



Potential role of a nutraceutical spice (*Allium hirtifolium*) in reduction of atherosclerotic plaques

Mahmoud Rafeian-kopaei¹, Mahtab Keshvari², Sedigheh Asgary^{2,*}, Maryam Salimi¹, Esfandiar Heidarian³

¹Medical Plants Research Center, Shahrekord University of Medical Sciences, Shahrekord, Iran

²Isfahan Cardiovascular Research Center, Isfahan Cardiovascular Research Institute, Physiology Research Center, Isfahan University of Medical Sciences, Isfahan, Iran

³Clinical Biochemistry Research Center, Shahrekord University of Medical Sciences, Shahrekord, Iran

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ABSTRACT

Introduction: Spices are now considered as agents that not only can prevent but may even treat chronic diseases. This study was aimed to investigate the effects of *Allium hirtifolium* as a hypolipidemic and anti-atherosclerotic substance in hypercholesterolemic rabbits.

Methods: Twenty four adult New Zealand male rabbits were divided randomly into 3 groups of 8 animals each and treated for 60 days as follows. Normal group received basal feed, while the two intervention groups were fed with hypercholesterolemic diet (1% cholesterol) and hypercholesterolemic diet plus *A. hirtifolium* extract, respectively. At the start and the end of the experiment, fasting blood was taken from all animals. Serum concentrations of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides (TG), apolipoproteins A and B, serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), high-sensitivity C-reactive protein (hs-CRP), glucose and insulin were measured at the end of supplementation period in all studied groups. Atherosclerotic plaque thickness of aorta to media was also determined in all groups.

Results: Rabbits fed only with high cholesterol diet showed increased atherosclerotic plaque thickness to media compared to the control group, while the group fed with hypercholesterolemia diet plus *A. hirtifolium* extract significantly decreased atherosclerotic plaque thickness to media, TC, TG, LDL-C, and significantly increased HDL-C compared to hypercholesterolemic diet group. Supplementation with *A. hirtifolium* extract did not cause any significant alteration in apolipoproteins, SGOT, SGPT, hs-CRP, glucose and insulin compared to the hypercholesterolemic diet group ($p > 0.05$).

Conclusion: Ethanolic extract of *A. hirtifolium* ameliorates fatty lesions in aorta and may reduce risk factors of cardiovascular diseases.

Implication for health policy/practice/research/medical education:

Ethanolic extract of *Allium hirtifolium* ameliorates fatty lesions in aorta and may reduce atherosclerosis risk factors. Therefore, its consumption may be useful in cardiovascular diseases.

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Introduction

Cardiovascular disease (CVD) is the leading cause of morbidity and mortality worldwide. Atherosclerosis (AS) is one of the major risk factors in the development of hypertension and cardiovascular diseases. It is the narrowing or occlusion cause of the arteries. Increased plasma cholesterol, low-density lipoprotein (LDL), and oxidized LDL (Ox-LDL) are other

important risk factors of this problem (1). Lowering LDL and cholesterol concentrations significantly reduces coronary heart disease (CHD) mortality (2). It has been established that diet therapy is the cornerstone in lowering total cholesterol (TC) and LDL-cholesterol (LDL-C) concentrations and in reducing the risk of CVD (3). The first step to reduce CVD is by lifestyle measures including dietary changes, in particular a

*Corresponding author: Sedigheh Asgary.
E-mail: sasgary@yahoo.com

reduction in the intake of total and saturated fat (4). Presently, search for new drugs, capable of regulating and reducing serum cholesterol and triglyceride (TG) levels have been the focus of attention with numerous reports showing remarkable activities of natural agents. The plant products are regarded as less toxic and free from side effects than synthetic agents (5,6). Also, it is well established that diet rich in vegetables and fruits can reduce CVD (7-11).

