ANTI-HYPERLIPIDEMIC ACTIVITY OF FENUGREEK (*TRIGONELLA FOENUM-GRAECUM*) SEEDS EXTRACT IN TRITON AND HIGH FAT DIET INDUCED HYPERLIPIDEMIC MODEL: A POTENT ANTI-ATHEROSCLEROTIC AGENT

Saxena B.[1*] and Saxena U.[2]

[1]Pharmacology Research lab, Department of Pharmaceutics, Meerut Institute of Engineering and Technology, Meerut-250005, India (* Author for correspondence)

[2]Department of Biotechnology, Indian Institute of Technology Guwahati, Guwahati-781039, Assam, India

Summary

Lipid lowering effect of fenugreek seed extract was evaluated in triton and high fat diet induced hyperlipidemic models of albino rats. Aqueous seed extract of fenugreek (120 mg/kg, *p.o.*) inhibited the elevation in plasma cholesterol in triton administrated rats. The aqueous seed extract at the same dose level significantly attenuated the elevated plasma total cholesterol, triglycerides, lipoprotein cholesterol (HDL, LDL and VLDL) in high-fat diet-induced hyperlipidemic rats. It also found to improve the atherogenic index (AI) in high fat diet model. The standard dose of Atrovastatin in the triton and Nanaka Gugullu in the high fat diet induced hyperlipidemic rats showed slightly better effects.

Keywords: Fenugreek (*Trigonella foenum-graecum*); Anti-hyperlipidemic; High-fat diet; Triton WR 1339; Atherosclerosis.

Author for correspondence
Email id: bsaxenapharm@gmail.com

Introduction

Hyperlipidemia is elevated level of triglycerides and cholesterol. It is one of the most common complications in increasing the risk of premature atherosclerosis, coronary and myocardial infarction [1], which in turn are major causes of cardiovascular (CV) morbidity and mortality [2, 3, 4]. Hyperlipidemia and reduced high-density lipoproteins (HDL-C) occur as a consequence of several interrelated factors that may be lifestyle, genetic, metabolic or other conditions that influence plasma lipoprotein metabolism [5]. The current anti-hyperlipidemic therapy includes principally statins and fibrates. The statins acts by inhibiting the biosynthesis of cholesterol while fibrates acts by enhancing the clearance of triglyceride rich lipoproteins [5]. The investigation of lipid lowering activity of natural products will be a useful strategy in the discovery of new lead molecules eliciting improved activity with fewer side effects. The plants extract maintaining the lipid metabolism can be used in treating hyperlipidemia of varied etiology. Additionally, drug having favorable effect on lipid profile would be beneficial in the treatment of lipid abnormalities and the accompanying premature atherosclerosis of cardiovascular disease.

Fenugreek (*Trigonella foenum-graecum*) is known as 'Methi' in Hindi and commonly used as a spice in cooking. Fenugreek is cultivated in India, Egypt, Middle East and North Africa. The seeds of the plant have been used as a traditional remedy for conditions including gastrointestinal disorders, gout, inflammation, hyperlipidemia and diabetes. Its seeds are used for their wound healing, carminative, tonic and aphrodisiac effects. Moreover, *Trigonella foenum-graecum* seeds and leaves are also said to have anti-diabetic activity [6]. In recent studies, it has received much scientific attention as a potential source of steroidal sapogenins. The seeds of *Trigonella foenum-graecum* contain the alkaloid trigonelline with mucilage, tannic acid, yellow coloring matter, fixed and volatile oils and a bitter extractive, diosgenin, gitogenin a trace of trigogenin and Vitamin A [7]. A curative dose of *Trigonella foenum-graecum* seeds also produces antiulcer and hypolipidemic effects [8]. However no study has been conducted on its preventive activity against hyperlipidemia and atherosclerosis. Thus in our present investigation the extract of seed of fenugreek are studied for its protective activity against hyperlipidemia and atherosclerosis.

Materials and Methods

Animals. Albino rats of both sexes were purchased from Central Drug Research Institute, Lucknow, Uttar Pradesh, India. The body weights of the animal ranged between 175 and 225 g. The rats were housed in polypropylene cages (one in each cage) at an ambient temperature of 25 ± 2 ^{o}C and 55-60 % relative humidity. The animals were acclimatized to in-house conditions and were fed a commercial pellet diet (Hindustan Lever Limited, Bangalore, India) and water ad libitum. Experimental protocol was undertaken in accordance with "Principles of laboratory animal care" (NIH publication number 85-23, revised 1985) guidelines.

