The effect of hydro alcoholic extract of Origanum vulgare on weight and serum lipid profile in male Wistar rats

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Abstract
Introduction:
Today, hyperlipidemia, followed by atherosclerosis, leads to cardiovascular diseases and even death in most countries. Antioxidants can reduce blood cholesterol and risks of cardiovascular diseases. Given that Origanum vulgare is full of antioxidants, we aimed to study the effects of Origanum vulgare extract (OVE) on serum lipid profile and weight.

Methods and Materials:
In this study, 28 male Wistar rats were divided into four groups (n=7). The control group received distilled water, three experimental groups received the hydroalcoholic OVE at different doses of 50, 75 and 100 mg/kg, respectively. Pretreatment with OVE was performed for 30 days orally through gavage. Two hours after the last gavage, the rats were weighed, then blood samples were collected to measure serum levels of cholesterol, triglyceride, low density lipoprotein (LDL) and high density lipoprotein (HDL). The data were analyzed using one-way ANOVA in SPSS.

Results:
The results of the current study showed that OVE reduced the levels of cholesterol, triglycerides, low density lipoprotein and increased the level of high density lipoprotein in the three groups receiving OVE (50, 75 and 100 mg/kg) compared to the control group. OVE did not cause changes in rats’ weight.

Conclusion:
It appears that OVE can exert a protective effect against cardiovascular diseases by reducing the levels of cholesterol, triglyceride, low density lipoprotein and increasing high density lipoprotein.

Keywords: Origanum, Cholesterol, Triglycerides, HDL, LDL

Introduction
Today, hyperlipidemia followed by atherosclerosis leads to cardiovascular diseases and even death in most countries. The relationship between high cholesterol levels and cardiovascular diseases was first revealed in 1984. Increased levels of low-density lipoprotein (LDL) has acute and chronic effects on the vessels (1). As one of the most common diseases of the present century, atherosclerosis leads to ischemia and finally myocardial infarction and brain stroke. Women are less at risk of developing the disease because of having less estrogen (2). The liver produces triglyceride from the fatty acids that it has synthesized or fatty acids obtained from the blood to place them in very-low-density lipoproteins (VLDL). LDL is the most
important cholesterol carrier in the blood and transfers cholesterol from the liver to the peripheral tissues. After leaving the liver, LDL goes to peripheral tissues and enters the cells through endocytosis and delivers its contents to the cells. Cholesterols transferred to tissues through LDL are cell’s general storage. High-density lipoprotein (HDL) carries cholesterol from tissues to the center (3). The presence of high levels of LDL and low levels of HDL accelerates atherosclerosis (4).

Free radicals are reactive species that are naturally produced in metabolic reactions and are removed by antioxidant defense system (5). An imbalance between the production of free radicals and antioxidant defense system causes oxidative stress (6). Free radicals cause oxidation of macromolecules such as lipids and proteins and damage vessel walls and increase the risk of cardiovascular diseases. Antioxidants can reduce the risk of atherosclerosis by removing free radicals (5).

Factors such as patient dissatisfaction with lipid-lowering medications, side effects caused by long-term and excessive consumption of these medications, diseases caused by hyperlipidemia and expenses imposed on patients have grown a tendency toward alternative and traditional treatments (7). Marjoram (Origanum vulgare) from Lamiacea (Labiatae) family has a world-wide distribution. This plant is more available in the north and northwest of Iran and is not found in warm regions of the south (8). Marjoram is also called Varzanjoosh Vahshi and Avihan Koohi in Persian (9).

This plant is used in Iranian traditional medicine as an antitussive, anti-inflammatory, analgesic, tonic, diaphoretic agent and a soother of the nervous system. It is also used for the treatment of diseases such as dysmenorrhea, rheumatoid arthritis, kidney diseases and impaired digestion (9). Antidiabetic (10), anxiolytic (11), and antioxidant (12) effects of marjoram are also proved. The most important compounds found in the extract of marjoram are phenolic monoterpenes thymol (35%) and carvacrol (32%). Phenolic thymol monoterpenes are known to cross the blood–brain barrier and exert antioxidant effects dose-dependently (12). Its other important compounds are rosmarinic acid, ericitrin, apigenin glucoside, and origanol that have significant antioxidant effects (9). There are other flavonol and flavonoid compounds, in addition to rosmarinic acid and ursolic acid, that have introduced marjoram as an effective antioxidant (13). Thymol, carvacrol, p-cymene and borneol inhibit the acetylcholinesterase enzyme dose-dependently (14). Ursolic acid is effective in inhibiting the blood hemolytic factors and has anti-cancer and anti-inflammatory effects (15). Oral administration of thymol reduced superoxide dismutase and glutathione peroxidase concentrations in rats (16). Due to its broad antioxidant activities, marjoram appears to be able to reduce free radicals and lipid oxidation. Therefore, the present study was conducted to investigate the effect of marjoram hydroalcoholic extract on body weight, serum lipoproteins, and blood cholesterol and triglycerides.

