European Journal of Integrative Medicine 18 (2018) 13–17

Contents lists available at ScienceDirect

European Journal of Integrative Medicine

journal homepage: www.elsevier.com/locate/eujim

Clinical trial

Does fenugreek (*Trigonella foenum-graecum*) seed improve inflammation, and oxidative stress in patients with type 2 diabetes mellitus? A parallel group randomized clinical trial

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ARTICLE INFO

Keywords:
Diabetes mellitus
Inflammation mediator
Oxidative stress
Phytotherapy
Randomized controlled trial
Trigonella
Fenugreek

ABSTRACT

Introduction: Fenugreek seed has not previously been investigated for its effect on oxidative stress and inflammation in human subjects. The aim of this study was to examine the effects of fenugreek seeds on selected inflammation and oxidative stress biomarkers in patients with type 2 diabetes mellitus (T2DM).

Methods: Forty-eight T2DM patients were randomly assigned to either an intervention or control group using a parallel group design. The intervention group received 15 g/day fenugreek seed powder. There was no placebo used in this study due to intervention food-based nature. Dietary intake and physical activity were assessed during the study. Blood samples were collected at the baseline and after 8 wk of the intervention to quantify intended variables.

Results: Fenugreek seed consumption resulted in a significant decrease in high-sensitivity C-reactive protein (hs-CRP) ($p = 0.012$) compared to the control group. Within-group analysis showed a significant increase in superoxide dismutase (SOD) activity ($p = 0.001$) among the fenugreek group without any changes in the control group. There was no significant effect of fenugreek seed on glutathione peroxidase (GPX) activity, total antioxidant capacity (TAC), serum interleukin (IL)-6 and tumor necrosis factor (TNF)-α.

Conclusion: The present study indicates some promising properties for fenugreek seed on biomarkers of inflammation and oxidative stress in T2DM patients, but further human research is needed. This trial was registered at www.irct.ir as IRCT2017021232294N2.

1. Introduction

Diabetes mellitus is one of the most prevalent metabolic disorders worldwide which affects 8.5% of the population- 422 million- in 2014. According to World Health Organization (WHO)'s forecasts, diabetes will be the 7th main cause of death in 2030 [1]. It has been reported that 10.3% of the population suffered from diabetes in 2016 in Iran [2]. Type 2 diabetes mellitus (T2DM), which accounts for 90–95% of cases with diabetes [3], results from insulin resistance, which typically develops in conditions of excessive fat mass and accumulation of secondary products of lipid metabolism [4]. Insulin resistance and increased blood glucose level in T2DM cause increased formation of free radical productions and reactive oxygen species (ROS) which trigger peroxidation of lipids, resulting in depletion of antioxidant enzymes [5]. The imbalance between oxidant and antioxidant compounds leads to the activation of stress pathways which activates the stress-induced kinases and induces the expression of pro-inflammatory cytokines in the liver and adipose tissue. Furthermore, increased oxidative stress [6] and acute-phase inflammatory markers [7] actively participate in development of insulin resistance. Inflammation and oxidative stress have a main role in macro- and micro vascular complications of T2DM such as diabetic nephropathy [8], retinopathy [9], and cardiomyopathy [10]. Thus, reducing inflammation and oxidative stress as early predictors of the occurrence of such complications should be considered in

Abbreviations: BMI, body mass index; GPX, glutathione peroxidase; HbA1C, hemoglobin A1C; hs-CRP, high-sensitivity C-reactive protein; IL, interleukin; SOD, superoxide dismutase; TAC, total antioxidant capacity; T2DM, type 2 diabetes mellitus; TNF, tumor necrosis factor; 4-OH-Ile, 4-hydroxyisoleucine

