کارگاه‌های آموزشی مرکز اطلاعات علمی

آموزش مهارت‌های کاربردی ISI در تدوین و چاپ مقالات

روش تحقیق گمی

آموزش نرم‌افزار برای پژوهشگران

Word
Evaluation of the effect of *Anethum graveolens* L. crude extracts on serum lipids and lipoproteins profiles in hypercholesterolaemic rats

*Yazdanparast R., Bahramikia S.*

*Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran*

Received: 28 Nov 2007 Revised: 28 Jan 2008 Accepted: 9 Feb 2008

**ABSTRACT**

*Background and the purpose of study:* *Anethum graveolens* L. (Umbilliferae) is used in Iranian folk medicine as an anti-hypercholesterolaemic plant. The present study was carried out to determine the effect of *Anethum graveolens* extract (AGE) on serum lipoproteins in hypercholesterolaemic rats and also to determine its mechanism of action to some extent on liver hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase activity.

*Methods:* The changes in serum triglyceride (TG), total cholesterol (TC), high density lipoprotein-cholesterol (HDL-C) and low density lipoprotein-cholesterol (LDL-C) were measured using enzymatic kits. Hepatic HMG-CoA reductase activity was indirectly measured by the HMG CoA/mevalonate ratio. The amount of total phenolic and flavonoid compounds were also determined by common colorimetric methods.

*Results:* Keeping the rats on a high-fat diet for 7 consecutive days increased the serum TC, TG and LDL-C levels significantly. However, the HDL-C level decreased relative to normal rats. Treatment of hyperlipidaemic rats with AGE (single daily dose of 1 ml, equivalent to 500 mg of the plant powder) and high-fat diet for up to 10 and/or 30 days reversed the serum lipid levels compared to rats which were fed only high-fat diet. In addition, our data indicated significant increase in HMG-CoA/mevalonate ratio as compared to rats which were fed high-fat diet after treatment with AGE for 30 days, indicating a decrease in the enzyme activity. Experiments showed that AGE has the phenolic and flavonoid content of 105.2 mg of gallic acid equivalents/g of the dried extract and 58.2 mg of catechin equivalents/g of the dried extract, respectively.

*Conclusion:* The cumulative results clearly indicate that *A. graveolens* possesses potent hypocholesterolaemic effects in rats probably mediated through the suppression of endogenous cholesterol biosynthesis by inhibition of the activity of HMG-CoA reductase.

**Keywords:** *Anethum graveolens*; Hypercholesterolaemic rats; Hypolipidaemic property; HMG-CoA reductase; Flavonoids content.

**INTRODUCTION**

Cardiovascular disease, currently the leading cause of death and illness in developed countries, will soon become the pre-eminent health problem worldwide (1-3). Atherosclerosis, a progressive disease characterized by the accumulation of cholesterol, low density lipoprotein-cholesterol (LDL-C) and fibrous elements in the large arteries, constitutes the single most important contributor to this growing burden of cardiovascular disease (4-5). Elevated levels of plasma total cholesterol (TC) and triglycerides (TG) have been implicated as causative factors in development of atherosclerosis and coronary heart diseases (CHD) (4-6). Efforts to develop effective and better hypolipidaemic drugs have led to the discovery of natural agents. Research in herbal medicine has increased in the world as an alternative solution to health problems. Many plant extracts have been shown to have hypocholesterolemic activity in rats and the effects of several extracts have been described (7-11). *Anethum graveolens* L. (Umbilliferae), known as dill, is an annual herb growing in the Mediterranean region, Europe, central, southern Asia and it is widely cultured in south eastern region of Iran. The plant is used both medicinally and as an aromatic herb and spice and cookery. Dill has been used traditionally for gastrointestinal ailments such as flatulence, indigestion, stomachache colic and to tract intestinal gas (12). The presence of flavonoids, phenolic compounds and essential oil in *Anethum graveolens* has been reported (13-16). Some pharmacological effects of the plant such as antimicrobial (17), antispasmodic (18), anti-secretary and mucosal protective effects have also been reported (12). The anti-hyper-
cholerasterolaemic and anti-hyperlipidaemic activities (TC, TG) of the crude extract have previously been reported (19). The present study was designed to determine the effects of the _Anethum graveolens_ extract (AGE) on serum lipoproteins profile in hypercholesterolaemic rats and possible mechanism of action of the crude extract on liver HMG-CoA reductase activity.

