J. Pharm. Res. 2021;20(1):8–15

Print ISSN: 0973-7200
Online ISSN: 2454-8405

Journal of Pharmaceutical Research

RESEARCH ARTICLE

Pharmacodynamic Interaction of Polyphenolic Rich Extract of Fenugreek Seeds with Oral Hypoglycemic Agents in Diabetes Induced Gastropathy

B Kishore Reddy[1], N Prem Kumar[1,*]

[1] *Department of Pharmaceutical Chemistry, Krupanidhi College of Pharmacy, Bangalore, Karnataka, India*

ARTICLE INFO

Article history:
Received 09.12.2020
Accepted 22.02.2021
Published 10.03.2021

[*] *Corresponding author.*
N Prem Kumar

https://doi.org/10.18579/jopcr/v20.1.kishore

ABSTRACT

Phytochemical constituents such as Trigonella foenum-graecum (fenugreek) medicinal herbs have proven to possess therapeutic potential in metabolic diseases such as diabetes mellitus. This study aimed to evaluate the pharmacodynamic interaction of polyphenol-rich fenugreek seed extract (PEFS) with routine oral hypoglycaemic drugs, pioglitazone, glipizide, and acarbose, in a streptozotocin-induced type 2 diabetic rat model of gastropathy-associated diabetes. The extract was obtained from a multistep methanolic and ethyl acetate extraction, and its effects were assessed using OGTT, blood glucose, lipid profiles, gastrointestinal motility, contractility assays, and antioxidant enzyme analysis. Findings showed that PEFS greatly enhanced glycaemic control, body weight, HDL, and antioxidant status, particularly when combined with low doses of OHAs. Gastrointestinal investigations revealed improved motility and normalised contractility of smooth muscles, which counteracted the frequent side effects of OHA. Combination therapy with PEFS-OHAs was found to have a synergistic effect without causing hypoglycaemia, suggesting its dose-reducing efficacy over traditional drugs. This study introduces a new integrative approach using fenugreek extract to improve the efficacy and safety of antidiabetic treatments. Owing to the common and frequently unreported use of herbal supplements with prescription medications, these results highlight the necessity for further research on herb-drug interactions to guide clinical practice.

Keywords: Fenugreek seed extract; Diabetes; Oral Hypoglycemic Drugs; Combination

INTRODUCTION

Medicinal herbs and their constituents are of crucial importance to human health. Fenugreek (Trigonella foenum-graecum), a one-year herb belonging to the Leguminosae (Fabaceae) family, is used both as a spice and vegetable traditionally and for medicinal purposes[1,2]. Its seeds contain fibre,[3] phospholipids, oleic acid, glycolipids,[4] choline, vitamins (A, B1, B2, C, niacin, nicotinic acid),[5] linoleic and linolenic acids, and other bioactive components[6]. Fenugreek has been reported to have various pharmacological activities, including anti-inflammatory, antioxidant, antimicrobial, anticholesterolemic, antidiabetic, anticancer, antiviral, demulcent, and hepatoprotective[3,7]. It also controls enzymatic functions, decreases inflammation and pain, reduces fat, enhances appetite and hormone secretion, and increases lactation. Diabetes mellitus (DM), a common metabolic syndrome, leads to both microvascular and macrovascular complications, thereby causing high morbidity and mortality[8]. Globally, the prevalence of diabetes was 6.4% in 2010 and is estimated to reach 7.7% (439 million adults) by 2030[9]. Type-2 diabetes mellitus (T2DM) accounts for 90–95% of DM cases. Chronic hyperglycaemia in diabetes increases the risk of myocardial infarction and other complications[10]. A healthy diet plays a key role in managing diabetes, with fenugreek offering potential benefits in blood glucose regulation[7].

Herbal remedies are becoming more popular owing to their low side effects; however, when they are mixed with pharmaceuticals, there are herb-drug interactions. Research estimates that 15–20% of prescription drug users also consume herbal supplements, but fewer than 40% report this to their doctors[11]. Physicians are often unaware of such interactions, but more research into their safety and efficacy is needed[12]. Oral hypoglycemic drugs (OHAs) like pioglitazone (P), glipizide (G), and acarbose (A) can produce gastrointestinal side effects[13–15]. This study investigated the pharmacodynamic interaction between the polyphenolic-

KRUPANIDHI COLLEGE OF PHARMACY

rich fenugreek seed extract Trigonella foenum-graecum (T. foenum-graecum) and OHAs in diabetes-induced gastropathy.

MATERIALS AND METHODS

Chemicals and equipment: Chemicals and equipment from well-known sources were used in this study.

Extraction of polyphenolic-rich T. foenum-graecum) Seeds: Polyphenolic-rich extract of T. foenum-graecum (PEFS) (Batch no FEN/RD/01) was procured from Green Chem Industry, Bangalore. For extraction, 100 g of fenugreek seeds was finely powdered and macerated with 80% methanol at room temperature for five days. The solution was filtered, and the solvent was evaporated. The residual residue was dissolved in water and the aqueous phase was washed repeatedly with petroleum ether until a transparent upper layer was formed. The lower aqueous phase was subsequently treated with glacial acetic acid-containing ethyl acetate (10 ml/L) and extraction was performed for 36 h at room temperature. The ethyl acetate layer was concentrated and the residue was lyophilised and stored at 70°C. The yield was approximately 6–8 g per 100 g of seed powder. Methanolic extract was used in the experiments[16].

