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Antidiabetic Effects of Fenugreek (*Trigonella foenum-graecum*) Seeds in the Domestic Rabbit (*Oryctolagus cuniculus*)

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ABSTRACT

Adult male rabbits were used to evaluate the effects of supplementation with fenugreek seeds on Body Weight (BW), plasma glucose and serum levels of cholesterol and insulin in alloxan-induced diabetic and in non-diabetic rabbits. Diabetic and non-diabetic groups of rabbits supplemented with fenugreek showed a slight increase in BW. Supplementation with fenugreek resulted in a significant decrease in glucose level of diabetic group of rabbits and a slight effect in non-diabetic rabbits. Diabetic groups of rabbits had significantly higher serum cholesterol levels compared to non-diabetic groups. Supplementation with fenugreek lowered the cholesterol level slightly in diabetic groups. The alloxan-treated groups of rabbits maintained significantly lower serum insulin level compared to the respective values obtained for the non-diabetic groups. Supplementation with fenugreek slightly increased serum insulin level in diabetic rabbits. The findings demonstrate the antidiabetic and insulin mimetic effects of fenugreek seeds in rabbits.

Key words: Rabbits, alloxan-diabetes, fenugreek, insulin

INTRODUCTION

Diabetes mellitus is a chronic metabolic disease characterized by derangements in the metabolism of carbohydrates, proteins and lipids caused by complete or relative insufficiency of insulin secretion and/or insulin action (American Diabetes Association, 2009). The disease is a major medical concern as it is costly in individual, social and economic terms and its global burden is increasing in most populations. Although, biguanides and sulphonylurea are valuable in the treatment of diabetes mellitus, their use is limited by limited action, pharmacokinetic properties, secondary failure rates and accompanying side effects (Baquer *et al*., 2011). Herbal medicine plays an important role in anti-diabetes treatment (Park *et al*., 2005). Fenugreek (*Trigonella foenum-graecum*) has been used as a source of antidiabetic compounds from its seeds, leaves and extracts in various model systems (Raju *et al*., 2001; Srinivasan, 2006; Khalki *et al*., 2010). Preliminary animal and human studies suggest possible hypoglycaemic and antihyperlipidemic properties of fenugreek seed powder (Basch *et al*., 2003). The antidiabetic properties of a soluble dietary fibre fraction of fenugreek in normal, type 1 or type II diabetic rats significantly improved oral glucose tolerance (Hannan *et al*., 2007). Fenugreek significantly reduced fasting blood sugar and improved

the glucose tolerance test. Further, this therapy also resulted in an improved serum lipid profile with significant reduction in serum total cholesterol, LDL and VLDL cholesterol and triglycerides (Raju *et al*., 2001).

Fenugreek seeds contain alkaloids (mainly trigonelline) and protein high in lysine and L-tryptophan. Its steroidal saponins (diosgenin, yamogenin, tigogenin and neotigogenin) and mucilaginous fibre are thought to account for many of the beneficial effects. The steroidal saponins are thought to inhibit cholesterol absorption and synthesis (Sauvaire *et al*., 1991) while the fibre may help lower blood glucose level (Ribes *et al*., 1986). Use of more than 100 g of fenugreek seeds daily can cause intestinal upset and nausea in humans, otherwise, fenugreek is extremely safe (Brinker, 1998).

Fenugreek seed activity has been attributed largely to saponin and high fibre content and is probably not related to its major alkaloid trigonelline. The glycaemic effects have been linked to delayed gastric emptying caused by the fibre content and to unidentified components that inhibit carbohydrate digestive enzymes (Al-Habori and Raman, 1998). Fenugreek administration may increase plasma insulin levels *in vivo*. Its major free amino acid, 4-hydroxyisoleucine, an insulinotropic compound isolated from seeds, increased the insulin release in glucose fed hyperglycaemic rats and humans (Sauvaire *et al*., 1998). It also repaired the liver and kidney damage caused by alloxan in diabetic rats (Thakran *et al*., 2004).

The hypocholesterolaemic effect of fenugreek has been attributed to increased conversion of hepatic cholesterol to bile acids and loss in the faeces of complexes of these substances with fenugreek fibre and saponins. Fenugreek administration has not been reported to cause any toxicological effects. Its regular consumption may therefore be beneficial in the management of diabetes and the prevention of atherosclerosis and coronary heart disease (Al-Habori and Raman, 1998). Experimental models of laboratory animals are usually adopted with the purpose of improving the understanding of the pathophysiological mechanisms of the diseases that affect mainly humans. The goal of this experiment was to evaluate the effects of supplementation of fenugreek seeds on BW, blood metabolites and insulin in diabetic and non-diabetic rabbits.

