

EFFECTS OF ALFALFA ON LIPOPROTEINS AND FATTY STREAK FORMATION IN HYPERCHOLESTEROLEMIC RABBITS

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ABSTRACT

Alfalfa (*Medicago sativa*) is a leguminous plant with high contents of phytoestrogen and saponin which are both useful in preventing cardiovascular disorders. This study was designed to evaluate the preventive effect of dietary alfalfa on the development and the progression of atherosclerosis in hyperlipidemic rabbits. Twenty male rabbits were obtained and kept under standard conditions. After 2 weeks of accommodation to the new place, the animals were semi-randomly distributed into four groups of five each. Animals in Group 1 received basic diet and the ones in Group 2 received the same diet to which 1% cholesterol was added. Groups 3 and 4 received similar diets as Groups 1 and 2 respectively, but supplemented with alfalfa. Each of the four groups was fed with its respected diet for a period of 12 weeks. Fasting blood samples were collected from all animals at the beginning and at the end of the study and blood factors were measured. At the end of the study samples from the right and the left coronary arteries as well as the aorta were collected from all animals for pathological evaluations. Though dietary alfalfa decreased total cholesterol, LDL and triglyceride and this effect was not significant, but dietary alfalfa significantly increased HDL. The formation of fatty streaks in the aorta, the right and the left coronary arteries were significantly reduced under the influence of dietary alfalfa. The indication that dietary alfalfa may have preventive effects on the progression of fatty streak formation calls for more studies to clarify the mechanisms of the effect.

Keywords: Atherosclerosis, phytoestrogen, alfalfa, rabbit.

INTRODUCTION

Cardiovascular diseases are still the leading cause of mortality in America, Europe and in most of the Asian countries. These disorders are mainly caused by the accumulation of the fat in the intima and media of the medium to large arteries (Clermont *et al.*, 2005).

Phytoestrogens are non-steroid, herbal origin compounds that have some biological activities similar to estrogen (Axelson *et al.*, 1984). The role of phytoestrogens in human nutrition and their physiological effects were first described in 1984. It was reported that high level of dietary phytoestrogen consumption could increase the levels of isoflavones in the blood and in the urine more than what endogenous estrogen could do. Therefore, it was concluded that isoflavones were biologically active and they could be used as sources for induction of estrogen like activities (Sirtory *et al.*, 1984).

The protective effects of estrogen on cardiovascular system have been demonstrated. There are also some evidences that isoflavones could play some roles in the protection of the cardiovascular system. Some epidemiologic investigations have indicated lower risks of coronary artery diseases among the populations with high

intake of phytoestrogens (Hertog *et al.*, 1995 and Aldercreatz *et al.*, 1993).

Alfalfa (*Medicago sativa*) is high in vitamin content and phytoestrogen level and it is used as a food additive in USA, Russia, China and North Africa (Barens and Sheaffer, 1995; Jayapal *et al.*, 2002; Elakovich and Hampton, 1988). About 47 mg of Coumestrol, a phytoestrogen can be extracted from 1 kg of Alfalfa (Elakovich and Hampton, 1988; Moravcova and Kleinova, 2001).

One study has suggested that alfalfa seeds can be added to the diet to reduce serum cholesterol concentration in patients with Type II hyperlipidemia (Molgard *et al.*, 1987). The current study was designed to evaluate the effect of alfalfa in prevention of the formation and progression of the atherosclerosis.

MATERIAL AND METHODS

Medicago Sativa collected before sprouting from Semirum District, Isfahan Province. The plant specimen was authenticated and deposited at the Herbarium of the College of Sciences, Isfahan University. The aerial part of the plant was dried in shade. Twenty albino male

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Table 1: Comparing mean differences of blood factors between groups before and after the treatment period.

