COMPARISON BETWEEN FENUGREEK AND LOVASTATIN IN RESTORATION OF ENDOTHELIAL FUNCTION IN AN EXPERIMENTAL OLD RAT MODEL

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Abstract- The aim of this study was to compare the effectiveness of Fenugreek (Trigonella foenum-graecum) with that of lovastatin in restoration of endothelial function in the aorta taken from aged N-Mari rats. For this purpose, 4 groups of old N-Mari rats were used (n=6), normal saline treated control group, lovastatin (10 mg/kg, orally) and fenugreek seed powder in normal saline suspension (100 or 500 mg/kg) were administered orally daily for 8 weeks. The rate of relaxation of ephedrine-precontracted aorta to acetycholine, the lipid profiles, and histological examinations of the aorta were compared between these two groups and with a control non-treated normal saline treated group.

The results showed that treatment with lovastatin and fenugreek produced significant reduction in LDL, VLDL triglyceride and total cholesterol, while HDL was increased as compared to control non-treated group. Lovastatin induced an increase in contraction/mg tissue weight. However, improvement in endothelial function was significantly increased in all treatment groups. The histological findings showed significant reduction in thickness and lipid deposits in the aorta in all treatment groups. The improvement in the epithelial function was correlated with LDL-cholesterol lowering and partly with the reduction in the thickness of the aortic intimal layer. This study demonstrated that fenugreek is as effective as lovastatin in reducing the features associated with atherosclerosis.

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Key Words: Fenugreek, lovastatin, endothelial function

INTRODUCTION

Atherosclerosis is the underlying pathological process that leads to the most prevalent causes of death due to coronary artery disease, myocardial and cerebral infarction in the developing and developed world. The characteristic cellular components in atherosclerotic plaques are lipid-laid foam cells, which are transformed macrophages or smooth muscle cells that are filled with cholesteryl esters as a result of over-ingestion of fat. These cells represent the hallmark of atherosclerosis (1). The atherosclerotic plaque grows slowly over time which consequently produces a lesion composed of foam cells, collagen and fibrin which can occlude coronary vessels. Clinically, a rupture of the endothelium over an active plaque, leads to activation of platelets and formation of occlusive thrombi. Treatment of hyperlipidemia causes slow regression of plaques, which lead to reduction of acute cardiac events or coronary diseases (2). On the other hand, supply of oxygen to the myocardium is increased by the dilatation of coronary arteries via the release of endothelium derived relaxing factor (EDRF, nitric oxide). This is mediated by activation of the endothelium (3). Lowering the serum lipids by use of a variety of pharmacological means have shown to have indirectly reduced the number of cardiovascular events in various multi-centre studies. However, it seems that the main target for the treatments available is the restoration of the endothelial function (3).

Lovastatin, a cholesterol-lowering agent, one of the first 3-hydroxy-3 -methylglutary l-coenzyme reducatase (HMG-CoA) inhibitors, was found to improve endothelial-mediated vasodilator response in both animal (4) and human studies (5). It was selected, in the present study, as the positive control agent.

On the other hand, there is a growing global interest in the herbal and other forms of traditional medicine, and herbs have long been an important source of effective drugs (6). Fenugreek (*Trigonella foenum-graecum*, Leguminosae) seeds has been primarily used for its anti-diabetic and high triglyceride lowering activity. However, there is less agreement upon its anti-atherosclerotic property (7). The anti-lipidemic action has been linked to its steroidal saponins content, which interfere with both

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absorption and synthesis of cholesterol (8). Generally, fenugreek does not lower the beneficial HDL-cholesterol level, while its sugar lowering action is related to its high fiber content (9).

The aim of the present study was to compare the effectiveness of fenugreek seed with that of lovastatin in restoring the endothelial function of the aorta in old N-Mari rat, an experimental model that was found, in preliminary experiments in our laboratories, to be a suitable model for such studies.

MATERIALS AND METHODS

Animals. At the start of these experiments, 9-month-old rats of N-Mari strain (average weight 230 g) were used. They were purchased from Tehran Animal Centre. The animals were housed in groups of three in PVC cages, had free access to water and standard laboratory chew, and maintained at 27±5 °C and 60±10% humidity. The lighting cycle was on 12 hourly day/night bases and lighting conditions were between 7 a.m. to 7 p.m.

