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Supplementation of (*Trigonella foenum-graecum* L.) Fenugreek Leaves Stimulates the Insulin Action in Streptozotocin-Induced Diabetic Rats

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Abstract

Trigonella foenum-graecum (Fenugreek) leaves exhibit antidiabetic and antioxidant properties. The present study was designed to elucidate the insulin stimulatory effect on supplementation of fenugreek leaves in streptozotocin-induced diabetes in rats. Supplementation of fenugreek leaves mixed with diet at doses of 0.5g and 1.0g/kg of body weight twice daily to diabetic rats for a period of 45days resulted in change in bodyweight, increase in weight of pancreas, enhances the insulin levels and a significant decrease in fasting blood glucose levels. Histopathological observations showed marked changes of the pancreas in treatment with the fenugreek leaves improved the functional state of the pancreatic β-cells and partially retained the damage caused by streptozotocin to the pancreatic islets. These findings of our study clearly indicate the insulin stimulatory effect of fenugreek leaves. The effect observed with the fenugreek leaves was better than that of glibenclamide (600 μg/kg bodyweight).

1. Introduction

Diabetes mellitus is a syndrome, initially characterized by a loss of glucose homeostasis resulting from defects in insulin secretion, insulin action both resulting in impaired metabolism of glucose and other energy-yielding fuels such as lipids and protein(WHO, 1985).

The mechanism of most of the herbals used to treat diabetes has not been defined (Bailey 1989). It has been attributed that the antihyperglycemic effect of these plants is due to their ability to restore the function of pancreatic tissues by causing an increase in insulin output or inhibit the intestinal absorption of glucose or to the facilitation of metabolites in insulin dependent processes. Hence treatment with herbal drugs has an effect on protecting β cells and smoothing out fluctuation in glucose levels (Jia et al. 2003 and Elder 2004).

Most of the plants have been found to contain substances like glycosides, alkaloids, terpenoids, flavonoids etc. that are frequently implicated as having antidiabetic effects. But little is known on the specific modes of action of these plant drugs or herbal formulation used for treating diabetes (Loew and Kaszkin 2002). Plant drugs (Halberstein 2005) and herbal formulation (Mitra et al. 1996) are frequently considered to be less toxic and more free from side effects than synthetic one.

Trigonella foenum-graecum l. (Leguminoseae) is an erect, strongly scented annual herb that is extensively cultivated as a food crop in India, the Mediterranean region, North Africa, and Yemen, Indigenous People use both leaves and seeds of this plant to treat diabetes mellitus, Fenugreek leaves are consumed widely in India as a green, leafy vegetable and are a rich source of calcium, iron, B-carotene, and vitamin K (Sharma 1986).

Previous studies have shown that the water extract of fenugreek leaves given both orally and intraperitoneally ex-hibits a hypoglycemic effect in normal and alloxan-treated diabetic rats (Al-Habori and Raman 1998) there are no available reports on the effect of supplementation with fenugreek leaves in experimental diabetes. Hence, we have planned to study whether supplementation with fenugreek leaves exerts stimulatory effect of insulin in streptozotocin (STZ) - induced diabetes.

2. Materials and Methods

Plant material

Fenugreek leaves were purchased from the local market, and their botanical identity was confirmed by a botanist from the Department of Botany, Annamalai University. The leaves were cleaned, dried, and finely powdered. The powder was used for feeding the rats; it was mixed with the diet at a level of 0.5 g and 1 g/kg of body weight.

Chemicals

Streptozotocin was purchased from Sigma chemical Co., St.Louis Mo. USA. All other chemicals and biochemicals used in our study were of high-grade quality.

Animals and Diet

All the animal experiments carried out by us were approved by the ethics committee of Annamalai University, Tamil Nadu, India. Adult female albino rats of the Wistar strain weighing 170-200g were obtained from the Central Animal House, Department of Experimental Medicine, Annamalai University, Rajah Muthiah Medical College & Hospital, Annamalai University and were used in the present study. They were housed in polypropylene cages (47×34× 20cm) lined with husk, renewed every 24h under a 12:12 h light dark cycle at around 22°C and had free access to tap water and food.

