The effect of an ethanol extract derived from fenugreek (*Trigonella foenum-graecum*) on bile acid absorption and cholesterol levels in rats

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The hypocholesterolaemic properties of an ethanol extract from defatted fenugreek (*Trigonella foenum-graecum*) seeds were investigated. Purification of the crude extract by dialysis produced an isolated component with haemolytic properties. The dialysate was also found to contain saponins demonstrated by thin-layer chromatography. Experiments *in vitro* employing the everted-sac technique showed that the ethanol extract had the ability to inhibit taurocholate and deoxycholate absorption in a dose-dependent manner. In two separate feeding experiments, hypercholesterolaemic rats were fed on 30 or 50 g ethanol extract/kg for a 4-week period. Reductions in plasma cholesterol levels ranged from 18 to 26% and a tendency for lower concentrations of liver cholesterol was observed. These results indicate that the ethanol extract from fenugreek seeds contained hypocholesterolaemic components which appear to be saponins that interact with bile salts in the digestive tract.

Fenugreek: Saponin: Hypercholesterolaemic rats

Research carried out on legumes has led to the belief that they are beneficial in lowering total cholesterol levels in humans (Madar & Odes, 1990). Fenugreek (*Trigonella foenum-graecum*) is a member of the family Leguminosae and is grown predominantly in Northern Africa, the Middle East and Asia. It is most commonly used for seasoning and is consumed in relatively small quantities. For centuries fenugreek has been used in folk medicine to heal ailments ranging from indigestion to baldness (Fazli & Hardman, 1968). Recent scientific reports indicate that fenugreek does indeed have therapeutic properties that may be beneficial in treating such diseases as diabetes and hypercholesterolaemia (Singhal *et al.* 1982; Madar, 1984; Sharma, 1984, 1986*a b*; Valette *et al.* 1984; Bhat *et al.* 1985; Ribes *et al.* 1986; Madar *et al.* 1988). Elevation of cholesterol levels in rats was prevented by adding 150, 300 or 600 g fenugreek seeds/kg to a hypercholesterolaemia-inducing diet (Sharma, 1984). In addition, diets enriched with fenugreek increased both fecal weight and excretion of bile acids and cholesterol. Bhat *et al.* (1985) reported that the administration of fenugreek to rats increased total bile output. This was attributed to increased conversion of cholesterol to bile acids by the liver. Fenugreek has been found to contain relatively large quantities of saponins (Valette *et al.* 1984; Sharma 1986*b*). Saponins are a heterogeneous group of amphiphilic compounds found mainly in plants. They are highly surface-active and have many diverse properties. Most saponins are haemolytic, can bind cholesterol, and form stable foams (Price *et al.* 1987). A crude saponin fraction isolated from fenugreek reduced serum cholesterol in rats (Sharma, 1986*a*). Saponins derived from lucerne (*Medicago sativa*) were also found to reduce plasma cholesterol levels by direct binding of dietary saponins with cholesterol in the digestive tract and subsequent excretion in the faeces (Malinow *et al.* 1977; Story *et al.* 1984). In contrast, Calvert *et al.* (1981) found no

indication that soya-bean saponins lowered cholesterol levels in hypercholesterolaemic men. Similar results were reported by Gibney *et al.* (1982) when commercial saponins were fed to rats and hamsters.

The aim of the work presented in the present study was to isolate a fraction of the fenugreek seed that would have a hypocholesterolaemic effect. The isolated fraction was tested both *in vitro* (everted-sac experiments) and *in vivo* using hypercholesterolaemic rats.

MATERIALS AND METHODS

Preparation of ethanol extract from ground fenugreek seeds

The ground fenugreek seeds were defatted in light petroleum (b.p. 40–60°) in a Soxhlet apparatus for 20 h. The seeds were then dried at room temperature over a 2–3 d period. The defatted seeds underwent a second extraction in absolute ethanol in a Soxhlet apparatus for an additional 30 h. The ethanol extract a was then dried under a vacuum in a rotary evaporator and the remaining material was ground into a fine powder. The dried ethanol extract (EtOH extract) was stored in a desiccator. This fraction was used in all *in vivo* studies with rats.

Preparation of dialysate

Powdered ethanol extract (5 g) was dissolved in 100 ml distilled water. This solution was placed in a dialysis tube and underwent extensive dialysis against distilled water for a 5 d period until the sac contents were a milky white colour. The dialysate (35 ml) was then defatted in a separating funnel with chloroform (70 ml). The procedure was carried out twice and excess chloroform removed. The dialysate was then lyophilized into a dry material.