MATERIALS AND METHODS

Twenty clinically healthy adult male rabbits were used in the study. The animals were accommodated in individual cages in a well ventilated animal house at the Department of Physiology under summer conditions. They were randomly assigned to 4 groups (A, B, C and D) of 5 animals each. The treated groups (A and B) were made diabetic by a single intravenous injection of 150 mg kg^{-1} alloxan monohydrate (Sigma, St. Louis, MO) dissolved freshly in 0.9% NaCl. Therapeutic measures were adopted to secure survival of rabbits by administration of glucose to tide over initial hypoglycaemia and injection of insulin during acute phase of hyperglycaemia. The non-diabetic groups received only 0.9% NaCl. Group A diabetic rabbits were fed fresh lucerne only and served as a diabetic control, group B was fed lucerne supplemented with fenugreek. Group C (non-diabetic rabbits) were fed lucerne and group D (non-diabetic rabbits) were fed lucerne supplemented with fenugreek. The fenugreek seed powder was administered daily at 2.5 g kg^{-1} orally to normal and alloxan-induced diabetic rabbits. The powder of fenugreek was dissolved in water and was administered by gavage. All animals were fed fresh lucerne (dry matter basis: CP 18%, ME 7.8 MJ kg^{-1}) and were offered tap water *ad libitum*. The rabbits were subjected to the experimental protocol for 8 weeks.

During the experimental period, the Body Weight (BW) of rabbits was measured by a digital balance at weekly intervals. Jugular blood samples (4 mL) were collected weekly at 7:00 a.m. using plastic syringes. One milliliter of blood was kept in a test tube containing sodium fluoride and after centrifugation, the plasma was used for glucose determination. The rest of the blood sample was left at room temperature for 3 h and then centrifuged (Hettich-Germany) at 3000 rpm for 15 min. Haemolysis-free serum samples were stored at -20°C for subsequent analyses. The concentrations of plasma glucose and serum cholesterol were measured by colorimetric methods using kits (Spinreact, S. A., Spain). Serum insulin level was determined by Radioimmunoassay kit (Beijing Institute of Atomic Research, Beijing, China).

For each group of animals, the mean values were computed during the course of experimental period. The data obtained are presented as Mean±SD. The Analysis of Variance (ANOVA) (SAS Institute, 1996) and Duncan's Multiple Range Test (DMRT) were used to evaluate the effects of fenugreek supplementation on the parameters investigated at $p<0.05$ and $p<0.001$.

RESULTS

The results of the effects of fenugreek supplementation on BW, plasma glucose, serum cholesterol and insulin concentrations with time-course changes in diabetic and non-diabetic rabbits are shown in Fig. 1-4.

Body Weight (BW): The diabetic groups of rabbits had slightly lower values of BW compared to the corresponding values of non-diabetic groups. Supplementation with fenugreek was associated with a slight increase in BW of diabetic and non-diabetic groups of rabbits. Figure 1 shows that, there was slight gradual increase in BW of diabetic and non-diabetic groups of rabbits supplemented with fenugreek.

Plasma glucose: Diabetic groups of rabbits maintained significantly ($p<0.001$) higher plasma glucose levels compared to the non-diabetic groups. The diabetic rabbits supplemented with fenugreek showed significantly ($p<0.001$) lower, glucose level than the values obtained for

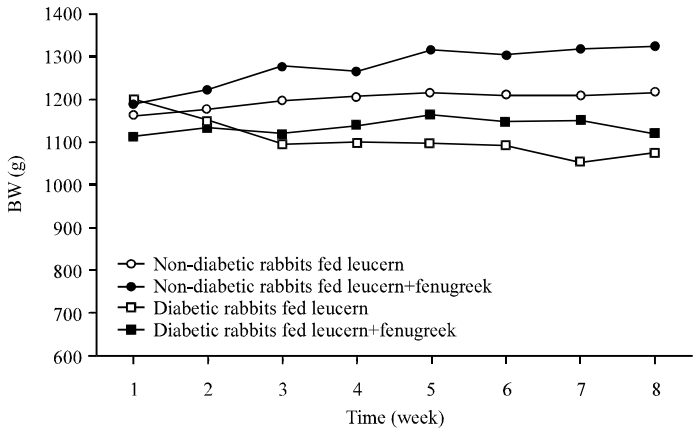

Fig. 1: Effect of supplementation with fenugreek on body weight (BW) in diabetic and non-diabetic rabbits

