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Nutrition Research 25 (2005) 1021–1028
NUTRITION RESEARCH
www.elsevier.com/locate/nutres

Antidiabetic property of fenugreek seed mucilage and spent turmeric in streptozotocin-induced diabetic rats

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Received 29 March 2005; revised 27 May 2005; accepted 14 September 2005

Abstract

The beneficial effect of feeding fenugreek (*Trigonella foenum graecum*) seed mucilage and spent turmeric (*Curcuma longa*) on diabetic status was studied in streptozotocin-induced diabetic rats. Diabetic rats lost weight but body weights were improved by feeding spent turmeric than fenugreek seed mucilage. In diabetic rats, a 30% improvement in urine sugar and urine volume profiles was observed with feeding fenugreek seed mucilage and spent turmeric. Fasting blood glucose showed a 26% and 18% improvement with fenugreek seed mucilage and spent turmeric feeding to diabetic rats, respectively. Fenugreek seed mucilage compared with tumeric was more effective in ameliorating diabetic state.

Keywords: Diabetes; Spent turmeric; Fenugreek seed mucilage; Urine sugar; Fasting blood glucose; Rat

1. Introduction

Diabetes mellitus is one of the major metabolic disorders, afflicting a large proportion of the population all over the world [1,2]. Diabetes is recognized for severe complications, which include diabetic nephropathy, neuropathy, and retinopathy [3-5]. In any form of management of diabetes with insulin or drug, diet is a common factor. With respect to diet, plants and foods of medicinal value have proved to be very useful and are in wide usage as they combine two basic central factors: food and medication [6,7]. Fenugreek seeds are known to improve diabetic status [8-12]. Medicinal properties of fenugreek such as

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0271-5317/$ – see front matter © 2005 Published by Elsevier Inc.
doi:10.1016/j.nutres.2005.09.012

hypocholesterolemic and hypolipdemic have also been studied [13,14]. In Southeast Asia, the water extract of fenugreek seeds is used in the management of diabetes and is known to improve kidney function during diabetes [15,16]. Similarly, turmeric has been known for centuries for its medicinal properties [17,18]. Most health beneficial effects are attributed to its active principle curcumin [19,20]. The active principle of turmeric, curcumin, was found to be beneficial in improving diabetic status and renal lesions [21].

Efficacy of turmeric to reduce blood sugar and the polyol pathway in diabetic rats was also reported [22]. However, not much is known about the biological functions of turmeric when curcumin is removed from the turmeric. The extracted curcumin from turmeric is marketed as a value-added nutraceutical and the material that remains is a by-product called spent turmeric. This spent turmeric is rich in dietary fiber (45%). Some of the polysaccharides of turmeric were shown to possess phagocytosis activating, reticuloendothelial potentiating, and anticomplementary activating activities [23]. Because spent turmeric is rich in dietary fiber and was also shown to possess many biologic activities, it has the potential to be used as a food supplement, including the preparation of diabetic foods.

Dietary fiber is well established as a beneficial food component for improving the complications of many diseases, including diabetes [24,25]. Besides the role in the insoluble matrix to slow absorption of glucose in the gastrointestinal tract, fermentation products of dietary fibers, namely, short-chain fatty acids (SCFAs) such as acetate, propionate, and butyrate, are gaining much interest in recent years [26,27]. We have shown that butyric acid supplementation to streptozotocin-induced diabetic rats improved diabetic status [28]. Butyric acid was reported to improve complications of many diseases and is also known to modulate activities of many key regulatory enzymes [29,30]. Thus, the present study was designed to investigate the beneficial effect of fenugreek seed mucilage and spent turmeric on diabetic status in streptozotocin-induced diabetic rats.

2. Methods and materials

2.1. Chemicals

Streptozotocin and *p*-dinitrosalicylic acid were obtained from Sigma (St Louis, Mo). Refined groundnut oil was used as a source of fat. GOD/POD kits were purchased from Span Diagnostics Limited, India. All other chemicals used were of analytical grade and obtained from Sisco Research Laboratories (Pvt Ltd), Mumbai, India.

