Comparative Analysis of Antidyslipidemic Effects of Fenugreek Seed Extract and Standard Pharmacological Therapy in Diet Induced Animal Model of Dyslipidemia: An Experimental Study

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Authors’ contributions

The concept of study, data analysis, drafting and finalizing of the results were done by author NZ. The article was critically reviewed and finally drafted by author SS. Finally reviewed and approved by author ZM. Laboratory/instrument based work was performed under the supervision of Mr. Moazzam Ali Shahid and assisted by authors AA, FA and UZ.

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ABSTRACT

Objectives: The objective of this study was to compare the antidyslipidemic effects of fenugreek seed extract in comparison to standard drugs used for dyslipidemia in an animal model of dyslipidemia.

Methods: It was an experimental study conducted on 42 male, 9 weeks old Wistar albino rats for a period of 7 weeks. Animals were randomly divided into 7 groups, out of which 6 groups were given lipid rich diet to induce dyslipidemia for 28 days while one group served as control given normal diet. After 28 days standard drugs alone as well as in combinations (statins 10 mg/kg and fibrates 100 mg/kg) and fenugreek seed extract in two different doses, 250 mg/kg and 500 mg/kg were administered once a day for 21 days to all the respective groups except positive (lipid rich diet) and negative control groups (on normal diet), both were administered normal saline. Baseline body
weights of all groups of animals were measured at the start of the study. At the end of the study period, body weights of all groups of animal groups were measured again and their blood was drawn through cardiac puncture for the assessment of TC, LDL-C, HDL, TGs, D/B, I/B and ALT. All the intervention groups were compared on the basis of above mentioned parameters and changes in weight.

**Results:** Amongst all the groups, lipid parameters (TC, LDL, TGs) showed a significant reduction and increase in HDL in the group on FSE in a dose dependent manner. Moreover, FSE also showed significant decrease in the levels of liver enzymes including ALT, D/B and in body weight when compared to other groups. While we did not find any significant change for urea, creatinine and I/B amongst all the groups.

**Conclusion:** It is concluded that the fenugreek seed extract showed superior effects when compared to traditional pharmacological therapy against dyslipidemia.

**Keywords:** Dyslipidemia; lipid rich diet; Atorvastatin; Wistar albino rats; Fenugreek seeds.

**ABBREVIATIONS**

TC- Total Cholesterol  
LDL-C – Low Density Lipoprotein Cholesterol  
HDL-C – High Density Lipoprotein Cholesterol  
TGs- Triglycerides  
D/B- Direct Bilirubin  
I/B- Indirect Bilirubin  
FSE- Fenugreek Seed Extract

**1. INTRODUCTION**

Dyslipidemia is a metabolic abnormality due to a persistent increase in the plasma levels of cholesterol and triglycerides, the most common form is hypercholesterolemia which is defined as a total cholesterol level more than 5.0 mmol/L or 190 mg/L. It is a frequent cause of various associated morbidities and mortalities all over the world; according to an estimate, one-third of ischemic heart diseases and 2.6 million (45%) deaths worldwide are secondary to hypercholesterolemia [1]. The total concentration of cholesterol present in blood is a major risk factor that leads to increase the risk of atherosclerotic and related morbidities, ischemic heart diseases, hypertension, stroke etc. [2]. Serum concentrations of low-density lipoprotein -cholesterol (LDL-C) and high-density lipoprotein -cholesterols (HDL-C) have opposite effects on CVD risk, consistent with the role of LDL particles in the progression of atherosclerosis while HDL particles has a protective role against atherosclerosis (Blesso and Fernandez, 2018).