24 rats were used, and divided into four groups of 6 animals. The first group was used as controls to whom 1 ml of normal saline was administered orally. The second group, positive control, was administered orally, via a feeding syringe, 10 mg/kg lovastatin (Sermic, Mexico) in normal saline. The third and fourth groups were used as test groups to whom 100 or 500 mg/kg fenugreek seed in normal saline was administered orally via feeding syringe. All treatments were administered once daily for 8 weeks.

Experimental Procedure. At the end of the experimental period, an anaesthetizing dose of pentobarbital sodium was injected and a blood sample was withdrawn from the tail of each animal for biochemical evaluation of serum lipids. The animals were then sacrificed by exsanguination. The thoracic aorta was removed, and sections were used for both pharmacological and histological tests.

Biochemical investigations. A 2 ml blood sample was withdrawn from the tail of the anaesthetised rats, centrifuged at 2000 rpm for five minutes, and the serum was separated for measurement of the lipid profile: LDL, HDL, VLDL, total cholesterol and triglyceride levels, using standard laboratory kits (Pars Azmoon), using RA-1000, Technicon Auto-analyzer, USA.

Pharmacological investigations. The pharmacological experiments were performed using the classical endothelium-mediated vasomotor relaxation response to acetylcholine in an ephedrine-induced pre-contracted aorta (10). In order to perform the pharmacological evaluations, approximately a 4 mm length of the aortic ring was removed from the thoracic part, weighed and placed in an oxygenated Tyrode solution maintained at 37°C. The Tyrode solution had the following composition (%w/v): NaCl 0.9, KCl 0.02, $MgCl_2$ 0.01, $CaCl_2$ 0.02, NaH_2PO_4 0.005, $NaHCO_3$ 0.01, Glucose 0.1 (11).

The aortic rings were then connected to an isometric transducer (model F60) and connected to a 4-pen Darco chart recorder. The aortic rings were suspended under 1 g tension, by a stainless steel triangular wire, which was slowly and carefully inserted into the lumen without damaging the endothelial layer. The following protocol was adopted: following the equilibration period of 1 h and washing with fresh Tyrode solution every 15 min, the aortic rings were exposed to 1 μM final bath concentration of ephedrine hydrochloride. The plateau level of contraction produced was recorded for all groups, from which the level of contraction/mg wet tissue weight was calculated and statistically compared. This concentration was the submaximal concentration that produced 75% of maximum contraction in the majority of aorta tested in the preliminary experiments. Endothelium-mediated relaxation was induced by the addition of acetylcholine (0.1 mM). The time taken for relaxation to the baseline tension was measured, and from which the rate of relaxation min^{-1} mg^{-1} tissue weight was calculated and statistically compared.

Histological investigations. The remaining section of the aorta was immersed in a phosphate buffered solution for histological examination. Using 4-μm thick histological sections, the samples were stained with haematoxylin and eosin. Three histological samples were prepared from each animal. Using a simple bench microscope (Olympus CH2), equipped with a projection arm and with aid of a graded graticule, the thickness of the aorta was recorded. The details of the method used for calculation of the thickness had been reported previously (12).

Statistical analysis. The data obtained were expressed as mean ± standard error of the means (SEM). The means between the different groups were compared using analysis of variance (ANOVA) for multiple-group comparison. Where ANOVA showed

significance (P<0.05), between two groups, then further comparisons using Newman-Keuls test was carried out.

RESULTS

Biochemical findings. The results from serum lipids values are summarized in table 1. The mean serum level of LDL, VLDL, triglyceride and total cholesterol in control rats were 90.75± 5.3, 10.2± 0.58, 51± 2.9 and 162.9± 2.1 mg/dl respectively. Treatment with 100 mg/kg fenugreek seed powder suspension, reduced LDL to 53.3±2.7, VLDL to 5.6±0.5, triglyceride to 28.2± 2.4 and total cholestrol to 120± 1.2 mg/dl. Treatment with 500 mg/kg fenugreek, also significantly reduced the total cholesterol level and other serum lipids (Table 1). However, the mean HDL serum level was significantly increased in both 100 and 500 mg/kg fenugreek seed suspension (61.8±1, P<0.001 and 46.8±1 mg/dl P<0.05, respectively) compared to control old rats..