2.2. Animals and diet

Three-month-old male Wistar rats weighing around 120 g were taken for the study. The study had approval of the Institutional Animal Ethical Committee (CFTRI). The rats were divided into two groups: one group served as control (6 rats each) and the other group as diabetic (15 each). Rats were assigned to those fed starch, fenugreek seed mucilage, and spent turmeric groups (6 groups of rats) to the following diets: starch-fed control (SFC), starch-fed diabetic (SFD), fenugreek seed mucilage–fed control (FFC), fenugreek seed mucilage–fed diabetic (FFD), spent turmeric–fed control (TFC), and spent turmeric–fed diabetic (TFD). The rats were fed the AIN-76 basal diet as presented in Table 1 [31].

Table 1
Composition of the diets fed to rats

Ingredients	SFC/SFD	Dietary groups FFC/FFD	TFC/TFD
		g/kg Diet	
Casein	200	200	200
AIN-76 Vitamin mix	10	10	10
AIN-76 Mineral mix	35	35	35
Choline chloride	2	2	2
Lipid	100	100	100
Corn starch	653	653	553
Fenugreek seed mucilage	–	25 g seed mucilage per rat per d	–
Spent turmeric	–	–	100

Diets are based the AIN-76.

The fenugreek seeds (*Trigonella foenum graecum*) were purchased from a local market. The seeds (25 g) were soaked in water (125 mL) overnight. The water was decanted and the extract was concentrated (about 10×) by flash evaporation at 35°C. Then, the extract was mixed as a paste with ingredients for the AIN-76 diet. This was done every day and fresh diet was given in the morning. Each rat received the water extract of 25 g of fenugreek seeds.

The spent turmeric (*Curcuma longa*) was obtained from Flavors and Essence Industry, Mysore, India. Principally, this was curcumin-free turmeric. The spent turmeric thus obtained was subjected to extraction (7-8 hours) with acetone to remove residual curcumin using a Soxhlet apparatus and washed with ethanol (95%) and air dried. A powder form of spent turmeric was added at a 10% level at the expense of starch to the AIN-76 diet and stored at 4°C and fresh diet was given every day.

2.3. Induction of diabetes

Diabetes was induced in rats by a single intraperitoneal injection of streptozotocin at 55 mg/kg body weight in freshly prepared citrate buffer (pH 4.5, 0.1 mol/L) injected in a volume of about 1 mL. Control rats received only citrate buffer. The diabetic status of rats was assessed by measuring fasting blood glucose. Soon after streptozotocin injection, glucose water (5%) was given to rats for 2 days [32].

2.4. Collection of urine and blood samples

Rats were maintained in metabolic cages and a 24-hour urine sample was collected under a layer of toluene. This was done on a weekly basis to examine the amount of sugar excreted in the urine. Blood was collected in tubes containing heparin (20 U/mL of blood), either from retro-orbital plexus during the experiment or from the heart at the time of euthanasia by ether anesthesia to measure fasting blood glucose.

2.5. Measurement of urine sugar and fasting blood glucose

The content of reducing sugar present in the urine was measured by the dinitrosalicylic acid method [33]. The rats were fasted overnight and the glucose level in the blood was measured by the glucose oxidase method [34] using a commercial kit.

2.6. Statistical analysis

Data are presented as the mean ± SEM. Comparisons between different groups were made with analysis of variance [35]. P values were considered significant at $P < .05$. All statistical tests were carried out using standard statistical software package available.

3. Results and discussion

High water intake is a characteristic symptom of diabetes. The control rats consumed 20 to 30 mL/d of water compared with 100 mL/d for the SFD rats (Table 2). Both the FFD and TFD groups consumed around 80 mL of water per day, which was statistically significant when compared with the SFD group of rats.

Diet consumption was followed in control and diabetic rats. The SFC rats consumed around 14 g/d diet whereas the SFD group was statistically significantly higher (Table 2). Consumption of diet was higher in the TFC group when compared with the SFC and was about 19 g/d, and the diabetic group (TFD) consumed around 20 g/d. The FFD group consumed around 16 g/d and was not different from the SFD. The higher intake of the spent turmeric diets (TFC and TFD) was observed in rats (Table 2). Dietary fiber–rich foods are known to be consumed in higher amounts, which may also be due to greater palatability [36,37].

