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## Effects of Germinated Seeds of *Trigonella foenum graecum* (Fenugreek) and Cholestyramine on Blood Lipids Profile and Aortic Fatty Streak in Rabbit

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**Abstract:** The aim of this investigation was to evaluate the combination effects of germinated seeds of *Trigonella foenum graecum* (TFG) and *Cholestyramine* on blood lipids profile and fatty streak forming in rabbits. Twenty five male rabbits were divided into 5 groups and received the following diets and drugs for 45 days: Group I: normal diet, Group II: high cholesterol diet, Group III: High cholesterol in additional Cholestyramine (200 mg bid), Group IV: high cholesterol diet in additional to germinated seeds of TFG powder (600 mg bid) and Group V: high cholesterol diet in additional to Cholestyramine and TFG powder. Blood samples were collected after overnight fasting on the first and the last day of test period and estimated for lipid profile. Also autopsy and aortic cross-sectional sampling was done for microscopic study after experiment. The serum cholesterol total, LDL and triglyceride levels of Group III, IV, and V were significantly increased less than levels of Group II but HDL level in Group III, IV and V increased more than Group II. The intensity of fatty streak was less in Group V. These results indicate the usefulness of combination of TFG and Cholestyramine in management of hyperlipidemia and atherosclerosis.

**Key words:** Atherosclerosis, *Trigonella foenum graecum*, cholestyramine, hyperlipidemia

### INTRODUCTION

Hypercholesterolemia is a major risk factor for the development of atherosclerosis, which in turn underlies most ischemic disease<sup>[1-3]</sup>. There are some drugs for preventing and treatment of hyperlipidemia including HMG Co A reductase inhibitors, bile acid resins and statins. The use of treatments, which can increase HDL while lowering LDL, will be of greater impact<sup>[2]</sup>. For this proposes there may be a shifting trend towards natural compounds such as plant stanols and phytoestrogens<sup>[4]</sup>. The leaves of *Trigonella foenum graecum* L. (Fabaceae) that commonly known as Shanbalileh in Iran, are used in Iranian meals but its seeds have pharmacological effects and are used in folk medicines for treatment of hyperlipidemia, cancer, arthritis, diabetes mellitus and some infections<sup>[5]</sup>. Several studies have reported the effects of TFG on hyperlipidemia<sup>[6-16]</sup>. However because of beneficial effects of combination therapy<sup>[4]</sup> we evaluated the combination effects of TFG and Cholestyramine on hyperlipidemia. The effectiveness of combined treatment of sodium-orthovanadate and seed powder of TFG were presented in amelioration of altered lipid metabolism during experimental type-I diabetes<sup>[17]</sup>.

### MATERIALS AND METHODS

**Plant material:** TFG was collected locally (Lorestan, Iran). Their seeds were separated and germinated in a wet field and then dried at room temperature and then powdered.

**Chemical and diet:** Cholestyramine was purchased from Iranian company (Poursina, Iran) and cholesterol was prepared from Merck Co. (Germany). Normal diet was the standard rabbit pellet diet and high cholesterol diet was the same in additional to cholesterol powder (1 g cholesterol per each 100 g pellet diet).

**Animal experiment:** Twenty five male albino rabbits of approximately same age having mean body weight 1800g were used after acclimatization for one week at laboratory conditions. The rabbits were divided randomly into 5 groups and were given normal diet and treatment for 45 days. The germinated TFG powder was given 600 mg twice a day and Cholestyramine 200 mg twice a day (8 pm and 8 am). The animal house temperature was maintained at 23±3°C. All efforts were made to minimize animal suffering and to reduce the number of animals used.

**Blood sampling and lipids estimation:** Five milliliter blood sample were obtained from the heart of each animal after overnight fasting in the beginning and the end of the experiment, placed into a tube centrifuged at 1500 rpm (Eppendorf 5810R, Germany) for 5 min, the serum were collected and then blood cholesterol, triglyceride, LDL and HDL were estimated by auto analyzer.

**Autopsy:** After drawing blood samples on the 45th day all the animals were killed by high dose of intravenous ketamine, aorta artery was removed immediately and washed in distilled water. They were cut in 3 sections (4-5  $\mu$ ) and stained with haematoxylin and eosin for macroscopic and microscopic study to determine intensity of fatty streak.

