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Anti-Hyperlipidemic and Hypercholesterolemic Properties of Germinated Fenugreek Seeds on Male Rat

Aml F. Elgazar

Department of Nutrition and Food Sciences, Faculty of Home Economics, Helwan University, Cairo, Egypt

Abstract: Hyperlipidemia and hypercholesterolemia are a problem disease for many societies and is one of the major risk factors for the development of cardiovascular diseases. The present study was conducted to investigate the hypolipidemic and hypocholesterolemic effects ofgerminated fenugreek seeds in rats fed high fat, cholesterol diet. Fenugreek seeds were germinated, dried, ground to fine powder and mixed with high fat, cholesterol diet at three different levels (5, 10 and 15%). Thirty five male rats weighing 150 ± 5g were divided into five groups of seven rats each. Group 1 served as normal control group and the remaining four groups fed high fat, cholesterol containing diets to induce hyperlipidemia and hypercholesterolemia. Group 2 kept as positive control group and groups 3, 4 and 5 fed high fat, cholesterol diet supplemented with 5, 10 and 15% of germinated fenugreek seeds, respectively. Food intake, body weight gain, % change of body weight, organs weight (liver, kidneys and heart) and its relative weight to body weight were determined. Serum total lipids (TL), triglycerides (TG), total cholesterol (TC), low density lipoproteins (LDL-c), high density lipoproteins (HDL-c), malondialdehyde (MDA), reduced glutathione (GSH) levels and activity of superoxide dismutase (SOD) and catalase (CAT) enzymes were assayed. Histopathological changes of heart in fed high fat, cholesterol diet and treated with germinated fenugreek seeds groups was studied. The present finding revealed thatgerminated fenugreek seeds significantly decreased body weight and amendment serum TL, TG, TC, LDL-c, HDL-c, MDA, GSH levels and activity of antioxidant enzymes (SOD, CAT), additionally, improvement of histopathological structure of heart compared with that fed high fat, cholesterol diet alone. Inconclusionthe present study concluded that fenugreek seeds were effective in reducing the total body weight and exhibited significant hypolipidemic and hypocholestermic effect, in a dose dependent manner.

Key words: Fenugreek Seeds · Rats · Lipid Profile · Antioxidant Enzymes

INTRODUCTION

Fenugreek (*Trigonella foenum graecum*) is an annual plant belongs to the family Leguminosae. Fenugreek seeds and its green leaves are used in food as well as in medicinal application that is the old practice of human history [1]. In India, Asian, African and other European countries, fenugreek plant is used as spice andhas many medicinal properties as antidiabetic effects and helpful in digestive disorders such as flatulence, dysentery, diarrhea, dyspepsia, chronic cough and enlargement of the liver and spleen [2]. Fenugreek seeds are considered as an appetizer and helps in digestion; improve growth performance and health [3]. It also has many therapeutic effects like antibacterial, anti-inflammatory, antipyretic and antioxidant properties [4]. The major medicinal properties of the fenugreek are anticarcinogenic, antiulcer, anthelmintic, antifertility activity, immunomodulatory effect and hypocholesterolemic [5].

Germinated fenugreek seeds were observed to be more beneficial than dried seeds because germination increases the bioavailability of different constituents of fenugreek seed [6]. Germinated fenugreek seeds had significantly higher contents of total protein and total lysine compared to dried seeds. Additionally, germination decreased dietary fiber and starch thereby raising the level of sugars [7].

Cardiovascular disease (CVD) is the most common cause of death in industrialized countries [8]. Major risk factors for cardiovascular disease are modifiable and/or non-modifiable factors. Modifiable risk factors include hypertension, hyperlipidemia, diabetes mellitus, obesity

Corresponding Author: Aml Fawzy Elgazar, 65 ElmatbaElahlia Street, Bolaq Abu Elaala, College of Home Economics, Helwan University, Cairo, Egypt. E-mail: dr_aml_fawzy@yahoo.com.

and high intake of saturated fat, physical inactivity, smoking and other lifestyles. Whereas, non-modifiable risk factors are family history of high blood pressure and/or premature CVD and old age [9].

Hyperlipidemia is a disorder, showing elevated levels of serum triglycerides (TG) and total cholesterol (TC) above the normal range. It is known, that plasma levels of TG are influenced by dietary composition, body weight, genetic factors and smoking. Similarly to the other risk factors, it is estimated that the contribution of genetic and environmental factors on plasma levels of TG are roughly the same [10]. Hyperlipidemia is a major cause for atherosclerosis and its associated conditions as coronary artery, ischemic cerebrovascular and peripheral vascular diseases [11,12].

The present study was undertaken to investigate the anti-hyperlipidemic and hypercholesterolemic effects of germinated fenugreek seeds experimentally on male rats fed high fat, cholesterol diet to induce hyperlipidemia and hypercholesterolemia.

