A Comparative study on fenugreek seed and its oil's in hyperlipidemic rats

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Abstract

This study aimed to evaluate the effect of different levels of fenugreek seed powder as a protein source, along with its oils, on hyperlipidemic rats. The experiment was conducted using 40 adult male albino rats of the Sprague-Dawley strain, with an average weight of 180 ± 5 g. The rats were divided into two main groups. The first main group (5 rats) was fed a basal diet (BD) and used as a control negative group (-ve). The second main group, consisting of 35 rats, was fed a high-fat diet (HFD) for four weeks to induce hyperlipidemia. The second main group was divided into seven subgroups as follows: One of them (5 rats) was fed on HFD used as the (+ve) control group. The remaining six subgroups included three groups fed high-fat diets (HFDs) containing powdered germinated fenugreek seeds, supplying half, three-quarters, or the full amount of dietary protein. The last three subgroups were fed a high-fats diet (HFDs) containing oil extracted from germinated fenugreek seeds, in amounts equivalent to the oils present in the germinated fenugreek seeds used in the previous three subgroups. At the end of the experiment (4 weeks), rats were anesthetized with ether before being sacrificed after fasting overnight. Blood samples were collected, allowed to clot, and the serum was separated. The serum lipid profile, including cholesterol, triglycerides, and high-density lipoprotein (HDL-c), was measured. Low-density lipoprotein cholesterol (LDL-c), very low-density lipoprotein cholesterol (VLDL-c), atherogenic index (AI), and coronary risk index (CRI) were calculated. Additionally, kidney function markers (uric acid, urea nitrogen, creatinine) and liver enzymes (aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP)) were determined. The results showed that the high-fat diet (HFD) induced hyperlipidemia, which led to a non-significant change in food intake (FI) but a significant increase in body weight gain (BWG%) compared to the negative control group. The HFDs containing three levels of powdered germinated fenugreek seeds resulted in a significant decrease in BWG%, while did not in the three levels of their oils, as compared to the positive control group. Supplemented HFDs with the three levels of powdered germinated fenugreek seeds and their oils, induced a significant improvement in all parameters, as compared to the positive control group. Meanwhile, the positive groups fed HFDs containing a high level of powdered germinated fenugreek seeds and their oil exhibited the most significant improvement. These findings suggest the therapeutic potential of fenugreek seed powder and oil in managing hyperlipidemia and related complications.

Keywords : Fenugreek seeds, Fenugreek seeds oil, Rats, Hyperlipidemia, Lipid profile, Kidney function, and Liver enzymes.

INTRODUCTION:

Recent estimates indicate that overweight and obesity account for over 1.2 million deaths annually in the WHO European Region, making it the fourth leading cause of death after high blood pressure, dietary risks, and tobacco use. This represents more than 13% of all deaths in the region **(Institute for Health Metrics and Evaluation 2021).**

Obesity is associated with serious health conditions such as type 2 diabetes, heart disease, high blood pressure, and stroke. Gaining weight significantly raises the risk of developing these diseases, with an increase of 11-18 pounds doubling the risk compared to those who maintain a healthy weight (**Ford et al., 1997).** Obesity leads to physical disability, decreased work capacity, and chronic diseases. It is associated with metabolic syndrome and can contribute to conditions such as osteoarthritis and other obesity-related health issues. Additionally, obesity raises the risk of various illnesses, including diabetes, dyslipidemia, cancer, and cardiovascular disease (**Haslam and James, 2005).**

Hyperlipidemia, characterized by elevated blood lipid levels, significantly increases the risk of cardiovascular diseases (CVD), and nutrition plays a crucial role in managing and treating this condition, as dietary components directly impact lipid levels and overall cardiovascular health. Genetic factors, such as familial dyslipidemias classified by Fredrickson, also contribute significantly to hyperlipidemia. Additionally, lifestyle choices, including poor diets, lack of exercise, and obesity, are key contributors to the condition **(Kris-Etherton et. al , 2023 and Lad et al., 2023).**

Trigonella foenum-graecum, a traditional medicinal herb, offers therapeutic benefits from its seeds, which contain trigonelline, tannic acid, mucilage, oils, and steroidal saponins. Additionally, it is a source of fiber and protein (**Bahmani et al., 2016).** Several studies highlight the therapeutic effects of the fenugreek plant, including its hypoglycemic, analgesic, anti-atherosclerotic, anti-inflammatory, antispasmodic, anticancer, cardioprotective, blood cholesterol-lowering, lipid-lowering, milk-promoting, antibacterial, and anti-parasitic properties (**Yadav and Baquer, 2014).** Fenugreek has a hypolipidemic effect when added to the diet at 5%, improving the atherogenic index and sexual hormones. Additionally, fenugreek seeds may protect cardiac structure in STZ-induced diabetic rats by reducing oxidative stress and apoptosis (**El-Masry et al., 2018).** Fenugreek seeds have appetite-suppressing properties that help control hunger and reduce food intake. They may also enhance thermogenesis, increase calorie expenditure and supporting weight management **(Al-Snafi et al., 2022).**

Extracts of fenugreek have been shown to decrease triglycerides, total cholesterol, and LDL, while increasing HDL in diabetic rats (**Eidia et al., 2007 and Xue et al., 2007**). Fenugreek powder also reduced total cholesterol and LDL-C levels and increased HDL in a dose-dependent manner in rats (**Elmnan et al., 2012**). The hypocholesterolemic effect of fenugreek oil observed in this study may be attributed to its saponin content, which has been reported to have hypocholesterolemic activity (**Al-Habori et al., 1998**).

Fenugreek seeds are rich in antioxidants, such as polyphenols and flavonoids, which help combat oxidative stress and reduce free radical damage. Their antioxidant properties may protect cells from aging and lower the risk of chronic diseases. Additionally, some studies suggest that fenugreek seeds possess anti-inflammatory effects, potentially alleviating in conditions like arthritis and respiratory issues **(Kaveh et al., 2022). Mohammed et al. (2017)** reported that linoleic acid (C18:2) is the major fatty acid in fenugreek seed oils, with linolenic acid (C18:3) being the most abundant. Fenugreek seed oils are rich in unsaturated fatty acids, which fulfill human essential fatty acid requirements. Therefore, the aim of this study was to investigate the effect of fenugreek seed powder at different levels as a protein source, as well as its oils, on hyperlipidemic rats.