Blood Factor (mg/dl)	High Chol / High Chol+ alfalfa (n=5)+	P value	Normal /Normal + alfalfa (n=5)	P value	High Chol/Normal (n=5)	P Value
FBS	9.15 ± 3.2	.677	6.60 ± 1.4	.750	31.60 ± 5.5	.136
HDL	-85.15 ± 7.11*	.001	4.400 ± 1.7	.843	*74.400 ± 7.8	.002
LDL	62.95 ± 14.8	.230	-17.800 ± 2.3	.715	*522 ± 22.3	.000
TG	69.7 ± 5.8	.174	-60.0 ± 8.9	.213	*187.800 ± 15.8	.001
Chol	17.8 ± 4.3	.762	-3 ± 1.2	.957	650.80 ± 27.4	.000

*p<0.05 is significant; FBS: Fasting Blood Sugar; HDL: High Density Lipoprotein; LDL: Low Density; Lipoprotein; TG: Triglyceride; Chol: Cholesterol.

Table 2: Prevalence of fatty streaks in the aorta, the right and the left coronary arteries at the end of study

Group/ diet		Normal diet	High cholesterol diet	Normal diet + alfalfa	High cholesterol diet + alfalfa
Right coronary	Mean ± SD	.51 ^b ± .42	2.2 ± 1.78 ^a	.29 ^b ± .083	.78 ^b ± .23
	Range	.74 - .08	1.2 - 3.19	0.1 - 0.58 ^b	.16-.83
Left coronary	Mean ± SD	.00 ± .00 ^b	1.53 ^a ± 1.2	0.58 ^b ± 0.17	.67 ^b ± .58
	Range	.00 - .00	.48 - 2.19	0 - 0	.00 - .00
Aorta	Mean ± SD	.00 ± .00 ^b	1.51 ± 1	0 ± 0 ^b	.39 ^b ± .17
	Range	.00 - .00	1.6 - 1.84	0 - 0	.08 - .68

^a: significant difference compared with normal control (p<0.05)

^b: significant difference compared with cholesterol normal control (p<0.05)

rabbits weighing 2-2.5 kg and 10-12 weeks old were purchased from Iran Pasteur Institute and were kept under standard conditions of light and temperature. The rabbits were fed with Super Fosskon, a standard basic rabbit chow composed of 14 g/kg protein, 150 g/kg fiber and 30 g/kg fat. After a two-week period of accommodation to the new place, the rabbits were semi-randomly distributed into four groups of five each. Animals in Group 1 received basic diet and the ones in Group 2 received the same diet to which 1% cholesterol was added. Groups 3 and 4 received similar diets as Groups 1 and 2 respectively, but supplemented with alfalfa. Each of the four groups was fed with its respected diet for a period of 12 weeks. High cholesterol diet was prepared by adding 1% cholesterol from Merck company to the diets. There was no restriction in water intake for all rabbits. Fasting blood samples were collected from all animals at the beginning and at the end of the study to measure blood biochemical factors.

At the end of the treatment period, all animals were anesthetized using pentobarbital (60 mg/kg BW, IV). Then, from each animal the right and the left coronary arteries were excised, rinsed with physiological saline and kept in 10% formalin. Microscopic slides were prepared from the tissue specimens by an experienced pathologist who was blind to the treatment types. Severity of fatty

streak formations were scored from zero to four (Fisher *et al.*, 1994).

In order to measure total cholesterol, HDL, LDL, triglyceride and fasting blood sugar a Hitachi auto-analyzer and Pars Azmoon kits were used. The collected data were analyzed using SPSS 15.0 for Windows and statistical tests including one way ANOVA test.

RESULT

There were not any significant differences in body weights of the rabbits either before or after the study. Changes in blood biochemical factors are shown in table 1. The results indicate that the dietary intake of 1% cholesterol did significantly increase both triglyceride and LDL in serum.

Data analysis of the blood biochemical factors in animals fed with high cholesterol diet supplemented with alfalfa indicated that dietary consumption of alfalfa could decrease total cholesterol, LDL, triglyceride and glucose. More interesting was the fact that due to the dietary supplementation of alfalfa, significant increase in HDL was observed (table 1).

