

## Compilation of Fenugreek Studies

<b>A fibre cocktail of fenugreek, guar gum and wheat bran reduces oxidative modification of LDL induced by an atherogenic diet in rats</b> .....	<b>pages 2-10</b>
<b>Improvement in the nutraceutical properties of fenugreek</b> .....	<b>pages 11-19</b>
<b>Effect of Supplementation of Traditional Medicinal Plants on Serum Lipid Profile in Non-Insulin Dependent Diabetics</b> .....	<b>pages 20-25</b>
<b>Therapeutic applications of Fenugreek.....</b>	<b>pages 26-32</b>
<b>Effect of Trigonella foenum-graecum seeds on glycemic control and insulin resistance in type 2 diabetes mellitus: a double blind placebo controlled study- fenugreek .....</b>	<b>page 33</b>
<b>Effect of Trigonella foenum-graecum (fenugreek) extract on blood glucose, blood lipid and hemorheological properties in streptozotocin-induced diabetic rats</b> .....	<b>page 34</b>
<b>Hypoglycaemic effect of fenugreek seeds in non-insulin dependent diabetic subjects</b> .....	<b>page 35</b>
<b>Relationship between structure and function of dietary fibre: A comparative study of the effects of three galactomannans on cholesterol metabolism in the rat</b> .....	<b>page 36</b>
<b>Soluble dietary fibre fraction of Trigonella foenum-graecum (fenugreek) seed improves glucose homeostatis in animal models of type 1 and type 2 diabetes by delaying carbohydrate digestion and absorption, and enhancing insulin action</b> .....	<b>page 37</b>
<b>Polysaccharide composition of a gel fraction derived from fenugreek and its effect on starch digestion and bile acid absorption in rats..</b> .....	<b>page 38</b>
<b>Fenugreek dietary fibre a novel class of functional food ingredient</b> .....	<b>pages 39-42</b>

# A fibre cocktail of fenugreek, guar gum and wheat bran reduces oxidative modification of LDL induced by an atherogenic diet in rats

Nandini Venkatesan,<sup>1,\*</sup> S. Niranjali Devaraj<sup>2</sup> and H. Devaraj<sup>3,\*</sup>

<sup>1</sup>Vaccine Research and Development Center, National Health Research Institutes, Miaoli County Taiwan R.O.C.; <sup>2</sup>Department of Biochemistry and Molecular Biology, University of Madras, Chennai, India; <sup>3</sup>Department of Zoology, School of Life Sciences, University of Madras, Chennai 600025, India

Received 2 March 2006; accepted 1 June 2006

## Abstract

**Background** LDL (low-density lipoprotein) oxidation is a key trigger factor for the development of atherosclerosis. Relatively few studies exist on the impact of dietary fibre on LDL oxidation. This study was undertaken to evaluate the influence of a novel fibre mix of fenugreek seed powder, guar gum and wheat bran (Fibernat) on LDL oxidation induced by an atherogenic diet.

**Method** Male Wistar albino rats were administered one of the following diets: (1) a control diet that was fibre-free (Group I); (2) an atherogenic diet containing 1.5% cholesterol and 0.1% cholic acid (Group II) or (3) an atherogenic diet supplemented with Fibernat (Group III). Peroxidative changes in low-density lipoprotein (LDL) and the oxidative susceptibility of LDL and the LDL + VLDL (very low-density lipoprotein) fraction were determined. As a corollary to the oxidative modification theory, the titer of autoantibodies to oxidised LDL (oxLDL) was determined at various time points of the study. In addition, plasma homocysteine (tHcy) and lipoprotein (Lp (a)), apolipoprotein (apoB), cholesterol, triglyceride, phospholipid and  $\alpha$ -tocopherol content of LDL were determined.

**Results** A decrease in malonaldehyde (MDA) content ( $p < 0.05$ ) and relative electrophoretic mobility (REM) of LDL was observed in the group III rats as compared to the group II rats. An increase in lag time to oxidation ( $p < 0.01$ ) and decrease in maximum oxidation ( $p < 0.01$ ) and oxidation rate ( $p < 0.01$ ) were observed in the LDL + VLDL fraction of group III rats. In group II rats, formation of autoantibodies to oxLDL occurred at an earlier time point and at levels greater than in the group III rats. Fibernat, had a sparing effect on LDL  $\alpha$ -tocopherol, which was about 51% higher in the group III rats than in the group II rats; apo B content of LDL was reduced by 37.6% in group III rats. LDL of group III rats displayed a decrease in free and ester cholesterol ( $p < 0.01$ ) as compared to that of group II. A decrease in plasma homocysteine ( $p < 0.01$ ) and an increase in GSH ( $p < 0.05$ ) were also observed in group III rats when compared with that of group II.

**Conclusion** Fibernat administration appears to combat oxidative stress resulting in a trend to lower oxidative modification of LDL. In addition, the cholesterol and apo B content of LDL were reduced significantly with a sparing effect on LDL  $\alpha$ -tocopherol. This novel fibre preparation could be an effective diet therapy and therefore needs further investigation.

**Key words:** dietary fibre, lipid peroxidation, oxidative modification, autoantibodies

\* Nandini Venkatesan and H. Devaraj are the corresponding authors.

Address for offprints: Nandini Venkatesan, Vaccine Research and Development Center, National Health Research Institutes, Keyan Road, Miaoli County, Taiwan R.O.C. (E-mail: 940714@nhri.org.tw)

## Introduction

Atherosclerosis and its complications constitute the most common cause of death in Western societies. Oxidative modification of LDL (low-density lipoprotein) is an important if not obligatory event in atherosclerosis [1]. Inhibition of LDL oxidation can reduce the risk of atherosclerosis independent of lowering plasma cholesterol levels [2]. A diet rich in fibre and vegetables has been shown to reduce atherogenesis and is often recommended as the first line of management.

Natural food products that can offer protection against LDL oxidation appear to have a promising role in the management of atherosclerosis [3]. Studies indicate that intake of some fibres impacts favourably on certain risk factors for atherosclerosis and even LDL oxidation [4]. Fibernat is a novel fibre mix containing soluble and insoluble fibre (fenugreek, guar gum and wheat bran in the ratio of 70:15:15, respectively). Our group has previously observed the beneficial effect of Fibernat intake on cholesterol homeostasis with reference to regulation of hepatic and plasma lipoprotein cholesterol concentrations and apolipoprotein B, E (apo B, E) receptor expression [5]. It was therefore of interest to determine the effect of Fibernat intake on some indicators of atherosclerotic risk and on the oxidative susceptibility of low-density lipoproteins.

Our first objective was based on the hypothesis that the oxidative susceptibility of LDL is influenced by the LDL concentration of pro-atherogenic apolipoprotein B (apo B) and  $\alpha$ -tocopherol, which has been reported to be anti-atherogenic although this does remain a topic of controversy. We observed whether Fibernat intake was able to alter the concentration of apo B and  $\alpha$ -tocopherol in LDL and hence have an influence on the oxidative modification of LDL. Oxidative modification to LDL was monitored by assaying the end products of lipid peroxidation such as conjugated dienes (CD), lipid peroxides (LPO), and REM of LDL. The oxidisability indices were determined in the LDL + VLDL fraction also.

Our other main objective was to determine the formation of autoantibodies to oxidised LDL at different time points of the experimental period. This was based on the hypothesis that oxidative change if any to LDL would result in an increase in circulating levels of autoantibodies to oxidised LDL. We assumed that an increased uptake of LDL and VLDL by the hepatic receptors [5] would decrease the "circulation residence" time of LDL + VLDL. This, in turn, might reduce the propensity of LDL to become oxidised. In addition, the levels of plasma homocysteine (tHcy) and Lipoprotein (a) [Lp (a)] were evaluated.

## Material and Methods

### *Experimental animals and diets*

All experiments and animal care were in accordance with the guidelines issued by the Indian Council of Medical Research and Indian National Science Academy (INSA) Guidelines for Care and Use of Animals in Scientific Research 2000. Guidelines as directed by the "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were also followed. Eighteen male albino rats (Wistar strain) were obtained from the Fredrick Institute of Plant Protection and Toxicology (FIPPAT) and were fed on a standard pellet feed (Hindustan Lever India Ltd., Bangalore, India) for an adaptation period of one week. Their average weights were  $120 \pm 10$  g and their average age was 2 months. They were then randomly divided into three groups of six animals each. Group I was fed a control diet; group II was fed an atherogenic diet containing 1.5% cholesterol and 0.1% cholic acid and group III was fed the above atherogenic diet + Fibernat (Table 1). The body weights of the rats were recorded every 48 h during the experimental period of 6 weeks. Food intake was recorded over two, 3-day periods. The atherogenic diet was based on the recommendations of Bajwa *et al.* [6] with an excess of Vitamin D<sub>2</sub> (1.25 million USP unit/kg of the diet i.e., 31.25 mg/kg). Although the rat as an animal model for atherosclerosis has been in decline because of the differences in response to diet (HDL being the major carrier of plasma cholesterol in rats) and its resistance to atherosclerosis, rats can be induced to develop atherosclerosis by administration of an excess of Vitamin D<sub>2</sub> [6]. At the end of the experimental period of 42 days, animals were sacrificed under light ether anaesthesia. Blood was drawn into tubes containing 0.1% EDTA.

Table 1. Composition of diets/kg

Diet group	Group I (Control) FF	Group II (Atherogenic diet)	Group III (Atherogenic diet + Fibernat)
Sucrose	550	534	434
Butter	250	250	250
Corn oil	140	140	140
Cholesterol	0	15	15
Cholic acid	0	1	1
Fibernat	0	0	100
Mineral mix	50	50	50
Vitamin mix	10	10	10

### *LDL isolation and characterisation*

Plasma separated from EDTA anti-coagulated fresh blood was used for the separation of the lipoprotein fractions. Lipoprotein separation was carried out by the method of Chung *et al.* [7] using a single discontinuous density gradient ultra centrifugation with solid potassium bromide (KBr) in a Beckman L8-60 model ultracentrifuge at 10 °C. A discontinuous NaCl/KBr density gradient was formed by adjusting the density ( $d$ ) of the plasma to 1.3 g/ml with KBr and layering normal saline  $d = (1.006 \text{ g/ml})$  over the adjusted plasma. The tubes loaded with the sample and gradient were immediately placed in a vertical rotor at room temperature and centrifuged at 10 °C with a slow start and then at  $100,000 \times g$ . At the end of the run, tubes were removed from the rotor and fractions were collected from each tube by bottom puncture using a 0.22 gauge needle. Plasma samples stained with oil Red'O' were used to qualitatively check the different lipoprotein classes. The "crude" LDL was collected, recentrifuged and obtained as a single, pure fraction.

### *LDL composition*

LDL composition was determined by estimating the free and esterified cholesterol, triglycerides and phospholipids by means of enzymatic kits. Apo B concentration of LDL was determined by the immunoturbidometric assay (K assay) using a polyclonal antibody that interacts with the apo B forming immune complexes that cause an increase in turbidity, which is directly proportional to the concentration of apo B. The  $\alpha$ -tocopherol content of LDL was estimated by the method of Desai [8] after subjecting the LDL thrice to hexane extraction to release the  $\alpha$ -tocopherol.

### *Lipid peroxidative changes in LDL*

#### *HPLC analysis of malonaldehyde*

Malonaldehyde, a lipid peroxidative product, was measured in isolated LDL using high performance liquid chromatography (HPLC) [9] with a Shim-Pack (CLC-NH<sub>2</sub>) column (size 25 cm length  $\times$  4.6 mm internal diameter). The absorbance was 270 nm and the range employed was between 0.04 and 0.32 absorbance units for full recorder scale. The mobile phase consisted of acetonitrile 0.03 M Tris, pH 7.4 (1:9 v/v). The flow rate applied was 2 ml/min.

#### *Conjugated diene concentrations*

Conjugated diene concentrations in LDL were monitored by differential absorbance spectra [10] and expressed as a ratio (A 233/A215).

### *REM of LDL*

Oxidative modification of separated LDL was assessed by observing LDL mobility in agarose gel electrophoresis using 0.5% agarose gel in barbital buffer medium (pH 8.6, 0.05 M). *In vitro* modification of LDL was performed by adding 0.5  $\mu\text{M}$  CuCl<sub>2</sub>·2H<sub>2</sub>O solution to 0.2 mg protein/ml LDL [4].

### *Detection of autoantibodies against oxidised LDL*

Plasma of animals was tested for the presence of oxidised LDL antibody (oLAb) by the method of Damasceno [11] with some modifications. Human LDL was oxidised *in vitro* as previously described [4]. Plates were coated with 10.0  $\mu\text{g/ml}$  oxLDL protein in carbonate/bicarbonate buffer, pH 9.4, and incubated overnight at 4 °C. The plates were blocked for 2 h with 50.0 g/L skim milk in PBS for 2 h and washed with PBS-Tween 20. The concentration of Tween 20 in PBS was 0.05%. Next, 50  $\mu\text{l}$  of serum (at a dilution of 1:50 (v/v) in PBS) was added to the wells and incubated at room temperature for 2 h. The plates were washed four times with PBS-T, and 50  $\mu\text{l}$  of horseradish peroxidase-conjugated polyclonal swine and anti-rabbit IgG antibody diluted 1:1000 in PBS (v/v) were added. The plates were then incubated for 40 min at room temperature, washed, and the colour was developed by the addition of o-phenylenediamine in 0.1 mol/L citrate/phosphate buffer, pH 5.0. After stopping the reaction by adding 2 N HCl, the optical density was measured with a microplate reader at 492 nm. The data are presented as % increase = [(value at defined time point – baseline value at day 0):baseline value at day 0]  $\times$  100.

### *Susceptibility of LDL + VLDL to oxidation*

The LDL + VLDL fraction was isolated by precipitation with heparin, magnesium chloride and sucrose [12]. Oxidation of the LDL + VLDL fraction was determined [13] using 100  $\mu\text{g}$  of protein from the LDL + VLDL fraction suspended in 5  $\mu\text{M}$  CuSO<sub>4</sub> solution. The Thiobarbituric acid-reactive substances (TBARS), formed were estimated by the routine Thiobarbituric acid (TBA) assay at 5-min intervals for two 1/4 hours [14] and expressed as nmoles MDA/mg protein. A graph depicting time against nmoles MDA formed was plotted and indices of oxidation such as lag time, oxidation rate and maximum oxidation were derived from this.

### *Estimation of plasma GSH*

Plasma GSH was evaluated by the method of Moron *et al.* [15].

### Assay of Lp (a) and tHcy

Plasma Lp (a) concentrations were measured using the commercially available latex agglutination assay (Biostat diagnostics Ltd., Stockport USA). An anti-Lp (a) antibody is adsorbed to latex particles and this antibody-latex suspension is incubated with plasma. The resulting agglutination that occurs when the Lp (a) in the plasma binds to the anti-Lp (a)/latex particle is detected by a change in absorbance. The axis homocysteine enzyme immunoassay was used for the quantitative determination of total L-homocysteine in the plasma. Prior to the immunoassay, protein bound homocysteine was reduced to free homocysteine and enzymatically converted into S-adenosyl L-homocysteine (SAH).

### Statistical analysis

Statistical analysis of the results obtained was done by One-Way ANOVA with Tukeys Post Hoc comparisons. A probability of 0.05 or less was considered as statistically significant.

## Results

### Food intake and body weight

Food intake (measured as total food consumption per cage) was similar between the dietary groups though it should be noted that the number of observations was small. Body weight gain was comparable among the three groups, which showed that Fibernat intake did not affect these two parameters.

### LDL composition

In response to Fibernat intake, a decrease in free and esterified cholesterol and apo B content was observed in Group III rats ( $p < 0.01$ ). LDL  $\alpha$ -tocopherol concentrations were significantly higher ( $p < 0.01$ ) in-group III rats as compared to group II rats (Table 2).

Table 2. LDL composition of rats fed diets that were fibre-free (Control), atherogenic or atherogenic + Fibernat supplemented diets

Parameters	Group I (Control)	Group II (Atherogenic diet)	Group III (Atherogenic diet + Fibernat)
Ester cholesterol (mg/dl)	24.2 ± 0.7	36.7 ± 2.9*	29.3 ± 3.2**
Free cholesterol (mg/dl)	8.0 ± 0.9	28.5 ± 2.7*	10.6 ± 1.6**
Triglycerides (mg/dl)	11.2 ± 4.8	20.8 ± 2.8*	18.3 ± 1.7
Phospholipids (mg/dl)	14.3 ± 1.5	42.3 ± 6.2*	35.7 ± 8.3
ApoB (mg/dl)	5.9 ± 0.9	11.7 ± 1.8*	7.3 ± 0.8**
$\alpha$ -tocopherol ( $\mu$ g/mg protein)	1.36 ± 0.01	0.63 ± 0.02*	0.95 ± 0.01**

Values are mean ± SD of six animals in each group.

\*Group II vs. Group I,  $p < 0.01$ ; \*\*Group III vs. Group II,  $p < 0.01$ .

### Lipid peroxidative changes in LDL

Table 3 depicts the LDL concentrations of malonaldehyde and conjugated dienes in the three groups of rats. Malonaldehyde concentrations were significantly lower ( $p < 0.05$ ) and conjugated diene concentrations did not show a significant decrease in Fibernat-fed rats as compared to rats fed the atherogenic diet alone.

### REM of LDL

Oxidative modifications of the LDL molecule were evaluated by observing the relative mobility of LDL isolated from the various groups of rats (Fig. 1). The LDL of rats fed the atherogenic diet alone (group II) showed an increase in the electrophoretic mobility which was comparable to the migration of *in vitro* oxidised LDL. The LDL of rats from group III migrated on par with that of group I.

Table 3. Malonaldehyde and conjugated diene concentrations in LDL of rats fed fibre and cholesterol free (control) diet, atherogenic diet and atherogenic diet + Fibernat

Group	Group I (Control)	Group II (Atherogenic diet)	Group III (Atherogenic diet + Fibernat)
MDA (nmol/mg LDL protein)	23.8 ± 1.8	28.5 ± 4.1*	24.1 ± 1.9***
Conjugated dienes (arbitrary units)	2.54 ± 0.9	4.91 ± 0.7**	3.89 ± 0.6

Values are mean ± SD of six animals in each group.

\*Group II vs. Group I,  $p < 0.05$ ; \*\*Group II vs. Group I,  $p < 0.01$ ; \*\*\*Group III vs. Group II,  $p < 0.05$ .

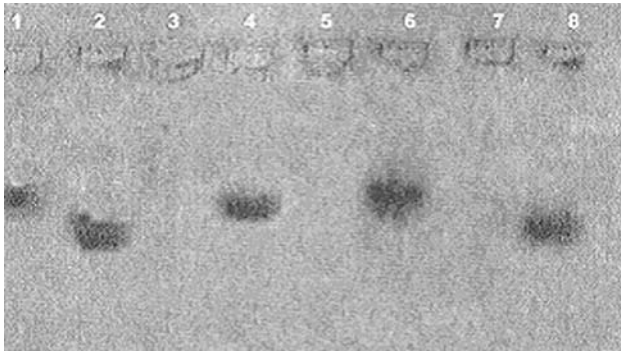


Fig. 1. Electrophoretic mobility of LDL. 30  $\mu$ g LDL (in terms of protein) was loaded in each well. Lane 1, LDL from group I rats; lane 2, LDL from group II rats; lane 4, LDL from group III rats; lane 6, native LDL; lane 8, 'in vitro' oxidised LDL. Results are representative of three experiments using different preparations of LDL.

#### Autoantibodies to oxidised LDL in the circulation

Day 0 was chosen as the baseline value. A significant increase in anti-oxLDL antibodies was observed in groups II and III rats from the 14th day of the experimental period as compared to baseline. However, in the Fibernat-supplemented group, oxLDL autoantibody levels were significantly lower than that of group II at the 28th and 42nd day of the treatment period. Autoantibodies against oxLDL were significantly higher in the group II rats at days 14, 28 and 42 of the treatment compared to groups 1 and III rats (see Fig. 2).

#### Susceptibility of LDL + VLDL to oxidation

LDL + VLDL from the group II rats had a significantly lower lag phase ( $p < 0.01$ ), a greater oxidation rate ( $p < 0.01$ ) and higher maximum oxidation ( $p < 0.01$ ) as compared to group I rats. In contrast, group III rats displayed a greater lag phase, ( $p < 0.01$ ), a lesser rate of oxidation ( $p < 0.01$ ) and

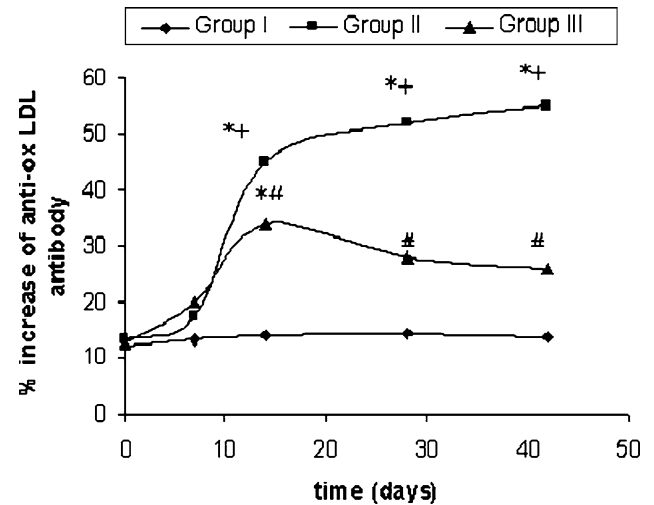


Fig. 2. Changes in levels of anti-oxidised LDL autoantibodies in plasma of rats fed a control diet, an atherogenic diet or an atherogenic diet + Fibernat for 42 days. Values are means,  $n = 6$ . \*Denotes significant difference from day 0, #denotes a significant difference between group II and III, +denotes a significant difference between groups II and I. Differences were assessed after subtracting the baseline values for each rat. % Increase = [value at defined time point - baseline value at day 0]:baseline value at day 0  $\times 100$ .

maximum oxidation was also significantly reduced ( $p < 0.01$ ) when compared to group II rats (Table 4).