Materials and Methods
This experimental study was carried out at Biology Research Center of Islamic Azad University of Zanjan in winter 2015. The subject of the study was approved by the Ethics Committee of Zanjan Islamic Azad University and registered under No. iaуз.REC.1393.83. A total number of 28 male Wistar rats, 8-10 month-old and 250-300 grams were procured from the Pasteur Institute of Iran and housed in suitable cages in Biology Research Center’s animal room at a temperature of 22±2 °C and 12 h light/dark cycles. The animals had free access to food and water. All ethical principles regarding laboratory animal care and use were respected. The animals were randomly divided into four groups, each
consisting of seven male rats. The control group received a regular diet and distilled water through gavage. The intervention groups received a regular diet with oral marjoram extract in doses of 50, 75 and 100 mg/kg for 30 days through gavage every day 10-11 AM. Doses were selected based on previous studies (17).

**Extraction:**
Marjoram was collected from pastures of Khodabande in Zanjan, Iran, and confirmed in terms of taxonomy by Department of Botany and Herbarium of Islamic Azad University of Zanjan. The collected plants were dried in the shade and then powdered. Extraction was done by soaking (18). To this end, 100 g of the powder was mixed with alcohol 70% in a flask, such that the solvent covered 2 cm above the powder. The flask opening was covered with aluminum foil and kept for 24 hours. During the 24 hours, the content of the flask was mixed with a glass stirrer every two hours. The mixture was then incubated at 50 °C in a rotary device to separate the solvent. After concentrating, the extract was put in an oven at 50 °C to obtain extract powder. Then, 50, 75 and 100 mg/kg doses were prepared from the extract at the time of gavage.

**Serum lipids and lipoproteins measurement:**
On day 30, two hours after the final gavage, animals were weighed and anesthetized by 400 mg/kg of chloral hydrate and blood samples were taken. Serums were extracted after centrifugation at 10000 rpm (g=17888) for 10 minutes and then frozen at -20 °C. Then the LDL, HDL, triglycerides and cholesterol were measured by MINDRIW-BS800 Autoanalyzer (19).

**Weighing animals:**
After each gavage with marjoram hydroalcoholic extract, the animals were weighed to determine the effect of the extract on their weight.

**Statistical analysis:**
All statistical analyses were performed using SPSS version 18.0. Cholesterol, triglycerides, LDL, HDL and body weight of the rats were evaluated using one-way ANOVA and Tukey post hoc test. The value of p<0.05 was considered statistically significant.

**Results**
Pre-feeding with marjoram extract significantly decreased the serum levels of triglycerides, LDL, and cholesterol, and significantly increased the serum levels of HDL. The significant difference between the group receiving 100 mg/kg of the extract with the groups receiving 50 and 75 mg/kg was dose-dependent, as no significant differences were observed in rats’ body weight before and after treatment with the extract compared to the control group.

The results showed that pre-feeding with marjoram extract for 30 days significantly decreased serum cholesterol at doses of 50, 75 and 100 mg/kg compared to the control group (p<0.001) (Table 1). Also, the serum triglycerides in each of the three doses of 50 mg/kg (p=0.027), 75 mg/kg and 100 mg/kg (p<0.001) significantly decreased after 30 days compared to the control group (Table 1). LDL serum levels in all three groups receiving the extract with doses of 50 mg/kg (p=0.016), 75 and 100 mg/kg (p<0.001) significantly decreased compared to the control group (Table 1). Pre-feeding with marjoram hydroalcoholic extract significantly increased the serum levels of HDL in all three doses of 50 mg/kg (p=0.016), 75 and 100 mg/kg (p<0.001) compared to the control group (Table 1). The body weight of the rats in the groups receiving 50, 75 and 100 mg/kg of marjoram hydroalcoholic extract was not significantly different compared to the weight of the control group before or after treatment. The body weight of the rats in the
groups receiving 50, 75 and 100 mg/kg of marjoram hydroalcoholic extract and the control group was not significantly different before and after treatment.