**MATERIAL AND METHODS**

**Plant material**
The aerial sections of _Anethum graveolens_ L. were collected from suburbs of Aleshtar city (Lorestan province, Iran) at the end of May 2006 and identified by Dr. F. Attar (Department of Biology, Faculty of Sciences, University of Tehran) and a voucher specimen (No. 11018) was deposited in the central herbarium of University of Tehran. The collected materials were dried at room temperature away from sun light, and the dried leaves were pulverized and kept at 8 °C for further use.

**Crude extract preparation**
The _Anethum graveolens_ powder (200 g) was defatted with hexane at room temperature. Then, the residue was extracted four times with ethanol (70 %, v/v) at room temperature. The combined extracts were concentrated under reduced pressure (70 %, v/v) at room temperature. The combined extracts were concentrated under reduced pressure and the volume was adjusted to 400 ml (equivalent to 500 mg plant powder). Aliquots were kept at -10 °C for further investigation.

**Determination of total phenolic and total flavonoid contents**
The total phenolic content of AGE was determined with the Folin–Ciocalteu’s reagent (FCR) according to a published method (20). Results were expressed as gallic acid equivalents (mg of gallic acid/g of the dried extract). The total flavonoid content of AGE was evaluated by colorimetric methods as described in the literature (21). Results were expressed as catechin equivalents (mg of catechin/g of dried extract).

**Experimental animal and diet protocols**
Male N-Mary rats weighing 200-250 g were allocated in groups of six per cage and maintained on a 10 h light/14 h dark cycle, at 22 ± 2 °C and relative humidity of 60 ± 5%. The animal had free access to water and normal or high-fat diet _ad libitum_. Chow with high-fat content, was made from normal pulverized chow (Protein, vitamin, carbohydrate) (48%), cholesterol (1%), sodium cholate (1%), dextrose (40%) and olive oil (10%). The cake was cut into pieces and dried at room temperature for 3 days before feeding to rats. The study lasted for 6 weeks. After one week of adaptation, the rats were randomly divided into three groups each of 6 animals as follows:

Group 1: received normal diet (NC)
Group 2: received high-fat diet (HFD)
Group 3: received HFD + _Anethum graveolens_ extract (HFD+AGE) (1 ml equivalent to 500 mg of the plant powder).

The dose of _Anethum graveolens_ in this study was chosen based on a concentration–response study which was established in our preliminary studies (data not shown). At time intervals of -7, 0, 10 days, the overnight fasted rats were anesthetized with diethyl ether and their blood samples were collected by cardiac puncture into glass tubes and centrifuged at 3000 rpm, 4 °C for 15 min. At the end of the study, the animals were fasted overnight and then sacrificed under diethyl ether anesthesia according to the guidelines for the care and use of experimental animals approved by state veterinary administration of University of Tehran. Blood samples and liver tissues were taken from the animals of all groups. Each liver tissue was immediately washed with saline, blotted on filter paper, weighted and was frozen until use. Prior to biochemical analyses, the liver samples were cut into small pieces and homogenized in Tris-HCl buffer (0.025 M, pH 7.5) with a homogenizer to give a 10% (w/v) liver homogenate. The homogenates were then centrifuged at 12000 rpm (Beckman) for 15 min at 4 °C and the supernatant was used for biochemical analyses. The protein concentration of each extract was determined by the method of Lowry et al (22) using bovine serum albumin as the standard. The serum samples were stored at -70 °C for biochemical analyses. Food intake was measured every day and body weight was recorded at weekly intervals.