MATERIALS AND METHODS

Materials

Rats and Diet: Thirty five male Sprague-Dawley rats weighing 150 ± 5g were purchased from the Laboratory Animal Colony, Ministry of Health and Population, Helwan, Egypt.Basal diet constituents were purchased from El-Gomhorya Company for Pharmaceutical and Chemical, Cairo, Egypt.

Fenugreek Seeds: Dried fenugreek seeds were purchased from the local market (Haraz market for herbs and medicinal plants), Cairo, Egypt.

Chemicals and Kits: Cholesterol, cholic acidand other chemicals were purchased from El-Gomhorya Company for Chemical and Pharmaceutical, Cairo, Egypt. Kits for biochemical analysis of serum total lipids (TL), triglycerides (TG), total cholesterol (TC), low density lipoproteins (LDL-C), high density lipoproteins (HDL-C), malondialdehyde (MDA), reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) were purchased from the Gamma Trade Company for Pharmaceutical and Chemicals, Dokki, Egypt.

Methods

Preparation of Basal Diet: The basal diet (AIN-93M) was prepared according to Reeves *et al*. [13]. Diet was formulated to meet the recommended nutrients levels for rats as shown in Table 1.

Table 1: Constituents of basal diet (AIN-93M)

Ingredient	Content (g/kg)
Casein	200.000
Corn starch	546.200
Sucrose	100.00
Soybean oil	50.000
Fibers	50.000
Mineral mix.	40.000
Vitamin mix.	10.000
L-Cystine	1•800
Choline chloride	2.000
Tert-Butylhydroquinone	0.008

Germination of Fenugreek Seeds: Dried fenugreek seeds were cleaned from broken seeds, dust and other foreign materials and then soaked in tap water for 12 hr. at room temperature. Soaked seeds were germinated in strainer with covering by wet cotton cloth for 48 hr. at room temperature with frequent watering. The germinated fenugreek seeds were rinsed in distilled water, then dried at 50-55 °C using oven under vacuum. The dried germinated seeds were ground to fine powder in an electric grinder and then stored in plastic containers for further use in room temperature.

Induction of Hyperlipidemia and Hypercholesterolemia: A Hyperlipidemia and hypercholestermia rat was done according to the method of Balkan*et al*. [14]. In briefly, basal diet was formulated with 1% cholesterol, 2% sheep fat and 0.5% cholic acid to enhance the enteral absorption of lipids.

Experimental Design: All animals were housed in healthy condition at room temperature, with 40–60% humidity, exposed to a 12:12-hr. light-dark cycle and fed on the basal diet, water was provided ad *libitum* for one week before starting the experiment for acclimatization. After acclimatization period rats were divided into five groups of seven rats each. Group I, feed on the basal diet and kept as negative control group (normal rats); the remaining four groups were feed on high fat, cholesterol containing– diets. Group 2 was representing as a positive control group; groups (3), (4) and (5) were feed on high fat, cholesterol containing – diets supplemented with 5, 10 and 15% of germinated fenugreek seeds, respectively.

At the end of the experimental period (4 weeks),diets were withheld from all rats for 12-hr., except of water, then all rats were sacrificed under light diethyl either. Blood samples were collected from the portal vein into dry clean centrifuge tubes. For serum separation, blood samples

were left at room temperature to get clot and then centrifuged for 15 minutes at 3000 rpm. Serum was carefully aspired using a needle and transfers into dry clean test tubes and kept frozen at -20ºC until chemical analysis. Organs such as liver, kidney and heart were removed and washed with saline solution, dried and then weighted to calculate relative organs weight to body weight. Heart of all animals was immersed in neutral buffered formalin 10% for histopathology examination.

Food intake, Body Weight Gain and Percent Change of Body Assay: Food intake (FI) was calculated every other day. The changes in body weight were determined by weighing the animals prior the experiment (IBW) and after four weeks (FBW). The biological value of the different diets was assessed by the determination of its effect on body weight gain (BWG)and percent change of body weight the end of the experimental period using the following formulas:

Body weight gain (BWG) = Final body weight - Initial body weight
% change of body weight = BWG/IBW

Biochemical Analysis:

Lipid Profile and Lipoprotein Cholesterol Assay: Serum total lipid (TL) levelwas determined colorimetric using spectrophotometer apparatus using kit instructions (Randox Co., Ireland) as described by Christopher [15]. Levels of serum triglycerides (TG), total cholesterol (TC), low density lipoprotein cholesterol (LDL-c) and high density lipoprotein cholesterol (HDL-c) were determined using the provided instructions kits (Analyticon ®Biotechnologies AG, Germany) as described by Fossati and Principe [16], Young [17], Wieland and Sidel [18] and Burstein *et al.* [19], respectively. Very low density lipoprotein cholesterol (VLDL-C) was calculated as described by Friedwald *et al.* [20] using the following equation:

VLDL-c (mg/ dL) = TG/5

Malondialdehyde Assay: Serum MDA level was determined as described byDraper and Hadley [21]. The principle of the methods is spectrophotometric measurement of the color produced by the reaction of thiobarbituric acid (TBA) with MDA. The concentration of MDA was then calculated as expressed as μmoles/dL.

