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Original Article

Physiology Section

Hypolipidemic Effects of Fenugreek and Atorvastatin-Comparative Study on High Fat Fed Dyslipidemic Rats

HEMAVARDHINI CHENNAPPAN SEKAR[1], GV LATHADEVI[2]

ABSTRACT

Introduction: Dyslipidemia is the current medical problem of utmost concern with an increased prevalence among the males between 31-40 years. Various extracts from fenugreek (*Trigonellafoenum-graecum*), methi (in Hindi), have known to have effect on the metabolic parameters, lipid lowing properties being one of them.

Aim: The objective of this study was to evaluate the efficacy of fenugreek, one of the commonest Indian spice in lowering the serum lipid levels and comparing its efficacy with lipid lowering properties of a pharmacological agent-atorvastatin so that it could be incorporated as a part of life style modifications among the dyslipidemic individuals.

Materials and Methods: Twenty four adult male Wistar rats were divided equally into four groups. Group A was kept as the control and fed on normal diet. Hyperlipidemia was induced in Groups B, C and D with high fat diet containing groundnut oil cake and dried coconut for 12 weeks. The hyperlipidemic rats were then subjected to the various hypolipidemic regimens. Group B- 2mL emulsion of fenugreek (1 gm/kg/day) orally, Group C- 2mL emulsion of atorvastatin (30 mg/kg/day) orally, Group D- 4mL emulsion of fenugreek and atorvastatin combined. The serum samples were analysed for lipid profile at the beginning and end of four weeks. The statistical analysis for the data collected was done using One-way Anova analysis in the software- SPSS Version 20.0.

Results: Administration of fenugreek to hyperlipidemic rats (Group B) showed a significant reduction of serum triglycerides, cholesterol and body weight ($p<0.05$) and a significant increase in the serum High Density Lipoprotein (HDL) levels ($p<0.05$). The serum Low Density Lipoprotein Cholesterol (LDL-C) values showed a statistical reduction in Group C ($p<0.01$) administered with atorvastatin monotherapy. In Group D the combination therapy with fenugreek and atorvastatin showed a statistical significant increase of High Density Lipoprotein Cholesterol (HDL-C) $p<0.01$.

Conclusion: Fenugreek is proved to be a potent hypolipidemic agent. Lifestyle modification with dietary supplement of fenugreek can be taken as an effective hypolipidemic element initially to combat the increasing risks of dyslipidemia. If they do not respond then the individuals can switch over to pharmacological agents like atorvastatin along with fenugreek to control dyslipidemia.

Keywords: Dietary supplement, Lifestyle modification, Metabolic disorder, Statins, Trigonellafoenum-graecum

INTRODUCTION

Dyslipidemia as defined by the abnormal levels of lipid parameters such as LDL-C, triglycerides, HDL-C and atherogenic indices in the blood, is the current medical as well as social problem of utmost concern leading to increasing morbidity and mortality. Studies have shown that regional disparity exists in India with the highest rates of hypercholesterolemia observed in Tamil Nadu (18.3%) among which the urban residents had the highest prevalence of lipid abnormalities compared to rural residents [1,2]. Studies have also revealed that dyslipidaemia is more common among the males aged 30-40 years leading to increased prevalence of young infarcts due to coronary artery disease in this group [3,4]. Current AACE guidelines on the prevention and management of dyslipidemia suggest that it is the major prerequisite for the development of coronary artery disease and may even be the primary risk factor occurring before other major risk factors comes into play [5].

Several methods for combating this life threatening metabolic disorder have been emphasized upon in various researches in the recent times. The armamentarium for managing it now includes “statins” that can decrease the LDL-C levels by up to 55% [6]. Statins are hypolipidemic agents with anti-atherosclerotic properties [7]. A study on comparison of safety and efficacy among statins showed that atorvastatin is more effective when compared to simvastatin and pravastatin in patients with hyperlipidemia [8,9]. Multiple clinical trials conducted across the world show that a significant number of patients on statins continue to have a high residual risk of coronary artery disease [10,11]. The various adverse effects associated with the statin group of drugs provide a clinical challenge in management of dyslipidemia with such pharmacological modalities.

