HYPOCHOLESTEROLEMIC AND ANTIATHEROSCLEROTIC EFFECT OF *ARTEMISIA AUCHERI* IN HYPERCHOLESTEROLEMIC RABBITS

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ABSTRACT

Atherosclerosis which results from gradual deposition of lipids in arteries is a leading cause of mortality worldwide. Diet is one of the most important factors underlying atherosclerosis. High-cholesterol diets enhance atherosclerosis and vegetarian diets are known to slow down the process. *Artemisia aucheri* is an herb of the Composite family. Many species of *Artemisia* have proven hypolipidemic and antioxidant properties. This study determine the effects of *Artemisia aucheri* on lipoproteins and atherosclerosis in hypercholesterolemic rabbits. Fifteen male rabbits were randomly divided into three groups. Normal diet group, high-cholesterol diet group (1% cholesterol) and *Artemisia aucheri* group (1% cholesterol diet supplemented with 100 mg/kg body weight the *Artemisi aucheri* every other day). Biochemical factors were measured at the start, end of the first and second months of the study. At the end of the study, the aorta were removed for assessment of atherosclerotic plaques. The results indicate that *Artemisia aucheri* significantly reduced the level of total cholesterol, LDL cholesterol and triglycerids and increased HDL cholesterol. The degree of atherosclerotic thickness was significantly reduced in the treated group. Therefore, *Artemisia aucheri* is one of the useful herbal medicine for preventation of atherosclerosis and more studies in this regard is recommended.

Keywords: Atherosclerosis, Lipoproteins, *Artemisia aucheri,* Hypercholesterolemia.

INTRODUCTION

Thickening and hardening of arteries and loss of elasticity of the inner arterial wall are among the hallmarks of atherosclerosis. This is a progressive condition which begins in childhood and its clinical manifestations surface in middle and old age. Atherosclerosis is the consequence of three principal biological processes, namely aggregation of smooth muscle cells, macrophages and T-lymphocytes, formation of connective matrix by smooth muscle cells and accumulation, in cells and in the connective tissue surrounding the cells of lipids which are basically in the form of cholesterol esters and free cholesterol (Zipes and Braunwald, 2004).

Epidemiological studies have demonstrated a positive significant relationship between plasma cholesterol concentration, smoking, hypertension, obesity, sex … with coronary artery diseases (Moarreaf, 2004). Given the increased risk of atherosclerosis with rising serum cholesterol levels, inhibitors of key enzymes involved in cholesterol biosynthesis (statins) are widely used to treat atherosclerosis. Although statins (Pravastatin, Lovastatin, Simvastatin, Fluvastatin, Atorvastatin, Cerivastatin) reduce cholesterol levels in coronary artery disease patients by inhibiting the activity of β-hydroxy-β-methyl-glutharyl coenzyme A (HMG-COA) reductase. However, as they also inhibit the synthesis of coenzyme Q which is involved in various metabolic processes including electron transfer in ATP synthesis in mitochondrial respiration chain, their use is associated with harms (Patrick and Uzick, 2001; Lankin *et al.,* 2003).

Hence much attention has been focused on the use of herbal drugs as substances with the fewest side-effects and pharmacological regulators of lipid metabolism (Bhandari *et al.*, 1998). *Artemisia aucheri* (Artemisia) is an herb of wide distribution in Iran. Its derivatives are used as appetizers, stimulants, and antiseptics and to distend canals and vessels and relieve rheumatic pains (Massry *et al.*, 2002). *Artemisia aucheri* is a long-lasting herb with a height of 25-30 cm. Studies have demonstrated the presence of flavonoids, santonin, and coumarin compounds in different parts of the herb. Bitter substances, lipids, and carbohydrates are among its other constituents. Given the remarkable pharmaceutical of this herb, the *Artemisia aucheri* was chosen studying its effects on serum lipoproteins and development of atherosclerosis.

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MATERIALS AND METHODS

Extract preparation: Artemisia aucheri was supplied in 2003 from Ghalhar region in Kashan and was verified by Mr. Asgarzadeh from the Research Center of Isfahan Province Natural Resources. The aerial parts were used and dried in the shade and then were subsequently ground to powder using a grinding machine (Erweka AV400AR, made in Germany).

An extract was prepared by dissolving herb powder in 96% and 70% ethanol. The solution concentrated in rotary apparatus and decanted with chloroform. The solution resulting dried under sterile conditions and suitable temperature.