Preparation of Fenugreek Seed Extracts. Dried and fresh batches of fenugreek seeds were washed in distilled water, dried and ground to fine powder. Twenty-five grams of the powder of fenugreek was taken and suspended in 500 ml of distilled water and kept for 16 hrs at 37^{0}C under stirring conditions. Finally the solution was taken and centrifuged at 12000 rpm for 10 min. The supernatant was obtained and was stored at 4ºC in refrigerator. Azeotropic mixture of the extract was made with methanol and acetic acid. This mixture was subjected to vacuum evaporation for 16 hrs. The dried material was re-dissolved in appropriate amount of sterile distilled water to get the appropriate concentration.

Triton induced hyperlipidemic model [9]. Adult male albino rats (both sex) were divided into 4 groups of 6 animals each. Group-1(vehicle control) received 0.3% w/v carboxy methyl cellulose (CMC) orally for one week. Group 2 and 3 were treated orally with 0.3% w/v CMC and the aqueous extract of fenugreek seed (120 mg/kg) (AEF-120) respectively for one week. Group 4 received atrovastatin, 1 mg/kg body weight once daily for one week. On seventh day, 200 mg/kg Triton WR 1339 (isoocctyl polyoxyethylene phenol) was injected (*i.p.*), to all the groups except 1st group immediately after drug administration. Blood was withdrawn from retro-orbital sinus for the estimation of plasma total cholesterol on seventh day after 24 hr of triton administration. Plasma was separated in cooling centrifuge (REMI, C24) by centrifuging at 2500 rpm for 10 min and plasma cholesterol was estimated [10].

Preparation of High fat diet. The composition of the high fat diet was adapted from Yugarani et al. [11]. The entire ingredient was individually weighed; wheat flour, milk powder and cholesterol were mixed well. Thereafter, yeast powder, sodium chloride and water were added to the above mixture. Dough was made and pellets of high fat diet were prepared using a manually

operated pelletizing machine. The pellets were baked at 100 ^{o}C for 3 hr and restored in airtight containers.

High fat diet model. The rats (of both sexes) were divided into four groups each comprising six animals. The rats of 1st group received normal diet and served as control. High fat diet (15 g/rat) was given to the rats of 2nd group (hyperlipidemic control) for seven weeks. The aqueous seed extract of fenugreek (120 mg/kg, *p.o.*) and an ayurvedic preparation containing Commiphora mukkkul, Navaka guggulu churna (400 mg/kg, *p.o.*) (NK-400) [12] as a reference were administered once daily to rats of 3rd and 4th groups respectively along with the high fat diet for seven weeks. After 7 weeks, the blood was collected from all the rats through the retro-orbital plexus under light anesthesia.

Biochemical estimations. The plasma was separated by centrifuging blood for 10 min at 2500 rpm and processed for estimation of plasma cholesterol [10], triglycerides [13] and plasma lipoproteins [14]. The atherogenic index was calculated according to Nikkila and Kekki, [15].

Statistical analysis. All the values of the experimental results were expressed as mean ± SEM (Standard Error Mean). Statistical analyses were performed by one way analysis of variance (ANOVA) followed by Tukey test. GraphPad Prism software was used for all statistical analyses.

Result and Discussion

The systemic administration of the surfactant triton to rats resulted in an enormous elevation of plasma cholesterol at 24 hr (Table 1). The aqueous seed extract of fenugreek (120 mg/kg, *p.o.*) showed a 20.42 % reduction in plasma cholesterol level in triton induced hyperlipidemic rats compared with untreated animals (Table 1). However, atrovastatin showed a 34.74 % reduction in plasma cholesterol level. Atrovastatin was employed as the standard drug in triton induced model as its lipid controlling mechanism is through inhibition of synthesis of cholesterol in the liver. Triton administration first causes sharp increase in plasma cholesterol level (phase I) followed by decreased hypercholesterolemia nearly to the control levels (phase II). The mechanism of the Triton induced hypercholesterolemia in phase I is thought to be due to increased hepatic synthesis of cholesterol through the ability of triton to interfere with the uptake of plasma lipids by the tissues [9].

