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Research Article

Antioxidant, Antidiabetic and Antihyperlipidemic Effects of *Trigonella foenum-graecum* Seeds

[1]Sarra Khlifi, [1]Houda Ben Jemaa, [1]Halima Ben Hmad, [2]Hajer Abaza, [1]Inchirah Karmous, [3]Abdelmajid Abid, [1]Anis Benzarti, [4]Jalila Elati and [1]Abdallah Aouidet

[1]High School of Sciences and Technology of Health, Tunis, Tunisia
[2]Laboratory of Clinic Biochemistry, Institute of Salah Azaiez, Tunis, Tunisia
[3]Institute of Nutrition and Alimentary Technology, Tunis, Tunisia
[4]Laboratory SURVEN, National Institute of Nutrition and Food Technology of Tunis, 176 Bab Souika, BP1006, Tunis, Tunisia

Abstract

Fenugreek (*Trigonella foenum-graecum*) seeds have been known as a traditional plant treatment for diabetes. The aims of this study were to determine the antioxidant activity of fenugreek seeds and to evaluate the antidiabetic and antihyperlipidemic effect of fenugreek seeds intake on diabetic patients. Sixty newly diagnosed diabetic patients were recruited for this study and have received two doses of fenugreek seeds (2.5 and 5 g) for 4 weeks. Phytochemicals analysis and antioxidant activity of *Trigonella foenum-graecum* seeds methanolic extract were also studied. The chemical analysis of fenugreek seeds exhibited a high level in total polyphenols (9.5 GAE g/g VM) and flavonoids (3.7 mg RE/g VM). Fenugreek seeds exertan important DPPH scavenging ability in terms of Trolox Equivalents (TE) with 1.03±0.008 mg TE/g VM and also exhibited a high antioxidant potential according to ABTS test. In Tunisian type 2 diabetic patients, the administration of fenugreek seeds (2.5 and 5 g) for 4 weeks improved blood glucose level in dose-dependent. Total cholesterol and triglycerides rate were significantly decreased by the dose of 5 g *Trigonella foenum-graecum* seeds. Moreover, daily oral intake of our treatment by diabetic subjects reduced significantly serum α-amylase activity. The present findings indicate that fenugreek seeds with abundant flavonoids and total polyphenols content may be a good adjuvant for the treatment of diabetes.

Key words: Hyperglycemia, lipid profile, triglycerides, fenugreek, blood glucose levels

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Corresponding Author: Sarra Khlifi, High School of Sciences and Technology of Health, Tunis, Tunisia Tel: 216-71578634 Fax: 216-71564481

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Diabetes is a serious disease growing at alarming rates around the world. It is reported to constitute the 6th leading cause of global mortality (Roglic and Unwin, 2010). Bouguerra *et al*. (2007) demonstrated that type 2 diabetes prevalence has more than doubled over a 15 years period in Tunisia. It is generally recognized that patients with diabetes are at risk for many severe complications, including diabetic retinopathy and cardiovascular diseases (Huxley *et al*., 2006).

Moreover, drugs therapies compensate partially metabolic dysfunctions in diabetes and do not correct the fundamental biochemical damage (Taylor and Agius, 1988). Natural products have been a source of medicinal treatments for several years ago and plant-based systems continue to play an vital role in the primary health care of approximately 80% of the world's undergoing development and developing countries (Kumar *et al*., 2014).

Therefore, it is prudent in this context to search new and interesting bimolecular compounds. Fenugreek (*Trigonella foenum-graecum*) leaves and yellow colored seeds were used as a spice as well as medicinal herb. In fact, fenugreek could be used for the treatment of metabolic disorders, respiratory and digestive problems (Bailey and Day, 1989).

Now a days, *Trigonella foenum-graecum* is still valued for its ability to ease digestive problems, but its intake could also be effective in managing type 2 diabetes. Experimental studies concluded that fenugreek not only helped to oppose the development of induced diabetes, it also had an "Anti-diabetic" effect in mice with established diabetes (Basch *et al*., 2003; Singh *et al*., 2010).