Experimental animals: Male Sprague Dawley rats weighing 200–300 g were kept in a well-ventilated animal facility at 25 ± 5°C under a 12:12 h light-dark cycle. The experimental protocols were approved by the Institutional Animal Ethics Committee (KCP/IAEC-27/2008-09), and all procedures adhered to the guidelines of the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA). The animals were maintained under standard conditions, and all handling was performed in accordance with Good Laboratory Practice (GLP).

Oral Glucose Tolerance Test (OGTT): OGTT was conducted in overnight-fasted (18 h) normal and diabetic rats, which were distributed into ten groups for different treatments. Glucose (2 g/kg, p.o.) was administered 30 min post-treatment, and blood samples were collected from the tail vein under light ether anaesthesia at 30,60 and 120-min. Blood glucose levels were estimated using Accu-Chek active glucose strips and a glucometer (Roche). A daily fresh fructose diet was composed of 660 g fructose, 100 g protein, 80 g fat, 0.04 g of zinc carbonate, 5 g of vitamin mixture, 5 g of mineral mixture and 150 g of cellulose all of commercial grade was prepared. For type 2 diabetes induction, rats received a daily high-fat diet (HFD) for two weeks, followed by intraperitoneal injection (i.p.) of streptozotocin (35 mg/kg) in 0.5 M citrate buffer (pH 4.4). After STZ administration, the rats were treated with a 5% glucose solution to avoid hypoglycaemia. Blood samples were collected from the tail vein, and blood glucose levels were recorded using a glucose diagnostic kit (Accu-Chek). Rats with non-fasting blood glucose ≥300 mg/dl were regarded as diabetic[17,18].

The rats were divided into six groups as follows:

Group NC: Normal control (saline/vehicle)

Group DC: Diabetic control

Group N + PEFG: Normal rats + Polyphenol-rich T. foenum graecum seed extract (200mg/kg)

Group N + P: Normal rats + Pioglitazone (10 mg/kg)[19]

Group N + G: Normal rats + Glipizide (5 mg/kg)[20]

Group N + A: Normal rats + Acarbose (5 mg/kg)[21]

Single-dose study: In the single-dose study, seven groups of rats were treated as follows: distilled water, extract (200 mg/kg), pioglitazone (10 mg/kg), glipizide (5 mg/kg), and acarbose (5 mg/kg). Blood was drawn 0, 1, 2, and 4 h after treatment[22].

Multidose study: In the multidose study, the same treatments were administered once a day for 10 days, and body weight and blood glucose were observed on days 1, 3, 7, and 10[22].

Gastrointestinal Motility Studies

Colonic Contractility and Charcoal Meal Transit in Small Intestine: At end of the treatment period, overnight-fasted rats from each group were orally administered 2 ml of charcoal meal (10% charcoal in 5% gum acacia). After 20 min, rats were euthanised by cervical dislocation. The abdomen was opened and the intestine was removed from the pyloric junction to the caecal end. The colon was isolated and suspended in perpetually aerated Tyrode's solution. The maximum distance travelled by the charcoal meal and the total length of the small intestine were measured. Gastrointestinal transit was determined as the ratio of the charcoal distance to the total length, expressed as a percentage[23].

Contractile Response of Colonic Smooth Muscle: Following cleaning, 1 cm lengths of the distal colon were mounted under 0.5 g resting tension in 40 ml organ baths filled with aerated Tyrode's solution. The temperature was maintained at 37°C. The tissues were equilibrated for 30 min prior to exposure to acetylcholine. A test dose of 100 ng acetylcholine was administered before obtaining the concentration–response curves. Isotonic contractile responses were obtained using a student's physiograph. The ED_{50} values of acetylcholine were obtained from log dose-response curves.

Gastric Fundus Preparation: Rats were euthanised and anaesthetised with thiopental sodium (50 mg/kg, i.p.). The stomach was removed, and the fundus was prepared as longitudinal muscle strips (15 × 3 mm) after the mucosal layers were removed. Each strip was mounted vertically under 1 g isometric tension in 10 ml Krebs solution at 37°C and was continuously oxygenated. The tissues were equilibrated for 60 minutes. Carbachol concentration–response curves were plotted to find ED_{50} values. Following steady-state contraction, isoprenaline (3 μmol) was cumulatively added. ED_{50} values were calculated from percent response vs. log

dose plots[24].

Gastric Emptying Time: Rats were euthanised by CO_2 asphyxiation. The stomach and proximal duodenum were removed and perfused with oxygenated Krebs bicarbonate buffer. Circular muscle rings were prepared from the fundus, antrum, and pylorus. Each ring was placed in 10 ml organ baths containing Krebs buffer at 37°C and attached to isometric force transducers. After 30 min of equilibration, bethanechol concentration-response curves (10–100 μM) were obtained. Each dose was administered for 3 min with rinsing between treatments. ED_{50} values were determined from log dose-response curves[25] .

The rats were divided into 11 treatment groups of six animals each.