None of the lipid lowering drugs can effectively control all the lipid parameters simultaneously, hence usually two drugs combination has to be given such as statins and fibrates are given to control LDL and TGs. It has been well documented that low-density lipoprotein cholesterol (LDL-C) increases the risk of major cardiovascular events and to reduce LDL, statins remain the first choice of treatment, prescribed worldwide [3]. Statins have been widely indicated for a variety of diseases including prevention and treatment of cardiovascular diseases, diabetes, hypertension etc. for a long term to achieve beneficial effects in terms of desired lipid levels (Davies et al. 2016). However due to the nonadherence to statin therapy or its resistance, many patients do not achieve LDL-C target levels, here comes the role of second line of antidyslipidemic drugs, includes Ezetimibe, fibrates and nicotinic acid, that are may be used alone or in combination with statins [4]. Given that the patient population thought to benefit from the use of statins will likely continue to increase, it is imperative that factors associated with long term use of statins such as patient costs, associated adverse effects (AEs) including hepatotoxicity, myopathies, cognitive dysfunction, memory loss etc [5,6,7]. Moreover as many of the patients are usually on polypharmacy due to associated co-morbid, statins are reported to cause drug-drug interactions and drug- food interactions, that has further limited their clinical use [8,9]. Hence it is highly pertinent to explore new agents with less drug-interactions and other potential toxic effects on health. Although herbal and natural products have been used to prevent and cure various ailments since ancient times worldwide. However their use has been tremendously increased and since last three or four decades, herbal derivatives have been scientifically tested and documented to possess beneficial effects on health and treatment of various diseases It is also estimated that about two-thirds of world population depends upon traditional medicine for primary medical needs. Fenugreek (Trigonella
foenum-graecum Linn.), a short-living annual medicinal plant belonging to Fabaceae family, is used extensively in various parts of the world as herb, food, spice, and traditional medicine [10]. Researchers have investigated multifaceted therapeutic benefits of fenugreek seeds against a variety of ailments including diabetes, cancer, hyperlipidemia, inflammation, neurotoxicity, hepatotoxicity, ulcers, wound, bacterial and fungal infections, weakness, and edema of the legs [11]. Several studies has reported hypolipidemic action of fenugreek seeds [12]. Therefore, the current study was undertaken to evaluate the dyslipidemic effects of fenugreek.

2. MATERIALS AND METHODS

This Experimental animal study was conducted at the animal house of Faculty of Pharmacy Ziauddin University Karachi from 1st November 2019 to 25th December 2019, while laboratory work was performed at Ziauddin University (MDRL-1). We purchased 9 weeks old, 42 male Albino Wistar rats, weighing 300-400 g from Liaquat National University and Hospital. After acclimating with the environment, animals were subdivided into seven groups (n=6/group) randomly and were kept on a standard animal diet bought from local supplier in sufficient quantity for the entire period (7 weeks) of the study and Water ad-libitum.

2.1 Procurement of Fenugreek Seeds

The fenugreek seeds were bought by a local supplier from the area of Saddar Market, Karachi Pakistan. The seeds were then brought to the department of botany in Karachi University, where it was checked, authenticated and given a voucher specimen 53.

2.2 Preparation of Herb Extract

*T. foenum graecum* (fenugreek seeds) were purchased in a quantity of 1000 g and ethanol was purchased in quantity of 2500 ml as we are going to prepare the herbal extract in ratio of 1:2.5. The seeds were washed with distilled water thoroughly and dried at 40°C. Fenugreek seeds were grounded to coarse powder in a laboratory mill and were soaked for 30 days in a volumetric flask containing 2500 mL of 90% ethanol, with intermittent shaking and stirring, followed by filtration of the mixture with Whitman filter paper. The filtrate was further processed using water bath at 60°C and was dried in an oven at 50°C until a well concentrated extract is produced. The extract was kept in an airtight bottle and stored in a refrigerator until used (Priya et al. 2011, Bukhari et al. 2008).

2.3 Drugs and Chemicals

Drugs used include Atorvastatin 20 mg (tab. Lipiget Getz Pharma) and Fenofibrate 67 mg (cap. Fenoget Getz Pharma). Other chemicals such as 90% ethanol and reagents used for the study were procured from the approved organization so that they do not contain any kind of harmful toxin or material.

2.4 Grouping of Animals

Animals were randomly selected for grouping as follows: (Total N=42 and 6 animals /group)

Group 1. Negative control, normal pellet diet (NPD) and 3 ml normal saline, PO.

Group 2. Positive control, high fat diet and 3 ml normal saline, PO

Group 3. High fat diet and 10 mg/kg atorvastatin, PO.

Group 4. High fat diet and fenofibrate 100 mg/kg, PO.

Group 5. High fat diet and combination of atorvastatin 10 mg/kg and fenofibrate 100 mg/kg, PO [13].

Group 6. High fat diet and fenugreek seed extract 250 mg/kg, PO [14].