#### Plasma GSH concentrations

Group II rats were depleted of GSH ( $p < 0.01$ ) compared to controls. Group III rats displayed plasma GSH concentrations that were significantly higher than that of group II ( $p < 0.05$ ) (Table 5).

#### Lp (a) and tHcy concentrations

Fibernat intake resulted in a significant lowering of plasma concentrations of homocysteine ( $p < 0.01$ ) (Table 5).

Table 4. The effect of Fibernat supplementation on indices of oxidation of LDL + VLDL

Group	Group I (Control)	Group II (Atherogenic diet)	Group III (Atherogenic diet + Fibernat)
Lag phase (min)	25.5 $\pm$ 1.7	15.0 $\pm$ 0.9*	60.5 $\pm$ 2.3**
Oxidation rate (nmoles MDA/ $\mu$ g LDL + VLDL protein/min)	0.052 $\pm$ 0.001	0.101 $\pm$ 0.007*	0.045 $\pm$ 0.008**
Maximum oxidation (nmoles MDA/ $\mu$ g LDL + VLDL protein)	9.5 $\pm$ 1.5	15.2 $\pm$ 2.7*	6.8 $\pm$ 0.9**

Values are indicated as mean  $\pm$  SD of six animals in each group.

\*Group II vs. Group I,  $p < 0.01$ ; \*\*Group III vs. Group II,  $p < 0.01$ .

Table 5. Plasma concentrations of GSH, Lp (a) and tHcy in rats fed fibre and cholesterol free (control), atherogenic or atherogenic + Fibernat supplemented diets

Group	Group I (Control)	Group II (Atherogenic diet)	Group III (Atherogenic diet + Fibernat)
Lp (a) (mg/dl)	19.6 ± 2.9	27.50 ± 4.5***	20.70 ± 1.3
tHcy (μmol/L)	2.03 ± 0.007	2.16 ± 0.09***	2.05 ± 0.07**
GSH (μg/mg protein)	10.95 ± 0.81	7.62 ± 0.62***	9.31 ± 1.30*

Values are mean ± SD of triplicate analysis.

\*Group III vs. Group II,  $p < 0.05$ ; \*\*Group III vs. Group II,  $p < 0.01$ ;

\*\*\*Group II vs. Group I,  $p < 0.01$ .

However, there was no significant difference in Lp (a) concentrations of Fibernat treated rats.

## Discussion

This study uses the rat as an atherosclerotic model due to its easy accessibility and availability. Despite its limitations, which have been stated earlier, several studies have employed the rat as an atherosclerosis model. Atherosclerosis is induced according to the method of Bajwa *et al.* [6]. Bennani-Kabachi *et al.* [16] have also used an excess of Vitamin D<sub>2</sub> along with a high cholesterol diet to induce atherosclerosis in rats. They conclude that Vitamin D<sub>2</sub> is an atherogenic agent which, when associated with hypercholesterolemia, allows the development of atherosclerotic lesions resembling the human plaque in the sand rat. The rat as an atherosclerotic model has been studied by other groups too [17], and the effect of Probuco/MaxE Supplementation has been studied in a hypercholesterolemic-diet induced rat model [18]. In our study, the experimental period required for the onset of atherosclerotic changes to manifest in the experimental rats was established by electron microscopic examination of the aortic tissues (Data appended).



Appended Figure. Electron micrograph of aortic tissue of atherogenic-diet fed rats showing dense connective tissue, collagen fibrils and elongated smooth muscle cells. Arrows indicate foam cell formation (×7650).

It is known that dietary factors influence plasma lipid levels and lipoprotein metabolism, thereby altering the atherogenicity of the lipoprotein profile [19]. Therefore, the main questions addressed in this investigation were whether Fibernat – a fibre cocktail of fenugreek seed powder, guar gum and wheat bran – could exert a potential antioxidant effect on circulating LDL and whether the LDL + VLDL fraction as a whole could display a resistance to oxidation *in vitro*. It was also determined whether inclusion of this fibre preparation in the diet could have a beneficial effect on some established indicators of atherosclerotic risk.

Reduced plasma triglyceride, total cholesterol, LDL and VLDL concentrations have been observed in Fibernat-fed animals in a previous study by our group [5]. In this study, LDL composition of cholesterol ester (CE), free cholesterol and apoB was increased significantly in the group II rats when compared to group I rats and group III rats. A cholesterol ester depleted LDL has been associated with a faster LDL turnover and a decreased risk for atherosclerosis. Conversely, cholesterol ester-enriched LDL (as was observed with atherogenic diet intake in untreated group II rats) is associated with an increased risk for atherosclerosis [20]. Previous studies have reported a positive correlation between soluble fibre intake and reduced CE content of LDL with reduction in atherosclerotic lesion extension [21]. Moreover, the  $\alpha$ -tocopherol composition of LDL in group II rats was significantly lower than group I and group III rats. A possible explanation for the  $\alpha$ -tocopherol sparing effect in Fibernat-treated rats could be related to the lower cholesterol content in group III rats. LDL particles containing less cholesterol could be less susceptible to oxidation and this could have led to a “sparing” effect on the  $\alpha$ -tocopherol of LDL as was observed in Fibernat-fed rats. This is particularly interesting since, usually, soluble fibre ingestion might affect the absorption of lipids and hence could affect the uptake of lipid soluble micronutrients like  $\alpha$ -tocopherol. In our study, however, LDL isolated from Fibernat-fed rats had higher  $\alpha$ -tocopherol concentrations.  $\alpha$ -tocopherol defends the LDL particle against oxidative damage by acting as a competitive substrate for oxidative attack. However, with prolonged oxidative stress,  $\alpha$ -tocopherol is depleted, thus rendering the substrate less defensive against oxidative damage. When observed with the hypothesis that LDL particles depleted of cholesterol ester are less susceptible to atherosclerosis [20], it is reasonable to speculate that the cholesterol ester depleted LDL particles (Group III rats) may have not been a major target for free radical formation and hence spared the  $\alpha$ -tocopherol in LDL. Possibly, the reason for the higher  $\alpha$ -tocopherol concentrations in the LDL particles from the Fibernat-fed rats could be attributed to this. In addition, group II rats displayed a significantly higher apo B content of LDL when compared to group III

rats. An increase in apo B is linked unequivocally to a greater risk of developing atherosclerosis in mice [22].

Recent studies report that dietary fibre supplementation retards oxidative stress in cardiac tissue of rats [23], reduces indices of lipid peroxidation in pigs fed a high fat diet [24] and in humans fed a mixture of a low fat diet, soluble fibre, soy and vegetable protein [25]. A lower indice of lipid peroxidation i.e., a lower MDA concentration in LDL was observed in group III rats when compared to group II rats. A slight though not statistically significant lowering of conjugated dienes was observed in the same group. Consistent with the increase in lipid peroxidative products that was observed in Group II rats, REM (on agarose gel) of LDL from these rats was greater. This is indicative of some degree of oxidative modification of LDL in this group of rats. Oxidative modification of LDL is characterised by a number of structural and compositional changes such as increased mobility, fragmentation of apo B, hydrolysis of phosphatidyl choline, derivatisation of lysine amino groups and generation of fluorescent adducts [26]. As opposed to this, with the decrease in formation of lipid oxidation products, REM of LDL from Fibernat-fed rats was comparatively less than group II rats. This probably indicates a reversal of atherogenic modifications to the LDL molecule when treated with Fibernat.

An increase in the *ex vivo* oxidation of lipoproteins is considered one way to assess predisposition to the *in vivo* development of oxidised LDL inside the arterial wall. Resistance of the LDL + VLDL fraction to oxidative modification *ex vivo* as evidenced by the increase in lag time to oxidation, a decrease in maximum oxidation and the oxidative rate was observed in Fibernat-treated rats but not in group II rats. An important determinant of LDL susceptibility to oxidation is its "residence" time in circulation. As the residence time and accumulation of lipoproteins increases (as in primary hyperlipidemia), the likelihood of them undergoing modification is greater as the oxidisable mass is correspondingly greater [27]. An up gradation in the expression of apo B, E receptors and an increase in uptake of LDL and VLDL were observed in Fibernat-fed rats [5]. This means an increased clearance of LDL and VLDL from plasma and hence less chances of them being oxidised. This probably explains the resistance to oxidation of LDL + VLDL fraction in Fibernat-fed rats.

The presence of autoantibodies against oxLDL has been considered a risk factor in coronary heart disease although conflicting reports do exist. Hence, as a corollary to the oxidative modification theory, we were interested in determining the development of autoantibodies to oxLDL at different time points of the study. Our observations revealed a basal level of anti-oxLDL antibody in all the rats at the start of the experiment. With change in diet, the rats in

groups II and III showed a sharp and early increase in anti-oxLDL antibodies. With regard to group III rats, however, changes in autoantibody levels were not significantly different at the 28th and 42nd days of the study as compared to baseline values. Fibernat supplementation (in consistence with our earlier observations) resulted in less formation of autoantibodies to oxLDL implying a possible suppression of LDL oxidation.

A similar observation was made by Damasceno *et al.* [11], wherein a soy-protein supplement served to suppress oxidation of LDL and hence the development of autoantibodies to oxLDL in rabbits. Further exploration of our finding is imperative for the determination of the degree of oxidation by using monoclonal antibodies specific for different degrees of oxidation of LDL such as minimally oxidised or highly oxidised LDL.

To explore the status of the oxidant:antioxidant balance, we measured the levels of the non-enzymatic antioxidant-GSH in the plasma of rats. A pro-oxidative effect of the high cholesterol diet was manifested as a reduction in the plasma GSH in group II animals as compared to groups I and III. An earlier study by our group correlates an atherogenic diet, elevated lipid peroxidation indices and a reduction in plasma GSH levels [28]. Accompanied by the amelioration in peroxidative changes, a corresponding increase in GSH levels was observed in Group III rats. It is probably a result of a decrease in lipid peroxidative products. Interesting studies have reported the antioxidant properties of lignin and grape pomace, as well as the soluble  $\beta$ -glucans of certain fibres [29, 30].

Homocysteine concentrations were higher in untreated atherogenic rats when compared to the Fibernat-treated atherogenic rats. An increase in homocysteine and lipoprotein (a) concentrations have been linked to the risk of developing atherosclerosis as they act in concert to dissociate apo (a) from Lp (a) [31], which impedes fibrinolysis and promotes atherosclerosis [32]. The increased tendency to develop atherosclerosis was evident from our observations of rat aorta by electron microscopy; group II but not group III displayed atherosclerotic changes in aorta (data for all experimental groups is not shown). Fibernat supplementation to rats appears to result in a significant decrease in tHcy. Lp (a) concentrations were lower but not significantly in Fibernat-treated rats. The observed reduction in homocysteine in our study appears to be in concert with other factors that predispose to atherosclerosis such as high lipid and lipoprotein concentrations. While there have been reports of increased intake of dietary fibre and a lowering of plasma tHcy in men [33], this cardiovascular risk factor is more amenable to reduction by folic acid, cobalamine and pyridoxine [34].

In summary, from these results, it is reasonable to conclude that in addition to the lowering of cholesterol and

apo B content of LDL, intake of Fibernat resulted in a sparing effect on  $\alpha$ -tocopherol content of LDL. Fibernat administration thus prevented the oxidative modification of LDL; the LDL + VLDL fraction also displayed a resistance to oxidative modification. Plasma antioxidant status with respect to GSH was enhanced. Interestingly, the plasma tHcy levels were lower as compared to untreated rats. Taken as a whole, these studies suggest that Fibernat intake could lower risk for atherosclerosis and other disorders of lipid metabolism. This particular fibre source therefore needs further investigation.

## Acknowledgments

The authors are grateful to the University Grants Commission for their Special Assistance Programme, Council of Scientific and Industrial Research, New Delhi, and Sterling Health Care Private Limited for providing study samples of Fibernat.

## References

- Witztum JL, Steinberg D: The oxidative modification hypothesis of atherosclerosis. Does it hold for humans? *Trend Cardiovasc Med* 11: 93–102, 2001
- Sato K, Niki E, Shimasaki H: Free radical mediated chain oxidation of low-density lipoprotein and its synergistic inhibition by vitamin E and vitamin C. *Arch Biochem Biophys* 279: 402–405, 1990
- Aviram M, Dornfeld L, Rosenblat M, Volkova M, Kaplan M, Coleman R, Hayek T, Presser D, Fuhrman B: Pomegranate juice consumption reduces oxidative stress, atherogenic modifications to LDL and platelet aggregation: studies in humans and in atherosclerotic apolipoprotein E-deficient mice. *Am J Clin Nutr* 71: 1062–1076, 2000
- Vergara-Jimenez M, Furr H, Fernandez ML: Pectin and psyllium decrease the susceptibility to LDL oxidation in Guinea pigs. *J Nutr Biochem* 10: 118–124, 1999
- Venkatesan N, Devaraj SN, Devaraj H: Increased binding of LDL and VLDL to apo B, E receptors of hepatic plasma membrane of rats treated with Fibernat. *Eur J Nutr* 42: 262–271, 2003
- Bajwa GS, Morrison LM, Ershoff BM: Induction of aortic and coronary atherosclerosis in rats fed a hypervitaminosis D, cholesterol containing diet. *Proc Soc Exp Biol Med* 138: 975–982, 1971
- Chung BH, Wilkinson T, Geer JC, Segrest JP: Preparative and quantitative isolation of plasma lipoproteins: rapid, simple discontinuous density gradient ultracentrifugation in a vertical rotor. *J Lipid Res* 21: 284–291, 1980
- Desai ID: Vitamin E analysis methods for animal tissues. *Meth Enzymol* 105: 138–147, 1984
- Esterbauer H, Zdravcevic SZ, Slater F: Detection of malonaldehyde by high-performance liquid chromatography. *Meth Enzymol* 105: 319–328, 1984
- Klein RA: The detection of oxidation in liposome preparation. *Biochim Biophys Acta* 210: 486–489, 1984
- Damasceno NRT, Goto H, Rodrigues FMD, Dias CTS, Okawabata FS, Abdalla DSP, Gidlund M: Soy protein isolate reduces the oxidizability of LDL and the generation of oxidized LDL autoantibodies in rabbits with diet-induced atherosclerosis. *J Nutr* 130: 2641–2647, 2000
- Burstein M, Scholnick HR: Lipoprotein – polyanion metal interactions. *Adv Lipid Res* 11: 68–108, 1973
- Wallin B, Rosengren B, Shertzer HG, Camejo G: Lipoprotein oxidation and measurement of thiobarbituric acid reacting substances formation in a single microtiter plate – its use for evaluation of antioxidants. *Anal Biochem* 208: 10–15, 1993
- Okhawa M, Ohishi N, Yagi K: Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem* 95: 351–358, 1979
- Moron MS, Defierre JW, Mannervik KB: Levels of glutathione reductase and glutathione-S-transferase activities in rat lung and liver. *Biochem Biophys Acta* 582: 67–74, 1979
- Bennani-Kabchi N, Kehel L, El Bouayadi F, Fdhil H, Amarti A, Saidi A, Marquie G: New model of atherosclerosis in insulin-resistant sand rats: hypercholesterolemia combined with D2 vitamin. *Atherosclerosis* 150: 55–61, 2000
- Joris I, Zand T, Nunnari JJ, Krolikowski FJ, Majno G: Studies on the pathogenesis of atherosclerosis. Adhesion and emigration of mononuclear cells in the aorta of hypercholesterolemic rats. *Am J Pathol* 113: 341–358, 1983
- Barbeau ML, Whitman SC, Rogers KA: Probuco, but not MaxEPA fish oil, inhibits mononuclear cell adhesion to the aortic intima in the rat model of atherosclerosis. *Biochem Cell Biol* 73: 283–288, 1995
- Grundy SM, Denke MA: Dietary influences on serum lipids and lipoproteins. *J Lipid Res* 31: 1149–1172, 1990
- Carr TP, Parks JP, Rudel LL: Hepatic ACAT activity in African Green monkeys is highly correlated to plasma cholesteryl ester enrichment and coronary artery atherosclerosis. *Arterioscler Thromb* 12: 1274–1283, 1992
- Cos E, Ramjiganesh T, Roy S, Yoganathan S, Nicolosi RJ, Fernandez ML: Soluble fiber and soybean protein reduce atherosclerotic lesions in guinea pigs. Sex and hormonal status determine lesion extension. *Lipids* 36: 1209–1216, 2001
- Veniant MM, Zlot CH, Walzem RL, Pierotti V, Driscoll R, Dichek D, Herz J, Young SG: Lipoprotein clearance mechanisms in LDL receptor-deficient “Apo B-48 only” and “Apo B-100 only” mice. *J Clin Invest* 102: 1559–1568, 1998
- Diniz YS, Cicogna AC, Padovani CR, Silva MD, Faine LA, Galhardi CM, Rodriguez HG, Novelli EL: Dietary restriction and fibre supplementation: oxidative stress and metabolic shifting for cardiac health. *Can J Physiol Pharmacol* 81: 1042–1048, 2003
- Rezar V, Pajk T, Logar MR, Janezic JV, Salobir K, Oresnik A, Salobir J: Wheat bran and oat bran effectively reduce oxidative stress induced by a high fat diets in pigs. *Ann Nutr Metab* 47: 78–84, 2003
- Jenkins DJ, Kendall CW, Vidgen E, Mehling CC, Parker T, Seyler H, Faulkner D, Garsetti M, Griffin LC, Agarwal S, Rao AV, Cunnane SC, Ryan MA, Connelly PW, Leiter LA, Vuksan V, Josse R: The effect of serum lipids and oxidized low-density lipoproteins of supplementing self-selected low-fat diets with soluble fiber, soy and vegetable protein foods. *Metabolism* 49: 67–72, 2000
- Steinbrecher Urs P, Zhang H, Loughheed M: Role of oxidatively modified low-density lipoproteins in atherosclerosis. *Free Rad Biol Med* 9: 155–168, 1990
- Araujo FB, Barbosa DS, Hsin CY, Maranhao RC, Abdalla DSP: Evaluation of oxidative stress in patients with hyperlipidemia. *Atherosclerosis* 117: 61–71, 1995
- Rajendran S, Kamaravelu P, Putschen DD, Devaraj SN: Protective effect of Tincture of Crataegus on oxidative stress in experimental atherosclerosis in rats. *J Clin Biochem Nutr* 20: 211–233, 1996
- Larauri JA, Ruperez P, Calixto FS: Antioxidant activity of wine pomace. *Am J Eno Viti* 47: 369–372, 1996
- Filipek J: The effect of the mushroom *Pleurotus ostreatus* on the lipid peroxidation of phosphatidylcholine liposomes. *Pharmazie* 47: 393

31. Wild SH, Fortmann SP, Marcovina SM: A prospective case control study of lipoprotein (a) size and risk of coronary heart disease in Stanford Five city project participants. *Arterioscler Thromb Vasc Biol* 17: 239–245, 1997
32. Sainani GS, Sainani R: Homocysteine and its role in the pathogenesis of atherosclerotic vascular disease. *J Assoc Phys India* 50: 5–8, 2002
33. Mennen LI, de Courcy GP, Guillard J-C, Ducros V, Bertrais S, Nicolas JP, Maurel M, Zarebska M, Favier A, Franchisseur C, Hercberg S, Galan P: Homocysteine, cardiovascular disease risk factors, and habitual diet in the French Supplementation with Antioxidant Vitamins and Minerals Study. *Am J Clin Nutr* 76: 1279–1289, 2002
34. Dusanond P, Eikelboom JW, Hankey GJ, Thom J, Gilmore G, Loh K, Qi Y, Klijn CJM, Langton P, Van Bockxmeer FM, Baker R, Jamrozik K: Homocysteine-lowering treatment with folic acid, cobalamin, and pyridoxine does not reduce blood markers of inflammation, endothelial dysfunction, or hypercoagulability in patients with previous transient ischemic attack or stroke. A randomized substudy of the VITATOPS trial. *Stroke* 36: 144–146, 2005

## Improvement in the nutraceutical properties of fenugreek (*Trigonella foenum-graecum* L.)

Surya Acharya<sup>1</sup>, Anchalee Srichamroen<sup>2</sup>, Saikat Basu<sup>3</sup>,  
Buncha Ooraikul<sup>4</sup> and Tapan Basu<sup>5</sup>

### Abstract

Acharya, S., Srichamroen, A., Basu, S., Ooraikul, B. and Basu, T.  
Improvement in the nutraceutical properties of fenugreek  
(*Trigonella foenum-graecum* L.)  
Songklanakarin J. Sci. Technol., 2006, 28(Suppl. 1) : 1-9

Fenugreek (*Trigonella foenum-graecum* L.) leaves and seeds have been used extensively for medicinal purposes. Fenugreek seed is known to exhibit anti-diabetic and anti-nociceptive properties and effects such as hypocholesterolaemic, anti-cancer and thyroxine-induced hyperglycaemia. Our research objectives have been to identify the chemical constituent(s) responsible for the health effects in human and to develop a strategy for improving these constituents in fenugreek plants. We have observed considerable variability among fenugreek genotypes. They differ in morphology, growth habit, biomass and seed production capability. Chemical constituents of the seed, e.g. saponins, fibre, protein, amino acids and fatty acid contents also differ markedly. This variability is most often overlooked or underestimated in clinical trials. Our research suggests that the genetic variability and the genotype by environmental interaction will play a significant role when the crop is used by the nutraceutical industry in Canada where high quality seed production

---

<sup>1</sup>Ph.D.(Agronomy), Agriculture and Agri-Food Canada Research Center, Lethbridge, AB T1J 4B1, Canada  
<sup>2</sup>Graduate Student in Food and Nutritional Science, <sup>3</sup>Graduate Student in Agronomy <sup>5</sup>Ph.D.(Biochemistry),  
Prof., Department of Agriculture, Food and Nutritional Science, University of Alberta, Edmonton, AB T6G 2P5,  
Canada <sup>4</sup>Ph.D.(Food Science), Prof., Faculty of Agro-Industry, Prince of Songkla University, Hat Yai, Songkhla,  
90110 Thailand.

