ISSN 0976 – 3333

Available Online at www.ijpba.info

International Journal of Pharmaceutical & Biological Archives 2012; 3(4):900- 904

ORIGINAL RESEARCH ARTICLE

Pharmacodynamic Interaction of *Fenugreek* Seeds Extract with Fluvastatin in Diabetes Induced Vascular Dysfunction

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Received 12 May 2012; Revised 09 Aug 2012; Accepted 18 Aug 2012

ABSTRACT

This study is mainly aimed to investigate the therapeutic potential of FG seeds in combination with the well-known hypolipidemic that is fluvastatin and hypoglycemic drugs pioglitazone and glipizide for the treatment of vascular dysfunction induced by diabetes mellitus. Clinical studies have already proven that fluvastatin therapy can significantly reduce the cardiovascular morbidity and mortality because of its lipid- and cholesterol-lowering potential. The long-term efficacy of pioglitazone and glipizide as single and combined therapy against the diabetes mellitus has been explored widely. In addition to their glucose-lowering effects, pioglitazones can modulate a number of cardiovascular risk factors via their Anti-inflammatory, anti-atherogenic and anti-thrombotic properties. Similarly, the therapy of glipizide is found to be highly potent for the treatment of diabetes mellitus, as the drug lowers blood glucose level very significantly. Although the combined therapy of standard drugs is found to be highly effective in normalizing the abnormal biochemical parameters in diabetic patients, the FG seed extract alone or in combination with hypoglycemic drugs, pioglitazone and glipizide is also observed to be very promising therapy for diabetes mellitus. The FG seed extract in combination with pioglitazone and glipizide shows the glucose-lowering effect. Earlier Fluvastatin was proved for normalizing endothelial dependent relaxation. Even the endothelial dependent relaxation was improved in treated groups. Thus, their combination is assumed to be a very potential treatment for vascular dysfunction induced by diabetes mellitus.

Key words: Hypolipidemic, Hypoglycaemic, vascular dysfunction, Fenugreek seed (FG).

INTRODUCTION

Endothelial dysfunction can be defined as the partial or complete loss of balance between vasoconstrictors and vasodilators, growth promoting and inhibiting factors, proatherogenic and anti-atherogenic factors, and pro-coagulant and anticoagulant factors. Endothelial dysfunction now regarded as an early pivotal event in atherogenesis [1].Endothelial dysfunction in the brachial artery highly correlates with endothelial dysfunction in the coronary circulation, which is emerging as an independent risk factor for cardiovascular disease [2].There is a strong association between endothelial dysfunction and oxidative stress and development of other microvascular complications [3].Nitric oxide is important substance secreted from endothelium so called endothelium-derived relaxing factor [4]. Nitric oxide not only produces vasodilation, but it also participates in various processes that are beneficial to the vasculature, such as the reduction of vascular smooth muscle cell migration and growth, platelet aggregation and thrombosis, monocyte and macrophage adhesion, and inflammation. There are various pharmacological and non-pharmacological stimuli that can be used to assess the production of nitric oxide in endothelial cells in vivo as well as its effect on the underlying vascular smooth muscle cells (endothelium-dependent and -independent vasodilation, respectively) [5].The main mechanism underlying endothelial dysfunction is oxidative stress other mechanism is production of AGEs and on turn producing Reactive oxygen species [6].and vascular endothelial dysfunction is leading cause for peripheral artery diseases [7].so association of both diabetes and endothelial vascular dysfunction may lead a patient to death. Many commercial drugs available which can modify the disease pattern. Among these drugs, there are two classes that are widely used in

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people with obesity and type 2 diabetes. These are the thiazolidenediones (TZDs) and the biguanides (metformin). TZDs directly reduce insulin resistance by enhancing insulin action in peripheral tissues and therefore, have been attractive candidates for studying the effects on vascular function of reducing insulin resistance. Several studies showed the significant impact of troglitazone on glycemia, insulin resistance, and traditional cardiovascular risk factors. There are two currently available TZDs, pioglitazone and rosiglitazone. They both significantly reduce insulin resistance and improve glycaemic control in people with type 2 diabetes. They also seem to have an overall positive effect on blood pressure, lipids, and fat distribution [8,9]. A good combination therapy can be used to get strict glycaemic control. Fluvastatin treatment for dyslipidaemia in patients with type 2 diabetes mellitus on insulin therapy is effective and well tolerated [10]. The hypoglycemic effect of fenugreek seeds is studied previously [11].

