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Effect of *Gundelia tournefortii* L. on some cardiovascular risk factors in an animal model

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ABSTRACT

Introduction: There is no certain result in the field of industrial pharmacy approaching to find effective drugs in prevention and treatment of atherosclerosis, like the control of lipid factors that are major risk factors of atherosclerosis. This study was designed to evaluate the effects of *Gundelia tournefortii* on atherosclerosis biomarkers by measuring some biochemical factors and formation of fatty streaks.

Methods: In this preclinical study, 20 male rabbits were randomly divided into 4 groups as normal diet, cholesterol diet, normal diet supplemented with *G. tournefortii* extract, and cholesterol diet supplemented with *G. tournefortii* extract. Fasting blood samples were taken at the first and end of the study. Lipoproteins, Apo lipoproteins, fasting blood sugar (FBS), low density lipoproteins (LDL), factor VII and C-reactive protein (CRP) were measured. For each animal, histopathology of right coronary artery, left coronary artery and aorta were also studied.

Results: Significant decrease in the fatty streak formation was observed in the group receiving cholesterol-rich diet supplemented with *G. tournefortii*. There were not any significant changes in measured biochemical factors except for significant reduction in factor VII when *G. tournefortii* was supplemented with high cholesterol diet.

Conclusion: *Gundelia tournefortii* decreases atherosclerosis in hypercholesterolemic diet especially by decreasing coagulating factors like factor VII. Hence, it might be beneficial in prevention of atherosclerosis.

Implication for health policy/practice/research/medical education:
Gundelia tournefortii is able to decrease atherosclerosis in hypercholesterolemic diet rabbits, hence, it might be beneficial in prevention of atherosclerosis.

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Introduction

Cardiovascular disease (CVD) due to atherosclerosis is the leading cause of morbidity and mortality in the world (1). Atherosclerosis is a complex disease, involving many cell types and circulating mediators and resulting in an inflammatory state (2). Atherosclerotic lesions form *de novo* from focal accumulation of lipoproteins, monocyte-derived macrophages, and lymphocytes within the arterial wall (2). These lesions can develop as early as the second decade of life and progress into clinical disease over time. However, despite recent advances in cardiology, atherosclerosis remains an important medical problem (3).

On the other hand people's inclinations for using herbal medicines have increased all over the world (4). Botanical dietary supplements (herbs) can ameliorate inflammatory process and prevent CVD at many steps in the process. Many herbs have antioxidant activity and can reduce low-density lipoprotein oxidation (4). *Gundelia tournefortii* is a spiny, thistle-like flowering plant of the genus *Gundelia*. It occurs in the semi-desert areas of Syria, Palestine, Iran, Iraq and Armenia (5). *G. tournefortii* is found as a wild herb growing during late winter and early spring on the hills in western and southern parts of Iran (6). *G. tournefortii* is used as an occasional food. In the Persian

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folk medicine, the stalk of plant has been considered to be hepatoprotective and blood purifier (6). The present study was designed to examine whether *G. tournefortii* could prevent atherosclerosis by effect on some biochemical factors and formation of fatty streaks.

Materials and Methods

Fresh edible stalk of *G. tournefortii* was collected during April 2006 from rural areas around Yasooj, southern Iran. The voucher specimen with code 1780 was deposited in the pharmacology department of medical science of Isfahan University.

Twenty albino male rabbits with the mean weight of 1500 ± 100 g and 10 weeks age were prepared from Pasteur's Institute. Animals were fed with normal diet (Super Fosskorn) for 2 weeks and then were randomly divided into four groups of 5 rabbits in each group. Also environmental characteristics (light, temperature and access to water) were similar. For 2 months these rabbits were fed with (*a*) normal diet, (*b*) 1% cholesterol diet, (*c*) normal diet supplemented with *G. tournefortii* powder and (*d*) 1% cholesterol diet supplemented with *G. tournefortii* powder. *G. tournefortii* powder was used 1/4 % daily.

Blood samples were collected from central ear artery of overnight fasted rabbits before starting experiment and at the end of study. Plasma cholesterol, triglyceride, high-density cholesterol (HDL-C), low-density cholesterol (LDL-C) and fasting blood sugar (FBS) levels in both, before experiment and at the end of study were determined using an automated enzymatic assay by auto analyzer Hitachi 902. Also Apo-lipoprotein A (ApoA), Apo-lipoprotein B (ApoB), oxidized LDL (Ox-LDL), C-reactive protein (CRP) and factor VII were measured at the end of experiment. ApoA and ApoB were measured enzymatically using standard PARS Azmun kits. Quantitative CRP was measured by Immunoturbidimetry method using auto analyzer Hitachi 902.

