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Ethanolic extract of *Artemisia aucheri* induces regression of aorta wall fatty streaks in hypercholesterolemic rabbits

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Artemisia aucheri is a native-growing plant which is widely used in Iranian traditional medicine. This study was designed to evaluate the effects of *A. aucheri* on regression of atherosclerosis in hypercholesterolemic rabbits. Twenty five rabbits were randomly divided into five groups of five each and treated 3-months as follows: 1: normal diet, 2: hypercholesterolemic diet (HCD), 3 and 4: HCD for 60 days and then normal diet and normal diet + *A. aucheri* ($100\ mg \cdot kg^{-1} \cdot day^{-1}$) respectively for an additional 30 days (regression period). In the regression period dietary use of *A. aucheri* in group 4 significantly decreased total cholesterol, triglyceride and LDL-cholesterol, while HDL-cholesterol was significantly increased. The atherosclerotic area was significantly decreased in this group. Animals, which received only normal diet in the regression period showed no regression but rather progression of atherosclerosis. These findings suggest that *A. aucheri* may cause regression of atherosclerotic lesions.

1. Introduction

Atherosclerosis, once believed to be a result of a slow, irreversible process resulting from lipid accumulation in arterial walls, is now recognized as a dynamic process with reversibility (Oka and Chen 2005). Studies in nonhuman primates and rabbits fed high cholesterol diets have revealed the possibility of lesion stabilization and even regression by an aggressive lipid lowering regimen (Shah 2002; Oka and Chen 2005). Furthermore, studies on thoracic aorta transplantation or transgenic mice carrying apolipoprotein E (apoE)-Mx1-*Cre* transgene have shown that long-term lipid normalization or stable expression of antiatherogenic proteins induces regression of advanced lesions (Reis et al. 2001; Raffai et al. 2005).

Recent studies by Nissen et al. (2003, 2004, 2006) show that atherosclerosis regression may be a realistic goal in some patients. Although the actual amount of plaque regression and compositional change is small, there may be substantial clinical benefit.

Despite the significant clinical benefits associated with statin therapy (up to 35% reduction in cardiac events), there is still an active search for effective antiatheriosclerotic agents or combinations that may improve the current standard of care (Nissen et al. 2003; Lima et al. 2004; Corti et al. 2004, 2007).

The World Health Organization has emphasized the importance of traditional indigenous medicine (Goleniowski et al. 2006) and hypercholesterolemia (principle risk factor of atherosclerosis) is among the most common health problems treated with traditional remedies (Gulcan et al. 2006). *Artemisia aucheri* (an endemic species) is widely distributed in desert area of Iran.

The essential oil of *A. aucheri* is rich in linalool (44.1%), genery acetate (10.7%), E-citral (9.7%) and Z-citral (7.7%) (Farzaneh et al. 2006). This plant has several therapeutic effects on different conditions like digestive disorters, hypercholesterolemia and antifungal activity.

This study was designed to evaluate the effects of *A. aucheri* on regression of atherosclerosis in male atherosclerotic rabbits. The atherosclerotic end point in this study was aortic atherosclerosis regression evaluated by biochemical and microscopic methods.

2. Investigations and results

The course of plasma lipid level and the changes for each treatment group are displayed in Figs. 1 and 2. The administration of cholesterol-enriched diet during the atherosclerosis induction period was associated with a significant increase in plasma TC, TG, LDL-C levels and a significant decrease in plasma HDL-C (Figs. 1, 2).

TC, TG and LDL-C decreased significantly and HDL-C increased significantly in the regression period. *A. aucheri* administration during the regression period (group 4) significantly decreased TC, TG and LDL-C, whereas this diet significantly increased HDL-C as compared with group 3. The grade of fatty streak formation in aorta of group 4 was significantly decreased as compared with group 2, whereas this grade was significantly increased in rabbits that use only ND in the regression period (group 3) as compared with group 2 (Fig. 3).