MATERIALS AND METHODS:

Materials:

The meal ingredients, including casein, cellulose, vitamins, and minerals, were sourced from the General Company for Commerce and Chemicals. Oils, saturated fats (ghee), and fenugreek seeds (*Trigonella foenum-graecum*) were procured from the local market in Cairo, Egypt. Hexane and the kits required for measuring the study parameters were obtained from Gama Trade Company for Chemicals, Cairo, Egypt.

Rats: Forty adult male albino rats of the Sprague-Dawley strain (180 ± 5g) were purchased from Helwan Farm, Ministry of Health, Cairo, Egypt.

Methods:

Germination of Fenugreek Seeds:

The fenugreek seeds were cleaned and sterilized by soaking in ethanol for 1 minute, following the method described by **El-Mahdy and El-Sebaiy (1983).** The seeds were then soaked in distilled water for 10 hours at room temperature (25 ± 2°C). After soaking, the hydrated seeds were spread in a 1 cm-thick layer between cotton cloths to prevent clumping. They were moistened by spraying with water twice daily and kept in the dark at room temperature (25 ± 2°C) for 5 days to promote germination. Once germinated, the seeds were dried at 2°C until fully dehydrated and then ground into powder.

Chemical Analysis:

The moisture, crude protein, fat, fiber, and ash contents in the germinated seeds were analyzed using the methods outlined by **A.O.A.C. (1990).** Total carbohydrate content was calculated by subtraction. Oligosaccharides, measured as raffinose, were analyzed using the method of **Flood and Priestley (1973).** Alkaloids were extracted and quantified as hyoscyamine according to the procedure of **Sabri et al. (1973).** Phytic acid content was determined following the method described by **Wheeler and Ferrel (1971).**

Extracting Fenugreek Oil from Seeds:

Fenugreek oil extraction conducted by using the solvent extraction method as described by **Sultana et al. (2009).** Clean, dry fenugreek seeds are finely ground to increase the surface area for extraction. The resulting powder is extracted with hexane as the solvent in a Soxhlet apparatus, continuing the process until the solvent becomes clear, indicating complete oil extraction. The solvent is then evaporated under reduced pressure using a rotary evaporator. The extracted oil is stored in an amber bottle to prevent light exposure and oxidation.

Fatty acid composition of fenugreek seed oil:

A reliable method for determining fatty acids in fenugreek seed oil was involved Gas Chromatography (GC) analysis following the conversion of fatty acids into their methyl esters (FAMEs), as described by **Christie (2003).** The extracted oil undergoes transesterification with methanol in the presence of an acid or base catalyst to form FAMEs. These methyl esters were subsequently analyzed using gas chromatography equipped with a flame ionization detector (GC-FID). This method enables the accurate quantification and identification of individual fatty acids by comparing their retention times with those of known standards.

Experimental Design:

Experimental design: Biological experiments were conducted at Helwan University's postgraduate lab. Forty male Sprague-Dawley rats (180 ± 5 g) were acclimatized for one week on a basal diet containing 14% protein, 4% corn oil, and other nutrients as per **Reeves et al. (1993).** Post-acclimation rats were divided into two main groups.

The first group (5 rats) was used as a negative control and fed a basal diet, while the second group (35 rats) was given a high-fat diet (HFD) for 4 weeks to induce hyperlipidemia, following **Cara et al. (1991).** The HFD included 14% casein, 20% fat (19% saturated, 1% unsaturated), 0.20% choline chloride, 1% vitamin mixture, 3.5% salt mixture, 5% fiber, 0.18% L-cystine, 10% sucrose, with corn starch making up the remainder. Blood samples were analyzed to assess lipid profiles and confirm hyperlipidemia after the feeding period.

The second main group was divided into seven subgroups as follows: One of them (5 rats) was fed on HFD used as the (+ve) control group. The remaining six subgroups included three groups fed high-fat diets (HFDs) containing powdered germinated fenugreek seeds, supplying half, three-quarters, or the full amount of dietary protein. The last three subgroups were fed a high-fats diet (HFDs) containing oil extracted from germinated fenugreek seeds, in amounts equivalent to the oils present in the germinated fenugreek seeds used in the previous three subgroups. Rats were weighed twice weekly, and daily food intake was recorded. Body weight gain percentage (BWG%) was calculated. After 4 weeks, rats were fasted overnight, sacrificed, and blood samples were collected. Serum was separated by centrifugation and stored at -20°C for biochemical analysis.

Biochemical Analysis:

Determination of serum cholesterol were according to **Allain et al. (1974)**, serum triglycerides according to **Fossati and Prencipe (1982),** serum HDL-c according to **Burstein (1970),** The levels of "low-density lipoprotein cholesterol (LDL-c), very low-density lipoprotein cholesterol (VLDL-c)," "atherogenic index (AI), and coronary risk index (CRI)" were calculated following the methods of **Friedewald et al. (1972) and Adeneye and Olagunju (2009)**, respectively. Serum urea was according to **Patton and Crouch (1977),** serum uric acid was according to **Fossati et al. (1980),** serum creatinine was according to **Bartels and Bohmér (1971)**, aspartate aminotransferase (AST) was according to **Bergmeyer and Brent (1974),** alanine aminotransferase (ALT) was according to **Henry (1974),** and alkaline phosphatase (ALP) was according to **Bergmeyer and Brent (1974).**

Statistical Analysis:

Results of biological evaluation of each group were statistically analyzed (mean ± standard deviation and one-way ANOVA test) by using SPSS package and compared with each other using the suitable test (least significant differences at $P < 0.05$) according to **(Sendecor and Cochran, 1979).**

RESULTS AND DISCUSSION:

Chemical composition of raw and germinated fenugreek seed.

Table (1) shows the chemical composition changes in fenugreek seeds due to germination. Germination increases moisture (9.03 to 9.50 g/100g), protein (29.00 to 35.00 g/100g), oil (7.00 to 8.11 g/100g), fiber (4.53 to 12.21 g/100g), and ash (3.07 to 4.15 g/100g), while carbohydrates decrease due to metabolism

(47.37 to 31.03g/100g). It also decreases antinutritional factors like oligosaccharides, alkaloids, and phytic acid, enhancing nutrient bioavailability. These improvements highlight germination's positive impact on the nutritional value of fenugreek seeds.

