Effects of *Urtica dioica* extract on lipid profile in hypercholesterolemic rats

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Objective: To investigate the effects of extract of *Urtica dioica*, a perennial herb in Iran, on lipid profile in hypercholesterolemic rats.

Methods: The effects of *Urtica dioica* extract were tested by using it as a supplement in a high-cholesterol diet. Male rats were fed a high cholesterol diet (10 mL/kg) for 4 weeks with *Urtica dioica* extract (100 or 300 mg/kg) or 10 mg/kg lovastatin supplementation to study the hypocholesterolemic effects of *Urtica dioica* on plasma lipid levels, hepatic enzymes activities, and liver histopathological changes.

Results: *Urtica dioica* extract at 100 and 300 mg/kg significantly reduced the levels of total cholesterol (TC), and low-density lipoprotein-cholesterol (LDL-C) and also markedly decreased liver enzymes and weight in animals with a high cholesterol diet. Hematoxylin and eosin staining showed that in the 100 mg/kg extract of *Urtica dioica* group, the appearance of the liver cells was similar to the control group, and steatosis and inflammation were not found. In the 300 mg/kg extract of *Urtica dioica* group, mild steatosis was observed but mononuclear inflammatory infiltration was not found.

Conclusion: The hepatic histopathological results reflect the correlation of *Urtica dioica* extract with both liver weight and the levels of plasma TC and LDL-C. These results indicate that *Urtica dioica* extract has hypocholesterolemic effects in the animal model.

Keywords: alcoholic extract, *Urtica dioica*, hypercholesterolemia, rats

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大荨麻提取物对高胆固醇血症大鼠血脂的影响

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目的：探讨大荨麻提取物对高胆固醇血症大鼠血脂的影响。

方法：雄性大鼠喂服高胆固醇饲料（10 mL/kg）4周，然后高胆固醇血症大鼠模型。同时给高胆固醇饲料中的高胆固醇饲料或10 mg/kg大荨麻提取物或10 mg/kg洛伐他汀。观察大荨麻提取物对大鼠血脂、肝功能和肝脏病理的影响。

结果：100、300 mg/kg大荨麻提取物可以显著降低高胆固醇血症大鼠总胆固醇、低密度脂蛋白胆固醇、高密度脂蛋白胆固醇，并可以降低丙氨酸氨基转移酶（alanine aminotransferase, ALT）和天冬氨酸氨基转移酶（aspartate aminotransferase, AST）活性。苏木精和伊红染色显示，100 mg/kg大荨麻提取物组肝脏组织未见明显脂肪变

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Lipid levels are a metabolic risk factor for cardiovascular disease (CVD) and abnormalities in plasma lipoprotein classes, and deregements in lipid metabolism rank among the most firmly-established and best-understood risk factors for atherosclerosis[1]. Plasma cholesterol levels are regulated by the absorption of dietary cholesterol, excretion of cholesterol via fecal sterols or bile acids, cholesterol biosynthesis, and removal of cholesterol from circulation. Numerous previous studies have reported on the beneficial effects of hepatic 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase and acyl-coenzyme A: cholesterol acyltransferase (ACAT) inhibitors on hypercholesterolemia and atherosclerosis[2-17]. Low-density lipoprotein (LDL) transfers cholesterol from liver to peripheral tissues, whereas high-density lipoprotein (HDL) facilitates the translocation of cholesterol from the peripheral tissues to the liver for catabolism. Therefore, HDL has a useful effect in reducing tissue cholesterol and an elevated ratio of serum total cholesterol to HDL cholesterol (HDL-C) is suggested with a decreased level of LDL cholesterol (LDL-C) to reduce the risk of cardiovascular diseases[14]. In recent decades, it was shown that a high cholesterol level and a high ratio of saturated and monounsaturated to polyunsaturated fatty acids in the blood predisposes patients to vascular diseases, whereas a high dietary intake of vegetables and fruits has the opposite effect[2-17].

_Urtica dioica_ is a common herb in most region of the world. It is used to treat rheumatic pain, urinary tract infections and bladder stone[23]. It has several pharmacological properties such as anti-inflammatory, antimicrobial, antioxidative activities and hepatoprotective and cardioprotective effects[3-12]. In previous studies, it has shown that _Urtica dioica_ decreased the lipid peroxidation and liver enzymes, and increased the antioxidant defense system activity in the carbon tetrachloride-treated rats[14, 15].

Thus, in this study we examined the hypocholesterolemic effects of chronic administration of alcoholic extract of _Urtica dioica_ in rats. It was predicted that it would show hypocholesterolemic effects on the lipid profile and liver tissue due to its properties.

1 Materials and methods

1.1 Materials

1.1.1 Animal Fifty male Wistar rats (250 to 300 g) were obtained from the Razi Institute (Karaj, Iran) and housed in groups of four per cage under standard laboratory conditions. They were kept at constant room temperature (21±2°C) under a normal 12-h light/12-h dark cycle with free access to food and water. All animal experiments were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) to minimize their suffering.