Treatment of rabbits: Fifteen male white New Zeeland rabbits weighing 2-2/5kg and 9 to 11 weeks old were purchased from Razi serum production and vaccine Research Institute in Karaj. They were acclimated in an air conditioned room at 22 ± 2°C for two weeks. Water and food (Super Fosskorn Standard Rabbit Chow) was given *ad libitum*. The rabbits were then randomly divided into three groups: normal diet group, high-cholesterol group (1% cholesterol) (Prasad, 1999) and *Artemisia aucheri* group [high-cholesterol diet supplemented with *Artemisia aucheri* by force feeding of 100 mg/kg body weight every other day)]. The experiment was conducted for 60 days.

Measurement of serum lipids: Blood samples were taken from the rabbits' hearts on day 0 (start of study), day 30 (middle of study), and day 60 (end of study) and the serum was used to determine levels of total cholesterol, triglyceride, LDL and HDL. The concentration of the afore-mention parameters was measured using bio-chemical test zistchem Dignostics kits (was purchased from Zistchem Chemical Co.) which utilized the colorimetric method (Rifai, 1999). The atherogenic index (AIP) which is a measure of the extent of atherosclerotic

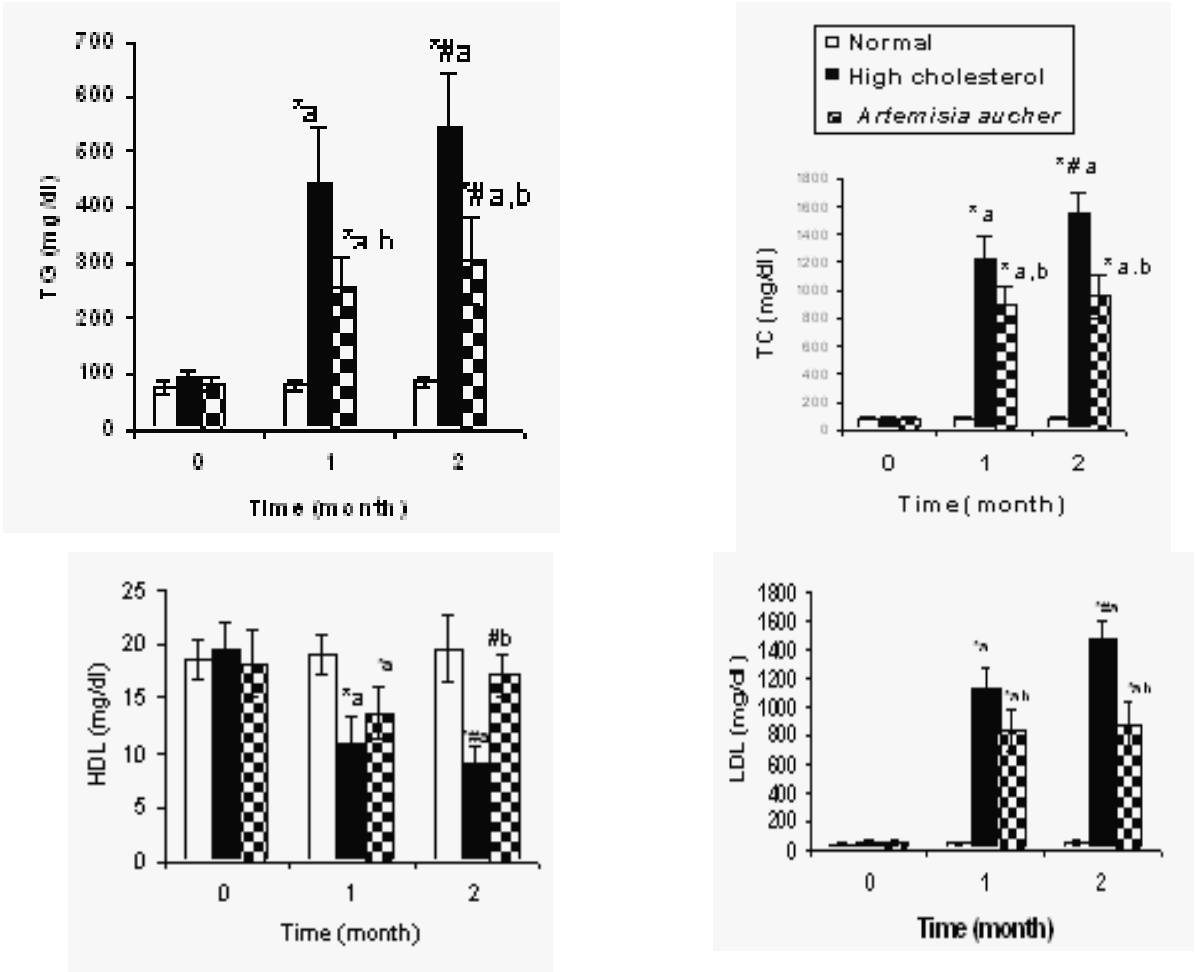

Fig. 1: Fasting plasma lipids and lipoproteins in studied groups. Data are expressed as Mean ± SD.
*P<0.05: Significance of the difference in biochemical factor at the end of the first and second months compared to the start of the study.
#P<0.05: Significance of difference in biochemical factor at the end of the second month compared to the end of the first month of the study
[a]P< 0.05: Significance of difference in biochemical factor in the high-cholesterol and *Artemisia aucheri* groups compared to normal diet group.
[b]P< 0.05: Significance of difference in biochemical factor in the *Artemisia aucheri* group compared to the high-cholesterol group