**Hepatic HMG-CoA reductase activity**
Hepatic HMG-CoA reductase activity was indirectly measured based on the HMG CoA/mevalonate ratio (22). HMG-CoA was determined by its reaction with hydroxylamine hydrochloride at alkaline pH and subsequent colorimetric measurement of the resulting hydroxamic acid by formation of complexes with ferric salts at 540 nm. Mevalonate was estimated by reaction with the same reagent but at pH 2.1. At this pH, the lacton form of mevalonate readily reacts with hydroxylamine hydrochloride to form the hydroxamate. The ratio of HMG-CoA to mevalonate is inversely proportional to HMG-CoA reductase activity, i.e. an increase in ratio indicates a decrease in activity.

**Biochemical analysis**
TG, TC, HDL-C, LDL-C and glucose in serum were determined using enzymatic kits (Pars Azmoon, Iran). Atherogenic index was calculated in term of the LDL/HDL cholesterol ratio. Serum
alanine transferase (SALT), aspartate transferase (SAST) and alkaline phosphatase (ALP) activities were assayed using the corresponding commercial kits according to the manufacture’s instructions (Pars Azmoon, Iran).

**Statistical analysis**
All values are expressed as mean ± S.D. The significance of differences between the means of the treated and untreated groups was calculated by unpaired Student’s t-test and P < 0.05 was considered significant.

**RESULTS**

Total phenolic and total flavonoids contents of A. graveolens extract
The total phenolic and flavonoid contents of AGE were determined and expressed in terms of gallic acid and catechin equivalents, respectively. The results show that the total phenol and total flavonoid contents of the AGE were 105.2 mg of gallic acid equivalents/g of the dried extract and 58.2 mg of catechin equivalents/g of the dried extract, respectively.

Dietary intake, body and liver weight
The daily food intake of the rats decreased in group II and III, from the 20th day of the experiment (Fig. 1). HFD apparently caused an increase in the body weight of rats despite their lower food intake. However, in group III the body weights of the plant treated rats decreased after the day of 20, as shown in Fig. 2. Liver weights were not significantly different between groups II and III (data not shown).

Effects of A. graveolens extract on lipid parameters
Statistical evaluation of rats serum lipid levels at the beginning of the study indicated that there was not significant difference between the serum levels of all rats (data not shown). However, feeding HFD for 7 days increased the serum levels of TC, TG and LDL-C among the rats of group II compared to the rats which were fed normal diet, but the serum level of HDL-C decreased (p < 0.01). Administration of AGE (at a single daily dose of 1 ml, equivalent to 500 mg of the plant powder) to rats for 10 consecutive days reduced the levels of TC, TG and LDL-C by 38.7%, 33.1%, and 66.5%, respectively, compared to rats which were fed HFD (p < 0.05) but the level of serum HDL-C increased by 24.6 % after 30 days of feeding which was, almost similar to the hypolipidaemic effect of AGE (Table 1).

The atherogenic index (AI, defined in term of LDL/HDL ratio) increased significantly after keeping the animals on HFD. However, it significantly decreased in AGE-treated rats (group III) compared to rats of group II (Table 1). The levels of fasting serum glucose for HFD groups increased compared to rats of normal group. Treatment of rats with AGE for a period of 10 and/or 30 days significantly decreased the serum glucose levels (Fig 3).

**Effects of A. graveolens extract on hepatic HMG-CoA reductase and serum hepatic enzymes**
This activity was measured indirectly in order to determine whether the decrease in cholesterol levels was due to suppression of this enzyme activity. As expected, feeding of HFD
Table 1. Changes in the serum lipid profile of rats which were fed normal diet (NC), high fat diet (HFD) and high-fat diet + extract (HFD+AGE).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>NC</th>
<th>HFD</th>
<th>HFD + AGE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 day 30 day</td>
<td>10 day 30 day</td>
<td>10 day 30 day</td>
</tr>
<tr>
<td>TC (mg/dl)</td>
<td>82.60 ± 4.72</td>
<td>92.01 ± 5.52</td>
<td>217.01 ± 11.12*</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>78.26 ± 9.61</td>
<td>83.11 ± 8.70</td>
<td>108.33 ± 9.70*</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>33.06 ± 3.22</td>
<td>37.36 ± 4.61</td>
<td>118.72 ± 32.12*</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>27.24 ± 2.20</td>
<td>25.30 ± 3.50</td>
<td>18.31 ± 1.02*</td>
</tr>
<tr>
<td>LDL/HDL</td>
<td>1.21 ± 0.11</td>
<td>1.47 ± 0.12</td>
<td>6.48 ± 0.23*</td>
</tr>
</tbody>
</table>