The pharmacodynamic interaction of *Fenugreek* seed extract (Rich in polyphenol) and fluvastatin in diabetic induced vascular dysfunction is not studied until now. Hence an attempt is made to study the effect of *Fenugreek* seed extract with fluvastatin in diabetes induced vascular dysfunction in rats.

MATERIALS AND METHODS

Collection of plant materials:

Fenugreek seed was collected from School of science, Gujarat University, Ahmadabad. It was identified and authenticated by Dr. Himanshu A. Pandya, Associate Professor, School of science, Gujarat University, Ahmadabad.

Preparation of extract:

Fenugreek seeds (100 g) were finely powdered, mixed with 80% methanol and kept at room temperature for 5 days. After 5 days it was filtered and the solvent was evaporated. The residue was dissolved in water and the aqueous layer was washed with petroleum ether several times until a clear upper layer of petroleum ether was obtained. The lower layer was then treated with ethyl acetate containing glacial acetic acid (10 ml/l).Extraction of polyphenols was carried out for 36 h at room temperature and the combined ethyl acetate layer was concentrated). The residue was lyophilized and stored. This yielded about 6–8 g per 100 g of seed powder. A Methanolic extract was prepared and used for studies.

Experimental animals:

Male Sprague dawley Rats weighing 150-180 g were housed at 25° ± 5°C in a well ventilated Animal house under 12:12 h light dark cycle. The animals were maintained under standard conditions in an animal house as per the guidelines of Committee for the Purpose of Control and Supervision on Experiments on Animals1359/ac/10/ (CPCSEA).

INDUCTION OF DIABETES:

The animals were fed with HFD once a day for two weeks followed by I.P injection of streptozotocin (35mg/kg) dissolved in 0.5M/l citrate buffer (pH: 4.4) after overnight fasting. STZ injected animals were then given 5% w/v glucose solution for 5-6 hours following the injection to prevent initial drug induced hypoglycemic mortality. The rats with non fasted plasma level 300mg/dl were considered diabetic. The blood Sample was collected from tail vein and blood glucose was checked using glucose diagnostic kit.

STANDARDISATION AND SELECTION OF LOW DOSE FOR STANDARD DRUGS:

Oral Glucose Tolerance Test: After overnight fasting (18hrs), a 0-min blood sample was taken from the tip of the tail of each rat of different groups. Glucose solution (2 g/kg P.O) was given after 30 min after the administration of the drug. Four more samples were taken at 30, 60, 90 and 120 min after glucose administration. All blood samples were checked with the help of accucheck glucometer. The oral glucose tolerance test will be performed on overnight fasted Sprague dawley rats. Rats will be divided into different groups as follows:

Group 1: Normal control, rats receive saline/vehicle.
Group 2: Diabetic control.
Group 3: *Fenugreek seeds* extract (200 mg/kg)
Group 4: Pioglitazone (10 mg/kg)
Group 5: Glipizide (5 mg/kg)

Effect of fenugreek seed extract alone and in combination with oral hypoglycemic agents in streptozotocin induced diabetic vascular dysfunction [12,13,14]:

Rats was divided into different groups (n=6) as follows and the treatment with the respective drugs was given for 8 weeks. The estimations were carried out on the 15th, 30th, 45th and 60th days of the treatment.

Group 1: Normal control, rats receive saline/vehicle.

Group 2: Diabetic control
Group3: FSEt (200 mg/kg *p.o*)
Group 4: Pioglitazone (10 mg/kg)
Group 5: Glipizide (5mg/kg)
Group 6: Pioglitazone (7.5mg/kg) plus Glipizide (3.75mg/kg)
Group 7: Pioglitazone(7.5 mg/kg)+ FSEt(200mg/kg)
Group 8: Glipizide (3.75 mg/kg) + FSEt (200 mg/kg)

STATISTICAL ANALYSIS:

Data are presented as the mean ± SE from 6 rats per group. Comparison of mean values among the various groups was performed by one way ANOVA. For the single comparison between the groups unpaired Student's t-test was used.