The animals were fed with a standard pellet diet (Kamadhenu Agencies, Bangalore, India).The pellet diet consisted of 22.02% crude protein, 4.25% crude oil, 3.02% crude fibre, 7.5% ash, 1.38% sand silica, 0.8% calcium, 0.6%phosphorus, 2.46% glucose, 1.8% vitamins and 56.17% carbohydrates. It provided a metabolisable energy of 3600kcal.

Induction of diabetes in rats

Diabetes was induced in rats by a single intraperitoneal injection of freshly prepared STZ (40mg/kg of body weight) in 0.1 M citrate buffer (pH 4.5) in a volume of 1 ml/kg (Fathy El-Fiky et al. 1996). Forty-eight hours after STZ injection. The blood glucose level of each rat was determined. Rats having a blood glucose range of 220-260 mg/dl were considered diabetic and included in the study.

Experimental design

In our study, we used a total of 36 rats (six normal and 30 STZ – induced diabetic surviving). The rats were divided into six groups of six rats each: Group 1, normal untreated rats; Group 2, STZ-induced diabetic rats; Groups 3 and 4, STZ-induced diabetic rats supplemented with fenugreek leaves (0.5 or 1 g/kg of body weight, respectively) daily in the diet for a period of 45 days (AnithaDevi et al. 2003); Group 5, STZ-induced diabetic rats given glibencamide (600 µg/kg of body weight) daily for 45 days (AnithaDevi et al. 2003); and Group 6, STZ-induced diabetic rats given insulin (6 units/kg of body weight) daily intraperitoneally for 45 days (AnithaDevi et al. 2004).The food intake was measured daily throughout the experiment.

Biochemical estimations

After the last treatment, the rats were fasted overnight and killed by cervical dislocation. Blood was collected in heparinized tubes and centrifuged at 5000 rpm for 10 min. Plasma was separated by aspiration, transferred into eppendorf tubes, and stored at-20°C for analysis. Blood glucose was estimated by the method of Sasaki et al. (1972). Plasma insulin assay was carried out by ELISA method using a Boehringer Mannheim Kit (Boehringer analyser Es 300), Mannheim, Germany.

Histopathological study

For histopathological study, two animals from each group were perfused with formalin (10%) and the tissues were separated and stored in 10% formalin. They were later dehydrated in graded alcohol, embedded in paraffin sectioned using a microtome, and stained with hemotoxylin and eosin (H&E).

Pancreas was removed immediately and fixed for histology. The histological observation was made under light microscope.

Statistical analysis

All the grouped data were statistically evaluated and the obtained data were analyzed by one way ANOVA followed by Duncan's Multiple Range Test (DMRT). P values<0.01 were considered as significant.

3. Results

Figure 1 shows the blood glucose levels in normal and experimental rats. There was a significant increase in the blood glucose levels in streptozotcin diabetic rats as compared to normal rats. Supplementation of fenugreek leaves in diabetic rats decreased blood glucose.

Figure 1. Effect of Fenugreek leaves on blood glucose in normal and STZ-diabetic rats. Each value is mean ± SD for 6 rats in each group. Values not sharing a common superscript differ significantly at p< 0.01 (DMRT).

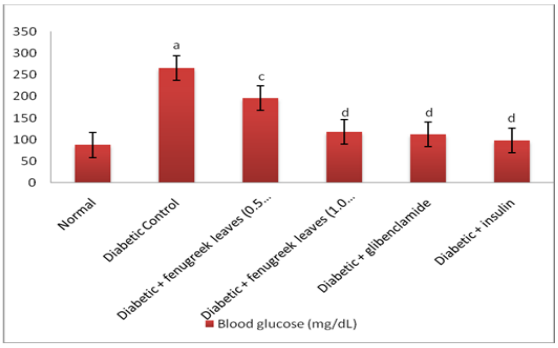


Figure 2 shows the levels of plasma insulin in normal and streptozotocin diabetic rats. Treatment with fenugreek leaves (0.5or1gm/kg body weight) enhances the plasma insulin levels in diabetic rats.

