World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 4.223

ASSESSMENT OF MODIFIED FENUGREEK ON BLOOD SUGAR LOWERING ACTION IN RATS

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Article Received on 19/03/2017 **Article Revised on 08/04/2017** **Article Accepted on 29/04/2017**

ABSTRACT

The present study was designed to compare the antidiabetic potentialities between standardized fenugreek powdered seed (FG) are modified fenugreek (FGM). HPTLC chromatographic fingerprint of both the samples were determined. Anti-hyperglycaemic activities of test drugs were evaluated on alloxan induced (120 mg/kg, i.p) diabetic rats. The glucose, cholesterol and triglycerides in blood were monitored. FGM (50-150 mg/kg, p.o) exhibited best potentiality in reducing blood glucose within 14 days treatment in comparison with FG. FGM also significantly ($p<0.5$) negated elevations of lipid profile in diabetic rats. The observed effects were also comparable with Glibenclamide (5 mg/kg). The present observation identified FGM for anti-hyperglycaemic and lipid lowering actions and substantiates its uses on diabetic population.

KEY WORDS: Diabetes, herbs, *Trigonella Foenum-graccum*, Blood glucose, blood lipids

INTRODUCTION

Fenugreek (*Trigonella foenum-graecum* L. family: Leguminosae) is one of the oldest medicinal plants, originating in India, China and Northern Africa. The leaves and seeds, which mature in long pods are used to prepare extracts or powders for medicinal use. Fenugreek seed is commonly used as a condiment and assumed to possess nutritive and has been used in folk medicine for centuries for a wide range of diseases including diabetes, fever and boils (Basch *et al.*, 2003). It is reported that the daily administration of either powdered or aqueous extract of fenugreek decreases the blood glucose level and increases glucose tolerance in rodents (Gupta *et al.*, 2001). Prasanna (2000) and Mitra and Bhattacharya (2006) reported that fenugreek powder showed significant anti-diabetic and dislipidaemic potentiality in Indian diabetic patients. The seeds of fenugreek contain a large quantity of folic acid, niacin, ascorbic acid, lysine, L-tryptophan, fatty acids, fibres, alkaloids (trigonelline) and saponins (disogenin, gitogenin, neogitogenin, homorientin saponaretin, neogigogenin, trigogenin etc) (Yoshikawa *et al.*, 1997). The hypoglycemic effects of fenugreek have been attributed to several mechanisms. Sauvaire *et al* (1998) demonstrated *in vitro* the amino acid 4-hydroxyisoleucine in fenugreek seeds increased glucose-induced insulin release in human and rat pancreatic islet cells. Unfortunately available human trial data of fenugreek for effectiveness on diabetes reported were varied from the doses of 25 to100 g in a twice daily dose for seed powder (Raghuram *et al.*, 1994; Sharma *et al.*, 1996; Bordia *et al.*, 1997). Consumption of 50-200 g of fenugreek daily is not only embarrassment to diabetic patient, but also creates some untoward side effects, like, nausea, vomiting, headache, dieresis etc (Basch *et al.*, 2003; Martins, 2014). Several pharmaceutical researches are undergoing to modify physicochemical properties of herbs through purification to improve their qualities and efficacies. In the present context a new simple method was adopted to improve the quality and efficacy of fenugreek. Further, it was standardized chemically and compared to fenugreek powder for its actions on diabetes and its complications in experiment animals.

MATERIALS AND METHODS

Plant materials collection and authentication

Fenugreek seed were collected from Salipur and properly identified by Botanical Survey of India, Howrah, West Bengal.

Test drug preparation

Fenugreek seeds were powdered (FG) and processed for purification and extraction (Sinha Mahapatra and Mandal, 2015). The powdered fenugreek seeds were soaked with raw milk (1:2 w/v) and kept overnight (12 h)

at 37ºC. Thereafter, washed with warm distilled water (37ºC), dried and again soaked with raw milk. This procedure was applied repeatedly 15 times. Finally, purified dried fenugreek powder (FGM) was kept in air dried container for further use.

