



Full Length Research Paper

ANTIDIABETIC AND HISTOPATHOLOGICAL ANALYSIS OF FENUGREEK EXTRACT ON ALLOXAN INDUCED DIABETIC RATS

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ABSTRACT

Fenugreek (Trigonella foenum-graecumL. Leguminosae) is widely used in Indian Ayurvedic medicine for the treatment of diabetes mellitus. Antihyperglycaemic effect of the two different doses (200 and 400 mg/kg) of the fenugreek extract was evaluated in this study. Blood glucose, liver profile, renal profile and total lipid levels were determined in alloxan induced diabetic rats after oral administration of a fenugreek extract. A comparable hypoglycemic effect was evidenced from the data obtained after 7 and 21 days of oral administration of the extract. The extract lowered the total cholesterol and serum triglycerides. Histopathological analysis of pancreas showed normal acini, and normal cellular in the islets of langerhans in the pancreas of normal control and Extensive damage to islets of langerhans and reduced dimensions of islets in alloxan induced diabetes. Restoration of islets of langerhans seen in diabetic rats treated with fenugreek extract. The results of this study clearly shows the hypoglycaemic activity of the extract

Key Words: *Alloxan, Fenugreek, Diabetes Mellitus.*

INTRODUCTION

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels. Several pathogenic processes are involved in the development of diabetes ^[1]. According to the recent estimation by International Diabetes Federations, India

has 50.8 million people with diabetes by 2010 that is predicted to increase 87 million by 2030, will still top the list. (www.eatlas.idf.org). Oxidative stress is currently suggested as mechanism underlying diabetes and diabetic complications. Enhanced oxidative stress and changes in antioxidant capacity, observed in both clinical and experimental diabetes mellitus are thought to be the etiology of chronic diabetic complications ^[2]. Long-term complications of diabetes include retinopathy with potential loss of vision; nephropathy leading to renal failure; peripheral neuropathy with risk of foot ulcers, amputations, and Charcot joints; and autonomic neuropathy causing gastrointestinal genitourinary and

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cardiovascular symptoms and sexual dysfunction. Patients with diabetes have an increased incidence of atherosclerotic cardiovascular, peripheral arterial and cerebrovascular disease. Hypertension and abnormalities of lipoprotein metabolism are often found in people with diabetes^[1]. Compared with synthetic drugs, drugs derived from the plants are frequently considered to be less toxic with fewer side effects. Therefore, the search for the more effective and safer antihyperglycemic agents becomes an area of active research^[3].

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^[4]. In India, fenugreek is commonly consumed as a condiment^[5] and used medicinally as a lactation stimulant^[6]. 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.

The extracts, powder and gum of fenugreek seeds and leaves have been reported to have anti-diabetic and hypocholesterolemic properties in both model animals and humans^[7-16]. Activity has been attributed largely to fenugreek's saponins^[17], high fiber content^[10], the amino acid 4-hydroxyisoleucine^[18] and the major alkaloid trigonelline^[7]. Anti-hyperglycemic effect was linked to delayed gastric emptying caused by the high fiber content, inhibiting of carbohydrate digestive

enzymes^[10] and stimulating of insulin secretion^[18]. Fenugreek seeds also lower serum triglycerides, total cholesterol (TC), and low-density lipoprotein cholesterol (LDL-C). These effects may be due to saponins, which increase biliary cholesterol excretion, in turn leading to lowered serum cholesterol levels^[10, 19]. The lipid-lowering effect of fenugreek might also be attributed to its estrogenic constituent, indirectly increasing thyroid hormone T4. Trigonella seeds have been shown to lower blood glucose levels and partially restore the activities of key enzymes of carbohydrate and lipid metabolism close to normal values in various animal model systems^[16, 21]. The components responsible and the mechanism by which Trigonella exerts these effects is not clearly understood. However, several studies have shown the presence of steroid saponins in Trigonella seeds^[17, 5]. 4-hydroxyisoleucine, a modified amino acid extracted and purified from fenugreek seeds also displayed an insulinotropic property in vitro, stimulated insulin secretion in vivo and improved glucose tolerance in normal rats and dogs and in rat model of type 2 diabetes mellitus^[12]. Besides 4-hydroxyisoleucine, arginine and tryptophan are the other amino acids having antidiabetic and hypoglycemic effect. In addition to this many trace elements, which are the components of Trigonella, have been found to possess antidiabetic effects.

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^[20]. In an animal study, the acute oral LD₅₀ was found to be >5 g/kg in rats, and the acute dermal LD₅₀ was found to be >2 g/kg in rabbits^[21]. 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^[22]. Moreover, there were no significant

hematological, hepatic, or histopathological changes in weanling rats fed fenugreek seeds for 90 days [23]. The present study was undertaken to study the Antidiabetic and histopathological analysis of fenugreek (25% w/w saponins) extract on alloxan induced diabetes mellitus.