Figure 2. Effect of Fenugreek leaves on plasma insulin in normal and STZ-diabetic rats. Each value is mean ± SD for 6 rats in each group. Values not sharing a common superscript differ significantly at p< 0.01 (DMRT).

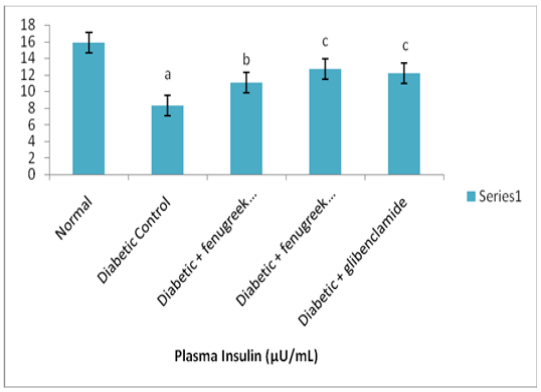


Figure 3 illustrates the change in bodyweight of STZ diabetic rats. In diabetic rats we found lose of weight when compared to normal rats. Supplementation of fenugreek leaves (0.5or1gm/kg bodyweight) to STZ treated diabetic rats improved in weight gain.

Figure 3. Effect of Fenugreek leaves on change in body weight of normal and STZ –diabetic rats. Each value is mean ± SD for 6 rats in each group. Values not sharing a common superscript differ significantly at p< 0.01 (DMRT).

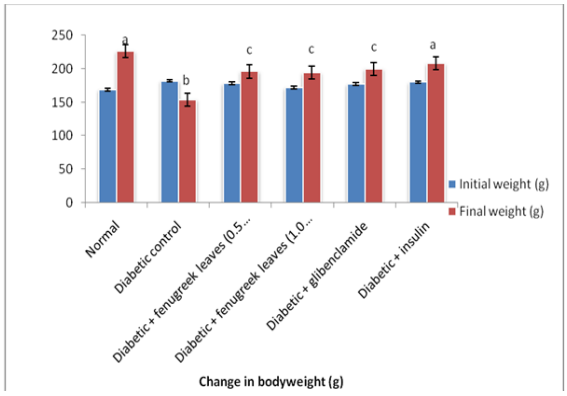


Figure 4 shows the effect of supplementation of fenugreek leaves and change in weight of pancreas in diabetic rats. There was a significant change in weight of pancreas in STZ treated diabetic rats compared to normal. Treatment with fenugreek leaves at doses of (0.5 or1 gm/kg bodyweight) improved the weight gain of pancreas in diabetic rats.

Figure 4. Effect of Fenugreek leaves on organ (pancreas) weight of normal and STZ-diabetic rats. Each value is mean ± SD for 6 rats in each group. Values not sharing a common superscript differ significantly at p< 0.01 (DMRT).

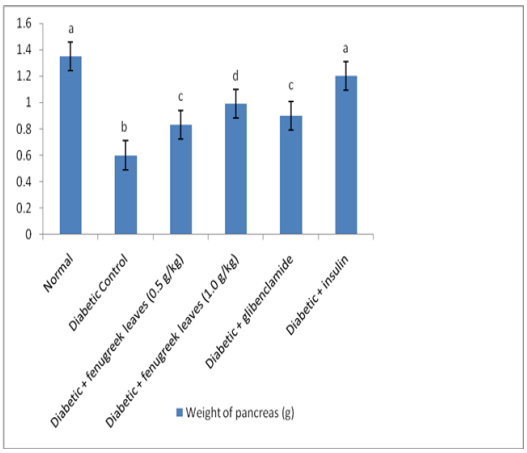


Figure 5 shows normal rats pancreas H&EXp100X normal acini with and cell

Figure 5. Normal pancreas – Normal acini with β cells.