Thin layer chromatography (TLC)

TLC was performed on Kieselgel (Oakenfull, 1981) with the following modifications in the solvent system: ethyl acetate–acetic acid–water (6:2:2, by vol.). Chromatograms were developed to the height of 60 mm and the spots were visualized by spraying with H_2SO_4 (100 ml/l) or anthrone reagent and heating at 110°.

Determination of the haemolytic activity of dialysate

The method of Segal *et al.* (1966) was used with the following modifications: heparinized rat blood (5 ml) was centrifuged (600 ***g***) and the plasma removed. The erythrocyte precipitate was washed four times and brought to a volume of 125 ml in an isotonic buffer containing: 3·16 g Na_2HPO_4, 0·76 g KH_2PO_4, 7·2 g NaCl/l water, pH 7·4. The erythrocyte suspension (2 ml) was added to varying amounts of dialysate and EtOH extract dissolved in the previously described buffer. The reaction mixture was brought to 4 ml with the same buffer. After 90 min the reaction mixtures were centrifuged (800 ***g***) for 5 min. Absorbance was measured in a spectrophotometer (Bausch & Lomb, Rochester, NY, USA) at 540 nm. Standard for total haemolysis was the absorbance reading of a reaction mixture containing distilled water under identical reaction conditions. The amount of haemolysis caused by various concentrations of dialysate was expressed as a percentage of total haemolysis. The haemolytic activity of the dialysate was also determined in the presence of albumin (125 mg bovine albumin/25 ml), plasma (1 ml/24 ml), cholesterol (1 g/l), and bile salts (taurocholic acid, sodium salt 25 mg/25 ml), obtained from Sigma Chemical (St Louis, Mo., USA).

Absorption experiments using the everted-sac technique

Experiments carried out using the everted-sac technique were based on the method described by Madar (1983) with the following modifications: male rats of the Hebrew University strain of approximate weight 200 g were decapitated and an abdominal incision

was made. A 240 mm section of the small intestine starting from 400 mm after the pyloric sphincter was removed and washed in saline (9 g NaCl/l). The intestine was then cut into three 80 mm pieces and each segment turned inside out (everted) with the aid of a Pasteur pipette. Each segment was then tightly tied at one end with a cotton thread and filled with 1 ml Kreb's-Henseleit buffer (KHB), pH 7·4. The open end of the sac was then ligated and placed into a 25 ml Erlenmeyer flask containing 6 ml KHB, radioactive bile salts or cholesterol (Amersham International Plc, Amersham, Bucks, UK), and the material to be tested (type and quantity noted for each experiment). The flasks were then gased with O_2–CO_2 (95:5, v/v), tightly stoppered, and incubated for 2 h at 37° in a shaking bath. At the end of the incubation period, the sac was removed from the flask and its content (inner serosal fluid) was measured. The volume of the outer fluid remaining in the flask was also measured. Samples (200 μl) from both the serosal fluid and mucosal fluid were transferred to mini vials containing 4 ml scintillation liquid. To prevent turbidity, 50 μl 0·1 M-HCl was added. Net radioactivity was measured in a scintillation counter (Beckman, Fullerton, CA, USA) and results were expressed as pmol. Each treatment was carried out in triplicate.

Feeding experiments

Animals. Male rats (Sabra strain, Hebrew University) weighing 130–150 g were housed in individual suspended stainless-steel cages in a controlled environment (22–24° and 12 h light–12 h dark) with food and water freely available. Animals were weighed once weekly and food intake was measured every 2 d. The animals were cared for under the guidelines set forth by the Animal Care of the Hebrew University, Jerusalem, Israel.

Experimental design. Two feeding experiments were carried out. In Expt 1, thirty rats were divided into three groups (ten rats each). Group 1 was fed on a standard diet and group 2 was fed on a cholesterol-enriched diet for a 9-week period. Group 3 was fed on a cholesterol-enriched diet for 5 weeks and then received the cholesterol-enriched diet with the addition of 30 g EtOH extract/kg for an additional 4 weeks. Expt 2 included forty rats fed on a high-cholesterol diet for a 9-week period and plasma cholesterol levels were then determined. The thirty rats with the highest cholesterol levels were chosen for the experiment. The rats were divided into three groups (ten rats per group) and received the following diets for a 4-week period. Group 1 continued to receive the high-cholesterol diet. Group 2 was fed on the high-cholesterol diet with the addition of 30 g EtOH extract/kg. Group 3 was fed on the high-cholesterol diet with 50 g EtOH extract/kg added. All diets' compositions are described in Table 1 and follow the guidelines for describing diets for experimental animals.