Group 7. High fat diet and fenugreek seeds extract 500 mg/kg, PO [14].

2.5 Standard Protocols for Animal Handling

All the animals were given twelve-hour light/dark cycle and before start of treatment animals were acclimatized with the environment and were given free access to water and normal diets.

3. EXPERIMENT

3.1 Induction of Dyslipidemia

The two chosen edible oils, Coconut oil and Banaspati ghee were administered at the dose of
10 ml/kg body weight to all the groups (except group 1 which is the negative control group) with feeding tube according to the protocol for 28 days. Body weight of all the animals was measured at baseline using the standard weighing machine.

3.2 Intervention

At 28th day (4 weeks) of respective lipid and normal diets were given to animals. All animals’ interventions according to their grouping were started for another 3 weeks through feeding tube in the mentioned concentrations. After the end of the total study period (7 weeks), all animals were anesthetized and 5 ml blood was drawn via cardiac puncture and collected in EDTA containing vacutainer tubes and was transferred to MDRL-1 for the analysis of lipid profile and Liver function tests (LFTs). After centrifugation of blood, serum was separated and the levels of Total cholesterol (make: human packing kit), Triglycerides (make: human packing), LDL-c (make: diasys packing kit), HDL cholesterol (make: human precipitation kit), ALT (make: diasys packing kit), D/B, I/B, Urea (make: diasys packing), D/B, I/B, Urea (make: labkit) were assessed by automated analyzer in order to evaluate the outcome of studied diets on aforementioned parameters.

3.3 Statistical Analysis

Data entry and analysis were conducted on SPSS version 20. To check the normality of the data Shapiro-wilk test was applied which showed all the variables were normally distributed except for TC, TGs and D/Hence ANOVA and post hoc analysis were applied for the intra and intergroup comparisons of all the variables, except for TC, TGs and D/B that were analyzed by Kruskalwillis test. P value less than 0.05 was considered as significant.

4. RESULTS

According to intergroup comparison there is significant difference in means for total cholesterol TC (p-value 0.000*), triglycerides TGs (p-value 0.000*), high density lipoprotein HDL (p-value 0.012*), low density lipoprotein LDL (p-value 0.000*), Alanine Amino Transferases ALT (p-value 0.007), indirect bilirubin I/B (p-value 0.012*), direct bilirubin D/B (p-value 0.001*) and body weight (p-value 0.000*) whereas the p-value for Urea and Creatinine are insignificant as shown in Table 1. While Fig. 1 is also representing the mean liver enzymes and bilirubin levels and Fig. 2 is displaying the mean weight of animals after respective interventions after the intergroup comparison.

Post hoc analysis displayed the results of intra group comparisons of all the groups and all the variables, it showed that regarding HDL there is significant difference amongst group 4 and group 7 (p-value 0.044) and group 5 and group 7 (p-value 0.025). While groups’ comparisons for LDL displayed significant p-value for group1 and group 7 (0.016), group 2 and group 6 (0.003), group 2 and group 7 (0.000), group 3 and group 7 (0.042), group 4 and group 6 (0.019), group 4 and group 7 (0.001) and group 5 and group 7 (0.033) respectively. On the other hand ALT (Alanine Amino transferases) has shown significant p-values for group 3 and group 6 (p-value 0.014), group 3 and group 7 (p-value 0.019).

When Urea and Creatinine were compared amongst all the groups, there was no significant difference amongst all the groups. However the intragroup comparison of I/D between all the groups found to be significant among group 2 and group 4 (p-value 0.004), groups 2 and group 6 (p-value 0.003), group 3 and group 4 (p-value 0.025), group 3 and group 6 (p-value 0.019) group 6 and group 7 (p-value 0.04). As far as body weight is concerned, after post hoc analysis we found out that there was significant difference between all the groups (p-value <0.05) excluding controls, Group 1 and group 5 (p-value 0.607), group 3 and group 4 (p-value 1.000), group 5 and group 6 (p-value 0.162), group 5 and group 7 (p-value 0.465), group 6 and group 7 (p-value 0.987).

