Dose Dependent Impact of Germinated Fenugreek Seed Flour Supplementation on Type 2 Diabetics

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ABSTRACT

The present study was conducted to examine the dose dependent effect of germinated fenugreek seed flour on type 2 diabetics. This case control clinical trial was conducted on 30 type 2 diabetic subjects who were equally divided into 3 groups: experimental group 1 (EG-1), experimental group 2 (EG-2) and control group (CG). The EG-1 subjects were supplemented with20 g germinated fenugreek seed flour in chapatti twice a day and EG-2 patients with 20 g germinated fenugreek seed flour with water four times a day for 60 days.The metabolic parameters studied were fasting glucose, post prandial glucose, serum cholesterol, serum LDL, serum HDL, serum triglycerides, and serum VLDL. Significant reduction was recorded in serum glucose (23%), serum cholesterol (6%), serum LDL (20%) and a slight decrease in serum triglycerides and VLDL. Serum HDL increased significantly by 23 per cent. During the cross over randomized clinical trial, all metabolic parameters except for HDL increased upon stopping the supplementation. Thus, it is concluded that fenugreek seed flour as a low GI food product leads to modest improvement in long-term glycemic and lipidemic control in type 2 diabetics.

Keywords: Type 2 diabetics,Germinated fenugreek seed, VLDL

INTRODUCTION

Recently, under the rapid economic development in advanced countries, lifestyle diseases including metabolic disorders are expanding. The principle of the basic therapeutic policy for these diseases consists of improvement of diet habits and enhancement of physical activities. However, it is difficult to control and change one's established life styles. In such cases, functional foods with therapeutic effects on metabolic disorders are very helpful for the improvement of lifestyle diseases. The importance of diet in diabetes can be traced back to the days of the ancient Ayurvedic physician Sushruta. He reported the consumption of wholesome foods, beside hereditary factors, to be a culprit for the development of diabetes. Indeed, before the discovery of insulin, diet was the only treatment (and was successful at the level of near starvation, for a limited period of time). Today, the pendulum has swung from the starvation diet of Allen to modern high carbohydrate and high fiber diet. Diet has been the sheet anchor in the management of diabetes.

Herbs and spices as medicine have been used for humans from the beginning of evolution; and one of them is fenugreek seeds. Fenugreek (*Trigonella foenum graecum*) is a common herb. Its seeds arecommonly used in Indian homes as a condiment. The seeds can be taken as such after overnight soaking in water or in powder form as a drink in water or buttermilk, 15 minutes before the meal. The fenugreek seed powder can also be incorporated in preparations such as chapati, adai, dhal and vegetables.

However, the seeds are bitter in taste due to presence of bitter saponins which limit their acceptability in foods (Sharma, 1986). It has been possible to debitter fenugreek seeds by employing germination (Shashikala, 1997). Fenugreek has been used for centuries but few studies confirm its efficacy in diabetes treatment. Earlier studies reported that sprouting or overnight soaking and washing of fenugreek seeds in running water removes the bitterness to a certain extent and makes possible its use in increased quantities for incorporation into various preparations which are commonly consumed. The beneficial effects of processing of fenugreek seed may be attributed to an increase in low methoxy salts of Calcium and Magnesium as well as proto-pectin. It is probable that formation of these constituents has a role to play in reducing the blood sugar levels or it may also be likely that an active, hypoglycemic principle might have increased during processing like soaking and germination (Hooda and Jood, 2003).

It may have beneficial effects in pancreatic and other tissues and may improve glucose and

carbohydrate absorption, as well as decrease insulin resistance. It is said that fenugreek, reveals a potential benefit in diabetes either in a mixture of water or milk products or in cooking (Madar, 1987).

Thus, the present study was taken up to investigate the effect of different doses of germinated fenugreek seed flour, on blood glucose and lipid profile in type 2 diabetics.

MATERIALS AND METHODS

Preparation of test sample

The cleaned seeds were soaked in tap water at the ratio of 1:5 (w/v) at room temperature for 12 hr. The water was changed after 6 hr.The soaked seeds were tied in a muslin cloth for germination in the dark at room temperature for 24 hr till the length of the sprout was about 1 cm. The germinated seeds were oven dried at 40^0C in aluminum trays for 6 hr. flour was prepared by grinding dried germinated fenugreek seeds in grinder. Flour was sieved with a metal sieve of 80 mesh size. Flour was packed in HDPE zip lock bags for distribution to experimental subjects.