Values are mean ± SD, for 6 rats.
*Significantly different from Group I (NC) (P< 0.01).
**Significantly different from Group II (HFD) (P< 0.05).

Table 2. Changes of hepatic enzymes activities in rats fed with: normal diet (NC), high-fat diet (HFD) or high-fat diet + extract (HFD+AGE)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>NC</th>
<th>HFD</th>
<th>HFD + AGE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>204.73 ± 14.40</td>
<td>512.71 ± 45.80*</td>
<td>337.81 ± 20.24**</td>
</tr>
<tr>
<td>SALP (IU/L)</td>
<td>17.80 ± 2.06</td>
<td>36.33 ± 3.50*</td>
<td>25.82 ± 0.91 **</td>
</tr>
<tr>
<td>SALT (IU/L)</td>
<td>24.91 ± 3.07</td>
<td>40.25 ± 3.91*</td>
<td>33.21 ± 2.61 **</td>
</tr>
<tr>
<td>HMG-CoA reductase***</td>
<td>2.42 ± 0.12</td>
<td>3.59 ± 0.24*</td>
<td>5.31 ± 0.39 **</td>
</tr>
</tbody>
</table>

Values are mean ± SD, for 6 rats.
*Significantly different from Group I (NC) (P< 0.01).
**Significantly different from Group II (HFD) (P< 0.01).
***HMG-CoA reductase was calculated based on of HMG-CoA / Mevalonate ratio.
SALP: Serum alkaline phosphatase; SALT: Serum alanine transaminase; SAST: Serum aspartate transaminase.

Figure 3. Effect of AGE on the blood glucose levels in high-fat diet rats after 10 and 30 days of treatment. Group I: normal diet (NC), group II: high-fat diet (HFD) and group III: high-fat diet + extract (HFD+AGE). Values are mean ± SD, for 6 rats. *Significantly different from Group I (P < 0.01). **Significantly different from Group II (P< 0.05).
significantly reduced the activity of HMG-CoA reductase (Table 2). On the other hand, the enzyme activity was further suppressed in AGE-treated rats (group III) by 47.9% compared to rats which were fed HFD (group II) (p < 0.01). The effects of AGE on serum aminotransferases (SAST, SALT) and ALP are shown in Table 2. Animals which were fed HFD, exhibited an elevation in serum AST, ALT and ALP levels compared to normal groups. Treatment with AGE for 30 days reduced activities of AST, ALT and ALP relative to animals of group II.