3. Discussion

This study clearly shows that administration of *A. aucheri* to rabbits induced regression of previous established lesions in

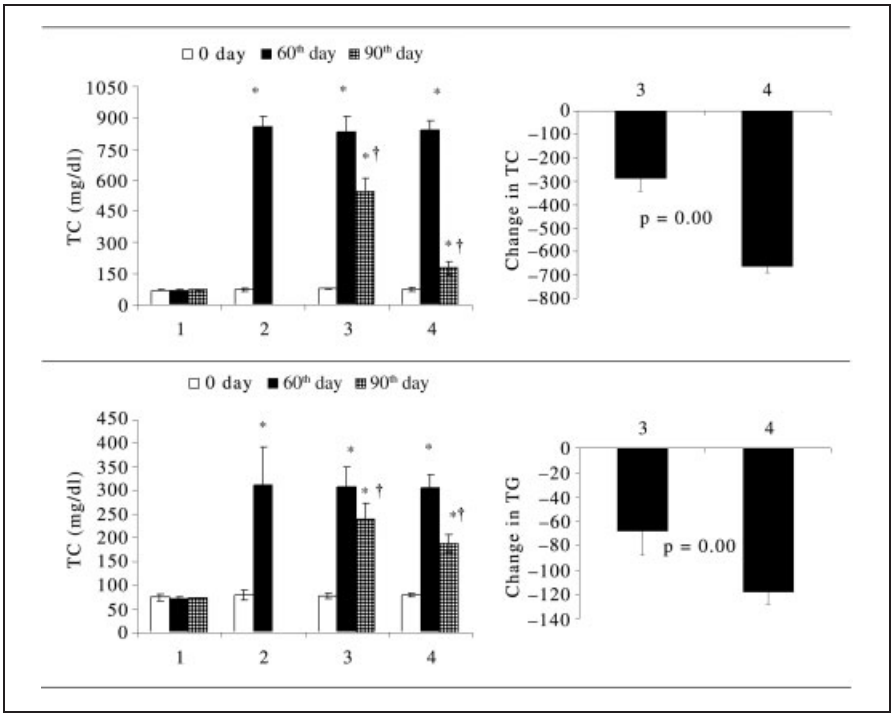

Fig. 1:
Effect of the different treatments on plasma TC (total cholesterol) and TG (triglycerid) levels are displayed as absolute value (left panels) and as changes vs. baseline in regression period (right panels).
[Normal diet (group 1), hypercholesterolemic diet (group 2), hypercholesterolemic diet for 60 days and then normal diet für 30 days (group 3) and hypercholesterolemic diet for 60 days and then normal diet + *Artemisia aucheri* (group 4)].
* $p < 0.05$: Comparison of values with respect to the group 1.
† $p < 0.05$: Comparison of values respect to the group 2