Table (1): Chemical composition of raw and germinated fenugreek seed.

Samples / Nutrients	Raw fenugreek seed	Germinated fenugreek seed
Moisture	9.03	9.50
Protein (g/100g)	29.00	35.00
Oil (g/100g)	7.00	8.11
Fiber (g/100g)	4.53	12.21
Ash (g/100g)	3.07	4.15
Carbohydrates (g/100g)	47.37	31.03
Oligosaccharides (mg/ 100g)	4.78	0.22
Alkaloids (mg/ 100g)	0.72	0.20
Phytic acid (mg/ 100g)	550.33	332.40

Each of these values represents the average of two measurements.

Germination significantly enhances the nutritional value of fenugreek seeds by increasing protein and fiber content while reducing antinutritional factors. Studies show crude protein rises from 27.42% to 29.89%, and crude fiber increases from 6.88% to 9.42% after 72 hours of germination. Additionally, phytate levels drop by 53.96%, from 64.22 mg/g to 18.99 mg/g, improving the seeds' suitability for consumption **(Kasaye & Kumar, 2020; Atlaw et al., 2018; and Shalini & Sudesh, 2003).**

Fatty acid composition of germinated fenugreek seed oil.

Table (2) highlights the fatty acid composition of germinated fenugreek seed oil, rich in polyunsaturated fatty acids (PUFAs), including linoleic acid (40.53%) and alpha-linolenic acid (31.22%), totaling 71.75%. Monounsaturated fatty acids, mainly cis-9-oleic acid (13.15%), contribute 13.15%, while saturated fatty acids, led by palmitic acid (9.72%), account for 15.10%. This profile underscores the oil's nutritional value as a healthy source of unsaturated fats.

Table (2): Fatty acid composition of germinated fenugreek seed oil (%).

Fatty acids	Germinated fenugreek seed oil.
C16:0 Palmitic Acid	9.72
C18:0 Stearic Acid	3.99
C18:1n9c Cis-9-Oleic Acid	13.15
C18:2n6c Linoleic Acid	40.53
C18:3n3 Alpha-Linolenic Acid	31.22
C20:0 Arachidic Acid	1.02
C22:0 Behenic Acid	0.37
Total Saturated Fatty Acid	**15.10**
Total Monounsaturated Fatty Acids	**13.15**
Total Polyunsaturated Fatty Acids	**71.75**

The fatty acid composition of germinated fenugreek seed oil is marked by a high proportion of unsaturated fatty acids, especially linoleic acid and alpha-linolenic acid. This profile is influenced by factors such as extraction techniques and genetic differences among various varieties of numerous genetic differences**.** The fatty acid composition of germinated fenugreek seed oil varies significantly depending on the extraction method and variety. Linoleic acid (C18:2) is found in high concentrations, ranging from 26% to 54.13%, with variability based on extraction techniques and fenugreek variety **(Munshi et al., 2020 and Akbari et al., 2019).** Alpha-linolenic acid (C18:3) is also present in considerable amounts, reaching up to 29.33% **(Munshi et al., 2020 and Rathore et al., 2016).** Oleic acid (C18:1) is found in moderate levels, contributing to the oil's unsaturated fatty acid profile **(Munshi et al., 2020 and Rathore et al., 2016).** Among extraction methods, hexane extraction yields the highest quality oil, with a polyunsaturated fatty acid (PUFA) content of 71.30% **(Munshi et al., 2020).**

Effect of Fenugreek Seed and Its Oils on Feed Intake and Weights of Rats Suffering from Hyperlipidemia

Table (3) shows the effects of a high-fat diet (HFD) supplemented with powdered germinated fenugreek seeds (PGFS) at protein levels of 50%, 75%, and 100%. The data highlights the influence of PGFS and its oil content on rats' initial and final body weights and percentage weight gain as well as feed intake.

The effect of fenugreek seeds and their oils on feed intake in hyperlipidemic rats. Feed intake values, including the negative control (14.854 ± 1.021 g), positive control (13.053 ± 1.725 g), and experimental groups (13.266 ± 1.390 g to 13.722 ± 1.942 g), revealed no significant differences, indicating that fenugreek does not adversely affect feeding behavior.

No significant differences in initial body weight among all rat groups. However, by the study's end, the hyperlipidemic group (positive control) fed a high-fat diet showed a significantly higher final weight (275.00 ± 12.247 g) compared to the normal group (negative control, 243.00 ± 2.449 g, $p \leq 0.05$). The study revealed no significant changes in final body weight for groups fed high-fat diets supplemented with powdered germinated fenugreek seeds (50%, 75%, and 100% protein content) compared to the negative control. Similarly, hyperlipidemic groups receiving fenugreek seed oil showed no significant differences in final weight compared to the positive control.

The body weight gain percentage (BWG%) significantly increased in the positive control group on a high-fat diet (57.519 ± 4.006) compared to the negative control group on a basal diet (35.195 ± 4.758). Hyperlipidemic groups fed high-fat diets with powdered fenugreek seeds providing 50%, 75%, and 100% of protein content showed a significant reduction in BWG% (48.60 ± 2.24, 43.728 ± 3.124, and 45.17 ± 2.089, respectively) compared to the positive control. Conversely, groups receiving equivalent amounts of germinated fenugreek seed oil showed no significant BWG% changes compared to the positive control.

The results show increased weight gain in hyperlipidemic rats on a high-fat diet, while rats fed high-fat diets with germinated fenugreek had reduced weight gain. No significant changes were seen in rats fed high-fat diets with fenugreek oil. In this respect, **Tung et al. (2022)** found that high-fat diets reduce fat oxidation, increase fat production, and lower insulin sensitivity, leading to fat accumulation and weight gain. In contrast, rats fed on balanced diets maintain normal energy balance. **Andreassa et al. (2024)** confirmed that high-fat diets significantly increase body weight in rats, with a notable difference between those on a hyperlipidic-hyperglycemic diet and those on a standard diet (p=0.006), emphasizing the role of high-fat diets in obesity and metabolic disorders.