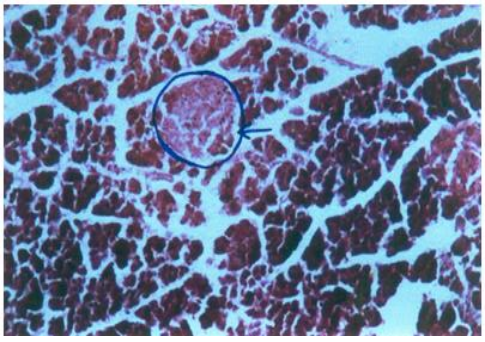


Figure 6 shows diabetic pancreas H&EX100X destroyed β cells of islets

Figure 6. Diabetic pancreas – Destroyed β cells of islets were seen

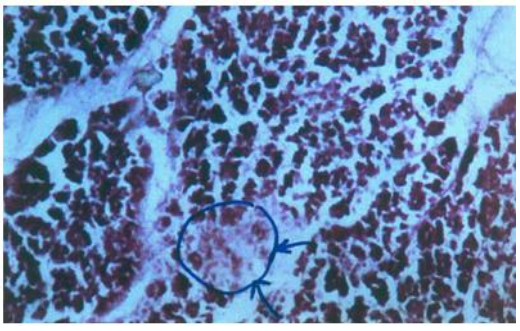


Figure 7 shows Diabetic + fenugreek leaves (0.5gm/kg body wt.) treated rats pancreas H&EX100X partially regenerated and cells

Figure 7. Fenugreek leaves (0.5 gm/kg) treated pancreas – partially regenerated β cells

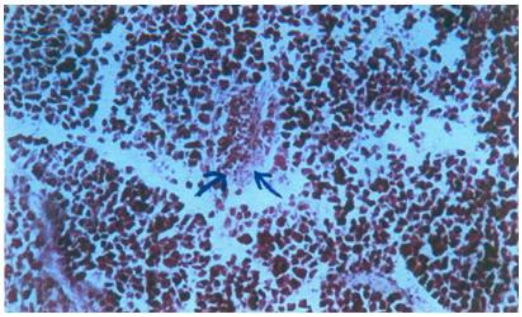


Figure 8 shows diabetic + fenugreek leaves (1 gm/kg body wt.) treated rats pancreas H&EX100X regenerated and cells of islets

Figure 8. Fenugreek leaves (1.0gm/kg) treated pancreas – Regenerated β cells

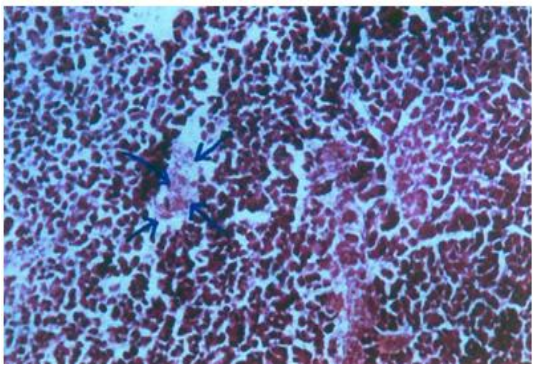


Figure 9 shows diabetic + Glibenclamide treated rat's pancreas H&EX100X Regenerating & cell