Analytical determinations

Sample collections. Blood samples were taken from rat tail tips and collected in polyethylene tubes that were pre-washed with heparin (400 U/ml) and dried overnight at 40°. All samples were centrifuged for 5 min at 2500 ***g***. The separated plasma was then removed and frozen at −20° until analysis was carried out. All experiments were terminated following an overnight fast. The animals were anaesthetized with Nembutal (0·2 ml/100 g body weight) and 5 ml of venous blood was collected. Livers were removed, weighed, and frozen at −20° for later analysis.

Determination of plasma cholesterol and triacylglycerol. Plasma cholesterol and triacylglycerol levels were measured by an enzymic assay (Allain *et al.* 1974; McGowan *et al.* 1983) using a prepared kit produced by Trace Scientific (Baulkham Hills, NSW, Australia).

Determination of liver cholesterol. Liver cholesterol was measured by the method of Searcy & Bergquest (1960) with the following modifications: 40–80 mg samples of liver

Table 1. *Composition of diets (g/kg)*

Diet...	Standard	High-cholesterol	High-cholesterol + EtOH extract (g/kg)	
Ingredients			30	50
Sucrose (670 g/kg)	667	639	620	604
Casein (160 g/kg)	159	153	148	144
Fat (170 g/kg)				
Oil	75	36	35	34
Butter	—	45	43	42
Cellulose	50	50	50	50
Vitamin mix*	10	10	10	10
Salt mix†	35	35	35	35
Methionine	3	3	3	3
Cholesterol	—	30	30	30
EtOH extract	—	—	30	50

EtOH extract, ethanol extract (for details, see p. 278).
* Vitamin mix AIN (American Institute of Nutrition, 1977, 1980).
† Mineral mix AIN (American Institute of Nutrition, 1977).

were saponified with 3 ml KOH (100 ml/l) in ethanol for 1 h at 65° in a water bath. Distilled water (3 ml) was then added to the cooled mixture. This was followed by a series of four extractions with light petroleum (b.p. 40–60°) using (ml) 7, 5, 5 and 5 respectively. The light petroleum extractions were pooled and dried under nitrogen. Cholesterol was dissolved in 1 ml light petroleum (b.p. 40–60°) and 20 μl samples were taken and dried under N_2. A colorimetric reaction was then carried out in 3 ml acetic acid saturated with $FeSO_4$ and 1 ml concentrated H_2SO_4. Values were measured at 490 nm on a spectrophotometer.

Statistical analysis

Results were analysed by one-way analysis of variance, ANOVA, and Duncan's multiple-range test or two-way analysis of variance with log transformed data and Duncan's multiple-range test.

RESULTS

Approximately 25 g EtOH extract/kg ground fenugreek seed was produced. One-fifth of the EtOH extract remained after dialysis. TLC was carried out in order to establish the purity and composition of the materials. The chromatograph of the EtOH extract contained many bands while the dialysate produced one thick band identified by both H_2SO_4 and anthrone reagent. TLC performed after acid-hydrolysis of the dialysate confirmed that the material contained a hydrophobic diethyl ether-soluble component and a water-soluble sugar component (values not shown).

Haemolytic activity of the dialysate

Tests of the haemolytic activity of the EtOH extract and dialysate were carried out to substantiate the presence of saponins in the fenugreek extractions. The crude EtOH extract (500 μg/ml) was unable to cause haemolysis of erythrocytes. In contrast, the dialysate was found to be a highly haemolytic material. Fig. 1 shows the haemolytic activity of the dialysate at various concentrations. The dialysate caused haemolysis in a dose-dependent manner. Maximum haemolysis (90%) was achieved at dialysate concentrations of 300 μg/ml. The haemolytic activity of the dialysate was also tested in the presence of

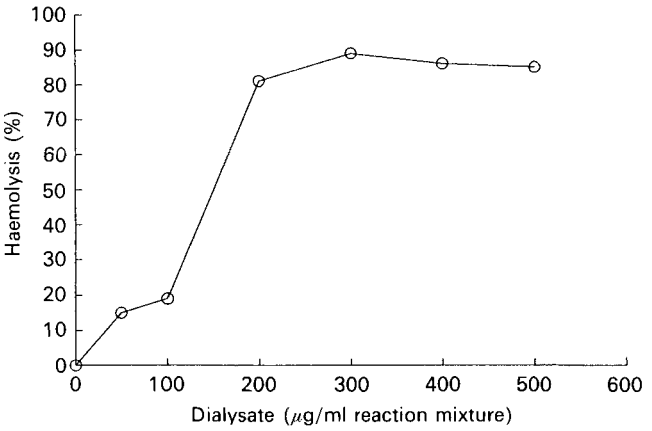

Fig. 1. Haemolytic activity of dialysate of ethanol extract from fenugreek (*Trigonella foenum-graecum*) seed at various concentrations. Results were expressed as percentage haemolysis calculated by comparison with absorbance measured for haemolysis caused by distilled water (100%). For details of procedures, see p. 278.