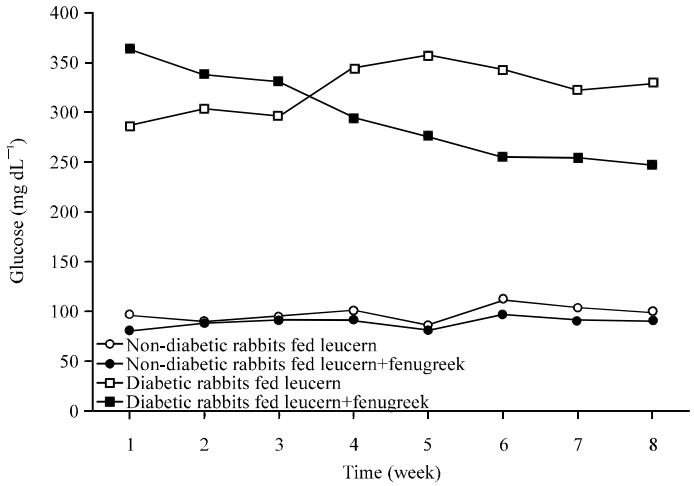

Fig. 2: Effect of supplementation with fenugreek on plasma glucose level in diabetic and non-diabetic rabbits

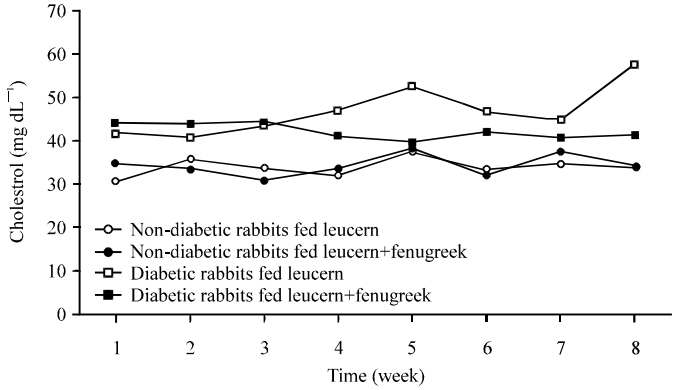

Fig. 3: Effect of supplementation with fenugreek on serum cholesterol level in diabetic and non-diabetic rabbits

respective group of diabetic rabbits fed lucerne only. However, supplementation with fenugreek resulted in a slight decrease in glucose concentration of non-diabetic group of rabbits. The diabetic group of rabbits supplemented with fenugreek showed progressive decrease in glucose concentration during the experimental period (Fig. 2).

Serum cholesterol: The diabetic groups of rabbits had significantly ($p<0.05$) higher values of cholesterol level than non-diabetic groups of rabbits. However, the diabetic and non-diabetic groups of rabbits supplemented with fenugreek had slightly lower values of cholesterol compared to the respective diabetic group fed lucerne only. The diabetic group of rabbits supplemented with fenugreek showed gradual decrease in cholesterol concentration (Fig. 3).

Serum insulin: The diabetic groups of rabbits had significantly ($p<0.001$) lower values of insulin concentration compared to the values measured for non-diabetic groups. Supplementation with fenugreek was associated with a slight increase in insulin concentration of diabetic rabbits (Fig. 4). However, there was no significant change in diabetic and non-diabetic groups of rabbits.

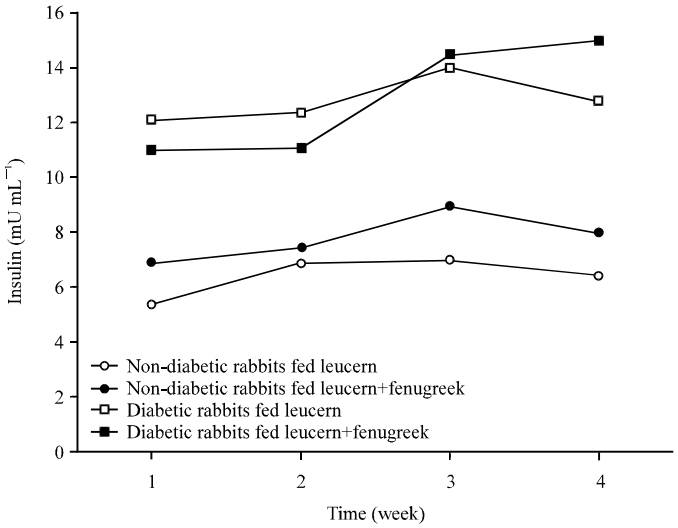

Fig. 4: Effect of supplementation with fenugreek on serum insulin level in diabetic and non-diabetic rabbits

DISCUSSION

The results indicate that regardless of supplementation with fenugreek, the diabetic groups tended to maintain lower BW compared to the non-diabetic groups. Also, it is apparent that fenugreek supplementation was associated with greater BW. This finding indicates that the mechanisms involved in maintenance of BW were enhanced when the rabbits were supplemented with fenugreek.