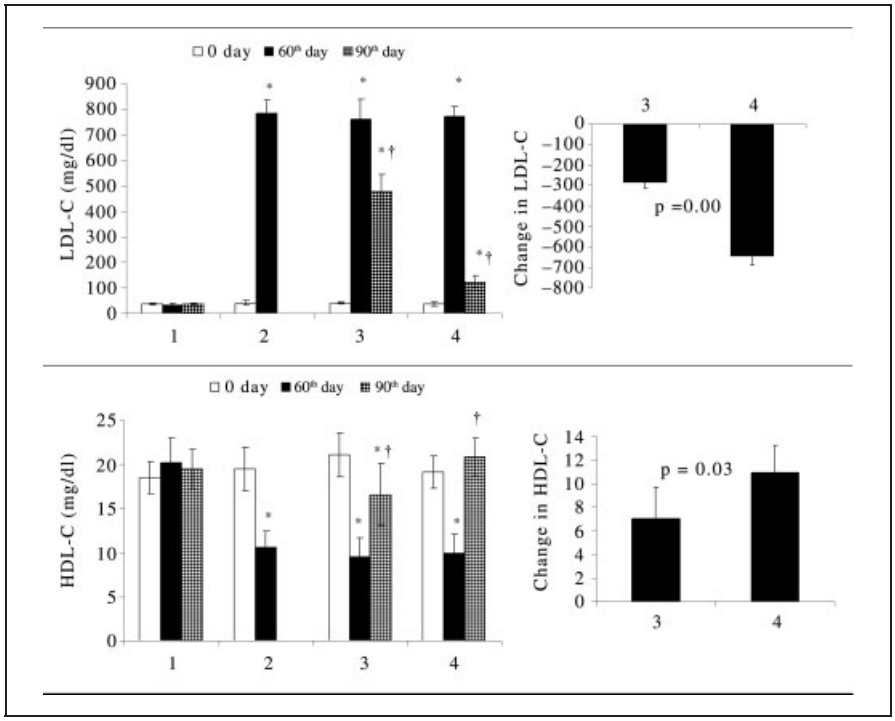

Fig. 2:
Effect of the different treatments on plasma lipoprotein level are displayed as absolute value (left panels) and as changes vs. baseline in regression period (right panels).
* $p < 0.05$: Comparison of values with respect to the group 1.
† $p < 0.05$: Comparison of values respect to the group 2

an experimental model of atherosclerosis (Fig. 4) which may be related to normalization of plasma lipid levels. Studies have indicated that HDL-C exerts beneficial effects by facilitating reverse cholesterol transport, namely, transporting cholesterol away from tissues and carrying it back to the liver where it can be eliminated (Nicholls et al. 2007; Spieker et al. 2002). Epidemiological studies have established elevated LDL-C levels as a major risk factor for atherosclerosis and LDL-C lowering has been identified as the primary goal of therapy for disease prevention (Grobee et al. 2004). Therefore, decrease in fatty streak formation may be related to the increase in HDL-C level and decrease in LDL-C level.

Rabbits that received only normal diet in the regression period showed no regression but rather progression of atherosclerosis. This has also been reported elsewhere (Thakur et al. 2001; Hayashi et al. 1999). Also there are studies about the regression and stabilization of atherosclerosis after removal of cholesterol from the diet; however, these studies showed that the morphological regression and restoration of vascular responses needs intensive lipid lowering or a much longer normolipidemic term, one of many years (Stroung et al. 1994; Harrison et al. 1987).

Intense lipid lowering through dietary manipulation has been previously shown to have salutary effects on plaque progression, inflammation, and plaque composition (Corti et al. 2004; Mcconnel et al. 1999; Helft et al. 2002). Intense lipid lowering is liable with using high-dose of statin. For example, in the Reversal (Reversal of Athero-

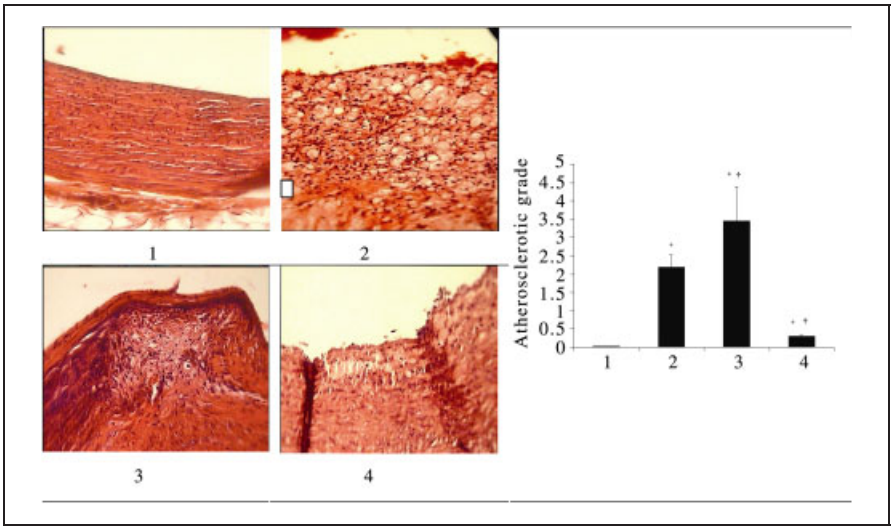

Fig. 3:
Histology of aorta and grade of atherosclerotic plaque in studied group.
* $p < 0.05$: Comparison of values with respect to the group 1.
† $p < 0.05$: Comparison of values respect to the group 2