Spices have shaped a large part of the world's history and they have been used for centuries, serving a variety of purposes in a wide variety of cultures. They have been used as flavor agents, as colorants to add special taste to dishes, and also as a preservative to prevent the growth of bacteria. But today, the importance of spices has become even more evident than they were throughout history. Due to their high antioxidant and anti-inflammatory properties, the common spices in today's diet have been demonstrated to also have medicinal value, and much of this potential has only been realized over the last 50 years (12).

Allium hirtifolium (Persian Shallot or *Moosir*), as a member of the Liliaceae family, belongs to the same biological genus as *Allium sativum* (garlic) and other onions, which has been traditionally used mainly as a spice from the ancient times. It has many different properties including antibacterial and anti-fungal properties (13,14), beneficial hematological influences (15), antioxidant properties (7,16), anti-*Helicobacter pylori* potential effect (17), and decreasing blood fibrinogen and factor VII (7). The constituents of *Allium* confirmed the presence of flavone and polyphenolic derivatives such as mannose-specific lectin (18), new furostanol saponins (19), selenium and sulfur species (20), and various flavonol glucosides (21). Most of the medicinal benefits of *Allium* such as reducing total plasma cholesterol, blood pressure and platelet aggregation are attributed to a sulfur compound known as allicin (22). Allicin is converted to allicin, pyruvate, and ammonia by the enzyme allinase, when the cloves are cut or crushed (23). Allicin was reported to possess diverse biological properties such as antimicrobial, antiparasite, and antifungal. It has been found that lipid peroxidation is inhibited and OH is scavenged (24). Presence of this aromatic and mineral compounds in *Allium hirtifolium* led to the hypothesis of whether supplementation with ethanol extracts of *A. hirtifolium* and high cholesterol diet could ameliorate apolipoprotein B (ApoB), TG, LDL-C, TC, hs-CRP, glucose (FBS), glutamic pyruvic transaminase (SGPT), glutamic oxaloacetate transaminase (SGOT), fatty streak formation, and insulin in hypercholesterolemic rabbit, which will be tested in the present study.

Materials and Methods

Collection of plant material and extraction

In this experiment, *A. hirtifolium* was collected from Chaharmahal & Bakhtiari province. In addition, Medicinal Plants Research Center of Shahrekord University of Medical Sciences identified the plant specimen.

Preparation of extract

A. hirtifolium was washed with tap water and cut into small slices. The slices were powdered after air-drying. Then 100 g of powder was added to 500 ml of 80% ethanol and the mixture kept for 48 h at 15-20° C. After filtering the extract,

we repeated the extraction twice and transferred the collected plant extract to the vacuum distillation unit and concentrated it. Then it was dried at a temperature of 40° C.

Animals and treatment

24 adult New Zealand male rabbits weighing 2010±234 gr were purchased from Razi Institute of Tehran, Iran. The animals were acclimatized under room temperature and were housed in cages under 12 h light/dark cycle according to the approved standards for laboratory animal care for two weeks, and had free access to water and a standard powdered purified diet that was purchased from Pars Animal Feed Co., Tehran, Iran which consisted of 15% protein, 40-50% carbohydrates, 2% vegetable fat, 15-25% fiber, 2.5% calcium carbonate and bisphosphate, 3.9% mineral mixture, and 0.5% vitamin mixture.

Rabbits were divided into three groups of eight rabbits each and *A. hirtifolium* extract was injected intraperitoneally once a day for 60 days as follows (25):

The first group was the control (normal);

the second group fed a high cholesterol diet (cholesterol suspended in olive oil and added to the diet 1% of food content daily with a normal diet); and the third group was fed high cholesterol diet (1% cholesterol in addition with a normal diet + *A. hirtifolium* extract (1g/kg BW). The study protocol was approved by the Medical Ethics Committee of the Isfahan Cardiovascular Research Center.