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https://doi.org/10.1016/j.eujim.2018.01.005
Received 4 December 2017; Received in revised form 13 January 2018; Accepted 13 January 2018

therapeutic approaches. Traditional medicines based on botanicals have had a long history of application across cultures [11], and in fact, plant sources have been the origin of almost half of today's modern pharmaceuticals [12]. *Trigonella foenum-graecum*, commonly known as fenugreek, named 'Shanbelileh' in Persian, has been a medicinal plant used as a potential anti-diabetic agent in traditional medicine since antiquity [13]. This plant is a member of Leguminosae family and the main medicinal part of the plant is seeds [14]. The hypoglycemic property of fenugreek has been shown in recent studies. Despite substantial heterogeneity in dose and duration of the interventions, it has been documented that fenugreek seed can decrease plasma glucose and hemoglobin A1C (HbA1c) in T2DM patients [15–19], but its effects on inflammation and oxidative stress are unknown in human subjects. Presence of complex array of important phytochemicals such as diosgenin [20], galactomannan [21] and 4-hydroxyisoleucine (4-OH-Ile) [22] in fenugreek seed, make it a possible agent to improve inflammation and oxidative stress in diabetes, as these effects have been demonstrated in some animal researches [23–25]. Thus, the aim of this study was to examine the effects of fenugreek seeds on some inflammation and oxidative stress biomarkers in patients with T2DM.

2. Subjects and methods

2.1. Study participants

This randomized controlled, parallel-designed clinical trial was conducted in Isfahan, Iran, from March 2017 to June, the same year. On the basis of the sample size formula suggested for randomized clinical trials, considering a type I error of 5% ($\alpha = 0.05$) and type II error of 10% ($\beta = 0.10$, power = 90%) and serum FPG level as the key variable [26], we determined a sample size of 21 persons for each group. A total of 560 clinical records of diabetic patients in Endocrinology and Metabolism Research Center, Isfahan University of Medical Sciences, were screened in order to identify 48 potential patients for the trial who met the inclusion criteria (Table 1). This recruitment number allowed for a predicted 15% loss. The Declaration of Helsinki guidelines were followed in conducting the study. The ethics committee of Isfahan University of Medical Sciences approved the study (code: IR.MUI.REC.1395.3.225) and the informed written consent was obtained from all participants. This study has been registered in IRCT (www.irct.ir) with the registration no. IRCT2017021232294N2.

Table 1
Inclusion-exclusion criteria of the study.

Inclusion criteria	Exclusion criteria
• T2DM (according to the American Diabetes Association (ADA) criteria) • 30–65 y • 1–10 y diabetic history • Body mass index (BMI) < 35 kg/m2	• Consuming fenugreek in any form outside the program protocol • Inadequate compliance to the intervention • Any side effects following the intervention
Lack of: • Insulin therapy • Pregnancy or lactation (women) • Menopause and hysterectomy (women) • Any other certain diseases like liver (e.g. fatty liver), renal, cardiac, hematologic (e.g. Thalassemia and coagulation disorders), thyroid and gastrointestinal diseases or using any certain medications other than anti-diabetic ones • Allergy to Fabaceae family herbs • Using dietary supplements and other medicinal plants for at least 3 months prior to the study • Smoking or using drugs	Any changes in the: • Inclusion criteria • Routine physical activity • Usual dietary intakes • Type and dose of anti-diabetic medications

2.2. Study procedure

The intervention and control groups were stratified for three variables: gender (male/female), age (< 45 and ≥45 y) and Body mass index (BMI) (< 25 and ≥25 kg/m^2) resulting in eight subgroups (tree design matching). The number of people assigned to each subgroup was determined based on an assessment of 200 random clinical records of patients, representative of T2DM patients being referred to the center. Random allocation for each subgroup was performed by the binary random block method held by an independent statistician, and patients allocated to the intervention (n = 24) and control (n = 24) groups. The allocation was concealed from the clinical recruitment staff until each patient had entered the trial and received a randomization code. Individuals in the intervention group consumed 15 g fenugreek seed powder − determined in accordance with the previous similar studies [18,27]- in doses of 5 g three times a day between the main meals, dissolved in the water, in addition to their routine drugs; whilst those in the control group only consumed their routine drugs. The duration of the study was 8 wk. There was no placebo used in this study due to various reasons; basically, this study was a food-based intervention and it was not possible to use a powder that resembles the color and the intense taste and smell of fenugreek seed powder. In addition, the dose of fenugreek seed powder used in this study was 15 g/day which would exceed 20 capsules a day if the investigators tended to use fenugreek seed powder as capsules. Besides, adding the fenugreek seed powder to a food (like bread, yogurt, biscuit and cookie) would have had its own issues as changing the taste and smell of that food (un-blinded study); possible chemical reaction of active components of fenugreek seed powder to that food; and changing the activity or structure of active components of fenugreek seed powder due to the thermal and maintenance processes of that food. So neither participants nor investigators were blinded to the treatment allocation (clinical laboratory personnel was blinded to the intervention groups). Fenugreek seeds were provided from a local herbal drug market, milled and packed in 210 g aluminum-based packages, sufficient for a period of 2-week consumption. Visits were scheduled every 2 weeks to supply next package and evaluate the possible side effects of consumption by questioning. Consumption compliance was assessed at every visit by a checklist supposed to be marked every day after fenugreek seed powder consumption. The chemical composition of fenugreek seed powder per 100 g, analyzed in 'Meyar Danesh Pars' laboratory according to the Association of Analytical Communities methods [28] was; 22.41% Protein, 41.83% Carbohydrate, 1.53% Fat, 24.2% Fiber, 6.85% Moisture, and 3.18% Ash.