Phytochemical analysis

The group analysis of fenugreek powder (FG) and purified fenugreek powder (FGM) extract were examined for alkaloids by Dragendroff's test, sterols and triterpenoids by Liberman Buchard test, saponins by Froth test, tannins by lead acetate test, carbohydrates by Fehling's test, reducing sugars by Benedict test, proteins by Millon's test, phenolics by ferric chloride test, flavonoids by aluminum chloride test and glycosides by Borntrager's test (Sinha Mahapatra *et al.*, 2016).

Test drug standardization by HPTLC fingerprints

Sample preparation

1 g of each test drug was refluxed with methanol to final concentration at 1 mg/ml and filtered for HPTLC analysis. For further hydroxylation, 1 g of each test drug was further refluxed with 25 ml of acidic ethanol (1M sulphuric acid) for an hour, cooled and filtered and incubated at 50ºC to neutralized (pH 7) with 25% ammonia solution. Then extracted in 10 ml of dichloromethanol and washed with 0.1 M sodium oxide solution and evaporated to dryness (Sur *et al.*, 2015). Finally, the dry extracted material was dissolved in 5 ml methanol and filtered for HPTLC analysis.

HPTLC analysis

The test samples was filtered and spotted (5 µl) on a pre-coated silica gel plates (Merck, KGaA $60F_{254}$, 10X10 cm) using Camag Linomat 5 applicator and processed in a solvent system (toluene: ethyl acetate: formic acid= 7:5:1) for 30 min. The densitometric scanning was performed on Camag TLC Scanner 3 at absorbance 280 nm (D2 lamp) operated by multi level winCATS planar chromatography manager (Sur *et al.*, 2016). The gallic acid and quercetin were used as markers. The obtained unknown peaks were individually marked as Rf and area percent were measured. The known peaks for gallic acid and quercetin were only quantified and compared.

Animals

Swiss albino mice and Wistar rats obtained from registered breeder and maintained in our animal house for at least 10 days prior to experimentation. Recognized guidelines for the care and use of the animals were followed (CPCSEA guidelines for laboratory animal facility, 2003). Permission for the experiments was obtained from the institute animal ethics committee. The room temperature was maintained at 23±2°C and humidity at 40-60%. A 12 hour light-dark cycle was also maintained. The animals were fed supplementary feed for rats (procured from Provimi, Bangalore) and water *ad libitum*. The food was withdrawn as per experimental protocol.

Acute toxicity studies

The homogenous suspension of two test drugs were prepared freshly, using 0.5% (w/v) carboxyl methyl cellulose (CMC) using a mortar and pestle. The different groups of mice were administered various doses (0.5-2 g/kg p.o.) of extracts. The mice were then critically observed for clinical symptoms, behavioural changes and mortality up to 72 h period following OECD guidelines No.423 (2001).

Selection of doses

The effective doses of blood sugar lowering action of the two test compounds was selected at 50, 100 and 150 mg per kg orally and therefore, all experiments were done with above three doses for better comparison (Sinha Mahapatra *et al.*, 2016).

Induction of experimental hyperglycemia in rats

In overnight fasted albino rats, diabetes was induced by a single intraperitoneal injection of alloxan monohydrate in ice cold saline at a dose of 120 mg/kg body weight (Sur *et al.*, 2004; Sinha Mahapatra *et al.*, 2016). The diabetic state was confirmed 72 h after alloxan injection by measuring fasting blood glucose (one-touch Accu-chek sensor glucometer) from their tail vein. Rats with fasting blood glucose ≥250 mg/dl were considered to hyperglycemia (clinically resembles to diabetes) and were used in this study. The diabetic rats were divided into nine groups of six animals in each and treated orally as follows:

Treatment schedule in rats

Groups	Test Drug	Dose (orally)
1	Normal control	2 ml/kg
2	Alloxan diabetic control (AD)	2 ml/kg
3	AD + Glibenclamide	5 mg/kg
4	AD + FG	50 mg/kg
5	AD + FG	100 mg/kg
6	AD + FG	150 mg/kg
7	AD + FGM	50 mg/kg
8	AD + FGM	100 mg/kg
9	AD + FGM	150 mg/kg