Corresponding e-mail: acharya@agr.gc.ca

Received, 11 February 2005      Accepted, 16 September 2005

is at present difficult. Our multi-disciplinary approach aims at understanding the processes involved in the genetic improvement of fenugreek and use the new knowledge to improve the crop. We have developed a fenugreek cultivar "Tristar" for western Canada that can produce very high quality forage and will now concentrate on producing cultivars having improved nutraceutical value. Our research results indicate that the variability for important traits in fenugreek have a genetic base, making selection for improved levels of these traits possible.

---

**Key words :** Fenugreek, cultivar, Tristar, nutraceutical, chemicals,  
*Trigonella foenum-graecum* L.

---

Fenugreek (*Trigonella foenum-graecum* L.) is an annual crop belonging to the legume family. This crop is native to an area extending from Iran to northern India, but is now widely cultivated in China, north and east Africa, Ukraine and Greece (Petropoulos, 2002). In parts of Asia, the young plants are used as potherbs and the seeds as a spice or as herbal medicine (Lust, 1986 and Petropoulos, 2002). The species name "*foenum-graecum*" means "Greek hay" indicating its use as a forage crop in the past (Petropoulos, 2002). According to Lust (1986) fenugreek is one of the oldest known medicinal plants in the recorded history.

Fenugreek leaves and seeds have been used extensively to prepare extracts and powders for medicinal uses (Basch *et al.*, 2003). Fenugreek is reported to have anti-diabetic, anti-fertility, anti-cancer, anti-microbial, anti-parasitic and hypocholesterolaemic, effects (Al-Habori and Raman, 2002). In India, fenugreek is used as a lactation stimulant (Tiran, 2003). Fenugreek seed in powder or germinated form exhibits anti-diabetic properties (Broca *et al.*, 2004; Devi *et al.*, 2003; Hannan *et al.*, 2003; Tahiliani and Kar, 2003a; Thakaran, 2003 and Vats *et al.*, 2003), hypocholesterolaemic effect (Suboh *et al.*, 2004; Thompson Coon and Ernst, 2003 and Venkatesan, 2003), anti-cancer effect (Devasena and Menon, 2003), effect on thyroxine-induced hyperglycaemia (Tahiliani and Kar, 2003b) and protective effect on ethanol toxicity (Thirunavukkarasu *et al.*, 2003). Hooda and Jood (2003), on the basis of their studies on the physiological, rheological and organoleptic characteristics of wheat-fenugreek supplemented blends have found encouraging results with respect to the increase in protein and fat contents of their

supplemented blends. But enhancing property of fenugreek is also known but Fugh-Berman (2003) correctly points out the lack of clinical evidence to support this claim.

Interest in cultivating this crop with potential nutraceutical value in temperate climates, such as that in western Canada, has increased because the crop is adapted to dryland growing conditions (Moyer *et al.*, 2003). This interest in fenugreek is further enhanced by the release of the first forage cultivar "Tristar" developed at the Lethbridge Research Centre (LRC) in close collaboration with researchers from Alberta Agriculture, Food and Rural Development.

Over the past ten years, LRC researchers have shown that fenugreek can be a very useful legume crop for incorporation into short-term rotations (Moyer *et al.*, 2003). Tristar fenugreek can produce high yield (Mir *et al.*, 1993) and high quality forage, can be grown for hay or silage (Mir *et al.*, 1998), contains animal growth promoting substances such as diosgenin not present in other forage legumes, but does not cause bloat (Mir *et al.*, 1997) like many forage legumes.

Fenugreek genotypes differ in morphology, growth habit, biomass and seed production capability. Chemical constituents of the seed such as saponins, fibre, protein, amino acids and fatty acid contents also differ markedly (Taylor *et al.*, 1997 and Taylor *et al.*, 2000). This variability is most often overlooked or underestimated in clinical trials. Our research suggests that the genetic variability and the genotype by environmental interaction will play a significant role when the crop is used by the nutraceutical industry in Canada, where high quality seed production is at

present difficult. Our multi-disciplinary approach aims at understanding the processes involved in the genetic improvement of fenugreek and to use this knowledge to improve the crop.

The new cultivar Tristar requires approximately 120 days to produce high quality seed. However under Prairie conditions growing seasons are short (~ 100 frost free days) and so it is necessary to develop cultivars with short maturity time and high seed yield. Attempt will be made to select for increasing the number of pods per node and the number of flowering nodes per plant to accentuate yield per hectare. Improvement in chemical constituents such as saponin, proteins, amino acids, fibers and galactomannans are important goals of our research, which has the potential to enhance nutraceutical qualities of this crop.

### Materials and Methods

Genetic investigations include a search for genotypes in the world collections for high yielding, early maturing types and generation of mutants that would combine determinate growth habit with high seed yield. The objectives of our multi-disciplinary research have been to identify the chemical constituent(s) that are responsible for the health effects in human and to develop a strategy for improving these constituents in fenugreek plants.

Fenugreek accessions were procured from Plant Gene Resources of Canada, Saskatoon and from Indian spice markets. Fenugreek seed of four cultivars, namely, Amber, F-70, F-86, L-3314, were obtained from Lethbridge Research Center, AB, Canada for chemical property determinations.

### Evaluation of new accessions

Seeds from new accessions collected from PGRC and Indian spice stores were seeded in early May in 2004 at LRC to determine seed yield potential. Eighty-three accessions were planted in an irrigated field while 65 of the accessions having adequate amount of seed were included in a dryland test. In both tests, the genotypes were planted as in a two times replicated randomized

complete block design. In each case, plots consisted of a three-meter long single row plot and 120 seeds were planted using a custom built forage seeder. After one month (mid June) the plots were scored for proportion of the row with plants. The irrigated and dryland plots were hand harvested on September 20 and October 11, 2004, respectively. After drying the material for one week indoors, the seed were separated from the rest of the plant, cleaned and weighed. Since all the lines did not have same number of plants the seed yield was adjusted using the observation on proportion of surviving plants in mid June.

### Mutation breeding

A mutation breeding study, using Tristar as base population, was initiated in the green house at LRC to look for mutants with desirable and beneficial phenological traits like determinate growth habit and/or high seed yield. EMS was used as the mutagenic agent at the level of 10, 20, 30, 40, 50, 100, 150, 200 and 300 mM concentrations. The seed were pre-soaked in water for 2, 4, 6, 8, 12, 16 and 24 h before applying different concentrations of EMS. Treated seed were planted individually in pots containing soil-free mix and were designated as  $M_1$  plants. After 85 d in the greenhouse set to cycle 16 h long days (22°C) and 8 h night (15°C) the plants were desiccated with 0.4% Reglone solution. The plants were then allowed to dry for 10 d before separating the seed for yield determination. Seed from selected  $M_1$  plants were again seeded in pots and allowed to produce  $M_2$  seed.

### Determination of crude proteins

Total nitrogen content of a 1 g ground seed sample was determined using Technicon Industrial nitrogen determination procedure 146/71A and the conversion factor of 6.25 was used to calculate the crude protein content (AOAC, 1995).

### Determination of saponin

Defatted and dried seed powder was transferred into a test tube, saponin were extracted with 5 mL of 80% ethanol and hydrolyzed for 2 h

with 2 mL of 1M sulfuric acid in 70% propanol. Water (3 mL) and 50 µg of 6-methyldiosgenin internal standard were added, extracted with methyl *tert*-butyl ether (3 x 1 mL) solvent, evaporated at 30°C in a Meyer N-EVAP apparatus (Organomation Associates, Berlin, MA), weight of the residue recorded and the residue dissolved in 1 mL of toluene. A portion (2 µL) was analyzed in a GC (Hewlett-Packard 6890) equipped with an HP-5 column (30 m x 0.32 mm i.d.), an HP 6890 series autoinjector, a flame ionization detector (FID, 300°C), and an electronic gas control. The split/splitless injection port, operated at 250°C, was equipped with a silanized glass liner (HP part 5181-3316). The sample was injected directly (30 s) at an initial oven temperature of 200°C and then ramped to 290°C at a rate of 1°C min<sup>-1</sup>. The carrier gas was helium with 2 mL min<sup>-1</sup> constant flow under electronic pressure control. Retention times and peak area counts were obtained with HP GC ChemStation software (ver. A.05.04). Confirmation of peak identity was obtained by mass spectrometry, using HP 5989A GC-MS as reported previously by Taylor *et al.* (2000). Sapogenin content

was expressed as % (w/w) and its composition was expressed as % relative GC peak area.

## Results and Discussion

### Seed yield

Seed yield of the accessions were variable in irrigated and dryland locations at Lethbridge (Table 1a and b) in 2004. Seed sizes and the color were also variable (Figure 1). Two of the accessions (L3172 and 3177) had determinate growth habit. Accession 3172 was 30 cm in height, matured very early and produced about 1000 kg ha<sup>-1</sup> seed yield whereas accession 3177 grew only 15 cm in height, matured very early and produced 650 kg ha<sup>-1</sup> seed yield. Although these determinate lines are not as high yielding as some other lines, they can be used as parents for transferring determinate nature to new cultivars.

Top and bottom five seed yielding accessions in the two growing conditions were different (Table 1a). Only one low yielding accession (L3068) was common among five top and five bottom seed producers. All the top five and four

**Table 1a. Top and bottom 5 seed yielding fenugreek lines grown under irrigated and dryland conditions at Lethbridge, Alberta in 2004.**

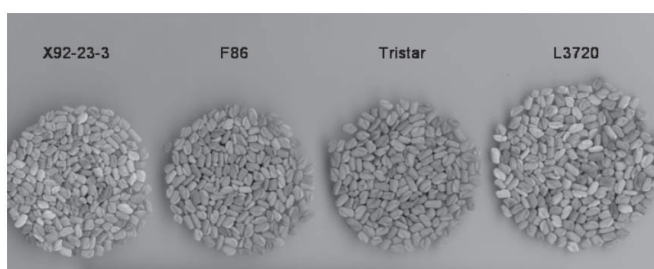
Irrigated		Dryland	
Accessions	Seed yield (kg ha <sup>-1</sup> )	Accessions	Seed yield (kg ha <sup>-1</sup> )
PI 211636	2881	PI 143504	4119
L3312	2621	L3308	3952
L3671	2452	F18	3937
L3720	2391	PI 138687	3320
X92-23-3	2374	F86	3207
L3678	261	L3708	1032
L3673	253	L3704	884
L3674	137	L3702	823
L3068 <sup>1</sup>	127	L3710	491
L3675	19	L3068	377
Mean	1254	Mean	1990
SE <sup>2</sup>	64	SE	104

<sup>1</sup> L3068 is the only common line in this summary table.

<sup>2</sup> SE = Standard Error

**Table 1b.** Five fenugreek lines that were top seed yielding under dryland and low yielding on irrigation or vice versa.

Accessions	Irrigated	Dryland
	Seed yield (kg ha <sup>-1</sup> )	Seed yield (kg ha <sup>-1</sup> )
L3718	737	2194
L3713	937	2564
L3693	1054	2216
L3699	1536	1407
L3720	2391	1731



**Figure 1.** Seed size and color variation among four fenugreek accessions.



**Figure 2.** Double pods in fenugreek mutant plant.

bottom seed producing lines were different in the two conditions. Fenugreek lines grown under dryland condition produced higher seed yield than under irrigation. This can be attributed to dryland adaptation of the crop. The correlation coefficient calculated between seed yield in dryland and

irrigated condition was positive and significant ( $r = 0.54$ ;  $p < 0.05$ ) indicating significant genotypic effect for seed yield. However, there were lines that did well under one condition but not in the other (Table 1b). The above observations indicate presence of genotype x environment interaction.

**Table 2a. Observed range of different morphometric parameters of the M<sub>1</sub> mutant plants generated in green house at LRC, Lethbridge, AB, Canada in 2004.**

EMS conc. (mM)	Plant height (cm)	No. of nodes	No. of pods	No. of single pods	No. of double pods	Pod length (cm)	No. of seed	Seed weight/plant (g)
10	18.8-67.6	6-21	6-25	0-14	0-12	0.3-15.9	9-336	0.44-4.2
20	30.3-81.2	8-19	4-18	0-13	0-7	1.3-19.8	68-271	0.56-4.1
30	31.3-74.4	9-24	6-21	0-17	0-7	0.5-19.5	56-306	0.28-2.8
40	32.4-72.2	11-27	5-22	0-17	0-6	0.4-21.6	59-324	0.47-2.8
50	30.2-65.8	5-24	5-27	1-15	0-7	0.2-21.1	3-176	0.03-3.4
100	33.2-72.7	12-23	6-36	1-26	0-9	0.7-24.3	5-190	0.01-2.5
150	28.7-75.1	11-29	8-67	1-29	0-19	0.6-21.6	4-289	0.05-2.4
200	25.7-73.6	7-26	4-90	0-36	0-27	0.2-18.8	5-1352	0.01-10.1
300	7.5-35.7	4-26	0-18	0-7	0-6	0.8-11.4	0-173	0-1.7

**Table 2b. Observed range of different morphometric parameters of selected M<sub>2</sub> mutant plants generated in green house at LRC, Lethbridge, AB, Canada in 2004.**

EMS conc. (mM)	Plant height (cm)	No. of nodes	No. of pods	No. of single pods	No. of double pods	Pod length (cm)	No. of seed	Seed weight/plant (g)
10	11.6-42.4	11-44	10-38	4-23	1-13	2.6-6.9	98-388	1.2-4.6
20	6.7-45.6	13-56	2-52	4-34	2-15	1.5-8.6	89-556	1.2-6.1
30	16.7-67.3	18-51	17-52	3-32	2-14	2.3-11.2	98-467	1.2-5.2
40	6.9-45.6	9-41	5-37	1-28	0-8	3.5-13.7	124-756	1.6-8.3
50	33.9-83.4	28-77	23-82	2-44	3-23	1.7-11.5	0-654	1.4-5.8
100	11.9-45.3	9-39	7-41	3-22	0-13	3.2-9.1	0-667	1.2-5.0
150	9.1-35.1	6-29	4-27	4-21	0-5	2.5-10.8	125-636	1.5-5.3
200	17.5-48.9	15-54	10-58	3-34	2-18	3.2-12.8	122-552	1.8-5.9
300	5.6-28.9	3-31	5-48	3-23	0-14	1.9-13.2	163-759	1.1-9.8

Seed yield of EMS treated plants and other morphometric parameters measured from M<sub>1</sub> and M<sub>2</sub> plants are presented in Table 2 a and b. The M<sub>1</sub> and M<sub>2</sub> plants were variable in spite of the fact that M<sub>2</sub> were selected for seed yield. Lower value for range of seed number and seed weight are higher in M<sub>2</sub> compared to M<sub>1</sub>. This may be due to the selection practiced for higher seed yield in M<sub>1</sub> generation. Stability of seed yield performance in the mutants will most probably be visible in later generations (M<sub>4</sub> or M<sub>5</sub>). In M<sub>2</sub> frequency of double pods increased. Since double pod characteristics (Figure 2) is linked to high diosgenin content, it is expected that some of these mutants may produce more diosgenin in addition to producing high seed yield (Petropoulos, 2002). High diosgenin pro-

ducing lines will probably be preferred by the nutraceutical industry. Raghuvanshi and Singh (1981) observed high heritability estimates and genetic advance for double pod trait implying that selection will be effective for improvement in this trait.

#### Chemical constituents

Crude protein content in seed of lines grown in Southern Alberta (Lethbridge) was significantly higher than that of the Indian line (Table 3). Protein content of the four Lethbridge grown lines was not significantly different. This was expected as these lines were selected for their ability to produce high forage yield and had very similar growth habit, morphological features and were grown under

**Table 3. Composition of Fenugreek Seeds (% , w/w, dry basis)<sup>1</sup>**

Seed components	Fenugreek Lines				
	Amber	F-70	F-86	L-3314	Indian
Crude proteins	31.6±0.8 <sup>a</sup>	28.7±0.3 <sup>b</sup>	30.1±0.5 <sup>ab</sup>	31.6±0.2 <sup>a</sup>	26.0±0.3 <sup>c</sup>
Soluble fiber	18.8±0.2 <sup>b</sup>	21.7±0.3 <sup>a</sup>	16.1±0.3 <sup>c</sup>	18.2±0.3 <sup>b</sup>	17.5±0.8 <sup>bc</sup>
Insoluble fiber	25.8±0.3 <sup>d</sup>	25.8±0.4 <sup>cd</sup>	32.3±0.5 <sup>a</sup>	27.4±0.6 <sup>bc</sup>	28.1±0.1 <sup>b</sup>
Sapogenins	0.4±0.0 <sup>a</sup>	0.3±0.0 <sup>b</sup>	0.3±0.0 <sup>b</sup>	0.3±0.0 <sup>b</sup>	0.5±0.0 <sup>a</sup>
Diosgenin	47.8±1.6 <sup>a</sup>	41.0±5.1 <sup>a</sup>	43.9±2.9 <sup>a</sup>	44.6±2.1 <sup>a</sup>	43.8±3.2 <sup>a</sup>

<sup>1</sup> Means sharing the same superscript in a row are not significantly different ( $p>0.05$ ) from one another.

**Table 4. Diosgenin levels from 10 accessions of fenugreek seed grown in 1998 and 1999 at Saskatoon, SK; Lethbridge, AB and Agassiz, BC. Table adopted from Taylor *et al.* (2002).**

Accession	Saskatoon		Lethbridge		Agassiz	
	1998 <sup>a</sup>	1999 <sup>a</sup>	1998 <sup>a</sup>	1999 <sup>a</sup>	1998 <sup>a</sup>	1999 <sup>a</sup>
19062	0.284	0.727	0.420	0.392	0.398	0.923
19063	0.411	0.347	0.642	0.246	0.347	0.458
19064	0.431	0.291	0.400	0.361	0.206	0.333
19065	0.519	0.402	0.478	0.355	0.528	0.391
19066	0.448	0.243	0.473	0.209	0.286	0.373
19067	0.685	0.663	0.453	0.800	0.354	0.906
19068	0.592	0.498	0.522	0.366	0.328	0.464
19069	0.542	0.352	0.539	0.248	0.383	0.302
19070	0.801	0.506	0.467	0.369	0.633	0.543
19071	0.730	0.555	0.535	0.341	0.747	0.613
Mean %	0.544	0.458	0.493	0.369	0.421	0.531

<sup>a</sup> Average values (N=3) determined by single analysis of three sub-samples of defatted and dried seed material.

uniform growing conditions. Seed used in this study had a high protein content as compared to 24% observed in seed from Egypt (Gerhartz, 1987), and 26% in seed from India (Sharma, 1986), but they were slightly lower in proteins than the 34% reported by Brummer *et al.* (2003). Differential crude protein content in seed in this experiment cannot be attributed directly to the genotypic potential of the lines. Higher levels of protein in seed grown in southern Alberta may have been due to better nutrient status of the Lethbridge soil compared to the soil where the seed was grown for the spice market in India.

Sapogenin content of Amber (0.4) and the Indian seed (0.5) were significantly higher ( $p<0.05$ ) than the other three lines tested (Table 3). The lines F-70, F-86 and L-3314 had about 0.3% sapogenin. Diosgenin was found to be the predominant steroidal sapogenins in all lines but were not significantly ( $p>0.05$ ) different from each other in the five lines tested. Diosgenin levels observed in these samples were within the range of values observed earlier by Taylor *et al.* (2002) in a more detailed study. In the Taylor *et al.* (2002) study, the same genotypes were grown in three locations in western Canada in two consecutive years (Table

4). In 1998, Saskatoon and Lethbridge sites produced higher mean value for diosgenin than 1999. Whereas 1999 harvested seed contained higher mean diosgenin than 1998 in Agassiz site indicating genotype x environment interaction for this trait. It is interesting to note that even within a location some genotypes did not follow the general yearly trend. For example, accessions 19062 and 19067 did better in 1999 than 1998 in Saskatoon and Lethbridge, respectively. Reverse trend was noticed for the accessions 19065 and 19070 in Agassiz.

### Conclusion

From our field and green house trials we can conclude that there is variability among fenugreek accessions for the traits studied. The accessions differ in their ability to produce good quality seed within 90 to 100 frost-free days. The present set of experiments identified genotypes with determinate growth habit and ability to produce high seed yield under western Canada growing conditions. Genotypes with determinate growth habit by themselves do not seem to be suitable for commercial seed production. These genotypes, however, may be valuable as parent material for improvement of high yielding cultivars in this crop. It is also encouraging to note that mutants are showing signs of high proportion of double or twin pods indicative of high diosgenin content in the seed. If these mutants are stable, we will be able to improve nutraceutical property of fenugreek.