2.3. Study variables

Baseline weight and height were measured with standard instructions (Seca, GmbH & Co. KG, Germany) and BMI was calculated as weight (kg) divided by height^2 (m^2). All patients provided 3 dietary records (on 2 weekdays and 1 weekend day) in 2nd, 4th and 6th intervention weeks. Simultaneously, Physical activity levels of patients were assessed by IPAQ (International Physical Activity Questionnaire-Short Form) [29] through those weeks. The average results of these three points were used for analyses. To obtain nutrient intakes of participants, we used Nutritionist-IV software (First Databank, San Bruno, CA, USA) modified for Iranian foods, and physical activity levels were calculated based on MET-min/week. Blood samples (10 mL) were collected at the baseline and after the 8-wk intervention and were centrifuged (D-78532; Hettich GmbH) at 3000 rpm for 10 min to separate serum. Serum samples were then stored at −70 °C before analysis. Serum concentrations of interleukin (IL)-6 and tumor necrosis factor (TNF)-α were measured using ELISA kits (Diaclone SAS, Besancon Cedex, France) with intra- and inter-assay CVs of 4.2% and 7.7%, respectively for IL-6 and of 3.3% and 9.0%, respectively for TNF-α. High sensitivity C-reactive protein (hs-CRP) was quantified using a similar

method (DRG Instruments GmbH, Germany) with intra- and inter-assay CVs of 4.4% and 3.3%, respectively. Serum total antioxidant capacity (TAC), glutathione peroxidase (GPX) activity and superoxide dismutase (SOD) activity were assessed using colorimetric assay kits (ZellBio GmbH, Germany) with intra- and inter-assay CVs < 8%. We performed the laboratory measurements in pairs (before/after intervention) and in the same analytical run to reduce systematic errors.

2.4. Statistical analysis

The analyses were performed on the basis of an intention-to-treat approach. Missing values were treated according to linear regression method. We used the Shapiro-Wilk test and histogram to examine the normal distribution of variables. Log transformation was conducted for non-normally distributed variables, but it could not convert the distributions of GPX, IL6, TNF-α, and hs-CRP to normal. Data on general characteristics and dietary intakes were compared between the 2 groups by Independent Student *t*-test. To determine the effects of fenugreek seed on TAC and SOD activity, we used analysis of covariance (ANCOVA). Final measures were used as dependent variables and two models were run (only adjusted for baseline values and both baseline values and dietary energy intake). Baseline and endpoint values were compared within each group using Paired *t*-test. For non-normally distributed variables, we used non-parametric tests; Mann-Whitney test and 1-sample Wilcoxon were used to compare median differences between groups and within groups respectively. $P < 0.05$ was considered significant. All statistical analyses were conducted by using the SPSS, version 20 (SPSS Inc).

3. Results

Among the 48 participated patients, five individuals (3 from the intervention group and 2 from the control group) dropped out of the study due to the reasons described in Fig. 1, but data from all of them were included in the analysis. General characteristics of patients in each group are shown in Table 2. There was not any significant difference between the two groups for the duration of diabetes and physical activity (Table 2). Mean (SD) age, weight and BMI of participants were 49.63(6.43) y, 79.73(12.95) kg and 29.02(3.61) kg/m^2, respectively.