All the animals were treated once daily for consecutive 14 days. The blood glucose was monitored 2 h after last dose given at 7^{th} day and 14^{th} day. After the experimental schedule, the rats were fasted overnight (16 h). Finally, blood was collected from heart under deep anaesthesia and serum was separated by centrifugation at 5000 rpm for 15 min and kept in –20ºC (Sinha Mahapatra *et al.*, 2016). The serum was further analyzed for biochemical estimations of total cholesterol and triglycerides using commercial kits (Span Diagnostics, India).

Statistical analysis

The data generated during the study were expressed as means ± standard error of mean. The data were analyzed statistically using software based statistical package (spss version 20, IBM, USA). The percent changes were also calculated.

RESULTS

Phytochemical analysis

The results of phytochemical group analysis of all individual test compounds and formulated test drugs were showed Table 1.

Table 1: Phytochemical screening of raw fenugreek (FG) and modified fenugreek (FGM).

	Reducing sugars	Non-reducing sugars	Proteins	Tannins	Alkaloids	Triterpenoids	Glycosides	Flavonoids	Phenolics	Saponins	Sterols
FG	-	+	-	-	+	-	-	+	+	+	+
FGM	-	+	+	-	+	-	+	+	+	+	+

FG= Fenugreek powdered; FGM= Modified fenugreek

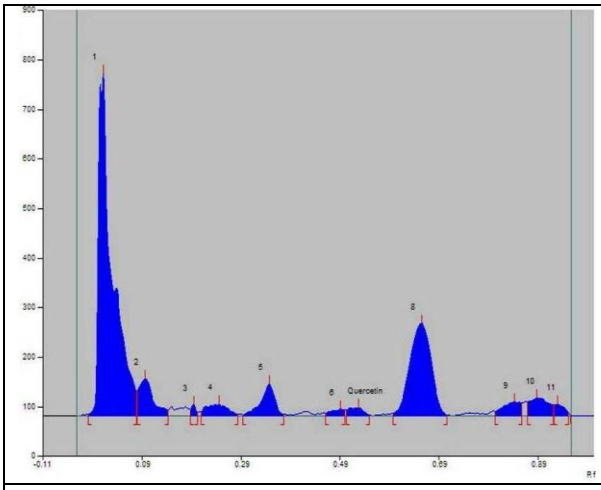

Fig. 1 (a): HPTLC of FG

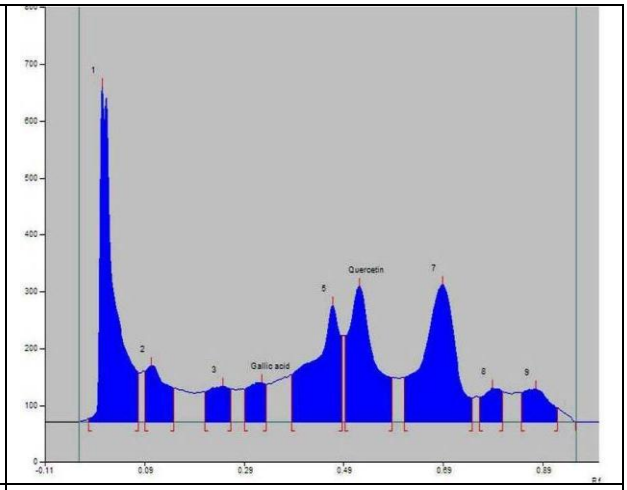

Fig. 1 (b): HPTLC of FGM

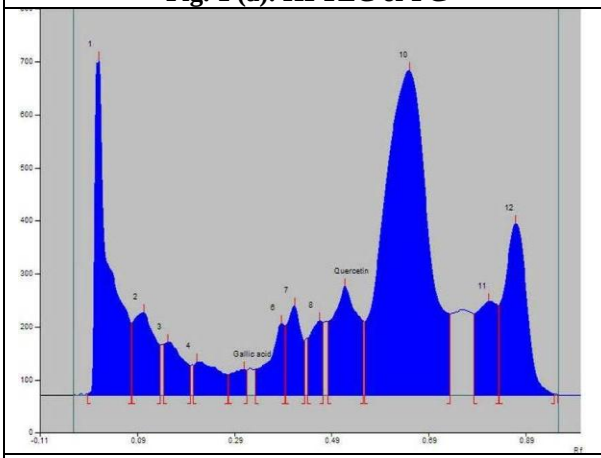

Fig. 1 (c): HPTLC of hydrolysed FG

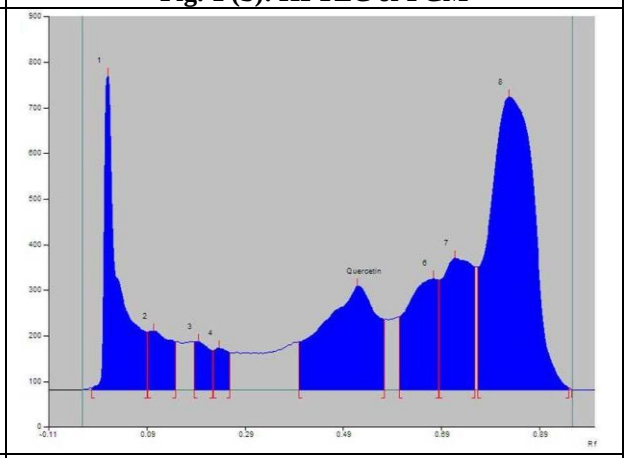

Fig. 1 (d): HPTLC of hydrolysed FGM

Fig 1: HPTLC chromatograms of raw fenugreek (FG) and modified fenugreek (FGM).

