Original Article

Effect of *Trigonella foenum-graecum* (fenugreek) extract on blood glucose, blood lipid and hemorheological properties in streptozotocin-induced diabetic rats

Wan-Li Xue PhD[1], Xuan-She Li MB[1], Jian Zhang MD[2], Yong-Hui Liu MD[2], Zhi-Lun Wang PhD[1] and Rui-Juan Zhang PhD[1]

[1]*Department of Public Health, School of Medicine, Xi'an Jiaotong University, Xi'an, China*
[2]*Department of Traditional Chinese Medicine, First Affiliated hospital, School of Medicine, Xi'an Jiaotong University, Xi'an, China*

Trigonella foenum-graecum (fenugreek) seeds have previously been shown to have hypoglycemic and hypocholesterolemic effects on type 1 and type 2 diabetes mellitus patients and experimental diabetic animals. The *Trigonella foenum-graecum* extract has now been investigated for its effects on general properties, blood glucose and blood lipid, and hemorheological parameters in experimental diabetic rats. Streptozotocin-induced diabetic rats were administrated by oral intragastric intubation separately with low dose (0.44 g/kg.d), middle dose (0.87 g/kg.d), high dose (1.74 g/kg.d) of *Trigonella foenum-graecum* extract, and Metformin HCl (0.175 g/kg.d) for 6 weeks. Compared with diabetic group, rats treated with *Trigonella foenum-graecum* extract had an increase in body weight and a decrease in kidney /body weight ratio ($p<0.05$). Compared with diabetic group, rats treated *Trigonella foenum-graecum* extract had lower blood glucose, glycated hemoglobin, triglycerides, total cholestrol and higher higher-density-lipoprotein-cholesterol in a dose-dependent manner ($p<0.05$). The plasma viscosity, whole blood viscosity of high shear rate (200 s^{-1}) and low shear rate (40 s^{-1}), erythrocyte sedimentation rate, whole blood reduction viscosity and platelet conglutination were significantly reduced in diabetic rats treated with high and middle doses of *Trigonella foenum-graecum* extract, but not in those treated with low dose of *Trigonella foenum-graecum* extract. It may be concluded that *Trigonella foenum-graecum* extract can lower kidney /body weight ratio, blood glucose, blood lipid levels and improve hemorheological properties in experimental diabetic rats following repeated treatment for 6 weeks.

Key Words: diabetes, *Trigonella foenum-graecum*, fenugreek, blood glucose, blood lipid, hemorheology

Introduction

Diabetes mellitus (DM), one of the major metabolic disorders, is characterized by high blood glucose levels due to the inability of body cells to utilize glucose properly. By the year 2010, the total number of people worldwide with DM will be as high as 239 millions. Regions with greatest potential are Asia and Africa, where DM incidence could rise to 2–3-folds of the present incidence.[1] Diabetes is recognized for severe complications such as diabetic nephropathy, neuropathy, and retinopathy.[2-4] The complications are major causes of morbidity and mortality in DM. Although insulin treatment and other chemical therapies can control the disease to various extents, the complications are very common, whose pathologic base is microangiopathy. Abnormalities in hemorheology have been related to the development of diabetic micro- and macroangiopathy. On the other hand, alterations in hemorheology might also be a consequence of underlying vessel wall injury in diabetic patients with manifest microangiopathy. [5-6]

Among various forms of treatments for DM, diet is of vital importance. Foods of medicinal value have been proved effective and thus are widely used as they combine two basic central factors: food and medication.[7-8] *Trigonella foenum-graecum* is cultivated throughout India and in certain regions of China. Its seeds are used as condiment in India, a supplement to wheat and maize flour for bread-making in Egypt, and one of the staple foods in Yemen. Its seeds are also be used as herbal medicine in many parts of the world for their carminative, tonic and aphrodisiac effects. Various reports have demonstrated that *Trigonella foenum-graecum* (fenugreek) seeds can lower blood glucose and cholesterol in type 1 and type 2 diabetics and experimental diabetic animals. [9-12] However, the effects of fenugreek seeds on hemorheology in normal and pathological states have not been reported yet, so we intended to investigate the effects of aqueous extracts of *Trigonella foenum-graecum* seeds on blood glucose, blood lipid and hemorheological properties in streptozotocin-induced diabetic rats.

Corresponding Author: Dr Wanli Xue, Department of Public Health, School of Medicine, Xi'an Jiaotong University, 76 West Yanta Road, Xi'an, Shaanxi, China 710061
Tel: 86 029 8265 5107; Fax: 86 029 8265 5103
Email: xwl0908@163.com

Materials and methods

Plant material

Trigonella foenum-graecum (Fenugreek) seeds were purchased from the local herbal market, cleaned, dried and finely powdered.