sclerosis with Aggressive Lipid Lowering) trial, median atheroma volume decreased (regressed) 0.4% in the high-dose statin group (80 mg atorvastatin) versus progressed 2.7% in the moderate-dose group (40 mg pravastatin) over an 18-months period (Nissen et al. 2004). As intense lipid lowering should only be considered in patients who are at very high risk of cardiovascular disease and high dose of statins are associated with an increased risk of myopathy. Consequently, careful attention to dosing recommendation is needed to minimize this risk (Nissen et al. 2006). The ability of *A. aucheri* to induce regression in low doses given for short time is an interesting result of this study. The mechanism leading to the beneficial effects on the vessel wall was not elucidated in this study. In addition to lipid profile improvement, potential effects of *A. aucheri* include antiinflammatory, antioxidant and antithrombotic effects. In a previous phytochemical study, the essential oil was obtained by hydro-distillation of air-dried samples and its chemical composition was identified by GC-MS. Oxygenated monoterpens were the major components of the oil. The essential oil of *A. aucheri* was rich in linalool (44.1%), geranyl acetate (10.7%), (E)-citral (9.7%) and (Z)-citral (7.7%). These components have been described to exert several interesting pharmacological activaties, namly, antioxidant (Farzaneh et al. 2006).
A. aucheri is used by patients in Iraq had good remission of diabetic symptoms with no side effects (Al-Waili 1986). Ribnicky et al. (2004) reported that ethanolic extract of *Artemisia dracunculus* appears to be safe and non-toxic and the no observed adverse effect level in rats was established at 1000 $mg \cdot kg^{-1} \cdot day^{-1}$ for 14 days. LD_{50} after acute intraperitoneal and oral doses of *Artemisia afra* were 2.45 and 8.96 $g \cdot kg^{-1}$ respectively. Although in this study 100 $mg \cdot kg^{-1} \cdot day^{-1}$ were used which is less than the doses regarded as safe for other *Artemisia,* species of the it is suggested that LD_{50} defermination and toxicological evaluation for this plant should be done (Mukinda and Syce 2007).
In conclusions it can be said that dietary use of *A. aucheri* can increase the frequency of regression of coronary lesions. The potential benefit of this plant deserves to be tested in other models and human clinical trials.

4. Experimental

4.1. Preparation of ethanolic extract

Aerial parts (stem, leaves and flowers) of *A. aucheri* were collected from Ghalhar (Kashan-Iran) at flowering stage in autumn 2004 and authenticated by Dr. Rahimnezhad at the Biology Department, School of Science, Isfahan University. The voucher speicmen was deposited in Isfahan University Herbarium under the number 14067.
Above-ground parts of the plant were dried for 10 days at room temperature. The dried plants were ground by an electric blender. *A. aucheri* plant powder (100 g) was soaked in 96% ethanol for 72 h, filtered, and concentrated by a distiller in a vacuum. The concentrated solution was decanted in three consecutive steps (once with 100 ml and twice with 50 ml of chloroform). The resulting solution was vaporized and desiccated in 50 °C under sterile conditions. An auburn coloured crude extract (4 g) was obtained and kept in a dark glass bottle at 4 °C until use (Erdemoglu et al. 2003).