Antioxidant Assay: Serum GSH level was measured by the method of Beutler *et al.* [22]. This method depends on spectrophotometric estimation at 412 nm. The serum concentration of GSH was calculated as expressed as μmoles/dl. Serum activity of SOD and CAT enzymes were determined by Autoanalyzer (Roche-Hitachi, Japan) using commercial kits according to the methods described by Hissin and Hiff [23] and Sinha [24], respectively.

Statistical Analysis: Data were expressed as mean ± standard deviation. The statistical analysis was performed using computerized statistical package of social sciences (SPSS) program (SPSS. 20 software version) with one-way analysis of variance (ANOVA) followed by Duncan's multiple range tests. $P < 0.05$ values were considered to be statistically significant.

RESULTS

The present data in Table 2 illustrates the effect of feeding on supplemented high fat, cholesterol diet with different levels (5, 10 and 15%) of germinated seeds on FI, BWG and % change of body weight in rats. It showed that ratsfed highfat, cholesterol diets (positive group) have no significant increase ($p< 0.05$) in FI, compared with that fed normal basal diet (normal group). Rats fed supplemented high-fat cholesterol diets with different levels of germinated fenugreek seeds have significant decrease in FI compared with that fed high-fat cholesterol diet alone. Mean and standard division values of FBW, BWG and % changes of body weight were increased significantly ($p<0.05$) in rats fed highfat, cholesterol dietcompared to that normal diet. No significant changes were detected in treated rats with low (5%) level of germinated fenugreek seeds. However, treated rats with 10 and 15% of germinated fenugreek seeds have significant decrease in FBW, BWG and % changes of body weight, compared to that of untreated rats (positive rats).

Table 3 shows weights and relative weights to body weight of liver, kidneys and heart of normal rats, positive rats (fed high fat, cholesterol diet) and rats fed high fat, cholesterol diet supplemented with different levels of germinated fenugreek seeds. The present data illustrated that rats fed high fat, cholesterol diet have significant increase ($p<0.05$) in organs (liver, kidneys and heart) weight and its relative to body weight, compared with that of rats fed normal diet. Treated rats with different levels of germinated fenugreek seeds have significant decrease ($p<0.05$) in liver, kidneys and heart weight as well as their relative to body weight, compared with that of untreated rats.

Table 2: Effects of germinated fenugreek seeds on FI, BWG and % change of body weight in hyperlipidemic and hypercholesterolemic rats

	Parameters as Mean ± SD				
Groups	FI (g)	IBW (g)	FBW (g)	BWG (g)	% Change of body weight (%)
G 1:Negative group (Normal)	23.14±0.90[a]	151.71±1.38	176.43±1.13 [b]	24.71±1.89[b]	15.91±1.17[b]
G 2: Positive group	24.00±1.00[a]	152.29±1.89	183.71±1.11 [a]	31.43±2.15[a]	20.75±1.64[a]
Treated groups with:					
G3: 5% GFS	21.71±1.25[b]	151.43±2.70	182.43±1.27 [a]	31.00±1.53[a]	20.49±1.40[a]
G4: 10% GFS	21.43±0.79[b]	151.57±2.64	177.86±1.77 [b]	26.29±3.09[b]	17.09±2.55[b]
G5: 15 % GFS	18.43±0.79[c]	152.14±2.12	177.29±1.38 [b]	25.57±1.27[b]	16.52±1.37[b]

Means ± SD with different superscripts in the same row are significant at $P< 0.05$

SD: Standard Division of mean

Table 3: Effects of germinated fenugreek seeds on organs (liver, kidneys and heart) weight and it'srelative to body weight in hyperlipidemic and hypercholesterolemic rats

	Parameters as Mean ± SD					
	Liver		Kidneys		Heart	
Groups	Weight (g)	Relative to body weight	Weight (g)	Relative to body weight	Weight (g)	Relative to body weight
G 1:Negative group (Normal)	3.33 ±0.27[cd]	1.89±0.16[c]	0.89±0.04[d]	0.50±0.02[c]	0.60±0.01[c]	0.34±0.01[c]
G 2: Positive group	4.08±0.13[a]	2.22±0.07[a]	1.30±0.06[a]	0.70±0.03[a]	0.71±0.01[a]	0.38±0.01[a]
Treated groups with:						
G3: 5% GFS	3.79±0.13[b]	2.08±0.08[b]	1.04±0.13[b]	0.57±0.07[b]	0.64±0.01[b]	0.35±0.01[b]
G4: 10% GFS	3.48±0.06[c]	1.91±0.14[c]	0.98±0.03[bc]	0.54±0.02[bc]	0.61±0.01[c]	0.34±0.04[c]
G5: 15 % GFS	3.23±0.21[d]	1.82±0.12[c]	0.92±0.05[cd]	0.51±0.03[c]	0.59±0.01[c]	0.34±0.01[c]