MATERIALS AND METHODS

Animal Care

Healthy adult male Wistar albino rats were purchased from Central Animal facility, Indian Institute of Science, Bangalore. They were kept for 1 week in our laboratories before the experiments for acclimatization to the laboratory conditions and fed with standard pellet diet (Amrut rat and mice feed, Pranav agro industries Ltd. Sangli, India) and water. Animal care and protocols were in accordance with and approved by the Institutional Animal Ethics Committee, Bapuji Pharmacy College, Davangere, Karnataka (Registration No. 105/1999/CPCSE). All rats were housed in polypropylene cages in a temperature (25±2°C) and humidity (60±10%) controlled room submitted to a 12-dark/light cycle (artificial lights, 7 a.m – 7 p.m.) and air exhaustion cycle (15 min/h). All procedures were carried out in accordance with the conventional guidelines for experimentation with animals. Prior to experimental treatments, animals were fasted overnight but were allowed free access to water. Six animals were used for each group of study.

Collection of Fenugreek extract

Fenugreek extract (25 % w/w saponins) was procured as a kind gift from Amruta Herbals Private Limited, Plot No. B-35/A, Sector C, Industrial Area, Sanwer Road Indore, MP - 452 015 (India).

Experimental Induction of Diabetes

Rats were injected intraperitoneally with freshly prepared solution of alloxan monohydrate in normal saline at a dose of 150 mg/kg BW [25]. Alloxan is capable of producing fatal hypoglycemia as a result of massive pancreatic insulin release rats were treated with 20% glucose solution (5–10 ml) orally after 6 h. The rats were then kept for the next 24 h on 5% glucose solution bottles in their cages to prevent hypoglycemia [26]. After 1 week, rats with moderate diabetes that exhibited glycosuria and hyperglycemia (i.e. blood glucose concentration >200 mg/dL) were taken for the experiment.

Animal Treatment Protocol

In the experiment a total number of 24 rats (18 diabetic rats, 6 normal rats) were used. The rats were divided into four groups of six each. Group I served as control animals and received 2 ml of distilled water [instead of fenugreek] by gastric intubation using force-feeding needle. Group II animals were treated with single intraperitoneal injection of alloxan monohydrate (150 mg/kg bw) after overnight fast for 12 h. The diabetic condition was assessed by measuring the blood glucose concentration 3 and 7 days after alloxan treatment. The rats with blood glucose level above 180 mg/dL and urinary sugar (+++) were selected for experimental study. Different doses of (200 and 400 mg/kg bw) fenugreek were assessed to find out the effective Antidiabetic dose in alloxan induced diabetic rats. The antidiabetic effect was assessed by giving the different doses of extract (200 and 400 mg/kg bw) daily for 21 days, to diabetic rats [blood glucose 200 mg/dl and urinary sugar (+++)] and studying their effects on fasting blood glucose and urine sugar. Group III animals were received the water suspension of fenugreek (200 mg/kg bw) once daily, for 21 days after the diabetic state was assessed in alloxan induced diabetic rats. Group IV animals were received the water suspension of fenugreek (400 mg/kg bw) once

daily, for 21 days after the diabetic state was assessed in alloxan induced diabetic rats. After the experimental period, all animals were sacrificed by cervical dislocation and biochemical studies were conducted on blood, plasma and liver samples of control and experimental animals.

Determination of the Blood Glucose Levels

Blood glucose concentrations (mg/100 mL) were determined using a Contour^{TS} Blood Glucose monitoring system (Serial No. 1511836, Bayer Polychem (India) Limited, India), based on the glucose oxidase method. Blood samples were collected from the tip of tail.

Blood Analysis

Biochemical parameters were determined in plasma using a Star 21Plus (Aspen Diagnostics, India). The parameters analysed are Cholesterol, Triglycerides, HDL-C, Total Protein, Bilirubin, ALT, AST, ALP, Creatinine and Urea.

Histopathological Examination

Pancreas was subjected for routine Histopathological examination and fixed in 10 % formal saline (10 parts of formaldehyde and 90 parts of normal saline). Tissues were processed and embedded in paraffin wax. Sections were cut at 5µm thickness and stained with Haematoxylin and Eosin [27]. Light microscopic examination of the sections was then carried out and micrographs produced using Vanox-T Olympus

photographing microscope. The histopathological examinations were reviewed by the pathologist.

Statistical Analysis

All the values are mean ± SEM, One way analysis of Variance (ANOVA) followed by multiple comparison test. ^ap<0.005, ^bp<0.01, ^cp<0.001 as comparison to normal group (Group I); *p<0.005, **p<0.01, ***p<0.001 as comparison to control group (Group II).