Extraction of aqueous plant material

1 kg of powdered *Trigonella foenum-graecum* (Fenugreek) seeds were boiled in 10000 ml distilled water for 30 mins. Then, the decoction was cooled for 30 mins at room temperature. Next, the cooled decoction was filtered through a coarse sieve twice. Finally, the filtrate was concentrated by flash evaporation at 358 ℃ to a thick paste (totally 190 g).

Chemicals

Streptozotocin (STZ) were purchased from Sigma Chemicals Co, St Louis, MO, USA. Metfomin (MF) was purchased from Shanghai Jiufu pharmaceutical Co. Ltd, China. TG, TC and HDL-C kits were purchased from Zhejiang Dongou Bio-Engineering Co, China. Glycated hemoglobin (GHb) kits were purchased from Nanjing Jiancheng Bioengineering Institute, China. All the chemicals used were of analytical grade and obtained from Xi'an Chemical Plant, China.

Animals

Adult male SD rats weighing 100-110 g were used throughout this study. All institutional guidelines were adhered to during the care and treatment of the animals used in the present study.

Induction of diabetes and study design

70 rats were fed with the diets enriched with sucrose (50%, w/w) and lard (30%, w/w) and cholesterol (2.5%, w/w) to induce insulin resistance for 8 weeks. All the 70 experimental rats were injected with STZ intravenously (25 mg/kg body wt) freshly prepared in 0.1M sodium citrate buffer (pH 4.5) after starved for 12 h. Other 10 control rats were given only vehicle. The diabetic state was confirmed 48 h after STZ injection by glucosuria and hyperglycaemia. Rats with a fasting blood glucose level higher than 11.1 mmol/L and positive urine glucose three times continually were included in the study. 55 diabetic rats were randomly divided into five groups: diabetic (D), diabetic treated with MF (D+MF), diabetic treated with low dose (0.44 g/kg.d) of *Trigonella foenum-graecum* seeds extract (TE) (D+TE1), middle dose (0.87 g/kg.d) of TE (D+TE2), high dose (1.74 g/kg.d) of TE (D+ TE3) . The rats in each group were administered manually by oral intragastric intubation with distilled water, MF and TE of different doses respectively. Such treatment was continued for six weeks, during which body weight was measured every week for each rat. The rats were fasted for 8 h and blood samples were collected by tail nipping and assessed for glucose by an electronic glucometer. Then, blood samples were collected from common iliac artery on euthanasia by ether anesthesia and kept in tubes containing heparin (20 U/mL of blood) and in tubes containing citrate to measure hemorheological parameters. Serum was separated for determining blood lipid. Samples were centrifuged for 5 min at 2500 g and red cells were separated to measure GHb. The kidneys were rapidly excised and weighed.

Measurement of parameters

Blood glucose was determined by a One-touch electronic glucometer. Both GHb and blood lipids were estimated by a commercial kit. Hemorheological parameters were measured by the method devised by Yang *et al* [13] with some modification. Whole blood viscosity corrected at 43 ± 1% was measured by a tubby rotational viscometer (Chinese Academy of Sciences, Transduction Technology Co., Beijing, China) at two different points on shear rates (200, 40 s^{-1}) five times respectively. The averages of the five values were then calculated. The same procedure was repeated in each blood sample. The final viscosity was estimated for each point by the average of the two re-