The chromatograms of FG and FGM showed distinct features of compounds and peaks. Fig. 1(a) represents HPTLC chromatogram of FG and Fig. 1(b) of FGM and after hydroxylation the chromatogram exhibited in Fig. 1(c) and Fig. 1(d) respectively. FG has 11 peaks and FGM has 9 peaks. The Rf values of gallic acid and quercetin were 0.33 and 0.5 respectively. FG has 1.3% quercetin but after modification it was quantified 17.8% in FGM. Moreover, 3.5% gallic acid also observed in FGM, while it was absent in FG. After hydroxylation, quercetin concentration was enhanced 1.3% to 7.8% in FG, whereas after modification, FGM exhibited 27% quercetin (Table 2).

Table 2: Compounds of raw fenugreek (FG) and modified fenugreek (FGM) in HPTLC.

	Rf (Area %)											
FG	0.01 (53.5)	0.1 (5.9)	0.19 (0.5)	0.25 (2.8)	0.35 (4.9)	0.49 (1)	0.5 (1.3)	0.66 (21.8)	0.84 (2.8)	0.89 (3.8)	0.93 (1.2)	
FGM	0.00 (24.4)	0.1 (5.8)	0.25 (3.9)	0.33 (3.5)	0.47 (15.9)	0.5 (17.8)	0.69 (21.9)	0.79 (3)	0.88 (4.2)			
FG hydroxylation	0.01 (14.1)	0.1 (4.9)	0.15 (4.8)	0.21 (2.4)	0.33 (1.1)	0.39 (3.2)	0.41 (3.6)	0.47 (2.7)	0.5 (7.8)	0.65 (40.6)	0.81 (5.6)	0.87 (10.8)
FGM hydroxylation	0.1 (14.8)	0.1 (4)	0.19 (2.2)	0.24 (1.8)	0.5 (27)	0.67 (10.2)	0.72 (11.7)	0.83 (38.8)				

Rf of gallic acid=0.33 and Rf of quercetin=0.5

Acute toxicity studies

Both the test drug was found to be safe up to 2 g/kg body weight dose in mice.

Anti-hyperglycaemic action

Alloxan elevated blood glucose up to 358.5% within 7 days and 490.9% within 14 days compared to normal control rats. The standard oral anti-hyperglycaemic agent, glibenclamide reduced blood glucose 41% within 7 days and 58.8% within 14 days. FG powdered and modified FGM lowered the blood glucose levels significantly ($p<0.05$) and dose dependently and in a similar fashions (Table 3).