At the end of study, after anaesthetization of the rabbits with pentobarbital 5% and cutting of chest each animal was sacrificed and gross anatomic examinations and pathologic investigations were performed on the subjects. Then, the right and the left coronary arteries as well as aorta were excised and kept in 10% formalin solution to be used for pathologic evaluation. Tissue specimens were sectioned and prepared using particular histological methods and were assessed by a pathologist with respect to the presence of fatty streaks. For each animal and from each tissue specimen, either right coronary artery, left coronary artery and aorta, three consecutive sections were prepared and mounted on one slide. Prepared tissue sections were observed using light microscope (7).

Statistical analysis

Results were presented as the mean ± SD. Differences in means of biochemical factors and fatty streak scores between groups were statistically analyzed by non-parametric one-way analysis of variance (ANOVA). *P* values <0.05 were considered statistically significant. A computer program (SPSS 13.0, SPSS Inc. Chicago, IL, USA) was used for statistical analysis.

Results

The severity of aortic atherosclerosis, as judged by gross grading, was more marked in the group fed only with cholesterol. The control group did not show any evidence of atherosclerosis. Histological findings showed significant increase in the fatty streak formation in rich cholesterol diet compared with normal diet group in both left and right coronary arteries ($P=0.01$, $P=0.01$, Respectively). In this study, significant decrease in the fatty streak formation was observed in the group receiving cholesterol-rich diet supplemented with *G. tournefortii*, compared with the group fed cholesterol rich diet (Figure 1) ($P<0.05$, $P<0.05$, respectively) (Table 1).

Statistically significant increase was found on some biochemical factors like Cholesterol, TG, HDL-C, LDL-C, FBS, ApoB and Factor VII of cholesterol-rich group compared with control group. Also the differences for ApoA, CRP and Ox-LDL were not significant between these two groups (Table 2).

At the end of experimental period, significant increase was observed in the amount of FBS in the group supplemented with *G. tournefortii*, compared with the control group (Table 2).

Factor VII significantly decrease and FBS significantly increase in the group fed cholesterol rich diet supplemented with *G. tournefortii*, compared with the group fed cholesterol rich diet (Table 2).

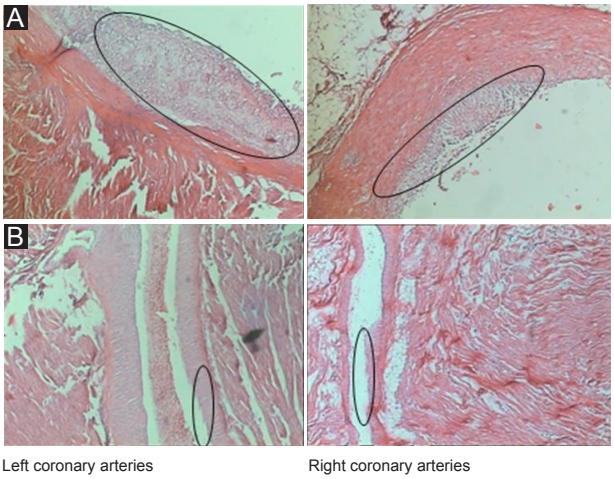

Figure 1. (A) Microanatomies of left and right coronary arteries in rich cholesterol diet. By x40 magnifications, high grade of fatty streak formation was observed. (B) Microanatomies of left and right coronary arteries in cholesterol-rich diet supplemented with *Gundelia tournefortii*. By x40 magnifications, a smooth surface, without any fatty streak level was observed.

Table 1. Histological results of aorta and right and left coronary according to the diet group

	Control	Control + *Gundelia tournefortii*	Cholesterol rich	Cholesterol rich + *Gundelia tournefortii*
Aorta	0±0	0±0	2.80±0.44[a]	1.8±0.83[b]
Right coronary artery	0±0	0±0	2.6±0.54[a]	1±0.7[b]
Left coronary artery	0±0	0±0	3±1[a]	1±0.7[b]

The mean ± SD of fatty streak is compared according to the diet group. Using *Gundelia tournefortii* significantly decreased the fatty streak formation in the group receiving cholesterol rich diet.
[a] Significantly different from control group ($P < 0.01$).
[b] Significantly different from cholesterol rich group ($P < 0.05$).

Table 2. Comparison of the mean ± SD of biochemical factors between the control group, cholesterol rich group, control group supplemented with *Gundelia tournefortii* and cholesterol rich group supplemented with *Gundelia tournefortii* at the end of experimental period

	Control diet	Cholesterol rich diet	Control diet+ G.t.	Cholesterol rich diet + G.t.
Chol (mg/dL)	41.7±5.26	1186.25±22.29[a]	41.7±5.26	1175.2±21.05
TG (mg/dL)	73.75±10.78	403.5±277.5[a]	73.75±10.78	253.6±115.9
HDL-C (mg/dL)	12±5.94	108.7±24.99[a]	12±5.94	116±13.98
LDL-C (mg/dL)	15±3.7	996.8±21.59[a]	15±3.7	1008.48±30.3
FBS (mg/dL)	45±22.31	80.5±2.51[a]	105.2±25.99[b]	96.2±8.64[c]
CRP (mg/dL)	4±4.96	2.5±1.91	4±4.96	5.2±8.34
ApoA (mg/dL)	30.75±3.77	39.75±5.31	30.75±3.77	43.4±3.5
ApoB (mg/dL)	70±20	75±32.96	70±20	72 ±28.08
Factor VII	224±8.32	440.5±45.98[a]	250±20.74	380.8±28.14[c]
Ox-LDL (mg/dL)	1.25±0.2	1.67±0.37	1.18±0.33	1.18±0.49

