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Assessment of the antidiabetic, antihyperlipidemic and antioxidant properties of *Trigonella foenum-graecum* Linnaeus, 1753 (Fenugreek) in alloxan-induced diabetic rats

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Abstract - Fenugreek (*Trigonella foenum-graecum* Linnaeus, 1753) is used in alternative medicine for treating diabetes mellitus and its associated symptoms. The aim of this study was to investigate the antidiabetic, antihyperlipidemic and antioxidant activities of fenugreek in alloxan induced diabetic rats. Diabetes was confirmed after 3 days of single subcutaneous injection of alloxan (120 mg/kg) in albino Wistar rats. Animal's standard diet was supplemented with fenugreek (5%) for 30 days. Rats were fasted overnight, weighed and then sacrificed on the 31st day of the experiment. Their bloods were collected and submitted to various biochemical measurements, including blood glucose, total cholesterol (TC), triglycerides (TG) and high-density lipoprotein-cholesterol (HDL-CH). Further, low-density lipoprotein-cholesterol (LDL-CH) was calculated from the data obtained. Oxidative enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) were assessed in serum and pancreas homogenate. The results showed that fenugreek supplementation in diabetic rats significantly decreased the levels of glucose (342.2 ± 23.9 mg/dl vs 171.0 ± 18.7 mg/dl; $p< 0.05$), TG (0.87±0.17g/l vs 0.49±0.09; $p< 0.05$), TC (0.95±0.11g/l vs 0.75±0.17; $p<0.05$) and LDL-CH (0.79±0.5g/l vs 0.53±0.15g/l; $p<0.05$) compared to diabetic control group. Furthermore, fenugreek increased the level of HDL-CH from 0.11±0.01g/l to 0.14±0.04g/l ($p<0.05$). These results are accompanied with improved weight gain of diabetic rats compared with the diabetic controls. Concerning oxidative stress, fenugreek significantly improved SOD, CAT and GPx activities both in serum and in pancreas homogenate. Fenugreek demonstrated significant antidiabetic, antihyperlipidemic, and antioxidant activities that may be due to its multiple effects involving both pancreatic and extra-pancreatic mechanisms. Fenugreek has the potential to be used as a dietary supplement for the management of diabetes.

Keywords: antihyperglycemic, antihyperlipidemic, alloxan, antioxidant enzymes, fenugreek

1. Introduction

Diabetes mellitus is a common disease and its prevalence is increasing worldwide. The World Health Organization estimates a prevalence of 347 million people with diabetes worldwide in 2013 (WHO, 2013). This prevalence is expected to double between 2005–2030 (Wild et al. 2004). Diabetes is characterized by hyperglycemia and disturbances in carbohydrate, protein, and lipid metabolism. Chronic hyperglycemia that occurs in diabetes is associated with long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels (American Diabetes Association, 2013). Hyperglycemia may perturb cellular antioxidant defense systems and damage cells. Free radicals are formed disproportionately in diabetes by glucose oxidation, nonenzymatic glycation of proteins, and the subsequent oxidative degradation of glycated proteins. Abnormally high levels of free radicals and the simultaneous decline of antioxidant defense mechanisms can lead to damage of cellular organelles and enzymes, increased lipid peroxidation, and development of insulin resistance (Henriksen et al. 2011). Diabetes is also associated with profound alterations in the plasma lipid and lipoprotein profile and an increased risk of premature atherosclerosis and cardiac failure (Betteridge et al. 1997). Clearly, hyperlipidemia (high in TC, TG, LDL-CH) and in vivo oxidation stress in diabetic patients deteriorate diabetic interrelated

complications. However, compounds that show antioxidant and anti-inflammatory potential may prevent pancreatic islet insulin-producing β cells from selective destruction (Jaidane et al. 2009), inhibit islet apoptosis, delay the occurrence of type 1 Diabetes (Hara et al. 2006) and alleviate diabetic interrelated complications.