Study subjects

Thirty type 2 diabetics (14 male and 16 female) aged 30-60 years, with body mass index (BMI) in the range of 18.5-32.3 kg/m2, were selected out of 100 patients attending the hospital (Mehta Charitable Hospital, Haldwani, Uttarakhand, India). They were selected under the supervision of the Chief Medical Officer on the basis of criteria that they were on mild medication and were not eating fenugreek on regular basis. Two subjects were on diet control, one subject was taking herbal preparation, 16 subjects were taking sulfonylurea, 10 subjects were taking biguanides, and one subject was on a combination of sulfonylurea and biguanides.

Approval to conduct the study was obtained from the Chief Medical Officer. All patients were informed about the purpose and nature of the study and a written informed consent was obtained from each subject.

Study design

Case control clinical trial: Three groups, experimental group 1 (EG-1), experimental group 2

(EG-2) and control group (CG) consisting of 10 subjects each were formed. The EG-1 patients were supplemented with 20 g germinated fenugreek seed flour in chapatti twice a day and EG-2 patients with 20 g germinated fenugreek seed flour with water four times a day and no supplementation was given to CG. The trial was conducted for a period of 60 days. All subjects were examined for biochemical parameters on day 0 and then on day 60 (i.e. at starting and ending supplementation). Comparison of all EG-1, EG-2, with CG was done to see the effect of supplemented test sample.

Follow up of subjects was done in order to know the number and reasons of dropouts during the study.

Study variables

Serum glucose was analyzed by enzymatic method using Eco-Pak Glucose kit; serum cholesterol by enzymatic method using Autozyme Cholesterol kit ; serum HDL cholesterol enzymatically using Autozyme HDL-cholesterol precipitating kit ; serum triglycerides (TG) were measured enzymatically using Autozyme Triglyceride kit ; serum VLDL and serum LDL were calculated on the basis of a formula.

Statistical analysis

Paired t-test was employed to find out the differences amongst the groups before and after the supplementation. Student's t-test was used to find out differences among EGs and CG during clinical trials (15).

RESULTS

The effect of feeding germinated fenugreek seed flour on serum glucose level, serum cholesterol, serum LDL, serum HDL, serum TG, and serum VLDL is shown in Table 1 and Table 2.

Decrease in the fasting blood glucose level in group I was 17.65 per cent however there was 18.49 per cent decrease in mean fasting blood glucose level in Group II. When interpreting results about changes in postprandial blood glucose level, there was significant difference from initial to final. In group I mean postprandial blood glucose was 244.10 mg/dl which decreased to 186.70 mg/dl after 60 days of GFSF supplementation. Whereas in group II mean initial postprandial blood glucose decreased from 239.00 mg/dl to

170.94 mg/dl. Significant difference was observed in initial mean blood glucose level to final mean blood glucose level in group I and group II both. In control group non-significant decrease in mean fasting blood glucose from 147.83 mg/dl to 146.50 mg/dl has been found. While decrease in postprandial glucose in control subjects was from 269.52 mg/dl to 266.60 mg/dl.

There was significant difference ($P< 0.05$) in the mean fasting blood glucose reduction between group I and group II. There was 17.65 per cent reduction in group I subjects whereas 18.49 per cent reduction in mean fasting blood glucose level was observed in group II subjects.

Results regarding mean postprandial blood glucose level showed that there was non-significant difference between group I and group II. On the other hand there was 23.51 per cent reduction in group I while 28.47 per cent reduction was observed in group II after 60 days of supplementation. This might be attributed to cumulative effect of GFSF intake on lowering blood glucose after every meal.