The results in the lovastatin treated group, showed that lovastatin (10 mg/kg orally, daily, for 8 weeks) reduced significantly (P<0.001) serum LDL, VLDL, triglyceride and total cholesterol to 48.3±7, 6.5±0.54, 32.3±3.7 and 140±1 mg/dl respectively. Conversely, serum HDL was significantly increased from 42± 0.7 in control group to 54±2.5 mg/dl (P<0.01) (Table 1).

The LDL/ HDL ratios were reduced from 2.16 in the control group to 0.89, 0.86 and 1.11 in lovastatin-, 100 and 500 mg/kg fenugreek-treated respectively.

Pharmacological findings. The general observation from the pharmacological investigations showed that the aorta taken from control rats had slower rate of relaxation per mg wet weight relative to aorta taken from treatment group. The mean submaximal contraction recorded with 1 μM ephedrine for aorta taken from the control rats was 175±6 mg min^{-1} mg^{-1} weight in the aorta taken from old rats, which was significantly (P<0.05) increased in lovastatin-treated group. In contrast, the level of contraction was non-significantly lower in fenugreek-treated group relative to control group (Table 2). On the other hand, the rate of relaxation in the aortic samples taken from 100 and 500 mg/kg fenugreek-treated rats were significantly faster (6.79±0.3 and 6.71± 0.5 $mg.min^{-1}mg^{-1}$ wet weight, respectivley, relative to 5.31± 0.9 $mg.min^{-1}mg^{-1}$ wet weight in the aorta taken from control rats (P<0.001)) (Table 2). The average weight of the aortic samples was 5.3 mg. The rate of relaxation recorded for the aorta taken from the lovastatin-treated group showed significant increase relative to control aged rats, 6.79 ± 0.3 mg $min^{-1}mg^{-1}$ wet tissue weight (P<0.001) (Table 2).

Table 1. The serum lipid profile of control, lovastatin (10 mg/kg), and fenugreek (100 and 500 mg/kg) treated old N-Mari rats

	Lipid type in mg/dl (SEM)				
Group	LDL	HDL	VLDL	TG	T. Chol
Control	90.75(5.3)	42 (0.7)	10.2 (0.58)	51 (2.9)	162.9 (2.1)
Lovastatin	48.3*** (4.35)	54** (2.5)	6.5*** (0.54)	32.3*** (2.7)	140** (1)
Fenugreek 100	53.3*** (2.7)	61.8***(1)	5.6*** (0.5)	28.2*** (2.4)	120.7*** (1.2)
Fenugreek 500	52.2*** (4.7)	46.8* (1)	6.3*** (0.5)	31.5*** (2.5)	105.3*** (2.1)

LDL= Low-density lipoprotein, HDL= High-density lipoprotein, VLDL= Very low-density lipoprotein, TG= Triglyceride, T. Chol= Total cholesterol

* P< 0.05 **P< 0.01 ***P< 0.001 relative to old control rats, n= 6, ANOVA followed by unpaired Newman-Keuls test

Table 2. The contractile responses to ephedrine (1 μM) and the rate of relaxation following administration of 10UM acetylcholine in the aorta taken from control, lovastatin (10 mg/kg), and fenugreek (100 and 500 mg/kg) treated old N-Mari rats (n= 6)

Group	Contraction/mg wet tissue weight Mean±SEM	Rate of relaxation in mg tension/min. mg tissue weight Mean±SEM
Control	175±6	5.31±0.9
Lovastatin	202*±4.2	6.79**±0.3
Fenugreek 100	164♦±3.6	6.33**±0.3
Fenugreek 500	146♦♦±5	6.71**±0.5

* P< 0.05 and **P< 0.01 relative to control normal saline treated group, ♦P<0.05 and ♦♦P< 0.01 relative to lovastatin-treated group

Histological findings. The general observation for the aorta taken from the control rats showed circular arrangement, and a thicker medial layer compared to treatments groups (Table 3). In addition, there were distinct areas of splitting due to intracellular lipid deposits within the intimal layer and extracellular foaming cells in the outermost layers (Photomicrograph 1A). The mean thickness of the aorta of the control rats was 0.95±0.017 mm. This was thicker and highly significant (P<0.001), compared to 0.088±0.016 mm in aorta taken from lovastatin-treated rats (photmicrograph 1B). While in the 100 and 500 mg/kg fenugreek-treated groups, the mean thickness was 0.89±0.016 and 0.81±0.015 mm, showing a significant reduction relative to aorta taken from control untreated rats (P<0.05 and <0.001 respectively) (Photomicrographs 1C and 1D).