The modern medicine draws its potential from the rich legacy of traditional medicine. Fenugreek (*Trigonellafoenumgrae*-cum) is one of the oldest medicinal plants, dating back to the Hippocrates and ancient Egyptian times and known to have immense therapeutic value as proven by several experiments on animal and human models [12]. It is also said to have a high levels of iron hence it can be an useful supplement in iron-deficient patients [13]. In vivo studies using fenugreek extracts has an inhibitory effect on the growth of cancer cells and reduces inflammation [14]. The other beneficial nutritive effects of fenugreek seeds include hypolipidemic and anti-diabetic effects [15]. Lipid lowering effects of fenugreek have been attributed to the various chemical extracts such as ethyl acetate extract which was found to have-Naringenin, the abundant flavonoid compound found to have significant hypocholesterolemic effects [16].

This study was aimed at determining the hypolipidemic potential of fenugreek in comparison to the pharmacological agents like atorvastatin in order to include fenugreek as part of the dietary modification in the hypolipidemic regimen taken up by the dyslipidemic individuals.

MATERIALS AND METHODS

A randomised control study was carried out in the animal facility of PSG Institute of Medical Sciences and Research (PSGIMSR), Coimbatore, Tamil Nadu, India, during the period of June 2014 to August 2014. The study was approved by the Institutional Animal Ethics Committee (IAEC) proposal number: 244/2014/IAEC and experiments were carried out according to the guidelines of CPCSEA, New Delhi. Twenty four healthy, six-month-old male Wistar rats, weighing 200-300 gm, were included in the study. Irritable and overweight rats were excluded. They were housed in steel cages under controlled temperature (22±2^{0}C) and humidity (55±5%) with a fixed 12 hours light-dark cycle. The rats were allowed to acclimatize for three days, and they were randomly divided into 4 groups-control Group A (n=6) Group B (n=6), Group C (n=6) and Group D (n=6)

Initial blood samples were taken by anesthetizing them using ether. The tail end was nipped and 2ml of blood was milked from each rat. The serum was separated by centrifuging the blood samples at 3000 revolutions for 5 minutes. The lipid profile parameters were total cholesterol, triglycerides, LDL-C, HDL-C, Very Low Density Lipoprotein (VLDL-C) were estimated by enzymatic calorimetric analysis.

High fat diet was prepared using dried coconut and ground nut oil cake. In the proportions such that for every 30 gm of rat feed per day, 12 gm of fat ingredients and 300 gm Vanaspati ghee as a cholesterol ingredient was added and mixed with the normal rat chow using water as binding agent. The mixture was then made into pellets and sterilized in microwave oven. The feed was prepared freshly once in two days. Group A was the control group and was fed on normal rat chow. Groups B, C and D were fed on the high fat diet for 12 weeks [17].

At the end of 12 weeks the serum samples were analysed for all the lipid profile parameters mentioned above to confirm the induction of hyperlipidemia. The fenugreek seeds were first ground into fine powder and weighed according to 1 gm/kg body weight of the rats. It was then emulsified in distilled water. Group B was administered with a 2 mL emulsion of fenugreek orally. In view of the small body size of rats, the whole fenugreek seeds were used instead of their extracts, the dosing regimen for fenugreek administration has been adjusted for the convenience of this study.

Group C was administered with a 2 mL emulsion orally containing 30 mg/kg of body weight of atorvastatin per day and Group D containing 4 mL emulsion containing fenugreek and atorvastatin in the combined form. The regimen was followed for four weeks [18,19].

At the end of four weeks the serum samples were collected and the lipid profile was analysed. The parameters analysed were, total serum cholesterol, serum triglycerides, LDL -C, HDL-C, VLDL- C, and LDL-C/HDL-C and total cholesterol/HDL ratio (atherogenic index) was calculated.