The other important conclusion is that for most traits studied there was evidence of genotype x environment interaction. This is important for a crop that has potential to be used as a nutraceutical. Such interaction indicates that seed produced in all environments will not be of similar quality and all genotypes will not produce high quality seed every year. To maintain high quality in nutraceutical product we need to produce stable cultivars showing least amount of genotype x environment interaction and continue monitoring quality every year.

### References

- Al-Habori, M. and Raman, A. 2002. Pharmacological Properties in Fenugreek - The genus *Trigonella* (1<sup>st</sup> edition) by G.A. Petropoulos (ed.), Taylor and Francis, London and New York, 10: 163-182.
- AOAC. 1995. Official Methods of Analysis. 16<sup>th</sup> ed. Assoc. Offic. Anal. Chem., Arlington, VA.
- Basch, E., Ulbricht, C., Kuo, G., Szapary, P. and Smith, M. 2003. Therapeutic applications of fenugreek. *Alt. Med. Rev.*, 8: 20-27.
- Broca, C., Breil, V., Cruciani-Guglielmacci, C., Manteghetti, M., Rouault, C., Derouet, M., Rizkalla, S., Pau, B., Petit, P., Ribes, G., Ktorza, A., Gross, R., Reach, G. and Taouis, M. 2004. The insulinotropic agent 1D1101(4-hydroxy-isoleucine) activates insulin signaling in rat. *Am. J. Physiol. Endocrinol. Metab.*, 287(3): E463-E471.
- Brummer, Y., Cui, W. and Wang, Q. 2003. Extraction, purification and physicochemical characterization of fenugreek gum. *Food Hydrocolloids*, 17(3): 229-236.
- Devasena, T. and Menon, V.P. 2003. Fenugreek affects the activity of beta-glucuronidase and mucinase in the colon. *Phytother Res.*, 17(9): 1088-1091.
- Devi, B.A., Kamalakkannan, N. and Prince, P.S. 2003. Supplementation of fenugreek leaves to diabetic rats-Effect on carbohydrate metabolic enzymes in diabetic liver and kidney. *Phytother Res.*, 17(10): 1231-1233.
- Fugh-Berman, A. 2003. "Bust enhancing" herbal products. *Obstet. Gynecol.*, 101(6): 1345-1349.
- Gerhartz, W. 1987. Ullmann's Encyclopedia of Industrial Chemistry (5<sup>th</sup> ed.) by W. Gerhartz, (ed.), VCH, Weinheim, Germany, A8: 597; A13: 110, 117 & 135-138.
- Hannan, J.M., Rokeya, B., Faruque, O., Nahar, N., Mosihuzzaman, M., Azad Khan, A.K, and Ali, L. 2003. Effect of soluble dietary fibre fraction of *Trigonella foenum-graecum* on glycemic, insulinemic, lipidemic and platelet aggregation status of Type 2 diabetic model rats. *J. Ethnopharmacol.*, 88: 73-77.

- Hooda S. and Jood, S. 2003. Physicochemical, rheological, and organoleptic characteristics of wheat-fenugreek supplemented blends. *Nahrung.*, 47(4): 265-268.
- Lust, J.B. 1986. *The Herb Book*, Bantam Books Inc., New York.
- Mir, P.S., Mir, Z. and Townley-Smith, L. 1993. Comparison of the nutrient and *in situ* degradability of fenugreek (*Trigonella foenum-graecum*) and alfalfa hays. *Can. J. Anim. Sci.*, 73: 993-996.
- Mir, Z., Acharya, S.N., Mir, P.S., Taylor, W.G., Zaman, M.S., Mears, G.J. and Goonewardene, L.A. 1997. Nutrient composition, *in vitro* gas production and digestibility of fenugreek (*Trigonella foenum-graecum*) and alfalfa forages. *Can. J. Anim. Sci.* 77: 119-124.
- Mir, Z., Mir, P.S., Acharya, S.N., Zaman, M.S., Taylor, W.G., Mears, G.J. and Goonewardene, L.A. 1998. Comparison of alfalfa and fenugreek silages supplemented with barley grain on performance of growing steers. *Can. J. Anim. Sci.* 78: 343-349.
- Moyer, J.R., Acharya, S.N., Mir, Z. and Doram, R.C. 2003. Weed management in irrigated fenugreek grown for forage in rotation with other annual crops. *Can. J. Plant Sci.*, 83: 181-188.
- Petropoulos, G.A. 2002. Fenugreek - The genus *Trigonella*, Taylor and Francis, London and New York.
- Raghuvanshi, S.S. and Singh, R.R. 1981. Inheritance of double-pod in fenugreek. *Ind. J. Agric. Sci.*, 51(3): 163-166.
- Sharma, R.D. 1986. Effect of fenugreek seeds and leaves on blood glucose and serum insulin responses in human subjects. *Nutr. Res.*, 6: 1353-1364.
- Suboh, S.M., Bilto, Y.Y. and Aburjai, T.A. 2004. Protective effects of selected medicinal plants against protein degradation, lipid peroxidation and deformability loss of oxidatively stressed human erythrocytes. *Phytother. Res.*, 18(4): 280-284.
- Tahiliani, P. and Kar, A. 2003a. The combined effects of *Trigonella* and *Allium* extracts in the regulation of hyperthyroidism in rats. *Phytomed.*, 10(8): 665-668.
- Tahiliani, P. and Kar, A. 2003b. Mitigation of thyroxine-induced hyperglycaemia by two plant extracts. *Phytother. Res.*, 17(3): 294-296.
- Taylor, W.G., Zaman, M.S., Mir, Z., Mir, P.S., Acharya, S.N., Mears, G.J. and Elder, J.L. 1997. Analysis of steroidal sapogenins from Amber fenugreek (*Trigonella foenum-graecum*) by capillary gas chromatography and combined gas chromatography-mass spectrometry. *J. Agri. Food Chem.*, 45: 753-759.
- Taylor, W.G., Elder, J.L., Chang, P.R. and Richards, K.W. 2000. Micro determination of diosgenin from fenugreek (*Trigonella foenum-graecum*) seeds. *J. Agric. Food Chem.*, 48: 5206-5210.
- Taylor, W.G., Zulyniak, H.J., Richards, K.W., Acharya, S.N., Bittman, S. and Elder, J.L. 2002. Variation in diosgenin levels among 10 accessions of fenugreek seeds produced in western Canada. *J. Agric. Food Chem.*, 50: 5994-5997.
- Thakaran, S., Salimuddin and Baquer, N.Z. 2003. Oral administration of orthovanadate and *Trigonella foenum-graecum* seed powder restore the activities of mitochondrial enzymes in tissues of alloxan-induced diabetic rats. *Mol. Cell Biochem.*, 247(1-2): 45-53.
- Thirunavukkarasu, V., Anuradha, C.V. and Viswanathan, P. 2003. Protective effect of fenugreek (*Trigonella foenum-graecum*) seeds in experimental ethanol toxicity. *Phytother. Res.*, 17(7): 737-743.
- Thompson Coon, J.S. and Ernst, E. 2003. Herbs for serum cholesterol reduction: a systematic view. *J. Fam. Pract.*, 52(6): 468-78.
- Tiran, D. 2003. The use of fenugreek for breast feeding women. *Complement Ther. Nurs. Midwifery*, 9(3): 155-156.
- Vats, V., Yadav, S.P. and Grover J.K. 2003. Effect of *Trigonella foenum-graecum* on glycogen content of tissues and the key enzymes of carbohydrate metabolism. *J. Ethnopharmacol.*, 85(2-3): 237-242.
- Venkatesan, N., Devaraj, S.N. and Devraj, H. 2003. Increased binding of LDL and VLDL to apo B, E receptors of hepatic plasma membrane of rats treated with Fibrinat. *Eur. J. Nutr.*, 42(5): 262-271.

## Effect of Supplementation of Traditional Medicinal Plants on Serum Lipid Profile in Non-Insulin Dependent Diabetics

Anita Kochhar\*, Malkit Nagi and Rajbir Sachdeva

*Department of Food and Nutrition, College of Home Science, Punjab Agricultural University, Ludhiana 141 004, Punjab, India*

*\*Telephone: 0161-2401960/328 Ext.(O), 9872453467 (M)*

*\*E-mail: dranita kochhar@yahoo.com*

**KEYWORDS** Bittergourd. Fenugreek Seeds. Jambu Seeds. NIDDM. Serum Lipids

**ABSTRACT** The effect of supplementation of powdered mixture of three traditional medicinal plants namely bittergourd, jambu and fenugreek seeds in raw and cooked form on serum lipid profile was studied in 60 non insulin dependent male diabetics. The patients were divided into two groups of 30 each. The patients of group I was given raw powdered mixture in the form of capsules and to the patients of group II this mixture was given in the form of salty biscuits. Supplementation of one gram of this powdered mixture for one and a half month period and then further increased to two gram to another one and a half month period to the diabetics significantly improved the serum lipid profile by lowering total, LDL-cholesterol, VLDL cholesterol level thus helping in retarding secondary complications of the disease.

### INTRODUCTION

The incidence of diabetes mellitus is increasing all over the world, affecting 150 million people. More than one fifth or 33 million of them are Indians according to International Diabetes Federation (IDF) India has been declared as "Diabetic Capital of the world" at the recent 2003 IDF Conference in Paris. The World Health Organisation (WHO) estimates a 170 percent increase from 84 to 228 million diabetes in developing countries by 2025. India will lead the pack followed by China and the United States (Bezbaruah, 2003).

Diabetes is associated with increased risk of developing CHD which appears earlier than with the general population, affects females almost as often as men and is more frequently fatal (NIH, 1995 and ADA 1988). Cardiovascular risk factors such as hypertension, low level of HDL and elevated level of triglycerides and have been shown to precede the onset of type I diabetes (Haffner, 1990 and Mykanen, 1993).

Medicinal plants and herbs are of great importance to the health of the individuals and communities. A scientific investigation of traditional herbal remedies for diabetes may provide valuable lead for the development of alternative drug and therapeutic strategies. Studies carried out at National Institute of Nutrition (NIN) revealed that fenugreek incorporated in the experimental diets of human subjects significantly reduces the serum

cholesterol, LDL/VLDL cholesterol and triglycerides level and they are likely to provide a significant reduction in LDL/VLDL fractions without altering the HDL fractions (Saibaba and Raghuram, 1997). The literature reports also indicate that daily ingestion of bittergourd improved the glucose tolerance in diabetic patients. Protein fraction gourdin extracted from bittergourd seeds reduces the blood sugar, cholesterol level and triglycerides levels in diabetic patients and have no side effects (Khanna, 1998). Similarly oral administration of jambu seeds in casein diets of rabbits significantly lowered the elevated post meal values of blood glucose, cholesterol, free fatty acids and triglycerides (Kedar and Chakrabarti, 1983).

Due to non acceptability of taste in their original form, it is not possible to continue the intake of these medicinal plants for longer period. Efforts are needed to develop some nutritional supplement using these traditional medicinal plants so that they can be incorporated in the diets of diabetics. Hence, the present study was planned to access the combined effect of powdered mixture of bittergourd, jambu seeds and fenugreek seeds on serum lipid profile in non insulin dependent diabetics.

### MATERIALS AND METHODS

**A. Procurement and Processing of Traditional Medicinal Plants:** The raw materials, bittergourd fruit (*Momordica*

*charantia*), fenugreek seeds (*Trigonella foenum graceum*) and jambu seeds (*Euglnia jambolana*) procured from local market of Ludhiana city in one lot and were used for the development of different products.

**Bittergourd:** Fresh, immature bittergourd fruit were washed in clean water and water was wiped off with clean muslin cloth and were cut into small pieces by stainless steel knife and then dried in oven at 60 to 65°C till complete drying and then converted to fine powder of 60 mesh sieve size in cyclotec mill. The fine powder of bittergourd was stored in airtight plastic container till further use.

**Fenugreek Seed:** Foreign materials were removed from fenugreek seeds and these seeds were washed in clean water to remove the dust. Then seeds were soaked in equal volume of water for overnight and excess water was drained off. The soaked seeds were dried in oven at 60-65°C till complete drying and ground to fine powder for preparation of products.

**Jambu Seeds:** The pulp of the jambu fruit was removed with stainless steel knife and seed coat was removed. The seed kernel were dried in an oven at 60-65°C till complete drying and ground to fine powder of 60 mesh sieve size in a cyclotec mill and stored in air tight plastic container and used for incorporation in various recipes.

The powdered form of fenugreek, bittergourd and jambu was mixed in equal proportion and salty biscuits using 500 mg. to 2 g of mixture of these medicinal plant were developed and were evaluated for organoleptic characters by twelve experts (staff and post graduate students) of Department of Food and Nutrition, College of Home Science, PAU, Ludhiana using nine point hedonic scale. On the basis of their judgement and composition, most acceptable level i.e. 500 mg. was selected for supplementation. The empty capsules of 500 mg. capacity were purchased from the medicine market and were filled with the prepared mixture. Filling was done using automatic filling machine and were given to the diabetic patients for three days for testing physiological functioning of gastrointestinal before starting the actual supplementation trial. No side effect and drug interaction was observed among subjects while combined mixture of these medicines were given to the diabetic subjects.

**B. Selection and Feeding of the Subjects.** Sixty NIDDM patients free from serious

complications were selected from PAU Hos:ital, Ludhiana. General information fo the subject is given in Table 1. After collecting and analyzing blood of selected 60 NIDDM subjects, were followed for one month period and no treatment was given during this period except the prescribed medicine which they were already taking and the period was treated as self control. After one month, fasting and post prandial blood samples were again collected and analysed. Then these subjects were divided into two groups of 30 each.

**Table 1: General information of the subjects**

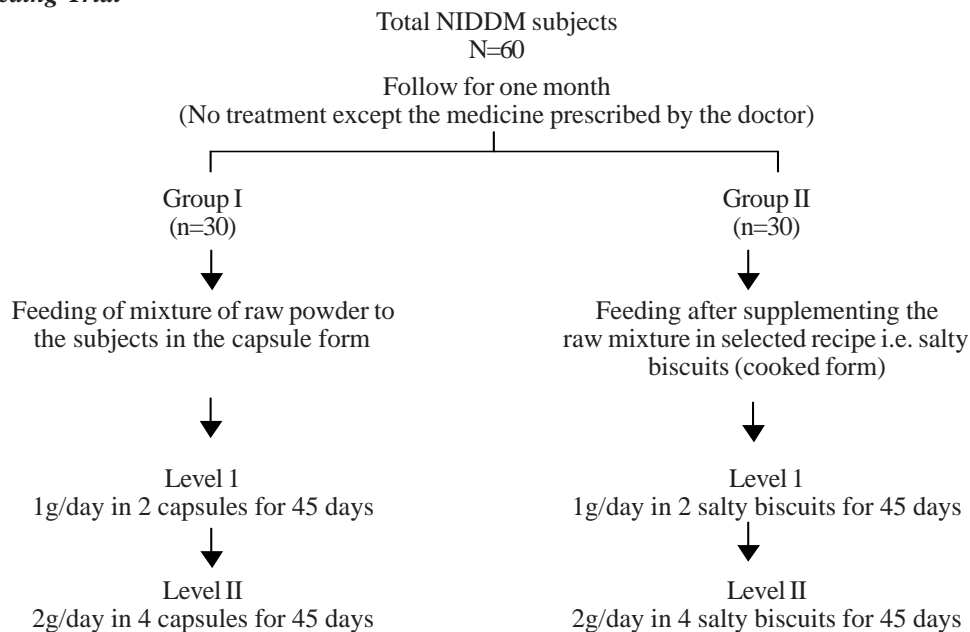
S. No.	Variables	Subjects (n=60)	
		No.	%
1.	<i>Sex</i>		
	Male	60	100
2.	<i>Age (Years)</i>		
	40-50	16	26.7
	50-60	34	56.7
	>60	10	16.6
3.	<i>Religion</i>		
	Sikh	35	58
	Hindu	25	42
4.	<i>Education</i>		
	Illiterate	05	8.3
	Primary to middle	10	16.7
	High School	25	41.7
	Graduate to P.G.	20	33.33
5.	<i>Occupation</i>		
	Service	50	83.33
	Retired	10	16.7
6.	<i>Family Type</i>		
	Nuclear	41	68.3
	Joint	19	31.7
7.	<i>Family Size</i>		
	Small (upto 4)	28	46.7
	Medium (4 to 8)	28	46.7
	Large (>8)	04	6.6
8.	<i>Activity</i>		
	Sedentary	56	93.3
	Moderate	04	6.7

All the subjects were married at the time of study

**Group I :** Patients of group I were fed raw powder mixture in the form of capsules.

**Group II :** Patients of group II were fed powdered mixture in the form of salty biscuits.

To both the groups the mixture was given at two different levels i.e. 1 gm and 2 gm daily. Feeding at each level was done for one and a half month period. To subjects of group I, two capsules at 1 gm level and 4 capsules at 2 gm level daily were given along with the lunch and dinner. Similarly to the subjects of group II, 2

**Feeding Trial**

biscuits at 1 gm level and 4 biscuits at 2 gm level were given along with lunch and dinner. These supplements were distributed to the patients on weekly basis to ensure regular consumption of supplementations as per instructions given to them. During herbal medicine treatment, the patients were taking their regular diabetic pills to avoid any complications.

**C. Collection of Blood Samples:** Five ml of fasting and post prandial (2 hours after meal) blood samples of sixty selected NIDDM subjects were collected in the beginning, after one month period, after 45 days feeding and after 90 days feeding period.

**D. Analysis of Blood Samples:** The serum was analysed for triglycerides, cholesterol, high density lipoprotein cholesterol, low density lipoprotein cholesterol by using standard methods.

Serum total cholesterol was analysed by BIOTRON BTR 820 Auto Blood Analyser using enzymatic method (Richmond, 1973). Serum high density lipoprotein cholesterol (HDL-C) was measured by using BIOTRON, BTR 820 using phosphotungstate method (Lopes-Virella, 1997). The value of serum low density lipoprotein cholesterol (LDL-C) was calculated based on Friedwald's equation (Friedewald, 1972).

$$\text{LDL-cholesterol} = \frac{\text{Total cholesterol} - \text{Triglycerides} - \text{HDL-C}}{5}$$

Serum very low density lipoprotein cholesterol (VLDL-C) was calculated based on equation

$$\text{VLDL} = \frac{\text{Triglycerides}}{5}$$

Serum triglycerides were estimated by using Autopack Reagent Kit by method of enzymatic DHBC Colourmetric Method (Fossati and Principle, 1992).

**Statistical Analysis:** The data on serum = lipid profile was analysed statically. The mean, standard error, analysis of variance, CD value, t-value and their test of significance was calculated using a computer package programme (Cheema and Singh, 1990).

**RESULTS AND DISCUSSION**

The serum lipid profile of diabetics before and after supplementation of medicinal plants mixture at two different levels is shown in Table 2. It was found that mean initial value of triglycerides, total cholesterol, LDL cholesterol and VLDL-C was on the higher side in both the groups than the desirable value given by Raghuram (1993). It was observed in the present study that as the

**Table 2: Serum lipid profile of diabetics before and after supplementation of medicinal plants\* mixture at two different levels (mg/dl).**

Serum lipid profile (mg/dl)	Group I (Raw form) n=30						Group II (Cooked form) n=30					
	Control			Supplemented			Control			Supplemented		
	Initial level	After one month	Level I	Level II	C.D. (P≤0.05)	Initial level	After one month	Level I	Level II	C.D. (P≤0.05)	Normal* range (mg/dl)	
Total triglycerides	180.7±2.56	177.0±2.60	166.5±2.73	148.7±2.66	7.53	179.3±2.92	177.6±2.87	171.8±2.89	161.6±2.75	11.05	<150	
Total cholesterol	211.8±4.26	208.5±4.21	195.2±4.08	179.7±3.88	11.73	209.0±3.62	205.8±3.36	198.3±3.49	187.9±3.48	9.96	<200	
HDL-C	37.3±0.93	40.1±0.96	48.8±0.83	58.5±0.86	2.56	35.1±0.56	37.1±0.63	42.9±0.61	50.9±0.58	1.70	30-70	
LDL-C	138.0±3.91	133.1±3.89	112.5±3.56	91.3±3.65	10.73	139.1±3.34	134.9±3.17	121.1±3.25	104.8±3.26	9.29	80-160	
VLDL-C	35.9±0.50	35.1±0.53	33.1±0.54	29.9±0.55	1.51	35.6±0.60	35.2±0.59	34.3±0.59	32.3±0.54	1.65	20-40	

Medicinal plants - mixture of fenugreek, jamun and bittergourd seeds powder

Control - without supplementation

Supplementation

Level I - 1g/day of medicinal plant mixture for 45 days.

Level II - 2g/day medicinal plant mixture for 45 days.

\*Raghuram (1993)

level of supplementation of these medicinal plants mixture increased, there was significant ( $P \leq 0.05$ ) decrease in serum triglycerides, total cholesterol, LDL-C, VLDL-C in both raw and cooked form but increase in HDL-C with the increase in supplementation of medicinal plants was observed in both the groups (Fig. 1, 2 and 3). Ratio of total cholesterol to HDL-C and LDL-C to HDL-C was also reduced with the supplementation of medicinal plants mixture in both the groups which further decreased the risk for cardiovascular disease in diabetic patients (Table 2). Percent decrease in total triglycerides (18 and 10%), total cholesterol (15 and 11%) and LDL-C (34 and 25%), VLDL-C (17 and 9%) and percent increase in HDL-C (36 and 31%) was found when powdered mixture of these medicinal plants was given in raw and cooked form (Table 3).

