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Endothelium-Dependent Attenuating Effect of *Trigonella foenum-graecum* on the Contractile Vascular Reactivity of Diabetic Rats

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ABSTRACT

The present study was undertaken to determine whether two-month treatment of streptozotocin (STZ)-diabetic rats with aqueous leaf extract of *Trigonella foenum-graecum* (TFG; 200 mg/kg; i.p.) could improve thoracic aortic responsiveness and to evaluate its endothelium dependency. For this purpose, vascular responses to KCl and noradrenaline (NA) were measured. Diabetic state significantly increased contractile responses to KCl and NA in aortic rings in both endothelium-intact and -denuded rings. Extract-treated diabetic rats showed a significant lower maximal contractile response to KCl only in endothelium-intact rings as compared to diabetic rings. It is concluded that intraperitoneal administration of aqueous leaf extract of TFG for two months could improve some functional indices of the vascular system in diabetic state and the integrity of the endothelium is essential for its beneficial effects. *Iran. Biomed. J. 9 (3): 129-133, 2005*

Keywords: *Trigonella foenum-graecum* (TFG), Diabetes mellitus, Streptozotocin, aorta,

INTRODUCTION

Fenugreek (*Trigonella foenum-graecum*) (TFG) is a plant with traditional medicinal use in diabetes and its beneficial effects have been demonstrated in diabetic animals and both insulin-dependent and non-insulin-dependent diabetic subjects [1]. Since mortality from cardio-vascular abnormalities including hypertension, atherosclerosis, microangiopathy, and congestive heart failure is almost three times more prevalent in the diabetic population than in the general population [2-3], finding new treatment strategies for attenuation of diabetic vascular complications has been always a mainstay in medicine. In this regard, fenugreek has been considered as an appropriate candidate. Hypoglycemic and anti-hyperglycemic effects of fenugreek seeds [4] and aqueous leaf extracts [5] have previously been reported in experimentally induced diabetic rats. Since, there is no strong scientific evidence for therapeutic and pharmacological properties of TFG, especially its leaf derivatives in vascular abnormalities of diabetic state, the present study was carried out to evaluate the protective effect of aqueous leaf extract of TFG on the contractile responsiveness of aortic rings of streptozotocin (STZ)-diabetic rats to KCl and noradrenaline (NA) and the possible involvement of endothelium in this respect.

MATERIALS AND METHODS

Preparation of TFG extract. Fresh fenugreek (TFG) was obtained from local grocery in April and systemically identified by the botanists in the Department of Biology (Shaheed Beheshti University, Tehran, Iran). Green leaves were separated, cleaned, and shade dried at room temperature and 125 g was grounded and mixed with 1,000 ml of boiling distilled water for a period of 15 min under continuous stirring. The obtained mixture was filtered twice through a mesh and the

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resulted liquid was dried at room temperature (30-34ºC) until a concentrated residue (67% w/w) was obtained (29 g). This stock extract was maintained at -20ºC until use. Fenugreek extract of lower concentrations was prepared by dilution of the stock with cold and sterile 0.9% saline solution.

Animal experiments. Male Albino Wistar rats (the Pasteur Institute of Iran, Tehran) weighing 223 ± 8 g (7-9 weeks old) were housed in an air-conditioned colony room on a light/dark cycle at 21 ± 3℃ and supplied with standard pellet diet and tap water ad libitum. Procedures involving animals and their care were conducted in conformity with the institutional guidelines of Shahed University (Tehran, Iran) and in accordance with the NIH guidelines for the care and use of laboratory animals.

The animals were randomly divided into four experimental groups: vehicle-treated control (n = 12), extract-treated control (n = 8), vehicle-treated diabetic (n = 10), and extract-treated diabetic (n = 10). Diabetes was induced by a single intra-peritoneal injection of STZ (60 mg/Kg) dissolved in cold 0.9% saline immediately before use. Control and extract-treated control animals received i.p. injection of normal saline solution and aqueous extract of fenugreek extract (200 mg/kg) respectively. The latter was also administered one other day to extract-treated diabetic animals from day +3 thereafter. Serum glucose level and body weight were measured one week before, and four and eight weeks after the experiment. Diabetes was verified by a serum glucose level higher than 250 mg/dl using glucose oxidation method (glucose oxidase kit, Zistchimie, Tehran, Iran). All treatments continued for two months.