An ethnobotanical survey was conducted in Tunisia on the use of hypoglycemic plants by diabetic patients has shown that the fenugreek is the second plant used by diabetics with an utilization percentage of 28.3% (Ben Othman *et al*., 2013).

The numbers of studies which evaluate the fenugreek effects in glucose level and lipid profile of diabetic patients were limited. In this study, the fenugreek seeds anti-diabetic propriety was evaluated in Tunisian patients newly diagnosed with type 2 diabetes. Blood glucose level, lipid profile and α-amylase activity in the serum of diabetic subjects were evaluated.

MATERIALS AND METHODS

Biological material: Yellow colored fenugreek seeds collected from Tunisia center in March, 2014 were used in this study. Fenugreek seeds were dried in the open air for two weeks and then crushed in a mortar with liquid nitrogen to obtain powder product. For each plant sample, 2 g powder was mixed with 20 mL of methanol (20%). After maceration for 24 h, the mixture was filtered. The recovered filtrate was then stored in glass bottles in the dark place at 4°C until use for the phenolic compounds assay and the evaluation of their antioxidant activities.

Patients: Sixty newly diagnosed type 2 diabetic patients were recruited from the Institute of Nutrition and Alimentary Technology (diabetes age is between some months and 5 years). The patients were treated by oral anti-diabetic- drugs (OAD) and followed by a clinical endocrinologist. Concerning the diet, participants are asked to keep their same spontaneous diet. The age of patients was between 40-65 years and sex ratio was 1. Twenty patients were considered as controls. These patients were matched with treated patients in the age and sex ratio.

Twenty others patients were considered as the first treated group with 2.5 g per day of fenugreek seeds (FS treated 1). The second treated group (FS treated 2) was served by 5 g of fenugreek seeds daily. Treatment duration was 4 weeks. Informed consent was signed by all patients for being included in this study.

Venous blood samples following 10–12 h of fasting were collected in heparinized tubes from each patient at the start and at the end of the study (after 4 weeks). Plasma was separated from the blood cells by centrifuging the blood at 1500 g for 15 min at 4°C and stored in aliquots at -80°C until analysis.

Biochemical analysis

Total phenolic determination: Folin-Ciocalteu reagent was used for the colorimetric measure of total phenolic content. Briefly, 0.5 mL of distilled water and 0.125 mL of Folin-Ciocalteu reagent were added to 0.125 mL of diluted sample extract. The mixture was shaken and allowed to stand for 6 min, before addition of 1.25 mL of 7% Na_2CO_3. The solution was then adjusted with distilled water to a final volume of 3 mL and mixed thoroughly. After incubation in dark, the absorbance at 760 nm was read versus the prepared blank. Total phenolic content of plant parts was expressed as milligrams of gallic acid equivalents per gram of dry weight of extract (mg DW) through the calibration curve with gallic acid (Singleton and Rossi Jr, 1965).

Total flavonoids content: The amount of flavonoids was estimated by the colorimetric method using an aluminum chloride ($AlCl_3$). The assay is based on the formation of a

colored complex (yellow) between the flavonoids and the aluminum chloride which absorbs at a wavelength of 415 nm. The yellow color intensity is proportional to the concentration of flavonoids in extracts.

For this assay, 500 µL of the extract to 500 µL of $AlCl_3$ (2% in methanol) are mixed and then incubated in the dark for 15 min at room temperature. Absorbance is measured at 415 nm. Flavonoids content is expressed in rutin equivalent per g of vegetal material (mg RE/g VM) from a standard range prepared with varying concentrations of rutin from 100 µg mL^{-1} to 6.5 mg mL^{-1} (Chang *et al*., 2002).