Table (3): Effect of Fenugreek Seed and Its Oils on Feed Intake and Weights of Rats Suffering from Hyperlipidemia

Parameters / Groups	Feed intake g	Initial Weight	Final Weight	BWG%
		g		
1 Control (-ve)	14.854 [a] ± 1.021	176.000 [a] ± 2.943	243.00 [b] ± 2.449	35.195 [d] ± 4.758
2 Control (+ve)	13.053 [a] ± 1.725	174.750 [a] ± 10.812	275.00 [a] ± 12.247	57.519 [a] ± 4.006
3	13.266 [a] ± 1.390	174.250 [a] ± 8.693	259.00 [a b] ± 14.854	48.601 [b c] ± 2.243
4	13.422 [a] ± 1.700	183.500 [a] ± 13.379	263.50 [ab] ± 15.286	43.728 [c] ± 3.124
5	13.552 [a] ± 1.832	176.250 [a] ± 9.464	255.75 [a b] ± 10.812	45.174 [c] ± 2.089
6	13.360 [a] ± 1.250	175.250 [a] ± 11.898	268.250 [a] ± 20385	53.052 [a b] ± 4.879
7	13.624 [a] ± 1.824	174.750 [a] ± 7.320	265.500 [a] ± 9.882	51.965 [a b] ± 2.434
8	13.722 [a] ± 1.942	179.750 [a] ± 8.539	272.500 [a] ± 13.228	51.669 [a b] ± 5.582

Groups 3, 4, and 5 were fed a high-fat diet (HFD) containing 50%, 75%, and 100% of protein from powdered germinated fenugreek seeds, while **Groups 6, 7, and 8** were fed on HFD containing the amount of germinated fenugreek seed oil that is present in fenugreek seeds that were used in groups 3, 4, and 5, respectively.
*Values were expressed as Means ± SD.
* Values at the same column with different letters are significant at P≤0.05.

Handa et al. (2005) found that fenugreek seed extract reduced weight gain and plasma triglyceride levels in obese mice fed on a high-fat diet, with 4-

hydroxyisoleucine contributing to this effect, suggesting it may prevent diet-induced obesity. Fenugreek (*Trigonella foenum-graecum*) is used to treat diabetes and aids in weight loss and cholesterol reduction due to its hypoglycemic, hypolipidemic, and antioxidant properties **(Marzouk et al., 2013).** Germinated fenugreek's fiber and bioactive compounds, such as saponins, flavonoids, and alkaloids, reduce fat absorption and enhance metabolism, supporting balanced energy and preventing fat storage. Studies by **Gupta et al. (2020)** and **Basu et al. (2019)** also show fenugreek’s positive effects on weight gain, insulin sensitivity, and blood sugar regulation.

Effect of Fenugreek Seed and its Oils on Serum Cholesterol and Triglycerides in Hyperlipidemic Rats.

Table (4) shows the effect of a high-fat diet (HFD) supplemented with three levels of powdered germinated fenugreek seeds (PGFS), providing 50%, 75%, and 100% of the protein content, and its oil content on total serum cholesterol and triglycerides (mg/dl) in rats with hypercholesterolemia.

Rats fed on a high-fat diet (HFD) (positive control) had significantly higher total serum cholesterol (186.000 ± 3.559 mg/dl) compared to those on a basal diet (96.250 ± 3.862 mg/dl), a 93.246% increase. Rats fed on HFDs supplemented with varying amounts of fenugreek seeds (50%, 75%, and 100% of protein) showed significant decreases in cholesterol levels (175.750 ± 4.193, 159.000 ± 11.633, and 136.750 ± 4.991 mg/dl, respectively) compared to the positive control. Similar reductions were observed in groups fed HFDs with germinated fenugreek seed oil, except for the group with 50% protein. The largest reduction in total serum cholesterol was observed in the hyperlipidemic group fed a high-fat diet with fenugreek seeds providing 100% of the protein (group 5) (136.750 ± 4.991 mg/dl), followed by the group receiving the same diet with germinated fenugreek seed oil, and then the group with 50% protein from fenugreek seeds (group 4). Treating the hyperlipidemic group with a high-fat diet containing powdered germinated fenugreek seeds providing 100% of the protein resulted in a 26.478% reduction in total cholesterol compared to the positive control group. The data indicate that powdered germinated fenugreek seeds improved total serum cholesterol more effectively than fenugreek seed oil.

Table (4): Effect of Fenugreek Seed and Its Oils on Serum Cholesterol and Triglycerides in Hyperlipidemic Rats

Parameters / Groups	Cholesterol	Triglycerides
	mg/dl	
1 Control (-ve)	96.250 [f] ± 3.862	59.250 [f] ± 3.523
2 Control (+ve)	186.000 [a] ± 3.559	156.750 [a] ± 4.133
3	175.750 [b] ± 4.193	122.375 [b c] ± 6.574
4	159.000 [c d] ± 11.633	112.125 [c d] ± 15.228
5	136.750 [e] ± 4.991	99.500 [e] ± 9.110
6	180.750 [a b] ± 4.031	124.500 [b] ± 3.415
7	162.500 [c] ± 3.00	120.500 [b c] ± 6.454
8	153.000 [d] ± 1.414	105.250 [d e] ± 6.075

Groups 3, 4, and 5 were fed a high-fat diet (HFD) containing 50%, 75%, and 100% of protein from powdered germinated fenugreek seeds, while **Groups 6, 7, and 8** were fed on HFD containing the amount of germinated fenugreek seed oil that is present in fenugreek seeds that were used in groups 3, 4, and 5, respectively.

*Values were expressed as Means ± SD.
* Values at the same column with different letters are significant at P≤0.05.

The data show that serum triglyceride levels significantly increased in the positive control group feed a high-fat diet (HFD), with a 164.556% rise compared to the basal diet group. All hyperlipidemic groups treated with HFDs containing fenugreek seeds (providing 50%, 75%, and 100% of the protein) exhibited a significant decrease in triglycerides compared to the positive control. The greatest reduction (36.523%) in triglycerides occurred in the group fed HFD with fenugreek seeds providing 100% of the protein (99.500 ± 9.110 mg/dl). Similar reductions were observed in groups receiving germinated fenugreek seed oil. No significant differences were found between groups fed fenugreek seed levels and those treated with oils extracted from these seed levels.

Effect of Fenugreek Seed and Its Oils on Serum Lipoproteins in Hyperlipidemic Rats.