4.2. Animals and diets

Twenty five 10-weeks old Male New Zealand white rabbits with the average weight of 2–2.5 kg were purchased from Razi Institute, Teheran, Iran. The rabbits were housed individually at 20 ± 3 °C with a 12-h: 12-h light/dark cycle. Animals were fed a normal rabbit chow (Super Fosskorn, Dam Pars Co., Tehran, Iran) for 2 weeks, then they were randomly divided into five groups of 5 animals. Each group of animals had its specific diet and regular drinking water *ad libitum*. As the control group, animals in group 1 received a normal diet (ND) throughout the experiment (90 days). Animals in group 2 received ND supplemented with 1% cholesterol which is considered to be a high cholesterol diet (HCD) and were killed after 60 days. They served as a control for evaluation the extent of aortic fatty streaks before *A. aucheri* administration. Animals in group 3 and 4 received HCD for 60 days and then ND and ND + *A. aucheri* (100 $mg \cdot kg^{-1} \cdot day^{-1}$),

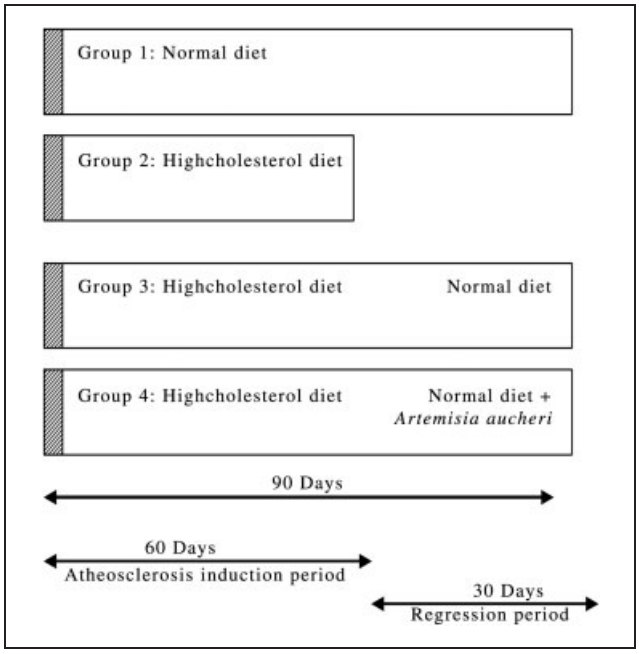

Fig. 4: Experimental design of study

respectively for 30 days. Each 100 mg of the plant extract was equal of 2.5 g *A. aucheri* dried powder. The study design is summarized in Fig. 4. Isfahan Cardiovascular Research Center Ethics Committee which is a member of Office for Human Research Protections, US Department of Health and Human Services, approved the present study, and the animals were handled according to guidelines of Isfahan University of Medical Sciences for Laboratory Animal Sciences for the care and use of laboratory animals.

4.3. Assay for lipids

Fasting blood samples were taken from a central ear artery of each rabbit on days 0, 60, 90 of the study to measure total cholesterol (TC), triglyceride (TG), high density lipoprotein (HDL-C), and low density lipoprotein (LDL-C). Lipid and lipoprotein levels were determined using an automated enzymatic assay by an auto-analyzer Hitachi 902-model and diaSys kits from Germany

4.4. Histological evaluation of atherosclerosis

At the end of the treatment period, the rabbits were killed with an overdose of pentobarbital (60 mg/kg iv) and gross anatomic examinations and pathologic investigations were performed. Aorta was 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 after slicing and staining with hematoxylin, atherosclerotic thickness was assessed on a scale of 1–4 as described by Chekanov et al. (2003). In this method the atherosclerotic thickness to media thickness was measured with an ocular grid.

4.5. Statistical analysis

Results are expressed as the mean ±SD. All analyses were performed using SPSS 13 statistical software. The Kolmogorov-Smirnov test was applied to test for a normal distribution. Data were analysed by univariate ANOVA. If a resultant fraction was found to be significant, i.e, established at $p < 0.05$, a post-ANOVA Duncan's test was used to specify pair-wise differences.

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