RESULTS AND DISSCUSSION

The pharmacodynamic interaction of *fenugreek* seed extract (Rich in polyphenol) and fluvastatin in diabetic induced vascular dysfunction is not studied until now. Hence an attempt is made to study the effect of *fenugreek* seed extract with fluvastatin in diabetes induced vascular dysfunction in rats and result is tabulated in table. The present study (OGTT) also indicates a profound effect of FSEt (Fenugreek seed extract). FSEt showed a significant increase in glucose tolerance when compared with diabetic control, which might be due to insulinotropic property *in vitro* and improved glucose tolerance in rat model of type 2 DM1[15]. In type 2 diabetic patients, the ingestion of 15 g of powdered (water soaked) fenugreek seed significantly reduced postprandial glucose levels during the glucose tolerance test [16]. The combined therapy of FSEt with glipizide showed more significance in reducing the postprandial glucose levels when compared with individual therapy. When the diabetic rats were treated for 10 weeks, the combination therapy (FSEt with glipizide) did not produce hypoglycemic effect but the combined therapy of (Oral hypoglycaemic agents) OHA's had produced hypoglycemia. The production of NO from the endothelial cells is stimulated by insulin [17]. Thus, in healthy non-diabetic subjects, insulin increases endothelium-dependent (NO-mediated) vasodilatation but endothelium- dependent vasodilatation is reduced in insulin-resistant subjects [18]. Insulin-mediated glucose disposal is correlated inversely with the severity of the impairment in endothelium-dependent vasodilation [19]. Drug therapies which increases insulin sensitivity (metformin and thiazolidinediones), improves endothelium-dependent vasodilatation [20,21].

Hyperglycemia induces a series of cellular events that increase the reactive oxygen species (ROS) production (such as superoxide anion) that inactivate NO (Nitric Oxide) to form peroxynitrite [22,23]. Diabetic rat endothelium produces an enhanced rate of superoxide anion generation [24]. It is important to underscore the fact that the superoxide is an initial oxygen radical species which may lead to secondary radicals or reactive oxygen species such as H_2O_2 or hydroxyl radicals [25]. The present study (FSEt with low dose of OHA's) indicates the significant improvement in endothelial dependent vasodilatation because the percentage relaxation produced by acetylcholine was more significant when these groups were compared with diabetic control. All the results are tabulated in (**Table 1, 2, 3 & 4**).

Table 1: Effect of FSEt and its combination with Oral Hypoglycemic Agents on OGTT in Normal rats

TIME	FBS	0 min	30 min	60 min	120 min
NC	84±1.1	103 .1±1.5	120.6±0.8 (↑16%)	114 .71 ±1.5 (↑10%)	86 .2± 0.6 (↓17%)
DC	205±2.5	214± 1.4	289 ±2.6 (↑35%)	288.3 ±3.0 (↑34.5%)	285.4±2.1 (↑33%)
FSEt	86.3 ±1.20	91 .33± 1.0	108.12± 4.1 (↑18.68%)	104.42±1.9 (↑14%) [a]	88 .90±0.8 (↓4%) [a]
P	85.6 ±1.20	93 .04 ±1.2	120.1±3.4 (↑29%)	11(↑20%)[c] 2.33±1.6	80.8 ±2.8 (↓3%) [a]
G	88.20 ±0.57	90.3 ±1.2	116.66 ±3.1 (↑28.8%)	102.12±0.6 (↑13%) [a]	84.99±0.5 (↓6%) [a]
P+G	89. 07 ±0.5	90.33± 0.5	106 ±0.5 (↑17.7%)	99 ±0.8 (↑9%) [a]	70.22± 0.9 (↓23%) [a]
P+ FSEt	86.1 ±0.5	90.32±0.88	110.9±0.9 (↑20%)	102 ±1.8(↑15%) [b]	85.77± 0.8 (↓5.1%) [a]
G+ FSEt	82.66± 0.3	86.66± 1.7	102.8±3.8 (↑15.9%)	96± 2.2 (↑11.6%) [a]	82.1± 0.7 (↓5%)[a]

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

Table 2: Effect of FSEt and its combination with Oral Hypoglycemic Agents on OGTT in Diabetic rats

TIME	FBS	0 min	30 min	60 min	120 min
NC	89.26 ±1.2	98.34± 1.9	104.1 ±2.0 (↑6%)	112.90± 0.9 (↑15.3%)	90.21± 2.1 (↑2.8%)
DC	188.06±3.9	212.11 ±0.8	249.2± 0.8 (↑17.5%)	298.14 ±0.8 (↑40%)	306.3±0.1 (↑44%)
FSEt	179.26 ±0.7	189.22±1.5	208.9± 3.3 (↑10.4%) [b]	222 ±1.2 (↑17%) [a]	186.1±1.7 (↓2%) [a]
P	184.43± 0.4	201.66 ±1.2	236.67 ±3.2 (↑17.4%) [c]	257.45 ±4.1(↑27%) [c]	212.7±5.0 (↑5.5%) [b]
G	197.26±0.9	203.67±2.1	229.88±2.4 (↑12.56%) [b]	243.5±4.3(↑20%) [a]	180±2.9 (↓12.4%) [a]
P+G	168.8.±0.5	174.29±2.3	198.74±0.8 (↑8%) [c]	208±1.9 (↑19%) [a]	143.1±1.8(↓18%) [a]
P+ FSEt	170.14±1.9	190.2±3.9 (↑11%)	211.67±4.0 (↑11%) [c]	248.15±2.8 (↑30%) [c]	194.8±1.8 (↑14%)[b]
G+ FSEt	182.88±1.5	189.12±1.7	209.37±2.2 (↑3%) [b]	219.76±2.1 (↑15%) [a]	187.4±0.6 (↓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 FSEt and its combination with Oral Hypoglycemics on Blood Glucose (mg/dl)