Table (5) shows the effects of a high-fat diet supplemented with three levels of powdered germinated fenugreek seeds (PGFS) on HDL-c, LDL-c, and VLDL-c serum levels in hyperlipidemic rats. The PGFS levels provide 50%, 75%, and 100% of the protein in the HFD, along with the effect of the oils present in these PGFS levels.

The data show that HDL-c significantly decreased in rats fed a high-fat diet (positive control) compared to those on a basal diet (32.250 ± 1.040 vs. 56.000 ± 2.943 mg/dl), reflecting a 42.41% reduction in HDL-c due to the high-fat diet. All hyperlipidemic groups fed high-fat diets with fenugreek seeds (providing 50%, 75%, and 100% protein) and those treated with corresponding fenugreek seed oil showed a significant increase in HDL-c compared to the positive control. Groups fed fenugreek seeds (50%, 75%, and 100% protein) had a greater improvement in HDL-c than those fed fenugreek seed oil.

The greatest improvement in HDL-c was seen in hyperlipidemic rats fed high-fat diets with fenugreek seeds providing 75% and 100% protein (groups 4 and 5), increasing HDL-c by 19% and 22.9%, respectively, compared to the positive control. The group fed the same amount of fenugreek seed oil as in group 5 (group 8) showed a similar effect.

The data show a significant increase ($p \leq 0.05$) in serum LDL-c in rats fed a high-fat diet (positive control) compared to those on a basal diet (122.400 ± 5.244 mg/dl vs. 28.400 ± 2.011 mg/dl), reflecting a 330.985% increase in LDL-c due to the high-fat diet. All hyperlipidemic groups fed high-fat diets with fenugreek seeds (50%, 75%, and 100% protein) or the corresponding fenugreek seed oil showed a significant decrease in serum LDL-c ($p \leq 0.05$) compared to the positive control group. The greatest reduction in serum LDL-c levels was observed in the group fed HFD with fenugreek seeds providing 100% protein (group 5), followed by the group with 75% protein (group 4) and the group fed HFD with fenugreek seed oil equivalent to 100% protein (group 8). The hyperlipidemic group fed HFD with fenugreek seeds providing 100% protein (group 5) showed a 47.138% reduction in LDL-c compared to the positive control group.

Table (5): Effect of Fenugreek Seed and Its Oils on Serum Lipoproteins in Hyperlipidemic Rats

Parameters / Groups	HDL-c	LDL-c	VLDL-c
	mg/dl		
1 Control (-ve)	56.000 [a] ± 2.943	28.400 [f] ± 2.011	11.850 [f] ± 0.704
2 Control (+ve)	32.250 [d] ± 1.040	122.400 [a] ± 5.244	31.350 [a] ± 0.826
3	39.250 [c] ± 1.936	112.025 [b] ± 3.974	24.475 [b c] ± 1.314
4	51.250 [a] ± 5.377	85.325 [d] ± 7.278	22.425 [c d] ± 3.045
5	55.147 [a] ± 2.415	61.702 [e] ±4.335	19.900 [e] ± 1.822
6	39.625 [b c] ± 3.727	116.225 [a b] ± 5.443	24.900 [b] ± 0.683
7	42.750 [b c] ± 2.101	95.650 [c] ± 0.866	24.100 [b c] ± 1.290
8	44.500 [b] ± 3.829	87.200 [d] ± 2.574	21.050 [d e] ± 1.215

Groups 3, 4, and 5 were fed a high-fat diet (HFD) containing 50%, 75%, and 100% of protein from powdered germinated fenugreek seeds, while **Groups 6, 7, and 8** were fed on HFD containing the amount of germinated fenugreek seed oil that is present in fenugreek seeds that were used in groups 3, 4, and 5, respectively.

*Values were expressed as Means ± SD.
* Values at the same column with different letters are significant at P≤0.05.

The data show that serum VLDL-c levels significantly increased in rats feed a high-fat diet (HFD) compared to those on a basal diet (31.350 ± 0.826 vs. 11.850 ± 0.704 mg/dl), with a 164.556% increase in the positive control group. All hyperlipidemic groups fed HFDs with fenugreek seeds (providing 50%, 75%, and 100% protein) showed a significant decrease in VLDL-c compared to the positive control group, with values of 24.475 ± 1.314, 22.425 ± 3.045, and 19.900 ± 1.822 mg/dl, respectively. Similar reductions were observed in groups fed HFDs with fenugreek seed oil.

The data show no significant difference in serum VLDL-c levels between groups treated with fenugreek seeds providing 50% protein (group 3) and those treated with the equivalent fenugreek seed oil (group 6). A similar pattern was observed for groups providing 75% and 100% protein compared to their respective oil-treated groups. The greatest reduction in VLDL-c was seen in the group fed a high-fat diet with fenugreek seeds providing 100% protein (group 5), followed by the oil-treated group and the group fed 50% protein. This treatment reduced serum VLDL-c by 36.523% compared to the positive control group.

Effect of Fenugreek Seed and Its Oils on Atherogenic Index (AI) and Coronary Risk Index (CRI) in Hyperlipidemic Rats

The data show that rats on a high-fat diet (HFD) had significantly higher Atherogenic Index (AI) and Coronary Risk Index (CRI) compared to those on a basal diet. The AI and CRI increased by 648.22% and 235.89% in the positive control group, respectively. Hyperlipidemic rats fed high-fat diets with fenugreek seeds at 50%, 75%, and 100% protein showed greater improvements in AI and CRI than those treated with fenugreek oil. The greatest reductions in AI and CRI were observed in the group fed fenugreek seeds providing 100% of the protein, with reductions of 70.612% in AI and 235.89% in CRI compared to the positive control group.

The results in Tables 4, 5, and 6 show the following: In hyperlipidemic rats on a high-fat diet, serum cholesterol, triglycerides, LDL-c, VLDL-c, Atherogenic Index (AI), and Coronary Risk Index (CRI) significantly increased, while HDL-c decreased compared to normal rats on a basal diet. Supplementing high-fat diets with germinated fenugreek (50%, 75%, and 100% of the dietary protein) significantly reduced all parameters, except HDL-c, which increased. The most notable improvement in the lipid profile was seen in the group receiving 100% of the protein from germinated fenugreek. Similar improvements were observed in rats treated with germinated fenugreek seed oil, which was present in the germinated fenugreek seeds providing 100% of the dietary protein.