DPPH assay: Antiradical activity was evaluated by measuring the scavenging activity of fenugreek methanolic extract on the 2,2-diphenyl-l-1-picrylhydrazil (DPPH) radical, using the method described by Brand-Williams *et al*. (1995). Ascorbic acid and quercitine were used as the standard in solutions ranging from 1 mg mL^{-1}. About 0.004% DPPH in methanol was prepared. After, 500 µL of this solution was assorted with standard solution and 500 µL of example solution to be analyzed separately. These solution mixtures were kept in the dark for 30 min and optical density was calculated at 517 nm using spectrophotometer. The blank was used as 500 µL of methanol with 500 µL of DPPH solution (0.004%). The optical density was recorded and percentage of inhibition (PI) was calculated as follows:

$$PI = \frac{A0 - At}{A0} \times 100$$

where, A0 is optical density of the blank and At is optical density in the presence of methanolic extract. The result is expressed in the IC_{50} (µg mL^{-1}) is the PI correspond to 50% (Brand-Williams *et al*., 1995).

ABTS assay: The free radical scavenging activity of FS methanolic extract was given by the method of ABTS (2.20 azinobis-3-ethylbenzthiazoline-6-sulphonic acid) radical cation decolorization assay described by Re *et al*. (1999) with minor modifications. The ABTS was dissolved in water to a 7 mM concentration. The ABTS radical cation (ABTS*+) was produced by reacting ABTS solution 2.45 mM potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12-16 h before use. The ABTS*+ solution was diluted with methanol to an absorbance at 734 nm and equilibrated at 23±2°C. Reagent blank reading was taken (A0). After addition of 1 mL of diluted ABTS*+ solution of FS extract, the absorbance was read 7 min after the first mixing (At). The free radical scavenging capacity offenugreek extract samples, calculated as percentage inhibition of ABTS*+ was evaluated against a Trolox standard curve, the activity was expressed in terms of µmol Trolox equivalent/gram vegetal material (Re *et al*., 1999).

Iron chelating power: It is a colorimetric test based on the chelating of the ferrous ion Fe^{2+} by ferrozine. An intense magenta color appears when the complex is formed. The complex formed with Fe^{2+} absorbs at 562 nm. Thus the capacity of the complex fading (iron/ferrozine) reflects the iron chelating power of FS methanolic extract (Sharma *et al*., 2015).

Chemicals: All the biochemicals and chemicals were purchased from Sigma Chemical Aldrich Germany.

Serum indices analysis: Glucose (G), Total Cholesterol (TC), triglycerides (TG) and HDL levels were determined by SYNCHRON LX® System(s), UniCel® DxC 600/800 System(s) and also Synchron® Systems Multi Calibrator.

Blood alpha-amylase activity was determined by using an automatic analyser (Cobas Mira) and assay kits obtained from Roche.

Statistical analysis: All phytochemicals assays were performed in triplicate. The results were expressed as Mean±SD (standard deviation).

Statistical analysis were realized with SAS software (Version 9.2, Cary, NC, USA). Duncan's multiple range test was used for comparison of dependent variables. The difference is considered significant for $p \leq 0.05$. The association between the different parameters was realized with Pearson correlation.

RESULTS

Phytochemical analysis and antioxidant activity: The FS methanolic extract showed an important phenolic and flavonoids levels, respectively (9.58±0.12 mg GAE/g VM and 3.70±0.02 mg RE/g VM) as summarized in Table 1. The results obtained showed that for the methanol fenugreek extract exhibited an interesting antioxidant power (1.23±0.003 mg TE/g VM), antiradical activity (1.03±0.008 mg ET/g) and an important iron chelating power (Table 2).

Effect of fenugreek seeds on bloodglucose level: Table 3 indicates that the mean glycemia level was not significantly lower in the group treated with the low dose of the FS compared to the control group (8.83 vs 8.24, p>0.05). However, the medium dose (5 g) of FS reduces significantly the glycemia (8.83 vs 6.45, p<0.05).