Means ± SD with different superscripts in the same row are significant at $P< 0.05$

SD: Standard Division of mean

Table 4: Effects of germinated fenugreek seeds on serum TL, TG and TC in hyperlipidemic and hypercholesterolemic rats

	Parameters as Mean ± SD		
Groups	TL (mg/dL)	TG mg/dL)	TC (mg/dL)
G 1:Negative group (Normal)	323.40± 0.89[e]	115.2±0.84[d]	55.80±0.84[d]
G 2: Positive group	435.40±1.14[a]	161.00±1.00[a]	96.40±1.14[a]
Treated groups with:			
G3: 5% GFS	388.80±0.84[b]	145.00±0.71[b]	84.40±1.34[b]
G4: 10% GFS	355.00±0.71[c]	131.20±1.64[c]	69.00±1.22[c]
G5: 15 % GFS	326.20±0.84[d]	113.80±1.10[d]	54.00±1.00[e]

Means ± SD with different superscripts in the same row are significant at $P< 0.05$

SD: Standard Division of mean

As shown in Table 4 results revealed that rats fed high fat, cholesterol diet (positive rats) have significant increase ($p<0.05$) in serum TL, TG and TC level compared to that of rats fed normal diet. Ratsfed high fat, cholesterol supplemented diet with different levels of germinated fenugreek seedshave significant reduction in serumTL, TG and TC level compared to that of positive rats. The higher amelioration of serum lipid profile was showed in rats fed high fat, cholesterol supplemented diet with the higher level (15%) of germinated fenugreek seeds compared to that of the other treated rats with 5 and 10 % of germinated fenugreek seeds.

Table 5: Effects of germinated fenugreek seeds on serum LDL-c, HDL-c and VLDL-c in hyperlipidemic and hypercholesterolemic rats

	Parameters as Mean ± SD		
Groups	LDL-c (mg/dL)	HDL-c (mg/dL)	VLDL-c (mg/dL)
G 1:Negative group (Normal)	11.94±0.43[d]	20.10±0.20[a]	23.74±0.96[d]
G 2: Positive group	53.36±1.89[a]	11.08±0.64[c]	31.38±0.85[a]
Treated groups with:			
G3: 5% GFS	38.92±0.08[b]	16.78±0.31[b]	29.60±0.16[b]
G4: 10% GFS	21.90±0.63[c]	19.42±0.28[a]	26.94±0.44[c]
G5: 15 % GFS	10.84±0.42[d]	20.14±0.99[a]	23.96±1.06[d]

Means ± SD with different superscripts in the same row are significant at $P< 0.05$

SD: Standard Division of mean

Table 6: Effects of germinated fenugreek seeds on serum MDA and GSH level and SOD and CAT activities ofhyperlipidemic and hypercholesterolemic rats

	Parameters as Mean ± SD			
Groups	MDA (μmol/dl)	GSH (nmol/l)	SOD (mmol/dl)	CAT (mmol/dl)
G 1:Negative group (Normal)	18.5857±0.73[d]	55.1143±0.90[b]	78.9714±1.04[b]	69.3429±0.97[a]
G 2: Positive group	60.5714±1.02[a]	33.96±1.08[e]	46.1857±0.76[e]	52.1857±0.68[d]
Treated groups with:				
G3: 5% GFS	45.2429±0.64[b]	41.4143±1.01[d]	57.9857±0.48[d]	58.1571±0.78[c]
G4: 10% GFS	31.3286±1.13[c]	47.07±0.46[c]	67.8786±1.02[c]	62.8000±0.26[b]
G5: 15 % GFS	19.2143±0.64[d]	56.2000±0.75[a]	81.0571±1.12[a]	68.9571±1.53[a]

Means ± SD with different superscripts in the same row are significant at $P< 0.05$

SD: Standard Division of mean

Results in Table 5 shows serum LDL-c, HDL-c and VLDL-c levels in normal rats, rats fed high fat, cholesterol diet alone and rats fed high fat, cholesterol supplemented diet with different levels of germinated fenugreek seeds. It demonstrated that untreated rats have significant increase in serum level of LDL-c and VLDL-c and decrease in HDL-c compared to that of normal rats. Germinated fenugreek seeds at the three levels induced significant amelioration in serum LDL-c, VLDL-c and HDL-c level rats compared to that of untreated rats. Increasingly improvement with increased added level of germinated fenugreek seeds to high fat, cholesterol diet.