Group NC: Normal control (saline/vehicle)

Group DC: Diabetic control

Group D + PEFS: Diabetic rats treated with PEFS

Group D + P: Diabetic rats treated with pioglitazone

Group D + G: Diabetic rats treated with glipizide

Group D + A: Diabetic rats treated with acarbose

Group D + G + P: Diabetic rats treated with glipizide (3.75mg/kg) + pioglitozone (7.5mg/kg)

Group D + G + A: Diabetic rats treated with glipizide (3.75mg/kg) + acarbose (3.75mg/kg)

Group D + PEFS + P: Diabetic rats treated with PEFS + pioglitazone (7.5mg/kg)

Group D + PEFS + G: Diabetic rats treated with PEFS + glipizide (3.75mg/kg)

Group D + PEFS + A: Diabetic rats treated with PEFS + acarbose (3.75mg/kg)

Parameters measured: Body weight was measured weekly. Blood glucose was approximated from tail vein blood by using Accu-Chek glucose strips[18]. Serum cholesterol was assessed by the modified Roeschlau method on a clinical chemistry analyzer, in which quinoneimine absorbance at 505 nm was directly related to cholesterol concentration[26]. For triglycerides, the Wako method with suitable incubation and measurement at 505 nm was employed[26].

Preparation of Stomach Homogenate and Estimation of Antioxidant Enzymes: The stomach tissues at the end of the experimental period were homogenized in 0.1 N cold perchloric acid containing EDTA and isopropyl homocholine, and then centrifuged at 10,000 rpm for 20 min at 4°C. Total protein content was estimated by the Lowry method, which includes the Folin–Ciocalteau reagent and absorbance measurement at 610 nm. Bovine serum albumin was used as the standard. Superoxide dismutase (SOD) activity was measured as the inhibition of NBT reduction by hydroxylamine auto-oxidation. Sodium carbonate, NBT, EDTA, and hydroxylamine hydrochloride were added to the reaction mixture, and absorption was measured at 560 nm to compute enzyme activity in units inhibiting 50% NBT reduction. The rate of hydrogen peroxide (H_2O_2) decomposition was measured spectrophotometrically at 240 nm. Catalase activity was assessed in an assay mixture containing 0.25 M phosphate buffer (pH 7). Lipid peroxidation was estimated by measuring thiobarbituric acid reactive substances (TBARS), primarily malondialdehyde (MDA), via formation of a coloured complex with TBA at 532 nm[27–31].

Statistical analysis: Values are expressed as the mean ± standard error (SE) from eight rats per group. Statistical analysis between more than two groups was performed using one-way analysis of variance (ANOVA). For comparisons between two groups, an unpaired Student's t-test was applied, and a p-value < 0.05 was considered statistically significant.

RESULTS

Impact of PEFS and Control Drugs on OGTT in Normal Rats: OGTT was performed on normal rats to compare the antihyperglycemic activity of PEFS with that of control oral hypoglycaemic drugs, Pioglitazone, Glipizide, and Acarbose, individually and in combination. The blood glucose levels of all groups decreased significantly two hours after glucose challenge (2 g/kg) compared with the control group. The combination groups utilised 75% of the normal dose of OHAs as a low dose when used with PEFS.

Effect of PEFS and OHAs on OGTT in Diabetic Rats: In STZ-induced diabetic rats, there was a marked decrease in blood glucose levels in groups receiving PEFS alone and in combination with Glipizide, Pioglitazone, or Acarbose, compared to the diabetic control group. Although single treatment with standard OHAs significantly decreased glucose levels, OHAs in combination had hypoglycaemic effects, reflecting an increased response to combination therapy.

Effect of PEFS and OHAs on Body Weight: Body weights at more than one time point showed normal rats to have a steady weight increase. However, diabetic control rats sustained weight loss throughout the experiment. PEFS and OHAs combined treatment groups showed non-significant body weight reduction compared to diabetic controls, whereas marked loss of weight was seen in OHAs alone-treated rats. Interestingly, the PEFS group and combinations of Pioglitazone + Glipizide (P+G) and Glipizide + Acarbose (G+A) showed significant improvement in body weight.

Effect of PEFS and OHAs on Blood Glucose Level: Fasting serum glucose levels were measured. The normal control group had stable glucose levels (122.5 ± 0.5 mg/dl to 125 ± 0.8 mg/dl by day 60). The diabetic control group had high glucose levels without spontaneous control. However, the groups treated with a combination of PEFS and regular OHAs had significantly lower serum glucose levels by day 60 than those receiving regular OHAs alone. The PEFS monotherapy also showed good glucose control by day 30. The OHA combination therapy caused hypoglycaemia by

Table 1: Effect of PEFS and its combination with oral hypoglycaemics on HDL (mg/dl) estimation

Groups	0th day	15th day	30th day	45th day	60th day	75th day
NC	40±0.2	41.56±0.2	42.7±0.2	43.20±0.4	44.8±0.1	45±0.2
DC	44±0.6	21.4±0.7	25.2±0.7	25.36±0.8	28±0.88	24±0.3
D + PEFS	43.1±0.4	33.1±0.4 [b]	35.5±0.5 [b]	37.2±0.4 [b]	38.4±0.1 [b]	40±0.1 [b]
D + P	41.1±0.8	31.8±1.04 [c]	33.6±1.4 [c]	36.2±0.2 [c]	37.9±1.2 [c]	35.3±1.8 [c]
D + G	42.1±0.4	30.3±0.5 [c]	34.06±0.8 [c]	35.5±0.4 [c]	34.4±1.8 [c]	37.7±1.2 [c]
D + A	43± 0.5	32.4±0.4 [c]	34.3±0.8 [c]	36.3±0.9 [c]	37.3±0.4 [c]	38.1±2.1 [b]
D + P+G	43.3±0.2	35.16±0.6 [b]	38.96±0.14 [b]	38.7±0.6 [b]	39.4±1.9 [b]	-
D + G+A	41.3± 0.5	34.4±0.8 [b]	38.6±0.34 [b]	38.4±0.07 [b]	39.0±0.01 [b]	-
D + P+PEFS	42.2± 0.4	36.5±0.7 [a]	39.13±0.4 [a]	39.6±0.6 [a]	41.3±0.5 [a]	42±1.4 [a]
D + G+PEFS	43.4±0.3	35.4±0.7 [a]	39.56±0.5 [a]	38±0.70 [a]	40.4±0.8 [a]	43±0.1 [a]
D + A+PEFS	42.8±0.7	37.5±0.4 [a]	39.23±0.2 [a]	39.9±0.9 [a]	42.1±0.4 [a]	43.6±1.6 [a]

All values are mean ± SEM, n=6, [a] p<0.001, [b]p<0.01, [c]p<0.05 when compared to Diabetic control group

day 60.

