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RESEARCH ARTICLE

Evaluation of anti-diabetic and anti-inflammatory activities of Fenugreek (*Trigonella foenum-graecum*) seed extracts on Albino Wistar Rats

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ABSTRACT

Background: Fenugreek (*Trigonella Foenum-Graecum*) is a compound used since ancient times in Indian folk medicine for several medicinal properties. It has been known to produce hypoglycemic and antioxidant actions. **Aim and Objective:** This study aimed to determine ethanolic extracts out of fenugreek seeds for the anti-diabetic including anti-inflammatory effects on Wister rats. **Materials and Methods:** Inbred Albino male and female Wistar rats were used for the study. The powdered seeds were extracted with 90% ethanol by Soxhelation (100 g) for 3–4 days. The diabetes was assessed after 72 h of alloxan-induced to rats by determining the blood sugar level. Rat Paw volume was measured to the ankle joint in drug-treated and untreated groups at 0 min, 30 min, 60 min, and 120 mins using carrageenan challenge measured mercury plethysmograph. **Results:** Fenugreek at 200 mg/kg dose and 400 mg/kg decreased blood glucose which was dose-dependent. The reductions in blood glucose levels (BGLs) were significant post 14th day in both groups. The anti-inflammatory activity Fenugreek 200 mg/kg did not significantly reduce paw volume. Fenugreek at the dose of 400 mg/kg demonstrated inhibition of paw volume to 39.076% at the end of 2 h which was lesser than standard drug Aspirin. **Conclusion:** *T. foenum-graecum* decreased blood glucose towards the end of 21 days reduced the BGLs like the standard drug Gliclazide. The anti-inflammatory actions of the extracts were not found to be significant at the dose of 200 mg/kg and moderate anti-inflammatory actions at the dose of 400 mg/kg compared to standard drug Aspirin.

KEY WORDS: Fenugreek; *Trigonella foenum-graecum*; Anti-diabetic; Anti-inflammatory; Ethanolic extracts

INTRODUCTION

Trigonella foenum-graecum (TFG) commonly known as fenugreek (Fabaceae), is an annual, herbaceous and aromatic plant. TFG (Linn.) belonging to the family Fabaceae is also known as Fenugreek. It is an aromatic, 30–60 cm tall, annual herb, cultivated throughout India.[1,2] It has nearly smooth erect annual Stipulates which are not toothed. Leaflets 2–2.5 cm long, oblanceolate oblong, toothed. Flowers 1–2, axillary, sessile. Calyx-teeth linear. Corolla is much exserted. Pod 5–7.5 cm long, with a long persistent beak, often falcate, 10–29 seeded, without transverse reticulations.[1,2] It is a nutritional source of minerals such as calcium, iron, carotene, and Vitamins.[3] Fenugreek is commonly used as a seasoning and in food preparations and possesses nutritive, restorative properties.[4] the major chemical constituents are phenolic compounds, galactomannan, diosgenin, quercetin, trigonelline, and 4-hydroxy isoleucine.[5] TFG has been used since ancient times in Indian folklore medicine for its several medicinal properties.[6] It has been used in hypoglycemic,

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hypocholesterolemic, antioxidant, antirheumatic, appetite stimulation, gastroprotective, and chronic inflammatory activity, and analgesic.[7-12] Fenugreek seeds contain 4.8% saponins in the form of diosgenin, Yamogenin, Tigogenin, Neotigogenin, Yuccagenin, Lilagenin, Gitogenin, Neogitogenin, Sarsapogenin, and Smilagenin. Fenugreek has been used in glucose-lowering and antioxidants by modulating the peroxisome proliferator-activated receptors.[13] Fenugreek contains about 8000 polyphenolic compounds, including phenolic acids, flavonoids, stilbenes, lignans, and polymeric lignans have been identified from whole plant foods. These compounds are secondary metabolites of the plants that act as a defense against ultraviolet radiation, oxidants, and pathogens.[14] Phenolic compounds possess anti-oxidative attributes, which may be useful in the treatment of some forms of chronic diseases.[15] It has been seen that polyphenol-rich diets provide significant protection against the development and progression of cancer, diabetes, cardiovascular problems, and aging.[16] Fenugreek powdered seed extract is used for as antioxidant which prevents oxidative damage in alloxan-induced diabetic rats.[17] Fenugreek seeds are known to exhibit antioxidant properties and prevent cellular structural damage due to oxidative stress. Based on the above medicinal properties, the present study has been undertaken to primarily investigate the anti-diabetic, and anti-inflammatory, properties of the fenugreek seed extract with a secondary objective of determining the dose at which the properties were significant.