albumin, plasma, cholesterol and bile salts. Experiments were conducted using a concentration of dialysate (150 μg/ml) that caused approximately 50% haemolysis and the results were expressed as the relative increase in absorbance in comparison with control test-tubes containing no dialysate. The addition of 2·5 mg albumin to 4 ml reaction mixtures strongly inhibited haemolysis (90%), while 5 mg of albumin totally inhibited haemolysis. Similarly, plasma (20 μl/reaction mixture) was able to inhibit 90% of the dialysate's haemolytic activity. Higher concentrations of plasma were unable to increase the inhibitory effect.

The addition of either cholesterol or bile salts did not inhibit haemolysis caused by 150 μg dialysate/ml. An attempt to improve results by boiling the cholesterol suspension mixed with the dialysate for 30 min before addition of the erythrocytes was unsuccessful.

Absorption of bile acids using the everted-sac technique

Fig. 2 compares the effects of the EtOH extract and other substances on [^{14}C]deoxycholic acid sodium salt (0·5 μmol/l) absorption. Fig. 2 shows the absolute quantity of radioactive material found inside the sacs (passed through the mucosa to the serosal side). Absorption of the bile salt was inhibited by 400 mg EtOH extract by 84%. Pectin (35%) and fenugreek mucilages (55%) were also effective in preventing the bile salts from entering the everted sac. Significant inhibition was observed when saponins from soya beans (60%) and lucerne (68%) were used. Similar results were observed using [^{14}C]taurocholic acid (values not shown). Table 2 shows the results of dose–response experiments using either [^{14}C]-taurocholic acid (0·2 μM) or [^{14}C]deoxycholic acid (0·5 μM). In both cases, as the quantity of EtOH extract increased, inhibition of absorption increased. The presence of 400 mg EtOH extract in the mucosal side reduced bile salt absorption by 84 and 87% for taurocholate and deoxycholate respectively.

The effect of EtOH extract on cholesterol absorption

Experiments to demonstrate the effect of EtOH extract on cholesterol absorption were carried out using 0·2 μM-[^{14}C]cholesterol with or without 100 mg EtOH extract and various concentrations of unlabelled cholesterol (0, 0·01 and 0·1 mM). The results (Table 3) show that the EtOH extract inhibited cholesterol absorption independently of the quantity of cholesterol added.

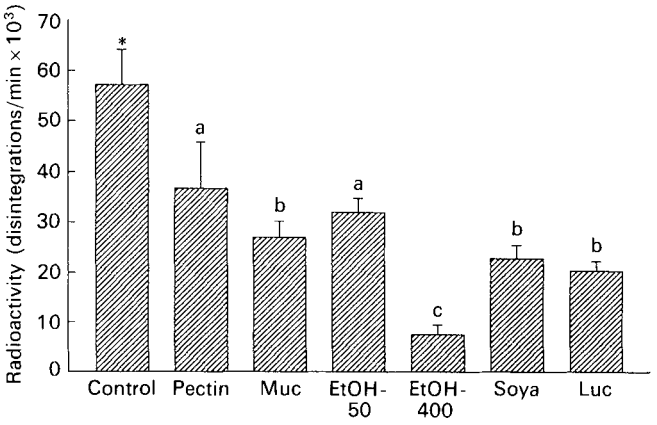

Fig. 2. Comparative absorption (total radioactivity in serosal fluid) of [^{14}C]deoxycholic acid, sodium salt (0·5 μM), in rat everted sac in the presence of pectin (200 mg orange pectin), Muc (200 mg fenugreek (*Trigonella foenum-graecum*) mucilages), EtOH-50, EtOH-400 (50 and 400 mg ethanol extract of fenugreek seeds respectively), Soya (100 mg soya-bean saponins), Luc (100 mg lucerne (*Medicago sativa*) saponins). Values are means with their standard errors represented by vertical bars for six experiments. a, b, c, Values with unlike superscript letters were significantly different. Mean values for control were significantly different from those of the fractions tested: * $P < 0{\cdot}05$.