The effects of PEFS and OHAs on HDL: HDL ratios were drastically reduced in the diabetic control group. Significant increments were observed in the groups receiving PEFS along with conventional OHAs (P+PEFS, G+PEFS, and A+PEFS). Moderate increases were observed in the PEFS alone, P+G, and G+A groups. Single OHAs treatments (P, G, and A) did not show any significant alteration in HDL levels. (Table 1)

Effect of PEFS and OHAs on Total Cholesterol and Triglycerides (TC and TG): Levels of total cholesterol and triglycerides were high in diabetic controls versus normal controls. Both TC and TG levels were significantly decreased in the PEFS combination groups (P+PEFS, G+PEFS, and A+PEFS). Groups treated with PEFS alone, P+G, and G+A showed a moderate decrease. No statistically significant changes were induced by individual treatments with Pioglitazone, Glipizide, or Acarbose. (Table 2 and Table 3)

Role of Antioxidants in Serum and Homogenate of Stomach

- **TBARS (Thiobarbituric Acid Reactive Substances)**

PEFS ($p < 0.001$), PEFS + G ($p<0.001$), PEFS+A ($p < 0.001$), and PEFS + P significantly lowered TBARS levels in stomach homogenates compared to diabetic controls. Comparable reductions were observed in the serum TBARS levels in the PEFS + G, PEFS + A, and PEFS + P groups. (Table 4)

- **Catalase Activity**

Catalase activity in the stomach homogenate increased substantially in the PEFS + G, PEFS + A, and PEFS + P groups ($p < 0.001$). All the other treatment groups also exhibited a significant increase ($p < 0.01$). In the serum, catalase activity was significantly higher in the PEFS + G and P + G groups than in the diabetic controls. (Table 4)

- **Superoxide Dismutase (SOD)**

PEFS + G, PEFS + P, and PEFS + A treatments significantly increased SOD content in stomach homogenates. Other groups, such as P, G, A, A+G, and P+G, also exhibited significant ($p < 0.01$) increases in kidney homogenate SOD content compared to diabetic controls. (Table 4)

Influence of PEFS on Colonic Contractility, Small Intestinal Transit, Gastric Fundus and Gastric Emptying Time Models: Diabetic control contractility of the colon and gastric fundus response were reduced. Functional restoration was considerable in the P + PEFS, G + PEFS, and A + PEFS groups. Moderate restorations were found in the PEFS, P+G, and G+A groups. Individual OHAs had no marked effect on any of the groups. (Table 5 & Table 6)

Small intestinal transit was slowed in diabetic controls, but markedly improved in the P + PEFS, G + PEFS, and A + PEFS groups. Gastric fundus contractility, challenged by bethanechol-induced contractions, was markedly impaired in diabetic rats and normalised by PEFS combination treatment. Pyloric contractility was abnormally elevated in diabetic rats and decreased significantly with the PEFS combination therapy. Antral contractility was not significantly different between the groups. PEFS treatment successfully reversed the fundic hypersensitivity observed in diabetes. (Table 5 & Table 6)

DISCUSSION

The current study assessed the antihyperglycemic and metabolic actions of T. foenum-graecum seed extract (PEFS) and conventional oral hypoglycaemic agents Pioglitazone, Glipizide, and Acarbose in normal and diabetic rat models. These results verified the considerable therapeutic potential of PEFS, supporting and extending previously published findings.

In the OGTT performed on healthy rats, PEFS reduced the blood glucose level two hours post-administration after

Table 2: Effect of PEFS and its combination with Oral Hypoglycaemics on Triglycerides (mg/dl) estimation

Groups	0^{th} day	15^{th} day	30^{th} day	45^{th} day	60^{th} day	75^{th} day
NC	94.1±0.8	95.8±0.4	96.6±0.6	98±1.3	96±0.1	96±0.2
DC	95.3±0.4	153±0.5	164.2±0.4	180±0.3	183±0.7	194.2±0.7
D + PEFS	95.1±0.6	145.3±0.8[b]	129±0.3[b]	123±0.34[b]	108±0.6[b]	110±0.5[b]
D + P	96.3±0.5	148±2.4[c]	133±0.3[c]	130±0.3[c]	115±0.14[c]	116±1.4[c]
D + G	95.4±1.2	149.3±0.7[c]	133.8±0.6[c]	132±0.1[c]	117±0.84[c]	126±0.8[c]
D + A	94.7±0.8	146.4±1.4[c]	131±0.45[c]	129±0.4[c]	114±0.3[c]	123.4±0.8[c]
D + P+G	98.4±0.5	134.1±1.2[b]	123±0.2[b]	113±0.1[c]	110±1.3[b]	-
D + G+A	92.4±0.6	143±1.49[b]	128±0.93[b]	121±0.39[b]	111±2.3[b]	-
D + P+PEFS	98±1.1	111.4±0.8[a]	112±2.4[a]	108±1.73[a]	104±0.98[a]	97±0.4[a]
D + G+PEFS	99.4±1.5	128.3±1.20[a]	118±1.3[a]	111±1.8[a]	102±0.31[a]	99.6±0.5[a]
D + A+PEFS	99.8±0.5	111.4±0.8[a]	107.2±0.7[a]	105±1.21[a]	101±1.9[a]	100±0.2[a]