Table 3: Anti-hyperglycaemic potency of raw fenugreek (FG) and modified fenugreek (FGM) on alloxan induced Wistar rats.

Group	Test Drug	Dose	Blood glucose		
			Day 0	Day 7	Day 14
1	Normal Control	2 ml/kg 0.5% CMC	68.1±5.34	69.3±6.08	68.8±7.13
2	Diabetes Control (Alloxan)	2 ml/kg0.5% CMC	67.3±5.35(a)	317.8±5.34(a)* (358.5%)	406.6±12.30(a)* (490.9%)
3	Glibenclamide	5 mg/kg	69.3±7.39(b)	187.5±8.09(b)* (-41%)	167.3±9.01(b)* (-58.8%)
4	Diab + FG	50 mg/kg	67.6±7.60(b)	303.1±10.28(b) (-4.6%)	289.8±7.67(b)* (-28.7%)
5	Diab + FG	100 mg/kg	68.0±8.36(b)	263.8±6.79(b)* (-16.9%)	254.5±12.92(b)* (-37.4%)
6	Diab + FG	150 mg/kg	68.5±6.53(b)	251.6±8.22(b)* (-20.8%)	223.8±9.51(b)* (-44.9%)
7	Diab + FGM	50 mg/kg	67.6±7.60(b)	284.7±7.51(b)* (-10.4%)	241.5±6.84(b)* (-40.6%)
8	Diab + FGM	100 mg/kg	68.0±8.36(b)	248.2±8.04(b)* (-21.9%)	204.9±9.03(b)* (-49.6%)
9	Diab + FGM	150 mg/kg	68.5±6.53(b)	219.4±6.19(b)* (-30.9%)	187.5±8.66(b)* (-53.8%)

Data are Mean ± SD (N=6); (a) means compared to normal control, (b) means compared to diabetic control on same day; Diab means alloxan induced diabetic control; * mean $p<0.05$

Lipid lowering action

Alloxan diabetic rats elevated serum cholesterol (158.6%) and triglycerides (181.9%) than normal control rats. Glibenclamide exhibited 28.8% reduction of cholesterol and 36.6% triglycerides within 14 days. FG powdered and modified FGM showed significant ($p<0.05$) diminution of total cholesterol and triglycerides in rat serum (Table 4).

Table 4: Anti-lipidemic potency of raw fenugreek (FG) and modified fenugreek (FGM) on alloxan induced Wistar rats.

Group	Test Drug	Dose	Total Cholesterol	Triglycerides
1	Normal Control	2 ml/kg 0.5% CMC	69.6±7.44	97.1±5.26
2	Diabetes Control (Alloxan)	2 ml/kg0.5% CMC	180.0±10.19(a)* (158.6%)	273.8±9.70(a)* (181.9%)
3	Glibenclamide	5 mg/kg	128.1± 10.16(b)* (-28.8%)	173.5± 14.33(b)* (-36.6%)
4	Diab + FG	50 mg/kg	151.3±7.99(b)* (-15.9%)	268.5±7.34(b) (-1.9%)
5	Diab + FG	100 mg/kg	144.5±11.46(b)* (-19.7%)	249.5±9.39(b)* (-8.8%)
6	Diab + FG	150 mg/kg	137.1±6.55(b)* (-23.8%)	224.1±7.57(b)* (-18.1%)
7	Diab + FGM	50 mg/kg	141.8±8.16(b)* (-21.2%)	216.5±10.94(b) * (-20.9%)
8	Diab + FGM	100 mg/kg	132.9±7.22(b)* (26.1%)	189.4±9.11(b)* (-30.8%)
9	Diab + FGM	150 mg/kg	121.6±5.08(b)* (-32.4%)	162.5±10.36(b)* (-40.6%)

Data are Mean ± SD (N=6); (a) means compared to normal control, (b) means compared to diabetic control on same day; Diab means alloxan induced diabetic control; * mean $p<0.05$