MATERIALS AND METHODS

This prospective study was one in the Department of Pharmacology, Mamata Medical College, Khammam. The study was approved by the Institutional Animal Ethics Committee with reference (No: Proposal No. IAEC/2012-17). Inbred Albino male and female Wistar rats, weighing 150–250 g were used for the study. The animals were sustained under standard laboratory conditions at 27–29°C, commercial pellet diet with water *ad libitum*, and normal photoperiod (12 h dark/12 h light). All the experiments were carried out between 09:00 and 13:00 h to avoid circadian variation and to maintain uniformity. Wistar rats were used for the evaluation of anti-diabetic, and anti-inflammatory. Freshly collected seeds were obtained from the local market. Fine powder of seeds was prepared by grinding. The extracts of powdered seeds with 90% ethanol were prepared by a process of continuous hot percolation process called Soxhlet extraction or Soxhelation (100 g) for 3–4 days.[18] Normal saline (1 ml)-used as a vehicle for all control groups. Alloxan monohydrate (150 mg/kg)-used to produce diabetes mellitus. (INR Chem, Bombay). Gliclazide (25 mg/kg)-used for the treatment group, in the treatment of diabetes (Glizid-40 Panacea Biotec Ltd) Carrageenan (0.1 ml)-used to induce inflammation. (Hi media Laboratories Pvt Ltd, Mumbai). Aspirin (50 mg/kg)-used as a standard drug in the treatment of inflammation. (Ecospirin-75, USV Pharma Ltd). Diabetes mellitus was caused by a single intraperitoneal injection of alloxan (150 mg/kg of body weight) dissolved in normal saline to the overnight fasted Wistar rats. The diabetes was assessed after 72 h of alloxan-induced to rats and measurement of the blood glucose level (BGL) at 0 days, 7th day, 14th day, and 21st day. The rats with BGL greater than 250 mg/dl were selected for the experimental studies. The diabetic rats were fed with *T. foenum-graecum* extract for 21 days in the concentration of 200 mg and 400 mg/kg respectively, to confirm the anti-diabetic and antioxidant activity. All the rats received alloxan, and the control group serves as a diabetic untreated. $n = 6$ animals in each group were divided as Group I-Control, Group II-Gliclazide (Standard), Group III-Test 1 (Fenugreek-200 mg/kg), Group IV-Test 2 (Fenugreek-400 mg/kg), Group V-Gliclazide+Test 2 Group VI-Gliclazide+Test 2+Vitamin C.

To study the acute and subacute phases of inflammation in rodents carrageenan is a widely used irritant or inflammogen or antiphlogistic agent. A 1% volume suspension of carrageenan is prepared freshly in normal saline and injected into the sub-plantar region of the left hind paw (usually 0.1 ml in rats and 0.025–0.05 ml in mice). In the control group animals, the only vehicle is injected. The test drug was usually administered orally, according to bodyweight within one hour prior to carrageenan challenge. A mark was made at the ankle joint of the tested rat. Paw volume up to the ankle joint was measured in drug-treated and untreated groups at 0 min, 30 min, 60 min, and 120 mins subsequently to the carrageenan challenge using a mercury filled plethysmograph. $n = 6$ animals in each group were divided as Group I-Control Group II-Aspirin (Standard), Group III-Test 1 (Fenugreek-200 mg/Kg), Group IV-Test 2 (Fenugreek-400 mg/kg), Group V-Aspirin+Test 2, Group VI-Aspirin+Test 2+Vitamin C. Statistical study of data was done using a one-way analysis of variance, followed by Dunnett's test. The $P < 0.05$ was considered significant.

