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With 1 table and 3 figures in the text

Trigonella foenum Hypoglycemic and Hypocholesterolemic Effects in Glucose-Induced Hyperglycemic Rats

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ملخص الدراسة

أجريت هذه التجربة لدراسة أثر نبات الحلبة على نسبة السكر والكلوسترول في الدم. أستخدمت في هذه التجربة عشرون جرذاً تراوحت أوزانها بين 90 - 120 جرام. قسمت إلى أربعة مجموعات تحتوى كل مجموعة على خمس جرذان. جُرع كل فرد من أفراد المجموعات 2 جم جلكوز/كجم وزن حى. حُقنت المجموعة ب بـ 10 مجم/كجم أنسولين داخل الغشاء البريتوني كما جُرعت المجموعتين ج و د بـ 400 و 800 مجم/ كلجم من مستخلص الحلبة الميثانولي على التوالي. أُخذت عينات دم من أفراد المجموعات الأربع عند الزمن صفر من بداية التجربة ثم كل فترة ساعتين بعد ذلك. أظهرت مقارنة قياس سكر الدم والكلوسترول للمجموعتين ج و د إنخفاضاً معنوياً ($P < 0.05$) بالنسبة لمستويات السكر والكولسترول لمجموعة الضبط أ التى تناولت جرعة الجلكوز فقط.

Summary

The hypoglycemic and hypocholesterolemic effect of *Trigonella foenum* (Fenugreek) was studied on twenty rats (90-120 g in weight). They were divided into four groups; all group members were each dosed with 200 mg/kg of glucose per Os. Group B then received 2mg/kg insulin I/P, group C and group D, respectively received 400 and 800 mg/kg of *T. foenum* methanolic extract per Os, blood samples were taken, glucose and cholesterol levels were measured. *T. foenum* showed hypoglycemic and hypocholesterolemic effect compared with the group which was loaded with glucose only (A).

Introduction

Fenugreek (*Trigonella Foenum*) is one of the oldest medicinal plants, originating in India and northern Africa. It is an annual plant that grows to an average height of two feet. The leaves and seeds which mature in long podes, are used to prepare extracts or powders for medicinal use.

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 (Yoshikawa *et al,* 1997). In India, fenugreek is commonly consumed as a condiment and used medicinally as a lactation stimulant (Patil, 1984). There are numerous other folkloric uses of fenugreek, including the treatment indigestion and baldness.

Materials and Methods

Plant Material and Extraction

The fenugreek seeds extract was prepared as follows:

One hundred grams of oven dried (45 °C) seeds were macerated in 70% methanol for 5 days. The filtrate was concentrated to dryness in vacuum and weighed, 70% methanolic extract was prepared.

Some minerals composition of *T. foenum* such as copper, zinc, iron and calcium were determined using atomic absorption spectrophotometer with computer readout after acid digestion of the sample according to AOAC (1990).

Amino acids analysis

Amino acids content of *T. foenum* seeds are shown in Fig. 1 as determined by SAYKAM amino acids analyzer (S 5200 sample injector, S. 4300 amino acids reaction module and S. 2100 solvent delivery system). Samples were prepared according to the method described by Sparkman *et al* (1958).

Experimental Animals

Experimental design

Twenty rats (90-120 g in weight) divided into four groups (A,B,C and D) were used. Rats were subjected to 18 hours of fasting prior to glucose administration. All groups received 2g/kg l.b.wt of 50% glucose solution administrated orally, group B received 10 mg/kg l.b.wt of insulin I/P, whereas groups C and D received orally 400 and 800 mg/kg l.b.wt of *T. foenum* methanolic extract, respectively.

Blood samples were taken from the eye vein at zero time and in two hours interval in plain vacutainers, centrifuged at 3000 rpm for 10 minutes and serum was separated and stored at -20 °C until analysis.

Determination of the blood glucose levels was done by glucose-oxidase principle (Beach and Turner, 1958), and cholesterol levels were determined using spectrophotometric method; Biosystem kits were used and the instructions of the manufacturer were followed.

Statistical analysis

Blood glucose and cholesterol levels were expressed in mg/dl as mean ± SEM. The data were statistically analyzed using ANOVA with multiple comparisons versus control group by Dunnett's method. The values of $P < 0.05$ were taken as significant.

Results

Table 1 shows some mineral contents of *T. foenum* seeds.