Table 3. The mean±SEM of the thickness (mm) of the aorta taken from control, lovastatin (10 mg/kg) and fenugreek (100 and 500 mg/kg) treated old N-Mari rats

Group	Thickness in mm
Control normal saline treated	0.95±0.017
Lovastatin (10 mg/kg, daily, 8 weeks)	0.88**±0.016
Fenugreek (100 mg/kg, daily, 8 weeks)	0.89*±0.016
Fenugreek (500 mg/kg, daily, 8 weeks)	0.81*♦***±0.015

*P< 0.5, **P< 0.01 and ***P< 0.001, relative to control group, ♦P< 0.05 relative to lovastatin-treated group

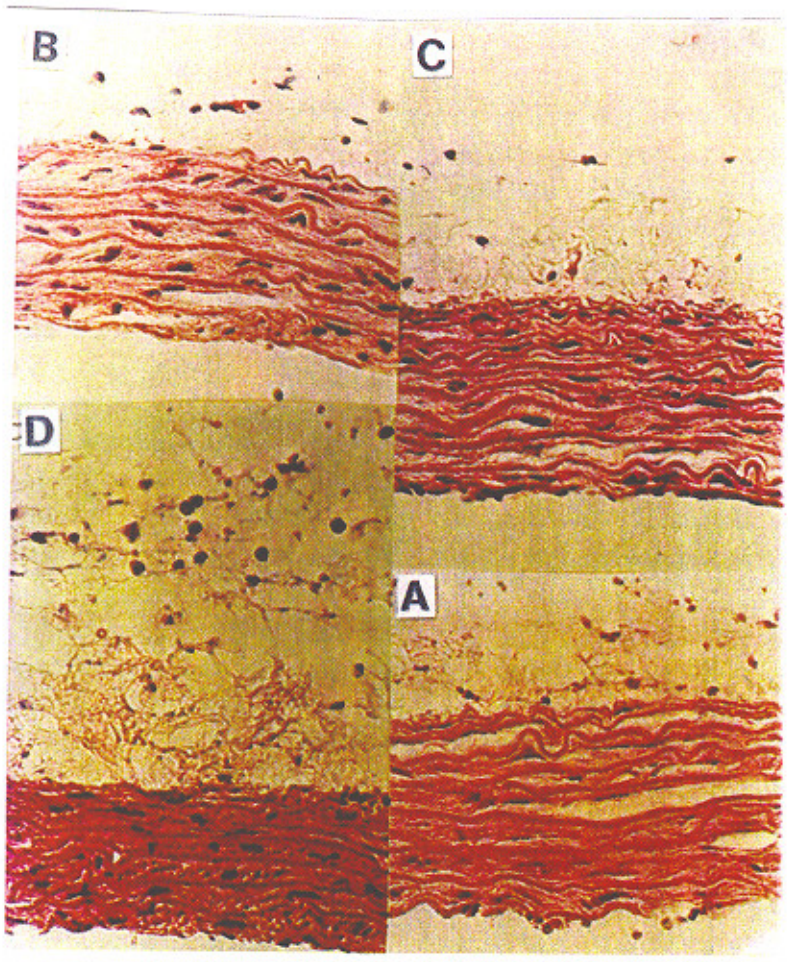

Fig. 1. A cross-sectional illustration of haematoxylin and eosin stained aortic samples taken from old rat (A), 10 mg/kg lovastatin (B), 100 and 500 mg/kg fenugreek-treated (C and D, respectively) (All× 336). For details refer to the text

DISCUSSION

The present study demonstrated that LDL-cholesterol level, LDL/HDL ratio, aortic endothelial dysfunction and the increased thickness with widespread splitting within the aortic intima, which are characteristics of old age and probably atherosclerosis were significantly improved by both the HMG-Co A reductase inhibitor lovastatin and fenugreek seed powder.