RESULTS

Estimation of BGLs was taken as a parameter for antidiabetic activity. Group- II treated with gliclazide (25 mg/kg) revealed a significant decrease in BGL after 14 days with ($P < 0.05$) from 0–21 days correlated to the control group. Plant extract Fenugreek-200 mg/kg (Group III) and Fenugreek-400 mg/kg (Group IV) treated groups revealed a decreased blood glucose which was dose-dependent. However, levels were found to be significantly reduced post 14th day in both groups. Group V was treated with Fenugreek-400 mg/kg and the standard drug showed a convincing reduction in BGL ($P < 0.001$) matched with the control and standard groups from 0 to 21 days. The possible reason would be a synergistic activity between the plant extract and standard drug. Group-VI received 400mg/kg of fenugreek, standard drug, and Vitamin C. There

Table 1: Effect of fenugreek (*Trigonella foenum-graecum*) seed extract on Blood glucose levels (mg/dl)

Groups	MEAN±SD			
	0 Day	7th Day	14th Day	21st Day
Group-I				
Control	288.3±1.86	294±3.95	314.8±6.49	314.8±6.49
Group-II				
Gliclazide	284.3±1.63	153.7±7.17	114.3±2.87*	87±2.44*
Group-III				
Test 1 (Fenugreek-200 mg/kg)	281±1.26	168.2±4.79	129.2±3.76*	96.5±2.07*
Group-IV				
Test 2 (Fenugreek-400 mg/kg)	279.5±1.04	161±2.19	122.5±2.88*	92.1±2.78*
Group-V				
Test 2+Gliclazide	277.8±1.72	150.7±5.92	116.3±3.50*	84.6±3.32*
Group-VI				
Test 2+Gliclazide+Vit C	276±1.09	142.7±4.84	106.3±4.08*	80.5±2.88*

*Significant difference

was a convincing reduction in BGL ($P < 0.01$) compared to control standard groups. This group is more efficacious than the standard group as depicted in Table 1.

The percentage of edema inhibition was taken as a parameter for anti-inflammatory activity. In Group-II, mice treated with aspirin (50 mg/kg) revealed a powerful inhibition of paw volume (paw edema volume reduced in standard group) starting from 30 to 120 min compared to the control group. Plant extract Fenugreek-200 mg/kg (Group III) did not show significant inhibition of paw volume. Fenugreek-400 mg/kg (Group IV) administered groups revealed a significant prevention of paw volume after 120 min. Group V was treated with Fenugreek-400 mg/kg and standard drug and Group VI received 400 mg/kg of fenugreek, standard drug, and Vitamin C, results showed significant inhibition of paw volume at the end of one hour and beyond given in Table 2.

The percentage of reduction of rat paw volume measurements revealed standard drug aspirin was able to reduce the volume to greater than 50% after 1 h of administration and thereafter. In group III no significant reduction of paw volume percentage was observed. In Group IV a significant percentage of reduction was found after 2 h. Similarly, in groups, V and VI significant reductions were found from the end of 60 min and beyond depicted in Table 3.

DISCUSSION

The results of the current study reveal that plant extract of Fenugreek at 200 mg/kg dose and 400 mg/kg reduced BGL which was directly proportional to the dose of drug. The reductions in BGLs were significant post 14th day in both groups. Group V treated with Fenugreek 400 mg/kg and standard drug Gliclazide showed a greater reduction in BGLs compared to control and standard groups from 0 to 21 days. The possible reason could be a synergistic activity between the plant extract and standard drug. As far as the anti-inflammatory activity Fenugreek 200 mg/kg did not significantly reduce paw volume. Fenugreek at the dose of 400 mg/kg demonstrated reduction of paw volume to 39.076% at the end of 2 h which was lesser than standard drug Aspirin. Aspirin reduced the paw volume by 50.63% at the end of 1 h and 58.46% at the end of the 2nd h [Table 3].

Laboratory manufactured hypoglycemic agents can produce certain side effects. Apart from that many are unsuitable for use in pregnancy.[19,20] Therefore, the quest for more efficient and safer hypoglycemic agents is an important concern for researchers. In this study when fenugreek extracts were used in doses of 400 mg/kg BGLs were significantly reduced. Various reports revealed that fenugreek (*T. foenum-graecum*) possesses abundant benefits revealed in various laboratory experiments. In reconciliation with the present results, the blood sugar lowering effect of fenugreek seeds has been experimentally proved in diabetes-induced rats, dogs, mice, and healthy volunteers, and Type-I and II diabetic cases.[21,22] Ingestion of fenugreek seeds in diabetic rats, with its major alkaloid component, enhances insulin affectability and decrease digestives enzymes activities subsequently, decrease the BGLs.[23] According to Shimon *et al*.,[24] the fenugreek has volatile oil, phenolic acids, and flavonoids, which are potent sources of antioxidant activity. Fenugreek decreases the rate of glucose absorption and may also lag gastric emptying, thereby preventing the increase in blood sugar levels following a meal.[25] Amino acid, 4-hydroxy isoleucine of seed fiber also powerfully stimulates insulin secretion in the cells, and which increases the glycolysis of cellular glucose.[26] A meta-analysis of clinical trials on the effect of fenugreek (*T. foenum-graecum*) intake in