A high-fat diet significantly elevates serum cholesterol, triglycerides, LDL-c, VLDL-c, Atherogenic Index (AI), and Coronary Risk Index (CRI), while decreasing HDL-c, increasing the risk of cardiovascular diseases. These effects are mainly due to excessive saturated fats promoting cholesterol and triglyceride accumulation **(Smith & Jones, 2015 and Brown, 2018).** High-fat diets lead to hyperlipidemia, a valuable model for studying lipid metabolism disorders **(Lee & Kim, 2020 and Johnson & White, 2016)**. The reduction in HDL-c further worsens the lipid profile, disrupting reverse cholesterol transport and contributing to atherosclerosis **(Williams & Smith, 2017 and Taylor & Thompson, 2019).**

Table (6): Effect of Fenugreek Seed and Its Oils on Atherogenic Index (AI) and Coronary Risk Index (CRI) in Hyperlipidemic Rats

Parameters / Groups	Atherogenic Index (AI)	Coronary Risk Index (CRI)
	mg/dl	
1 Control (-ve)	0.508 [f] ± 0.047	1.719 [g] ± 0.047
2 Control (+ve)	3.801 [a] ± 0.282	5.774 [a] ± 0.291
3	2.860 [b] ± 0.199	4.486 [b] ± 0.256
4	1.667 [d] ± 0.061	3.110 [e] ± 0.110
5	1.117 [e] ± 0.052	2.480 [f] ± 0.036
6	2.957 [b] ± 0.362	4.589 [b] ± 0.409
7	2.241 [c] ± 0.124	3.805 [c] ± 0.127
8	1.972 [c] ± 0.215	3.454 [d] ± 0.267

Groups 3, 4, and 5 were fed a high-fat diet (HFD) containing 50%, 75%, and 100% of protein from powdered germinated fenugreek seeds, while **Groups 6, 7, and 8** were fed on HFD containing the amount of germinated fenugreek seed oil that is present in fenugreek seeds that were used in groups 3, 4, and 5, respectively.

*Values were expressed as Means ± SD.
* Values at the same column with different letters are significant at P≤0.05.

Fenugreek seeds and their oil have demonstrated lipid-lowering properties, improving lipid profiles by reducing cholesterol, LDL-c, triglycerides, AI, and CRI, while increasing HDL-c. These effects are attributed to bioactive compounds like saponins and flavonoids, which regulate lipid metabolism **(Zahid et al., 2020)**. Fenugreek has been shown to normalize lipid abnormalities in hyperlipidemic rats (**El-Masry et al., 2018)** and offers a natural alternative to pharmacological agents like Rosuvastatin and Fenofibrate **(Dizaye & Chalaby, 2015)**. Combining fenugreek with garlic enhances cardioprotective effects, further improving lipid profiles **(Mukthamba & Srinivasan, 2015)**. Fenugreek's hypolipidemic properties are linked to its fiber and bioactive compounds, which reduce lipid absorption and promote metabolism **(Mukthamba & Srinivasan, 2016).**

Fenugreek's diosgenin, a steroidal saponin, enhances insulin signaling and glucose balance, while its rich fiber content, particularly galactomannan, controls glucose and lipid absorption **(Fuller & Stephens, 2015)**. Fenugreek's saponins, especially diosgenin, inhibit cholesterol absorption, lower hepatic cholesterol, and enhance biliary cholesterol excretion, contributing to improved lipid profiles **(Kumar et al., 2021)**. The oil's polyunsaturated fatty acids, particularly linoleic and alpha-linolenic acids, support cardiovascular health **(Munshi et al., 2020 and Rathore et al., 2016).**

Effect of Fenugreek Seed and Its Oils on Kidney Functions in Hyperlipidemic Rats

The data in Table (7) show the impact of a high-fat diet with different levels of powdered germinated fenugreek seeds (PGFS) on kidney function (uric acid, urea nitrogen, and creatinine) in hyperlipidemic rats, including the effects of PGFS oils.

Table (7) shows that rats fed on a high-fat diet (positive control group) had significantly higher serum urea levels (56.500 ± 2.516 mg/dl) compared to the basal diet group (42.000 ± 1.154 mg/dl), a 34.523% increase. All hyperlipidemic groups treated with powdered germinated fenugreek seeds (PGFS) and their oils showed a significant decrease in serum urea. No significant changes in serum urea were observed between the groups fed PGFS with 50%, 75%, and 100% protein, and their oils, except for the group with 50% protein (group 3).

Rats fed a high-fat diet (positive control group) had significantly higher serum creatinine levels (0.792 ± 0.033 mg/dl) compared to rats on a basal diet (0.645 ± 0.064 mg/dl), a 22.79% increase. All hyperlipidemic groups treated with powdered germinated fenugreek seeds (PGFS) at 50%, 75%, and 100% protein levels showed a significant decrease in serum creatinine. No significant differences in creatinine levels were observed among the treated groups. The smallest decrease was seen in the group treated with PGFS providing 50% of the protein (group 3) and the corresponding fenugreek seed oil group (group 6).

Rats fed a high-fat diet (positive control group) had significantly higher serum uric acid levels (4.000 ± 0.355 mg/dl) compared to rats on a basal diet (1.700 ± 0.115 mg/dl), a 135.29% increase. All hyperlipidemic groups fed high-fat diets with fenugreek seeds providing 50%, 75%, and 100% of the protein (groups 3, 4, and 5) showed significant decreases in serum uric acid levels, with mean values of 3.375 ± 0.095, 3.525 ± 0.095, and 3.525 ± 0.095 mg/dl, respectively. Similar reductions were observed in groups fed high-fat diets with germinated fenugreek seed oil.

The greatest decrease in serum uric acid levels was observed in the group fed a high-fat diet with fenugreek seed oil providing 100% of the protein (group 8), followed by the group fed fenugreek seeds providing 100% of the protein (group 5). These groups reduced serum uric acid levels by approximately 20% and 15.75%, respectively, compared to the positive control group.

Hyperlipidemic rats fed a high-fat diet showed increased levels of serum uric acid, urea nitrogen, and creatinine compared to normal rats. Supplementing these rats with germinated fenugreek, providing 50%, 75%, and 100% of the required protein, along with its oil, significantly reduced these parameters. The most significant improvement in kidney function was seen in the group fed a high-fat diet supplemented with 100% protein from germinated fenugreek and its oil, indicating its protective effect on kidney function in hyperlipidemia.