**Table 3: Percent change in serum lipid profile of the diabetic subjects when medicinal plants given in raw and cooked form.**

Serum Lipid Profile	Raw(%)	Cooked(%)
Triglycerides	18	10
Total cholesterol	15	11
LDL-C	35	25
VLDL-C	17	9
HDL-C	36	31

Studies reported that diabetic state, resulting from an impaired secretion and sensitivity of insulin may be responsible for high triglycerides level in serum than normal individuals, as the insulin stimulated the synthesis of adipose tissue by agency of lipoprotein lipase (Matshushita, 1982). Similar decrease in triglycerides and total cholesterol level of the diabetics were observed by feeding fenugreek seeds by various workers (Sharma, 1996). Fenugreek seed's gum and saponin fractions showed a hypocholesterolemic effect. Saponin of fenugreek may compete with cholesterol at binding site or interfere with cholesterol biosynthesis in liver. Soluble fibres like gums, pectins, mucilages may block cholesterol absorption in the intestine (Lansky, 1993). Presence of dietary fibre in fenugreek seeds, bittergourd and jambu seeds may affect serum cholesterol by reducing cholesterol and bile acid absorption by altering the metabolism and ratio of bile acid absorbed, by changing the intestinal secretion and hepatic production of lipoprotein (Chen, 1986). Gel forming properties

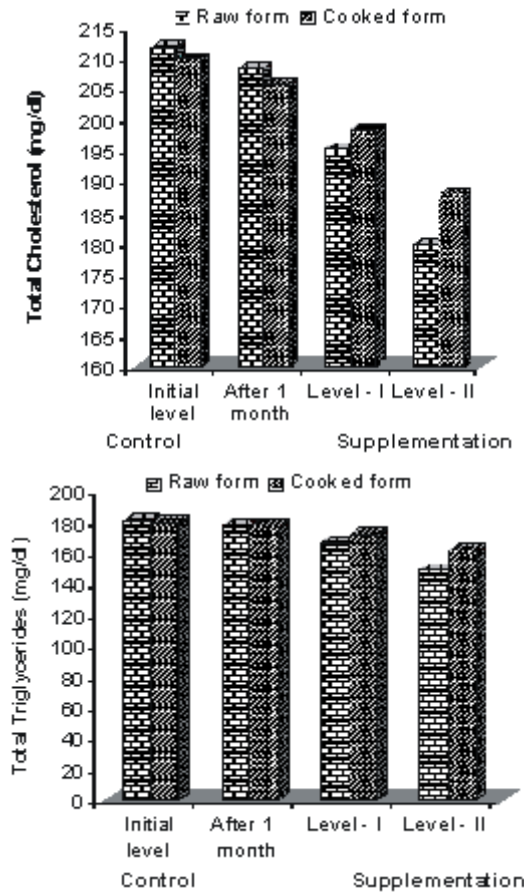


Fig. 1. Effect of supplementation of medicinal plants in raw and cooked form on total triglycerides and total cholesterol at different levels.

of some soluble dietary fibres maintain a more acidic pH of chyme which increases cholesterol ester formation. Since cholesterol are not absorbed directly by the mucosal cell which may explain cholesterol lowering effect (Chang, 1983).

Similarly, a significant increase in HDL-Cholesterol level in diabetic rats by fenugreek seeds has been reported earlier (Khosla, 1995). HDL-Cholesterol is considered to have anti-atherogenic properties, since there is negative correlation between HDL-cholesterol and risk of cardiovascular disease, HDL-C transports cholesterol from peripheral tissues to the liver thereby reducing the amount stores in tissue and decreasing the likelihood of getting atherosclerotic plaques (Eder, 1982).

Similar decrease in plasma LDL-C was observed with fenugreek seeds in diabetic rats.

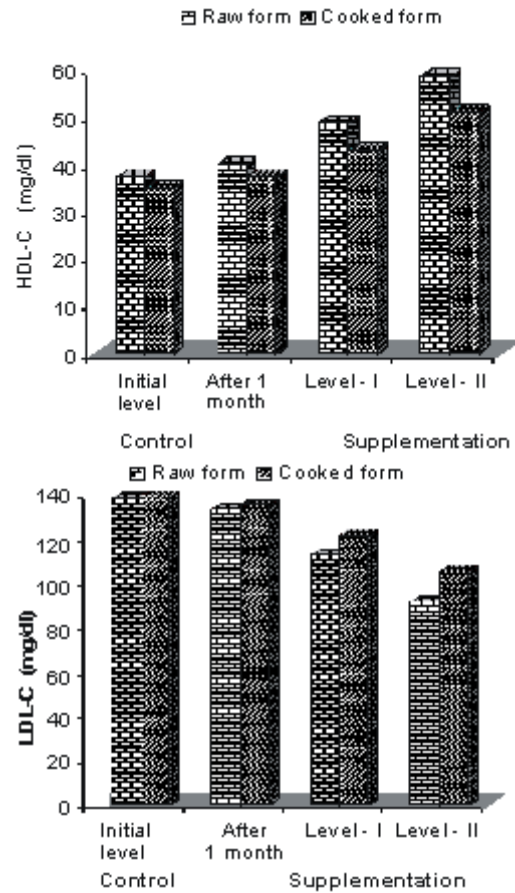


Fig. 2. Effect of supplementation of medicinal plants in raw and cooked form on HDL-C and LDL-C at different levels.

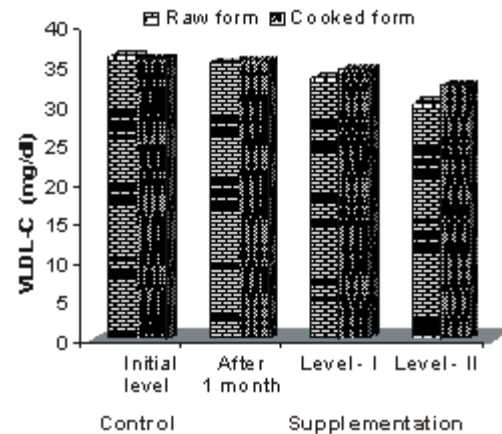


Fig. 3. Effect of supplementation of medicinal plants in raw and cooked form on VLDL-C at different levels.

Dietary fibre present in fenugreek seeds exerts a reduction mainly in LDL-cholesterol. The effect of dietary fibre on lipoprotein cholesterol is due to its association with absorption and transport of lipids (Kirby, 1981; Story, 1982). Results of the present study coincide with the result of various workers who showed a hypolipidemic effect of fenugreek seed and observed that there was significant reduction in total cholesterol, LDL, VLDL-C and triglycerides.

The results of the present study suggested that mixture of powdered bittergourd, fenugreek seeds and jambu seeds had a significant effect in lowering serum lipid profile.

### CONCLUSION

Supplementation of these medicinal plants mixture both in raw and cooked form showed a significant improvement in the serum lipid profile by lowering total, LDL-cholesterol, VLDL-cholesterol and triglycerides and by increasing HDL-cholesterol level thus helping in retarding the secondary complications. However the decrease in serum lipid profile was most significant ( $P \leq 0.01$ ) with raw form.

The traditional medicinal plants used in the present study quite acceptable by the diabetic subjects. So diabetic patients should be encouraged to include these medicinal plants in their daily diet to control lipid profile. Efforts can also made to commercialize these in the form of capsules for the convenience of the patients as it become difficult to consume these plants as such and preparation of the product with these medicinal plants is also a tedious process.

### REFERENCES

- ADA: Consensus development conference on insulin resistance. *Diabetes Care*, **21**: 310-14 (1988).
- Bezbaruah, S.: The New Face of Diabetes – Striking the Young. In: *India Today*, **10**: 14-22 (2003).
- Chang, M.L.W.: Dietary pectin: Effect on metabolic processes in rats. Unconventional sources of dietary fibre. *American Chemical Society*, Washington DC. pp. 143-154 (1983).
- Cheema, H.S. and Singh, B.: *CPCSI-A Computer Programme Package for the Analysis of Commonly Used Experimental Designs*. Punjab Agricultural University, Ludhiana. (1990).
- Chen, W.J.L. and Anderson, J.W.: Hypocholesterolemia effects of soluble fibres. pp 275-286. In: *Dietary Fibre; Basic and Clinical Aspects*. G. V. Vahouny and D. Kritchevsky (Eds.) Plenum Press, New York (1986).
- Eder, H.A. and Gidez, L.I.: The Clinical significance of plasma high density lipoprotein. *Med. Clin. North Am.*, **66**: 431-440 (1982).
- Fossati, P. and Principe, L.: Quantitative determination of triglycerides in serum or plasma by enzymatic DHBC colorimetric method *Clin. Chem.*, **28**: 2077 (1982).
- Friedewald, W.T., Levy, R.I. and Friedrickson, D.S.: Estimation of plasma or serum low density lipoprotein cholesterol concentration without use of preparative ultracentrifuge. *Clin. Chem.*, **18**: 499 (1972).
- Haffner, S.M., Stern, M.P., Hazuda, H.P. and Patterson, J.K.: Cardiovascular risk factors in confirmed prediabetic individuals. Does the clock for coronary heart disease start ticking before the onset of clinical diabetes? *JAMA*, **263**: 2893-98 (1990).
- Kedar, P. and Chakrabarti, C.H.: Effect of jambolan seed treatment on blood sugar, lipid and urea in streptozotocin induced diabetes in rabbits. *Ind. J. Physiotherap. 27*: 135-40 (1983).
- Khanna, P.: *Gourdin and its Effects. The Origin of Herbal Drug*. Department of Botany, University of Rajasthan, Jaipur (1998).
- Khosla, P., Gupta, D.D. and Nagpal, R.K.: Effect of *Trigonella foenum graecum* (fenugreek) on serum lipids in normal and diabetic rats. *Ind. J. Pharmacol.*, **27**: 89-93 (1995).
- Kirby, R.W., Anderson, J.W., Sielong, B., Rees, E.D., Chen, W.J.L., Miller, R.E. and Kay, R.M.: Dietary bran intake selectively lowers serum low density lipoprotein cholesterol concentrations in hypocholesterolemic men. *Am. J. Clin. Nutr.*, **34**: 824-29 (1981).
- Lansky, P.S.: Plants and cholesterol. *Acta Horticulture*, **332**: 131-36 (1993).
- Lopes-Virella, M.F., Stone, P., Ellis, S. and Cohwel, J.A.: Quantitative determination of HDL-Cholesterol in serum or plasma by phosphotunstate method *Clin. Chem.*, **23**: 882 (1997).
- Matshushita, K., Saito, N. and Ostuji, F.: Factors influencing serum lipid level in patients with diabetes mellitus. *J. Nutr.*, **40**: 79-90 (1982).
- Mykkanen, L., Kuvisisito, J., Pyorala, K. and Laakso, M.: Cardiovascular disease risk factor of type 2 diabetes mellitus in elderly subjects. *Diabetologia*, **36**: 553-59 (1993).
- NIH: *Diabetes in America*. 2<sup>nd</sup> Edn. National Institute of Diabetes, Digestive and Kidney Disease. Washington DC Publ. No. 951468 (1995).
- Raghuram, T.C., Pasricha, S. and Sharma, R.D.: *Diet and Diabetes*. National Institute of Nutrition, ICMR, Hyderabad (1993).
- Richmond, W.: Quantitative determination of cholesterol in serum or plasma by enzymatic method. *Clin. Chem.*, **19**: 1350 (1973).
- Saibaba, A. and Raghuram, T.C.: Fenugreek the wonder seed. *Nutrition*, National Institute of Nutrition, ICMR, Hyderabad, **31**: 21-25 (1997).
- Sharma, R.D., Sarkar, A., Hazra, D.K., Misra, B., Singh, J.B., Maheshwari, B.D. and Sharma, S.K.: Hypolipidemic effect of fenugreek seeds. A chronic study in non insulin dependent diabetic patients. *Phytotherapy Research*, **10**: 332-34 (1996).
- Story, J.A. and Kelley, M.J.: Dietary fibre and lipoproteins. pp 229-36. In: *Dietary Fibre in Health Disease*. G. V. Vahouny and D. Kritchevsky (Eds.) Plenum Press, New York (1982).

## Therapeutic applications of Fenugreek - Fenugreek

Ethan Basch

### Abstract

Fenugreek has a long history of medical uses in Ayurvedic and Chinese medicine, and has been used for numerous indications, including labor induction, aiding digestion, and as a general tonic to improve metabolism and health. Preliminary animal and human trials suggest possible hypoglycemic and antihyperlipidemic properties of oral fenugreek seed powder.

(Altern Med Rev 2003;8(1):20-27)

### Historical Uses of Fenugreek

Fenugreek (*Trigonella foenum-graecum* L. Leguminosae) is one of the oldest medicinal plants, originating in India and Northern Africa. An annual plant, fenugreek grows to an average height of two feet. The leaves and seeds, which mature in long pods, are used to prepare extracts or powders for medicinal use. Applications of fenugreek were documented in ancient Egypt, where it was used in incense and to embalm mummies. In modern Egypt, fenugreek is still used as a supplement in wheat and maize flour for bread-making. (1) In ancient Rome, fenugreek was purportedly used to aid labor and delivery. In traditional Chinese medicine, fenugreek seeds are used as a tonic, as well as a treatment for weakness and edema of the legs. (2) In India, fenugreek is commonly consumed as a condiment (2) and used medicinally as a lactation stimulant. (3) There are numerous other folkloric uses of fenugreek, including the treatment of indigestion and baldness. The possible hypoglycemic and antihyperlipidemic properties of oral fenugreek seed powder have been suggested by the results of preliminary animal and human trials.

### Active Constituents

The fraction of fenugreek that contains the testa (i.e., the portion of the fenugreek seed with the peculiar smell and bitter taste) and the endosperm of the defatted seeds (i.e., the "A" subfraction) are thought to be associated with the hypoglycemic effects of fenugreek. These effects have not been observed in studies of lipid extracts. (4,5) It is possible fenugreek lowers lipids because it contains saponins that are transformed in the gastrointestinal tract into sapogenins. Fenugreek seeds contain 50-percent fiber (30-percent soluble fiber and 20-percent insoluble fiber) that can slow the rate of postprandial glucose absorption. This may be a secondary mechanism for its hypoglycemic effect.

### Mechanisms of Action

The hypoglycemic effects of fenugreek have been attributed to several mechanisms. Sauvaire et al demonstrated in vitro the amino acid 4-hydroxyisoleucine in fenugreek seeds increased glucose-induced insulin release in human and rat pancreatic islet cells. (6) This amino acid appeared to act only on pancreatic beta cells, since the levels of somatostatin and glucagon were not altered. In human studies, fenugreek reduced the area under the plasma glucose curve and increased the number of insulin receptors, although the mechanism for this effect is unclear. (7) In humans, fenugreek seeds exert hypoglycemic effects by stimulating glucose-dependent insulin secretion from pancreatic beta cells, (8) as well as by inhibiting the activities of alpha-amylase and sucrase, (9) two intestinal enzymes involved in carbohydrate metabolism.

Fenugreek seeds also lower serum triglycerides, total cholesterol (TC), and low-density lipoprotein cholesterol (LDL-C). (10-14) These effects may be due to sapogenins, which increase biliary cholesterol excretion, in turn leading to lowered serum cholesterol levels. (10-15-17) The lipid-lowering effect of fenugreek might also be attributed to its estrogenic constituent, indirectly increasing thyroid hormone [T.sub.4].

### Fenugreek in the Treatment of Diabetes Type 2 Diabetes

In animal and several small, human trials, fenugreek seeds have been found to lower fasting serum glucose levels, both acutely and chronically.

Gupta et al reported the results of a small randomized, controlled, double-blind trial to evaluate the effects of fenugreek seeds on glycemic control. (18) Twenty-five patients with newly diagnosed type 2 diabetes received either 1 g daily of a hydroalcoholic extract of fenugreek seeds or "usual care" (dietary discretion and exercise). After two months, mean lasting blood glucose levels were reduced in both groups without significant differences between groups (148.3 mg/dL to 119.9 mg/dL in the fenugreek group versus 137.5 mg/dL to 113.0 mg/dL in the "usual care" group). There were no significant differences between groups in mean glucose tolerance test values at the study's end. The authors did note differences between groups in the area under the curve for blood glucose and insulin levels. This study suggests that fenugreek seed extract and diet/exercise may be equally effective strategies for attaining glycemic control in type 2 diabetes. However, the trial may have been too small or brief to detect significant mean differences between groups. In addition, it is not clear if mean glucose values would have normalized without intervention, and design and methods were not well described, which limits the clinical relevance of these results.

Raghuram et al reported the results of a randomized, controlled, crossover trial of fenugreek seeds in 10 patients with type 2 diabetes. (7) The doses of these patients' antidiabetic drug, glibenclamide, ranged from 2.5-7.5 mg per day; both medication dose and dietary intake were stabilized prior to the actual study periods. The patients were given either 25 g powdered fenugreek seeds in two equal doses with meals or meals without fenugreek supplementation for 15 days. The fenugreek powder was added to the experimental diet in the form of dietary fiber, resulting in higher fiber content in the experimental diet than in the control diet. Five diabetic patients were randomized to receive fenugreek during the first 15-day period; the other five received it during the second period. Subjects were then crossed over an additional 15 days with no washout period. In the fenugreek-treated patients, statistically significant mean improvements were reported for glucose-tolerance test scores and serum-clearance rates of glucose (control group, 153 [+ or -] 11.92 mg/mL/min; fenugreek group, 136.4 [+ or -] 6.36 mg/mL/min). The absolute difference in glucose between the two groups was not mentioned. Larger studies with washout periods are needed to confirm these results.

Sharma and Raghuram conducted two randomized, controlled, crossover studies in patients with type 2 diabetes. (19) The doses of 15 patients' antidiabetic drug, glibenclamide/glipizide/ metformin, were reduced by 20 percent and both medication dose and dietary intake were stabilized for one week prior to the actual study

periods. In the first study, subjects ate meals with or without 100 g of defatted fenugreek seed powder, divided into two equal doses, for 10 days. Patients were then crossed over an additional 10 days. Seven of the 15 patients received the fenugreek diet first; there was no washout period.

The second study had a similar study design, except the duration of the study was 20 days and the total subject number was five (three patients received the fenugreek diet first). (19) Significant mean improvements in fasting blood-glucose levels and glucose-tolerance test results were described in the fenugreek-treated patients. The reduction in fasting blood glucose ranged from 179 [+ or -] 24 mg/dL to 137 [+ or -] 20.2 mg/dL ( $p < 0.05$ ) in the first study and from 157 [+ or -] 22.2 mg/dL to 116 [+ or -] 17.1 mg/dL ( $p < 0.05$ ) in the second study. The 24 hour urinary glucose excretion in both studies was statistically significant. The fenugreek-treated patient group also reported subjective improvements in polydipsia and polyuria.

Neeraja and Rajyalakshmi presented a poorly designed, complex case series including six men with type 2 diabetes and six without diabetes. (20) The cases suggest fenugreek reduced postprandial hyperglycemia primarily in subjects with diabetes, but less so in subjects without diabetes. This effect might be more pronounced if raw seeds rather than boiled seeds had been used.

Results from several additional case series (21-24) also suggest fenugreek seeds may improve glycemic control in type 2 diabetes. As with all case series, however, the lack of controls increases the possibility the results obtained were due to confounding from other interventions. Although the results of some of these case series are promising, the conclusions drawn from them are preliminary. The studies conducted to date have been methodologically weak, lacking adequate descriptions of blinding, randomization, baseline patient characteristics, statistical analysis, and standardization data for the therapy used. Demonstrating the efficacy of fenugreek has also been confounded by inconsistencies in the preparations, dosing regimens, and outcome measures used in the trials. Moreover, none of the investigations have been conducted over the long term. Additional study of fenugreek is warranted in this area before firm conclusions can be drawn.

#### Type 1 Diabetes

Sharma et al conducted a randomized, controlled, crossover trial in 10 patients with type 1 diabetes. (25) Over a 10-day period, the subjects were served meals that contained 100 g fenugreek seed powder in two divided doses each day (lunch and dinner) or meals without fenugreek. At the study's end, significant improvement was noted in the fenugreek group in several parameters, including a 54-percent reduction in 24-hour urine glucose levels and mean reductions in glucose-tolerance test values and fasting serum-glucose levels (from 15.1 [+ or -] 2.4 mMol/L to 10.9 [+ or -] 2.75 mMol/L;  $p < 0.01$ ). Although these data are intriguing, they cannot be considered definitive. This study suggests fenugreek may aid with insulin secretion, as suggested by animal studies, since typically these patients have little or no endogenous insulin production. More studies in people with type 1 diabetes are warranted.

Table 1 summarizes the fenugreek studies for diabetes, while Table 2 describes the scoring procedure for these studies.

#### Effects on Lipid Lowering

In the Sharma et al trial on type 1 diabetes cited above, (25) small but statistically significant reductions were noted in TC (approximately 1.3 mMol/L;  $p < 0.001$ ) and in LDL-C levels (approximately 1.0 mMol/L;  $p < 0.01$ ), but the level of high-density lipoprotein cholesterol (HDL-C) remained unchanged. Without an adequate description of blinding and randomization, the results of this study can only be considered preliminary.

Several case series have also found hypocholesterolemic effects associated with oral fenugreek. Sharma et al investigated 15 nonobese, asymptomatic, hyperlipidemic adults. (26) After the subjects had ingested 100 g defatted fenugreek powder per day for three weeks, their triglyceride (TG) and LDL-C levels were lower than baseline values. Slight decreases in HDL levels were also noted.