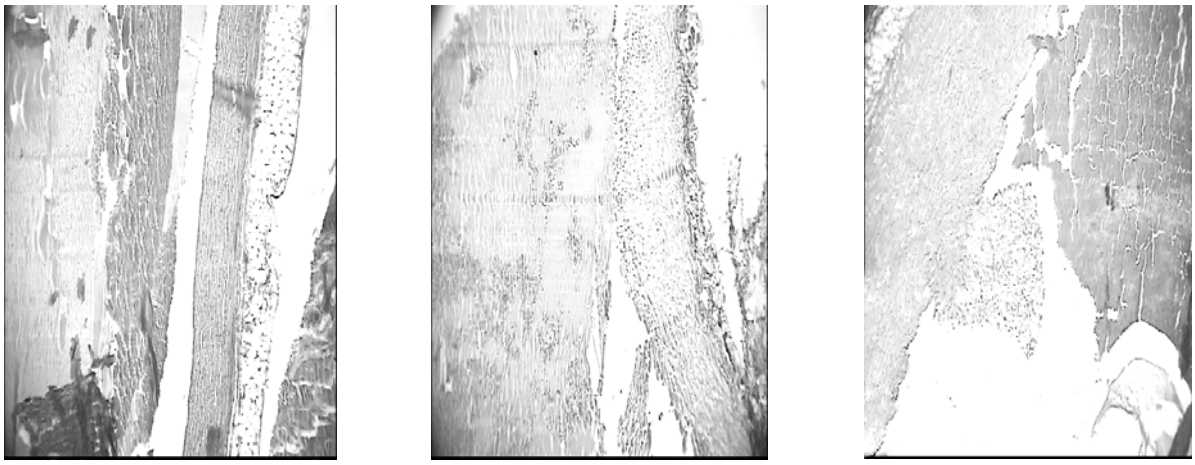

Fig. 2: Aortic intima cross-section in the three groups under study
a: High-cholesterol group (10×), highly protruding atherosclerotic plaque
b: Artemisia aucheri group (10×), foam cells in intima, c: Normal diet group (10×), normal aortic wall.

lesions based on plasma lipids was determined in all three groups. The atherogenic index was calculated using the formula AIP=log (TG/HDL) (Dobiasova, 2004).

Assessment of the severity of atherosclerotic lesions: At the end of the study, chloroform was used to anesthetize the rabbits while blood samples were drawen. Following chest incision, the animals' hearts and aortas were excised to study fatty streaks. After slicing and staining with hematoxylin, microscopic slides were prepared and aortic cross-section was used to grade atherosclerotic plaques according to reference(Chekanov, 2003) on a 1-4 scale:

Grade 1: Plaque less than half as thick as the media with some form of endothelial dysfunction

Grade 2: Plaque at least half as thick as media with accumulation of intracellular lipid, macrophages, and smooth muscle cells.

Grade 3: Plaque as thick as the media with an abundance of macrophages, smooth muscle cell, and connective tissue.

Grade 4: Plaque thicker than the media with a large extracellular intimal lipid core and inflammatory cell infiltration.

STATISTICAL ANALYSIS

Serological and histological results have been expressed as mean ± standard deviation. One-way ANOVA and Duncan tests were used for compare mean values within and between the groups. Data analysis was performed using SPSS software. P values less than 0.05 were considered to be significant.

