Effect of *Amirkabiria odoratissima* mozaffarian on the Development and Progression of Fatty Streaks in Hypercholesterolemic Rabbits

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New studies have introduced atherosclerosis as an inflammatory disease. Different factors affect the development of inflammation that can be linked to free radicals, hypertension, diabetes mellitus, genetic changes, hypercholesterolemia and some of the microorganisms. Using anti-inflammatory drugs can be useful in preventing atherosclerosis. Finding drugs from natural sources and without side-effects can be useful. *Amirkabiria odoratissima* (umbelliferae) is from Iranian flora and is used traditionally as an anti-inflammatory drug. This study was designed to assess the effects of this plant on the development and progression of fatty streaks. Male rabbits were fed: (a) rabbit chow; (b) 1% cholesterol diet; (c) cholesterol diet supplemented with the plant; (d) normal diet supplemented with the plant. After 12 weeks, the animals were sacrificed and the aorta, right and left branches of coronary arteries were dissected and histologically processed. Before and after the end of the study, biochemical factors were measured. The amounts of cholesterol, LDL, HDL, triglyceride and fasting blood sugar (FBS) were determined by enzymatic methods, quantitative CRP was determined by turbidimetric methods, malondialdehyde (MDA) and antioxidant capacity were determined by spectrophotometric methods. The results indicate that there is a significant difference between the groups supplemented with herbal medicine and others (*p* < 0.05) in the mean grade of fatty streak of right and left coronary artery and aorta. At the end of the 12-week period cholesterol, LDL, and CRP were significantly reduced in the groups which received herbal medicine (*p* < 0.05). The data suggests that *Amirkabiria odoratissima* Mozaffarian has beneficial effects to prevent development of fatty streak; however, further studies are needed to understand the mechanisms whereby this plant exerts its anti-atherosclerotic effects. Copyright © 2004 John Wiley & Sons, Ltd.

Keywords: atherosclerosis; inflammation; *Amirkabiria odoratissima* rabbit.

**INTRODUCTION**

Cardiovascular diseases remain the major cause of mortality in the United States, Europe and most Asian countries (Braunwald, 1997; Breslow, 1997). Efforts have always been in progress to better understand the mechanisms which lead to cardiovascular disease, especially atherosclerosis. New studies have referred to atherosclerosis as an inflammatory condition. Various factors affect the start and advance of inflammation. These include free radicals, hypertension, diabetes mellitus, genetic alterations; hypercholesterolemia, high plasma homocysteine levels and certain microorganisms, namely herpes virus and chlamydia (Rose et al., 1973; National Cholesterol Education Program, 1993; Vanhoutte et al., 1993; Jackson et al., 1997; Libby et al., 1997). Natural homeostatic responses develop in reaction to changes in the endothelial function. Monocytes, lymphocytes and macrophages infiltrate the site of damage (Jamison et al., 1986; van der Wal et al., 1989). The inflammatory process is regulated by the release of inflammatory factors such as IL-1, CD-14 and TNF (Libby et al., 1996; Raines et al., 1996). Therefore the use of anti-inflammatory drugs can prevent or reduce complications of atherosclerosis.

Despite their beneficial effects, piroxicam, diclofenac, ketoprofen and tenoxicam have several side-effects on different body systems (Katleen et al., 1999). Hence finding drugs of herbal origin with no side-effects is of high priority. *Amirkabiria odoratissima* Mozaffarian (umbelliferae) has analgesic and anti-inflammatory properties. Studies have established the presence of 3', 4', 7-trihydroxy flavonol, rutin, caffeic acid and phthalides in *Amirkabiria odoratissima* Mozaffarian. Phthalides occurring in this herb are among the constituents of volatile oils and may have anti-inflammatory effects. *Amirkabiria odoratissima* Mozaffarian is known in traditional Iranian medicine as a herb with anti-inflammatory properties.

The present study was conducted to evaluate the effect of this herb on the development and progression of atherosclerosis.
that anti-inflammatory medications may decrease the incidence of atherosclerotic damages.

Amirkabiria odoratissima Mozaffarian is a herbal species with applications in traditional medicine due to its anti-inflammatory properties. Studies of total extracts of this herb have shown the presence of 3', 4', 7-trihydroxy flavonol, caffeic acid and phthaldic acids (Aamans, 1989). Owing to their special stereoeomposition, flavonoids are able to interact with prostaglandin A2 receptor and with prostaglandin E2 receptor in membrane of arterial smooth muscle cells. Moreover, reduction in cholesterol, LDL and oxidation changes of these lipoproteins has also prevented the progression of atherosclerosis. The presence of caffeic acid and O-quinol groups account for the antioxidant properties of Amirkabiria odoratissima Mozaffarian. As shown in Table 2, increased antioxidant property was observed in rabbits which were on a diet containing Amirkabiria odoratissima Mozaffarian (Kaovaljii, 1986). Therefore the conclusion can be made in view of the anti-inflammatory properties of Amirkabiria odoratissima Mozaffarian that it can be used to prevent the start and progression of atherosclerosis.