Table 1: Some mineral contents of *T. foenum* seeds

Mineral	Value (ppm)
Fe	9.031
Ca	1.278
Zn	0.354
Cu	40.014

Fig. 2 shows that there was no significant difference in serum glucose levels among the different groups at zero time. After two hours, group A rats which had received glucose only, showed significant ($p < 0.05$) high levels of serum glucose compared with other groups. Group B, which had received insulin, showed the lowest level of glucose whereas group D members which had received 800 mg/kg of *T. foenum* extract showed significant ($p<0.05$) low levels of glucose compared with those of group C ones which had received 400mg/kg of *T. foenum* extract.

After four hours, group A rats showed the highest glucose level, while group B ones showed the lowest level. There was no significant difference between group B and D rats in serum glucose while group C rats showed no significant difference in serum glucose level compared with those of group A rats.

As shown in Fig. 3, there was no significant difference of serum cholesterol level among all groups at zero time. After two hours, group B rats, showed the lowest cholesterol level compared with other groups, while group A ones showed the highest level.

Group D and group C rats showed no significant level of serum cholesterol compared with that of group B rats both of them showed significant ($P < 0.05$) low cholesterol level compared with that of group A rats.

Discussion

Trigonella foenum methanolic extract showed hypoglycemic effect on glucose-induced hyperglycemia, this result agrees with what reported by Ethan *et al* (2003). The hypoglycemic effect of fenugreek was shown to be attributed to several mechanisms; Sauvaire *et al* (1998) demonstrated *in vitro* the insulin releasing property of the amino acid 4-hydroxyisoleucine, which is one of the major constituents of fenugreek seeds. 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 reduces the area under the plasma glucose curve and increases the number of insulin receptors, although the mechanism of this effect is unclear as reported by Raghuram *et al* (1994). In humans, fenugreek seeds exert hypoglycemic effects by stimulating glucose-dependent insulin secretion from pancreatic beta cells (Ajabnoor and Tilmisany, 1998), as well as by inhibiting the activities of two intestinal enzymes, involved in carbohydrate metabolism, alpha-amylase and sucrose (Amin *et al,* 1987).