All values are mean ± SEM, n=6, [a] p<0.001, [b]p<0.01, [c]p<0.05 when compared to Diabetic control group

Table 3: Effect of PEFS and its combination with Oral Hypoglycemics on Total Cholesterol (mg/dl) estimation

Groups	0^{th} day	15^{th} day	30^{th} day	45^{th} day	60^{th} day	75^{th} day
NC	58.2±0.8	58.8±0.4	62.6±0.8	61±0.5	64.8±0.2	64.8±0.12
DC	60±0.4	110.7±0.6	116.6±0.8	117±2.5	119±0.7	128.5±0.2
D + PEFS	61.1±0.6	101.6±1.7[b]	95.6±2.8[b]	81±0.5[b]	79±0.7[b]	82.4±0.8[b]
D + P	62.4±0.5	104.6±1.2[c]	95.6±2.4[c]	90.3±1.3[c]	86±1.4[c]	95±0.5[c]
D + G	62.5±1.2	102.7±0.4[c]	96±1.7[c]	98±1.6[c]	92±0.4[c]	91±0.6[c]
D + A	59.4±0.8	100.6±1.4[b]	91±3.4[b]	86±1.6[c]	82±9.6[c]	87±0.2[c]
D + P+G	58.2±0.5	67.3±1.7[a]	76.6±0.8[a]	72±1.1[b]	71±0.9[b]	-
D + G+A	59.9±0.6	102.3±1.4[b]	85±1.7[b]	78.6±0.8[b]	72±0.8[b]	-
D + P+PEFS	60.8±1.1	96.3±2.6[b]	75.3±0.8[a]	70±0.5[a]	67±0.2[a]	77.8±0.5[b]
D + G+PEFS	61.5±1.5	68.66±2.2[a]	71.66±1.2[a]	72±2.4[a]	70±2.5[a]	74±0.4[a]
D + A+PEFS	62.8±0.5	69.06±1.4[a]	73.6±1.2[a]	70±0.5[a]	67±0.2[a]	72.7±0.5[b]

All values are mean ± SEM, n=6, [a] p<0.001, [b]p<0.01, [c]p<0.05 when compared to Diabetic control group

Table 4: Effect of PEFS and its combination with Oral Hypoglycemics on antioxidant estimation in stomach homogenate

Groups	SOD	CATALASE	TBARS
NC	4.32+ 0.27	2.83±1.29	4.01±0.02
DC	1.9±0.4	0.73±0.40	7.56±0.01
D + PEFS	3.6±0.41[b]	1.1±0.4[b]	5.60.07[b]
D + P	2.9±0.8[b]	1.9±0.61[b]	5.16±0.16[b]
D + G	2.7±0.11[c]	1.4±0.3[c]	4.18±0.08[c]
D + A	2.8±0.11[c]	1.4±0.11[c]	5.9±0.14[c]
D + P+G	4.7±0.64[a]	2.9±0.82[a]	4.5±0.02[a]
D + G+A	3.8±0.32[b]	2.8±0.14[b]	4.6±0.09[b]
D + P + PEFS	4.8±0.21[a]	2.9±0.41[a]	4.5±0.14[a]
D + G + PEFS	5.4±0.9[a]	3.2±0.14[a]	5.6±0.13[a]
D + A+ PEFS	5.2±0.2[a]	2.7±0.21[a]	5.5±0.12[a]

All values are mean ± SEM, n=6, [a] p<0.001, [b]p<0.01, [c]p<0.05 when compared to Diabetic control group

Table 5: Effect of PEFS and its combination with OralHypoglycemics on colonic contractility

Groups	Contractile response of acetylcholine ED_{50} (μg)	Charcoal meal colon to exogenous transit in small intestine (%)
NC	1.18 ± 0.03	80.5 ± 3.01
DC	15.83 ± 2.37	59 ± 4.91
D + PEFS	6.9±0.82[b]	68±2.56[b]
D + P	4.4 ± 0.49[c]	70.5 ± 3.51[b]
D + G	6.1 ± 0.73[b]	73.16 ± 3.31[b]
D + A	6.5±0.54[b]	71±0.9 [c]
D + P+G	5.8±0.68[b]	64±1.53[b]
D + G+A	6±0.91[c]	62±0.98[c]
D + P+ PEFS	5.2±0.6[a]	61±2.1[a]
D + G+ PEFS	13.05 ± 0.82[a]	67.5 ± 1.23[a]
D + A+ PEFS	6.1±0.5[a]	73±1.4[a]

p value: [a]p < 0.001 when compared with control. [b]p < 0.001 when compared with diabetic control. [c] p < 0.01 when compared with diabetic control

Table 6: Effect of PEFS and its combination with Oral Hypoglycemics on contractile response of Gastric emptying time and Gastric fundus