Table 2: Effect of fenugreek (*Trigonella Foenum Graecum*) seed extract on Carrageenan induced Rat paw edema reduction (min)

Groups	Mean±SD			
	0 min	30 min	60 min	120 min
Group-I				
Control	2.53±0.08	2.78±0.11	3.16±0.10	3.25±0.05
Group-II				
Aspirin	2.11±0.07	1.81±0.11*	1.56±0.10*	1.35±0.12*
Group-III				
Test 1 (Fenugreek-200 mg/kg)	2.2±0.22	2.28±0.11	2.13±0.08	2.05±0.10
Group-IV				
Test 2 (Fenugreek-400 mg/kg)	2.3±0.10	2.11±0.07	2.01±0.07	1.98±0.11*
Group-V				
Test 2+Aspirin	2.26±0.16	1.98±0.07	1.86±0.08*	1.80±0.10*
Group-VI				
Test 2+Aspirin+Vitamin C	2.31±0.16	1.91±0.09	1.81±0.09*	1.68±0.11*

*Significant difference

Table 3: Effect of fenugreek (Trigonella Foenum Graecum) seed extract on the Percentage of inhibition of Rat paw edema

Groups	0 Min	30 Min	60 Min	120 Min
Group-II				
Aspirin	16.600	34.892	50.632*	58.46*
Group-III				
Test 1 (Fenugreek-200 mg/kg)	13.043	17.985	32.594	36.923
Group-IV				
Test 2 (Fenugreek-400 mg/kg)	9.090	24.100	36.392	39.076*
Group-V				
Test 2 +Aspirin	10.671	28.776	41.139*	44.615*
Group-VI				
Test 2+Aspirin+Vit C	8.695	31.294	42.721*	48.307*

*Significant difference

diabetic patients had found fenugreek seeds convincingly decreased the fasting blood glucose, post-load glucose was also decreased and glycated hemoglobin was reduced significantly compared to untreated subjects.[27] Animal studies also found that Fenugreek seed soluble fiber and saponin fractions decrease the absorption of glucose from the gastrointestinal tract. This effect is produced due to reduction of rate of enzymatic digestion of amylase and glycosidase.[28] *T. foenum-graecum* appears to inhibit the diabetic effects on GLUT4 transporters. Saponins of the fenugreek seeds and alkaloids and trigonelline inhibit intestinal glucose uptake *in vitro*.[29] Fenugreek extract showed some degree of anti-inflammatory action. The existence of flavonoids saponins salicylate and alkaloids are the compounds responsible for their anti-inflammatory activity.[27,30,31] The probable mechanism of action is that flavonoids act as viable inhibitors of cyclooxygenase, lipoxygenase, and nitric oxide synthase as well as being antioxidants.[32,33] The seeds of the plant have a gastroprotective effect an important factor concerning the gastrointestinal disturbance caused by non-steroidal anti-inflammatory drugs.[34] Although the degree of the anti-inflammatory effect produced was only moderate at the dose of 400 mg/kg which is lesser as compared to the standard drug Aspirin. Our results confirm the findings of Ahmadiani *et al*.,[31] and Vyas *et al*.,[35] who reported that fenugreek phenolic extract has a potential anti-inflammatory activity.

The strength of this study is that it confirmed the traditional use of natural remedies of domestic plants-origin for the treatment of diabetes mellitus. However, its limitations are because of small number of animals with limited time observation done. We definitely require a large scale and multicentric clinical trials in order to establish the usefulness Longitudinal studies and definitive clinical trials are still required to base the usefulness of this natural drug *T. foenum-graecum* seeds along with diet as a modulator in the treatment of diabetes mellitus.

CONCLUSION

This study within its limitations found *T. foenum-graecum* seed extracts in ethanol decreased BGLs which was proportionate to the strength of dose. Fenugreek seed extracts at the dose of 400 mg/kg on the conclusion of 21 days reduced the BGLs similar to the standard drug Gliclazide. The anti-inflammatory actions of the extracts were not found to be convincing at the dose of 200 mg/kg and moderate anti-inflammatory actions at the dose of 400 mg/kg compared to standard drug Aspirin.

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