Drugs interfering with cholesterol biosynthesis were shown to be active in phase I, while those interfering with cholesterol excretion and metabolism were active in phase II triton induced hyperlipidemia [16, 17, 18, 19, 20]. The treatment with atrovastatin resulted in a slightly better effect than fenugreek. These results indicate that fenugreek seed extract may interfere with cholesterol biosynthesis as triton accelerates the hepatic synthesis of cholesterol [21].

Table 1: Effect of aqueous seed extract of fenugreek (AEF-120) in triton induce hyperlipidemic model

Groups	Plasma Cholesterol (mg/dL)	Reduction (%)
Control	102.11±8.31	--
Triton 200 mg/kg	498.13 ± 2.4*	--
AEF-120	396.41 ± 2.3$^{*@}$	20.42
Atrovastatin (1 mg/kg)	325.08 ± 10.6$^{*@a}$	34.74

Value are expressed as Mean ± SEM (n=6). $^{*}P$<0.05 compared with Control. $^{@}P$<0.05 compared with triton treated group. ^{a}P<0.05 compared with AEF-120 (One way ANOVA followed by tukey test).

Table 2: Effect of aqueous seed extract of fenugreek (AEF-120) on the body weights in HFD model.

Group	Body weight (gm.)		
	Initial weight	After 4 weeks	After 7 weeks
Control (Normal feed)	221.12 ± 8.13	236.47± 7.03	254.15±8.66
HFD	208.97 ± 4.28	306.36±4.72*	389.51±7.21*
AEF-120	196.28± 10.39	249.73±8.95$^{@}$	269.62±8.11$^{@}$
Nanaka Guggulu	201.22±6.12	247.52±3.15$^{@}$	262.99± 6.81$^{@}$

Value are expressed as Mean ± SEM (n=6). *P<0.05 compared with Control. $^{@}$P<0.05 compared with HFD group (One way ANOVA followed by tukey test)

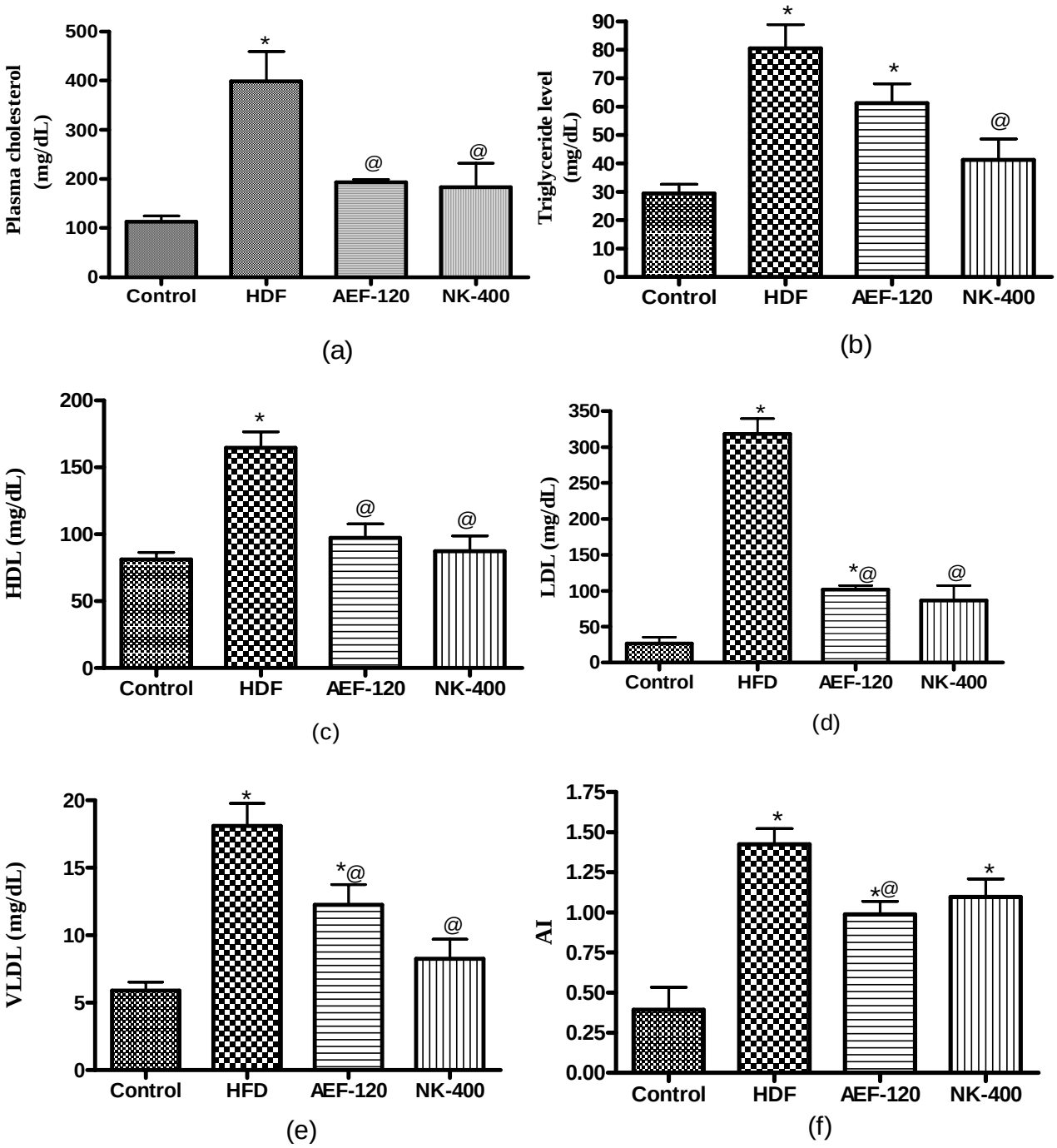
Triton induced hypercholesterolemia, though simple and rapid for evaluating hyperlipidemic compounds, is rather artificial. Hence the lipid controlling potential of fenugreek seed extract was further validated in high fat diet-induced hyperlipidemic rat model. Feeding the high fat diet (HFD) to the animals for 7 weeks was found to increase the body weight (Table 2), plasma cholesterol, triglycerides, lipoproteins (HDL LDL and VLDL) significantly ($P<0.05$) (Fig 1a-e). Administration of the aqueous extract of fenugreek along with a high fat diet to rats showed a significant decrease ($P<0.05$) in HDF induced increased body weight, total cholesterol, HDL, TG, LDL, VLDL to that of control body weight (Fig 1a-e). Additionally HDF increased the AI and aqueous extract of fenugreek decreased the HDF induced increase in AI (Fig 1f).