In a later study, normalization of lipid profiles was observed in 60 patients with type 2 diabetes whose diets were supplemented with 25 g powdered fenugreek seeds per day for 24 weeks. (27,28) While mean TC, LDL-C, and TG levels decreased by 14-16 percent during the study period, mean HDL-C levels increased by 10 percent. Similarly, Sowmya and Rajyalakshmi observed significant reductions in TC and LDL-C levels in 20 adults with hypercholesterolemia who received 12.5-18.0 g powdered, germinated fenugreek seeds for one month, although no changes in HDL-C, very-low-density lipoprotein (VLDL), or TG levels were observed. (29)

In another study, Sharma also reported a decrease in total cholesterol levels in five diabetic patients treated with fenugreek seed powder (25 g orally per day) for 21 days. (30) Bordia et al studied the effects of fenugreek seed powder (2.5 g administered twice daily for three months) in a subgroup of 40 subjects. (22) In the subjects who had coronary artery disease and type 2 diabetes, significant decreases in the TC and TG levels were observed, with no change in HDL-C level. The methodology for this study was not clearly documented.

Most available studies are case series lacking proper controls, randomization, or blinding. Further, double-blind research is warranted.

#### Safety/Adverse Effects

A review of the literature on fenugreek reveals no reports of clinically significant harmful adverse effects. Although fenugreek has traditionally been considered safe and well tolerated, some side effects have been associated with its use. Caution in using fenugreek is warranted in patients known to be allergic to it or who are allergic to chickpeas because of possible cross-reactivity. (3) Fenugreek contained in curry powder was found to be an allergen in a patient who reported severe bronchospasm, wheezing, and diarrhea. (31)

Other reported side effects include transient diarrhea and flatulence. (23,27,30) and dizziness. (32) Hypoglycemia is an expected effect; therefore, care should be taken to monitor blood glucose levels when beginning supplementation. (19,21-25,32-34) Decreased body weight has also been reported and attributed to decreases in [T.sub.3]. (35) Because fenugreek preparations can contain coumarin derivatives, there is a theoretical risk prothrombin time (PT) or the international normalized ratio (INR) might be increased, which, in turn, increases the risk of bleeding. (36) Fenugreek should not be used during pregnancy because of its potential uterine stimulating properties observed in early animal studies. (37)

#### Potential Drug Interactions

Products rich in fiber (such as fenugreek fiber) can interfere with the absorption of oral medications because its fiber is mucilaginous and has high viscosity in the gut.

Prescription medications, therefore, should be taken separately from fenugreek-containing products. Because concomitant use of fenugreek with other hypoglycemic agents might lower serum glucose levels more than expected, the level should be monitored closely. (19,21-25,32,33) An aqueous extract of fenugreek reduced potassium levels in a small group of healthy subjects 14 percent. (15) Consequently, fenugreek may precipitate hypokalemia when used in combination with some diuretics, laxatives, mineralocorticoids, or other hypokalemic agents. (32) Fenugreek is also purported to contain an estrogenic constituent. Decreases in the serum level of [T.sub.3] and in the [T.sub.3]/[T.sub.4] ratio, as well as an increase in the serum level of [T.sub.4], have been observed in mice and rats given fenugreek. (35)

**Toxicology**

Toxicological evaluation of 60 diabetic patients who took powdered fenugreek seeds at a dose of 25 g per day for 24 weeks disclosed no clinical hepatic or renal toxicity and no hematological abnormalities. (27) In an animal study, the acute oral [LD.sub.50] was found to be >5 g/kg in rats, and the acute dermal [LD.sub.50] was found to be >2 g/kg in rabbits. (38) In another animal study, fenugreek powder failed to induce any signs of toxicity or mortality in mice and rats who received acute and subchronic regimens. (39) Moreover, there were no significant hematological, hepatic, or histopathological changes in weanling rats fed fenugreek seeds for 90 days. (40)

**Dosage**

Defatted powdered fenugreek seeds (100 g), divided in two equal doses, (25) have been used to treat type 1 diabetes. Fenugreek seed powder in capsule form (2.5 g twice daily for three months) (22) and in seed powder (25 g divided into two equal doses) (7,27,28) have been used to treat type 2 diabetes. Fenugreek has also been used to treated hyperlipidemia, both as seed powder in capsule form (2.5 g twice daily for three months) (22) and as defatted powdered seeds (100 g divided in two equal doses). (25) Commercially, fenugreek is available in seed powder capsules, teas, and pulverized seeds that can be mixed in water.

**Conclusions and Future Direction**

The incidence of type 2 diabetes is increasing dramatically worldwide, resulting in large measure from the increasing prevalence of obesity. (41) In addition, research is uncovering the importance of the "pre-diabetic" state or metabolic syndrome, when insulin resistance gives rise to impairment of glucose metabolism. (41,42) Unfortunately, patients who have metabolic syndrome or diabetes are at greatly increased risk of cardiovascular morbidity and mortality. (42,43) Thus, dietary supplements that can modulate glucose homeostasis and potentially improve lipid parameters would be desirable. This is especially true for diabetes prevention in patients with metabolic syndrome. These patients already manifest abnormalities of glucose handling and could benefit from a low-risk, inexpensive, food-based intervention aimed at normalizing their metabolic milieu. Fenugreek is a dietary supplement that may hold promise in this regard. The data generated to date are sparse but will hopefully lead to the development of well-designed, adequately powered, randomized, clinical trials evaluating the effect of fenugreek seed powder on measures of insulin resistance, insulin secretion, and cholesterol metabolism.

Table 1. Summary of Fenugreek Studies for Diabetes

Condition Treated (Primary or Secondary Outcome)	Evidence/ Study Type	Author; Year	N	Statistically Significant Results?
Type 2 diabetes, hyperlipidemia	Randomized, controlled, double-blind study	Gupta, 2001	25	Yes
Type 2 diabetes	Randomized, crossover study	Raghuram, 1994	10	Yes
Type 2 diabetes	Randomized, crossover study	Sharma, 1990	15	Yes
Type 2 diabetes	Case series with matched controls	Neeraja, 1996	12	Yes
Type 1 diabetes, hyperlipidemia	Randomized, crossover study	Sharma, 1990	10	Yes
Condition Treated (Primary or Secondary Outcome)	Quality of Study 0-2=poor 3-4=good 5=excellent	Magnitude of Benefit (how strong is the effect?)	Absolute Risk Reduction	Number of Patients Needed to Treat for One Outcome
Type 2 diabetes, hyperlipidemia	3	None	NA	NA

Type 2 diabetes	1	Large	NA	NA
Type 2 diabetes	1	Small	NA	NA
Type 2 diabetes	1	Medium	NA	NA
Type 1 diabetes, hyperlipidemia	1	Large	NA	NA
Condition Treated (Primary or Secondary Outcome)	Comments			
Type 2 diabetes, hyperlipidemia	Improved fasting glucose and GTT with fenugreek seeds or diet/exercise, without differences between groups. Altered AUC and insulin resistance with fenugreek.			
Type 2 diabetes	Improved peripheral glucose utilization with fenugreek seed supplementation.			
Type 2 diabetes	Improvement in reported diabetic symptoms.			
Type 2 diabetes	Improvement of acute glycemic response, most notable with raw fenugreek seed powder.			
Type 1 diabetes, hyperlipidemia	Fasting blood glucose levels and GOT improved; serum insulin levels unchanged.			

Table 2. Jadad Score Explanation

Item	Score *
Was the study described as randomized (this includes words such as randomly, random, and randomization)?	0/1
Was the method used to generate the sequence of randomization described and appropriate (table of random numbers, computer-generated, etc)?	0/1
Was the study described as double blind?	0/1
Was the method of double blinding described and appropriate (identical placebo, active placebo, dummy, etc)?	0/1
Was there a description of withdrawals and dropouts?	0/1

Deduct one point if the method used to generate the sequence of randomization was described and it was inappropriate (patients were allocated alternately, or according to date of birth, hospital number, etc).

Deduct one point if the study was described as double blind but the method of blinding was inappropriate (e.g., comparison of tablet vs. injection with no double dummy).

\* Points are added, and a score [greater than or equal to] 4 is considered high quality methodologically. Based on Jadad AR, Moore RA, Carroll D, et al. Assessing the quality of reports of randomized clinical trials: Is blinding necessary? *Control Clin Trials* 1996;17(1):1-12.

#### References

- (1.) Morcos SR, Elhawary Z, Gabrial GN. Protein-rich food mixtures for feeding the young in Egypt. 1. Formulation. *Z Ernährungswiss* 1981;20:275-282.
- (2.) Yoshikawa M, Murakami T, Komatsu H, et al. Medicinal foodstuffs. IV. Fenugreek seed. (1): structures of trigoneosides Ia, Ib, IIa, IIb, IIIa, and IIIb, new furostanol saponins from the seeds of Indian *Trigonella foenum-graecum* L. *Chem Pharm Bull (Tokyo)* 1997;45:81-87.
- (3.) Patil SP, Niphadkar PV, Bapat MM. Allergy to fenugreek (*Trigonella foenum graecum*). *Ann Allergy Asthma Immunol* 1997;78:297-300.
- (4.) Ribes G, Sauvaire Y, Baccou JC, et al. Effects of fenugreek seeds on endocrine pancreatic secretions in dogs. *Ann Nutr Metab* 1984;28:37-43.
- (5.) Ribes G, Sauvaire Y, Da Costa C, et al. Antidiabetic effects of subfractions from fenugreek seeds in diabetic dogs. *Proc Soc Exp Biol Med* 1986; 182:159-166.
- (6.) Sauvaire Y, Petit P, Broca C, et al. 4-Hydroxyisoleucine: a novel amino acid potentiator of insulin secretion. *Diabetes* 1998;47:206-210.
- (7.) Raghuram TC, Sharma RD, Sivakumar B, et al. Effect of fenugreek seeds on intravenous glucose disposition in non-insulin dependent diabetic patients. *Phytother Res* 1994;8:83-86.
- (8.) Ajabnoor MA, Tilmisany AK. Effect of *Trigonella foenum graecum* on blood glucose levels in normal and alloxan-diabetic mice. *J Ethnopharmacol* 1988;22:45-49.
- (9.) Amin R, Abdul-Ghani AS, Suleiman MS. Effect of *Trigonella foenum graecum* on intestinal absorption. Proc. of the 47th Annual Meeting of the American Diabetes Association (Indianapolis U.S.A.). *Diabetes* 1987;36:21 la.
- (10.) Stark A, Madar Z. The effect of an ethanol extract derived from fenugreek (*Trigonella foenum-graecum*) on bile acid absorption and cholesterol levels in rats. *Br J Nutr* 1993;69:277-287.
- (11.) Petit P, Sauvaire Y, Ponsin G, et al. Effects of a fenugreek seed extract on feeding behaviour in the rat: metabolic-endocrine correlates. *Pharmacol Biochem Behav* 1993;45:369-374.
- (12.) Al-Habori M, Al-Aghbari AM, Al-Mamary M. Effects of fenugreek seeds and its extracts on plasma lipid profile: a study on rabbits. *Phytother Res* 1998;12:572-575.
- (13.) Al-Habori M, Raman A. Antidiabetic and hypocholesterolaemic effects of fenugreek. *Phytother Res* 1998; 12:233-242.
- (14.) Valette G, Sauvaire Y, Baccou JC, Ribes G. Hypocholesterolaemic effect of fenugreek seeds in dogs. *Atherosclerosis* 1984;50:105-111.
- (15.) Sauvaire Y, Ribes G, Baccou JC, et al. Implication of steroid saponins and sapogenins in the hypocholesterolemic effect of fenugreek. *Lipids* 1991;26:191-197.
- (16.) Varshney IP, Sharma SC. Saponins and sapogenins: part XXXII. Studies on *Trigonella foenum-graecum* Linn. seeds. *J Indian Chem Soc* 1966;43:564-567.
- (17.) Sidhu GS, Oakenfull DG. A mechanism for the hypocholesterolaemic activity of saponins. *Br J Nutr* 1986;55:643-649.
- (18.) Gupta A, Gupta R, Lal B. Effect of *Trigonella foenum-graecum* (fenugreek) seeds on glycaemic control and insulin resistance in type 2 diabetes mellitus: a double blind placebo controlled study. *J Assoc Physicians India* 2001;49:1057-1061.
- (19.) Sharma RD, Raghuram TC. Hypoglycaemic effect of fenugreek seeds in non-insulin dependent diabetic subjects. *Nutr Res* 1990;10:731-739.
- (20.) Neeraja A, Rajyalakshmi P. Hypoglycemic effect of processed fenugreek seeds in humans. *J Food Sci Technol* 1996;33:427-430.
- (21.) Madar Z, Abel R, Samish S, Arad I. Glucose-lowering effect of fenugreek in non-insulin dependent diabetics. *Ear J Clin Nutr* 1988;42:51-54.
- (22.) Bordia A, Verma SK, Srivastava KC. Effect of ginger (*Zingiber officinale* Rosc.) and fenugreek (*Trigonella foenum-graecum* L.) on blood lipids, blood sugar and platelet aggregation in patients with coronary artery disease. *Prostaglandins Leukot Essent Fatty Acids* 1997;56:379-384.
- (23.) Sharma RD. Effect of fenugreek seeds and leaves on blood glucose and serum insulin responses in human subjects. *Nutr Res* 1986;6:1353-1364.
- (24.) Sharma RD, Sarkar A, Hazra DK, et al. Use of fenugreek seed powder in the management of non-insulin dependent diabetes mellitus. *Nutr Res* 1996;16:1331-1339.

- (25.) Sharma RD, Raghuram TC, Rao NS. Effect of fenugreek seeds on blood glucose and serum lipids in type I diabetes. *Eur J Clin Nutr* 1990;44:301-306.
- (26.) Sharma RD, Raghuram TC, Dayasagar Rao V. Hypolipidaemic effect of fenugreek seeds. A clinical study. *Phytother Res* 1991;3:145-147.
- (27.) Sharma RD, Sarkar A, Hazra DK, et al. Toxicological evaluation of fenugreek seeds: a long term feeding experiment in diabetic patients. *Phytother Res* 1996;10:519-520.
- (28.) Sharma RD, Sarkar DK, Hazra B, et al. Hypolipidaemic effect of fenugreek seeds: a chronic study in non-insulin dependent diabetic patients. *Phytother Res* 1996; 10:332-334.
- (29.) Sowmya R Rajyalakshmi R Hypocholesterolemic effect of germinated fenugreek seeds in human subjects. *Plant Foods Hum Nutr* 1999;53:359-365.
- (30.) Sharma R. An evaluation of hypocholesterolemic factor of fenugreek seeds (*T foenum graecum*) in rats. *Nutr Rep Int* 1986;33:669-677.
- (31.) Ohnuma N, Yamaguchi E, Kawakami Y. Anaphylaxis to curry powder. *Allergy* 1998;53:452-454.
- (32.) Abdel-Barry, JA, Abdel-Hassan IA, Jawad AM, al-Hakiem MH. Hypoglycaemic effect of aqueous extract of the leaves of *Trigonella foenum-graecum* in healthy volunteers. *East Mediterr Health J* 2000;6:83-88.
- (33.) Mishkinsky J, Joseph B, Sulman FG. Hypoglycaemic effect of trigonelline. *Lancet* 1967;2:1311-1312.
- (34.) Sewell AC, Mosandl A, Bohles H. False diagnosis of maple syrup urine disease owing to ingestion of herbal tea. *N Engl J Med* 1999;341:769.
- (35.) Panda S, Tahiliani P, Kar A. Inhibition of triiodothyronine production by fenugreek seed extract in mice and rats. *Pharmacol Res* 1999;40:405-409.
- (36.) Lambert JR Cormier A. Potential interaction between warfarin and boldo-fenugreek. *Pharmacotherapy* 2001;21:509-512.
- (37.) Abdo MS, al-Kafawi AA, Experimental studies on the effect of *Trigonella foenum-graecum*. *Planta Med* 1969;17:14-18.
- (38.) Opdyke DL. Fenugreek absolute. *Food Cosmet Toxicol* 1978; 16:S755-S756.
- (39.) Muralidhara, Narasimhamurthy K, Viswanatha S, Ramesh BS. Acute and subchronic toxicity assessment of debitterized fenugreek powder in the mouse and rat. *Food Chem Toxicol* 1999;37:831-838.
- (40.) Rao PU, Sesikeran B, Rao PS, et al. Short term nutritional and safety evaluation of fenugreek. *Nutr Res* 1996;16:1495-1505.
- (41.) Yale JF. Prevention of type 2 diabetes. *Int J Clin Pract Suppl* 2000;113:35-39.
- (42.) No authors listed. Executive summary of the third report of The National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III). *JAMA* 2001;285:2486-2497.
- (43.) Ford ES, Giles WH, Dietz WH. Prevalence of the metabolic syndrome among US adults: findings from the third National Health and Nutrition Examination Survey. *JAMA* 2002;287:356-359.

Catherine Ulbricht, PharmD--Senior Attending Pharmacist,  
Massachusetts General Hospital; Adjunct Clinical Professor,  
Massachusetts College of Pharmacy; Assistant Clinical Professor,  
Northeastern University; and Assistant Clinical Professor,  
University of Rhode Island.

Correspondence address: 1130 Massachusetts Avenue,  
Cambridge, MA 02138-5204;  
e-mail: kate@naturalstandard.com

Ethan Basch, MD--Editorial Board of Harvard Health Publications  
and the Journal of Herbal Pharmacotherapy; Chief Editor,  
Massachusetts General Hospital Primer of Outpatient Medicine;  
Advisory Boards, Integrative Medicine Alliance and CancerSource

Michael Smith, ND--Associate Dean for Research at the Canadian  
College of Naturopathic Medicine; serves on National Advisory  
Group on Complementary and Alternative Medicine and HIV/AIDS  
panel.

Philippe Szapary, MD--Assistant Professor of Medicine, Division of  
General Internal Medicine, University of Pennsylvania where he  
conducts clinical trials of CAM therapies in cardiovascular disease.

Grace Kuo, PharmD--Faculty, Baylor College of Medicine; teaches  
at the University of Houston College of Pharmacy.

COPYRIGHT 2003 Thorne Research Inc.  
COPYRIGHT 2003 Gale Group

**Effect of Trigonella foenum-graecum seeds on glycaemic control and insulin resistance in type 2 diabetes mellitus: a double blind placebo controlled study - fenugreek - Abstract**

A Gupta

Gupta A, Gupta R, Lal B. J Assoc Physicians India 2001;49:1057-1061.

**OBJECTIVES:** To evaluate the effects of Trigonella foenum-graecum (fenugreek) seeds on glycemic control and insulin resistance, determined by HOMA model, in mild to moderate type 2 diabetes mellitus we performed a double blind placebo controlled study. **METHODS:** Twenty five newly diagnosed patients with type 2 diabetes (fasting glucose < 200 mg/dl) were randomly divided into two groups. Group I (n=12) received 1 gm/day hydroalcoholic extract of fenugreek seeds and Group II (n=13) received usual care (dietary control, exercise) and placebo capsules for two months. **RESULTS:** At baseline both the groups were similar in anthropometric and clinical variables. Oral glucose tolerance test, lipid levels, fasting C-peptide, glycosylated haemoglobin, and HOMA-model insulin resistance were also similar at baseline. In group 1 as compared to group 2 at the end of two months, fasting blood glucose (148.3 +/- 44.1 to 119.9 +/- 25 vs. 137.5 +/- 41.1 to 113.0 +/- 36.0) and two hour postglucose blood glucose (210.6 +/- 79.0 to 181.1 +/- 69 vs. 219.9 +/- 41.0 to 241.6 +/- 43) were not different. But area under curve (AUC) of blood glucose (2375 +/- 574 vs 27597 +/- 274) as well as insulin (2492 +/- 2536 vs. 5631 +/- 2428) was significantly lower (p < 0.001). HOMA model derived insulin resistance showed a decrease in percent beta-cell secretion in group 1 as compared to group 2 (86.3 +/- 32 vs. 70.1 +/- 52) and increase in percent insulin sensitivity (112.9 +/- 67 vs 92.2 +/- 57) (p < 0.05). Serum triglycerides decreased and HDL cholesterol increased significantly in group 1 as compared to group 2 (p < 0.05). **CONCLUSIONS:** Adjunct use of fenugreek seeds improves glycemic control and decreases insulin resistance in mild type-2 diabetic patients. There is also a favourable effect on hypertriglyceridemia.

COPYRIGHT 2002 Thorne Research Inc.

COPYRIGHT 2002 Gale Group

A service of the National Library of Medicine  
and the National Institutes of Health

My NCBI

[\[Sign In\]](#) [\[Register\]](#)

All Databases PubMed Nucleotide Protein Genome Structure OMIM PMC Journals Books

Search PubMed for 17392143[uid] Go Clear [Save Search](#)[Limits](#) [Preview/Index](#) [History](#) [Clipboard](#) [Details](#)

Display AbstractPlus Show 20 Sort By Send to

All: 1 Review: 0

 1: [Asia Pac J Clin Nutr.](#) 2007;16 Suppl 1:422-6.[Links](#)**Effect of Trigonella foenum-graecum (fenugreek) extract on blood glucose, blood lipid and hemorheological properties in streptozotocin-induced diabetic rats.**[Xue WL](#), [Li XS](#), [Zhang J](#), [Liu YH](#), [Wang ZL](#), [Zhang RJ](#).

Department of Public Health, School of Medicine, Xi'an Jiaotong University, 76 West Yanta Road, Xi'an, Shaanxi, China 710061. xwl0908@163.com

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 administered 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 cholesterol 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<sup>-1</sup>) and low shear rate (40 s<sup>-1</sup>), 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.