RESULTS AND DISCUSSION

Within the context of present study, antidiabetic activity of the fenugreek extract was investigated by using in vivo assay techniques in order to evaluate folk medicine practices in India.

Blood Glucose Levels

Single dose intra-peritoneal (i.p) treatment of rats with alloxan monohydrate (150 mg/kg) significantly (p <0.001) increases the blood glucose as shown in table 1. The fenugreek extract 200 and 400 mg/kg oral route significantly (p <0.001) attenuated alloxan induced increased blood glucose level from 7 to 21st day of study. The effect of alloxan alone treatment significantly increased blood glucose level from 87 to 250.1 mg/dl on 14th day of study. Trigonella have been shown to lower blood glucose levels and partially restore the activities of key enzymes of carbohydrate and lipid metabolism close to normal values in various animal model systems [16,21].

	Group I	Group II	Group III	Group IV
0 th Day	98.3±6.52	87.00±5.01	90.23±2.89	85.95±4.32
7 th Day	93.05±3.18	215.00±8.093 ^c	205.6±15.26	220.7±12.36
14 th Day	90.85±4.12	243.5±23.16 ^c	164±13.28 ^{**}	140.5±8.49 ^{**}
21 st Day	85.75±4.98	250.1±32.22 ^c	130.3±4.06 ^{**}	129.66±11.18 ^{***}
28 th Day	99.25±2.01	248.2±20.26 ^c	118.9±5.51 ^{***}	96.7±3.67 ^{***}

Table 1: Effect of Fenugreek extract on Blood glucose (mg/dL) in normal and diabetic rats after 21 days.

Effect on Lipid Components

In diabetic rats the administration of fenugreek extract exhibited a very highly significant hypolipidemic effect ($p < 0.001$) and represented in the table 2. The hypolipidemic effect of fenugreek extract was most pronounced at the higher dose (400 mg/kg) and less marked as the dose (200 mg/kg) of fenugreek extract decreased, while both doses of fenugreek extract caused a very highly significant decrease in serum cholesterol, triglyceride, HDL-C and LDL-C levels. Fenugreek seeds also lower serum triglycerides, total cholesterol (TC), and low-density lipoprotein cholesterol (LDL-C). These effects may be due to saponin, which increase biliary cholesterol excretion, in turn leading to lowered serum cholesterol levels. (10, 19) The lipid-lowering effect of fenugreek might also be attributed to its estrogenic constituent, indirectly increasing thyroid hormone T4.

The most common lipid abnormalities in diabetes are hypertriglyceridemia and hypercholesterolemia [28-29]. Hypertriglyceridemia is also associated in metabolic consequences of hypercoagulability, hyperinsulinemia, insulin resistance and glucose intolerance [30]. Repeated administration of the extract for 21 days significantly ($P < 0.01$; $P < 0.05$) improved hypertriglyceridemia and hypercholesterolemia, the observed hypolipidemic effect may be due to decreased cholesterologenesis and fatty acid synthesis [31]. Significant ($P < 0.001$) lowering of total cholesterol and rise in HDL-cholesterol is a very desirable biochemical state for prevention of atherosclerosis and ischaemic conditions [32]. It is well known that diabetics especially in India often have unfavorable HDL levels. It will also be of interest in future work to examine the mechanism of the changes in HDL.

Groups	Cholesterol (mg/dL)	Tryglicerides (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)
Groups I	76.54±3.06	26.50±1.08	50.41±3.56	17.46±0.84
Groups II	140.42±2.68 ^c	130.23±6.51 ^c	32.77±0.98 ^b	78.20±4.61 ^c
Groups III	96.93±1.42 ^{***}	52.46±2.26 ^{***}	47.73±1.88 ^{***}	31.87±2.65 ^{***}
Groups IV	77.45±3.64 ^{***}	28.97±1.61 ^{***}	49.03±4.23 ^{***}	20.52±2.47 ^{***}

Table 3: Effect of Fenugreek extract on lipid levels in normal and diabetic rats after 21 days.

Effect on Liver Function

The effect of fenugreek extract on liver functions was represented in the table 3. The Bilirubin, ALT, AST and ALP levels were significantly elevated ($p < 0.001$) in alloxan induced diabetes. The rats treated with 200 and 400 mg/kg

fenugreek extract showed significant ($p < 0.001$) reduction in the elevated levels of liver enzymes in a dose dependent manner. Total protein level was decreased significantly ($p < 0.01$) and after 21 days fenugreek extract increased the level significantly ($p < 0.01$).