Blood sampling and analyses

Before the beginning and end of the study, the animals were fasted for 12 hours, and blood samples of rabbits were taken from the central ear artery. The blood taken from the rabbits was poured in two separate tubes to prepare serum. Tubes with specific number and date were centrifuged for 20 minutes at 3500 rpm in order to prepare serum. For biochemical analyses, the fasted blood samples were collected to determine serum concentrations of lipid parameters (TC, HDL-C, LDL-C and TG), liver enzymes (SGOT and SGPT), insulin, glucose and apo A and apo B. Serum insulin level was determined with ELISA method using a commercial kit (Monobind Inc., CA, USA). Other evaluated biochemical factors were measured by routine enzymatic methods using commercial kits (Pars Azmoon, Tehran, Iran) on a Hitachi 902 autoanalyzer (Tokyo, Japan).

Atherosclerotic lesion evaluation

After blood collection, the animals were anesthetized with chloroform and the aorta was dissected and washed with physiologic serum and stored in formalin 15%. Sections of aorta were stained with Haematoxylin and Eosin to determine the grade of the atherosclerotic plaque. Atherosclerotic lesions were graded according to the Chekanov index and the thickness was assessed in the following categories (26).

Grade 1: Plaque thickness less than half that of the aorta media (moderate forms of malfunction);

Grade 2: Plaque thickness almost half that of the aorta media (abundant connective tissue in the plaque);

Grade 3: Plaque thickness equal to that of the aorta media; and

Grade 4: Plaque thickness more than that of the aorta media.

Total flavonoids determination

The amount of total flavonoids in the extract was determined using colorimetric method as described by Chang and colleagues. 0.5 ml of the extract or rutin (standard flavonoid compound) was mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate, and 2.8 ml of distilled water and left at room temperature for 30 min. The absorbance of the reaction mixture was measured at 510 nm using rutin solutions at concentrations of 25 to 500 ppm in methanol. The experiment was repeated for three times. Total flavonoids were expressed in terms of rutin equivalent (mg/g), which is a common reference compound (27).

Statistical Analysis

Statistical analyses were conducted using SPSS software version 13.0 (SPSS Inc., Chicago, Illinois, USA). Between-group comparisons of biochemical factors were carried out using Kruskal-Wallis test. Post-hoc multiple comparisons were made using Dunn's test. A p-value of < 0.05 was considered as statistically significant.

Results

Analyzing *A. hirtifolium* total flavonoid content showed that in 100 ml of *A. hirtifolium* extract was 69 (g /100 ml naringenin equivalent).

Histological sections of aorta artery stained from the three groups after 60 days are shown in Figure 1 and Table 1. Normal diet group had completely normal arteries without any lesion in intima or media. The mean diameter of atherosclerotic plaque thickness of aorta to media was 0.33 ± 0.51 in the control group, which is equal to degree 1 in Chekanov scale (Figure 1). In high cholesterol group atheroma plaque was

formed with macrophages filled with fat creating foamy cells. Plaque thickness was also increased to more than half of media thickness, equal to degree 4 of Chekanov scale (Figure 1b, c). In the *A. hirtifolium* extract group some endothelial dysfunction along with a few foam cells and macrophages was seen in the intimal surface of the aorta artery and plaque degree was 1 (Figure 1d).

In the group fed with hypercholesterolemia diet plus *A. hirtifolium* extract compared to hypercholesterolemic diet group, TC, TG, and LDL-C significantly decreased, and HDL-C significantly increased. While Supplementation with hypercholesterol diet plus *A. hirtifolium* extract did not cause any significant alteration in apolipoproteins, SGOT, SGPT, hs-CRP, glucose or insulin. ($p > 0.05$; Table 2).

Discussion

A. hirtifolium is widely consumed as a component of the diet in many populations. It is widely believed that *A. hirtifolium* has beneficial effects on health and even curative potential against a range of debilitating conditions and diseases (28). *A. hirtifolium* is one of these safe plants used as a spice for more than 2000 years and has been shown to produce multi-systemic beneficial actions including antibacterial and anti-fungal properties (13,14), beneficial hematological influences (15), antioxidant properties (7,16), anti-Helicobacter pylori potential effect (17) and decreasing blood fibrinogen and factor VII (7).