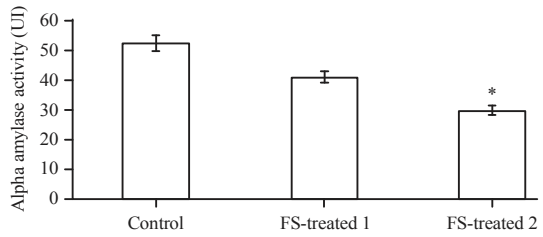

Fig. 1: Effect of fenugreek treatment on alpha amylase activity, Control: Controls group, FS-treated 1: Treated group with 2.5 g of fenugreek seeds, FS-treated 2: Treated group with 5 g of fenugreek seeds, *The difference was statistically significant for p<0.05 (FS-treated 2 vs control group)

Table 1: Total polyphenols contents and flavonoids levels in fenugreek seeds

	Fenugreek seeds levels
Total polyphenols	9.58±0.12 mg GAE/g VM
Flavonoids	3.70±0.02 mg RE/g VM

Data are expressed as Means±SD, GAE: Gallic acid equivalents, VM: Vegetal material and RE: Rutin equivalents

Table 2: Antiradical activity, iron chelating power and antioxidant power with ABTS method of fenugreek seeds extracts

	Fenugreek seeds
DPPH (mg ET/g)	1.03±0.008
Iron chelating power (mg EDTA E/g VM)	0.28±0.01
ABTS mg TE/g VM	1.23±0.003

Data are expressed as Means±SD, EDTAE: EDTA equivalents, TE: Trolox equivalents and VM: Vegetal material

Effect of fenugreek seeds on alpha-amylase activity: Fenugreek seeds intake by type 2 diabetic subjects significantly decreased the serum α-amylase activity for the medium dose (Fig. 1).

Effect of fenugreek seeds on lipid concentration: Table 3 shows that total cholesterol and triglycerides concentrations in plasma were significantly reduced only by the medium FS dose in the diabetic patients. The HDL concentration was also improved after the administration of 5 g of FS.

Correlation study: A negative correlation (r = -0.98, p<0.001) was noted between alpha amylase activity and FS dose, phenolic and flavonids levels, antioxidant power, iron chelating power and antiradical activity (Table 4).

DISCUSSION

Trigonella foenum-graecum was traditionally used as medicinal plant. Many experimental studies have reported that the fenugreek seeds have antidiabetic and antihyperlipidemic effects (Al-Habori *et al*., 1998; Basch *et al*., 2003). Clinical studies on FS effects were restricted.

The first objective of this work was to evaluate the phytochemical composition of the FS. In the present study, the FS methanolic extract showed an important total phenolic and flavonoids contents (9.58±0.12 mg EAG/g VM and 3.70±0.02 RE/g VM).

Many studies have reported that the FS had shown that the fenugreek seeds are rich in total phenolic and flavonoids contents (Jayawardena *et al*., 2015; Kaviarasan *et al*., 2007).

The obtained results demonstrated that the FS showed an important ability to neutralize the free radicals such as DPPH radicals with 1.03±0.008 mg T E/g V M. According to the ABTS test 1.23±0.003 mg TE/g VM.

Table 3: Effect of fenugreek treatment on biological parameters

Biological parameters (mmol L^{-1})	Glucose levels	Cholesterol	Triglycerides	HDL cholesterol
1	8.83±0.57^{a}	4.99±0.41^{a}	2.07±0.12^{a}	0.51±0.02^{b}
2	8.24±0.45^{a}	4.57±0.32^{a}	1.88±0.27^{a}	1.02±0.17^{a}
3	6.45±0.08^{b}	2.35±0.11^{b}	1.21±0.21^{b}	1.04±0.12^{a}

Data are expressed as Means±SD, ^{a,b}In the same column, the values followed by different letters are statistically different for p<0.05, 1: Controls group, 2: Treated group with 2.5 g of fenugreek seeds and 3: Treated group with 5 g of fenugreek seeds

Table 4: Correlation between biological parameters, FS phytochemicals composition and antioxidant power