It is recommended that in vivo studies be conducted to better understand the mechanisms which give Amirkabiria odoratissima Mozaffarian its anti-atherosclerotic properties.

### Table 2. Comparison of biochemical variables in groups 1, 2, 3, and 4 at the end of the 4-week dietary course, one-way ANOVA test

<table>
<thead>
<tr>
<th>Group</th>
<th>A-OX %</th>
<th>MDA µmol/l</th>
<th>CRP mg/dl</th>
<th>FBS mg/dl</th>
<th>HDL mg/dl</th>
<th>LDL mg/dl</th>
<th>TG mg/dl</th>
<th>Chl mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>76.25 ± 8.42</td>
<td>0.22 ± 1.1</td>
<td>5.42 ± 1.68</td>
<td>6.65 ± 9.75</td>
<td>7.13 ± 75.19</td>
<td>10.74 ± 14.2</td>
<td>15.67 ± 80.5</td>
<td>11.58 ± 40.75</td>
</tr>
<tr>
<td>2</td>
<td>10.43 ± 67.6</td>
<td>0.06 ± 0.96</td>
<td>0.73 ± 3.4</td>
<td>9.35 ± 91</td>
<td>8.29 ± 21.6</td>
<td>10.74 ± 14.2</td>
<td>64.6 ± 22.67</td>
<td>49.2 ± 22.53</td>
</tr>
<tr>
<td>3</td>
<td>19.36 ± 48.8</td>
<td>0.43 ± 1.23</td>
<td>3.11 ± 4.36</td>
<td>11.42 ± 83.4</td>
<td>2.28 ± 21.8</td>
<td>12.65 ± 14.68</td>
<td>149.12 ± 221</td>
<td>884.23 ± 2806</td>
</tr>
<tr>
<td>4</td>
<td>65.2 ± 15.3</td>
<td>0.24 ± 1.27</td>
<td>2.88 ± 2.23</td>
<td>25 ± 80.2</td>
<td>3.04 ± 21.6</td>
<td>858.89 ± 2740</td>
<td>295.44 ± 236.8</td>
<td>1113.33 ± 2305</td>
</tr>
</tbody>
</table>

**REFERENCES**


MATERIALS AND METHODS

Trimmed branch tips of *Amirkabiria odoratissima* Mozaffarian were obtained from the Chahar Mahal Bakhtiari region. After identity verification by the Pharmacognosy Department of the Isfahan School of Pharmacy, the powdered form of the herb was prepared. Twenty white male rabbits with a mean weight of 2-2.5 kg and mean age of 10 weeks were purchased from the Iran Pasteur Institute and were kept in standard temperature and light conditions. Their basic diet consisted of super fooskorn standard rabbit chow, which contained 14 g/kg protein, 150 g/kg fiber, and 30 g/kg fat. The rabbits were randomly divided into four groups of five animals. The first and second groups were given a normal diet and a high cholesterol diet, respectively. The third group was given a normal diet supplemented with *Amirkabiria odoratissima* Mozaffarian, and the fourth group was given a high cholesterol diet supplemented with *Amirkabiria odoratissima* Mozaffarian. Every rabbit received 5 g dried *Amirkabiria odoratissima* Mozaffarian on a daily basis (the first and second groups served as control groups). Before and after receiving the diet for a period of 14 weeks, biochemical tests (LDL, HDL, total cholesterol, triglyceride, and fasting blood sugar (FBS), CRP, Malondialdehyde (MAD), and antioxidant capacity) were determined. CRP was measured via turbidimetric and MAD and antioxidant capacity was measured using the spectrophotometric method with a Shimadzu machine (Miki et al., 1987; Kostner et al., 1997). High-cholesterol diet was prepared by supplementing daily rabbit chow with cholesterol (purchased from Merck) dissolved in diethyl ether (1% of weight). At the end of the 14-week period, the rabbits were anesthetized using 5% pentobarbital solution. After collecting blood, right and left coronary arteries and aorta were excised and kept in 10% formalin. Microscopic tissue specimens were prepared and studied by a pathologist with respect to the presence of fatty streaks and were graded on a scale of 0 to 4 (Nematbakhsh et al., 1998). All data were compared using one-way variance analysis. The Duncan test was applied to assess the difference between the groups. *P* values less than 0.05 (*p* < 0.05) were considered meaningful. The Mann–Whitney test was used to assess the difference between the groups, which had received high-cholesterol and normal diets.