PMID: 17392143 [PubMed - indexed for MEDLINE]

Display AbstractPlus Show 20 Sort By Send to

**Related Links**

- ▶ Soluble dietary fibre fraction of Trigonella foenum-graecum (fenugreek) see [Br J Nutr. 2007]
- ▶ Effect of Trigonella foenum graecum (Fenuareek) on blood glucose in [Indian J Physiol Pharmacol. 1995]
- ▶ Hypoglycaemic and antihyperalcaemic effects of Trigonella foenum-graecum [J Ethnopharmacol. 1997]
- ▶ Trigonellafoenum graecum (fenuareek) seed powder improves glucose T [Mol Cell Biochem. 2001]
- ▶ Effect of soluble dietary fibre fraction of Trigonella foenum graecum [J Ethnopharmacol. 2003]

[See all Related Articles...](#)[Write to the Help Desk](#)[NCBI](#) | [NLM](#) | [NIH](#)[Department of Health & Human Services](#)[Privacy Statement](#) | [Freedom of Information Act](#) | [Disclaimer](#)



You have **Guest** access to ScienceDirect  
[Find out more...](#)

Login: [Register](#)

Home	Browse	My Settings	Alerts	Help
<b>Quick Search</b>	Title, abstract, keywords	Use of Fenugreek seed powder	Author	<input type="text"/> e.g. j s smith
search tips	Journal/book title	<input type="text"/>	Volume	<input type="text"/>
			Issue	<input type="text"/>
			Page	<input type="text"/>
			Clear	

## Nutrition Research

Volume 10, Issue 7, July 1990, Pages 731-739

[Result list](#) | [previous](#) < 3 of 4 > [next](#)

**SCOPUS**  
refine your res

[Abstract](#) | [Abstract + References](#) | [PDF \(433 K\)](#)

Add to my Quick Links | E-mail Article

doi:10.1016/S0271-5317(05)80822-X [Cite or Link Using DOI](#)  
 Copyright © 1990 Published by Elsevier Inc.

# Hypoglycaemic effect of **fenugreek seeds** in non-insulin dependent diabetic subjects

Ph.D. R.D. Sharma <sup>a</sup> and M.D., Ph.D. T.C. Raghuram <sup>a</sup>

<sup>a</sup>National Institute of Nutrition Indian Council of Medical Research Jamai Osmania, Hyderabad-500 007 India  
 Accepted 5 February 1990. Available online 2 June 2006.

## Abstract

**Fenugreek seeds** (Trigonella foenum graecum), a commonly used condiment in Indian homes, were evaluated for hypoglycaemic property. In a metabolic study, 15 non-insulin dependent diabetic patients were given randomly, in a cross over design, diets with or without 100 g of defatted **fenugreek seed powder**, each for 10 days. Incorporation of **fenugreek** produced a significant fall in fasting blood glucose levels and an improvement in glucose tolerance test. Insulin responses were significantly reduced. There was a 64% reduction in 24 hr urinary glucose excretion with significant alterations in serum lipid profile. Serum total cholesterol, LDL and VLDL cholesterol and triglyceride levels decreased without any alteration in HDL cholesterol fraction with **fenugreek** diet.

Incorporation of **fenugreek seeds** for 20 days in the diets of 5 diabetic patients resulted in similar changes of higher magnitude in all the above parameters.

**Key words:** Diabetes; dietary fiber; diet therapy; blood glucose; lipids; condiments

To whom reprint requests should be addressed

## Nutrition Research

Volume 10, Issue 7, July 1990, Pages 731-739

[Result list](#) | [previous](#) < 3 of 4 > [next](#)

Home	Browse	My Settings	Alerts	Help
------	--------	-------------	--------	------



[About ScienceDirect](#) | [Contact Us](#) | [Terms & Conditions](#) | [Privacy Policy](#)

Copyright © 2007 Elsevier B.V. All rights reserved. ScienceDirect® is a registered trademark of Elsevier B.V.

### Purchase the full-text article

- PDF and HTML
- ALL references
- All images
- All tables



### Related Articles in ScienceDirect

- [Use of Fenugreek seed powder in the management of non-i... Nutrition Research](#)
- [Effect of fenugreek seeds and leaves on blood glucose a... Nutrition Research](#)
- [Hypocholesterolaemic effect of Fenugreek seeds in dogs Atherosclerosis](#)
- [Effects of a fenugreek seed extract on feeding behaviou... Pharmacology Biochemistry and Behavior](#)
- [Antidiabetic property of fenugreek seed mucilage and sp... Nutrition Research](#)

[View More Related Articles](#)

[Request Permission](#)

[View Record in Scopus](#)

[Cited By in Scopus \(34\)](#)

**SCOPUS**  
refine your res

## Relationship between structure and function of dietary fibre: A comparative study of the effects of three galactomannans on cholesterol metabolism in the rat

**Authors:** Evans, A. J.<sup>1</sup>; Hood, R. L.<sup>1</sup>; Oakenfull, D. G.<sup>1</sup>; Sidhu, G. S.<sup>1</sup>  
**Source:** *British Journal of Nutrition*, Volume 68, Number 1, July 1992, pp. 217-229(13)  
**Publisher:** CABI Publishing



Online ISSN:  
1475-2662

[< previous article](#) | [next article >](#) | [view table of contents](#)

### ◀mark item

**Key:** **F** - Free Content **N** - New Content **S** - Subscribed Content **T** - Free Trial Content

### Abstract:

Male adult rats were fed on diets containing 80 g/kg galactomannans with different galactose (G): mannose (M) ratios/kg. The galactomannans were compared with purified cellulose (Solkaflor) and the animals were also fed on a basal diet free from fibre. All diets contained cholesterol (10 g/kg) and sodium cholate (2 g/kg). The three galactomannans were fenugreek gum (1G:1M), guar gum (1G:2M) and locust-bean gum (1G:4M). In comparison with the fibre-free and Solkaflor diets, all three galactomannans lowered the concentrations of cholesterol in both liver and blood plasma. The galactomannans also decreased the rate of hepatic synthesis of cholesterol. Dietary galactomannans increased caecal volatile fatty acids, particularly propionic, increased the weight of the caecum and its contents and increased the amount of water in the faeces. The increase in propionic acid production was significantly related to a decrease in caecal pH, but not to changes in plasma cholesterol or hepatic cholesterol synthesis. These effects were significantly influenced by chemical composition and structure of the galactomannan; they were most evident when the proportion of galactose in the galactomannan was highest (i.e. fenugreek gum). The three galactomannans also differed markedly in their effects on the viscosity of the digesta, but the galactomannan which gave the highest viscosity was least effective in lowering plasma cholesterol. A separate experiment with perfused loops of small intestine *in vivo* showed that the most effective galactomannan, fenugreek gum, had no direct effect on cholesterol absorption.

**Keywords:** Galactomannans; Dietary fibre; Cholesterol; Rat

**Document Type:** Research article

**DOI:** 10.1079/BJN19920079

**Affiliations:** 1: CSIRO Division of Food Processing, Food Research Laboratory, PO Box 52, North Ryde, NSW, 2113, Australia

**The full text article is not available for purchase.**

The publisher only permits individual articles to be downloaded by subscribers.

[< previous article](#) | [next article >](#) | [view table of contents](#)

[Back to top](#)

**Key:** **F** - Free Content **N** - New Content **S** - Subscribed Content **T** - Free Trial Content



A service of the National Library of Medicine  
and the National Institutes of Health

My NCBI

[\[Sign In\]](#) [\[Register\]](#)

All Databases PubMed Nucleotide Protein Genome Structure OMIM PMC Journals Books

Search PubMed for 17313713[uid] Go Clear [Save Search](#)

[Limits](#) [Preview/Index](#) [History](#) [Clipboard](#) [Details](#)

Display AbstractPlus Show 20 Sort By Send to

All: 1 Review: 0

1: [Br J Nutr.](#) 2007 Mar;97(3):514-21.

CAMBRIDGE

Journals Online

[Links](#)

**Soluble dietary fibre fraction of *Trigonella foenum-graecum* (fenugreek) seed improves glucose homeostasis in animal models of type 1 and type 2 diabetes by delaying carbohydrate digestion and absorption, and enhancing insulin action.**

[Hannan JM](#), [Ali L](#), [Rokeya B](#), [Khaleque J](#), [Akhter M](#), [Flatt PR](#), [Abdel-Wahab YH](#).

School of Biomedical Sciences, University of Ulster, Coleraine, Northern Ireland, UK.

*Trigonella foenum-graecum* (fenugreek) seeds have been documented as a traditional plant treatment for diabetes. In the present study, the antidiabetic properties of a soluble dietary fibre (SDF) fraction of *T. foenum-graecum* were evaluated. Administration of SDF fraction (0 x 5 g/kg body weight) to normal, type 1 or type 2 diabetic rats significantly improved oral glucose tolerance. Total remaining unabsorbed sucrose in the gastrointestinal tract of non-diabetic and type 2 diabetic rats, following oral sucrose loading (2 x 5 g/kg body weight) was significantly increased by *T. foenum-graecum* (0 x 5 g/kg body weight). The SDF fraction suppressed the elevation of blood glucose after oral sucrose ingestion in both non-diabetic and type 2 diabetic rats. Intestinal disaccharidase activity and glucose absorption were decreased and gastrointestinal motility increased by the SDF fraction. Daily oral administration of SDF to type 2 diabetic rats for 28 d decreased serum glucose, increased liver glycogen content and enhanced total antioxidant status. Serum insulin and insulin secretion were not affected by the SDF fraction. Glucose transport in 3T3-L1 adipocytes and insulin action were increased by *T. foenum-graecum*. The present findings indicate that the SDF fraction of *T. foenum-graecum* seeds exerts antidiabetic effects mediated through inhibition of carbohydrate digestion and absorption, and enhancement of peripheral insulin action.

PMID: 17313713 [PubMed - indexed for MEDLINE]

Display AbstractPlus Show 20 Sort By Send to

**Related Links**

- ▶ Effect of soluble dietary fibre fraction of *Trigonella foenum graecur* [J Ethnopharmacol. 2003]
- ▶ Effect of *Trigonella foenum-graecum* (fenuareek) extract on blood glucose, [Asia Pac J Clin Nutr. 2007]
- ▶ Characterization of the hypoglycemic effects of *Trigonella foenum graecum* seed [Planta Med. 1995]
- ▶ *Trigonellafoenum graecum* (fenuareek) seed powder improves glucose T [Mol Cell Biochem. 2001]
- ▶ Aqueous extracts of husks of *Plantago ovata* reduce hyperglycaemia in type 1 ; [Br J Nutr. 2006]

[See all Related Articles...](#)

[Write to the Help Desk](#)

[NCBI](#) | [NLM](#) | [NIH](#)

[Department of Health & Human Services](#)

[Privacy Statement](#) | [Freedom of Information Act](#) | [Disclaimer](#)

# Polysaccharide Composition of a Gel Fraction Derived from Fenugreek and Its Effect on Starch Digestion and Bile Acid Absorption in Rats

Zecharia Madar\*<sup>†</sup> and Ilan Shomer<sup>‡</sup>

Department of Biochemistry and Human Nutrition, Faculty of Agriculture, Hebrew University of Jerusalem, Rehovot 76100, Israel, and Department of Food Science, Agricultural Research Organization, The Volcani Center, P.O. Box 6, Bet Dagan 50250, Israel

The soluble gel fraction of fenugreek seeds constituted the major portion of the seed coat (including the endosperm) polysaccharides, most of which consisted of galactomannan with mannose:galactose ratio of 1.5:1. The relatively small amount of the insoluble cell wall included mainly cellulose (as glucose) and pectin (as galacturonic acid). In vivo nutrition experiments in rats and in vitro studies using inverted gut showed that the gel fraction decreased both digestion and absorption of starch and uptake of bile acid (taurocholate and deoxycholate). Whereas 600 mg of the gel fraction was required to inhibit 50% of starch digestion, as little as 80 mg inhibited 50% of bile salt uptake. The present study indicated that the gel fraction, i.e., galactomannan, in the fenugreek seed is the factor which may be of potential benefit of fenugreek seeds in controlling plasma glucose and cholesterol levels.

## INTRODUCTION

The seeds of fenugreek (*Trigonella foenum-graecum*), an annual plant of the legume family, are reported to have hypoglycemic and hypocholesterolemic effects (Shani et al., 1974). Ground seeds of fenugreek offered to diabetic rats reduced the postprandial glucose levels (Madar, 1984). Similar results were reported in alloxan-diabetic dogs (Ribes et al., 1986). Recently, it was shown that supplementation of ground seed reduced the plasma glucose levels in normal and diabetic subjects (Madar et al., 1988; Sharma, 1986a). The mechanism by which fenugreek may modulate plasma glucose is by delaying gastric emptying and direct interference with intestinal glucose absorption (Madar, 1984). In addition, a significant improvement in insulin response and a reduction in cholesterol levels were obtained in diabetics but not in healthy subjects (Sharma, 1986b).

Several investigators have reported that the ground seed of fenugreek has hypoglycemic and hypocholesterolemic effect, but the factors responsible for this activity have not been identified. Sharma (1986b) showed that the whole seed had the greatest effect in reducing plasma glucose, followed by gum isolate and extracted seeds. Fenugreek seeds were studied in relation to formation, metabolism, and enzymatic hydrolysis of galactomannan (Dey, 1978; Meier and Reid, 1977; Reid, 1971; Reid and Davis, 1977; Reid and Meier, 1970). It seems that the galactomannan is the factor which reduces the plasma glucose. In the present study the polysaccharide composition of fenugreek was determined, considering the specific fraction involved with the effect on both in vitro (using inverted gut) and in vivo (using rats) starch digestion and bile salts absorption using the inverted gut technique. In addition, the hypoglycemic effect of this fraction in vivo was evaluated in normal rats.

## MATERIALS AND METHODS

**Isolation of the Seed Fractions for Sugar Analyses.** Dry seeds of fenugreek were purchased from the local market, soaked in distilled water for 24 h at room temperature, and used for the various experiments (see Scheme I). The polysaccharides of the soaking water fraction (fraction B), as well as of the other soluble fractions, were obtained as a precipitate of alcohol-insoluble solids (AIS) in a solution of 70% ethanol stirred for 15 min and centrifuged at 3000g for 15 min. The soaked seeds (fraction A) were separated by hand and treated as follows: the seed coat (including the endosperm) was crushed with a mortar in the presence of water (1:4, respectively) and filtered through a nylon sieve of 40 mesh, diluted to achieve complete dissolution, and centrifuged at 5000g. The AIS of the supernatant combined with the washing water of the nonfiltrated matter was freeze-dried (fraction C). The precipitate of the filtrate (fraction D) was also freeze-dried. The nonfiltrated matter of the seed coat, including the endosperm, was washed several times with distilled water and then freeze-dried (fraction E). The AIS fractions and the insoluble matter were freeze-dried, and  $\approx 10$  mg of the dry matter was used for sugar analysis. The alditol acetates of the acid-hydrolyzed polysaccharides were analyzed by gas-liquid chromatography (GLC) according to the method of Slonker (1971). Pectin (as galacturonic acid) was analyzed according to the method of Blumenkrantz and Asboe-Hansen (1973).

**Isolation of Gel Fraction and Alcoholic Gel Fraction for in Vitro and in Vivo Studies.** Fraction A was separated into two fractions: the seed coat and cotyledons. The seed coat was crushed in the presence of water (1:4) and filtrated through a sieve of 40 mesh. The resultant filtrate was called the "gel fraction". Ethanol was added to the gel fraction to yield a final concentration of 70% ethyl alcohol, and the mixture was stirred for 15 min and centrifuged for 15 min at 3000g. The alcoholic precipitate was lyophilized for later use.

**Animals.** Male Hebrew strain rats (Sabra), weighing 150-180 g, were used for the experiment. The animals were housed in a controlled environment (22  $\pm$  2 °C and 12-h light-dark cycle). A regular laboratory diet that met the American Institute of Nutrition Recommendations (1980) was provided ad libitum.

**Determination of Bile Acid Absorption Using the Inverted Sac Technique.** The preparation of the inverted sac was described previously (Madar, 1983). Briefly, male rats were decapitated, and the intestine was removed by cutting of both the upper end of the duodenum and the lower end of the ile-

\* Address correspondence to this author.

<sup>†</sup> Hebrew University of Jerusalem.

<sup>‡</sup> Agricultural Research Organization.

# Fenugreek dietary fibre a novel class of functional food ingredient

KRISHNA KUMAR IM, BALU P. MALIAKEL

R & D Laboratory, Akay Flavours & Aromatics Ltd,  
Ambunadu, Malayidamthuruthu PO., Cochin-683561, Kerala, INDIA

Observational and epidemiological studies have substantiated beyond doubt that the food rich in fruits and vegetables have a vital role to play in maintaining ones good health conditions. Subsequent scientific query on various fruits, vegetables, spices and herbs for the active principles responsible for their wonderful efficacy resulted in the discovery of many phytochemicals. Presently, attempts have been going on in both academia and industries to derive such novel phytonutrients or so-called Nutraceuticals, with sufficient efficacy data and toxicological information to enable one to supplement them in appropriate levels to keep up the normal cellular functions and hence to prevent diseases. Moreover, many of such plant secondary metabolites in optimum levels are found to act as essential micronutrients capable of preventing a variety of disease states. Curcumin is one such plant derived chemical, beyond doubt, whose efficacy in various types of Cancer and Cardiac health have been substantially proved with concomitant research activities initiated by both academic and industrial organisations (1, 2). "Eat more Fiber", every one might have heard it several times before. Fiber, plant derived complex carbohydrates, is yet another class of indispensable food component whose deficiency over a long period of time may contribute to various disorders and diseases. The term Dietary Fiber, is mainly associated with non-digestible, but fermentable carbohydrate mixtures and lignins, which are neither hydrolyzed nor absorbed in the upper part of the gastrointestinal tract. Total Dietary Fiber (TDF) is a unique blend of Soluble fiber (SF) and Insoluble fiber (IF) fractions by nature. Cellulose, lignins, and some hemicelluloses form the IF fraction and pectins, gums, mucilages and some other hemicelluloses include the SF fraction. Dietary Fiber has received greater attraction in recent years and the horizon of health research studies related to fiber intake seems to be expanding. According to AC Nielsen's Label Trends report, there has been a substantial growth in product range featuring the fiber content and its health benefits in their labels. Presently, consumers are also more aware of the fiber benefits due to peer-reviewed research publications and dietary guidelines of prestigious institutes and associations such as American Heart Association, American Diabetes Association, National Cancer Institute and USDA (3). The recommendations for dietary fiber intake has been substantiated in many chronic diseases such as cardiovascular diseases, diabetes, obesity, colon cancer and other diverticular diseases, especially in the context of relatively fiber-deficient western diet.

## INSOLUBLE AND SOLUBLE FIBRE

The major functions of IF fractions can be attributed to their passive water holding capacity and non-digestibility, which may help to increase the bulk and shortens the transit time of the stool through the intestinal tract (4). So it can be of benefit to those who struggle with constipation or irregular stools. It may also be useful to people having watery stools by absorbing the excess water and helping it to solidify. Furthermore, the presence of insoluble fiber fractions in food require more chewing and which may lead to less fat absorption. Fiber can also stimulate the growth of the colonic micro flora, which in

turn, contributes to the increase in biomass. Soluble fiber, on the other hand is completely or partially fermentable in the large intestine by the action of colonic bacteria to produce short chain fatty acids such as butyrate, propionate, acetate etc. The short chain fatty acids contribute toward a variety of health benefits ranging from colonic health to cardiac health through well-characterised biochemical pathways (5). Thus soluble fiber increases the viscosity of the stomach contents, reduces the fatty acid and other nutrient absorption, regulates blood-sugar and reduces cardiac risk factors such as cholesterol, triglycerides, C-reactive proteins etc (6). FDA has approved a label claim for the soluble fiber isolated from oat bran and psyllium husk for improving the cardiac health and heart attack risk. Thus, a typical diet should contain both SF & IF fractions, intimately mixed and ingested together to get maximal nutritional benefits. However, an average man in a developed country is taking only 50 percent of the average quantity required by human body for its proper functioning. It will be interesting to know the relative percentage of fiber in some of the common food that we take (Table 1) (7). It can be seen that the fiber content is very low despite their high calorie value, which is a dangerous situation.

Item	Total dietary Fiber content per 100g (%)	Major type of Fiber
Potato	0.5	Insoluble
Banana	0.5	Insoluble
Tomato	0.9	Insoluble
Greens	1.1	Insoluble
Apple	0.9	Insoluble
Grape	3.0	Insoluble
Corn Flakes	1.0 - 1.2	Insoluble
Psyllium Husk	20 - 24	Soluble
Oat Bran	15 to 20	Soluble & Insoluble
Fenugreek seeds	45 - 50	Soluble & Insoluble

Table 1. Nature and content of fiber in some common food items

Some of the sources of dietary fiber from which both soluble and insoluble fiber can be commercially prepared are given in Table 2 (8).

Source	Soluble Fiber (%)	Insoluble fiber (%)	Total Dietary fiber (%)	Protein (%)
Soya Bean	16 - 18	2 - 5	18- 20	40- 45
Wheat	1- 2	9-12	13- 15	10- 12
Oats	5- 8	5- 8	10-15	15- 20
Guar seeds	22- 25	12- 15	38- 40	25- 28
Psyllium seed	22-25	1-3	22-26	3- 5
Fenugreek seed	25-28	20-24	48-50	25-30

Table 2. Common sources of dietary fiber

Fenugreek seeds, one of the widely used spices, probably is the richest source of both types of fiber.