Groups	0th day	15th day	30th day	45th day	60th day	75th day
NC	124±0.8	123.2±0.12	126±0.24	128±0.5	122±0.5	123±0.8
DC	265±0.5	273.5±0.2	291.8±0.9	332.8±0.4	395.2±0.5	418±0.4
FSEt	241±0.1	248.4±0.8 [b]	235.8±0.5 [b]	228.4±0.6 [b]	219.4±0.5 [b]	139±0.9
P	245±0.8	252.5±0.5[c]	241.2±1.8 [c]	234.6±0.8 [c]	225.8±0.6 [c]	148±0.7
G	270±0.5	261±0.6 [c]	248.1±2.3 [c]	224.7±0.4 [c]	194.4±0.4 [c]	143±0.4
P+G	241±0.5	222.8±1.7 [a]	202.5±0.7 [a]	161.1±0.5 [a]	89.1±0.5 [a]	-
P+FSEt	251±0.7	237.8±0.5[b]	228.6±1.2 [b]	218.8±0.5 [b]	183.5±1.5 [b]	137±0.1
G+FSEt	262±0.6	244±0.4 [a]	229.4±0.8 [b]	194.7±0.6 [a]	164.7±0.8 [a]	131±0.1

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: Comparison of relaxant responses in treated groups:

TREATMENT	Percentage relaxant response to Acetylcholine			SNP
	0.1 (µM)	0.3 (µM)	(1.0 µM)	
NORMAL (Mean ±SEM)	28.35 ±3.458	32.37 ±3.876	56.56 ±4.467	101.00 ±1.98
DIABETIC (Mean ±SEM)	9.07 ±2.345	14.76 ±3.234	20.67 ±4.778	103.00 ±2.87
FSEt (Mean ±SEM)	17.61[b] ±0.253	21.45[b] ±0.773	24.88[b] ±0.197	105.00 ±3.12
PIO (Mean ±SEM)	12.82[c] ±0.287	16.52[c] ±0.545	20.01[c] ±0.345	102.00 ±1.87
GLIP (Mean ±SEM)	15.54[c] ±0.548	17.64[c] ±0.550	22.98[c] ±0.791	101.00 ±2.34
PIO+GLIP (Mean ±SEM)	16.21[b] ±0.454	22.81[b] ±0.657	35.87[b] ±0.965	103.00 ±3.05
FSEt+PIO (Mean ±SEM)	15.20[b] ±0.505	27.60[b] ±0.724	37.98[b] ±0.876	101.00 ±1.05
FSEt+GLIP (Mean ±SEM)	16.43[b] ±0.327	25.70[b] ±0.479	34.76[b] ±0.876	103.98 ±3.76
FSEt+FLU (Mean ±SEM)	14.54[a] ±0.347	24.74[a] ±0.733	36.34[a] ±0.879	101.00 ±1.89
P+FLU (Mean ±SEM)	12.86[a] ±0.291	20.87[a] ±0.539	32.90[a] ±0.678	103.00 ±2.67
G+FLU (Mean ±SEM)	14.70[a] ±0.305	23.53[a] ±0.783	38.57[a] ±0.375	102.00 ±2.23

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.

CONCLUSION

Endothelial dysfunction associated with insulin resistance appears to precede the development of over hyperglycemia in patients with type 2 diabetes mellitus. Therefore, endothelial dysfunction may be a critical early target for the prevention of atherosclerosis and CVD in patients with diabetes mellitus or insulin resistance. Therefore preventive therapy with combination of FSEt with OHA's (low dose) may prevent the endothelial dysfunction in diabetic patients (prediabetic state) due to free radical scavenging activity, metabolic control.

ACKNOWLEDGEMENT

The authors wish to thank Chairman, Executive Director, Gopalak vikas mandal; GHB College of Pharmacy, Aniyad for their facilities provided for this study.

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