RESULTS

Pathological assessment of aorta and right and left coronary arteries (Table 1) showed that rabbits which had received a normal diet, and those which had received normal diet supplemented with *Amirkabiria odoratissima* Mozaffarian did not develop any fatty streaks, whereas the rabbits which had received high-cholesterol diet had 3 ± 0.7, 2.4 ± 1.1, and 2.8 ± 0.8 fatty streaks in their aorta, right and left coronary arteries, respectively. The rabbits which had received high-cholesterol diet supplemented with *Amirkabiria odoratissima* Mozaffarian, had 2.2 ± 1.3, 2.2 ± 1.1, and 1.8 ± 0.8 fatty streaks in their aorta, right and left coronary arteries, respectively, displaying a marked reduction in the number of fatty streaks, which was statistically significant. Assessment of biochemical factors at the end of the 14-week period showed that cholesterol, LDL and CRP levels were significant reduced in the groups which received cholesterol diet supplemented with *Amirkabiria odoratissima* Mozaffarian. Anti-oxidant capacity in the group receiving high-cholesterol diet was significantly lower than in the group, which received a normal diet. HDL levels were not significantly affected by intake of *Amirkabiria odoratissima* Mozaffarian. Statistical analysis of biochemical factors before the 14-week period did not show any significant difference between the four groups in respect of any of the biochemical factors; nor was there any difference between the four groups in respect of weight (*p* < 0.05).

DISCUSSION

Recent studies have demonstrated that atherosclerosis is an inflammatory disease. The role of *Amirkabiria odoratissima* Mozaffarian as a herbal anti-inflammatory drug in preventing the formation and development of fatty streaks in laboratory animals was studied. Our results showed that *Amirkabiria odoratissima* Mozaffarian can prevent the formation and development of fatty streaks. As shown in Table 1, no fatty streaks had been formed in the aorta, right and left coronary arteries of the animals which received normal diet, or normal diet supplemented with *Amirkabiria odoratissima* Mozaffarian, whereas in animals which were on a high cholesterol diet fatty streaks in stage 3 were seen in the aorta, right, and left coronary arteries, respectively (*p* < 0.001). Animals on a high-cholesterol diet supplemented with *Amirkabiria odoratissima* Mozaffarian had 2.2 ± 1.3, 2.2 ± 1.0, and 1.8 ± 0.86 fatty streaks in stage 2 in their aorta, right, and left coronary arteries, respectively (*p* < 0.001). Results show that intake of *Amirkabiria odoratissima* Mozaffarian significantly inhibited the formation and progression of fatty streaks. These observations strengthen the belief

<table>
<thead>
<tr>
<th>group</th>
<th>Diet</th>
<th>Aorta (mean ± SD)</th>
<th>Right coronary artery (mean ± SD)</th>
<th>Left coronary artery (mean ± SD)</th>
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<tbody>
<tr>
<td>1</td>
<td>Normal diet</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
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<tr>
<td>2</td>
<td>Normal diet + herb</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>3</td>
<td>High-cholesterol diet</td>
<td>3 ± 0.7*</td>
<td>2.4 ± 1.1*</td>
<td>2.8 ± 0.8*</td>
</tr>
<tr>
<td>4</td>
<td>High-cholesterol diet + herb</td>
<td>2.2 ± 1.3*</td>
<td>2.2 ± 1.1*</td>
<td>1.8 ± 0.8*</td>
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*p* < 0.0001, herb: *Amirkabiria odoratissima* Mozaffarian.

that anti-inflammatory medications may decrease the incidence of atherosclerotic damages.

Amirkabiria odoratissima Mozaffarian is a herbal species with applications in traditional medicine due to its anti-inflammatory properties. Studies of total extracts of this herb have shown the presence of 3',4',7trihydroxy flavonol, caffeic acid and phthalides (Amams, 1989). Owing to their special stereocorrelation, flavonoids which are in the aglyconic form have fast intestinal absorption and remarkable anti-inflammatory properties. Previous studies have shown that these flavonoids function by inhibiting the metabolism of arachidonic acid and lipoxygenase. The presence of 4-hydroxy group rotation of the B-flavonoid cycle, thus enhancing the inhibiting power of 5-lipoxygenase (Harborne, 1994).

The fact that intake of Amirkabiria odoratissima Mozaffarian has led to a significant reduction of CRP in animals which received normal or high-cholesterol diet, suggests that this herb prevents the formation of fatty streaks through anti-inflammatory mechanisms. Moreover, reduction in cholesterol, LDL and oxidation changes of this lipoproteins has also prevented the progression of atherosclerosis. The presence of caffeic acid and O-quinol groups account for the anti-oxidant properties of Amirkabiria odoratissima Mozaffarian. As shown in Table 2, increased anti-oxidant property was observed in rabbits which were on a diet containing Amirkabiria odoratissima Mozaffarian (Kaovacljii, 1986). Therefore the conclusion can be made in view of the anti-inflammatory properties of Amirkabiria odoratissima Mozaffarian that it can be used to prevent the start and progression of atherosclerosis.

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P-value

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