## FENUGREEK AS A NOVEL SOURCE OF DIETARY FIBER

Fenugreek (*Trigonella Foenum-gracium*) is an annual leguminous herb possessing wonderful medicinal values. The dried seeds are aromatic and bitter, which have been used traditionally in India, China, Egypt and in some parts of Europe for its well known carminative, galactagogue, antibacterial, anti-inflammatory, insulinotropic, and rejuvenating effects. The major constituents of fenugreek seeds have been identified as proteins 20 to 25 percent,

dietary fiber (45 to 50 percent), mucilaginous soluble fiber (20 to 25 percent), fixed fatty acids and essential oils 6 to 8 percent and steroidal saponins 2 to 5 percent. In addition to these main components, some minor components like alkaloids (trigonelline, cholin, gentianine, carpaine etc), free unnatural amino acids (4-hydroxyisoleucine), and individual spirostanols and furastanols like diosgenin, gitogenin, yamogenin etc have also been identified, isolated and characterised as the principal components responsible for its varying biological effects (9). Various solvent extraction techniques have been developed over the years to commercially extract the total fiber from fenugreek (10). The major challenge in the process is to remove the saponins and other phytochemicals present in fenugreek seeds, which are responsible for the bitter taste and the characteristic aroma of fenugreek seeds. A tasteless and odourless fiber fraction is indispensable for its applications in functional foods and nutraceuticals. Moreover, the fenugreek saponins are implicated for the body weight gain when supplemented to rats for hypercholestermia (11). The soluble fiber derived from fenugreek seeds has been identified chemically as galactomannans just like the other soluble fiber of guar seeds, psyllium husk etc (12). Galactomannans are biopolymers formed by the linear core poly (1→4)-β-D-mannan backbone to which varying degrees of D-galactosyl substituents are attached via 1,6-glycosidic linkages (Figure 1).

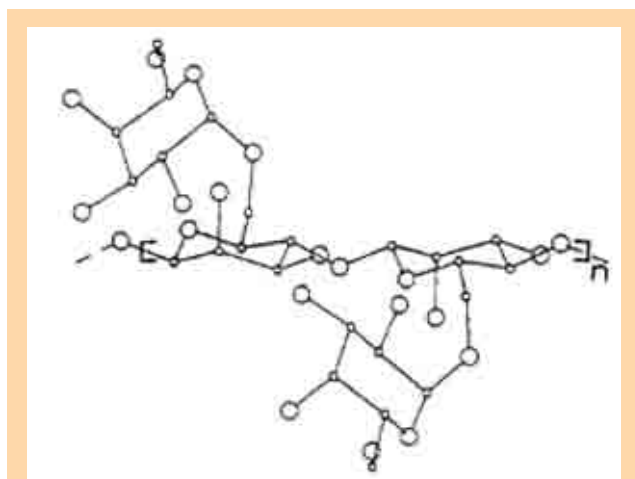


Figure 1. An average repeating unit of fenugreek galactomannans. (Adapted from Ref.12)

The ratio of mannans to galactose, molecular weight and the mode and place of linkages of galactose moieties to the mannan backbone are usually different in galactomannans derived from various legumes. For example, the one derived from locust beans have Mannans to Galactose ratio of 4:1, taragum has ratio 3:1, guar seed galactomannans have 2:1 and the fenugreek galactomannan has the maximum amount of galactose, the ratio being 1:1. That means, the galactose and mannose residues in fenugreek gum are uniformly linked and hence provide maximum hydration and solubility. This is again the reason for the low viscosity of fenugreek gum solutions and desegregation of molecular aggregates in solution at any given temperature. These properties make it an excellent ingredient for various food applications over the other natural hydrocolloids (13).

## NUTRACEUTICAL APPLICATIONS

A great deal of information has been gathered about the medicinal and therapeutic properties of total dietary fiber and the soluble fiber fractions made up of mainly galactomannans. The improvement in the serum biochemical profile of human and non-human primates, reduction in total serum cholesterol and triglycerides, raise in the high-density lipoprotein cholesterol level, management of glycemic indices and obesity etc are some of the effects that have been investigated in detail. For instance, when the galactomannans have been compared for their efficacy in cholesterol management, it was found that the

chemical composition and the structure of galactomannan has a significant influence; as it is most evident when the galactose content was the highest. Thus, fenugreek gums with the highest galactose content (galactose to mannose ratio, G:M is 1:1) showed the maximum decrease of cholesterol in both liver and blood plasma, as compared to guar gum and locust bean gum whose G:M are 1:2 and 1:4 respectively (14). Another aspect of modern interest in fiber research is its plausible development as a novel dietary ingredient for functional foods. For example, the supplementation of soluble fiber through bread was found to be more effective than powder in blood glucose reduction (15).

Below is a list of major metabolic syndromes and other disorders where dietary fiber, especially from fenugreek has been found to play a major role in controlling and/or preventing the disease states. The biochemical mechanisms of action of the dietary fiber in each case have also been tracked to some extent to understand its side effects and toxicity.

- **Constipation and irregularity:** Fiber-deficiency is now linked to a higher risk of digestive conditions. Insoluble fiber improves health in the intestinal tract by increasing stool volume and stimulating normal bowel contractions thus reducing passage-time through the colon (16). Fenugreek fiber that are incompletely or slowly fermented by microflora in the large intestine promote normal laxation and are integral components of diet plans to treat constipation and prevent the development of diverticulosis and diverticulitis. It can provide bulk to the waste, holds water, softens the stool and minimises the transit time through the intestine, which helps to maintain constant and steady stool time.
- **Obesity:** The prevalence of obesity has increased rapidly worldwide and the importance of considering the role of diet in the prevention and treatment of obesity is widely acknowledged. Dietary fiber is associated with a lesser degree of weight gain in observational studies. In this context, any diet with high fiber, low fat and high protein content will be of great significance (17). It was noticed that dietary fiber and protein rich food could increase secretion of the anorexigenic and insulinotropic hormone, glucagon-like peptide-1 (GLP-1) to improve glucose tolerance and reduce weight gain (18). Debitterized, defatted total dietary fiber derived from fenugreek may be one of the most suited functional food ingredient as it contains 60 to 70 percent dietary fiber (in which 30 to 40 percent soluble fiber, and remaining insoluble fiber), and 20 to 25 percent protein. If taken 30 min prior to meal, it can effectively suppress the appetite by initiating the sense of satiety. The insoluble fiber content in the food also demands more chewing which helps to reduce the fat absorption. In short, a fiber-rich meal is processed more slowly, which promotes earlier satiety, and is frequently less calorically dense and lower in fat and added sugars. All of these characteristics are features of a dietary pattern to treat and prevent obesity.
- **Diabetes Mellitus:** This is an area where significant amount of work has been done to establish the efficacy of fiber, especially the soluble part of the fenugreek dietary fiber on blood and serum glucose management and insulin production. Administration of 100 g fenugreek powder containing 50 percent dietary fiber for a period of 10 days have resulted a 25 percent decrease in the blood glucose level among the type II diabetes patients (19). SF fraction has been shown to reduce postprandial elevation in blood glucose level of Type 2 model diabetic rats by delaying the digestion of sucrose. When fenugreek soluble fiber was administered orally twice daily at a dose of 0.5 g/ kg for 28 days, it lowered the serum fructosamine level with no significant change in the insulin level as compared with the control. It is concluded that soluble fiber has a beneficial effect on dyslipidemia and has a tendency to inhibit platelet aggregation in Type 2 model diabetic rats (20). Recently, it was further found that the soluble fiber of fenugreek can improve glucose homeostasis by delaying carbohydrate digestion and absorption, and enhancing insulin action (21). An explanation could be the extensive gel formation and low viscosity of the resulting gels inside the intestine, which may delay the gastric emptying and decrease the intestinal

transit time of the food mass. Glucose trapped inside the gel may leaches out slowly and prevents the sudden raise of blood-glucose level, which may also help to avoid fatigue and over eating. Viscous and gel-forming properties of soluble DF inhibit macronutrient absorption, reduce postprandial glucose response, and beneficially influence certain blood lipids (22).

- **Cholesterol Management:** Substantial scientific information's and clinical data are now available on the efficacy of dietary fiber, especially the soluble counter part such as beta-glucans or galactomannans in the management of hypercholesterolemia. Among the various galactomannans studied, Fenugreek derived galactomannans have shown to have the maximum efficacy in lowering the plasma cholesterol level due to its unique structure of galactose to mannose 1:1 ratio (13). Moreover, soluble fiber fractions selectively reduce only the dangerous low-density lipoproteins and triglycerides; keep the good high-density cholesterol intact (23). In a human study conducted on 60 individuals with diabetes and high cholesterol and triglycerides level showed a significant lowering in blood glucose, LDL cholesterol and triglycerides level up on the regular administration of 25 g of fenugreek fiber powder containing nearly 50 percent fiber content. However, HDL level had no decrease. High LDL and triglycerides are implied in the development of plaques in the arteries, which contribute to atherosclerosis and other coronary heart diseases. Thus the intake of soluble fiber may provide an alternative, or at least an adjunct, to drastic therapeutic interventions like cholesterol synthesis inhibitors. The biochemical mechanism of soluble fiber as a hypolipemic agent can be delineated primarily to its capacity to bind bile acids, which are therefore excreted rather than recycled to the blood; this reduces blood cholesterol, which is taken up to reestablish an adequate supply of bile acids (24). In addition, soluble fiber may be fermented in the colon by bacteria to produce short-chain fatty acids, which can act to reduce cholesterol synthesis (25). There is also evidence for soluble fiber's capacity to directly regulate LDL metabolism.
- **Hypertension:** It is apparent from the efficacy of dietary fiber in conditions such as hypercholesterolemia, gastrointestinal tract related disorders and blood-glucose levels that, it should also have a positive control on the blood pressure or hypertensive patients. A very recent study shows that prevention of hypertension and improved blood pressure control can be achieved through dietary modification. Meta-analysis suggests that an increase of fiber intake of approximately 17 g/day will decrease systolic blood pressure by 1.15 mmHg and diastolic blood pressure by 1.65 mmHg, with soluble fiber showing a stronger effect than insoluble fiber. The same is true with proteins also. In a large population study, it is found that an increase in 37 g/day of protein leads to a decrease in mean systolic and diastolic blood pressure by approximately 3 and 2.5 mmHg, respectively. Thus either supplementation of proteins and fiber or the incorporation of legumes, a protein- and fiber-rich food, may seem to be a feasible approach to hypertensive patients (26).
- **C-reactive protein levels:** C-reactive proteins are a group of proteins produced in the liver whose levels are found to be the latest markers for the onset of cardiac diseases, diabetes and other inflammatory diseases like arthritis. Both insoluble and soluble fiber appears to be protective against high C-reactive protein levels. In a study conducted to examine the reduction in inflammation from a diet supplemented with fiber compared with a diet naturally high in fiber, it is found that fiber intake of about 30 g/d either from a diet naturally rich in fiber or from a supplement can reduce the levels of CRP (27). However more research is required at this stage to more clearly elucidate the differential effect seen in lean Vs obese individuals and whether modification of dietary fiber may be helpful in modulating inflammation and its consequent cardiovascular consequences.
- **Lung Function:** Increased intake of dietary fiber, could boost lung health, and may reduce the risk of chronic obstructive pulmonary

disease (COPD), suggests a new research report (28). COPD mainly affects smokers, and is the number five cause of death worldwide. It is characterized by chronic inflammation in the small airways of the lung and leads to excessive mucus production, excessive fibrous connective tissue development (fibrosis), and degradation of proteins. So far, there is no cure for this disease. Lung function was measured by the volume of air that could be forcibly blown out in one second, the so-called forced expiratory volume (FEV). The researchers reported that people with the highest average fiber intake had a FEV that was 60.2 ml higher than subjects with the lowest average fiber intake. Being the first study to report such findings, more studies are clearly necessary to further support the association. Moreover, mechanistic studies are needed to explain how fiber may exert a beneficial effect.

- **Colon cancer & Bowel disorders:** Since the fenugreek fiber are gentle and laxative, which contribute to reduce the chance of colon cancers and irritable bowel syndromes very often related to low fiber diet. The short-chain fatty acids like butyrate, produced by the anaerobic bacterial fermentation of dietary fiber within the colonic lumen, are thought to protect against colon carcinogenesis. A recent study has confirmed the antiproliferative effects in tumor progression, and short chain fatty acid production is safe and without consequences for the normal epithelium growth (29).
- **Gallstones:** Nearly 30 percent of the obese patients treated with hypoenergetic diets for weight reduction are found to develop gallstone disease. A double-blind clinical trial conducted to compare the effect of rational diet supplemented with the soluble fiber isolated from Psyllium plantago for the prevention of gallstone disease in obese subjects undergoing a weight-reduction diet showed some beneficial effect of fiber supplementation to prevent gallstone disease development among obese patients (30). The formation of soluble gels inside the intestine has shown to send message to brain to empty itself. Regular emptying of gall bladder may help to reduce the chance of gall-stone formation.

## APPLICATIONS IN FOOD INDUSTRY

As one of the rich source of natural dietary fiber, fenugreek has established itself in the modern food ingredient or functional food sector. As a hydrocolloid, the soluble fiber from fenugreek, referred to as fenugreek gum, provide texture, appeal, gelling, thickening, emulsifying, stabilising and encapsulating properties. Thus dietary fiber, especially soluble fiber can find their way into nutrition and cereal bars, yogurts, dairy products and nutritional beverages. Plain powders of soluble fiber or total dietary fiber can be mixed with fruit juices, other spice mixes and seasonings. As direct supplements, it can formulate as tablets or capsules along with the other vitamins and nutrients. It may also be applied to milk shakes, soups, dressings, sweets and candies. It may also be used to fortify bakery flour for pizza, bread, pizza, bagel, muffins, cake mix, noodles, tortilla & flat bread, fried and baked corn chips etc. Flour fortified with 8 to 10 percent SDF has already been used to prepare bakery foods like pizza, bread, muffins, and cakes. It has also been proved when fiber fortified flour was used for making oil fried snacks, 8 to 15 percent of less oil absorption only takes place which is really appreciable in terms of unwanted fat intake. Some of the food items where fenugreek fiber can be used for various purposes are summarised in the Table 3 given below.

Use	Function
Dessert gels	Gelation
Low-calorie Jellies	Gelation
Pel-foams (canned)	Fat stabilization, thickening, suspending, gelation
Fish gels	Gelation
Syrups	Suspension, bodying
Fruit drink powders and frozen concentrates	Bodying
Pastries, pizzas, barbecue sauces	Puffing effects
Imitation milk	Bodying
Imitation coffee creams	Bodying, fat stabilization
Whipped toppings (artificial)	Stabilize emulsion, overrun
Puddings (instant)	Emulsion stabilization
Air-treatment gels	Gelation
Toothpastes	Blander
Lotions	Bodying emollient

Table 3. Some applications of fiber in food formulation

## DIETARY RECOMMENDATIONS

According to National Academies' Institute of Medicine, adults of 50 years or younger should have an intake of nearly 38 g for men and 25 g for women on a daily basis. And for men and women over 50 years of age, can have an average daily intake of 30 and 21 g respectively. In this regard, it will be interesting to note that the fiber intake of an average American is only about 10 g per day (31). Typical fast food today offers even less amount of fiber, but high fat and carbohydrate content to make the situation really harmful. Though one can eat more grains and whole-grain products, fruits, vegetables, beans, peas, nuts and seeds for fiber balanced diet, fortification of the ingredients of food such as flours will be a better option so that people can have their beloved food with enough fiber. Fenugreek derived dietary fiber, being the conventional non toxic spice ingredient, assumes significance here. Optimum intake of fenugreek dietary fiber recommended for the above discussed health effects, as seen from the various independent study reports published in peer-reviewed scientific journals may be as follows.

5 to 10 g per day is generally recommended for efficacy, although excess consumption up to 100 g per day does not imply any significant negative effect upon health. The dosages may vary depending on the food habits of the individuals. For constipation and irregularity, dosage of 5 g per day especially prior to bedtime will be beneficial. Those who have obesity issues are recommended to take the carbohydrate out from their diet and to boost the fiber and protein intake. Consumption of 10 g fiber per day 1hr prior to meal will be of great help to reduce the appetite and to control food intake.

The same dosage is also recommended for the hypercholesteremic patients for managing the cholesterol and triglyceride levels. 10 g dosages in two or three times a day is found to be very much helpful for blood sugar management among diabetes patients. For those who does not have any health issues can also add about 5 to 10 g of fiber per day to maintain good metabolism and hence good health. Since both SF and IF fractions of dietary fiber plays a major role in all these effects, fenugreek derived fiber will be a better choice as it contains both types in high percentage.

Custom made fiber containing varying levels of SF and IF fractions or SF fraction alone is also currently available from fenugreek to meet the personalised demands depending on ones health condition.

Dieticians and nutritionists can help a patient to identify the specification best suit their needs.

However, new users are advised to start with small dosages and then to increase slowly to the desired dosage levels so as to allow the natural bacteria in your digestive system to adjust to the change. Also, drink plenty of water as the fiber works best when it absorbs water, making your stool soft and bulky.

## CONCLUSION

We are passing through a time and enjoying a life style, which demands the adoption of what Hippocrates, has been said some 2500 years ago, "let food be thy medicine and medicine be thy food". A large body of scientific evidence is currently available on a range of natural medicinal food or nutraceuticals to elucidate how this concept of resonance between food and medicine is possible. It is only a matter of segregating various functional ingredients and blending them in appropriate levels to have the food a medicinal touch and vice versa. Dietary fiber may be one such functional ingredient whose appropriate concentrations can have positive effect in maintaining good health and preventing many of the metabolic syndromes very often found across the globe. Some of the sources of dietary fiber and their commercial production from such sources have already been achieved. Guar gum, psyllium husk and oats bran are the major sources currently exploited to maximum level. In this regard, fenugreek seeds offer great potentiality being the richest source of both soluble and insoluble fiber and one of the traditionally used medicinal and culinary spices. If processed properly, fenugreek fiber

can be applied to all kinds of food formulation for fiber fortification. However, more rigorous scientific research has yet to be performed to get wide acceptance to this old herb to exploit its potentiality in functional foods and nutraceuticals to maximum extent.

## REFERENCES AND NOTES

1. B.B. Aggarwal, A. Kumar et al., *Anticancer Res.*, 23(1A), pp. 363-398 (2003).
2. I.M. Krishna Kumar, P. Balu Maliakel, *AgroFood Industry Hi- Tech*, 18(5), pp. 52-53 (2007).
3. Soluble Fiber takes the spot light: By Sharon Palmer; <http://www.foodproductdesign.com/articles/462/77h913442084032.html> Posted on: 08/02/2007
4. J.L. Casterline, C.J. Oles et al., *J. Agric. Food Chem.*, 45(7), pp. 2463-2467 (1997).
5. J.M. Wong, R. de Souza et al., *J Clin Gastroenterol.*, 40(3), pp. 235-243 (2006).
6. D.L. Topping, *Nutrition Reviews.*, 49(7), pp. 195-203 (1991).
7. E.K. Ba?garin, *Vopr Pitan.*, 75(3), pp. 42-44 (2006).
8. *Mayo Clin Health Lett.*, Aug; 25(8), p. 3 (2007).
9. P.D. Trivedi., K. Pundarikakshudu et al., *J AOAC Int.*, 90(2), pp. 358-363 (2007).
10. R.D. Sharma, et al *Nutrition Res.*, 10, pp. 731-739 (1990).
11. P.R. Petit, Y.D. Sauvaire et al., *Steroids*, 60, pp. 675-680 (1995).
12. B.K. Songt, W.T. Winter et al., *Macromolecules*, 22, pp. 2641-2644 (1989).
13. Y. Brummer.,W. Cui et al., *Food Hydrocolloids*, 17(3), pp. 229-236 (2003).
14. A.J. EvansR.L Hood et al., *Br. J. Nutr.*, 68(1), pp. 217-229 (1992).
15. S.R. Glore, D. Van Treeck et al., *J Am. Dietetic Ass.*, 94(4), pp. 425-436 (1994).
16. J.F. Johanson, *MedGenMed.*, 9(2), pp. 25-30 (2007).
17. I. Abete, D. Parra et al., *Clin Nutr.*, PMID: 18308431 (2008).
18. R.A. Reimer, J.C. Russell, *Obesity*, 16(1), pp. 40-46 (2008).
19. E. Basch, C. Ulbricht et al., *Altern Med Rev.*, 8(1), pp. 20-27 (2003).
20. J.M. Hannan, B. Rokeya et al., *J Ethnopharmacol.*, 88(1), pp. 73-77 (2003).
21. J.M. Hannan, L. Ali et al., *Br J Nutr.*, 97(3), pp. 514-521 (2007).
22. S. Ou, K. Kwok et al., *J Agric Food Chem.*, 49(2), pp. 1026-1029 (2001).
23. P.T. Boban, B. Nambisan et al., *Br J Nutr.*, 96(6), pp. 1021-1029 (2006).
24. M.A. Levrat, M.L. Favier et al., *J.Nutr.*, 124(4), pp. 531-538 (1994).
25. M. Comalada, E. Bailón et al., *J Cancer Res Clin Oncol.*, 133(3), p. 211 (2007).
26. Y.P. Lee, I.B. Puddey et al., *Clin Exp Pharmacol Physiol.*, 35(4), pp. 473-476 (2008).
27. D.E. King, B.M. Egan et al., *Arch Intern Med.*, 167(5), pp. 502-506 (2007).
28. H. Kan, J. Stevens et al., *Am J Epidemiol.*, 167(5), pp. 570-578 (2008).
29. A. Schatzkin, T. Mouw et al., *Am J Clin Nutr.*, 85(5), pp. 1353-1360 (2007).
30. S. Morán, M. Uribe et al., *Rev Gastroenterol Mex.*, 62(4), pp. 266-272 (1997).

Readers interested in having a complete list of references are kindly invited to write to the author at [Krishnakumar.IM@akay-group.com](mailto:Krishnakumar.IM@akay-group.com)