On the basis of dietary records, we observed that patients in the control group consumed larger amounts of vitamin C in comparison with the fenugreek group (p = 0.033). No significant differences were

Table 2
General characteristics of type 2 diabetes patients who received either fenugreek seed powder in addition to their routine drugs or just their routine drugs.[a]

Variables	Fenugreek group[b](n = 24)	Control group[c] (n = 24)	P value[d]
Age (y)	49.79 (6.00)	49.46 (6.95)	0.86
Male (%)	54.2	54.2	1.00
Weight (kg)	80.50 (14.76)	78.96 (11.11)	0.69
Height (cm)	166.06 (9.11)	164.98 (7.57)	0.66
BMI (kg/m^2)	29.02 (93.59)	29.02 (3.71)	0.99
Duration of diabetes (y)	4.19 (2.48)	5.00 (2.66)	0.32
Physical activity (min/wk)	2897.02 (6667.70)	2159.74 (3641.35)	0.38
Sitting time (min/d)	298.54 (144.48)	258.98 (85.39)	0.49

[a] All values are means (SDs) except for male presented as a percentage. BMI, Body mass index.
[b] Consumed 15 g fenugreek seed powder daily in addition to their routine drugs.
[c] Consumed only their routine drugs.
[d] Calculated by independent *t*-test and chi-square test for quantitative and qualitative variables respectively.

Table 3
Nutrient intakes of type 2 diabetes patients who received either fenugreek seed powder in addition to their routine drugs or their routine drugs alone.[a]

Variables	Fenugreek group[b] (n = 24)	Control group[c] (n = 24)	P value[d]
Energy (kcal/d)	1895.77 (547.47)	1932.15 (567.65)	0.82
Carbohydrate (g/d)	291.62 (98.90)	290.30 (88.27)	0.96
Protein (g/d)	65.19 (18.47)	60.27 (18.62)	0.36
Fat (g/d)	56.80 (21.34)	64.19 (25.82)	0.32
Dietary fiber (g/d)	18.81 (7.29)	18.79 (5.89)	0.99
Selenium (mg/d)	0.078 (0.039)	0.084 (0.036)	0.53
Zinc (mg/d)	7.03 (2.12)	6.92 (2.59)	0.74
Vitamin C (mg/d)	114.07 (61.34)	149.69 (70.77)	0.033
Vitamin E (mg/d)	2.42 (1.11)	2.77 (1.63)	0.55
β-Carotene (μg/d)	474.89 (709.52)	326.57 (280.83)	0.69

[a] All values are means (SDs).
[b] Consumed 15 g fenugreek seed powder daily in addition to their routine drugs.
[c] Consumed only their routine drugs.
[d] Calculated by independent *t*-test.

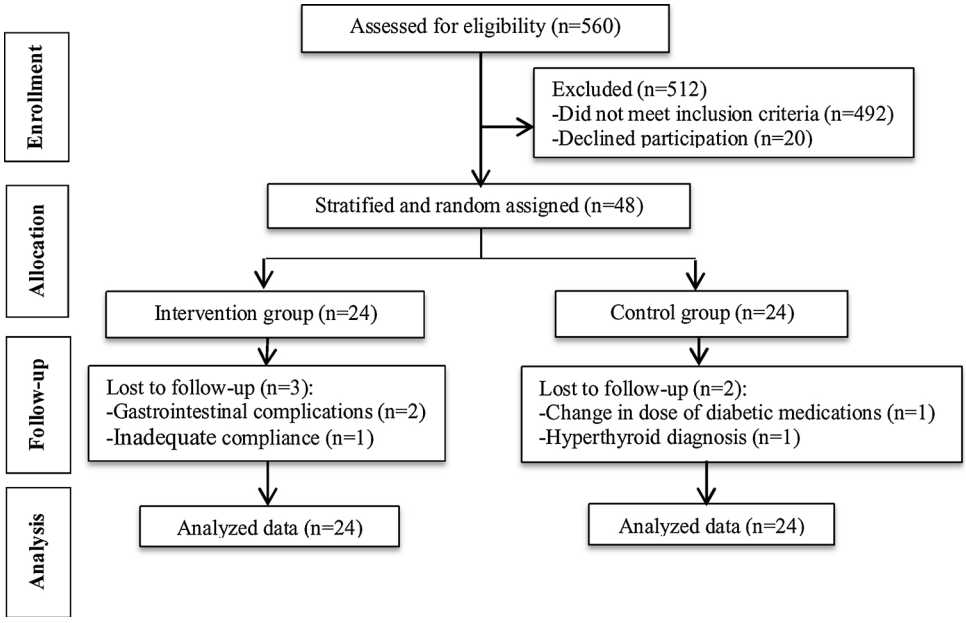

Fig. 1. Participant flow diagram.