**Statistical analysis:** All analysis was done using the SPSS for Windows. Results are expressed as Mean $\pm$ SD. One way ANOVA and Scheffe test performed for statistical comparison.  $p < 0.05$  was considered significant.

## RESULTS

**Effect on blood total cholesterol:** In rabbits with normal diet no significant change was observed after 45 days however animals with high cholesterol diet have shown significant increase in cholesterol level. Also there was significant increase in cholesterol level of the animals treated with Cholestyramine, TFG powder and both of them together (Table 1).

**Effect on blood triglyceride:** In the Group I we had not significant change in triglyceride level after 45 days but it increased in Group II, III, IV and V significantly (Table 2). Also we can see significant differences between Group II and III and between Group II and V.

**Effect on blood LDL:** In the Group I there was no significant change in LDL level after 45 days. However significant decrease was detected in other groups after experiment as compared to their initial value (Table 3) but this increase was in Group III, IV and V.

Table 1: Effect of oral administration of germinated of TFG powder and Cholestyramine on blood total cholesterol (mg dL<sup>-1</sup>) in rabbits after 45 days

Groups	Diet	Treatment	Day 0	Day 45
I	Normal	-----	44.2 $\pm$ 8.46	51.6 $\pm$ 7.16
II	High cholesterol	-----	42.8 $\pm$ 9.41	1682.6 $\pm$ 89.3**
III	High cholesterol	Cholestyramine	50.6 $\pm$ 14.6	1274.2 $\pm$ 143.7**a,b
IV	High cholesterol	TFG	62.2 $\pm$ 11.2	1298.4 $\pm$ 105.2**a,b
V	High cholesterol	Cholestyramine+TFG	52.4 $\pm$ 7.1	1055.8 $\pm$ 217.4**a,b

Values are mean $\pm$ SD (n=5). \* $p < 0.001$ , a: compared to Group I, b: compared to Group II

Table 2: The effect of oral administration of germinated TFG powder and Cholestyramine on blood triglyceride (mg dL<sup>-1</sup>) in rabbits after 45 days

Groups	Diet	Treatment	Day 0	Day 45
I	Normal	-----	75.2 $\pm$ 5.84	71.6 $\pm$ 9.5
II	High cholesterol	-----	64.6 $\pm$ 10.6	321.2 $\pm$ 29.2**a
III	High cholesterol	Cholestyramine	70.8 $\pm$ 11.8	189.2 $\pm$ 58.4**a,b
IV	High cholesterol	TFG	73.2 $\pm$ 8.52	211 $\pm$ 47**a,b
V	High cholesterol	Cholestyramine+TFG	55 $\pm$ 11.9	190.8 $\pm$ 78.6**a,b

Values are mean $\pm$ SD (n=5). \* $p < 0.05$ , \*\* $p < 0.001$ ; a: compared to Group I, b: compared to Group II

Table 3: The effect of oral administration of germinated TFG powder and Cholestyramine on blood LDL (mg dL<sup>-1</sup>) in rabbits after 45 days

Groups	Diet	Treatment	Day 0	Day 45
I	Normal	-----	19.2 $\pm$ 3.96	18 $\pm$ 3.16
II	High cholesterol	-----	18.6 $\pm$ 8.8	510.6 $\pm$ 38.96**
III	High cholesterol	Cholestyramine	19.8 $\pm$ 11.7	348 $\pm$ 22.51**a,b
IV	High cholesterol	TFG	30.8 $\pm$ 5.3	342.2 $\pm$ 45.76**a,b
V	High cholesterol	Cholestyramine+TFG	25.8 $\pm$ 6.2	238.4 $\pm$ 33.63**a,b

Values are mean $\pm$ SD (n=5). \* $p < 0.05$ ; a: compared to Group I, b: compared to Group II

Table 4: The effect of oral administration of germinated TFG powder and Cholestyramine on blood HDL (mg dL<sup>-1</sup>) in rabbits after 45 days

Groups	Diet	Treatment	Day 0	Day 45
I	Normal	-----	25.2 $\pm$ 2.6	23.6 $\pm$ 4.2
II	High cholesterol	-----	21.2 $\pm$ 2.8	34.4 $\pm$ 5.3
III	High cholesterol	Cholestyramine	27.6 $\pm$ 1.4	48.5 $\pm$ 7.7**a,b
IV	High cholesterol	TFG	28.3 $\pm$ 3.2	61.4 $\pm$ 8.2**a,b
V	High cholesterol	Cholestyramine+TFG	22.1 $\pm$ 5.2	66.6 $\pm$ 10**a,b