Experimental procedure. For this purpose, the routine protocol was applied as described before [6-7]. Briefly, after being anesthetized, descending thoracic aorta was carefully excised and placed in a Petri dish filled with cold Krebs solution containing (in mM): NaCl, 118.5; KCl, 4.74; $CaCl_2$, 2.5; $MgSO_4$,1.18; KH_2PO_4 , 1.18; $NaHCO_3$, 24.9 and glucose, 10.0. The aorta was cleaned of excess connective tissue and fat and cut into rings of approximately 4 mm in length. One ring of each pair was left intact, and in the other ring, endothelium was mechanically removed by gently rotating it on a glass rod. Aortic rings were suspended between the bases of two triangular-shaped wires. One wire was attached to a fixed tissue support in a 50 ml isolated tissue bath containing Krebs solution (pH 7.4) maintained at 37℃ and continuously aerated with a mixture of 5% CO_2 and 5% O_2. A cotton thread was attached to the other end of each wire and to a F60 isometric force transducer, which was connected to A/D board of the IBM-compatible computer. Recording and analysis of data was performed using the software Physiograph I (Behineh Arman Co., Tehran, Iran). The rings were allowed to equilibrate for 90 min under a resting tension of 2 g before experiments were begun. During equilibration period, the rings were washed every 30 min. Successful removal of the endothelium was confirmed by loss of acetylcholine (10^{-5} M)-induced relaxation in preconstricted rings by NA (10^{-6} M). Then, concentration-response curves were obtained with KCl and thereafter with NA in aortic rings with or without endothelium. In this respect, KCl (10-50 mM) and NA (10^{-9}-10^{-4} M) were added in a cumulative manner until a maximum response was achieved. The sensitivity to the agonists was expressed as pD_2, which is the negative logarithm of the concentration of the drug required to produce 50% of maximum response. After each experiment, the aortic rings were dried at 45℃ for 5 min, weighed, and cross-sectional area (CSA) was calculated using the following formula: Cross-sectional area (mm^2) = weight (mg) × [length (mm) × density (mg/mm^3)]$^{-1}$. The density of the preparations was assumed to be 1.05 mg/mm^3 [8].

Drugs and chemicals. Noradrenaline and acetylcholine-HCl were purchased from Sigma Chemical Co. (St. Louis, Mo., USA). Streptozotocin was obtained from Upjohn Company (France). All other chemicals were purchased from Merck (Germany) and Temad (Tehran, Iran). All drugs except STZ were freshly dissolved in Krebs' solution. STZ was freshly dissolved in 0.9% saline solution.

Data and statistical analysis. All values were given as mean ± SEM. Contractile responses to NA and KCl were expressed as grams of tension per cross-sectional area of tissue. Statistical analysis was carried out using student's paired *t*-test and one-way analysis of variance (ANOVA) followed by Tukey post-hoc test. $P<0.05$ was considered significant.

RESULTS

Body weight, serum glucose, and cross-sectional area. Body weight and serum glucose level were measured before and at different weeks after the

Table 1. Body weight and serum glucose level of control, diabetic, and TFG extract-treated diabetic rats at different weeks.

	Body weight (g)			Serum glucose (mg/dl)		
	Week 0	**Week 4**	**Week 8**	**Week 0**	**Week 4**	**Week 8**
Control	238.1 ± 4.2	267.5 ± 5.9	294.3 ± 4.9	102.7 ± 4.1	96.8 ± 3.5	107.4 ± 4.1
Control + TFG_{200}	237.7 ± 5.8	248.4 ± 4.3	258.3 ± 5.4	99.8 ± 5.6	91.7 ± 4.2	87.6 ± 4.8
Diabetic	241.7 ± 5.4	190.7 ± 6.7**	176.6 ± 6.5***	104.7 ± 4.7	379.8 ± 15.7***	391.5 ± 18.9***
Diabetic + TFG	227.3 ± 5.2	214.9 ± 7.1*	211.7 ± 6.7**	96.5 ± 5.8	281.7 ± 12.9**	241.9 ± 13.1**