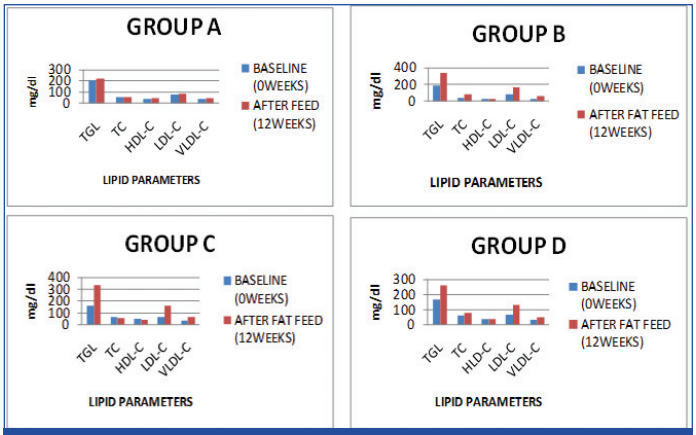
STATISTICAL ANALYSIS

Results were expressed as mean±standard deviation. The mean values of analyses between the different groups were compared using One-Way ANOVA analysis using the software (statistical package for social sciences) SPSS version 20.0. The p-valve of less than 0.05 was considered significant.

RESULTS

The mean values of the lipid parameters of Group A, B, C and D were analysed at baseline and following 12 weeks of their respective feeds. Group A fed on normal feed showed static lipid parameters whereas Groups B, C and D showed significant increase in their lipid parameter values following the high fat feed [Table/Fig-1a-d].

The mean values of the lipid profile parameters in Group B (fenugreek -FG) animals at 12 weeks of high fat feed and at four



[Table/Fig-1]: (A) Comparison of lipid values pre and post normal diet in Group A. (B) Comparison of lipid values pre and post post high fat feed in Group B. (C) Comparison of lipid values pre and post post high fat feed in Group C. (D) Comparison of lipid values pre and post post high fat feed in Group C.

weeks following fenugreek administration was analysed. The values after four weeks with Fenugreek Seeds (FG) in high fat fed rats indicated a significant ($p<0.05$) reduction in serum total cholesterol and triglycerides. The LDL-C, VLDL-C and atherogenic index also showed a significant ($p<0.01$) reduction in this group of fenugreek fed rats. After fenugreek therapy the HDL-C showed a significant ($p<0.05$) increase in them [Table/Fig-2].

On analysing the lipid profile parameters after four weeks of atorvastatin in Group C rats and the lipid parameter profiles of Group D rats on combination therapy of both atorvastatin and fenugreek, the following results were obtained. There was a statistical ($p<0.01$) reduction in the total cholesterol, triglycerides, VLDL-C, HDL-C/LDL-C ratio and Total cholesterol/HDL-C ratios in both the groups. The LDL-C values showed a statistical reduction in Group C ($p<0.01$), and Group D ($p<0.05$) respectively. The HDL-C values also showed a statistical increase in Group C ($p<0.05$) and Group D ($p<0.01$) respectively [Table/Fig-3,4].

Comparison of the lipid parameters among the three groups B, C, and D fed on fenugreek, atorvastatin and combination of fenugreek

Lipid Parameters Group B	After Fat diet (Week 12)	After Fenugreek (Week 4)
Triglycerides (mg/dL)	88.4±41.3	65±40*
Total cholesterol (mg/dL)	333.3±87.5	225±121.4*
HDL-C (mg/dL)	31.5±13.9	33.6±13.2*
LDL-C (mg/dL)	169.7±96.12	90.28±67.8**
VLDL-C (mg/dL)	66.66±26.58	45±24.2**
LDL/HDL ratio	4.5±3.9	2.1±2.1**
Total Cholesterol/HDL ratio	2.3±0.7	1.7±0.7**

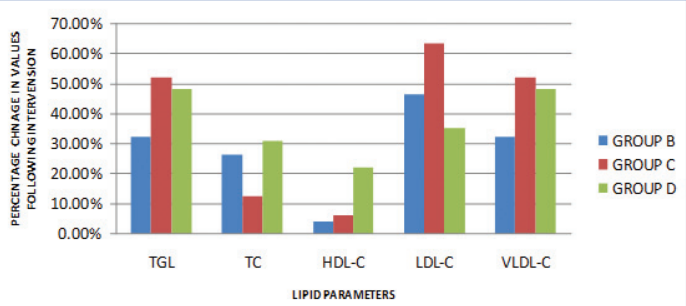
[Table/Fig-2]: Comparison of lipid parameters pre and post fenugreek administration in Group B.
*p<0.05, **p<0.01, there is a significant difference in the lipid parameters.HDL – High Density Lipoprotein, LDL- Low Density Lipoprotein, VLDL- Very Low Density Lipoprotein