1.1.2 Chemicals Cholic acid was purchased from Merck. The other drugs used in this study were lovastatin (Tehran Chemie Pharmaceutical Co., Tehran, Iran), propylthiouracil (Iran Hormone, Tehran, Iran), ketamine (Rotexmedica, GmbH, Germany), and xylazine (Loughrea Co., Galway, Ireland).

1.1.3 Preparation of extract _Urtica dioica_ was collected from Qazvin and authenticated by Qazvin Agriculture and National Resources Research Center, Iran (Voucher No. 957). Leaves were dried in the shade and followed by grinding into fine powder. Then, the powder was extracted by using maceration with ethanol. In the maceration method, 100 g of the powder was macerated in 1 L ethanol (volume fraction is 70%) for 3 days and subsequently, the solution was filtered and concentrated in a rotary evaporator at 50°C. The yield of the extract was 10% in weight fraction.

1.2 Experimental methods

1.2.1 Rat model of hypercholesterolemia In rats, hypercholesterolemia was induced by daily gavage administration of 10 mL/kg body weight (BW) of a cocktail containing in 1 L peanut oil, 100 g cholesterol, 30 g propylthiouracil, and 100 g cholic acid over a period of 28 days. The test compounds were administered simultaneously with the cocktail[14].

The rats were divided into five groups of ten animals each. Rats in control group received peanut oil orally (10 mL/kg) which served as control. Rats in untreated group were given the cocktail only as described above. Rats in lovastatin group were administered cocktail plus lovastatin (10 mg/kg). While rats in _Urtica dioica_ extract groups were administered cocktail plus 100 mg/kg _Urtica dioica_ extract or cocktail plus 300 mg/kg _Urtica dioica_ extract.

1.2.2 Laboratory testing The lipid profile was analyzed by using commercially available kits for HDL-C, LDL-C, total cholesterol (TC), and triglycerides (TG) (Pars Azmoon, Iran) and expressed in mmol/L. Plasma alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were analyzed by using biochemical analysis kits (Pars Azmoon, Iran) and expressed in U/L.

Twenty-four hours after the last gavages, rats were anesthetized with an intraperitoneal injection of ketamine (60 mg/kg) and xylazine (6 mg/kg). Blood samples were taken from left ventricle of the
heart and collected into test tubes to obtain plasma. Samples were subsequently centrifuged for 10 min (4 000 × g). Plasma was separated and used for the assessment of TC, HDL-C, LDL-C, TG levels and AST and ALT activities.

1.2.3 Liver histopathological assessment The livers were removed, weighed, and then cut into small pieces. Liver weight (LW) as well as BW was recorded. Tissue weights were normalized to whole body weight.

Liver sections were fixed in 10% formaldehyde, dehydrated in gradual ethanol (50% to 100%), cleared in xylene and embedded in paraffin. Sections (4 to 5 μm thick) were prepared and stained with hematoxylin and eosin (HE) dye for histopathological examination and observed under a microscope at a magnification of 100×.

1.3 Statistical analysis The data were expressed as mean ± standard deviation (SD) and were compared using one-way analysis of variance (ANOVA) followed by multiple comparison test of Tukey-Kramer. Results with P < 0.05 were considered significant.

2 Results

2.1 Effects of alcoholic extract of *Urtica dioica* on plasma lipid profile Feeding a high cholesterol diet in rats in the untreated group for 28 days resulted in hypercholesterolemia, as evidenced by the plasma TC and LDL-C levels, which were higher than those in the control group (P < 0.01, Table 1). The levels of TC in the *Urtica dioica* extract groups at 100 and 300 mg/kg and the lovastatin group were reduced by 22.9%, 11.4%, and 14.6%, respectively as compared with the untreated group. However, the differences between these groups in comparison with the control group were significant (P < 0.01).

LDL-C levels in the *Urtica dioica* extract groups at 100 and 300 mg/kg and the lovastatin group decreased by 21.8%, 12.5%, and 9.4% respectively as compared with the untreated group (Table 1). The differences between the *Urtica dioica* extract groups, and the lovastatin group in comparison with the control group were significant (P < 0.01) (Table 1). The differences between all groups were not significant in regards to HDL-C and TG levels.

2.2 Effects of alcoholic extract of *Urtica dioica* on plasma AST and ALT activities The measured plasma AST and ALT activities in rats that consumed the *Urtica dioica* extract-supplemented high cholesterol diet and lovastatin are shown in Table 2. The differences of plasma AST and ALT activities between the untreated group and the control group were significant (P < 0.01). Plasma AST in the *Urtica dioica* groups at 100 and 300 mg/kg were reduced as compared with the untreated group by 85% and 70.4% respectively. The difference between these groups in comparison with control was not significant (Table 2). Plasma ALT activity in the lovastatin group was reduced by 76.4% as compared with the untreated group. The difference between the lovastatin group and the control group was not significant (Table 2).

Moreover, *Urtica dioica* extract at doses of 100 and 300 mg/kg had a decreased plasma ALT activity as compared with the untreated group by 50.3% and 41%, respectively. Also, the difference between these groups in comparison with control was not significant (Table 2). Plasma ALT activity was reduced in the lovastatin group by 48% as compared with the untreated group. Moreover, the difference between this group and the control group was not significant (Table 2).