Values are mean $\pm$ SD (n=5). \* $p < 0.05$ , \*\* $p < 0.001$ ; a: compared to Group I, b: compared to Group II

**Table 5: The effect of oral administration of germinated *TFG* powder and Cholestyramine in intensity of fatty streak in aorta artery of rabbits after 45 days**

Groups	Diet	Treatment	Pathologic gadding	Mean±SD
I	Normal	-----	0,0,0,0,0	0
II	High cholesterol	-----	4 <sup>+</sup> ,3 <sup>+</sup> ,4 <sup>+</sup> ,4 <sup>+</sup> ,2 <sup>+</sup>	3.2±0.4 <sup>ab</sup>
III	High cholesterol	Cholestyramine	2 <sup>+</sup> ,3 <sup>+</sup> ,4 <sup>+</sup> ,3 <sup>+</sup> ,0	2.2±0.58 <sup>ab</sup>
IV	High cholesterol	<i>TFG</i>	3 <sup>+</sup> ,1 <sup>+</sup> ,4 <sup>+</sup> ,2 <sup>+</sup> ,1 <sup>+</sup>	2.3±0.58 <sup>ab</sup>
V	High cholesterol	Cholestyramine+ <i>TFG</i>	1 <sup>+</sup> ,2 <sup>+</sup> ,3 <sup>+</sup> ,1 <sup>+</sup> ,2 <sup>+</sup>	1.8±0.37 <sup>ab</sup>

Values are mean SD (n=5). \*P<0.05; 0: Not any lesion, 1: Mild fatty streak, 2: Moderate fatty streak, 3: Severe fatty streak, 4: Severe fatty streak, all over the aorta

**Effect on blood HDL:** HDL had not significant change in Group I but it rose significantly in other groups (Table 4). There were significant differences in HDL levels between Group II and IV and between Group II and V.

**Effect on fatty streak:** The intensity of fatty streak was less in Group III, IV and V than Group II (Table 5).

### DISCUSSION

The aim of this study was to evaluate the antihyperlipidemic activity of germinated seeds of *TFG* individually and in combination with Cholestyramine. Our data shows that the powder of germinated seeds of *TFG* or Cholestyramine decreases the total cholesterol and LDL levels in hypercholesterolemic rabbits significantly and combination of them is more effective. Cholestyramine- or *TFG*-treated hypercholesterolemic rabbits also showed decrease in triglyceride level as compared to the corresponding control group but synergistic effect was not seen. The HDL level was increased in *TFG* treated Group but it not changed by Cholestyramine. Hannan showed that the soluble dietary fibre of *TFG* decrease the triglyceride and LDL and increased HDL in diabetic rat<sup>[12]</sup>. Supplementation of fenugreek leaves lowered the lipid profile in streptozotocin-induced diabetic rats<sup>[6]</sup>. Also the other experiments show that *TFG* has hypolipidemic effects in animals<sup>[6, 7, 9-14]</sup>. But in human subjects the consumption of germinated of *TFG* seeds powdered reduced LDL but no significant changes were found in HDL, VLDL and Triglyceride<sup>[8]</sup>. In diabetic patients, serum total cholesterol, LDL and VLDL and triglyceride were significantly reduced but HDL remained unchanged<sup>[14]</sup>. High levels of total cholesterol, LDL and triglyceride are major coronary risk factors and increase of HDL is associated with a decrease in coronary risk<sup>[1]</sup>. In the present study administration of Cholestyramine and germinated seeds of *TFG* lowered both total cholesterol and LDL and increase HDL level in the rabbits. In addition both *TFG* and Cholestyramine reduced the fatty streak forming. This effect may be due to antihyperlipidemic or antioxidant activity of *TFG*<sup>[18]</sup>. It has been previously shown that the hypolipidemic action of soluble dietary fraction of *TFG* could be the result of

retardation of carbohydrate and fat absorption due to the presence of bioactive fiber in the agent<sup>[19]</sup>. Also hypocholesterolemic components of *TFG* seeds may be due to saponins that interact with bile acids in the digestive tract<sup>[6]</sup>.

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