Table 2. *Effects of various amounts of ethanol extract from fenugreek* (Trigonella foenum-graecum) *seed* on bile salt absorption in rat everted sac*†

(Mean values with their standard errors for three experiments, *n* 9)

	[^{14}C]deoxycholic acid (pmol)				[^{14}C]taurocholic acid (pmol)			
	Serosal side		Mucosal side		Serosal side		Mucosal side	
Ethanol extract (mg)	Mean	SE	Mean	SE	Mean	SE	Mean	SE
0	162^{a}	5	1467^{a}	98	118^{a}	36	780	39
50	95^{b}	50	1876^{b}	72	ND		ND	
100	57^{c}	10	1955^{bc}	71	71^{ab}	9	916	29
200	34^{c}	7	2202^{bc}	84	37^{b}	3	924	30
400	21^{c}	1	2267^{c}	80	19^{c}	2	877	30
800	21^{c}	2	2152^{bc}	106	19^{c}	3	891	27

^{a,b,c} Values with unlike superscript letters were significantly different ($P < 0{\cdot}05$).
ND, not determined.
* For details of preparation, see p. 278.
† For details of procedures, see pp. 278–279.

Effect of EtOH extract in hypercholesterolaemic rats

Incorporation of 30 g EtOH extract/kg into a hypercholesterolaemic diet in Expt 1 (Table 4) significantly increased growth variables in rats when compared with animals fed on a standard diet. In contrast, similar food intake, weight gain and liver weights were observed in rats fed on a high-cholesterol diet and a high-cholesterol diet containing 30 g EtOH extract/kg. In Expt 2, no significant differences in growth variables were observed (values not shown).

Table 3. *Effect of ethanol extract from fenugreek* (Trigonella foenum-graecum) *seed* on cholesterol absorption in rat everted sac using various concentrations of unlabelled cholesterol*†

(Mean values with their standard errors for three experiments)

		[^{14}C]cholesterol (pmol)			
		Serosal side		Mucosal side	
Cholesterol (mM)	Ethanol extract (mg)	Mean	SE	Mean	SE
0	0	29[a]	9	1580	242
0	100	8[b]	1	1930	117
0·01	0	12[ab]	2	1144	133
0·01	100	9[b]	2	1665	100
0·1	0	11[ab]	3	1423	66
0·1	100	6[b]	2	1700	103

Values with unlike superscript letters were significantly different (two-way analysis of variance was carried out on log transformed data; $P < 0·05$).

* For details of preparation, see p. 278.

† For details of procedures, see pp. 278–279.

Table 4. *Expt 1. Effects of a high-cholesterol diet and a high-cholesterol diet supplemented with 30 g ethanol extract from fenugreek* (Trigonella foenum-graecum) *seed (EtOH extract)/kg* on growth variables in rats*

(Mean values with their standard errors for ten rats)

	Body wt (g)				Wt gain (g)		Food intake (g/d)		Liver wt (g/kg body-wt)	
	Initial		Final							
Diet†	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Standard	149	2·5	413[b]	6·8	264[b]	6·1	20·2[b]	0·3	2·83[b]	0·13
High-cholesterol	150	3·0	447[a]	4·1	297[a]	5·0	22·6[a]	0·3	3·60[a]	0·12
30 g EtOH	149	2·8	437[a]	4·1	288[a]	8·8	22·4[a]	0·4	3·27[a]	0·11

[a, b] Values with unlike superscript letters were significantly different ($P < 0·05$).

* For details of preparation, see p. 278.

† For details of composition, see Table 1.

Plasma triacylglycerols and cholesterol levels in rats fed on diets enriched with 30 g EtOH extract/kg

Fasting and post-prandial plasma cholesterol and triacylglycerol levels are shown in Table 5. Fasting cholesterol levels were significantly higher in rats fed on the high-cholesterol diet and remained higher following meal intake. Rats fed on a high-cholesterol diet with 30 g EtOH extract/kg had fasting cholesterol levels similar to rats fed on the standard diet. Post-prandial cholesterol levels in rats fed on the 30 g EtOH extract/kg diet also tended to be lower than those fed on the high-cholesterol diet and closer to rats fed on the standard diet, but results were not significantly different. There was no significant difference found in fasting triacylglycerol levels among the groups but post-prandial triacylglycerol levels were higher in rats fed on the standard diet. All three groups had similar food intake during the meal tolerance test.