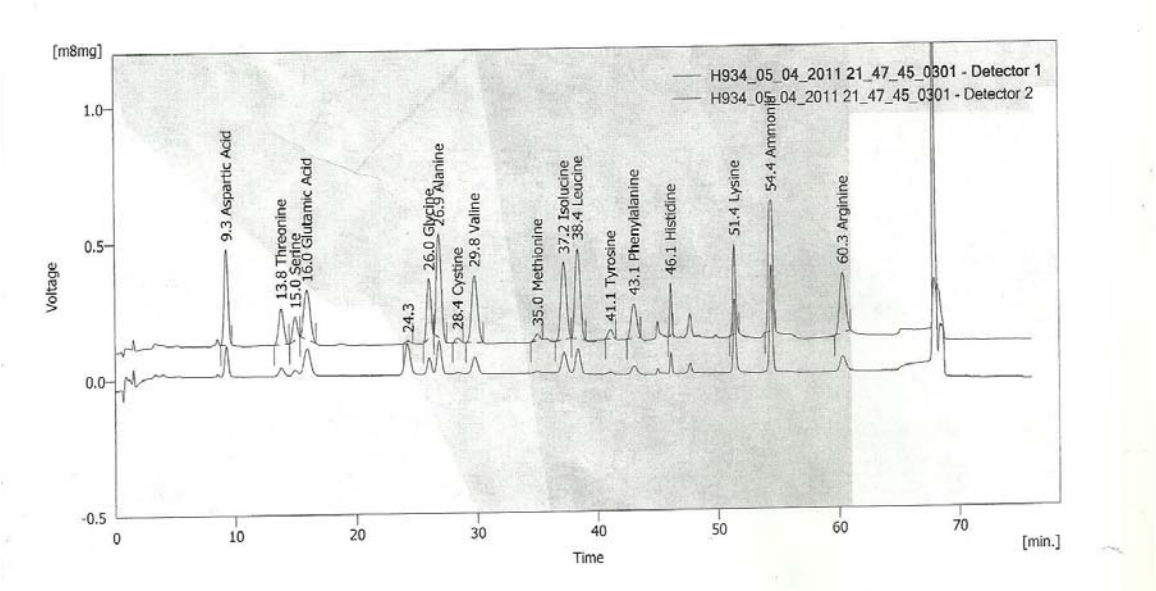

Fig. 1: Amino acids content of *T. foenum*

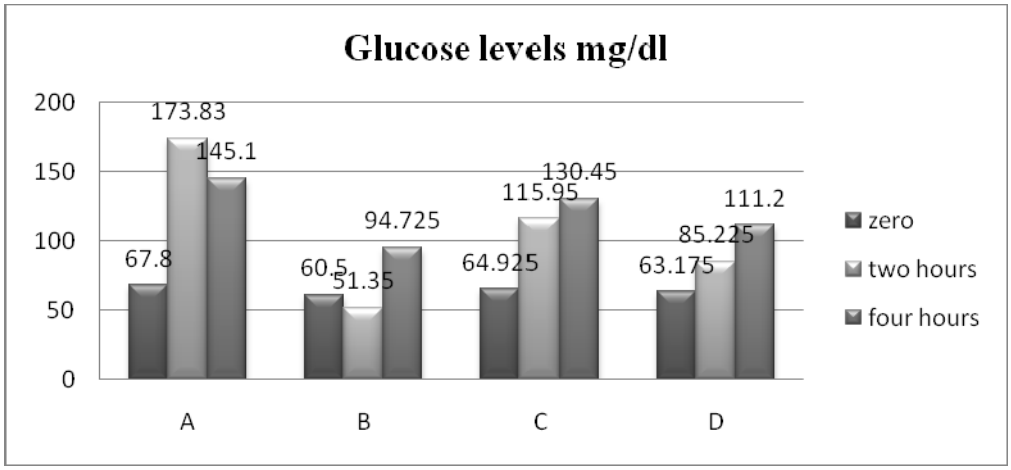

Fig. 2: Serum glucose levels (mg/dl) of different groups at different times.

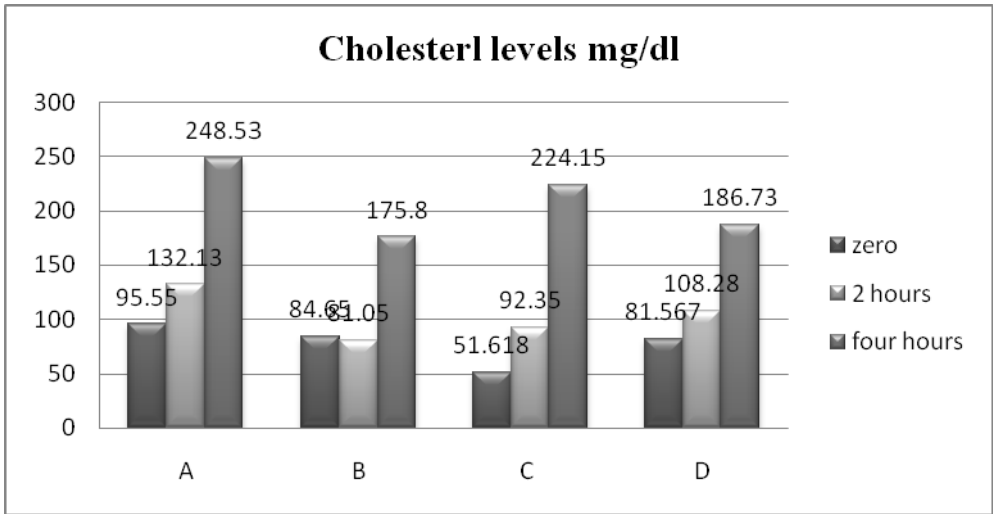

Fig. 3: Serum cholesterol levels (mg/dl) of different groups at different times.

Trigonella foenum seeds have some hypocholestrolemic activity, this is credited to both the galactomannas and the saponins. Galactomannas decreases uptake of bile acids, lowers blood and liver concentration of cholesterol and decreases hepatic cholesterol synthesis. The soluble dietary fraction of seeds significantly decrease the atherogenic lipids in type 2 diabetic rats (Hannan *et al*, 2003). Saponins also seem to interact with bile in the GIT.

Hypocholesterolemic effect of *T. foenum*, observed in this study, could be attributed to the ratio of lysine: arginin (0:2) as was reported by amino acids analysis result. Rajamohan and Kurup (1997) studied the effect of globulin fraction with lysine: arginin (0:7) on cholesterol metabolism, in rats fed cholesterol free and cholesterol containing diet. They concluded with significantly low concentrations of cholesterol in the serum. Hepatic diversion of cholesterol to bile was increased suggesting acid synthesis and increased faecal excretion of bile acids and sterols. Also significantly high activity of lipoprotein lipase was observed. The latter study showed that low lysine: Arginin ratios of a protein exert hypocholesterolemic effects.

Fenugreek seeds also lower serum triglycerides, total cholesterol (TC), and low-density lipoprotein (LDL) cholesterol (Stark and Madar, 1993). These effects may be due to sapogenins, which increase biliary cholesterol excretion and in turn, leading to low serum cholesterol levels (Stark and Madar, 1993; Sauvaire *et al*, 1991; Sidhu and Oakenfull, 1986).

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