Due to numerous injuries and occasionally fatal disease caused in diabetic patients, there is a need to find the treatment methods and ways to reduce and prevent it. Presently, with regard to the problems of preparing and injecting insulin and other glucose-lowering drugs and also considering the side effects of synthetic drugs, the use of herbal medicines has attracted the attention of researchers. Fenugreek (*Trigonella foenum-graecum Linnaeus*, 1753) is used as a condiment, as a supplement to ''bsissa'', a local diet preparation largely consumed in Tunisia and as a constituent of the daily diet in several African countries.

Since non processed herbs are usually more tolerated than extracted or formulated products and patients prefer to consume plants as salad, spice, and so forth, in this work, we aimed to investigate the effect of fenugreek powder supplemented to rat diet on glycemic, lipidemic and antioxidant status of diabetic rats.

2. Materials and methods

2.1. Plant material

Fenugreek seed used in the present study was purchased from the local herbal market and identified by Professor Abderrazek Smaoui at Laboratory of Medicinal and Aromatic Plants. Fenugreek seeds were cleaned, dried, finely powdered in a grinding machine. In the present study, powdered fenugreek seeds were mixed with standard diet (5%).

2.2. Animals

Thirty-two male Wistar albino rats weighing 200-230 g and averaging 12 weeks old were obtained from Pasteur Institute of Tunisia and used in this study. They were housed in polypropylene rat cages in an animal room with controlled lighting (12 h light/12 h darkness) and temperature (22 ± 2∘C). The animals were given standard pellets diet and water *ad libitum*. All the animal experiments were performed according to the recommendations of ethic committee of Tunis University for care and International Council of Laboratory Animal Science. Induction of diabetes was carried out in rats by administering a subcutaneous injection of 120 mg/kg of freshly prepared alloxan solution (alloxan monohydrate, Sigma Chemicals Co.), dissolved in acetate buffer (pH 5.5) prepared immediately before use. The control groups were injected only with the same volume of acetate buffer as the diabetic groups received. Alloxan induces diabetes through destructing Langerhans islands of the pancreas. Therefore, a large amount of insulin is released from the pancreas cells after the injection. In order to prevent hypoglycemic shock, during the first 24 h after the alloxan injection, the rats received 10% dextrose instead of water (Shahidi et al. 2001; Kwon et al. 2003). Induction of diabetes was confirmed by measuring fasting blood glucose (FBG) three days after alloxan injection. Rats with blood glucose level of 250mg/kg or higher were considered to be diabetic (Ghorbani et al. 2013).

2.3. Groups of study

The experiment was carried out on four groups; each group contained 8 rats as follows:

Group I. Normal control
Group II. Diabetic control
Group III. Normal control fed with diet enriched with fenugreek (5%, w/w)
Group IV. Diabetic rats treated fed with diet enriched with fenugreek (5%, w/w)

The treatment was initiated two days after alloxan injection and continued for 4 weeks. At the end of the study, animals were fasted overnight and anesthetized with chloroform. Blood samples were collected from the animal's heart and the serum was separated by centrifugation (3000 rpm at 40 °C for 15 min) and stored at -30 °C for different biochemical analyses.

2.4. Estimation of body weight

The body weights in experimental animals were determined before the study and at 7, 14, 21 and 28 days after the study by a digital balance (Entris®, precision 10mg). These weights were determined at the same time during the morning.

2.5. Estimation of blood glucose

Throughout the 4-week treatment period, fasting (12 hours) blood glucose was measured before the study and at 7, 14, 21 and 28 days after the study on lateral tail vein. Blood samples were analyzed using an ACCU-Check glucose meter (Roche, Mannheim, Germany).

2.6. Estimation of blood lipid profile

Lipid in serum concentration including TG, TC and HDL-CH were determined with the use of commercially available enzymatic kits from Biomaghreb Analyticals (Tunisia). LDL-CH was estimated by Friedwald method as follows:

LDL cholesterol = total cholesterol- HDL cholesterol- (Triglyceride ÷ 5)

All assessment assays and kits were performed in accordance with the manufacturer instructions and protocols.

2.7. Level of antioxidant enzymes activities in pancreas tissue and serum

Pancreas tissue homogenates of the control and experimental groups of animals were prepared with 0.1 M Tris–HCl buffer (pH 7.4) at 4°C using a tissue homogeneiser. The resulting tissue homogenates were used for biochemical measurements.