Table 5. *Expt. 1. The effect of hypercholesterolaemic diets* with or without 30 g ethanol extract from fenugreek* (Trigonella foenum-graecum) *seed† (EtOH extract)/kg on plasma cholesterol and triacylglycerol levels following a meal tolerance test‡*

(Mean values with their standard errors for ten rats)

Diet...	Standard		High-cholesterol		High-cholesterol + 30 g EtOH extract/kg	
	Mean	SE	Mean	SE	Mean	SE
Food intake§ (g)	8·7	0·4	9·1	0·9	8·4	0·6
Fasting cholesterol (mmol/l)	2·03[b]	0·09	2·47[a]	0·2	1·83[b]	0·06
Fasting triacylglycerols (mmol/l)	1·34	0·05	1·40	0·18	1·30	0·09
Post-prandial cholesterol (mmol/l)	1·97[b]	0·12	2·72[a]	0·17	2·26[ab]	0·19
Post-prandial triacylglycerols (mmol/l)	2·67[a]	0·02	1·89[b]	0·18	2·07[ab]	0·25

[a,b] Values with unlike superscript letters, were significantly different ($P < 0·05$).
* For details of composition, see Table 1.
† For details of preparation, see p. 278.
‡ Cholesterol and triacylglycerol levels measured 5 h after termination of food intake.
§ Food intake during first 2 h of meal tolerance test.

Table 6. *Expt. 2. Effects of a high-cholesterol diet or a high-cholesterol diet enriched with 30 or 50 g ethanol extract from fenugreek* (Trigonella foenum-graecum) *seed† (EtOH extract)/kg on plasma triacylglycerol and cholesterol levels, and liver cholesterol levels*

(Mean values with their standard errors for ten rats)

Diet‡...	High-cholesterol		High-cholesterol + EtOH extract (g/kg)			
			30		50	
	Mean	SE	Mean	SE	Mean	SE
Initial cholesterol (mmol/l)	2·61	0·2	2·63	0·17	2·67	0·18
Final cholesterol (mmol/l)	2·45	0·26	2·17	0·16	2·08*	0·13
Final triacylglycerols (mmol/l)	1·02	0·15	1·01	0·08	1·07	0·11
Liver cholesterol (mg/g liver)	43·4	5·4	34·6	5·4	36·8	4·9
Total liver cholesterol (mg)	683	93	573	94	590	81

Mean values was significantly different from initial value: * $P < 0·05$.
† For details of preparation, see p. 278.
‡ For details of composition, see Table 1.

Expt 2. Triacylglycerol and cholesterol levels in rats fed with various levels of EtOH extract

Initial cholesterol levels in all three groups were almost identical. Although there were no significant differences in the results, a definite trend was observed towards lower cholesterol levels when EtOH extract was introduced into the diet (Table 6). After 4 weeks of consuming a 50 g EtOH extract/kg diet, initial plasma cholesterol levels were significantly reduced. Triacylglycerol levels were similar in all three groups. Total liver cholesterol and liver cholesterol concentrations were not significantly different among the groups but there was a tendency in the groups fed on EtOH extract to have lower liver cholesterol levels than the rats fed on the high-cholesterol diet.

DISCUSSION

The present study focuses on the contribution of an EtOH extract derived from ground fenugreek seeds in lowering cholesterol levels in hypercholesterolaemic rats. In addition, attempts were made to clarify the mechanism of action of the crude extract in the rat intestine. Purification of the EtOH extract by dialysis and subsequent TLC showed that the dialysate contained substances that corresponded with the chemical structure of saponins (Birk, 1969). The dialysate was found to have haemolytic activity, similar to saponins isolated from other plants. The addition of albumen or plasma inhibited haemolysis, while cholesterol did not. Although experiments with lucerne saponins show that they directly interact with cholesterol (Gestetner *et al.* 1971), soya-bean saponins do not form complexes with cholesterol (Birk, 1969). These results indicated that the saponins present in fenugreek, similar to soya-bean saponins, do not directly interact with cholesterol. Using the everted-sac technique, attempts were made to clarify the mechanisms by which the EtOH extract from fenugreek acts as a hypocholesterolaemic agent. When compared with a wide range of materials with the potential to bind bile salts, the EtOH extract exhibited a strong inhibitory effect on bile salt absorption (Fig. 2).

The EtOH extract was capable of inhibiting bile salt absorption in a quantitative manner (Table 2), but the mechanism that causes this effect is still not clear. It is possible that large mixed micelles were formed containing bile salts and saponins and these large molecules were not available for absorption (Sidhu & Oakenfull, 1986). On the other hand, the inhibition of absorption may be primarily mechanical, due to the presence of a physical barrier interfering with absorption. The EtOH extract used in these experiments contained many substances other than saponins. Because of these impurities it is not possible, at this time, to determine the component responsible for inhibition of bile acid absorption. We believe that the saponin fraction is the main contributor to this effect. Although the EtOH extract was found to inhibit cholesterol absorption significantly (Table 3), the addition of large quantities of unlabelled cholesterol did not influence absorption in a dose-dependent manner. This implies that an active process of complex formation between the cholesterol and the EtOH extract did not occur. If complexes were formed between the cholesterol and the EtOH extract, as the relative concentration of radioactive material decreased on the mucosal side of the sac, the relative amount of [^{14}C]cholesterol absorbed should also decrease. It appears that the method of cholesterol absorption through the mucosa was diffusion and that inhibition of absorption was, for the most part, mechanical and independent of micelle formation.