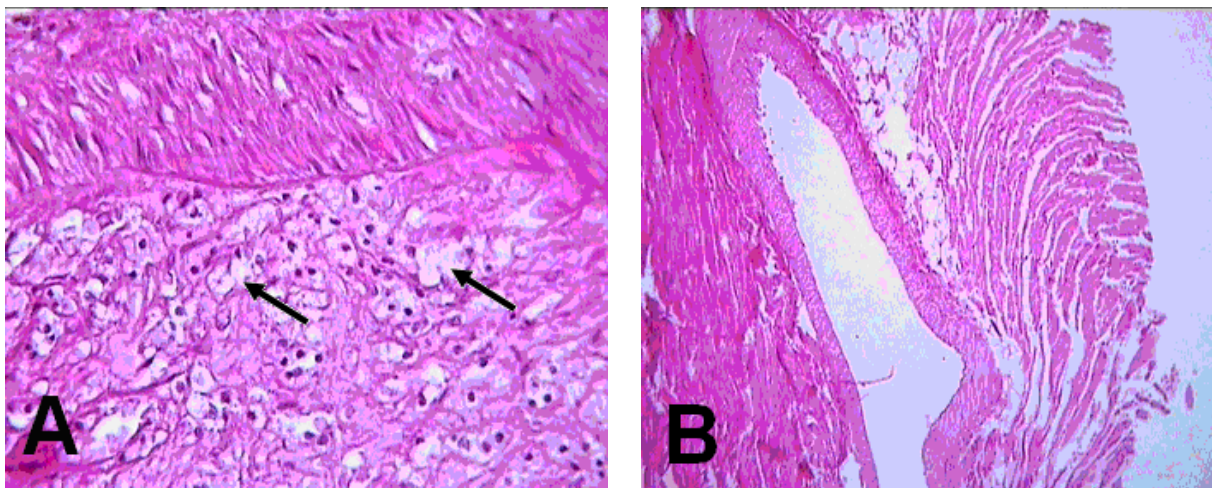


Fig. 1: A: Histology of left coronary of a hypercholesterolemic rabbit. Arrows point to the fatty streak formations. B: Histology of left coronary of a hypercholesterolemic rabbit treated with alfalfa added to the diet.

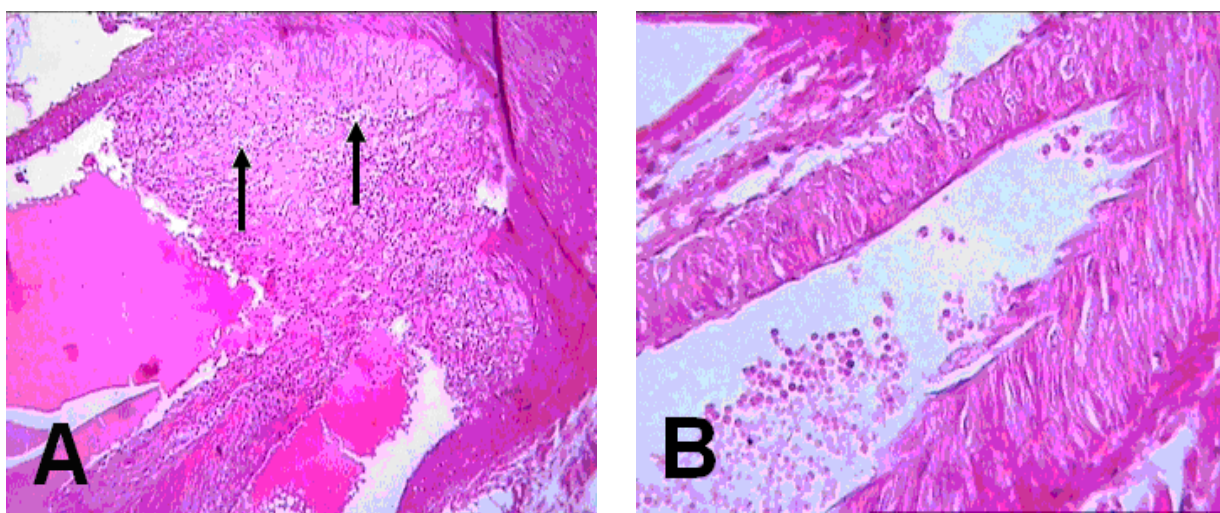


Fig. 2: A: Histology of right coronary of a hypercholesterolemic rabbit. Arrows point to the fatty streak formations. B: Histology of right coronary of a hypercholesterolemic rabbit treated with alfalfa added to the diet.