	G	TC	TG	HDLC	AA
Dose	r = 0.18, p = 0.165	r = 0.14, p = 0.277	r = -0.16, p = 0.204	r = -0.03, p = 0.781	r = -0.98, p<0.0001*
PTP	r = 0.18, p = 0.165	r = -0.14, p = 0.277	r = -0.16, p = 0.204	r = -0.03, p = 0.781	r = -0.98, p<0.0001*
FTF	r = 0.18, p = 0.165	r = -0.14, p = 0.277	r = -0.16, p = 0.204	r = -0.03, p = 0.781	r = -0.98, p<0.0001*
AARA	r = 0.18, p = 0.163	r = -0.14, p = 0.281	r = -0.16, p = 0.207	r = 0.03, p = 0.787	r = -0.98, p<0.0001*
IICP	r = 0.18, p = 0.165	r = -0.14, p = 0.277	r = -0.16, p = 0.204	r = -0.03, p = 0.781	r = -0.98, p<0.0001*
AAP	r = 0.18, p = 0.163	r = -0.14, p = 0.280	r = 0.16, p = 0.206	r = -0.03, p = 0.786	r = -0.98, p<0.0001*

Dose: Fenugreek seeds dose, TP: Total polyphenols, TF: Total flavonoides, ARA: Antiradical activity, ICP: Iron chelating power, AP: Antioxidant power, G: Glycemia, TC : Total cholesterol, TG: Triglcerides, HDLC: HDL cholesterol, AA: Alpha amylase activity and *Statistically significant differences (p<0.05)

Kaviarasan *et al*. (2007) demonstrated that FS extract acted as a scavenger of DPPH and ABTS radicals with respectively an IC_{50} value of 0.350 and 0.117 mg mL^{-1}. The iron chelating power of our FS methanolic extract was 0.28±0.01 mg EDTA/g VM.

The results obtained from other studies demonstrate that the extracts of *Trigonella foenum-graecum* had an effective capacity for iron binding (Subhashini *et al*., 2011).

The second purpose of this study was to evaluate the beneficial effect of FS administration on diabetic patients. The dose of fenugreek seeds was established based on previous trials which have demonstrated its safety, the dose of fenugreek seeds was equated to the therapeutic dose suggested for humans and has been exposed to safety evaluation (Rao *et al*., 1996).

Previous randomized clinical trial demonstrated that fenugreek seeds improved significantly glucose metabolism at two different doses (5 and 10 g) (Rafraf *et al*., 2014).

The innovation in this study was to evaluate other lower dose (2.5 g) besides the dose of 5 g and to determine the effect of FS intake by type 2 diabetic subjects on lipid profile and serum α-amylase activity.

These results demonstrated that treatment with 2.5 g of FS reduces glycemia but without any significant difference. When administrated at a medium dose (5 g) our data confirm that glucose levels were significantly decreased.

A meta-analysis of clinical trials on the effect of fenugreek (*Trigonella foenum-graecum* L.) intake in diabetic patient had reported that fenugreek significantly changed fasting blood glucose by -0.96 mmol L^{-1} , 2 h postload glucose was also decreased significantly by (-2.19) mmol L^{-1} and HbA1c was reduced significantly by -0.85 compared to untreated subjects (Neelakantan *et al*., 2014).

Total fenugreek raw seeds, extracted seeds powder, cooked seeds (25 g) and gum isolate of seeds (5 g) reduced postprandial glucose levels. These findings suggest that the gum fraction could be responsible of acute effects of FS without the exclusion of longer term effect of other fenugreek components on glycemia (Neelakantan *et al*., 2014).

The mechanisms by which fenugreek may reduce blood glucose levels have not been well explained in humans. Animal studies also denoted that FS soluble fiber and saponins fractions diminished the absorption of glucose from the gastrointestinal tract by reducing the rate of enzymatic digestion such as α-amylase and α-glycosidase (Hamden *et al*., 2011).