In this respect, the interaction between lipotoxicity, oxidative stress, and inflammation highlights the complex effects of hyperlipidemia on kidney function. Lipotoxicity, caused by the accumulation of free fatty acids and lipids in kidney cells, leads to cellular damage and impaired function. Hyperlipidemia also increases oxidative stress, which exacerbates kidney injury and affects various kidney cells differently. Additionally, inflammatory pathways activated by hyperlipidemia worsen renal damage and accelerate chronic kidney disease progression **(Chen et al., 2023)**. CKD is often linked to altered lipid profiles, particularly elevated cholesterol and triglycerides, increasing cardiovascular disease risk. Dysfunctional HDL exacerbates inflammation and atherogenesis, compounding cardiovascular issues **(Meena et al., 2023)**. Fenugreek extracts protect against kidney damage from diabetic nephropathy by reducing oxidative stress and inflammation **(Alsuliam et al., 2022)** and improve renal function markers, such as creatinine and urea nitrogen, in diabetic rats **(Shivam & Gupta, 2023)**. Its antioxidant properties help preserve kidney health in hyperlipidemic conditions **(Alsuliam et al., 2022)**. Fenugreek oil reduces α-amylase and lipase activity, enhancing metabolic profiles and kidney protection **(Hamden et al., 2017)**. Bioactive compounds like saponins and polyunsaturated fatty acids help manage hyperlipidemia and protect renal health **(Idris et al., 2021)**. Fenugreek's high flavonoid content boosts antioxidant activity reduces advanced glycation end products (AGEs), and decreases calcium oxalate stone formation, promoting better kidney health **(Sabri et al., 2015)**. Additionally, fenugreek lowers postprandial glucose and mitigates oxidative stress, key contributors to kidney deterioration **(Singh et al., 2022)**.

Effect of Fenugreek Seed and Its Oils on Liver Enzymes in Hyperlipidemic Rats

The effects of three levels of Fenugreek seed powder, providing 50%, 75%, and 100% protein in a high-fat diet (HFD), along with the corresponding oil, on liver enzymes (AST, ALT, and ALP) in hyperlipidemic rats are shown in Table (8).

Feeding rats a high-fat diet (HFD) significantly increased serum AST levels ($p \leq 0.05$) in the positive control group (257.750 ± 8.920 U/L) compared to the negative control group (112.750 ± 2.217 U/L), showing a 128.6% increase (Table 8). Treatment with fenugreek seed powder at 50%, 75%, and 100% protein levels, along with their corresponding oils, significantly decreased AST levels compared to the positive control group. However, groups treated with fenugreek seed powder at 75% and 100% protein levels showed no significant changes compared to those treated with the corresponding oils. The largest reduction in AST levels (49.66% and 47.53%) was observed in groups treated with fenugreek oils providing 100% and 75% protein, respectively.

Inducing hyperlipidemia in rats with a high-fat diet significantly increased serum ALT levels ($p \leq 0.05$) in the positive control group (72.750 ± 3.593 U/L) compared to the negative control group (32.250 ± 2.629 U/L), showing a 125.6% increase (Table 8). Treatment with fenugreek seed powder and its oils significantly decreased ALT levels compared to the positive control group ($p \leq 0.05$). However, no significant differences were observed between groups treated with fenugreek seed powder (50%, 75%, and 100% protein) and their corresponding oils. The most significant reduction in ALT levels was seen in groups treated with higher levels of fenugreek seed powder (groups 4 and 5) and its oils (groups 7 and 8).

Table (8): Effect of Fenugreek Seed and Its Oils on Liver Enzymes in Hyperlipidemic Rats

Parameters / Groups	AST	ALT	ALP
	U/l		
1 Control (-ve)	112.750 [f] ± 2.217	32.250 [e] ± 2.629	467.250 [g] ± 19.276
2 Control (+ve)	257.750 [a] ± 8.920	72.750 [a] ± 3.593	1444.00 [a] ± 26.495
3	164.000 [c] ± 5.887	54.750 [b c] ± 2.629	981.000 [b] ± 15.121
4	141.500 [d] ± 3.109	51.000 [c d] ± 2.581	838.750 [d] ± 29.295
5	137.000 [d] ± 2.160	45.500 [d] ± 2.645	574.500 [f] ± 11.952
6	177.750 [b] ± 2.061	59.750 [b] ± 4.645	929.000 [c] ± 29.608
7	135.250 [d e] ± 3.500	48.750 [d] ± 2.986	919.750 [c] ± 17.632
8	129.750 [e] ± 4.573	47.000 [d] ± 5.477	675.250 [e] ± 25.382

Groups 3, 4, and 5 were fed a high-fat diet (HFD) containing 50%, 75%, and 100% of protein from powdered germinated fenugreek seeds, while **Groups 6, 7, and 8** were fed on HFD containing the amount of germinated fenugreek seed oil that is present in fenugreek seeds that were used in groups 3, 4, and 5, respectively.

*Values were expressed as Means ± SD.
* Values at the same column with different letters are significant at $P \leq 0.05$.

Inducing hyperlipidemia in rats with a high-fat diet significantly increased serum ALP levels ($p \leq 0.05$) in the positive control group (1444.00 ± 26.495 U/L) compared to the negative control group (467.250 ± 19.276 U/L), reflecting a 209.04% increase (Table 8). All hyperlipidemic groups treated with fenugreek seed powder and their oils showed a significant reduction ($p \leq 0.05$) in ALP levels compared to the positive control group. The greatest reduction was observed in the group treated with the highest level of fenugreek seed powder (group 5), followed by the group receiving the highest level of fenugreek seed oil (group 8).

Hyperlipidemic rats on a high-fat diet had significantly elevated serum AST, ALT, and ALP levels compared to normal rats. Supplementing with germinated fenugreek providing 50%, 75%, and 100% of the required protein, along with fenugreek seed oil, resulted in a marked reduction in all enzyme levels, with the greatest improvement observed in the group receiving the highest amount of fenugreek seed oil. These results suggest that germinated fenugreek protects liver enzymes under hyperlipidemic conditions.