DISCUSSION

Alloxan is a known diabetogen that extremely used for induction of hyperglycemia in experimental animals. It is cytotoxic for pancreatic β-cells (Szkudelski, 2001). Damage of pancreatic β-cells results the symptoms like Type 2 diabetes in animals within 72 hours (El-Missyry *et al.*, 2004). *Trigonella foenum-graecum* is commonly known as fenugreek or methi and extensively used in many preparations of Ayurveda to treat diabetes and its related complication (Basch *et al.*, 2003). Previously, it was also reported that fenugreek have antioxidant and hypoglycemic action on alloxan induced diabetic rats (Gupta *et al.*, 2001; Prasanna, 2000). There are also considerable evident that fenugreek seed contains polyphenols and has antioxidant action (Kaviarasan *et al.*, 2004). In this study, alloxan induced rats showed hyperglycemic actions, while, fenugreek exhibited anti-hyperglycaemic action and supported the previous reports (Raghuram *et al.*, 1994). Furthermore, in humans, fenugreek seeds exert hypoglycemic effects by stimulating glucose-dependent insulin secretion from pancreatic beta cells, as well as by inhibiting the activities of alpha-amylase and sucrase, two intestinal enzymes involved in carbohydrate metabolism (Raghuram *et al.*, 1994, Sauvaire *et al.*, 1998). It is also assumed that saponins in fenugreek seeds participates the essential role for its lipid lowering action (Yoshikawa *et al.*, 1997; Mitra and Bhattacharya, 2006).

Present study demonstrated that purification of raw fenugreek seed not only altered the chemical identities but also improved its bioactivities related to therapeutic potentialities. HPTLC studies revealed that purification with milk eliminated some unessential compounds as well as enriched some of the essential components. After modification, the test compound (FGM) enhanced quercetin content from 1.3% to 17.8%. Therefore, this modification is able to improve bioactivities of quercetin (bioactive flavonoids) more than 1000 times. Like quercetin, there are also other unidentified compounds present in fenugreek that may be changed its bioactivities. This purification process could facilitate to reduce the effective daily dose of raw fenugreek powder in diabetes subjects from 100 g to 100 mg only.

The purification process also reflected in bioactivities like, blood sugar and blood lipids lowering efficacies in diabetic conditions in rats. Interestingly, modified fenugreek has more or less similar or better efficacy in comparison to oral Sulfonylurea, glibenclamide. Shorr *et al* (1996) reported the risk of hypoglycaemia in the elderly after using Glibenclamide, that are no more safe as predicted before.

Furthermore, uncontrolled diabetes is one of the prime risk factor of coronary diseases, might be due to inefficiency of insulin that resulted in impaired lipid metabolism and ultimately raise the blood cholesterol and triglycerides (Sur and Hazra, 2017). The abnormal high concentration of serum lipids in the diabetic subject is mainly due to increase in the mobilization of free fatty acids from the peripheral fat droplets, since insulin inhibits the hormone sensitive lipase (Arvind *et al.*, 2002). Experimentally, alloxan induced diabetic hyperglycaemia is accompanied by increase in serum cholesterol and triglycerides. In this study, treatment with fenugreek and modified fenugreek demonstrated a significant decline in total cholesterol and triglycerides in diabetic rats. Although, modified fenugreek (FGM) has shown maximum anti-hyperglycaemic action and proved to be more effective on alloxan-induced lipid lowering metabolisms than eventually common use of

glibenclamide. These active chemical constituents may aid absorption of active principles responsible for hypoglycaemic and hypolipidemic properties. Fenugreek is an edible common spice and available throughout the world, therefore, its use as antidiabetic agent is known and popular. But, still some modification can improve its quality as also bioactivities and eliminate some untoward effects. More research is required to further purify and identify other active moieties of modified fenugreek that may be responsible for more effective antidiabetic agent in near future.

ACKNOWLEDGEMENTS

The authors would like to express gratitude to the Principal, Institute of Pharmacy and Technology, Salipur for providing support for conducting the research.

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