of clinical and laboratory characteristics except for MDA.

Table 2

Baseline characteristics of the participants in the BJ group and the placebo group.

Variables		Groups of study		P-value*
		BJ group (n=19)	Control group (n=19)	
Sex (M/F)	Total	16/3	15/4	.999
Age (year) ^a	Total	54.08 ± 9.23	53.89 ± 8.78	.971
Duration of diabetes (year)	Total	6.42 ± 3.45	6.44 ± 3.17	.981
Hypoglycemic medications (%)	Metformin	16 (84.2 %)	17 (89.4 %)	.631
	Sulfonylurea	11 (57.89 %)	12 (63.15 %)	.74
	Thiazolidinedione	1 (5.2 %)	0 (0.0 %)	.311
	Meglitinide	1 (5.2 %)	0 (0.0%)	.311
	DPP-4 inhibitor	0 (0.0 %)	1 (5.2 %)	.311
	α-glucosidase inhibitor	2 (10.52 %)	2 (10.52 %)	> .999
Anti-hyperlipidemic medications (%)	Yes	10 (52.6 %)	13 (68.42 %)	.319
	No	9 (47.3 %)	6 (31.5 %)	
Anti-hypertensive medications (%)	Yes	7 (36.8 %)	8 (42.1 %)	.74
	No	12 (63.1 %)	11 (57.8 %)	
Metabolic characteristics				
Height (cm)	Total	164.73 ± 7.73	159.57 ± 8.02	.051
Weight (kg)	Total	76.81 ± 10.85	78.34 ± 13.2	.699
BMI (kg/m ²)	Total	28.28 ± 2.77	30.93 ± 5.4	.067
WC (cm)	Total	92.68 ± 11.79	96.42 ± 11.63	.332
HC (cm)	Total	104.57 ± 6.84	109.52 ± 10.65	.099
WHR	Total	0.88 ± 0.09	0.87 ± 0.04	.866
MET (h/d)	Total	39.82 ± 4.60	39.24 ± 4.68	.439
Inflammatory factors				
hs-CRP (ng/mL)	Total	1.35 ± 0.9	1.52 ± 1.06	.788
MDA (μM)	Total	1.27 ± 0.37	0.87 ± 0.45	.008

IL-6 (pg/mL)	Total	4.3 ± 0.84	4.25 ± 0.49	.835
TNF-α (ng/mL protein)	Total	5.08 ± 1.37	4.48 ± 1.55	.216
NF-κβ (pg/mg protein)	Total	0.54 ± 0.64	0.52 ± 0.36	.810
*Based on independent <i>t</i> test for continuous data and Chi square test for categorical variables. All trial arms were well matched in respect to baseline characteristics, except for plasma concentration of MDA and TNF-α.				
WC waist circumference, BMI body mass index, WHR waist to hip ratio, MET metabolic equivalent of tasks, hs-CRP high-sensitive C-reactive protein, MDA malondialdehyde, IL-6 interleukin-6, TNF-α tumor necrosis factor-alpha, NF-κβ nuclear factor kappa B.				
^a Mean ± SD (all such values).				

No major side effects were reported following consuming beetroot concentrated in the subjects who completed the study.

Anthropometric parameters and dietary analysis

All anthropometric parameters including weight, BMI, WC, HC, and WHR showed no statistically significant differences between the BJ group and control group at baseline and at the end of the intervention. Moreover, no significant differences were observed between the two groups in terms of physical activity at baseline and at the end of the trial.

Based on the 3-day food recalls obtained throughout the trial, no significant changes were found in total energy, protein, carbohydrate, total fat, saturated fatty acids, monounsaturated fatty acids, polyunsaturated fatty acids, cholesterol, vitamin E, C, β-carotenoid, α-carotenoid, selenium, and fiber between groups at baseline and at 12 weeks. Also, these parameters did not significantly alter within each group during the study (Table 3).