Result showed significant decrement in triglycerides level in both group I (126.72 mg/dl to 114.80 mg/dl) and in group II (125.10 mg/dl to 110.68 mg/dl) after consumption of GFSF for 60 days. However, decrease in control group was found to be non – significant from 212.25 mg/dl to 197.10 mg/dl after 60 days. There was significant reductions in total cholesterol level in group I and group II both after consumption of GFSF for 60 days. There was decrease in total cholesterol from 275.09 mg/dl to 247.28 mg/dl in group I and from 192.40 mg/dl to 163.25 mg /dl in group II. On the other hand there was slight decrease in control group from 179.13 mg/dl to 178.33 mg/dl, however this decrease was found to be non-significant. HDL cholesterol was found to increase non-significantly in group I (66.57 – 68.24 mg/dl) and group II (47.74 mg/dl to 50.49 mg/dl) after GFSF intake for 60 days. While in control group slight increase was observed from 43.28 mg/dl to 43.80 mg/dl however, this increment was found to be non-significant. The LDL cholesterol of group I decreased significantly to 149.42 mg/dl from 178.25mg/dl after 60 days. Similar result was observed in group II where LDL cholesterol reduced significantly from 114.05 mg/dl to 89.86 mg/dl after consumption of GFSF for 60 days. Non -significant decrease in LDL cholesterol from 93.38 mg/dl to 92.50 mg/dl was observed in control group. There was non-significant reduction in VLDL cholesterol in group I from 30.16 mg/dl to 28.55 mg/dl and in group II from 30.68 mg/dl to 22.90 mg/dl after 60 days intake of GFSF. Non – significant reduction from 42.47 mg/dl to 42.00 mg/dl was observed in control group. The total Cholesterol to HDL ratio is a number that is helpful in predicting an individual's risk of developing atherosclerosis. The number is obtained by dividing the total cholesterol value by the value of the HDL cholesterol. High ratios indicate higher risk of heart attacks and vice-versa. TC/HDL ratio decreased significantly from 4.13 to 3.60 in Group I and in group II from 4.03 to 3.23 after 60 days of the GFSF intake. Whereas, in control group non-significant reduction was observed from 4.13 to 4.07. The LDL/HDL ratio is more important ratio than Total Cholesterol/HDL because LDL is a measure of bad cholesterol and HDL is a measure of good cholesterol whereas the total cholesterol is sum of HDL, LDL and VLDL. The LDL/HDL ratio of group I decreased significantly from 2.67 to 2.18 and of group II from 2.38 to 1.77 after 60 days of GFSF supplementation. In control group slight decrease from 2.15 to 2.11 was also observed. Results in this study proved that germinated fenugreek seed flour is effective as hypolipidemic agent. This effect may be due to its content of antioxidants, carotenoids, polyphenolic compounds and flavonoids and some components like saponins (which can bind cholesterol), amino acid 4-hydroxyisuolcine, dehydroxy phenylalanine (which increase antioxidant activity) and also to dietary fiber in fenugreek. The aforementioned explanation was in agreement with the finding of Narender *et al.*(2006).

DISCUSSION

High levels of total cholesterol and, more importantly, LDL-cholesterol in blood are major coronary risk factors (NCEPEP, 1994). Sowmya and Rajyalakshmi (1999)reported decrease in the levels of total cholesterol with reduction in the LDL fraction after feeding of germinated fenugreek seeds 12.5 g and 18.0 g per day. Earlier experiments also showed similar results with decrease in levels of total cholesterol corresponding to a decrease in the LDL fraction (Sharma *et al.,* 1990). Fenugreek has been shown to bring about a hypocholesterolemic effect through different mechanisms. One such mechanism is increased excretion of fecal bile acids and neutral sterols with depletion of cholesterol stores in the liver (Sharma, 1986). Dietary fenugreek stimulates bile formation in the liver and the conversion of cholesterol into bile salts or the fiber potentially reduces the rate of diffusion towards the absorptive mucosal surface and has been shown to alter glucose and cholesterol

absorption (Anderson and Chen, 1979) or the soluble fiber increases the viscosity of the digest and increases the thickness of the unstirred layer in the small intestine or inhibits uptake of cholesterol and bile acids. Soluble fiber is an excellent substrate for fermentation by the microorganisms in the large bowel. The volatile fatty acids produced by fermentation enter the blood stream and appear to suppress hepatic cholesterol synthesis. It is also reported that cholesterol lowering activity of the galactomannan depends on the D-galactose, D-mannose ratio. Fenugreek seeds contain diosgenin and trigonellin in the form of glycosides. These saponins can form complexes with cholesterol in the intestine reducing its absorption. It has also been reported that the ingested fenugreek saponins are partially hydrolyzed to diosgenin in the gastrointestinal tract and that diosgenin interferes with cholesterol absorption (Sauvaire *et al.,* 1991). Another possibility for the lowering effect could be based on amino acid pattern of fenugreek proteins.

In the present study administration of GFSF to type 2 diabetics also showed a significant reduction in the levels of triglycerides, serum total cholesterol, LDL fraction and VLDL fraction. However, with regard to the HDL fraction no significant changes were observed. Earlier experiments with raw fenugreek showed a significant reduction in the levels of serum total cholesterol, LDL/VLDL fractions without altering HDL fraction (Prasanna, 2000). Similar results were also reported with fenugreek fed in combination with some herbs for four weeks in Non-Insulin Dependent Diabetes Mellitus (NIDDM) patients (Praveen *et al.,* 1987), where no significant changes in the levels of HDL cholesterol, VLDL cholesterol and serum triglycerides were reported. Lowering of total and LDL cholesterol levels will also reduce their ratio with HDL cholesterol, thus, reducing the risk for coronary artery disease. This could be an important step in the management of hypercholesterolemic patients. Moosa *et al.* (2006) reported that administration of fenugreek seed powder of 25 g orally twice daily for 3 weeks and 6 weeks produces significant ($P<0.001$) reduction of serum total cholesterol, triglycerides and LDL-cholesterol in hypercholesterolemic group but the change of serum HDL-cholesterol was not significant. The quality and quantity of protein in the diets have a direct effect on the levels of cholesterol. Generally plant protein appears to lower cholesterol level (James, 2004). The plant protein in fenugreek is 26 -35per cent, so it might exert a lipid lowering effect (Sharma, 1986). Narender *et al.* (2006) mentioned that amino acid 4-hydroxyisoleucine present in fenugreek seeds significantly decreased the plasma triglycerides, total cholesterol and free fatty acids by 33, 22 and 14% respectively, meanwhile HDL cholesterol/total cholesterol ratio increased by 39%.