**DISCUSSION**

*Anethum graveolens* has been reported to have a variety of biological effects including anti microbial, anti secretory, mucosal protective and hypolipidaemic activity. For the hypolipidaemic effects, scientific data on its efficacy is scarce. In the present study, we examined whether the AGE is capable to improve the lipoprotein profile in rats. It has been reported that hyperlipidaemia (increased level of TG, TC and LDL-C) is an important risk factor for development and progression of CHD (24-26). In this study, administration of AGE to hyperlipidaemic rats significantly lowered serum TG, TC and LDL-C levels. Epidemiological studies have also shown that high HDL-C levels could potentially contribute to anti-atherogenesis, including inhibition of LDL-oxidation to protect the endothelial cells from the cytotoxic effects of oxidized LDL (27, 28). Our results clearly showed that AGE is capable of increasing the serum level of good cholesterol (i.e., HDL-C) in the treated rats relative to group II rats. In addition, LDL/HDL ratio has direct correlation with the incidence of cardiovascular diseases. A logical therapeutic strategy to prevent or treat atherosclerosis and reduce the incidence of CHD events is to target the hyperlipidaemia by diet/or lipid-lowering drugs. Phytochemicals, especially the phenolic compounds and flavonoids of fruits and vegetables, have been proposed as the major bioactive compounds which provide the health benefits in diets which are rich in plant-foods. Several clinical trials have documented beneficial modifications of the LDL/HDL ratio after intake of flavonoid-containing food products. Weggemans and Trautwein (29) have reported that flavonoids intake decreased LDL-C and increased HDL-C in hypercholesterolaemic individuals. Independent studies have confirmed the presence of phenolic compounds mainly flavonoids in *A. graveolens* (14-17). Considering these facts, it may be possible that these active principles are responsible for lowering TC and LDL-C and elevating HDL-C in group III rats. The possible underlying mechanism by which AGE can exert its lipid lowering activities is not completely elucidated. At the moment, several fundamental mechanisms have been proposed. A decrease in cholesterol absorption from the intestine, through binding to bile acids and an increase in faecales bile acids excretion, has been considered as the mechanism of action of *Momordica charantia* (30). The same mechanism might be behind the mode of action of *A. graveolens* leaves extract in decreasing the TC level among the treated rats. In addition, the observed hypotriglyceridemic effect might be due to a decrease in fatty acids synthesis (31), enhanced LDL receptors, activation of LCAT and lipases (32) and also inhibition of acetyl-CoA carboxylase (33). On the other hand, *A. graveolens* leaves extract may improves hypercholesterolemia by modification of lipoprotein metabolism mainly through enhancement of the LDL receptors and therefore, increase in the uptake of LDL (34).

Another mechanism involved in lowering of TC might be related to the suppression of cholesterol biosynthesis by decrease in the HMG-CoA reductase activity which is the rate-limiting enzyme in the cholesterol biosynthetic pathway. In the present study, HMG-CoA reductase activity was indirectly measured in terms of the ratio of HMG-CoA to mevalonate (23). The ratio is inversely proportional to HMG-CoA reductase activity, meaning that an increase in the ratio indicates a decrease in the enzyme activity. The rats in group II showed a slight increase in HMG-CoA/mevalonate ratio as compared to rats in group I. This is mainly due to inhibition of reductase activity by the exogenous cholesterol in the diet. However, the AGE-treated rats showed further decrease in the HMG-CoA reductase activity, thus suggesting a possible interaction with the enzyme resulting in lower TC levels. These properties might be attributed to flavonoid content of the AGE. Such an effect has been demonstrated by flavonoids isolated from *E. officinalis* in hypercholesterolaemic rats (35). Further elaborated work is in progress to prove this claim.

Serum transaminases (AST and ALT) and alkaline phosphatase (ALP) have long been considered as sensitive indicator of hepatic injury (36). Injury to the hepatocytes alters their transport functions and membrane permeability, leading to the leakage of enzymes from their cells (37). This leakage causes an increase in levels of serum ALT, AST and ALP (38). Which may explains the increase in levels of SALT, SAST and SALP observed among Group II rats of this study. Treatment with AGE (Group III rats)
appears to protect animals against hepatic injury due to high-fat diet as suggested by the near-normal levels of SALT, SAST and SALP among the rats of this Group.

On the basis of results, it is concluded that AGE possesses hypolipidaemic and hypo-cholesterolemic activities which is probably mediated through suppression of HMG-CoA reductase activity. The observed properties apparently validate the folk medicinal use of this herb. Further scientific efforts are certainly required to establish the exact mechanism of action using the purified active component(s) of the AGE.

ACKNOWLEDGMENT

The authors appreciate the Research Council of University of Tehran and Iran National Science Foundation for the joint financial support of this investigation.

REFERENCES


کارگاه‌های آموزشی مرکز اطلاعات علمی

آموزش مهارت‌های کاربردی ISI در تدوین و چاپ مقالات

روش تحقیق کمی

آموزش نرم‌افزار برای پژوهشگران