Table 3
Anthropometric and dietary factors in the BJ group and the placebo group.

Factors	Time	BJ group	Control group	P**
Weight (kg)	before	76.81 ± 10.85	78.34 ± 13.2	.699
	after	77.07 ± 11.84	78.02 ± 13.98	.823
	P*	.571	.599	
BMI (kg/m ²)	before	28.28 ± 2.77	30.93 ± 5.4	.067
	after	28.32 ± 30	30.74 ± 5.51	.102
	P*	.835	.384	
WC (cm)	before	92.68 ± 11.79	96.42 ± 11.63	.332
	after	93.13 ± 12.54	100.5 ± 6.53	.486
	P*	.385	.354	
WHR	before	0.88 ± 0.09	0.87 ± 0.04	.866
	after	0.89 ± 0.1	0.87 ± 0.04	.444
	P*	.071	.166	
MET (h/day)	before	39.82 ± 82	39.24 ± 4.68	.198
	after	39.01 ± 5.24	38.88 ± 3.15	.872
	P*	.198	.872	
Energy (kcal/d)	before	1478.49 ± 384.4	1299.3 ± 306.04	.100
	after	1459.33 ± 351.53	1308.62 ± 233.3	.128
	P*	.233	.635	
Carbohydrate (g/d)	before	212.84 ± 61.58	190.85 ± 59.71	.271
	after	211.07 ± 57.74	183.76 ± 61.43	.167
	P*	.867	.448	

Mean ± SD (all such values)

P*: Based on paired t test for within group comparison.

P**: Based on independent samples T Test for between group comparison.

MET, metabolic equivalent of tasks; **BMI**, body mass index; **WC**, waist circumference; **WHR**, waist to hip ratio.

Factors	Time	BJ group	Control group	p**
Protein (g/d)	before	64.24 ± 17.82	62.38 ± 17.47	.746
	after	62.37 ± 20.22	52.55 ± 14.80	.96
	p*	.756	.037	
Fat (g/d)	before	37.70±15.98	31.52 ± 9.26	.153
	after	36.01±13.46	27.37 ± 8.88	.065
	p*	.658	.132	
Cholesterol (mg/d)	before	2.36 ± 0.34	2.16 ± 0.24	.470
	after	2.16 ± 0.30	2.19 ± 0.28	.766
	p*	.264	.677	
MUFA (g/d)	before	12.67 ± 8.32	9.33 ± 3.13	.110
	after	12.18 ± 7.50	9.95 ± 2.46	.125
	p*	.672	.542	
PUFA (g/d)	before	8.93 ± 6.73	5.32 ± 3.63	.057
	after	8.84 ± 5.67	5.18 ± 1.97	.061
	p*	.954	.905	
SFA (g/d)	before	12.95 ± 5.80	12.94 ± 4.45	.993
	after	12.31 ± 7.51	12.19 ± 4.89	.950
	p*	.732	.583	
Fiber (g/d)	before	10.23 ± 3.86	12.43 ± 4.05	0.096
	after	9.76 ± 2.71	9.61 ± 3.84	0.891
	p*	.504	.120	
Vitamin E (mg/d)	before	0.64 ± 0.41	0.64 ± 0.50	.984
Mean ± SD (all such values)				
P*: Based on paired t test for within group comparison.				
P**: Based on independent samples T Test for between group comparison.				
MET , metabolic equivalent of tasks; BMI , body mass index; WC , waist circumference; WHR , waist to hip ratio.				