Groups	Contractile response of BethanecholED_{50} (μg)			Contractile responseof CarbacholED_{50} (μg)
	Fundus	Antrum	Pylorus	
NC	0.45±0.1	0.06±0.9	0.2±0.8	0.82±0.018
DC	0.15±0.2	0.03±0.8	0.1±0.6	0.64±0.01
D + PEFS	0.04±0.5 [b]	0.03±0.2 [b]	0.3±0.8 [c]	0.52±0.05[c]
D + P	0.18±0.6 [c]	0.04±0.8 [C]	0.25±0.7 [b]	0.49±0.03[b]
D + G	0.25±0.9[c]	0.04±0.8 [b]	0.12±0.4 [c]	0.51±0.03[b]
D + A	0.23±0.4 [c]	0.05±0.5[a]	0.18±0.8 [c]	0.55±0.02[c]
D + P+G	0.34±0.9 [b]	0.046±0.8 [b]	0.19±0.4 [b]	0.50±0.01[b]
D + G+A	0.54±0.8[a]	0.038±0.3 [b]	0.29±0.5 [a]	0.60±0.02[c]
D + P+ PEFS	0.38±0.4[c]	0.02±0.5 [b]	0.3±0.4[a]	0.61±0.03[a]
D + G+ PEFS	0.32±0.9 [a]	0.03±0.5[b]	0.5±0.4[a]	0.6±0.02[a]
D + A+ PEFS	0.37±0.4[a]	0.036±0.4 [a]	0.4±0.8[a]	0.63±0.01[b]

p value: [a]p < 0.001 when compared with control. [b]p < 0.001 when compared with diabetic control. [c] p < 0.01 when compared with diabetic control

glucose administration, and this effect was further potentiated when PEFS was administered together with OHAs at 75% of their usual doses. This finding is in agreement with Broca et al., who found that 4-hydroxyisoleucine, a bioactive constituent in fenugreek, released insulin in a dose-dependent manner[32]. Concurrently, Bnouham et al. showed the potential of fenugreek to lower blood glucose levels through mechanisms such as delaying glucose absorption and modulating insulin[33].

In diabetic rats, PEFS single administration and combined with OHAs caused significant reductions in blood glucose levels compared to diabetic controls. Single-agent OHA treatment was efficacious; however, its combination with PEFS demonstrated improved glycaemic control and induced hypoglycaemia in certain instances. This is in agreement with the synergistic hypothesis of Jayaraj et al., who found that the combination of herbal extracts, such as fenugreek, with conventional drugs improved therapeutic effects while reducing the dosages of drugs needed[34].

Body weight is an indicator of metabolic well-being in diabetes models. In the present study, diabetic control rats showed consistent weight loss, whereas the combination therapy groups (PEFS + Pioglitazone or Glipizide) showed improved weight gain. This is likely due to the metabolic and nutrition-supportive action of fenugreek's soluble fibres, as explained by Basch et al., who reported weight control and enhanced glycaemic management of fenugreek's mucilaginous fibre content and slow gastric emptying[35].

Fasting glucose levels were lowered considerably by PEFS alone by day 30, and even more drastically when combined with OHAs by day 60. Hannan et al., support these observations with their study that fenugreek seed fiber considerably enhanced glucose tolerance in diabetic rats[36]. The combination regimens caused hypoglycaemia in some cases, reproducing the intensified insulinotropic and glucose-lowering effects reported by Jayaraj et al. and Gupta et al[34,37].

As for lipid profiles, HDL was significantly lowered in diabetic controls but increased considerably in groups treated with PEFS and OHAs. The dual OHA- and PEFS-only groups also showed a moderate increase in HDL levels. This aligns with the findings of Raghuram et al.. and Sowmya and Rajyalakshmi, who both indicated that supplementation with fenugreek resulted in increased levels of HDL and decreased serum cholesterol levels in diabetic patients and hyperlipidaemic individuals[38,39].

These findings validate PEFS's multifaceted therapeutic potential in glycaemic control as well as in enhancing lipid metabolism and maintaining body weight in diabetic models. With combined use of adjunct therapy to traditional OHAs, PEFS seems to augment antidiabetic effectiveness while potentially reducing side effects, thus representing a compelling argument for its adoption as an adjunct treatment in the management of diabetes.

CONCLUSION

In conclusion, this study demonstrated the efficacy of fenugreek, particularly PEFS, in improving glucose homeostasis and lipid profiles in normal and diabetic rats. The interaction of PEFS with conventional OHAs resulted in greater antihyperglycemic activity, validating the hypothesis of synergistic therapy for diabetes treatment. In addition, PEFS alone was found to have encouraging outcomes, particularly in managing blood glucose and body weight improvement in diabetic animals. These findings indicate the therapeutic value of fenugreek in the management of diabetes.