Abbreviations: G.t, *Gundelia tournefortii*; Chol, cholesterol; CRP, C-reactive protein; FBS, fasting blood sugar; TG, triglyceride.
[a] Significantly different from control group ($P < 0.05$); [b] Significantly different from control group ($P < 0.05$); [c] Significantly different from cholesterol rich group ($P < 0.05$)

Discussion

In this study we showed that use of *Gundelia* tournefortii with high cholesterol diet decreases significantly fatty streak formation in left and right coronary arteries. It seems that *G. tournefortii* exerts its effect by reduction of coagulation factor VII, because factor VII significantly decreased in *G. tournefortii* group supplemented with high cholesterol diet.

In the present study we observed that dietary treatment of rabbits with high cholesterol diet caused hyperlipidemia which was in accordance with a previous investigation (8). Using *G. tournefortii* caused non-significant decrease of all lipid factors in cholesterol rich diet supplemented with *G. tournefortii.*

It has been shown that antioxidant compounds can reduce Ox-LDL one of atherosclerosis risk factors. *G. tournefortii* has an antioxidant component but Ox-LDL was not changed in diet with *G. tournefortii* in our study (9).

Also supplementing *G. tournefortii* with normal diet did not affect fatty streak formation but significantly increased FBS.

In high cholesterol diet, fatty streak formation has increased in left and right coronary (10,11). In rich-cholesterol diet group supplemented with *G. tournefortii,* the formation of fatty streak was inhibited (Table 1). Scopoletin component in *G. tournefortii* prevented fatty streak formation (12). In our study in diet with *G. tournefortii* the amount of factor VII was decreased. Factor VII, a serine-protease zymogen, is the protein component of the macromolecular complex which initiates the extrinsic pathway of the coagulation cascade (13). A close relation between blood coagulation and atherosclerosis is supported by studies revealing the presence of specific coagulation proteins within an atherosclerotic lesion (14,15). Tissue factor and factor VII, of which the complex is the principal initiator of coagulation in vivo, are expressed on macrophages and vascular smooth muscle cells within the arterial vessel wall and atherosclerotic lesion (16,17). Both proteins potentially participate in multiple pro-atherogenic processes such as migration and proliferation of smooth muscle cells (18), inflammation, and angiogenesis (19). In addition to the single effects of each protein, the local interaction between macrophage/smooth muscle cells-derived tissue factor and factor VII may provide a catalytic complex for subsequent generation of thrombin and fibrin, of which the latter is also detectable in atherosclerotic lesions (20,21).

David Green showed positive association of factor VII with CC-IMT (Common carotid intima-media thickness). This association between factor VII and IMT was observed in age/gender/race adjusted analyses, but was attenuated after adjustment for BMI, total cholesterol and

HDL-cholesterol, thus factor VII is not an independent risk factor for carotid thickness (22). Elevated levels of factor VII have been reported in persons with carotid wall thickening (23), and in men with coronary events (24). Factor VII is increased in women with coronary atheroma (25) and common factor VII haplotypes contribute to the risk of myocardial infraction in women (26). In our study, presumably, *G. tournefortii* exerted its effects on inhibition of fatty streak formation by decreasing factor VII.

Also another study recorded fluvastatin was able to inhibit the formation of fatty streak without any decrease in cholesterol amount and this was like *G. tournefortii* effect (27). Studies with *Cynara scolymus* L. which is from the same family as *G. tournefortii* has previously shown that it has anti-atherosclerosis property because it contains antioxidant component and it can decrease cholesterol by inhibition of its synthesis. Also it can inhibit oxidation of LDL cholesterol, so it is a suitable plant for food diet improvement (28-30).

Conclusion

The results of the present study suggest that the *G. tournefortii* extract by inhibiting the fatty streak formation as the first step of atherosclerosis can be considered as a candidate for a new drug for these purposes. Active component of *G. tournefortii* extract needs further investigation.

Authors' contributions

All contributed to the study. LR, MK, AMA, ZH, GH, RD and SA acquired data and prepared the drafting. MK and SA revised it critically for important intellectual content and submitted it. All read and confirmed the article for publication.

Conflict of interests

The authors declared no competing interests.

Ethical considerations

Ethical issues (including plagiarism, misconduct, data fabrication, falsification, double publication or submission, redundancy) have been completely observed by the authors.

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