Factors	Time	BJ group	Control group	p**
	after	0.63 ± 0.39	0.52 ± 0.22	.272
	p*	.948	.343	
Vitamin C (mg/d)	before	1.74 ± 0.51	1.91 ± 0.32	.236
	after	1.58 ± 0.54	1.76 ± 0.47	.296
	p*	.230	.266	
Selenium (mg/d)	before	0.671 ± 0.02	0.06 ± 0.31	.583
	after	0.05 ± 0.02	0.04 ± 0.03	.464
	p*	.183	.204	
β carotenoid (μg/d)	before	1.62 ± 0.78	1.74 ± 0.78	.656
	after	1.65 ± 0.84	1.25 ± 0.57	.120
	p*	.920	.128	
α-carotenoid (μg/d)	before	0.47 ± 0.31	0.38 ± 0.21	.284
	after	0.39 ± 0.44	0.40 ± 0.14	.935
	p*	.233	.635	
Mean ± SD (all such values)				
P*: Based on paired t test for within group comparison.				
P**: Based on independent samples T Test for between group comparison.				
MET , metabolic equivalent of tasks; BMI , body mass index; WC , waist circumference; WHR , waist to hip ratio.				

Oxidative stress and inflammatory factors

As presented in Table. 4, serum MDA levels at the end of the interventional period did not show any significant changes between groups during study ($P = .810$). The result of this comparison was also non-significant after adjusting for the effects of baseline values. BJ consumption could not change statistically the MDA levels after intervention in comparison with baseline ($P = .144$); however, changes in MDA levels were significant in control group ($P = .001$).

Table 4
Oxidative stress and inflammatory markers in the BJ group and the placebo group.

variables	Group	Before	After	Mean changes	P*	P**	P***
hs-CRP (ng/mL)	BJ	1.35 ± 0.9	1.15 ± 0.65	-0.19 ± 0.9	.469	.12	.280
	Control	1.52 ± 1.06	1.75 ± 1.9	0.19 ± 1.35	.836		
IL-6 (pg/mL)	BJ	4.3 ± 0.84	4.2 ± 0.7	-0.1 ± 0.48	.385	.001	.001
	Control	4.25 ± 0.49	5.09 ± 0.56	0.83 ± 0.24	.001		
TNF- α (pg/mL protein)	BJ	5.08 ± 1.37	3.8 ± 1.44	-1.28 ± 2.31	.026	.001	.001
	Control	4.48 ± 1.55	10 ± 2.93	5.51 ± 3.37	.001		
NF- κ B (ng/mg protein)	BJ	0.56 ± 0.57	0.52 ± 0.23	-0.03 ± 0.65	.798	.006	.005
	Control	0.52 ± 0.36	0.89 ± 0.47	0.36 ± 0.62	.021		
MDA (μ M)	BJ	1.27 ± 0.37	1.41 ± 0.33	0.14 ± 0.4	.144	.810	.399
	Control	0.87 ± 0.45	1.39 ± 0.32	0.51 ± 0.47	.001		
Serum NO (μ M)	BJ	26.21 ± 16.42	34.89 ± 17.09	8.57 ± 23.93	.13	.124	.128
	Control	25.42 ± 9.52	27.73 ± 9.7	2.42 ± 15.97	.536		
<i>Note</i> , hs-CRP; high sensitive-C reactive protein, IL-6; Interleukin-6, TNF- α ; Tumor necrosis factor-alpha, NF- κ B; Nuclear factor-kappa B, MDA; Malondialdehyde.							
P* Based on paired t test for within group comparison							
P** Based on independent-samples t test for between group comparison							
P*** Based on ANCOVA model that regressed changes from baseline on treatment group, and baseline value of the outcome							

With regard to serum hs-CRP levels, supplementation with BJ could not have a significant effect compared to control group, both in the pre-adjustment analysis and after adjusting the baseline values ($P = .12$, and $P = .28$, respectively). Also, intra-group comparison with paired t-test disclosed that hs-CRP did not change in both studied groups compared to beginning of the trial.