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Groups	Total Protein (g/dL)	Bilirubin (mg/dL)	ALT (U/L)	AST (U/L)	ALP (U/L)
Groups I	6.07±1.12	0.361±0.06	78.94±6.01	240.51±28.46	235±12.56
Groups II	5.58±0.98 ^b	0.618±0.08 ^c	123.10±8.38 ^c	376.30±16.30 ^c	346.56±18.32 ^c
Groups III	5.83±0.56 [*]	0.423±0.12 ^{***}	93.56±3.36 ^{***}	263.51±10.21 ^{***}	261.51±6.71 ^{***}
Groups IV	6.11±1.13 ^{**}	0.312±0.05 ^{***}	81.61±9.10 ^{**}	245.83±12.58 ^{***}	231.85±10.56 ^{***}

Table 2: Effect of Fenugreek extract on liver profile in normal and diabetic rats after 21 days.

Effect on Kidney Function

The renal function markers like Creatinine and urea were elevated in the alloxan induced diabetic rats when compared with the normal rats. The Creatinine level increased from 1.05 to 3.12 mg/dl in diabetic control group. Where as the 200 mg

of fenugreek it self reduces the levels to 1.73 and the activity of the fenugreek is dose dependent. Serum urea showed drastic elevation in diabetic control group whereas the fenugreek extract at concentration 200 mg/kg itself showed significant (p<0.001) reduction.

Groups	Creatinine (mg/dL)	Urea (mg/dL)
Groups I	1.05±0.21	30.48±2.49
Groups II	3.12±0.89 ^c	57.21±6.82 ^c
Groups III	1.73±0.45 ^{***}	34.76±1.40 ^{***}
Groups IV	1.26±0.17 ^{***}	32.18±2.28 ^{***}

Table 4: Effect of Fenugreek extract on kidney function in normal and diabetic rats after 21 days.

Histopathological Analysis of Pancreas

Photomicrographs of pancreas (Figure 1) showed normal acini, and normal cellular in the islets of langerhans in the pancreas of normal control (A).

Extensive damage to islets of langerhans and reduced dimensions of islets (B), restoration of normal cellular population size of islets with hyperplasia by PCC (C & D).

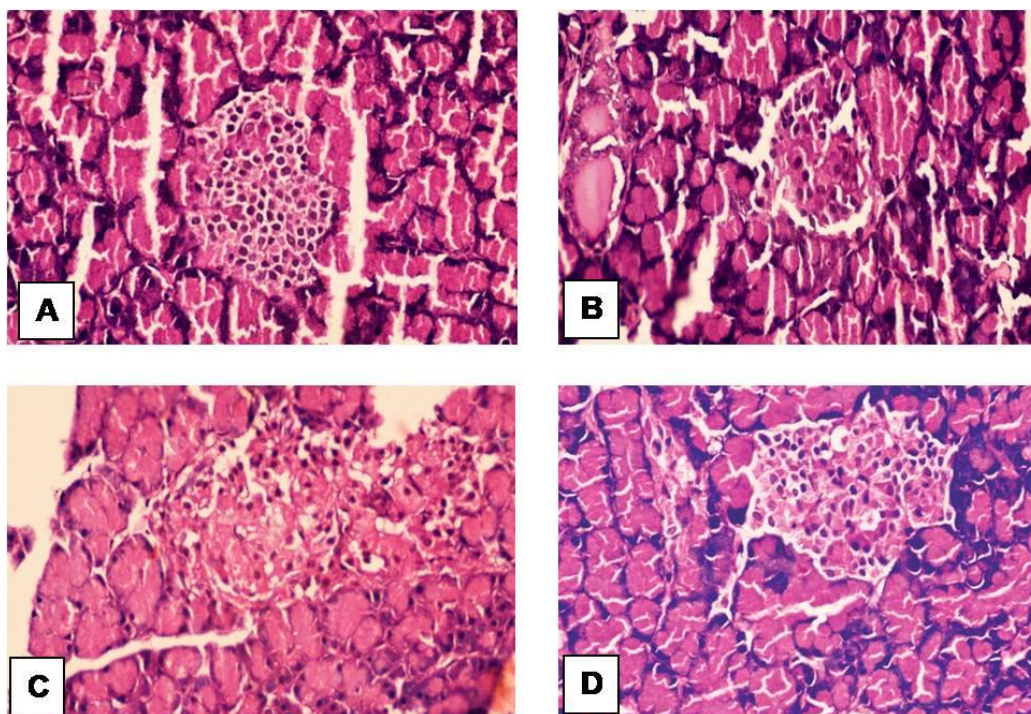


Figure 1: Effect of oral administration of fenugreek (21 days) extract on normal and diabetic rats

Conclusion

Present study reveals that fenugreek extract has beneficial effects on blood glucose level as well as improving kidney, liver function and hyperlipidaemia due to diabetes. On the other hand, fenugreek has a favorable effect to inhibit the histopathological changes of the pancreas in alloxan induced diabetes. Therefore, fenugreek may provide new alternatives for the clinical management of diabetes and the consumption of fenugreek can prevent the complications of hyperglycemia associated with diabetes.

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