Table 6 shows the serum MDA and GSH level and activity of SOD and CAT enzymes of normal control group, fed high fat, cholesterol diet group and fed high fat, cholesterol supplemented diet with germinated fenugreek seeds groups. The present results showed significant increase in serum MDA level and decrease in GSH level and activities of SOD and CAT enzymes in rats fed high fat, cholesterol diet alone, compared to that fed normal diet. Co-administration of high fat, cholesterol diet with different levels of germinated fenugreek seeds caused significant decrease ($p<0.05$) in serum MDA level and increased GSH level and activity of SOD and CAT enzymes, compared with that fed high fat, cholesterol diet alone.

Heart sections of normal rats show apparently normal heart muscle and blood vessel (Fig. 1). In contrast, heart sections of rats fed high fat, cholesterol diet reveale ddilated blood vessel with thick muscle wall as shown in Fig. 2. Heart section of treated rats with 5% germinated fenugreek seeds showed congested blood vessel as shown in Fig. 3. Normal histological structure in heart sections was showed in rats fed high fat, cholesterol-diet supplemented with 10 and 15% germinated fenugreek seeds as shown in Fig. 4.

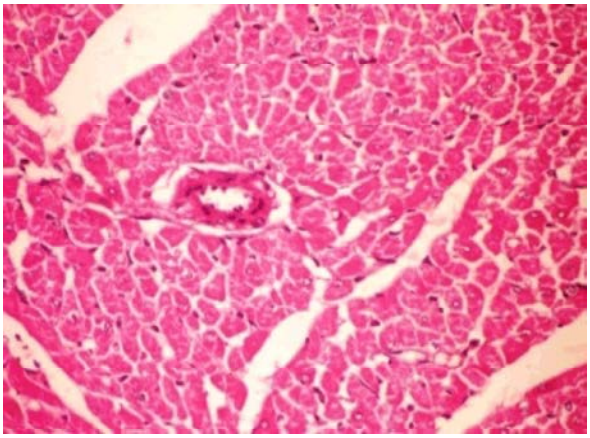

Fig. 1: Heart section of normal rats showing apparently normal heart muscle and blood vessel (H&E X 400)

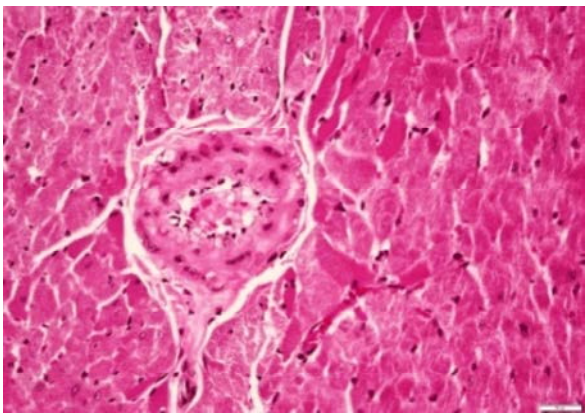

Fig. 2: Heart section of positive control rats showing dilated blood vessel with thick muscle wall (H&E X 400)

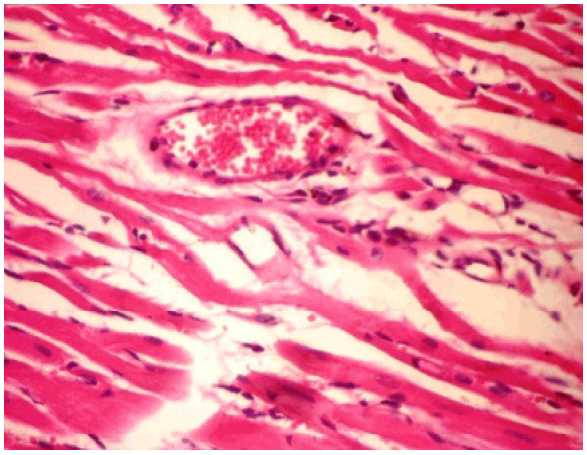

Fig. 3: Heart section of treated rats with 5% germinated fenugreek seeds showing congested blood vessel (H&E X 400)

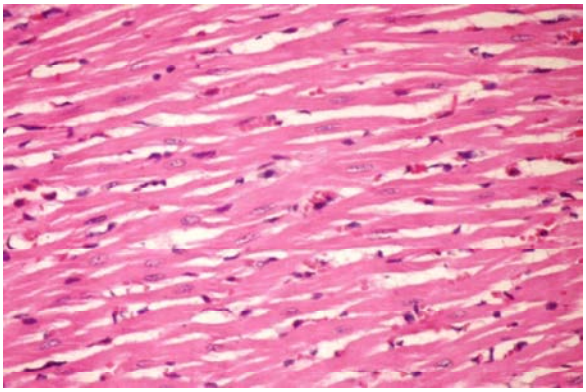

Fig. 4: Heart section of treated rats with 10 and 15% germinated fenugreek seeds showing no histopathological changes (H and E x 400)