Present study revealed that the fenugreek seeds exhibit its antihyperglycemic effect by stimulating glucose-dependent insulin secretion from pancreatic β-cells. It inhibits the activities of α-amylase and sucrose. *Trigonella foenum-graecum* could successfully inhibit the diabetic effect on GLUT4 transports and returns them to normal levels. The saponins present in the *Trigonella foenum-graecum* seeds and also saponins compounds like diasgenin, alkaloids and trigonelline inhibit intestinal glucose uptake *in vitro* (Rani *et al*., 2014).

In diabetic rats, the ingestion of trigonelline, a major alkaloid component of fenugreek, enhanced insulin sensitivity and reduceddigestives enzymes activities, consequently decrease blood glucose levels (Hamden *et al*., 2013). In a test of acute effects in healthy subjects, trigonelline decreased the early glucose response during an OGTT (Basch *et al*., 2003).

In addition, a novel amino acid derivative extracted from fenugreek seeds, 4-hydroxyisoleucine is found exclusively in plants and has been extracted and purified from FS. This amino acid stimulates glucose-dependent insulin secretion and ameliorates glucose tolerance (Jette *et al*., 2009; Singh *et al*., 2010). Vanadium is a trace metal which possesses anti-diabetic effects, especially when used in combination with fenugreek or insulin. When used together, fenugreek significantly decreases the toxicity of vanadium (Baquer *et al*., 2011). Treatment with insulin, vanadate and fenugreek corrected the altered levels of antioxidant enzymes. This treatment also decreased the rate of gastric emptying and slowed carbohydrate absorption. Additionally, treatment decreased blood glucose levels and partially restored the activities of key enzymes involved in carbohydrate and lipid metabolism to near normal levels (Neelakantan *et al*., 2014).

Moreover, *Trigonella foenum-graecum* reduced insulin, insulin resistance and fasting plasma glucose (DeFronzo, 1999).

In addition, this study showed that 5 g FS intake by type 2 diabetic patients resulted in a significant reduction in cholesterol levels and TG. Previous work studied human subjects who ingested 100 g of defatted fenugreek powder per day for three weeks and unregistered that their triglycerides (TG) and LDL-C levels were lower than baseline values (Sharma *et al*., 1990).

Trigonella foenum-graecum reduced significantly total cholesterol while, increasing good cholesterol High Density Lipoprotein (HDL) (DeFronzo, 1999).

The FS beneficial effect was confirmed in humans by several trials, which demonstrate regulator effects in cholesterol levels in association with oral fenugreek intake (Basch *et al*., 2003).

Kassaian *et al*. (2009) studied the effect of FS on lipid profiles in type 2 diabetic patients showed that this treatment reduces serum triglycerides, total cholesterol and LDL cholesterol (Kassaian *et al*., 2009). In fact, it has reported

that FS intake by diabetic rats decreased lipase activity in the intestine and improved lipid absorption from the intestine consequently decrease the hypercholesterolemia and hyperlipidemia (Hamden *et al*., 2010).

These findings proved that fenugreek seeds intake by type 2 diabetic subjects significantly decreased the serum α-amylase activity.

Many natural α-amylase inhibitors have been reported to decrease plasma glucose through delaying glucose absorption and retarding the release of glucose from dietary complex carbohydrates (Sudha *et al*., 2011).

Hamden *et al*. (2011) proved that administration of fenugreek terpenes with omega-3 in diabetic rats significantly inhibited capital enzymes related to diabetes such as α-amylase activity by 46 and 52% and maltase activity by 37 and 35%, respectively in plasma.

CONCLUSION

In our study, the intake of 5 g of fenugreek seeds for 4 weeks has been very interesting for newly diagnosed type 2 diabetes mellitus to correct lipid disorders and glucose levels. These effects may be due to the abundance of FS on bioactive molecules. Therefore, the use of medicinal plants besides the drugs treatment and a correct alimentary diet could be efficient in the prevention of diabetes complications especially cardiovascular diseases.

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