Figure 9. Glibenclamide (600 µg/kg) treated pancreas – Regenerating β cells

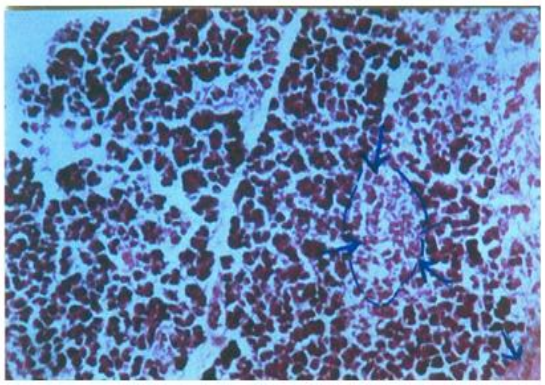
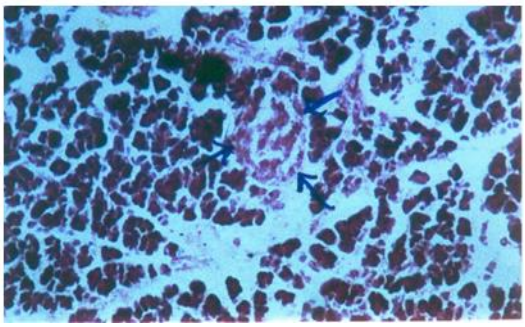


Figure 10 shows Diabetic + Insulin treated rat pancreas H&EX100X Well preserved & cells of islets

Figure 10. Diabetic +insulin treated rat pancreas- Well preserved β cells of islets



4. Discussion

At present, the treatment of diabetes mainly involves a sustained reduction in hyperglycaemia by the use of biguanides, thiazolidinediones, sulphonylureas, Dphenylalanine derivatives, meglitinides and á-glucosidase inhibitors in addition to insulin. However, due to unwanted side effects the efficacies of these compounds are debatable and there is a demand for new compounds for the treatment of diabetes (Jackson and Bressler 1981; Thirunavukkarasu et al. 2003). Hence, plants have been suggested as a rich, as yet unexplored source of potentially useful antidiabetic drugs. However, only a few have been subjected to detailed scientific investigation due to a lack of mechanism-based available in vitro assays (Saxena and Vikram 2004). Fenugreek (*Trigonella foenum graecum L., Leguminosae*), as mentioned before is one of the oldest medicinal plants, its aqueous extracts of seeds and leaves of fenugreek have been shown to possess hypoglycaemic activity and are nontoxic (Abdel-Barry 2000).

Fenugreek (*Trigonella foenum-graecum*) (TFG) is a plant with traditional medicinal use in diabetes and its beneficial effects have been demonstrated in diabetic animals and both insulin-dependent and non- insulin-dependent diabetic subjects (Al-Habori et al. 2001).

Hypoglycemic and antihyperglycemic effects of fenugreek seeds (Alarcon-Aguilara et al. 1998) and aqueous leaf extracts (Abdel-Barry et al. 1997) have previously been reported in experimentally induced diabetic rats. Since, there is no strong scientific evidence for therapeutic and pharmacological properties of TFG. We have studied the insulin stimulatory effect of fenugreek leaves on streptozotocin induced diabetes rats.

Streptozotocin which is a naturally occurring chemical discovered in strain of soil microbe is particularly toxic to the insulin producing beta cells of the pancreas in mammals. It is used in medicine for treating certain cancers of the islets of Langerhans and used in medical research to

produce permanent animal model of Type I diabetes (Brentjens and Saltz 2001).

Streptozotocin-induced diabetes provides a condition of insulinopenia and has been described as a useful experimental model to evaluate the activity of hypoglycemic agents (Bailey and Flatt 1986). The use of a lower dose of STZ produced an incomplete destruction of pancreatic β – cells even though rats became permanently diabetic (Aybar et al. 2001). We have observed decreased blood glucose levels in STZ - diabetic rats treated with fenugreek leaves. Since the supplementation of fenugreek leaves with the food reduced the blood glucose potently in streptozotocin-diabetc rats it may be assumed that the diabetic state is not severe. Thus, the fenugreek leaves supplemented may act by a direct stimulation of insulin secretion in remaining β-cells. This effect could be attributed to due to the presence of flavonoids, xanthophylls, metals chelating agents (chromium, manganese and magnesium) (Fatima et al. 2004) and glucoside in fenugreek leaves, that stimulate insulin secretion.