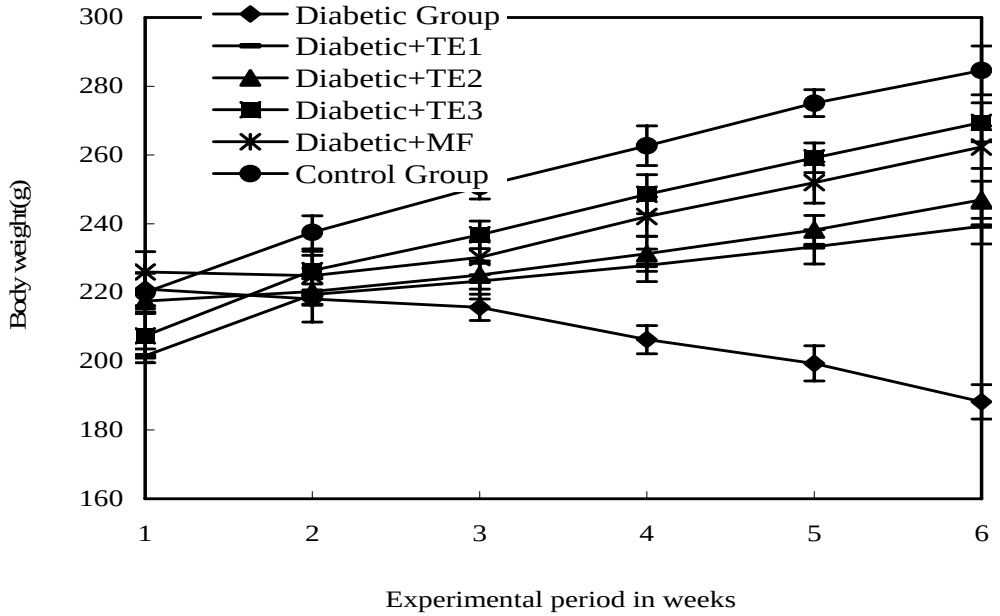

Figure 1. Effects of aqueous extraction of *Trigonella foenum-graecum* (TE) treatment on body weight in STZ-induced diabetic rats. TE was administered to diabetic rats for 6 weeks. Body weight of all groups were measured each week. MF, Metformin HCl.

peated tests. Plasma viscosity was estimated at the highest shear rate of 200 s^{-1} by the average of five measurements. All measurements were performed at 37 °C. Kidney /body weight ratio was expressed in percentages.

Statistical analysis

The results were analyzed for statistical significance by one way ANOVA test using computerised software, SPSS version 11.0, SPSS Ireland, 79 Old Kilmainham Road, Dublin 8, USA.

Results and discussion

The average body weight of all rats was about 220 g at the beginning of the treatment. Diabetic rats treated with TE and MF registered a weight gain, while untreated diabetic rats showed a progressive reduction in body weight (Fig 1). This finding is in agreement with previous reports.[14-15] The progressive increase in weight suggests that fenugreek seeds can attenuate the toxicity of STZ, particularly at high dose. It might be possible that treatment with TE can lead to a better utilization of nutrients in the diet and thus a gain in weight.

Meanwhile,the average kidney/body weight ratio of TE-treated diabetic rats (0.98±0.16%, 0.80±0.16%, 0.76±0.10%) were significantly lower than that of untreated diabetic rats (1.03±0.12%) during the experiment. Moreover, there was also a significant correlation ($p < 0.01$) between the dosage groups (Fig 2). Diabetes may produce some alterations to the kidney. One change is the hyperperfusion and hyperfiltration of glomerulus. Another change is the enhanced effect of non-enzymatic glycation under high blood glucose, which leads to an increase in the number of GHb then an accumulation of advanced glycation end (AGEs) products in glomerulus basement membrane.[16] All these alteration result in kidney hypertrophy and damage. The renoprotective effect observed in the present study may due to the the reduction in non-enzymatic glycation,

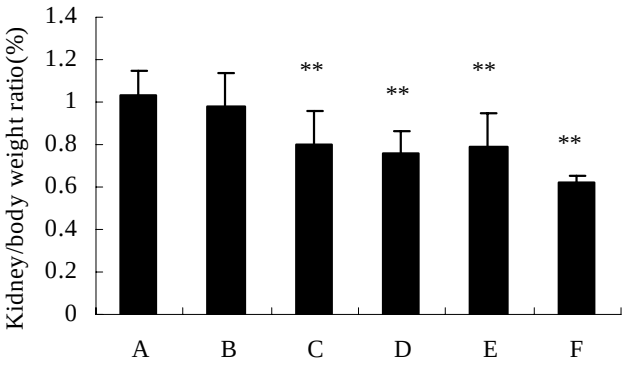

Figure 2. Effects of aqueous extraction of Trigonella foenum-graecum (TE) treatment on kidney/body weight ratio in STZ-diabetic rats. A, B, C, D, E, F denote diabetic rats and diabetic rats treated with low dose (0.44 g/kg.d) of TE (D+ TE1), middle dose (0.87 g/kg.d) of TE (D+ TE2), high dose (1.74 g/kg.d) of TE, diabetic rats treated with Metformin HCl, and control rats, respectively. TE was administered by oral intragastric intubation to diabetic rats for 6 weeks. Asterisks are used to mark groups that showed significant differences from diabetic group (*p <0.05; ** p <0.01). The standard error in each mean is shown by bars.