DISCUSSION

The present study was undertaken to study the hypolipimic and hypocholesterolemic effects of germinated fenugreek seeds experimentally on rats fed high fat, cholesterol diet. The present results showed that rats fed high-fat, cholesterol diets have no significant change ($p< 0.05$) in FI and significant increase in FBW, BWG and % changes of body weight compared to that fed normal diet. As notice, the resultant weight higher in positive rats was confirmed by the significant increase in organs (liver, kidneys and heart) weight and their relative weight to body weight which could be as a result of the high fat and caloric intake of the high fat, cholesterol diet. The present result is in agreement with Ramulu *et al.* [25] who indicated that there are no significant differences in food intake between control and experimental groups fed high fat diet. Kusnoki *et al.* [26] reported that high fat diet is considered to be an important factor in the development of obesity, leading to accumulation of body fat even in the absence of an increase in caloric intake in fed rats with high fat diet. Picchi *et al*. [27] observed that the high-fat diet led to greater gain of hepatic fat compared to control. Rezq and El-Khamisy [28] showed that rats fed high fat, cholesterol-diet have significant increase in relative weight of liver, heart and kidneys to body weight compared to normal rats. Macfarlane [29] demonstrated that high-fat content is harmful being high in energy and therefore leading to overweight. Jakobsdottir *et al.* [30] and Haghshenas *et al.* [31] observed that the average body weight and liver weight was higher in rats fed high fat diet than rats fed normal diet. de Castro *et al.* [32] reported that young rats fed high fat diet have higher mean liver weight and mesenteric, retroperitoneal and epididymal fat deposits compared to normal group.

On the other hand, high fat, cholesterol supplemented diet with different levels of germinated fenugreek seeds caused significant reduction in FI, compared to that fed high fat, cholesterol diet alone. However, treated rats with 10 and 15% of germinated fenugreek seeds have significant decrease in FBW, BWG and % changes of body weight, compared to that fed high fat, cholesterol diet. These results are in accordance with Omi *et al.* [33] who showed normal rats fed supplemented diet with fenugreek seeds have a significant decrease in total body weight. The reduction in body weight may be attributed to inhibition effect of fat accumulation caused by fenugreek seed [34]. Toshiaki *et al*. [35] showed that feeding the highfat diet markedly increased tissue weight in adipose tissue compared to the lowfat diet.

The fenugreek seed extract significantly reduced adipose tissue weights and liver weight. These results suggest that fenugreek seed extract decreased lipid accumulation in the liver induced by a high-fat diet. Geetha *et al.* [36] showed that animals fed diets containing various levels of fenugreek seed extract have significant reduction in food intake and body weight. Also, Abor [37] observed that weight gain was significantly decreased in treated rats with both powder and aqua extract fenugreek as compared to hyperlipidemic rats. Food intake was insignificantly different among all experimental groups as compared to hypolipidemic rats. The significant lowering in food intake and body weight gain in rats fed high fat, cholesterol diet containing different levels of germinated fenugreek seeds may be due to its content of soluble fiber. Several literature indicated that dietary fibre reduces weight gain, especially soluble and viscous ones, because it have the capacity to slow down and limit food intake or increase satiety and diminish the absorption of nutrients in the small intestine [38]. In a human study by Johansson *et al.* [39] demonstrated that the consumption of a highfibre evening meal resulted in less food intake the following day. Soluble fiber fraction of fenugreek seeds reduces the rate of enzymatic digestion and the absorption of glucose from the gastrointestinal tract [40]. In addition to, fenugreek seeds modulate plasma glucose levels by delaying gastric emptying and by interference with glucose absorption at the gastrointestinal system and it exerts an inhibitory effect on intestinal carbohydrate digestion [41]. Recently, Abor [37] reported that fenugreek outburst out the carbohydrates from the body before they enter the blood stream resulting in weight loss. This fiber forms a gelatinous structure which may have effects on slowing the digestion and absorption of food from the intestine and create a sense of fullness in the abdomen, promotes weight loss.