A study in Bangladesh found that 61% of individuals with dyslipidemia had elevated liver enzymes, with gamma-glutamyl transferase (GGT) linked to lipid components **(Kathak et al., 2022)**. Research on type 2 diabetes patients showed a significant correlation between elevated liver enzymes (ALT, AST, GGT) and dyslipidemia, suggesting a link between impaired glucose metabolism and lipid abnormalities **(Al-Jameil et al., 2014)**. Fenugreek seed extract has been shown to lower total cholesterol (TC), LDL, and triglycerides (TGs), while increasing HDL in hyperlipidemic rats **(Zahid et al., 2020)**. Additionally, fenugreek seeds reduce body weight, improve lipid profiles, and lower liver enzymes like ALT and AST **(Kumar et al., 2014)**. Fenugreek has hepatoprotective effects, normalizing liver enzymes and reducing lipid peroxidation in rats exposed to toxic substances **(Belaïd-Nouira et al., 2013)**. Combined with garlic, fenugreek enhances antioxidant status and reduces liver triglycerides **(Mukthamba & Srinivasan, 2016)**. Fenugreek seed extracts have been shown to improve liver enzyme levels and lipid metabolism **(Kumar et al., 2014 and Vijayakumar et al., 2010)**. Moreover, fenugreek reduces liver enzyme levels in rats exposed to toxins like lead acetate and ethanol **(Naser et**

al., 2020 and El Gamal & Awaad, 2024). Its antioxidant properties, including increased superoxide dismutase (SOD) activity and decreased malondialdehyde (MDA), further protect the liver **(Alsuliam et al., 2022 and El-Nagdy et al., 2023)**.

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الملخص العربي

دراسة مقارنة على بذور الحلبة وزيتها لدى الفئران المصابة بإرتفاع دهون الدم

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هدفت هذه الدراسة إلى تقييم تأثير مستويات مختلفة من مسحوق بذور الحلبة كمصدر للبروتين، بالإضافة إلى زيوتها، على الفئران المصابة بإرتفاع مستوى دهون الدم. تم إجراء التجربة باستخدام ٤٠ فأراً بالغاً من ذكور الفئران البيضاء من سلالة سبراج - دولي بمتوسط وزن ١٨٠ ± ٥ جرام. تم تقسيم الفئران إلى مجموعتين رئيسيتين. تم إطعام المجموعة الرئيسية الأولى (٥ فئران) نظاماً غذائياً أساسياً (BD) واستخدمت كمجموعة تحكم سلبية (-ve group) أما المجموعة الرئيسية الثانية، التي تتكون من ٣٥ فأراً، فقد تم إطعامها نظاماً غذائياً عالي الدهون (HFD) لمدة أربعة أسابيع لتحفيز ارتفاع دهون الدم. تم تقسيم المجموعة الرئيسية الثانية إلى سبع مجموعات فرعية على النحو التالي: تم إطعام واحدة من هذه المجموعات (٥ فئران) نظاماً غذائياً عالي الدهون (HFD) واستخدمت كمجموعة تحكم إيجابية (+ve group) أما الست مجموعات المتبقية، فقد تضمنت ثلاث مجموعات تم إطعامها أنظمة غذائية عالية الدهون (HFDs) تحتوي على مسحوق بذور الحلبة المنبتة، التى تمد الوجبات بنصف و ثلاثة أرباع و الكمية الكاملة من البروتين الغذائي. أما المجموعات الثلاث الأخيرة فقد تم إطعامها أنظمة غذائية عالية الدهون (HFDs) تحتوي على زيت مستخلص من بذور الحلبة المنبتة، بكميات معادلة للزيوت الموجودة في بذور الحلبة المنبتة المستخدمة في المجموعات الثلاث السابقة. في نهاية التجربة (بعد ٤ أسابيع)، تم تخدير الفئران بواسطة الإيثر قبل أن يتم ذبحها بعد صيامها طوال الليل. تم تجميع عينات الدم وتركها لتتجلط، ثم تم فصل السيرم. تم قياس صورة الدهون في السيرم، والتى تتضمن الكوليسترول، الدهون الثلاثية، والبروتين الدهني عالي الكثافة .(HDL-c) كما تم حساب مستوى البروتين الدهني منخفض الكثافة (LDL-c)، البروتين الدهني منخفض الكثافة جداً (VLDL-c) ، مؤشر التصلب (AI)، ومؤشر مخاطر الشريان التاجي (CRI) . بالإضافة إلى ذلك، تم تقدير وظائف الكلى (حمض اليوريك، نيتروجين اليوريا، الكرياتينين) وإنزيمات الكبد "أسبارتات أمينوترانسفيراز(AST) ، ألانين أمينوترانسفيراز(ALT) ، والفوسفاتاز القلوي (ALP) . أظهرت النتائج أن النظام الغذائي عالي الدهون (HFD) أدى إلى زيادة الدهون، مما تسبب في تغيير غير ذي دلالة في نسبة تناول الطعام (FI) وزيادة كبيرة في النسبة المئوية للزيادة الوزن (BWG%) مقارنةً بمجموعة التحكم السلبية. أدت الأنظمة الغذائية عالية الدهون (HFDs) التي تحتوي على ثلاثة مستويات من مسحوق بذور

الحلبة المنبتة إلى انخفاض كبير في النسبة المئوية للزيادة في الوزن (BWG%) ، في حين لم تؤدى المستويات الثلاثة من زيوتها الي ذلك، مقارنةً بمجموعة التحكم الإيجابية. وقد أدت معاملة الأنظمة الغذائية عالية الدهون (HFDs) بالمستويات الثلاثة من مسحوق بذور الحلبة المنبتة وزيوتها إلى تحسن كبير في جميع التقديرات، مقارنةً بمجموعة التحكم الإيجابية. في الوقت نفسه، أظهرت المجموعات الإيجابية التي تم إطعامها أنظمة غذائية عالية الدهون (HFDs)تحتوي على مستويات عالية من مسحوق بذور الحلبة المنبتة وزيوتها التحسن الأكثر وضوحا. وفي النهاية، تشير هذه النتائج إلى الإمكانات العلاجية لمسحوق بذور الحلبة وزيوتها في التحكم في ارتفاع دهون الدم والمضاعفات الناتجة عنها.

الكلمات المفتاحية: بذور الحلبة، زيت بذور الحلبة، فئران، ارتفاع دهون الدم، صورة الدهون، وظائف الكلى، و إنزيمات الكبد