The diabetic groups of rabbits maintained significantly higher plasma glucose levels compared to the non-diabetic groups. This confirms findings reported earlier (Hollenbeck, 1993). The groups of diabetic rabbits supplemented with fenugreek showed significantly lower values of glucose than the respective group of diabetic rabbits fed lucerne only. This hypoglycaemic effect is thought to be largely due to its high content of soluble fibre which acts to decrease the rate of gastric emptying, thereby delaying absorption of glucose from the small intestine (Yin *et al.*, 2003). The seeds of fenugreek contain about 50% fibre, of which 20% is fibre similar to guar gum which is a known hypoglycaemic agent. The high level of fibre interferes with carbohydrate absorption. Saponin compounds diosgenin, alkaloids and trigonelline were shown to be associated with inhibition of intestinal glucose uptake in vitro (Al-Habori *et al.*, 2001). The protein fraction of the seeds contains the amino acid 4-hydroxyisoleucine which stimulates insulin production (Sauvaire *et al.*, 1996). Owing to its particular insulinotropic action, 4-hydroxyisoleucine might be considered as a novel secretagogue with potential role in treatment of diabetes (Baquer *et al.*, 2011). The hypoglycaemic effect of fenugreek seed is mediated, at least in part, by the activation of an insulin signaling pathway in adipocytes and liver cells (Vijayakumar *et al.*, 2005).

The hypoglycaemic effect of fenugreek reported in rabbits in the current study is in agreement with previous findings (Satyanarayana *et al.*, 2007). However, Swanston-Flatt (1989) did not report significant alteration in plasma glucose level. Furthermore, Raju *et al.* (2001) reported that fenugreek seed improves glucose homeostasis in alloxan-diabetic rat tissues by reversing the altered glycolytic, gluconeogenic and lipogenic enzymes.

Diabetic groups maintained significantly higher serum cholesterol levels (Fig. 3). This confirms findings reported earlier in diabetic rabbits (Raju *et al.*, 2001). Furthermore, the diabetic group

supplemented with fenugreek had lower serum cholesterol level. The reduction in cholesterol level may be attributed to the effect of the saponin present in fenugreek seeds which has been shown to lower cholesterol levels (Szkudelski *et al.*, 1998). Also, the fibre in general enhances faecal excretion of bile acids and cholesterol. Other studies reported reduction in serum cholesterol levels associated with fenugreek in rabbits (Al-Habori and Raman, 1998; Satyanarayana *et al.*, 2007). Prasanna (2000) reported in a double blind study, reduction of serum cholesterol in rats receiving fenugreek. Therefore, the role of fenugreek seed powder in diabetic state can be attributed to the change of glucose and lipid metabolizing enzyme activities to normal values, thus stabilizing glucose homeostasis.

The administration of fenugreek was associated with a slight increase in serum insulin level of diabetic rabbits. This response may be related to regenerative effect of fenugreek on pancreatic tissue. Nelson *et al.* (1991) reported regenerative effect of fibre on pancreatic tissue in dogs with alloxan-induced diabetes and stimulation of B cells to produce more insulin. The protein fraction of the seeds contains the amino acid 4-hydroxyisoleucine which has been proven to stimulate insulin release (Sauvaire *et al.*, 1996). However, Swanston-Flatt (1989) reported that, fenugreek seeds did not affect insulin level.

The data presented clearly demonstrate the antidiabetic and insulin mimetic effects of fenugreek seeds in rabbits. Consumption of fenugreek seeds as dietary supplement may be beneficial in management of diabetes in humans. The literature does not reveal significant harmful adverse effects of fenugreek. However, caution is warranted in diabetic patients who are allergic to fenugreek (Basch *et al.*, 2003). Recent studies revealed an antifertility effect of fenugreek seeds in female rabbits and more of a toxicity effect in male rabbits (Kassem *et al.*, 2006). Further research is required to clarify aspects pertaining to the safety of fenugreek being a potential alternative medicine.

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