Table 1: A comparison of cholesterol, triglycerides, LDL and HDL amounts

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>50 mg/kg dose</th>
<th>75 mg/kg dose</th>
<th>100 mg/kg dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol *</td>
<td>94.86±1.56</td>
<td>83.14±2.03</td>
<td>51.00±1.69</td>
<td>43.86±1.45</td>
</tr>
<tr>
<td>Triglyceride *</td>
<td>76.00±1.31</td>
<td>70.71±1.30</td>
<td>60.00±1.04</td>
<td>42.00±1.23</td>
</tr>
<tr>
<td>LDL *</td>
<td>58.71±1.15</td>
<td>52.14±1.59</td>
<td>38.57±1.43</td>
<td>29.14±1.47</td>
</tr>
<tr>
<td>HDL *</td>
<td>19.43±1.06</td>
<td>25.86±1.28</td>
<td>36.57±1.70</td>
<td>46.14±1.10</td>
</tr>
</tbody>
</table>

* Significant difference compared to the control group (p<0.05)

Table 2. Rat’s body weight the weight of rats before and after treatment with the extract (no significant difference to the control group)

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>50mg/kg</th>
<th>75mg/kg</th>
<th>100mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before treatment</td>
<td>271.77±4.01</td>
<td>272.08±3.85</td>
<td>273.34±3.98</td>
<td>274.02±3.80</td>
</tr>
<tr>
<td>After treatment</td>
<td>281.71±3.80</td>
<td>282.74±3.56</td>
<td>284.79±3.81</td>
<td>284.69±3.89</td>
</tr>
</tbody>
</table>

Discussion

Pre-feeding with Marjoram hydroalcoholic extract in doses of 50, 75 and 100 mg/kg, decreased the cholesterol, triglycerides, and LDL in blood and increased HDL serum levels, while it had no effect on body weight. Marjoram mechanism might be explained by the presence of flavonoids (13), as in wild celery (Kelussia odoratissima) which exerts lipid-lowering effects by reducing the activity of 3-hydroxy-3-methylglutaryl-CoA enzyme and increasing the liver receptors (20). Asgari et al. showed that oral administration of amaranthus reduced triglycerides, cholesterol, and LDL, and increased HDL. They attributed the hypolipidemic effect of the plant to its lipid peroxidation lowering properties and scavenging oxygen free radicals (21), which concurred the present study. Brown et al. reviewed 67 clinical trials in a meta-analysis and showed that soluble fiber reduced total cholesterol and LDL dose-dependently (22). ElRokh et al. also showed that ginger extract decreased the amount of cholesterol, triglycerides, and LDL. They attributed these effects to the presence of polyphenols and flavonoids that have antioxidant properties (23), which is consistent with results of the present study.

Kazemi et al. also found that marjoram has androgenic effects at high doses (40 mg/kg) and strengthens the hypothalamic-pituitary-gonadal axis. The researchers attributed these effects to the plant’s antioxidant compounds (24). Marjoram has also been proven to reduce anxiety and exert analgesia in rats due to its flavonoids and alkaloids (11). A study proved that marjoram prevents LDL oxidation, attributed to the high levels of polyphenols (flavonoids and rosmarinic acid) (25). Antioxidants lower serum lipids and lipoproteins via cholesterol biosynthesis inhibition. Cholesterol is regulated at the beginning of its pathway, i.e. at HMG-CoA (Hydroxymethyl glutathione, coenzyme A) phase. The main phase of cholesterol biosynthesis is where hydroxymethyl glutathione coenzyme A converts to mevalonate influenced by hydroxymethyl glutathione coenzyme A reductase and Nicotinamide Adenine Dinucleotide Phosphate Hydrogen (NADPH), where cholesterol-lowering drugs act (26). Marjoram has powerful antioxidant properties due to its phenolic compounds such as rosmarinic acid, caffeic acid and Protocatechuic acid and the corresponding glycosides (27). The total phenols and flavonoids are also abundant in marjoram.
Flavonoids have antimicrobial, anti-inflammatory, anti-fever and antioxidant properties. Flavonoid intake reduces the risk of cardiovascular diseases (27). Phenolic compounds with high molecular weight such as tannin, have a great ability to remove free radicals and have an antioxidant activity like Trolox, which depends on the number of aromatic rings and nature of mobile hydroxyl groups. Since marjoram has high molecular weight phenolic compounds, it has unique antioxidant activities (27).

**Conclusion**

Marjoram appears to have protective effects against cardiovascular diseases by decreasing cholesterol, triglycerides, and LDL and increasing HDL. Due to the proven antioxidant properties of marjoram, its extract might also decrease cholesterol, triglycerides, and LDL, and increase HDL for the same reason. More extensive studies are needed to confirm these effects. It is suggested that future studies examine marjoram therapeutic effects on rats with a high-cholesterol diet. The extraction of antioxidant compounds of the plant and the examination of their effect on lipid profile individually might reveal the exact mechanism of the effect of this herb on blood lipids.

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**Conflict of interest**

The Authors declare that there is no conflict of interest in this paper.

**References:**