Table 4
Superoxide dismutase activity (SOD) and total antioxidant capacity (TAC) at the baseline and following an 8-wk intervention in type 2 diabetes patients who received either fenugreek seed powder in addition to their routine drugs or routine drugs alone.[a]

Variables	Fenugreek group[b](n = 24)		Control group[c] (n = 24)		P value[d]	P value[e]
	Before	After	Before	After		
SOD activity (U/ml)	19.80 (8.64)	31.90 (14.51)	22.95 (8.46)	25.91 (9.36)	0.099	0.10
P value[f]	0.001		0.28			
TAC (mmol/L)	0.55 (0.31)	0.46 (0.09)	0.49 (0.29)	0.46 (0.18)	0.80	0.72
P value	0.19		0.49			

[a] All values are means (SDs). SOD, Superoxide dismutase; TAC, Total antioxidant capacity.
[b] Consumed 15 g fenugreek seed powder daily in addition to their routine drugs.
[c] Consumed only their routine drugs.
[d] Calculated by ANCOVA, adjusted for baseline values.
[e] Calculated by ANCOVA, adjusted for baseline values and dietary energy intakes.
[f] Calculated by paired *t*-test.

seen between the two groups in terms of other nutrients (Table 3). Due to lack of any correlation between dietary vitamin C intake and desired outcomes, vitamin C was not considered as a confounding factor in the statistical analyses.

Within-group analysis showed a significant increase in SOD activity ($p = 0.001$) in the fenugreek group (Table 4). As seen in Table 5, there was a significant difference in change of serum hs-CRP between the two groups ($p = 0.012$). We did not find any significant effect of fenugreek seed consumption on GSH activity, TAC, serum IL6 and TNF-α (Tables 4 and 5). No major side effects were reported consuming fenugreek seed powder except two patients having mild abdominal symptoms (abdominal distention or nausea) desired to leave the study.

4. Discussion

As our results show, fenugreek seed consumption for 8 wk in T2DM patients resulted in a significant decrease in hs-CRP. Endpoint value of SOD activity compared to the baseline was significantly increased within the fenugreek group, while there was no change in this variable for the control group. To the best of our knowledge, this study is the first to examine the effects of fenugreek seed on biomarkers of oxidative stress and inflammatory factors in human subjects. Animal trials investigating the effect of fenugreek seed on oxidative stress have some heterogeneity in the type of fenugreek seeds preparation, type of induced diabetes, and type of tissues for markers assessment. Some of these studies have shown that fenugreek seeds can reduce oxidative stress markers and increase antioxidant status [24,30]. In the study of Haghani et al., fenugreek seeds enhanced SOD and CAT activity but could not change GPX activity [31]. Some studies have not shown any improvement in oxidative stress markers or antioxidant enzymes activity following fenugreek seed intervention [23]. Although a decrease in plasma oxidative stress markers and an increase in plasma antioxidant molecules level were seen after fenugreek seed consumption in the study of Pradeep et al., but plasma antioxidant enzymes activity seemed to decrease; These results were different in other tissues in the same study [25]. So the results of diabetic animal studies on the effect of fenugreek seeds on oxidative stress seem to be disparate. In the single human study on healthy overweight subjects with normal baseline oxidative/antioxidant profiles, no effect of fenugreek seed on oxidative stress was reported [32]. But as it is clear, antioxidant effect of an intervention is better shown in oxidative conditions like diabetes and obesity. Anti-inflammatory effects of fenugreek seeds have been documented only in limited animal studies. Joshi et al., showed that treatment with hydro-alcoholic extract of fenugreek seeds can reduce serum IL6 and TNF-α, dose-dependently in an alloxan-induced type-II diabetic rat model [24]. Retinae showed marked inhibition in the expression of inflammatory biomarkers (TNF-α and IL1β) following hydro-alcoholic extract of fenugreek seeds intervention in the study of Gupta et al., on streptozotocin-induced diabetic rats [30].