Rats fed high fat, cholesterol diet have significant increase in serum level of TL, TG, TC, LDL-c and VLDL-c and decrease in HDL-c compared to that fed normal diets. These results were confirmed by histopathological examination of heart which revealeddilated blood vessel with thick muscle wall. The present results are in accordance with Gregorio *et al.* [42] who reported that serum TG, TC, LDL-c and VLDL-c increased significantly in rats fed high cholesterol diet, compared to rats fed normal diet. Puskas *et al.* [43] showed intracellular lipid accumulation in cardiomyocytes in response to cholesterol diet. Cohn [44] revealed that hyperlipidemia in rats is characterized by the rise in triglyceride-rich lipoproteins after a high fat diet. Zhen-Yu and Xiao-Qi and [45] demonstrated that lipid metabolism in rats feed high fat diet presented disorder and the level of serum TC and triglycerides increased significantly, compared with those in the normal group. Rezq and El-Khamisy [28] reported that high fat, cholesterol diet caused significant increase in serum TL, TG, TC, LDL-c and VLDL-c and decrease in HDL-c levels. Gupta [46] indicated that cholesterol diet increased mean serum TG, TC Triglyceride, LDL and VLDL levels significantly in rabbits fed high fat diet. The elevation in serum lipid profile and lipoprotein levels in rats fed high fat, cholesterol diet might be attributed to the activity of lipoprotein lipase which augmented in hypercholesterolemic animals. Lipase transforms VLDL to LDL-c and lead to increase serum concentration of LDL-c [47]. An uptake of LDL-c is depended on receptors in plasmatic membrane and these are reduced in number when the cell has enough cholesterol. This may have happened in hepatic cells of the animal fed cholesterol-supplemented diets, explanatory their higher LDL-c concentration [48]. Rats fed high fat, cholesterol diet supplemented with different levels (5, 10 and 15%) of germinated fenugreek seeds have significant ameliorate in serum TL, TG, TC, LDL-c, VLDL-c and HDL-c compared to that fed high fat, cholesterol diet alone. These results were confirmed by histopathological examination of heart which revealedcongested blood vessel in heart section of treated rats with 5% germinated fenugreek seeds showed. However, normal histological structure in heart sections was showed in rats fed high fat, cholesteroldiet supplemented with 10 and 15% germinated fenugreek seeds. This improvement indicate that supplementation of diet with fenugreek quickened the process of achieving eulipidemic state. These results are in accordance with Sowmya and Rajyalakshmi [49] who revealed that consumption of the germinated fenugreek seeds at two different levels (12.5 g and 18.0 g) resulted in a hypocholesterolemic effect in the hypercholesterolemic adults of both sexes in the age range of 50-65 years. Between the two levels, higher levels consumption of germinated fenugreek seed resulted in a significant reduction in total cholesterol and LDL levels. Bahram *et al.* [50] showed that serum TG, TC and LDL-c levels of group fed high cholesterol diet in additional to germinated seeds powder were significantly decreased compared to that fed high cholesterol diet alone. Abu-Saleh *et al.* [51] reported that administration of fenugreek seed produces a significant reduction of total serum TC, TG and LDL-c in hypercholesterolemia group. Reddy and Srinivasan [52] showed that rats fed fenugreek seeds powder mixed with hypercholesterolemia inducing diet caused decrease in

serum LDL-c and VLDL-c levels. Gupta [46] observed that fenugreek significantly lowered serum TC, TG and LDL-c levels and raised the HDL-c level than the control group. Recently, Sharma and Choudhary [53] showed that fenugreek seeds significantly decrease serum TC, TG, LDL-c and VLDL-c and atherogenic index and increase serum HDL-c level as compared to that fed high fat diet.

The responsible factors for amendment effect of germinated fenugreek seeds on serum lipid profile and lipoprotein level have so far not yet been established. However, it has been suggested that these finding may be related to the higher content of saponins in fenugreek seeds that known to have hypocholesterolemic effects [54]. Petit *et al.* 55] demonstrated that steroid saponins extracted from fenugreek seeds decrease total plasma cholesterol and lipid in rats. The hypocholesterolemic and hypolipidemic effect of saponins may be due to its ability to prevent the lipid absorption either in the intestine or bind to bile acids during enter hepatic re-absorption mechanism [41], increased the biliary cholesterol excretion [56] and form insoluble complex with lipids [34]. On the other hand, the hypolipidemic and hypocholesterolemic effect of fenugreek seeds could be attributed to its dietary fiber which have a key role in reducing the cholesterol levels through increase fecal excretion of bile acids and salts as well as inhibitors of hepatic cholesterol biosynthesis by short chain fatty acids produced by bacterial fermentation of soluble dietary fiber in the lower parts of large intestine [57]. Germination of the fenugreek seeds improves the soluble fiber content of the seeds, thus improving their effect on cholesterol [49]. The crude fiber content of fenugreek seeds is one of the most lowering cholesterol agents [41,58]. Ramulu *et al.* [25] demonstrated that the hypocholesterolemic effect of germinated fenugreek seeds is not related to the decrease in cholesterol biosynthesis but are by increased rate of catabolism of cholesterol. However, there is indirect evidence in terms of increased fecal content in experimental rats, which is as a result of increased excretion of fecal bile acids and neutral sterols. This could be due to high content of soluble and in soluble dietary fiber. Dietary fibre (galactomannan) in fenugreek seeds form a viscous gel in the intestine and inhibit glucose and lipid absorption [59]. Additionally, fenugreek seeds contain the phenolic compounds, mainly flavonoids that have hypolipidemic effect [60]. In epidemiological studies, polyphenol and flavonoid-rich extract from fenugreek seeds have been shown to possess the hypolipidemic effect due to their antioxidant defense [61]. Also, the lipid lowering effect of fenugreek seeds is due to its action on the adipocytes and the liver cells, which leads to decreased triglycerides and cholesterol synthesis in addition to an enhanced LDL receptor mediated LDL uptake [62]. Triglyceride lowering effect may be due to the pectin component of the fenugreek extract that absorbs the bile acids [63]. Vijayakumar *et al.* 64 nsuggested that the lipid-lowering effect of thermo stable extract of fenugreek seeds is due to inhibition of fat accumulation and up regulation of the low-density lipoprotein receptor. The hypolipidemic effect of the fenugreek seeds could be attributed to the presence of 4-hydroxy isoleucine, an atypical, branched chain amino acid [65]. Also ethyl acetate content of fenugreek decreases total cholesterol [66].