The level of IL-6, TNF- α , and NF- κ B significantly decreased in the BJ group compared with the control group after intervention. As the results of Table 4 indicate, when we adjusted the analysis for baseline values, above-mentioned findings remained unchanged. Although, the IL-6 and NF- κ B levels reduced in the BJ group in comparison with baseline, but it was not statistically significant. Also, intra-group

comparison indicated a significant reduction in TNF- α in BJ group ($P = .026$). These three inflammatory factors were significantly increased in the control group compared to the beginning of the study. No significant change was observed either in intra-group comparisons or in inter-group comparisons for plasma NO; however, the mean differences increased more in the BJ group compared with the control group after 12 weeks (8.57 ± 23.93 in the BJ group vs. 2.42 ± 15.97 in the control group).

Discussion

Systemic inflammation and oxidative stress play a key role in the pathogenesis of metabolic diseases such as type 2 diabetes mellitus which can aggravate the disease condition and its associated complications (26). So, targeting inflammation pathways could possibly be a component of the new therapeutic strategies to prevent and control metabolic disorders, including T2DM (27). Inflammation in patients with T2DM is caused by elevated levels of glucose, which can accelerate several mechanisms including increased intracellular production of advanced glycation end products (AGEs), formation of mitochondrial reactive oxygen species, activation of nuclear factor κ B (NF- κ B), and activation of protein kinase C (20, 28). Beetroot is a rich source of phytochemical compounds that may provide health-related benefits associated with antioxidant, anti-inflammatory and other physiological effects, particularly for disorders characterized by chronic inflammation (29). This important vegetable contains highly amounts of nutritious and bioactive compounds including macronutrients, micronutrients, ascorbic acid, carotenoids, phenolic compounds, flavonoids, betalains, alkaloids, terpenoids, coumarins, volatile constituents and tannins (30). Betalains are one of the most effective compounds in beetroot that inhibit production of inflammatory mediators, such as cytokines and chemokines, and also phagocyte cells via interfering with the NF- κ B pathway (30). It seems that betalains, in particular betanin may inhibit lipoxygenase enzyme and cyclooxygenase expression by activating PPARs which are involved in the inflammatory response (31). In addition to betalains, beetroot has other excellent antioxidant constituents including ascorbic acid, rutin, epicatechin, caffeic acid, and nitrate which has antioxidant property by scavenging oxidative-inducing molecules (32, 33).

This randomized controlled trial revealed that concentrated BJ consumption can improve some inflammatory markers in patients with diabetes. The significant improvement in three inflammatory markers including serum concentration of IL-6, and levels of TNF- α and NF- κ B in PBMC resulted from 12 weeks of supplementation with BJ. The anti-inflammatory properties of beetroot have been demonstrated in several in vivo studies. Treatments of rats with beetroot extract for 28 days could reduce the activity of IL-6, TNF- α , and NF- κ B (34). Also, it has been reported that BJ supplementation for 28 days had beneficial effect against inflammation through interfering with pro-inflammatory signaling cascades in rats (35). It is interesting to note that the anti-inflammatory effects of betalains were comparable or greater than several phenolic compounds such as cyanidin-3-O-glucoside, lycopene, β -caroten, and even nonsteroidal anti-inflammatory drugs (Ibuprofen, Vioxx and Celebrex) (36). Similarly, in a clinical trial, administration of betalain-rich oral capsules made from beetroot extracts for 10 days decreased the pro-inflammatory cytokines; tumor necrosis factor-alpha (TNF- α) and interleukin-6 (IL-6) in osteoarthritis affected patients, which leads to reduction in pain (37). Similar protective effect was seen in another trial when

hypertensive patients treated with 250 ml raw beet juice for 2 weeks. Inflammatory parameters, hs-CRP and TNF- α , decreased significantly in raw BJ in comparison with cooked beet; while, intracellular adhesion molecule-1, vascular endothelial adhesion molecule-1, IL-6, and E-selectin showed no significant changes between raw BJ and cooked beet groups (16). Despite few studies investigating the effects of BJ on inflammatory factors, our findings are consistent with available research indicating the anti-inflammatory activity of beetroot. Collectively, this clinical trial provides preliminary evidence supporting the anti-inflammatory effects of beetroot by decreasing inflammatory factors except for hs-CRP. One of the reasons for the lack of effectiveness of BJ consumption in comparison with control group could be that the baseline values of hs-CRP were not high enough to make a significant change between groups.