RESULTS

Figure one shows alterations in TG, TC, LDL and HDL concentration at the end of the first and second months compared to the start of the experiment in all three groups. At the end of the first and second months of the experiment TG, TC and LDL in the high-cholesterol group are increased compared to the normal diet group ($P<0.05$) while HDL decreased in this group as compared to the normal diet group ($P<0.05$). At these time TG, TC and LDL in the *Artemisia aucheri* group are decreased compared to the high-cholesterol diet group ($P<0.05$) while HDL increased in this group as compared to the high-cholesterol diet group ($P<0.05$). Fig. 2 represents atherogenic index in the three groups at three different times. The three groups are not significantly different in respect of this index at the start of the experiment. Over time, however, this index increases in the high-cholesterol and *Artemisia aucheri* groups. This increase is less notable in the *Artemisia aucheri* group, showing a significant difference between the two groups ($P<0.05$). Figure 6 represents atherosclerotic changes of aortal intima in the three groups under study. The results of atherosclerotic plaque grading in the three groups is shown in fig. 3. No atherosclerotic plaques are seen in the group receiving a normal diet. By contrast, the high-cholesterol group displayed visibly protruding atherosclerotic plaques. Foam cells are observed in the aortal intima of the *Artemisia aucheri* group. Atherosclerotic plaque grade in the *Artemisia aucheri*

group decreased significantly compared to the high-cholesterol group (P<0.05).

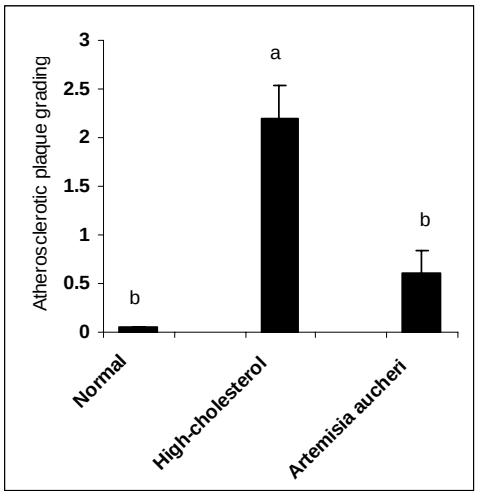

Fig. 3: Atherosclerotic plaque scores in the three groups under study
a: significance of difference compared to normal diet group
b: Significance of difference compared to the high-cholesterol group

DISCUSSION

The significant decrease in TC, TG and LDL and the significant increase in HDL in rabbits receiving *Artemisia aucheri* as compared to the high-cholesterol group is suggestive of the effectiveness of this extract in moderating the dyslipidemic condition arising from a high-cholesterol diet (P<0.05). In 1998, Zhong reported that *Artemisia capilaris* extract decreases TC levels and increases HDL levels in hyperlipidemia mice by improving metabolism (Zhong, 1998). Weng and colleagues reported in 1994 that *Artemisia scoparia* reduces atherosclerotic lesion in hypercholesterolemic rabbits by deceasing TC and TG levels (Weng *et al.*, 1994). Pathology results indicate that *Artemisia aucheri* extract significantly reduces arterial wall atherosclerotic lesions, when compared to the high-cholesterol groups (P<0.05). Lesions in animals receiving extract are limited to foam cells and aggregation of inflammatory cells in the intima, whereas visibly protruding atherosclerotic plaques are seen on the inner surface of arteries in the high-cholesterol group. Statistical analysis of data from atherosclerotic plaque grading based on the severity of aortal lesions in rabbits shows no significant difference between the *Artemisia aucheri* group and the normal diet group, suggesting the effectiveness of *Artemisia aucheri* extract in offsetting the effect of a high-cholesterol diet down to levels comparable with the normal group. On the other hand, calculation of the atherogenic index, another measure of atherosclerotic lesions based on plasma lipids, shows this index to be significantly different between the *Artemisia aucheri* and normal groups (P<0.05), leading one to expect lesions in the *Artemisia aucheri* group not to have decreased to levels in the normal group; hence there is an inconsistency with the results from atherosclerotic plaque grading. This suggests that decrease in lesions in the group receiving *Artemisia aucheri* extract has likely been due to factors other than the effect of the extract on plasma lipoproteins. These factors may be related to the effect of *Artemisia aucheri* on inflammatory and antioxidant reactions in atherosclerosis. Extracts of several species of Artemisia have been shown to possess anti-inflammatory and antioxidant properties.

Artemisia capillaris has anti-inflammatory effects and results in dilation of blood vessels. Capillarisin isolated from this herb has potent antioxidant properties and curbs oxidative damage in rat hepatocytes exposed to butyl hydro-peroxide (Marrif et al, 1995; Wang et al, 1999). *Artemisia scoparia* reduces production of free radicals by inhibiting lipoxygenase(Teng and Pal, 2003). In 2003, Kim reported that *Artemisia apiacea* extract inhibits lipid peroxidation and increases the activity of antioxidant enzymes (Kim et al, 2003). In 2002, Kim reported that *Artemisia asiatica* extract has strong antioxidant properties and prevents hepatic fibrosis in rats (Cheong et al, 2003). Given the documented antioxidant and anti-inflammatory effects of the extracts of several *Artemisia* species, *Artemisia aucheri* can be expected to display similar properties. Further studies in this regard are warranted.

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