The model used in the experiments *in vivo* was based on diet-induced hypercholesterolaemia. Rats fed on the standard diet had a slightly lower weight gain than rats fed on both a high-cholesterol diet or a high-cholesterol diet supplemented with EtOH extract (Table 4). Possible explanations for this difference may be changes in taste of the diets or differences in energy density. The standard diet was included to provide information on the effectiveness of the hypercholesterolaemia-inducing diet rather than serve as a control diet, therefore, this small difference has little relevance. The high-cholesterol diet serves as the control diet for diets containing EtOH extract, and no differences in growth were observed among these diets in either experiment. Rats fed on 30 g EtOH extract/kg had cholesterol levels similar to those of rats fed on the standard diet, indicating that the EtOH extract was able to prevent an increase in plasma cholesterol levels despite the large quantity of cholesterol present in the diet. The fact that post-prandial cholesterol levels of rats fed on high-cholesterol diets were slightly increased probably is a direct consequence of the quantity of the cholesterol in the diet. This increase indicates that the EtOH extract at 30 g/kg concentration only partially, if at all, inhibited cholesterol

absorption. Experiments with lucerne saponins, both *in vivo* and *in vitro*, indicate that direct interaction occurs with cholesterol and insoluble complexes are produced that could limit intestinal cholesterol absorption (Malinow *et al.* 1977; Story *et al.* 1984). It is important to note that lucerne saponins may have very different chemical structures and physical properties from those found in fenugreek (Malinow, 1984; Price *et al.* 1987). The results of the meal tolerance test coincide with the results of the experiments *in vitro* which indicated that inhibition of cholesterol absorption in the intestine by the EtOH extract was primarily mechanical. In Expt 2 (Table 6) a definite trend towards lower liver cholesterol levels in rats fed on the EtOH extract was observed. This further indicates that the EtOH extract may prevent accumulation of cholesterol in the liver. The results of these experiments show that the addition of the EtOH extract from fenugreek seeds affects cholesterol metabolism in laboratory rats. It appears that the saponins present in the extract may be responsible for its hypocholesterolaemic properties. In concurrence with the findings of Sharma (1984) and Bhat *et al.* (1985), the lower plasma and liver cholesterol concentrations found in rats fed on the EtOH extract may have been caused by increased excretion of faecal bile salts and a subsequent increase of conversion of cholesterol to bile salts. Furthermore, based on the results of all the experiments discussed here, one can conclude that fenugreek contains biologically-active components which do not directly interact with cholesterol. Nevertheless, these components had a hypocholesterolaemic effect in a manner that requires further elucidation. Experiments *in vivo* with the dialysate fraction isolated from fenugreek seeds should provide additional evidence to further support these conclusions.

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REFERENCES

Allain, C. C., Poon, L. S., Chan, C. S. G., Richmond, W. & Fu, P. C. (1974). Enzymatic determination of total serum cholesterol. *Clinical Chemistry* **20**, 470–475.

American Institute of Nutrition (1977). Report of the American Institute of Nutrition *Ad Hoc* committee on standards for nutritional studies. *Journal of Nutrition* **107**, 1340–1348.

American Institute of Nutrition (1980). Second report of the *Ad Hoc* Committee on standards for nutritional studies. *Journal of Nutrition* **110**, 1726.

Bhat, G. B., Sambaiah, K. & Chandrasekhara, N. (1985). The effect of feeding fenugreek and ginger on bile composition in the albino rat. *Nutrition Reports International* **32**, 1145–1151.

Birk, Y. (1969). Saponins. In *Toxic Constituents of Plant Foodstuffs*, pp. 169–210 [I. E. Liener, editor]. New York and London: Academic Press.

Calvert, G. D., Blight, L., Illman, R. J., Topping, D. L. & Potter, J. D. (1981). A trial of the effects of soya-bean flour and soya-bean saponins on plasma lipids, faecal bile acids and neutral sterols in hypercholesterolaemic men. *British Journal of Nutrition* **45**, 277–281.