Oxidative stress is recognized as one of the principal mechanisms in the pathogenesis of diabetes mellitus and its complications. Oxidative stress results from reactive oxygen species (ROS) accumulation which leads to imbalance in the normal redox status. Increased production of AGEs, activation of hexosamine pathway and protein kinase C are known to be possible causes of the condition typically referred to as oxidative stress (29, 38). Betalain-containing foods protect cellular components from oxidative agents through scavenging the ROS and show antioxidant activities (29, 30). So, beetroot may be used as a beneficial strategy to augment endogenous antioxidant defence systems, helping to preserve cellular components from oxidative damage (29). In an animal study, beetroot extract has been effective in inhibiting ROS formation, decreasing lipid peroxidation, protein oxidation, DNA damage, and reducing MDA levels (39). Interestingly, another study demonstrated that red beetroot extract increased the activity of antioxidant enzymes such as glutathione S transferase (GST) and NAD(P)H Quinone Dehydrogenase 1 (NQO1) (40). It has been reported that betanin (extracted from beetroot) increased the activity of nuclear factor (erythroid-derived 2)-like 2 (Nrf2), which is responsible for the transcription of several endogenous antioxidant enzymes (41). In spite of previous studies, daily consumption of concentrated BJ to patients with diabetes for 12 weeks had no effect on plasma MDA as an oxidative stress parameter in present research. There are several possible reasons for failure to obtain significant differences in MDA levels including small number of subjects, low baseline levels of MDA in the study population, short duration of intervention, low dosage of concentrated BJ, and also the presence of confounding factors that may prevent the effect of BJ on oxidative stress parameters.

To our knowledge, no studies to date have investigated the effects of concentrated BJ consumption on oxidative stress and inflammatory markers in patients with diabetes to compare with the results of the present study. So, the main strength of this trial was to investigate the effects of concentrated BJ consumption on oxidative stress and inflammatory markers in patients with T2DM for the first time. Moreover, we measured some inflammatory parameters (TNF- α and NF- κ B) in peripheral blood mononuclear cells. The limitation of this trial was that although the number of subjects in each studied group was enough for statistical analysis in this study, but larger study groups would be desirable. Also, this study encountered some difficulties due to a lack of placebo for the control group. On the other hand, our study period was simultaneous with the COVID-19 pandemic which resulted in limitations with the number of participants.

Conclusion

This trial suggests that daily administration of 24 ml concentrated beetroot juice decreases the concentrations of IL-6, TNF- α , and NF- κ B which are involved in the pathogenesis of T2DM and its associated complications. To the best of our knowledge, this study is first to address the effects of concentrated BJ in ameliorating oxidative stress and inflammation in patients with T2DM. Since there are limited studies, more trials are warranted.

Declarations

Ethics approval and consent to participate

The Ethics Committee of Shahid Beheshti University of Medical Sciences has approved the study protocol (IR.SBMU.nnftri.Rec.1399.035). A written informed consent form was signed and dated by subjects and investigators at the beginning of the study (in Persian). Participation was free, and a patient could withdraw, at whatever point the person feels they were unable, to continue. The personal information of participants was kept secret before, during, and after the trial.

Consent for publication

A written consent to publish the information and data of the participants was obtained.

Availability of data and materials

The datasets used during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interest.

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Author's contribution

L K, V B and G S conceptualized and designed the study and wrote the manuscript; L K and G E collected data; L K, and G E provided the study administration works. L K, G S, V B, M H interpreted the data, provided professional comments, and critically revised the manuscript for intellectual content and data accuracy. All authors had access to the study data and reviewed and approved the final manuscript.

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Figures

Figure 1

Study flow diagram