For the three variable which were not normally distributed as I mentioned earlier, the analysis was carried out by applying Kruskal willis test. The results for TC, TGs and D/B showed that they all are having significant difference between all the groups having the p-values 0.008, 0.007 and 0.050 respectively. After all the comparisons most significant decrease in all the lipid parameters was shown in Group 7 (700 mg FSE).
### Table 1. Mean± SD of Various Lipid parameters and Body weights of animals (N=42)

| Hematological Parameter | Group 1 Negative Control 3 ml N.S n=6 | Group 2 Positive Control 3 ml N.S n=6 | Group 3 10 mg/kg Atorvastatin n=6 | Group 4 100 mg/kg Feno fibrate n=6 | Group 5 Atorv.+Feno. n=6 | Group 6 250 mg/kg FSE n=6 | Group 7 500 mg/kg FSE n=6 | P value |
|-------------------------|----------------------------------------|---------------------------------------|----------------------------------|------------------------------------|-------------------------|---------------------------|---------------------------|--------|
| TC                      | 136.00 ± 2.0                           | 148.00± 2.6                           | 127.66± 2.0                      | 130.00± 2.6                        | 123.00± 2.6            | 124.66± 3.5               | 122.33±3.2                | 0.000*  |
| TGs                     | 100.00 ± 2.0                           | 123.00± 4.5                           | 81.33± 4.0                       | 67.00±2.6                          | 72.33± 6.5             | 76.66± 1.5                | 73.06± 1.0                | 0.000*  |
| HDL                     | 32.66 ± 4.7                            | 30.66± 1.1                            | 30.00 ± 1.7                      | 27.33 ± 6.8                        | 26.33 ± 5.1            | 36.00 ± 3.0               | 38.66 ± 1.5                | 0.019*  |
| LDL                     | 83.33 ± 3.9                            | 92.73± 3.2                            | 81.40 ± 3.8                      | 89.26 ± 8.0                        | 81.86 ± 2.6            | 75.33 ± 2.5               | 69.03 ± 3.2                | 0.000*  |
| Urea                    | 22.30± 2.8                             | 22.80± 1.6                            | 20.56± 1.5                       | 24.70± 8.4                         | 19.10± 6.4             | 22.00± 2.8                | 17.46± 1.3                | 0.516   |
| Creatinine              | 0.59± 0.12                             | 0.53± 0.06                            | 0.41±0.06                        | 0.47± 0.11                         | 0.55± 0.07             | 0.53± 0.11                | 0.62± 0.11                | 0.257   |
| ALT                     | 55.00± 3.6                             | 66.67± 10.2                           | 73.33± 7.37                      | 65.66± 3.51                        | 61.66± 5.50            | 50.66± 8.96               | 51.66± 4.72                | 0.007*  |
| D/B                     | 0.15± 0.08                             | 0.11± 0.01                            | 0.20± 0.04                       | 0.30± 0.10                         | 0.18± 0.09             | 0.19± 0.05                | 0.12± 0.02                | 0.001*  |
| I/B                     | 0.35± 0.06                             | 0.20± 0.01                            | 0.25± 0.09                       | 0.44± 0.07                         | 0.34± 0.06             | 0.44± 0.04                | 0.27± 0.03                | 0.012*  |
| Body wt.                | 392.67±2.944                           | 416.17±1.329                          | 408.17±0.408                     | 405.83±1.169                       | 404.63±1.128           | 384.50±2.429              | 385.83±0.753               | 0.000*  |

* p value<0.05 =significant
Fig. 1. Comparison of mean Liver Enzymes and Bilirubin levels of various intervention groups