REFERENCES

1. Basu TK, Srichamroen A. Health Benefits of Fenugreek (Trigonella foenum-graecum leguminosse). *Bioactive Foods in Promoting Health*. 2010;p. 425–435. Available from: https://doi.org/10.1016/B978-0-12-374628-3.00028-1.
2. Acharya SN, Thomas JE, Basu SK. Fenugreek: an "old world" crop for the "new world". *Biodiversity*. 2006;7(3-4):27–30. Available from: https://dx.doi.org/10.1080/14888386.2006.9712808.
3. Roberts KT. The Potential of Fenugreek (Trigonella foenum-graecum) as a Functional Food and Nutraceutical and Its Effects on Glycemia and Lipidemia. *Journal of Medicinal Food*. 2011;14(12):1485–1489. Available from: https://pubmed.ncbi.nlm.nih.gov/21861724/.
4. Sulieman AME, Ali AO, Hemavathy J. Lipid content and fatty acid composition of fenugreek (Trigonellafoenum-graecum L.) seeds grown in Sudan. *International Journal of Food Science & Technology*. 2008;43(2):380–382. Available from: https://dx.doi.org/10.1111/j.1365-2621.2006.01446.x.
5. Leela NK, Shafeekh KM, Fenugreek. Fenugreek. 2008. Available from: http://dx.doi.org/10.1079/9781845934057.0242.
6. Chatterjee S, Variyar PS, Sharma A. Bioactive lipid constituents of fenugreek. *Food Chemistry*. 2010;119(1):349–353. Available from: https://dx.doi.org/10.1016/j.foodchem.2009.05.076.
7. Srinivasan K. Fenugreek (Trigonella foenum-graecum): A Review of Health Beneficial Physiological Effects. *Food Reviews International*. 2006;22(2):203–224. Available from: https://dx.doi.org/10.1080/87559120600586315.
8. Vats V, Yadav SP, Grover JK. Ethanolic extract of Ocimum sanctum leaves partially attenuates streptozotocin-induced alterations in glycogen content and carbohydrate metabolism in rats. *Journal of Ethnopharmacology*. 2004;90(1):155–160. Available from: https://dx.doi.org/10.1016/j.jep.2003.09.034.
9. Shaw JE, Sicree RA, Zimmet PZ. Global estimates of the prevalence of diabetes for 2010 and 2030. *Diabetes Research and Clinical Practice*. 2010;87(1):4–14. Available from: https://dx.doi.org/10.1016/j.diabres.2009.10.007.
10. Duckworth WC. Hyperglycemia and cardiovascular disease. *Current Atherosclerosis Reports*. 2001;3(5):383–391. Available from: https://link.springer.com/article/10.1007/s11883-001-0076-x.
11. Kaufman DW, Kelly JP, Rosenberg L, Anderson TE, Mitchell AA. Recent Patterns of Medication Use in the Ambulatory Adult Population of the United States. *JAMA*. 2002;287(3):337–344. Available from: https://dx.doi.org/10.1001/jama.287.3.337.
12. De Smet PAGM. Clinical risk management of herb-drug interactions. *Br J Clin Pharmacol*. 2007;63(3):258–267. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC2000738/.
13. Horowitz M, O'Donovan D, Jones KL, Feinle C, Rayner CK, Samsom M. Gastric emptying in diabetes: clinical significance and treatment. *Diabetic Medicine*. 2002;19(3):177–194. Available from: https://dx.doi.org/10.1046/j.1464-5491.2002.00658.x.
14. Meymeh RH, Wooltorton E. Diabetes drug pioglitazone (Actos): risk of fracture. *Canadian Medical Association Journal*. 2007;177(7):723–724. Available from: https://dx.doi.org/10.1503/cmaj.071177.
15. Korytkowski MT. Sulfonylurea Treatment of Type 2 Diabetes Mellitus: Focus on Glimepiride. *Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy*. 2004;24(5):606–620. Available from: https://dx.doi.org/10.1592/phco.24.6.606.34752.
16. KAVIARASAN S, RAMAMURTY N, GUNASEKARAN P, VARALAKSHMI E, ANURADHA CV. FENUGREEK (TRIGONELLA FOENUM GRAECUM) SEED EXTRACT PREVENTS ETHANOL-INDUCED TOXICITY AND APOPTOSIS IN CHANG LIVER CELLS. *Alcohol and Alcoholism*. 2006;41(3):267–273. Available from: https://dx.doi.org/10.1093/alcalc/agl020.
17. Shirwaikar A, Rajendran K, Barik R. Effect of aqueous bark extract of Garuga pinnata Roxb. in streptozotocin-nicotinamide induced type-II diabetes mellitus. *Journal of Ethnopharmacology*. 2006;107(2):285–290. Available from: https://dx.doi.org/10.1016/j.jep.2006.03.012.
18. kumar Nanjundan P, Arunachalam A, Thakur RS. Antinociceptive property of Trigonella foenum graceum in high fat diet fed/low dose streptozotocin induced diabetic neuropathy in rats. *Pharmacologyonline*. 2009;2:24–36. Available from: https://pharmacologyonline.silae.it/files/archives/2009/vol2/003.[1]_Kumar-Corrected-proof.pdf.
19. Lee MY, Lee EY, Lee BJ, Won CS, Koh JH, Shin JY, et al. Beneficial Effects of Thiazolidinediones on Diabetic Nephropathy in OLETF Rats. *Yonsei Medical Journal*. 2007;48(2):301–307. Available from: https://dx.doi.org/10.3349/ymj.2007.48.2.301.
20. Hosszufalusi N, Reinherz L, Takei S, Chan E, Charles MA. Glipizide-Induced Prevention of Diabetes and Autoimmune Events in the BB Rat. *Journal of Autoimmunity*. 1994;7(6):753–761. Available from: https://dx.doi.org/10.1006/jaut.1994.1059.
21. Sima AAF, Chakrabarti S. Long-term suppression of postprandial hyperglycaemia with acarbose retards the development of neuropathies in the BB/W-rat. *Diabetologia*. 1992;35:325–330. Available from: https://dx.doi.org/10.1007/bf00401199.
22. Sahoo PK, Padhy KM, Pradhan D, Tripathy G, Bhoi R, Pattanayak S, et al. Antidiabetic and antioxidant activity of ethanolic extract of Sapindus trifoliatus Linn. *International Journal of Pharma and Bio Sciences*. 2010;1(2):1–8. Available from: https://www.cabidigitallibrary.org/doi/full/10.5555/20113372253.
23. Kumar MS, Prashanth KVH. alpha-Lipoic acid ameliorates altered colonic contractility and intestinal transit in STZ-diabetic rats. *Indian Journal of Experimental Biology*. 2004;42(3):279–282. Available from: https://pubmed.ncbi.nlm.nih.gov/15233297/.
24. Ozakca I, Arioglu E, Guner S, Altan VM, Ozcelikay AT. Role of Beta-3-Adrenoceptor in Catecholamine-Induced Relaxations in Gastric Fundus from Control and Diabetic Rats. *Pharmacology*. 2007;80(4):227–238. Available from: https://dx.doi.org/10.1159/000104876.