The activities of three antioxidant enzymes: SOD, CAT and GPx were determined in pancreas homogenate and in serum. SOD activity was measured according to the method of Beyer and Fridovich (Beyer et al. 1987). CAT activity was determined using the method of Aebi (Aebi et al. 1984) and GPx activity was measured according to the method of Flohe and Gunzler (Flohe and Gunzler, 1984).

2.8. Protein quantification in pancreas homogenate

Protein content in pancreas samples was assayed as described by Lowery et al. (Lowry et al. 1951), using bovine serum albumin as standard.

2.9. Statistical analysis

Statistical analysis was performed with Statistica^{TM} software, using one-way analysis of variance (ANOVA). Statistical assessments of differences between mean values were performed by Duncan's multiple range test at $P \leq 0.05$.

3. Results and discussion

3.1. Acute toxicity in rats

Rats administrated fenugreek did not develop any clinical signs of toxicity either immediately or during treatment and no mortality occurred.

3.2. Effects of Fenugreek on body weight and plasma glucose

There was a significant increase in blood glucose and decrease in body weight in alloxan induced group II diabetic animals as compared to the normal ones indicating the impaired glucose metabolism. Fenugreek supplemented diet significantly lowered the blood glucose and elevated body weight when compared with the untreated diabetic group. Table 1 shows body weight and blood glucose levels of different animal groups at the beginning and at the end of the experiment. There was no significant difference in body weight and blood glucose levels between groups before induction of diabetes. At the end of the experimental period, the body weight of the untreated (249.5±8.3g) and fenugreek-treated diabetic rats (221.5±10.3g) were lower than control rats (264.3±10.2g). Considering plasma glucose levels, untreated diabetic animals had significantly (P <0.05) elevated plasma glucose levels (342.2 ± 23.9mg/dl) compared with control animals (88.6 ± 4.8mg/dl). Supplementation diet with fenugreek to diabetic rats decreased the plasma glucose levels of the treated groups (171.0 ± 18.7mg/dl) compared with the untreated diabetic group (171.0 ± 18.7mg/dl; P <0.05).

This beneficial effect on glycemic status is expected to happen as it has been confirmed with repeated studies (Sajad Arshadi et al, 2015; Gaddam et al. 2015). Antihyperglycemic effect of medicinal plants

is achieved by different mechanisms including decreasing glucose absorption from intestine, enhancing insulin secretion from beta cells, increasing glucose uptake by tissues, inhibiting glucose production in liver, and increasing pancreatic tissue regeneration and/or presence of insulin-like agents in plants (Hui et al, 2009; Shanmugasundaram et al. 1981). The antihyperglycemic effect of fenugreek has been hypothesized to be due to the aminoacid 4-hydroxyisoleucine which acts by the enhancement of insulin sensitivity and glucose uptake in peripheral tissues (Singh et al. 2010). Other components of *Fenugreek s*eeds are known to have hypoglycemic effects include arginine, tryptophan, ascorbic acid, niacin, nicotinic acid, chromium, copper, magnesium, manganese, zinc, gentianine, choline and quercetin (Thakran et al. 2004).

Table 1: Body weight and plasma glucose levels of different animal groups at the beginning and end of the study

Groups	Plasma glucose (mg/dl)		Body weight (g)	
	Beginning	**End**	**Beginning**	**End**
Group I	86.6 ± 3.6 a	88.6 ± 4.8 a	214.2±10.7 a	264.3±10.2 a
Group II	344.4 ± 35.0 b	342.2 ± 23.9 b	217.5±6.1 a	186.7±10.2 b
Group III	85.6 ± 3.6 a	85.4 ± 7.3 a	205.5±11.0 a	249.5±8.3 a
Group IV	337.2 ± 29.8 b	171.0 ± 18.7 c	210.0±11.4 a	221.5±10.3 c

Data are expressed as mean ± SD of measurements from 8 rats. Groups are labelled as follows: Group I: normal control, Group II: diabetic control, Group III: Normal control fed with diet enriched with fenugreek (5%, w/w), Group IV: Diabetic rats treated fed with diet enriched with fenugreek (5%, w/w).
Different letters in the same raw indicate significant differences at $P \leq 0.05$ as determined by Duncan's multiple range tests.