Fasting blood glucose was measured in rats at the end of the experiment (Table 2). The fasting blood glucose level was around 120 mg/dL in all control groups and the SFD group had a fasting blood sugar level of 302 mg/dL. Improvements (26% reduction) in fasting blood glucose level were observed in the FFD group compared with the SFD rats. The TFD group also showed an improvement of 18 % in fasting blood glucose level compared with the SFD group.

Table 2
Effect of fenugreek seed mucilage and spent turmeric on food and water intake and fasting blood glucose in diabetic rats

Dietary groups*	Food intake (g/24 h)	Water intake (mL/24 h)	Fasting blood glucose (mg/dL)
SFC	13.8 ± 0.8	22.5 ± 1.1	125.3 ± 05.7
SFD	18.8 ± 1.3[a]	101.3 ± 6.3[a]	302.0 ± 07.9[a]
FFC	12.8 ± 2.0	27.6 ± 4.7	119.3 ± 04.6
FFD	16.0 ± 0.9[b,NS]	83.4 ± 4.7[b]	222.4 ± 13.2[b]
TFC	18.6 ± 0.4[a]	32.3 ± 1.1	122.8 ± 05.6
TFD	20.3 ± 1.3[b,NS]	82.0 ± 4.5[b]	247.7 ± 17.1[b]

Values (mean ± SEM; n = 6 control; n = 9 diabetic groups) in columns having different superscripts are significantly different ($P < .05$) when compared with controls (SFC, FFC, TFC). NS indicates not significant compared with SFD.

[a] Compared with SFC.

[b] Compared with SFD.

* See Table 1 for dietary treatment descriptions.

Table 3
Effect of fenugreek seed mucilage and spent turmeric on body weight in control and diabetic rats

Group*	Body weight (g)		
	Initial	Final	Gain
SFC	116.3 ± 3.8	212.0 ± 15.7	95.7 ± 11.8
SFD	124.1 ± 2.3	123.4 ± 7.1[a]	−0.76 ± 4.07[a]
FFC	120.0 ± 7.3	232.0 ± 3.1	112.0 ± 4.20
FFD	116.6 ± 2.7	162.3 ± 8.6[b]	45.7 ± 5.90[b]
TFC	119.3 ± 6.5	234.0 ± 6.9	114.7 ± 00.4
TFD	120.2 ± 2.1	161.0 ± 5.6[b]	40.8 ± 03.5[b]

Values (mean ± SEM; n = 6 control; n = 9 diabetic groups) in columns having different superscripts are significantly different ($P < .05$) when compared with controls.

[a] Compared with SFC.

[b] Compared with SFD.

* See Table 1 for dietary treatment descriptions.

The average body weight of all rats was about 120 g at the beginning the experiment and the control rats continued to increase weight uniformly to the end of the experiment (Table 3). The diabetic rats lost weight and the SFD rats were the lowest (123 g).

Excretion of urine was monitored weekly in rats and the controls excreted around 20 mL/d. Polyurea condition prevailed in the SFD rats and urine output was about 90 mL/d (Fig. 1). Both fenugreek seed mucilage and spent turmeric feeding to diabetic rats resulted in improvement of urine output (around 70 mL/d) in the diabetic groups (FFD/TFD).

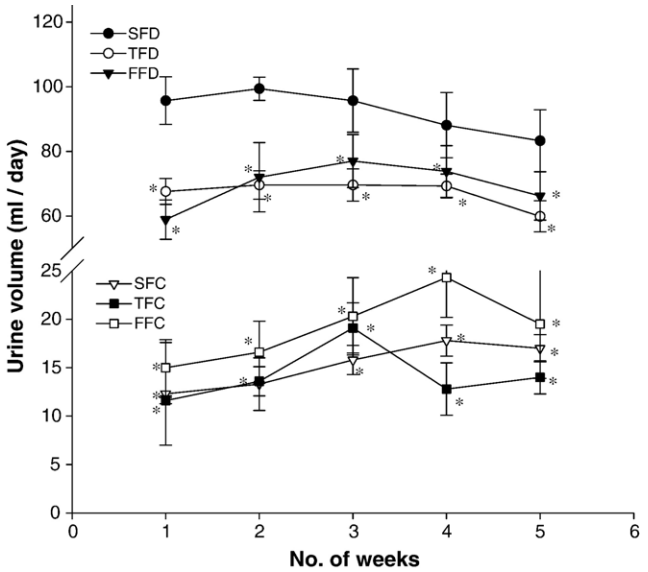

Fig. 1. Effect of fenugreek seed mucilage and spent turmeric on urine volume (mL/24 h) in control and diabetic rats. Values are mean ± SEM (n = 6 rats for controls, SFC, FFC, and TFC; n = 9 rats for diabetic groups, SFD, FFD, and TFD). Asterisk indicates significant difference ($P < .05$) from the SFD group for all time intervals. See Table 1 for dietary treatment descriptions.