*P<0.05, ** P<0.01, *** P<0.001 (In comparison with control).

experiment (Table 1). At the end of two months, the body weight of the diabetic rats was found to be significantly decreased as compared to data one week before the experiment (P<0.01). Diabetic rats had also elevated serum glucose level more than that of control rats. Treatment of diabetic rats with fenugreek extract at a dose of 200 mg/kg caused a significant lower glucose serum level and a significant higher body weight in extract-treated diabetic rats as compared to diabetic group (P<0.05). Furthermore, there was a significant reduction in cross-sectional area of aortic rings in diabetic group, and extract-treated diabetic group showed some improvement.

Contractile responses to KCl and NA. Cumulative addition of KCl (10-50 mM) and NA (10^{-9}-10^{-4} M) to the organ bath resulted in concentration-dependent contractions in aortas of all groups (Figs. 1 and 2). The contractile responses to KCl at concentrations higher than 20 mM in diabetic rats were found to be significantly higher than control rats both in the presence (Fig. 1A) or absence of endothelium (Fig. 1B), and treatment of diabetic rats with TFG caused a significant reduction in contractile response to KCl (50 mM) only in endothelium-intact rings. The contractile responses to NA at concentrations higher than 10^{-7} M in vehicle-treated diabetic rats were found to be significantly higher than vehicle-treated control rats both in the presence (Fig. 2A) or absence of endothelium (Fig. 2B), and treatment of diabetic rats with TFG caused a significant reduction in contractile response to NA only in endothelium-intact rings. Furthermore, treatment of control rats with TFG leaf extract did not produce any significant changes in response to KCl and NA in both endothelium-intact and-denuded aortic rings.

DISCUSSION

The results of the present study demonstrated that aortas from 2-month STZ-diabetic rats are more responsive to the contractile effect of α-adrenoceptor agonist NA and to non-specific agent KCl both in the presence and absence of endothelium than those from corresponding controls. Similar results showing the increased vascular responsiveness to contractile agents in STZ-diabetic rats have been reported in most previous studies [8, 9]. This increased vascular smooth muscle responsiveness in diabetic rats could be attributed to deficient endothelial activity [8-9], enhanced phosphoinositide (PI) metabolism [10],

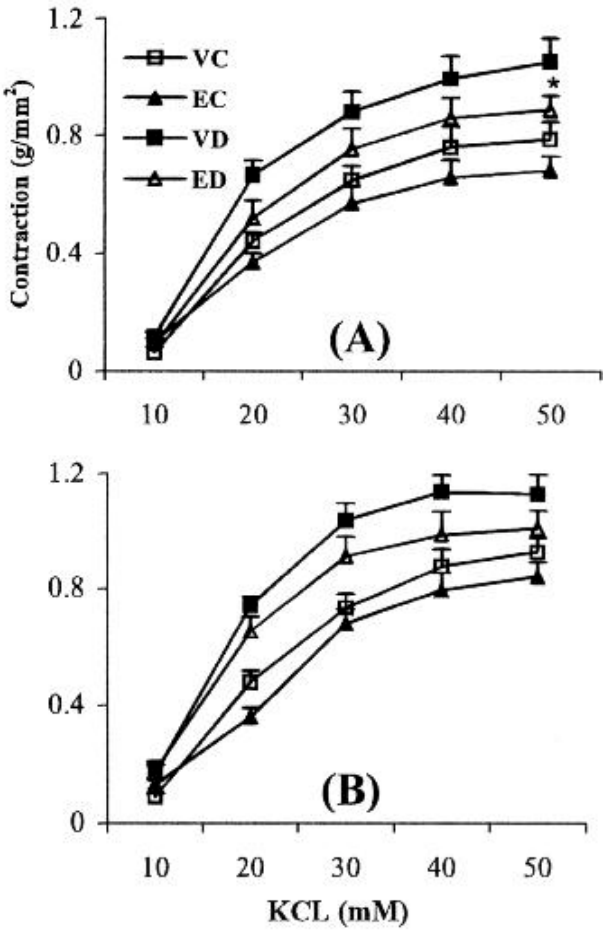

Fig. 1. Cumulative concentration-response curves for KCl in aortic preparations 2 months after the experiment in the presence **(A)** or absence **(B)** of endothelium. Contractile responses are expressed as grams of tension per cross sectional area (mm^2). Data are shown as means ± SEM. *P<0.05 (Compared to VD) (Refer to text for abbreviations).