With regard to serum MDA and GSH level in experimental group, the present results showed significant increase in serum MDA level and decrease in GSH level and activities of SOD and CAT enzymes in rats fed high fat, cholesterol diet, compared to that fed normal diet. The present results are in agreement with Moussa [67] who indicated that hyperlipidemia induces oxidative stress and the malondialdehyde (MDA) is one of the products in lipid peroxidation. Plasma MDA levels increased markedly in animals with obesity and diabetes mellitus. Denisenko and Novgorodtseva [68] showed that animals fed high fat diet inhibits blood enzyme activity of reduced glutathione and activate lipid peroxidation (MDA). Amirkhizi *et al.* [69] indicated that hypertriglyceridemia results in obese rats contribute to the alteration in the oxidant-antioxidant balance, suggesting that an increase in the bioavailability of free fatty acids can increase lipid peroxidation. Hussein *et al*. [70] showed significant increase in liver MDA and decrease in GSH, CAT and SOD in high fat diet induced hyperlipidemia. The present result provides a perfect correlation between lipid peroxidation products and decreased level of GSH and activities of CAT and SOD enzymes which play an important role in the antioxidant system. A decrease in the activity of these enzymes, as seen in serum of high fat diet induced hyperlipidemia and hypercholesterolemia in rats, can lead to the excessive availability of superoxide and peroxyl radicals, which in turn generate hydroxyl radicals, resulting in the initiation and propagation of more lipid peroxidation products. Hypercholesterolemia disturbed the oxidant-pro-oxidant balance in favor of prooxidation [71]. The efficiency of this defense system is apparently weakened in hypercholesterolemia, resulting in ineffective scavenging of free radicals which lead to tissue damage [72]. On the other hand, Zhang *et al.* [73] demonstrated that a high fat

diet results in release of free fatty acids by lipoprotein lipase from increased serum triglycerides cause lipotoxicity, which results in insulin receptor dysfunction. The release of excessive free fatty acids provokes lipotoxicity, as lipids and their metabolites create oxidative stress. Additionally, lipid alterations have been considered as contributory factors to oxidative stress in obesity [74]. Increased production of reactive oxygen species as well as reduced antioxidant defense mechanisms have been suggested to play a role in both humans and animal models of obesity [69]. Co-administration of high fat, cholesterol diet with three different level of germinated fenugreek seeds caused significant decrease ($p<0.05$) in serum MDA level and increased GSH level and activities of SOD and CAT enzymes, compared with that fed high fat, cholesterol diet alone. These results are in convention with Anuradha and Ravikumar [75] that showed fenugreek seeds normalize the elevated lipid peroxidation and improved susceptibility to oxidative stress associated with depletion of antioxidants in diabetic rats. The antioxidant properties of fenugreek seeds might be relate to its content of phenolic and flavonoid compounds. Acharya *et al.* [76] reported that medicinal properties of fenugreek seeds are associated with its phytochemicals such as galactomannans, phenolic compounds, alkaloids, proteins, vitamins (A, B1, C and nicotinic acid) and volatile oils. Vishnu *et al*. [77] reported that fenugreek seeds have antioxidant activity and produce beneficial effects such as neutralization of free radicals and enhancement of antioxidant properties. The antioxidant activity is very well correlated with the content of phenolic components [78]. Phenolic compounds are efficient scavenger of free radicals as well as transition metal ion chelating agents. Flavonoids possess a chemical structure with particular hydroxyl position in the molecule that is considered to be involved in proton donating and radical scavenging mechanism [79]. The higher content of total phenolic and flavonoid compounds in the fenugreek seeds extracts means the greater it's the antioxidant capacity [80]. Germinated fenugreek seeds were shown to be rich in polyphenols [81] and considered to be more beneficial due to its content of flavonoids and polyphenols [82]. These results highlighted that higher contents of phenolic and/or flavonoid led to higher antioxidant activity. Thus, germination can lead to the development of such functional foods that have a positive effect on the human organism and that help in maintaining the health [83].

CONCLUSIONS

In conclusion, the results of this study demonstrated that germinated fenugreek seeds were effective for in ameliorate body weight, blood lipid and cholesterol and the antioxidant-defense system and inhibition of lipid peroxidation in rats that feed on the food high in fat, cholesterol diet. However, the hypolipidemic and hypocholestermic effect of germinated fenugreek seeds need to be confirmed by characterizing the active ingredient(s) of this plant as well as its mechanism(s) of action.

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