3.3. Effect of fenugreek on serum lipids

Diabetes is often associated with dyslipidemia, a main risk factor of cardiovascular diseases. Therefore, the levels of serum triglyceride and cholesterol are usually elevated in diabetic patients (Patel et al. 2008)

Here, we investigated the effect of a diet containing 5% fenugreek on lipidemic status of diabetic rats. Table 2 shows the level of serum lipids in study groups. In addition to reducing blood glucose, an insulin-mimetic agent should be able to normalize dyslipidemia. There was a significant elevation in the level of TG (0,87±0,17 g/l versus 0,45±0,07 g/l, P <0.05) and TC (0,95±0,11 g/l versus 0,60±0,07g/l, P < 0.05) in diabetic control rats as compared with normal group. Our results showed that *fenugreek powder supplemented to rat diet* extract at dose of 5 % was able to cause a significant decrease in TG and TC levels in animal serum. The levels of TG and TC in fenugreek treated group were 0,49±0,09 g/l versus 0.40±0.11g/l in diabetic control group and 0,75±0,17g/l versus 0.63± 0.09 g/l in diabetic control group, respectively. The hyperlipidemia observed in the untreated diabetic rats in the present study could indicate an increase in the mobilization of free fatty acids from the peripheral fat depots. This could result from the uninhibited actions of lipolytic enzyme lipase caused by insulin deficiency characteristic of the diabetic state (Omolola et al. 2014). The increased levels of CH and TG were brought back to near normal by the treatment with fenugreek. This observed restoration of the alloxan evoked changes in the serum lipid profile shows the protective nature of fenugreek. Nevertheless, the lipid-lowering compounds in fenugreek seeds could be of a polyphenolic nature. Indeed, Wilox et al. (2001) provided evidence that naringenin not only decrease cholesterol biosynthesis but also inhibit acyl transferase (ACAT), a key enzyme involved in the esterification and absorption of cholesterol, secretion of hepatic LDL-CH, and cholesterol accumulation in the arterial wall. Naringenin is in fact a well known flavonoid which was detected in fenugreek extract (Belaid-Nouira et al. 2012). Others compounds were also detected like 4-hydroxyisoleucine, an atypical branched-chain amino acid derived from fenugreek (Jetté et al. 2000) also detected in fenugreek (Belaid-Nouira et al. 2012). This effect could explain the significant increase in plasmatic HDL-CH following TC decrease when fenugreek seeds are administrated to alloxan-treated rats.

Numbers of studies have shown that steroid saponin extracted from fenugreek seeds has the ability to modify cholesterol status by its capacity to bind both cholesterol and bile acids (Basu et al. 2010). Diosgenin, a steroidal sapogenin extracted from fenugreek seeds, has in fact been shown to reduce TC as well as LDL-CH in high-cholesterol fed quails (Al-Matubsi et al. 2011). On the other hand, trigonelline, an alkaloid isolated from fenugreek seeds, was found able to normalize the rate of lipogenesis in streptozotocin induced hyperglycemic rats by stimulating hepatic lipogenic enzymes (El-Soud et al. 2007).

Table 2: Comparative levels of serum triglycerides, cholesterol, HDL-CH and LDL-CH in different rat groups

Groups	Serum lipids (g/l)			
	Triglycerides	**Cholesterol**	**HDL-CH**	**LDL-CH**
Group I	0,45±0,07 a	0,60±0,07 a	0.15±0.07 a	0.47±0.11
Group II	0,87±0,17 b	0,95±0,11 b	0.11±0.10 b	0.79±0.50
Group III	0,40±0,11 a	0,63±0,09 a	0.18±0.04 a	0.38±0.13
Group IV	0,49±0,09 a	0,75±0,17 c	0.14±0.04 a	0.53±0.15

Data are expressed as mean ± SD of measurements from 8 rats. Groups are labelled as follows: Group I: normal control, Group II: diabetic control, Group III: Normal control fed with diet enriched with fenugreek (5%, w/w), Group IV: Diabetic rats treated fed with diet enriched with fenugreek (5%, w/w).
Different letters in the same raw indicate significant differences at $P \leq 0.05$ as determined by Duncan's multiple range tests.