The endothelium is an important modulator of vasomotor tone through the release of endothelium derived relaxing factor (nitric oxide). Nitric oxide is released by endothelium upon stimulation by various endogenous substances including acetylcholine (13). In atherosclerosis, the integrity of this pathway is impaired (14, 15). This may be due to direct injurious effects of elevated oxidized LDL cholesterol on the endothelial cells (5). In clinical studies, vasoconstriction responses may play a part in the pathogenesis of ischemia (5), in which paradoxical vasoconstriction was obtained following challenge with acetylcholine. In animal studies similar observations were reported in both *in vivo and* in *vitro* (16,17) conditions. The present study has shown that lovastatin and fenugreek at both 100 and 500 mg/kg significantly improved the vasorelaxatory responses to acetylcholine. This action, was found to be correlated with their cholesterol lowering and partly with the reduction of aortic intimal thickness. It is not possible to suggest, from these findings, whether this improvement was reversal to complete normalization, but at least it can be suggested it attenuated the endothelial function. Whether longer period of drug administration would result in more improvement in the dilator responses need to be tested. However, the results from this study are encouraging, considering the short period of 8 week treatment of this study. Furthermore, the results from the two doses of fenugreek used did not show a significant dose dependent effect in the both cholesterol lowering and intimal thickness, suggesting 100 mg/kg daily dose to be sufficient for such studies. Especially noteworthy was that the HDL level did not increase with increase of the dose of fenugreek, the reason for this unexpected observation could not be explained from the findings of this study, and further work is needed to elucidate this aspect. Perhaps higher serum concentration of active components of fenugreek seem to induce inhibitory effects upon enzymes is involved in the synthesis of this useful lipid.

There is accumulating evidence to suggest that oxidation of LDL particles is important in the pathogenesis of atherosclerosis and endothelial dysfunction (18). This is consistent with the theory that by-products from oxidation environment, possibly oxidized LDL, destroy nitric oxide, account for the diminished endothelium-dependent vasodilatation. Perhaps, both lovastatin and fenugreek via reduction of LDL and increase in HDL level together with reduction in foam cells within the intimal layer prepare the ground for effective reduction of production and/or scavenging of free radical action and restoration of endothelial function (19).

Despite these similarities between lovastatin and fenugreek, there were some differences which deserve consideration. The extent of contraction following lovastatin treatment was found to be significantly increased relative to fenugreek which was reduced relative to control group. The importance of this difference needs further investigation. On the other hand, the rate of relaxation, relative to control goup, in the lovastatin-treated group was significantly greater. In contrast, the extent of reduction of aortic thickness was non-significantly greater in the fenugreek-treated group. This finding partly agrees with the suggestion that normalization of endothelial function may be independent of its morphological change (20). However, the overall observations suggest parallel cholesterol lowering with that of morphological changes. The relative contribution of each of these changes on the improvement of endothelial function can not be separated on the basis of our findings. However, it may be assumed that the more reduction in the lipid-filled foam cells can reduce the amount of oxidizeable substrates within the wall of the aorta and hence less free radical production (21).

The results from the measurements of the lipid levels in old control rats showed normal lipid levels. However, the overall results, using both lovastatin and fenugreek seed powder, demonstrated that these drugs are effective in alleviating the features associated with atherosclerosis namely the reduction in lipid levels, restoration of endothelial function and reduction in the thickness of the aorta. There is a possible additional advantage that is inherent in fenugreek, besides being as effective as lovastatin in reducing the features associated with atherosclerosis, it has an established primary use as an antihyperglycemic agent (9). Whether more beneficial effects can be obtained with fenugreek in cases where both of these metabolic abnormalities (atherosclerosis and diabetes) occur simultaneously, need to be tested.

It is now clear that cholesterol lowering has beneficial effect in the coronary artery disease and changes in the functional status of the arterial wall can go hand in hand with the reduction in degree of stenosis. What is the clinical relevance of these findings? Although we recognise that extrapolation of data from animal studies to man should be very guarded, the present study demonstrated that cholesterol lowering using lovastatin and fenugreek in this experimental model, not only was beneficial in restoring endothelial function, but also was accompanied with significant regression of atherosclerotic plaque in the wall of the aorta. This observation suggests that other factors beside reduction in the LDL lowering may contribute to the beneficial improvement in the endothelial function of anti-lipidemic agents, the significance of each of these effects deserves further investigation.

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