Our histological results indicate that *A. hirtifolium* intake reduced atherosclerotic lesion in aorta significantly compared to hypercholesterolaemic group. In addition, it significantly decreased TC, TG, LDL-C, and significantly increased HDL-C compared to the hypercholesterolemic diet group.

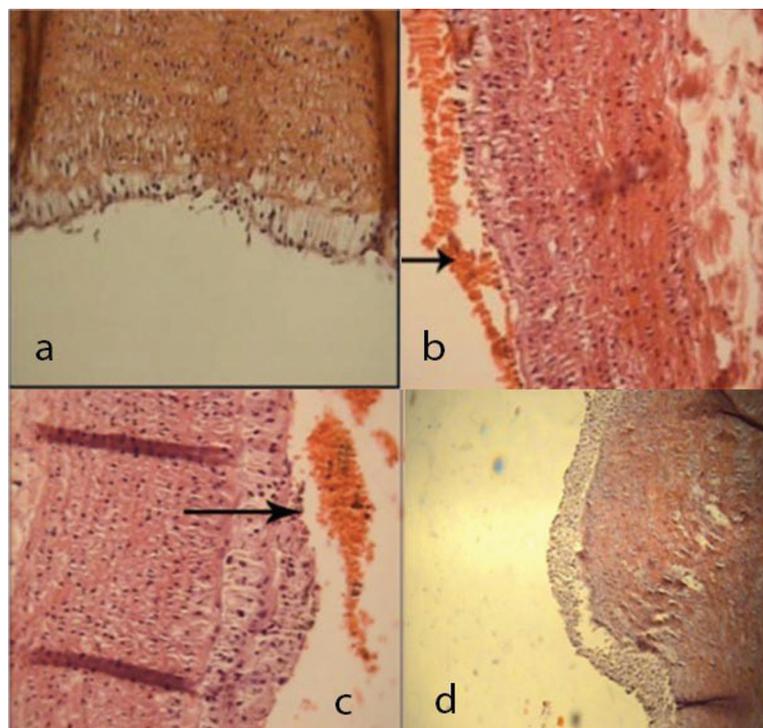


Figure 1. Histology of aorta and grade of atherosclerotic plaque in studied groups: a: Normal diet, b and c: 1% cholesterol in addition with a normal diet, d: 1% cholesterol in addition with a normal diet + *A. hirtifolium* extracts (1g/kg BW)

Table 1. Rate of plaque thickness to media thickness from the three groups after 60 days

Group	Normal	High-cholesterol (1%),	High cholesterol (1%) + <i>A.hirtifolium</i>
Rate Plaque thickness to media thickness	0.33±0.55	2±0.66	0.44±0.77
Grade of atherosclerotic plaque	1	4	1

Table 2. Effect of *Allium hirtifolium* on measured factors in experimental groups at the end (60 days) of the study, (n=24, 8 rabbits for each group)

Group	Normal	High-cholesterol (1%),	High cholesterol (1%) <i>A.hirtifolium</i>	P Value
TG (mg/dl)	42.7± 2.7	126.86±65.17	125.63±30.23	≤ 0.001
TC (mg/dl)	47.57± 1.98	282.48± 6.10	218.04± 4.13	≤ 0.001
HDL (mg/dl)	20.47± 5.44	10.33± 2.92	9.31± 2.37	≤ 0.001
LDL-C (mg/dl)	20.05±1.09	1348±54.60	1309±45.79	≤ 0.001
Apo B (mg/dl)	24.2±5.3	33.8±10.06	52.3±6.71	≥ 0.05
Apo A (mg/dl)	54.5±3.6	39.5±13.3	38.2±12.8	≥ 0.05
hsCRP (mg/lit)	13.75±24.3	5.51±5.54	5.4±3.01	≥ 0.05
SGOT (Iu/ml)	3.09 ±33.7	32.7 ±102.3	44.8 ±91.6	≥ 0.05
SGPT (Iu/ml)	39± 5.09	90.1±18.9	47.7±12.7	≥ 0.05
FBS (mg/dl)	33.3±125.7	74.6±171.6	22.8±155.1	≥ 0.05
Insulin (Iu/ml)	25.8±36.5	5.9±27	7.3±24.2	≥ 0.05