Elevated circulating lipid levels may be the outcome of inhibitory effect of high dietary fat intake on lipogenesis [22]. The treatment of hyperlipidemic rats with fenugreek seed extract brought down the elevated plasma total cholesterol and triglycerides. It improves the HDL-C levels as shown by reduced atherogenic index (Fig 1.f.).

Similar to Nanaka Guggulu, the standard drug used, the extract may have enhanced the breakdown of lipids, thus modifying the altered lipid metabolism induced by high fat-diet. Increase in HDL levels shows the intensive conversion of LDL to HDL and clearance of circulating lipids. A significant reduction in the atherogenic index in fenugreek seed extract treated group demonstrates the protective efficacy of the extract against atherogenesis. Consequently the lipid regulating efficacy of fenugreek seed extract would be beneficial in the prevention of plaque formation leading to atherosclerosis and congestive heart failure.

The hypolipidemic effect of fenugreek is might be largely due to its high content of soluble fiber, which acts to decrease the rate of gastric emptying thereby delaying the absorption of lipid from the small intestine. The finding of the study reveal that the seed extract of fenugreek can effectively control the blood levels in dyslipidemic conditions by interfering with biosynthesis of cholesterol and utilization of lipids.

Fig. 1. Effect of aqueous extract of fenugreek on plasma cholesterol (a), triglyceride (b), HDL (High density lipoprotein) (c), LDL (low density lipoprotein) (d), VLDL (very low density lipoprotein) (e), AI (Atherosclerotic Index) (f) in rats High Fat diet model. Values are expressed as Mean ± SEM (n=6). *P<0.05 compared with Control. $^{@}$P<0.05 compared with HFD group (One way ANOVA followed by Tukey test).



Conclusion

Aqueous extract of fenugreek seed have lipid lowering effect in triton and high fat diet model of hyperlipidemia. Therefore it will be beneficial in the treatment of lipid abnormalities and the accompanying premature atherosclerosis of CV disease.

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