The formation of fatty streaks in aorta and the right and the left coronary arteries were significantly reduced in animals that received high cholesterol diet supplemented with alfalfa as compared with high cholesterol diet without alfalfa (table 2 and figs. 1 and 2).

DISCUSSION

Atherosclerosis is caused due to alterations in the intima and the media of medium and large arteries as a result of fat accumulations (Clermont *et al.*, 2005). Based on evidences, researchers have recently paid attention to the roles of dietary phytoestrogens in preventing and curing cardiovascular disorders. Under the influence of purified phytoestrogen, relaxation was observed in the coronary arteries excised from rabbits in a dose dependent mode through a calcium-antagonism mechanism (Figtree *et al.*, 2000).

Since alfalfa, a leguminous plant contains high levels of isoflavones which have estrogenic properties, its dietary supplemental intake has been recommended. Hence, the role of aerial part of alfalfa in the formation and progression of the fatty streaks in rabbits treated with high cholesterol diet was considered in the design of this study. As it was hypothesized, dietary intake of alfalfa prevented the formation and progression of fatty streaks. In addition, dietary consumption of alfalfa was associated with decreases in total cholesterol, LDL, triglyceride, glucose and an increase in HDL.

Epidemiological studies have reported decreases in the occurrence of cardiovascular diseases in the communities where high intakes of dietary isoflavones were common (Helena *et al.*, 1966 and Adlercreutz, 1998). Studies in both human and animal models have reported that dietary soy protein supplementations containing phytoestrogens

could improve lipid profile and blood pressure (Sirtory *et al.*, 1984; Teede *et al.*, 2001 and Honore *et al.*, 1997). The results of some interventional studies have provided evidence that isoflavones improve systemic arterial compliance but has no effect on flow mediated dilation (Squadrito *et al.*, 2002 and Nestel *et al.*, 1999).

Phytoestrogen extracts obtained from soy and alfalfa called Coumestrol has anti-oxidation protective capability for LDL. This protective effect of the extracts has been attributed to the presence of flavonoids in soy and alfalfa extract (Whitten, 2001). Nogowski *et al.*, (1999) have reported that Coumestrol could increase muscles triglyceride in ovariectomized rats. However, reports indicate that Coumestrol in a non-significant mode decreased glucose level in serum. It is also reported that alfalfa seeds could lower both LDL and apolipoprotein B concentrations as much as 18% in the patients with Type II hyperlipoproteinemia (Molgard *et al.*, 1987).

In this study, though not significant but decreases in total cholesterol, LDL, triglyceride and glucose were observed. The increase in HDL observed in the animals fed with high cholesterol diet supplemented with alfalfa was significant. These results may be attributed to the low amount of Coumestrol per kilogram of alfalfa that was reported to be 47 mg (Naftolin and Stanbury, 2002; Skibola and Smith, 2000).

An increase either in the amount of alfalfa dietary intake or in the length of the interventional period could possibly enhance the efficacy of the effects of alfalfa on the evaluated biochemical blood factors. The fact that in this study, high cholesterol diet consumption supplemented with alfalfa could effectively prevent formation of fatty streaks may be related to alfalfa effects either on apolipoproteins or on the other new risk factors. Apolipoprotein B is a new recognized risk factor and possibly more important than other risk factors which is well worth to be considered in investigations regarding cardiovascular diseases.

The cardioprotective effects of alfalfa may also be related to its contents of saponins. Saponins are only available in herbs and are absorbed through digestive system. It is reported that saponins in alfalfa could reduce serum cholesterol in rats and in monkeys (Colodny *et al.*, 2001).

In addition, dietary alfalfa may well sustain lots of vitamins such as A, B, C and K. Therefore, besides having propounded preventive and therapeutic effects on cardiovascular disorders, it may be quite suitable as a dietary supplement. Clinical trials would better elucidate the above mentioned effects of alfalfa and its possible side effects.

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