Loss of body weight and the weight of pancreas were reduced in the diabetic rats. The decrease in body weight could be due to dehydration and the catabolism of fats and proteins seen during diabetes mellitus (Hakim et al. 1997). Supplementation of fenugreek leaves improved body weight (Anitha Devi et al. 2003) and organ (pancreas) weight. This is due to the effect of fenugreek leaves supplementation and its ability to reduce hyperglycaemia (Anitha Devi et al. 2003)

The glucose lowering activity observed in the diabetic animal may be due to the stimulation of β cells of pancreatic islets, also may be mediated through stimulation of insulin release resembling the oral hypoglycemic sulfonyl ureas or peripheral glucose utilization (Ivorra et al. 1988). This may be due to the presence of some hypoglycemic agents present in the fenugreek leaf powder, which were similar to insulin or oral hypoglycemic drugs.

Insulin levels were found to be increased significantly upon treatment with fenugreek leaves to the diabetic rats. An increase in the insulin levels reflects the regeneration of β – cells from the damaged pancreas by supplementation of fenugreek leaves. Gomes et al. (1995) have stated that after low dose of STZ-treatment, there should be many surviving β – cells and cell regeneration is also possible. Das et al. (1996) have shown that the damage caused by STZ (45 mg/kg) to the pancreas of Sprague Dawley rats was reversed by the A. marmelos leaf extract treatment. These finings are in line with our results, as we have used a lower dose of streptozotocin (45 mg/kg) for the induction of diabetes in rats.

Histopathological examination of the pancreas showed shrunken islets and degranulation of β-cell in the STZ-treated rats. An inadequate mass of functional pancreatic β-cell cause hyperglycaemia (Bonner-Weir 2000). Endocrine pancreas is considered to be a slowly renewed tissue (Finegood et al. 1995). Pancreatic β-cell replicates at a rate of 3% per day and β – cell mass gets doubled in one month if the cell death in negligible. Complete replacement of β-cell population occurs when the replication rate approaches the β – cell death rate. The rate of β-cell death is higher in rats treated with streptozotocin and this does not match with the replication rate. Hence, β-cell population is reduced with a subsequent decrease in insulin secretion (Hellerstrom et al. 1988).

Supplementation of fenugreek leaves partially retains the damage and increase insulin secretion by regeneration of the β-cells and the pancreatic islets in streptozotocin induced diabetes in rats. Expansion of islets were also noted, which reflects the ability of the extract to replicate and increase β-cell mass. Bonner-Weir (2000) have shown that increased β-cell mass secretes more insulin and reduce blood glucose levels. This action of the fenugreek leaves can be attributed to its antioxidant activity by scavenging free radicals and improving the antioxidant status (Annida and Stanely Mainzen Prince 2004) thereby resulting in islet cell expansion.

Earlier studies have shown that plant extracts and antioxidants were beneficial on the STZ-treated pancreas. Shanmugasundaram et al. (1990) have reported that two water soluble extracts of Gymnema sylvestre leaves orally administered to STZ-diabetic rats partially regenerated the damaged endocrine pancreas, such that the insulin content and islet number were increased. Kaneto et al. (1999) have reported that N-acetyl L-cysteine (NAC) treatment preserved the β-cell function, increased β-cell mass, increased insulin content and m RNA content in STZ-induced diabetic mice. Epicatechin, a phenolic compound increased β-cell population in alloxan diabetic rats (Chakravarthy et al. 1981). Also probucol – an antioxidant and anti – hyperlipidemic agent partially restored β-cell function through acting as an antioxidant in STZ-induced diabetic APA hamsters (Atsushi et al. 2003). Aegle marmelos fruit extract administration partially reversed the damage caused by STZ to the endocrine pancreas (Kamalakkannan and Stanely Mainzen Prince 2006).

In summary, the results of our study clearly indicate that supplementation of fenugreek leaves decrease blood glucose and increase insulin secretion by regeneration of the β-cells and the pancreatic islets in streptozotocin induced diabetes in rats.

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