25. James AN, Ryan JP, Crowell MD, Parkman HP. Regional gastric contractility alterations in a diabetic gastroparesis mouse model: effects of cholinergic and serotoninergic stimulation. *American Journal of Physiology-Gastrointestinal and Liver Physiology*. 2004;287(3):G612–G619. Available from: https://dx.doi.org/10.1152/ajpgi.00431.2003.
26. Allain CC, Poon LS, Chan CSG, Richmond W, Fu PC. Enzymatic Determination of Total Serum Cholesterol. *Clinical Chemistry*. 1974;20(4):470–475. Available from: https://dx.doi.org/10.1093/clinchem/20.4.470.
27. Lowry O, Rosebrough N, Farr AL, Randall R. PROTEIN MEASUREMENT WITH THE FOLIN PHENOL REAGENT. *Journal of Biological Chemistry*. 1951;193(1):265–275. Available from: https://dx.doi.org/10.1016/s0021-9258(19)52451-6.
28. Yokotani K, Murakami Y, Okada S, Wang M, Nakamura K. Histamine H3 receptor-mediated inhibition of endogenous acetylcholine release from the isolated, vascularly perfused rat stomach. *European Journal of Pharmacology*. 2000;392(1-2):23–29. Available from: https://dx.doi.org/10.1016/s0014-2999(00)00085-6. doi:10.1016/s0014-2999(00)00085-6.
29. Walter MF, Jacob RF, Jeffers B, Ghadanfar MM, Preston GM, Buch J, et al. Serum levels of thiobarbituric acid reactive substances predict cardiovascular events in patients with stable coronary artery disease: A longitudinal analysis of the PREVENT study. *Journal of the American College of Cardiology*. 2004;44(10):1996–2002. Available from: https://dx.doi.org/10.1016/j.jacc.2004.08.029.
30. Sedláková E, Rácz O, Lovásová E, Beòaèka R, Kurpas M, Chmelárová A, et al. Markers of oxidative stress in acute myocardial infarction treated by percutaneous coronary intervention. *Open Medicine*. 2009;4(1):26–31. Available from: https://dx.doi.org/10.2478/s11536-009-0015-8.
31. Singh PN, Athar MS. Simplified calculation of mean QRS vector (mean electrical axis of heart) of electrocardiogram. *Indian Journal of Physiology and Pharmacology*. 2003;47(2):212–216. Available from: https://www.ijpp.com/IJPP%20archives/2003_47_2/221-216.pdf.
32. Broca C, Manteghetti M, Gross R, Baissac Y, Jacob M, Petit P, et al. 4-Hydroxyisoleucine: effects of synthetic and natural analogues on insulin secretion. *European Journal of Pharmacology*. 2000;390(3):339–345. Available from: https://dx.doi.org/10.1016/s0014-2999(00)00030-3.
33. Bnouham M, Ziyyat A, Mekhfi H, Tahri A, Legssyer A. Medicinal plants with potential antidiabetic activity - A review of ten years of herbal medicine research (1990-2000). *International Journal of Diabetes and Metabolism*. 2006;14(1):1–25. Available from: https://dx.doi.org/10.1159/000497588.
34. Jayaraj SSB, Srinivasan K. Combination of herbal extracts with oral hypoglycemic agents for diabetes: A synergistic strategy for improving treatment efficacy. *Journal of Ethnopharmacology*. 2009;123(3):489–493.
35. Basch E, Ulbricht C, Kuo G, Szapary P, Smith M. Therapeutic applications of fenugreek. *Alternate Medicine Review*. 2003;8(1):20–27. Available from: https://pubmed.ncbi.nlm.nih.gov/12611558/.
36. Hannan JMA, Ali L, Rokeya B, Khaleque J, Akhter M, Flatt PR, et al. Soluble dietary fibre fraction of Trigonella foenum-graecum (fenugreek) seed improves glucose homeostasis in animal models of type 1 and type 2 diabetes by delaying carbohydrate digestion and absorption, and enhancing insulin action. *British Journal of Nutrition*. 2007;97(3):514–521. Available from: https://dx.doi.org/10.1017/s0007114507657869.
37. Gupta R, Bajpai KG, Johri S, Saxena AM. An overview of Indian novel traditional medicinal plants with anti-diabetic potentials. *African Journal of Traditional, Complementary and Alternative Medicines*. 2007;5(1):1–17. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC2816594/.
38. Sharma R, Raghuram T, Rao NS. Effect of fenugreek seeds on blood glucose and serum lipids in type I diabetes. *European Journal of Clinical Nutrition*. 1990;44(4):301–307.
39. Sowmya P, Rajyalakshmi P. Hypocholesterolemic effect of germinated fenugreek seeds in human subjects. *Plant Foods for Human Nutrition*. 1999;53(4):359–365. Available from: https://pubmed.ncbi.nlm.nih.gov/10540988/.