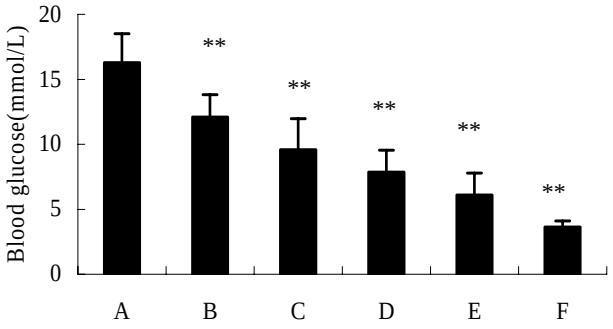

Figure 3. Effects of aqueous extraction of Trigonella foenum-graecum (TE) treatment on blood glucose levels in STZ-diabetic rats. A, B, C, D, E, F denote diabetic and diabetic treated with low dose (0.44 g/kg.d) of TE (D+ TE1), middle dose (0.87 g/kg . d) of TE (D+ TE2), high dose (1.74 g/kg.d) of TE , diabetic treated with Metformin HCl, and control, respectively. TE was administered by oral intragastric intubation to diabetic rats for 6 weeks. Asterisks are used to mark groups that showed significant differences from diabetic group (*p <0.05; ** p <0.01). The standard error of each mean is shown by bars.

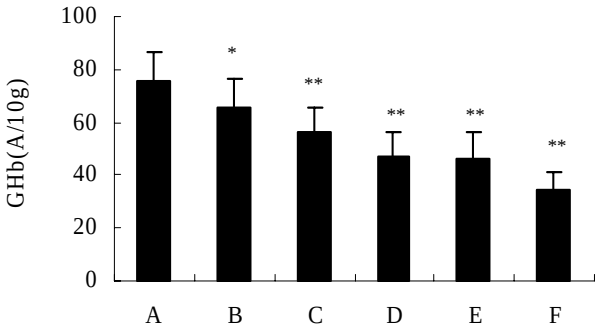

Figure 4. Effects of aqueous extraction of Trigonella foenum-graecum (TE) treatment on glycated hemoglobin (GHb) levels in STZ-diabetic rats. A, B, C, D, E, F denote diabetic and diabetic treated with low dose(0.44 g/kg.d) of TE (D+ TE1), middle dose(0.87 g/kg.d) of TE (D+ TE2), high dose (1.74 g/kg.d) of TE , diabetic treated with Metformin HCl, and control, respectively. TE was administered by oral intragastric intubation to diabetic rats for 6 weeks. Asterisks are used to mark groups that showed significant differences from diabetic group (*p <0.05; ** p <0.01). The standard error of each mean is shown by bars.

which inhibits the overproduction of AGEs in diabetic rats. The renoprotective role of TE on kidney damage needs to be studied in greater detail.

The blood glucose and GHb levels of each group are presented in Fig 3 and Fig 4. STZ-injection resulted in a nearly five fold increase in the fasting blood glucose levels in SD rats. TE-treated diabetic rats showed significant reduction ($p < 0.01$) in fasting blood glucose and GHb levels compared with diabetic rats. In addition, with increasing TE dosage, there was a progressive decrease ($p < 0.05$) in these two parameters. The diabetic rats treated with MF had a fasting blood glucose level of 4.42 - 7.78 mmol/L and GHb level of 36.3 - 56.2 A/10g. The blood glucose level of D+TE3 group was as high as that of MF group. It is likely that the beneficial effect of TE is due to some of the bioactive compounds present in it, including 4-hydroxy isoleucine, a novel amino acid known to facilitate insulin secretion.[17] In addition, the soluble dietary

Table 1. Blood lipids levels of control, STZ-diabetic and diabetic treated rats after six week treatment with TE and MF†

Parameters	Diabetic	Diabetic +TE1	Diabetic +TE2	Diabetic +TE3	Diabetic +MF	Control
TG/mmol/L	1.41 ± 0.13	1.19 ± 0.22**	0.84 ± 0.17**	0.67 ± 0.14**	1.17 ± 0.17**	0.59 ± 0.10**
TC/mmol/L	2.21 ± 0.26	1.94 ± 0.18**	1.37 ± 0.20**	1.09 ± 0.16**	1.94 ± 0.21**	0.99 ± 0.12**
HDL-C/mmol/L	0.68 ± 0.16	1.03 ± 0.19**	1.46 ± 0.12**	1.79 ± 0.20**	0.94 ± 0.20**	1.21 ± 0.13**

† The values represent the mean ± SD; Each group consisted of nine to ten animals; Experimental groups were statistically compared with the corresponding values of the diabetic control; TE, aqueous extraction of *Trigonella foenum graecum*; MF, Metformin HCl; TG, triglycerides; TC, cholestrol total; HDL-C, higher-density-lipoprotein-C. * Values are statistically significant at $p<0.05$. **Values are statistically significant at $p<0.01$