3.4. Effect of Fenugreek on pancreatic and plasmatic oxidative enzymes

The common chronic damage in diabetes patients is closely related to elevated oxidative/inflammatory activities with a continuum of tissue insults leading to more severe cardiometabolic and interrelated complications (Ndisang et al. 2010). In the present study, we also investigated the antioxidant effects of fenugreek in plasma and homogenate pancreas of alloxan-induced diabetic rats. To our knowledge, no studies compared oxidative enzymes activities in plasma and tissues. Our results show that untreated diabetic rats showed significantly reduced SOD ($p<0.05$), GPx ($p < 0.05$) and CAT ($p < 0.05$) activities in plasma and pancreas homogenate, compared to the non-diabetic control group. The enriched diet with fenugreek for four weeks produced significant increases in SOD ($p < 0.05$), CAT ($p < 0.05$) and GPx ($p < 0.05$) activities both in plasma and in pancreas homogenate.

The oxidative stress induced by alloxan arises due to a compromise in natural antioxidant mechanism and an increase in oxygen free radical production (El-Demerdash et al. 2005). Indeed, free radical scavenging enzymes such as SOD and CAT protect the biological system from oxidative stress (Del Rio et al. 2005). SOD catalyses the reaction in which superoxide anion is converted to hydrogen peroxide and oxygen while CAT is a haem-containing ubiquities enzyme that detoxifies H_2O_2 into water and oxygen (Selvan et al. 2008). The reductions observed in the activities of SOD and CAT in the diabetic control group suggest their excessive utilization in attenuating free radicals generated during the metabolism of alloxan. This observation has already been reported in diabetic animals (Onyeka et al. 2013). Increase in their activities is an indication of their ability to scavenge ROS, thus contributing to the protective effect against oxidative stress and preventing further damage to membrane lipids.

Table 3: Effect of fenugreek and/or alloxan treatment on SOD, CAT and GPx activities in plasma and pancreatic homogenates of treated rats

Groups	Pancreatic homogenate			Plasma		
	SOD U/mg protein	CAT (10^{-3} mM.min^{-1}.mg^{-1} protein)	GPx (Mol H_2O_2.min^{-1}.mg^{-1} protein)	SOD U/mg protein	CAT (10^{-3} mM.min^{-1}.mg^{-1} protein)	GPx (Mol H_2O_2.min^{-1}.mg^{-1} protein)
Group I	20.2±2.5 a	50.6±5.0 a	2.3±0.4 a	259.45±6.32 a	3.96±0.283 a	937.00±37.12 a
Group II	11.3±1.2 b	26.2±2.9 b	0.6±0.2 b	158.45±5.07 b	1.91±0.26 b	764.62±30.27 b
Group III	23.6±2.1 a	51.5±4.4 a	2.5±0.5 a	259.07±31.4 a	4.46±0.44 c	795.37±58.3 c
Group IV	20.2±3.4 a	37.1±4.9 c	1.2±0.2 c	249.05±13.37 a	3.82±0.47 a	789.69±312.5 c

Data are expressed as mean± SD of measurements from 8 rats. Groups are labelled as follows:
Group I: normal control, Group II: diabetic control, Group III: Normal control fed with diet enriched with fenugreek (5%, w/w), Group IV: Diabetic rats treated fed with diet enriched with fenugreek (5%, w/w). Different letters in the same raw indicate significant differences at $P \leq 0.05$ as determined by Duncan's multiple range tests.

4. Conclusion

In conclusion, the present study demonstrated that the hypoglycemic and antihyperlipidemic activities exhibited by fenugreek were effective enough to alleviate alloxan-induced diabetes in experimental rats. The beneficial effect of diet supplemented with fenugreek is presumably attributed to its potent hypoglycemic and antihyperlipidemic properties, as well as antioxidant potential. Further studies using different doses and for different periods are obviously needed to determine the dose required on the protective effects of fenugreek in humans and to make its use suitable as an effective functional food with therapeutic potential.

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