Fig. 2. Comparison of mean body weights of various intervention groups

5. DISCUSSION

Herbal treatments are emerging as an alternative therapy in various disorders across the world because of their robust effects safety, low budget and ecofriendly nature [15]. Since ancient times, fenugreek has been also employed for various ailments [16]. As we already know lipid disorders require treatment for longer periods, cost effective and relatively non-toxic effective therapeutic agents are needed [16]. In this regard the current experimental study highlighted the significant antidyslipidemic effects of fenugreek seed extract as compared to conventional antidyslipidemic therapy in an animal model of dyslipidemia. The group treated
with fenugreek seed extract significantly reduced total cholesterol, triglycerides, LDL and body weight and simultaneously increased HDL as shown in Table 1. Our results are similar to various studies including Sharma MS et al. [17] that assessed the effects of fenugreek seed extract in comparison to atorvastatin and showed a reduction in the levels of TC, LDL with a significant increase in the levels of HDL in experimentally induced hyperlipidemic rabbits [14]. These findings are consistent with various studies including a study conducted by Olfa et al. that reported analogous results of effects of FSE on high cholesterol fed rats [18]. Another study showed decrease of serum TG, LDL-cholesterol, and body weight when fat supplemented fed mice were treated with FSE for 15 days [19]. N. Takella et al. also conducted a study on rats which showed similar antidysslipidemic effects i.e. decrease in LDL along with increase in HDL levels [20]. Alcoholic fenugreek seed extract has been used on hyperlipidemic rats to see dyslipidemic properties of fenugreek which showed to decrease lipid parameters while increasing HDL [21]. The most striking reason behind reduction of lipid parameters is the presence of fibers and saponins in the fenugreek seeds, as fibers causes cholesterol reduction due to its ability of physical adsorption while saponins inhibits cholesterol absorption from intestine. Another possible explanation for the reduction in LDL is the ability of FSE to increase the LDL receptors in the body which causes increase uptake of LDL; the same mechanism of action by virtue of which statins also reduces LDL [19].

Another documented mechanism for antidysslipidemic effects of fenugreek seeds is due to its constituents such as diosgenin that has the ability to form large micelles from bile acids and saponin molecules in the small intestine, and in this way the cholesterol absorption is inhibited by directly excreting cholesterol in feces [22]. Diosgenin is also believed to reduce the content of triglycerides and total cholesterol through reducing the mRNA expression levels for lipid synthesis [23]. Another study suggests that the triglyceride lowering property of fenugreek is because of its pectin component that can absorb the bile acid [17]. The possible reason behind the role of fenugreek in increasing the HDL is on the account of the action of lecithin cholesterol acyltransferase that favors the incorporation of a greater amount of cholesterol in HDL and increased cholesterol uptake by the liver as mentioned by Nagamma et al. [24]. Fig. 1 shows the means of ALT and D/B is increased when atorvastatin and fenofibrates are used, which is in accordance with the previous studies. One such study conducted by Mahmoud H. et al., showed significant increase in ALT and D/B after the use of atorvastatin for 3 months [25]. However, the elevation of ALT because of statins is usually reversible and become normal when the therapy is discontinued [26]. Our study also illustrated a significant reduction in the levels of ALT and D/B in rats administered fenugreek seed extract on 250 mg and 500 mg (groups 6 and 7), these results are parallel to the study of Kandhara et al., that exhibited a significant decrease ($P < 0.05$) in the serum AST, ALT and D/B levels in rats that were administered 20 and 40 mg/kg of FSE [27]. Another research displayed similar beneficial effects of FSE, i.e. improved serum aspartate amino transferase (AST), alanine amino transferase (ALT) and lactate dehydrogenase (LDH) levels [28].

Our study also illustrated that the treatment with fenugreek seeds causes reduction in body weight in dose dependent manner and this finding is parallel to a study conducted by Kumar, et al. that suggested that the significant reduction in body weight with the use of FSE is due to decrease in the hormone leptin, secreted from adipose tissue that regulates the appetite with a subsequent reduction in the weight [28]. Another study reported the decrease in obesity after the use of ethanolic fenugreek seed extract on mice who were on high fat diet and the reason given in that study is that there is inhibition of accumulation of fat which causes reduction in body weight [29].

6. CONCLUSION
On the basis of all aforementioned facts, fenugreek seed extract has profound beneficial effects on all lipid parameters as a single antidysslipidemic and hepatoprotective agent along with better safety profile. Besides fenugreek has also shown to be effective in weight reduction.

7. FUTURE RECOMMENDATION
In the future it may be considered as an alternative lipid lowering agent, however further experimental and clinical trials are warranted to strengthen our results of experimental study.

8. LIMITATIONS
In this study the foremost limitation was that the we observed the animals only for 21 days after...
intervention, other than observing them for longer period.

9. STRENGTHS

To the best of our knowledge this is the first study in which various doses of fenugreek seed extract have been administered on Wistar rats and compared with the traditional drugs alone and in combination.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The study was approved by Animal Ethics committee Ziauddin University and was allotted Protocol No. 2018-005. Animals were dealt through all procedures according to CARE guidelines 2010 [30].

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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