Lipid Parameters Group C	After Fat diet (Week 12)	After Atorvastatin (Week 4)
Triglycerides (mg/dL)	62.2±21.7	52.3±24.5**
Total cholesterol (mg/dL)	333.3±132.9	158±37.6**
HDL-C (mg/dL)	47.1±23.4	49.2±23.5*
LDL-C (mg/dL)	161±54.1	58.7±28.5**
VLDL-C (mg/dL)	66.6±17.5	31.6±7.5**
LDL/HDL ratio	5.7±2.7	1.9±1.3**
Total Cholesterol/HDL ratio	2.3±0.9	1.6±0.8**

[Table/Fig-3]: Comparison of lipid parameters pre and post atorvastatin administration in Group C.
*p<0.05, **p<0.01, there is a significant difference in the lipid parameters. HDL – High Density Lipoprotein, LDL- Low Density Lipoprotein, VLDL- Very Low Density Lipoprotein

Lipid Parameters Group D	After Fat diet (Week 12)	After Fenugreek + Atorvastatin (Week 4)
Triglycerides (mg/dL)	81.4±18.1**	56.2±20.5**
Total cholesterol (mg/dL)	258.3±120**	133.3±112.5**
HDL-C (mg/dL)	37.6±19.3*	48.4±25.1**
LDL-C (mg/dL)	132.33±90.48**	85.34±81.25*
VLDL-C (mg/dL)	51.66±24**	26.66±22.5**
LDL/HDL ratio	5.3±5**	2.7±3.3**
Total Cholesterol/HDL ratio	2.8±1.7**	1.6±1.3**

[Table/Fig-4]: Comparison of lipid parameters pre and post combination therapy administration in Group D.
*p<0.05, **p<0.01, there is a significant difference in the lipid parameters.HDL – High Density Lipoprotein, LDL- Low Density Lipoprotein, VLDL- Very Low Density Lipoprotein.



[Table/Fig-5]: The comparison of the lipid profile parametersbetween Group B, Group C and Group D following four weeks of interventional hypolipidemic diet.
TGL-Triglycerides, TC-Cholesterol, HDL – high density lipoprotein, LDL- low density lipoprotein, VLDL- very low density lipoprotein

Groups	Base Line Weight (0 Weeks)	After High Fat Diet (12 Weeks)	After Hypolipidemic Regimen (4 Weeks)
A	333.1±18.4	338.3±18.2*	342.16±16
B	281±41.6	355.8±27.3**	347.6±31.8*
C	311.3±21.3	321.5±24.7**	306.6±15.6
D	271.8±13.8	323±18.8**	305.5±41.5**

[Table/Fig-6]: Average weight of the rats at different stages of the experiment.
*p<0.05, **p<0.01

and atorvastatin showed that fenugreek significantly contributed in the reduction of total cholesterol, triglyceride, LDL-C and VLDL-C cholesterol over the period of four weeks. There was a highly significant increase in the HDL cholesterol with the combination therapy of fenugreek and atorvastatin [Table/Fig-5].

Following the high fat feed, there was a statistically significant($p<0.01$) increase in the body weights of the rats in Groups B, C and D. Group A rats also showed an increase in body weight, since it is a growing animal. In Group B after fenugreek therapy there was a statistically significant decrease in weight with $p<0.01$ where as in Group C after atorvastatin therapy there is a gross reduction in weight which is not statistically significant. In Group D with combination therapy there is a statistical significant reduction in weight with $p<0.01$ [Table/Fig-6].