Fazli, F. R. Y. & Hardman, R. (1968). The spice, fenugreek (*Trigonella foenumgraecum* L.): its commercial varieties of seed as a source of diosgenin. *Tropical Science* **10**, 66–78.

Gestetner, B., Assa, Y., Henis, Y., Birk, Y. & Bondi, A. (1971). Lucerne saponins, IV. Relationship between their chemical constitution and haemolytic and antifungal activities. *Journal of the Science of Food and Agriculture* **22**, 168–172.

Gibney, M. J., Pathirana, C. & Smith, L. (1982). Saponins and fibre, lack of interactive effects on serum and liver cholesterol in rats and hamsters. *Atherosclerosis* **45**, 365–367.

McGowan, M. W., Artiss, J. D., Strandbergh, D. R. & Zak, B. (1983). A peroxidase-coupled method of the colorimetric determination of serum triglycerides. *Clinical Chemistry* **29**, 538–542.

Madar, Z. (1983). Demonstration of amino acid and glucose transport in chick small intestine everted sac as a student laboratory exercise. *Biochemistry Education* **11**, 9–11.

Madar, Z. (1984). Fenugreek (*Trigonella foenum graecum*) as a means of reducing postprandial glucose level in diabetic rats. *Nutrition Reports International* **29**, 1267–1273.

Madar, Z. & Odes, H. S. (1990). Dietary fiber in metabolic disease. In *Dietary Fiber Research*, pp. 1–54 [R. Paoletti, editor]. Basel: Karger.

Madar, Z., Abel, R., Samish, S. & Arad, J. (1988). Glucose-lowering effect of fenugreek in non-insulin dependent diabetics. *European Journal of Clinical Nutrition* **42**, 51–54.

Malinow, M. R. (1984). Saponins and cholesterol metabolism. *Atherosclerosis* **50**, 117–119.

Malinow, M. R., McLaughlin, P., Papworth, L., Stafford, C., Kohler, G. O., Livingston, A. L. & Cheeke, P. R. (1977). Effect of alfalfa saponins on intestinal cholesterol absorption in rats. *American Journal of Clinical Nutrition* **30**, 2061–2067.

Oakenfull, D. (1981). Saponins in food: a review. *Food Chemistry* **6**, 19–40.

Price, K. R., Johnson, I. T. & Fenwick, G. R. (1987). The chemistry and biological significance of saponins in foods and feedingstuffs. *CRC Critical Reviews of Food Science and Nutrition* **26**, 27–135.

Ribes, G., Sauvaire, Y., Da Costa, C., Baccou, J. C. & Loubatieres-Mariani, M. M. (1986). Antidiabetic effects of subfractions from fenugreek seeds in dogs. *Proceedings of the Society for Experimental Biology and Medicine* **182**, 159–166.

Searcy, R. L. & Bergquist, L. M. (1960). A new color reaction for quantitation of serum cholesterol. *Clinica Chimica Acta* **5**, 192–199.

Segal, R., Mansour, M. & Zaitschek, D. V. (1966). Effects of ester groups in the haemolytic action of some saponins and sapogenins. *Biochemical Pharmacology* **15**, 1411–1416.

Sharma, R. D. (1984). Hypocholesterolemic activity of fenugreek (*T. foenum graecum*): an experimental study in rats. *Nutrition Reports International* **30**, 221–232.

Sharma, R. D. (1986*a*). An evaluation of hypocholesterolemic factor of fenugreek seeds (*T. foenum graecum*) in rats. *Nutrition Reports International* **33**, 660–677.

Sharma, R. D. (1986*b*). Effect of fenugreek seeds and leaves on blood glucose and serum insulin responses in human subjects. *Nutrition Research* **6**, 1353–1364.

Sidhu, G. S. & Oakenfull, D. G. (1986). A mechanism for the hypocholesterolaemic activity of saponins. *British Journal of Nutrition* **55**, 643–649.

Singhal, P. C., Gupta, R. K. & Joshi, L. D. (1982). Hypocholesterolemic effect of Trigonella foenum graecum. *Current Science* **51**, 136–137.

Story, J. A., LePage, S. L., Petro, M. S., West, L. G., Cassidy, M. M., Lightfoot, F. G. & Vahouny, G. V. (1984). Interactions of alfalfa plant and sprout saponins with cholesterol in vitro and in cholesterol-fed rats. *American Journal of Clinical Nutrition* **39**, 917–929.

Valette, G., Sauvaire, Y., Baccou, J. C. & Ribes, C. (1984). Hypocholesterolemic effect of fenugreek seeds in dogs. *Atherosclerosis* **50**, 105–111.

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