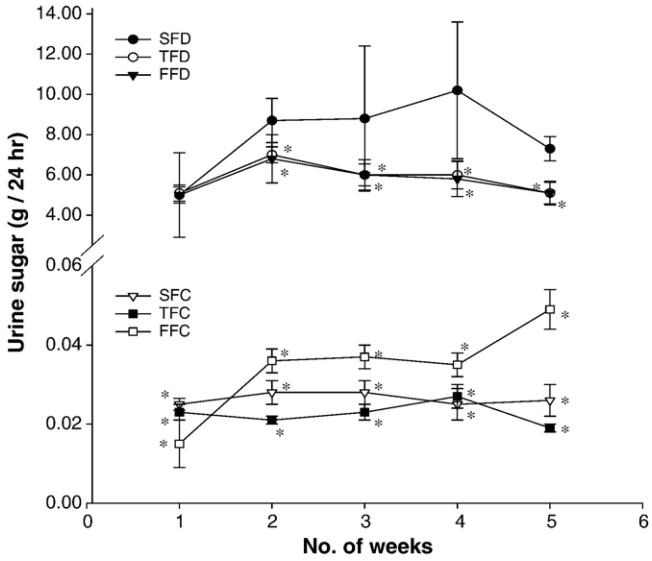

Fig. 2. Effect of fenugreek seed mucilage and spent turmeric on urine sugar (g/24 h) in control and diabetic rats. Values are mean ± SEM (n = 6 rats for controls, SFC, FFC, and TFC; n = 9 rats for diabetic groups, SFD, FFD, and TFD). Asterisk indicates significant difference ($P < .05$) from the SFD group for all time intervals. See Table 1 for dietary treatment descriptions.

Urine sugar in rats was measured weekly (Fig. 2). The control rats demonstrated low levels of sugar in the urine (mg quantities). The diabetic rats (SFD) excreted around 8 g/d during the experimental period. The excretion of sugar in the urine was reduced in both of the FFD and TFD rats, which was around 6 g/d.

The results presented in this study provide some evidence that feeding fenugreek seed mucilage and spent turmeric to the diabetic rat improved diabetic status, as assessed by water consumption, gain in body weight, urine output, urine sugar, and fasting blood glucose. A 26% improvement in fasting blood glucose was observed at the end of the experiment in FFD rats compared with the starch control diabetic rats. It is likely that the beneficial effect of fenugreek seed mucilage is due to some of the bioactive compounds present in the mucilage, including 4-hydroxy isoleucine [38]. 4-Hydroxy isoleucine is a novel amino acid known to facilitate insulin secretion [38]. Spent turmeric feeding at the 10% level also improved the diabetic status of rats by 18%. The effect of spent turmeric in rats may be mainly due to the high amount of dietary fiber. The dietary fiber in this product is both soluble and insoluble. These fibers would facilitate a slower absorption of glucose in the gastrointestinal tract [24,25]. The effect of fermentation products of dietary fiber such as SCFAs (acetate, propionate, and butyrate) should also be considered in the amelioration of diabetic status [26,27]. We have shown earlier that butyric acid supplementation alleviates diabetic status [28]. Besides the actions of dietary fiber and SCFAs in ameliorating diabetic status, a role for bioactive compounds present in fenugreek seed mucilage and spent turmeric on other aspects of diabetes, including kidney damage, needs to be studied in greater detail.

Acknowledgments

Research fellowships were received from the University of Mysore, Mysore and Council of Scientific and Industrial Research, New Delhi. The authors appreciate the financial assistance from the Department of Science and Technology, New Delhi, India, for this research (DST/SP/SO/B67/98).

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