Table 1. Impact of germinated fenugreek seed flour supplementation on biochemical parameters in type 2 diabetics

Parameters (mg/dl)	Group	Baseline value	Final value	Difference	S/NS
Fasting Blood Glucose	EG-1	159.29 ± 11.59	131.16 ± 22.50	28.13	S
	Control	147.83 ± 37.58	146.50 ± 31.85	1.33	NS
P. P. Blood Glucose	EG-1	244.10 ± 21.48	186.70 ± 27.33	57.40	S
	Control	269.52 ± 26.17	266.6 ± 26.70	2.92	NS
Triglycerides	EG-1	126.72 ± 16.53	114.80 ± 21.80	11.92	S
	Control	212.25 ± 75.62	197.10 ± 81.57	15.15	NS
Total cholesterol	EG-1	275.09 ± 11.80	247.28 ± 12.77	27.81	S
	Control	179.13 ± 27.07	178.33 ± 27.44	0.8	S
HDL-C	EG-1	66.57 ± 5.5	68.24 ± 5.54	1.67	NS
	Control	43.28 ± 5.92	42.8 ± 5.63	0.48	NS
LDL-C	EG-1	178.25 ± 10.85	149.42 ± 11.75	28.83	S
	Control	93.38 ± 23.87	92.50 ± 23.58	0.88	NS
VLDL-C	EG-1	30.16 ± 6.28	28.55 ± 2.99	1.61	NS
	Control	42.47 ± 14.95	41.00 ± 14.95	1.47	NS
TC/HDL ratio	EG-1	4.13 ± 0.32	3.60 ± 0.33	0.53	S
	Control	4.13 ± 0.63	4.07 ± 0.63	0.06	NS
LDL/HDL ratio	EG-1	2.67 ± 0.24	2.18 ± 0.29	0.49	S
	Control	2.15 ± 0.59	2.11 ± 0.59	**0.04**	NS

* Mean±SD; S/NS = significant/non significant (P≤0.05)

Table 2. Impact of germinated fenugreek seed flour supplementation on biochemical parameters in type 2 diabetics

Parameters (mg/dl)	Group	Baseline value	Final value	difference	S/NS
Fasting Blood Glucose	EG-2	134.55 ± 18.65	109.66 ± 5.76	24.89	S
	Control	147.83 ± 37.58	146.50 ± 31.85	1.33	NS
P. P. Blood Glucose	EG-2	239.00 ± 24.54	170.94 ± 19.86	68.06	S
	Control	269.52 ± 26.17	266.6 ± 26.70	2.92	NS
Triglycerides	EG-2	125.10 ± 8.65	110.68 ± 12.13	14.42	S
	Control	212.25 ± 75.62	197.10 ± 81.57	15.15	NS
Total cholesterol	EG-2	192.40 ± 24.57	110.68 ± 12.13	81.72	S
	Control	179.13 ± 27.07	178.33 ± 27.44	0.80	S
HDL-C	EG-2	47.74 ± 4.74	50.49 ± 4.58	-2.75	NS
	Control	43.28 ± 5.92	42.8 ± 5.63	0.48	NS
LDL-C	EG-2	114.05 ± 22.49	89.86 ± 17.33	24.19	S
	Control	93.38 ± 23.87	92.50 ± 23.58	0.88	NS
VLDL-C	EG-2	30.16 ± 6.28	28.55 ± 2.99	1.61	S
	Control	42.47 ± 14.95	41.00 ± 14.95	1.47	NS
TC/HDL ratio	EG-2	4.03 ± 0.34	3.23 ± 0.22	0.8	S
	Control	4.13 ± 0.63	4.07 ± 0.63	0.06	NS
LDL/HDL ratio	EG-2	2.38 ± 0.26	1.77 ± 0.23	0.61	S
	Control	2.15 ± 0.59	2.11 ± 0.59	0.04	S

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