Apolipoprotein B (ApoB), apolipoprotein A (ApoA), triglycerides (TG), lowdensity lipoprotein cholesterol (LDL-C), total cholesterol (TC), hs-CRP (high-sensitivity C-reactive protein), glucose (FBS), glutamic pyruvic transaminase (SGPT), glutamic oxaloacetate transaminase (SGOT).

Plants are complex chemicals with medicinal properties that act on multiple pathways maintaining fatty streak (29). Recent studies have investigated the beneficial effects of flavonoids present in fruits and plant derived-foods such as common onion (*Allium cepa*) and *A. hirtifolium* in the prevention of cardiovascular diseases (30). Furthermore, the findings showed that bulbs of *A. hirtifolium* had high concentrations of quercetin, isorhamnetin, organosulfur compounds, allicin or diallyl thiosulfinate and their glycosides (31).

Furthermore, recent advances in the understanding of the biological importance of endogenous H₂S has shed light on the potential role of the gas in atherosclerosis. Wang et al. first reported a direct correlation between endogenous H₂S and atherosclerosis in apoE_{2/2} mice (32). Some studies have suggested that H₂S may hinder the development of atherosclerosis by inhibiting vascular smooth muscle cell proliferation, adhesion molecules expression in endothelial cells and foam cell formation (32-34).

Alliin (diallyl thiosulfinate), rapidly converted from alliin by allinase in crushed fresh garlic cloves, is a major component and thiosulfinate compound responsible for the biological activity of garlic (35). However, under certain circumstances, alliin or garlic extract may also work as an immunosuppressant to down-regulate inflammatory responses and inhibit the interaction of T-cells with the endothelial cells (36). Since inflammation has an important role in atherosclerosis development, significant reduction in inflammatory lesion may be due to anti-inflammatory effect of *A. hirtifolium*.

Fattorusso et al. reported that *A. hirtifolium* contains the highest level of total flavanols among the onion varieties (19). We found that acetone in ethanolic extract of *A. hirtifolium*

had high flavonoid content 69 (mg of quercetin equiv/100 g of sample). Quercetin is an important constituent of the flavonoid family and is found in high concentrations in shallot. The anti-angiogenic activity of quercetin was also documented (37).

Supplementation with hypercholesterol diet plus *A. hirtifolium* extract significantly decreased TC, TG, LDL-C, and increased HDL-C, compared to the hypercholesterol diet group. However, Asgari et al. showed that intake of *A. hirtifolium* and *Sesamum indicum* reduced SGPT, fibrinogen, TC and LDL-C values in comparison with hypercholesterolemic diet group while no significant changes on factor VII and ApoB were observed (38).

Our findings indicated that supplementation with *A. hirtifolium* extract did not cause any significant alteration in apolipoproteins, SGOT, SGPT, hs-CRP, glucose, and insulin as compared to the hypercholesterolemic diet group. While Mehdi et al. showed hydroalcoholic extract of Persian shallot significantly decreased serum levels of FBS and HbA1c in treated groups (in a dose-dependent manner) (40). A study showed that hepatoprotective effects of hydroalcoholic extract of *A. hirtifolium* (Persian shallot) in diabetic rats significantly decreased serum levels of liver enzymes (AST, ALT, ALP and LDH) in treated groups in a dose-dependent fashion (41).

Conclusion

These results suggest that ethanolic extracts of *A. hirtifolium* can reduce risk factors and alter fatty lesions in aorta.

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Authors' contributions

MK, SA, MS, and EH wrote prepared the manuscript, and MRK edited it.

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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