DISUSSION

In the present study there was a significant reduction in all the lipid parameters in Group B, Group C and Group D when compared to the control group (Group A). This decrease indicated that supplementation of diet with fenugreek in Group B, atorvastatin in Group C and combination of fenugreek and atorvastatin in Group D enhanced the process of achieving eulipidemia. Another significant effect was an increase in HDL-C level which is considered as good cholesterol has a protective effect against heart diseases. Indicating the intensive conversion of LDL-C to HDL –C and clearance of circulating lipids. Administering fenugreek extracts to study group, showed a significant reduction in the atherogenic index. This lipid regulating property would be beneficial in atherosclerosis and coronary artery disease by preventing plaque formation. This study reveals the beneficial effect of fenugreek and atorvastatin in atherosclerosis. Hypolipidemic effects of fenugreek have also been observed in other experimental animals like rabbits [20].

Stark A and Madar Z have also found out the significant reduction in total cholesterol, triglycerides and very low density lipoproteins after treating for eight weeks with fenugreek seeds soaked in hot water [21]. The chemical sapogenins (protodiscin) in fenugreek powder increase the biliary cholesterol excretion and decrease the lipid levels in the body [22]. The triglyceride lowering effect may be due to the pectin component of fenugreek that absorbs the bile acids [23].

The hypolipidemic effect of fenugreek seeds could be attributed to an aminoacid, 4 hydroxyisoleucine with an atypical branched chain present in fenugreek. Fenugreek acts on adipocytes and liver cells decreasing triglycerides and cholesterol synthesis in addition to an enhanced Low Density Lipoprotein (LDL) receptor mediated lipid intake [24].

Atorvastatin significantly decreased all lipid profile parameters and atherogenic index in Group C and Group D as compared to group A. The lipid lowering effect of atorvastatin is due to inhibiting HMG-COA reductase in the liver cells and is also decreases serum LDL cholesterol via enhanced LDL receptor mediated LDL uptake [25].

Currently used statins like atorvastatin, rosuvastatin and cimvastatinetc, have known to produce liver and muscle toxicity. In addition to these toxic effects, they also produce hypothyroidism, renal insufficiency and life threatening infections [7].

From the study it was found that fenugreek when administered alone as a dietary supplement in the high fat fed rats showed a statistically significant reduction in the serum LDL, total cholesterol and triglyceride and a significant increase in the HDL cholesterol. However, the combination therapy of fenugreek with atorvastatin also showed a higher level of significance when compared to Group B.

Herbal products like fenugreek are economical and easily available. Belguith HO et al., in their study proved the antioxidant effect of fenugreek with no toxic and adverse effects [26]. So, fenugreek could be used as a lipid lowering agent in dyslipidemic individuals.

LIMITATION

The antioxidant property of fenugreek and the histopathological examination of the organs like aorta and liver could be done. However, this was not possible due to financial constraints. Fenugreek is commonly used in daily cooking, a research study on human beings can also be carried out but recruiting them from the clinical trial registry was not possible. Fenugreek seeds should be used with caution in hypersensitive individuals and diabetic patients on medication.

Future recommendations can be based on this study; fenugreek can be added as a supplement for uncontrolled dyslipidemic patients on statins.

CONCLUSION

From the above results it was proved that Fenugreek is a potent hypolipidemic agent with a significant increase in the HDL cholesterol and reduction in other lipid parameter values and its combination with atorvastatin showed to be more potent in altering the serum lipids. Lifestyle modification with dietary supplement of fenugreek can be taken as an effective hypolipidemic element initially to combat the increasing risks of dyslipidemia. Hence, the simplest and the safest way remains the combination of dietary modification along with certain therapeutic intervention for the effective management of dyslipidemia.

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PARTICULARS OF CONTRIBUTORS:

1. Student, PSG Institute of Medical Sciences and Research, Coimbatore, Tamil Nadu, India.
2. Professor, Department of Physiology, PSG Institute of Medical Sciences and Research, Coimbatore, Tamil Nadu, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:

Dr. GV Lathadevi,
Professor, Department of Physiology, PSG Institute of Medical Sciences and Research, Peelamedu,
Coimbatore-641004, Tamil Nadu, India.
E-mail: lathaphysio1965@gmail.com

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