The possible beneficial effects of fenugreek seeds on oxidative stress and inflammation might be explained by three main bioactive compounds. Diosgenin −a steroidal saponin- can restore antioxidant status and reduce lipid peroxidation levels [33] and inhibit macrophage-derived inflammatory mediators [34]. 4-OH-Ile −a novel branched-chain amino acid in fenugreek- present strong inhibition on reactive oxygen species (ROS) production and related inflammation [22]. Fiber from fenugreek has also been demonstrated to decrease serum LDL oxidation and homocysteine and increases glutathione and α-tocopherol [35]. Moreover, Galactomannan may suppress inflammation and oxidative stress by down-regulating catabolic pathway of histidine to urocanic acid and also by reducing the levels of the metabolites of glycerophospholipid [21].

In the current study, we tried to regard and control most possible confounders, as we set strict inclusion-exclusion criteria, stratified the two interventional groups for three variables (age, sex, and BMI) and

Table 5
Inflammatory factors and glutathione peroxidase (GPX) activity at the baseline and following an 8-wk intervention in type 2 diabetes patients who received either fenugreek seed powder in addition to their routine drugs or just their routine drugs.[a]

Variables	Fenugreek group[b](n = 24)			Control group[c](n = 24)			P value[d]
	Before	After	change	Before	After	Change	
IL-6 (pg/ml)	1.16 (0.28–3.70)	0.76 (0.11–13.50)	−0.62 (−5.6 to 0.66)	1.35 (0.45–27.10)	0.76 (0.25–3.40)	−0.59 (−7.1 to 0.5)	0.69
P value[e]	0.004			0.000			
TNF-α (pg/ml)	5 (2.30–46.90)	2.76 (0.40–10.60)	−3.43 (−38.2 to −0.8)	5 (1.30–279.60)	2.30 (0.53–250.80)	−3.12 (−252.4 to 86.5)	0.84
P value	0.000			0.000			
hs-CRP (mg/L)	0.025 (0.001–0.190)	0.020 (0.001–0.150)	−0.010 (−0.180 to 0.019)	0.025 (0.001–0.150)	0.030 (0.004–0.130)	0.002 (−0.040 to 0.120)	0.012
P value	0.011			0.55			
GPX activity (U/ml)	320.60 (18.75–843.75)	372.15 (112.50–731.20)	36.18 (−375.00 to 516.15)	308.50 (37.50–881.25)	341.85 (37.50–879.00)	80.38 (−398.75 to 447.75)	0.47
P value	0.44			0.18			

[a] All values are medians (mins-maxes). IL-6, Interleukin-6; TNF-α, Tumor necrosis factor; hs-CRP, high-sensitivity C-reactive protein; GPX, Glutathione peroxidase.
[b] Consumed 15 g fenugreek seed powder daily in addition to their routine drugs.
[c] Consumed only their routine drugs.
[d] Calculated by Mann-Whitney test, comparison of change values between the two groups.
[e] Calculated by 1-sample Wilcoxon test.

evaluated physical activity and dietary intake during the study. Given the fact that other oxidative stress and inflammatory conditions like obesity [36], the duration of diabetes [37], menopause [38], most of the non-communicable diseases [39], smoking or using drugs [40] and dietary antioxidants can interrupt the results, we considered them in this study. A limitation of this study might be the short duration of intervention and the small sample size of the study. Additionally, there was no specific biomarker evaluating fenugreek seed consumption and the compliance was assessed using a self-reported method.

5. Conclusion

Overall, the conclusion of the present study indicates some promising properties for fenugreek seed on biomarkers of inflammation and oxidative stress in T2DM patients, but further human research are needed to achieve more decisive results.

Conflict of interest

None of the authors have any financial/commercial conflicts of interest to declare.

Acknowledgements

The financial support for design, data collection, analysis, and development of the manuscript came from the School of Nutrition & Food Science, Isfahan University of Medical Sciences, Isfahan, Iran. The authors are grateful to the staff of Endocrine & Metabolism Research Center, Isfahan University of Medical Sciences, Isfahan, Iran for their assistance in this project and to all study subjects for their participation in this study.

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