Table 2. Hemorheological parameters of control, STZ-diabetic and diabetic treated rats after six week treatment with TE and MF†

Parameters	Diabetic	Diabetic +TE1	Diabetic +TE2	Diabetic +TE3	Diabetic +MF	Control
Whole blood viscosity of high shear rate($200s^{-1}$) (mpa·S)	5.20 ± 0.65	4.83± 0.81	3.50 ± 0.61**	2.97 ± 0.40**	3.15 ± 0.49**	2.98 ± 0.39**
Whole blood viscosity of low shear rate($40s^{-1}$) (mpa·S)	10.3± 1.42	9.02± 1.31*	7.36± 1.08**	6.31± 0.73**	7.27 ± 1.11**	6.54 ± 0.82**
Erythrocyte sedimentation rate (ESR)(mm/h)	13.1± 3.59	9.67± 2.65**	6.90± 2.51**	5.10± 2.60**	6.20± 2.66**	4.60± 1.17**
Whole blood reduction viscosity	11.4± 1.89	10.1± 1.85	8.26± 1.06**	5.89± 1.22**	7.11± 1.61**	5.79± 1.79**
Plasma viscosity (mpa·S)	4.08± 0.84	3.61± 0.69	2.88± 0.47**	2.20± 0.23**	2.23± 0.25**	2.66± 0.48**
Platelet conglutination (%)	32.7± 5.27	31.4± 4.75	22.6± 4.65**	26.9± 4.82**	25.0± 3.80**	26.0± 4.47**
Hematocrit(HCT) (%)	35.8 ± 4.18	33.8 ± 4.76	35.8 ± 3.88	34.1 ± 4.63	35.6 ± 4.93	37.2 ± 2.94

† The values represent the mean ± SD; Each group consisted of nine to ten animals; Experimental groups were statistically compared with the corresponding values of the diabetic control; TE, aqueous extraction of *Trigonella foenum graecum*; MF, Metformin HCl; TG, triglycerides; TC, cholestrol total; HDL-C, higher-density-lipoprotein-C. * Values are statistically significant at $p<0.05$. **Values are statistically significant at $p<0.01$

fibers present in TE could inhibit absorption of glucose in the gastrointestinal tract.

Table 1 shows the levels of triglycerides (TG), total cholestrol (TC) and higher-density-lipoprotein-cholesterol (HDL-C) in each group. TG and TC levels of TE-treated diabetic animals were lower than those of untreated diabetic animals ($p<0.05$) and revealed a dose-dependent increase ($p<0.05$). These results are consistent with these previous findings.[18] Several studies show that an increase in HDL-C is associated with a decrease in coronary risk yet most of the drugs that decrease TC also decrease HDL-C.[19] In the present study, TE, while lowering TG and TC, significantly increased the concentration of HDL-C in a dose-dependent manner ($p<0.01$). TE may reduce TG and TC by decreasing the non-esterified fatty acids (NEFA) in diabetic rats. NEFA may influence platelet aggregation and vascular changes by accelerating the rate of prostacyclin in plasma.[20-21] The hypolipidemic action could also be the result of retardation of carbohydrate and fat absorption due to the presence of bioactive fibre in the agent.[18]

In diabetes, various hemorheological abnormalities have been widely reported, including increased high shear rate of whole blood viscosity and whole blood reduction viscosity which reflect the decreased erythrocyte deformability, low shear rate of whole blood viscosity , erythrocyte sedimentation rate(ESR) and platelet conglutination which reflect the increased erythrocyte and platelet aggregation.[22-25] Hemorheological parameters obtained in the present study demonstrated that high and low shear rates ($200s^{-1}$, $40\ s^{-1}$) of whole blood viscosity decreased significantly in high and middle dose of TE-treated diabetic rats compared to diabetic animals ($p<0.01$). Similar results were obtained with ESR, whole blood reduction viscosity, plasma viscosity and platelet conglutination ($p<0.01$) (Table 2). These changes might be due to the reduction in NEFA. Hence, the agent not only helps to combat hyperglycemia, but also helps to prevent

dyslipidemia-an important risk factor for the microvascular complications of diabetes. But low dose of TE did not show significant difference compared to diabetic group. Moreover, there was no statistical difference in hematocrit (HCT) between groups. It is necessary to conduct further study on it.

In conclusion, the results have revealed a well defined and to some extent, a dose-dependent role of TE in suppressing blood glucose, GHb, blood lipid levels and kidney / body weight ratio in diabetic rats. In addition, TE could improve hemorheological parameters in diabetic rats. These hemorheological effects may play a beneficial role in the improvement of microcirculation in the early stages of diabetes complications.

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Refereces

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