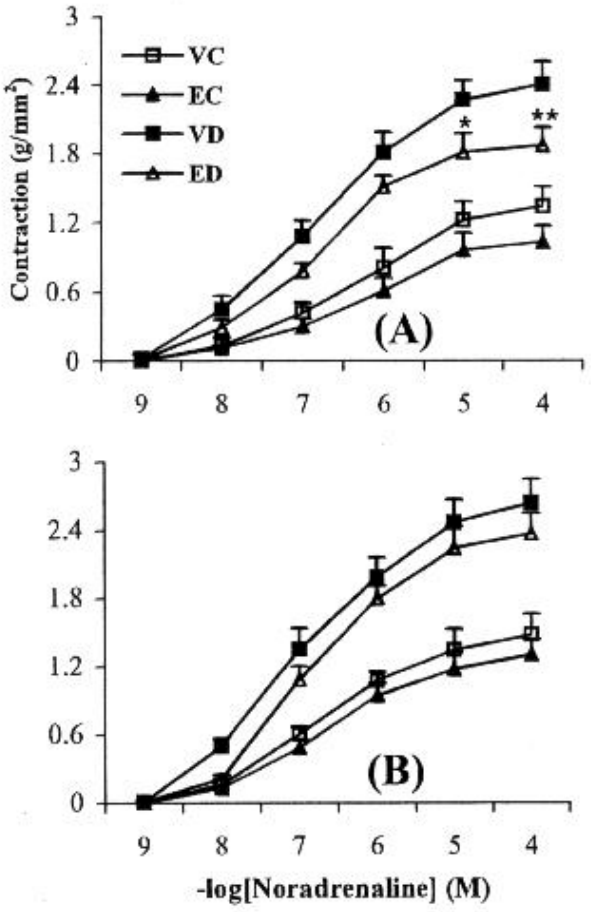

Fig. 2. Cumulative concentration-response curves for NA in aortic preparations 2 months after the experiment in the presence **(A)** or absence **(B)** of endothelium. Contractile responses are expressed as grams of tension per cross sectional area (mm^2). Data are shown as means ± SEM. $^{*}P<0.05$, $^{**}P<0.01$ (Compared to VD) (Refer to text for abbreviations).

enhanced sensitivity of calcium channels [6], and increased sensitivity to adrenergic agonists [11].

Furthermore, oxidative stress is increased due to excessive production of oxygen-free radicals and decreased antioxidant defense systems [12-13].

In this study, it was hypothesized that aqueous leaf extract of fenugreek (TFG) could attenuate the increased responsiveness of aortic rings in diabetic state. Our results demonstrated that fenugreek extract at a dose of 200 mg/kg could partially counteract the increased contractile response of endothelium-intact aortic rings of diabetic rats following NA and/or KCl application. The beneficial effect of chronic fenugreek extract treatment on NA- and KCl-induced contractions was specific for aortas of diabetic rats, because the extract treatment did not produce any significant change in control preparations. The results also directly indicate that TFG extract treatment did not significantly change the sensitivity of vascular smooth muscle of diabetic rats to NA and KCl. Since advanced stage glycosylation end products (AGE) accumulate in diabetes [14] and can inactivate NO [15], it is possible that TFG extract directly inhibits AGE production and prevents NO quenching induced by AGE. Therefore, the ameliorating effect of TFG extract on vascular responsiveness may be closely attributed to its glycosidic compounds with hypoglycemic and anti-hyperglycemic properties [16]. Further investtigations are warranted to study the related mechanisms.

These data indicate that chronic treatment of diabetic rats with aqueous leaf extract of fenugreek could partially prevent the development of changes in vascular reactivity as observed in untreated diabetic rats.

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