### Table 1: Effects of alcoholic extract of *Urtica dioica* on plasma lipid profile in hypercholesterolemic rats

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>TC (μmol/L)</th>
<th>LDL-C (μmol/L)</th>
<th>TG (μmol/L)</th>
<th>HDL-C (μmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10</td>
<td>3.70 ± 0.10</td>
<td>1.85 ± 0.10</td>
<td>2.05 ± 0.20</td>
<td>1.30 ± 0.10</td>
</tr>
<tr>
<td>Untreated</td>
<td>10</td>
<td>2.65 ± 0.10 **</td>
<td>3.25 ± 0.10 **</td>
<td>2.60 ± 0.50</td>
<td>1.25 ± 0.05</td>
</tr>
<tr>
<td>Lovastatin (10 mg/kg)</td>
<td>10</td>
<td>2.55 ± 0.05 ** △</td>
<td>2.94 ± 0.07 ** △</td>
<td>2.30 ± 0.30</td>
<td>1.03 ± 0.04</td>
</tr>
<tr>
<td>Extract of <em>Urtica dioica</em> (100 mg/kg)</td>
<td>10</td>
<td>7.48 ± 0.50 ** △△</td>
<td>2.58 ± 0.05 ** △△</td>
<td>2.21 ± 0.05</td>
<td>1.10 ± 0.10</td>
</tr>
<tr>
<td>Extract of <em>Urtica dioica</em> (300 mg/kg)</td>
<td>10</td>
<td>8.52 ± 0.02 ** △△</td>
<td>2.84 ± 0.01 ** △△</td>
<td>2.60 ± 0.05</td>
<td>1.20 ± 0.10</td>
</tr>
</tbody>
</table>

** P < 0.01, vs control group, △ P < 0.05, △△ P < 0.01, vs untreated group.

### Table 2: Effects of alcoholic extract of *Urtica dioica* on plasma AST and ALT activities and LW/BW ratio in hypercholesterolemic rats

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>AST (U/L)</th>
<th>ALT (U/L)</th>
<th>LW/BW ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10</td>
<td>55 ± 30</td>
<td>94 ± 6</td>
<td>0.040 ± 0.008</td>
</tr>
<tr>
<td>Untreated</td>
<td>10</td>
<td>248 ± 39 **</td>
<td>173 ± 30 **</td>
<td>0.051 ± 0.004 9 **</td>
</tr>
<tr>
<td>Lovastatin (10 mg/kg)</td>
<td>10</td>
<td>59 ± 10</td>
<td>90 ± 7</td>
<td>0.050 ± 0.003 5 **</td>
</tr>
<tr>
<td>Extract of <em>Urtica dioica</em> (100 mg/kg)</td>
<td>10</td>
<td>12 ± 10</td>
<td>85 ± 19</td>
<td>0.057 ± 0.001 5</td>
</tr>
<tr>
<td>Extract of <em>Urtica dioica</em> (300 mg/kg)</td>
<td>10</td>
<td>74 ± 9</td>
<td>102 ± 4</td>
<td>0.065 ± 0.002 4</td>
</tr>
</tbody>
</table>

** P < 0.01, vs control group.
2.3 Effect of alcoholic extract of *Urtica dioica* on LW/BW ratio  The ratios of LW to BW in the untreated and lovastatin groups were significantly higher than that in the control group (P < 0.01) (Table 2). Moreover, there were no differences in LW/BW ratios between the *Urtica dioica* groups (100, 300 mg/kg) and the control group (Table 2).

2.4 Effects of alcoholic extract of *Urtica dioica* on histopathological changes  In the control group, a normal lobular pattern, hepatic cells with well-preserved cytoplasm, a prominent nucleus, and a well-visualized central vein were observed in liver sections (Figure 1A). In the untreated group, microvesicular fatty changes, small-droplet fat in hepatocytes with centrally located nuclei, mononuclear inflammatory infiltration, and venous congestion were observed (Figure 1B). In the lovastatin group, small-droplet fat in hepatocytes of zone III and mononuclear inflammatory infiltration were observed (Figure 1C). In the 100 mg/kg extract of *Urtica dioica* group, the appearance of the cells was similar to the control group. Steatosis and inflammation were not found (Figure 1D). In the 300 mg/kg extract of *Urtica dioica* group, mild steatosis was observed but mononuclear inflammatory infiltration was not found (Figure 1E).

![Figure 1](HE stained section of the rat liver (Light microscopy, ×100)
A: Control group; B: Untreated group; C: Lovastatin (10 mg/kg) group; D: Extract of *Urtica dioica* (100 mg/kg) group; E: Extract of *Urtica dioica* (300 mg/kg) group.

3 Discussion

Rats are generally considered to be resistant to naturally occurring and experimentally induced atherosclerosis[10]. High-dose of dietary cholesterol combined with bile acids and experimentally induced hypothyroidism have been demonstrated to lead to the development of atherosclerotic lesions in rats[17]. Therefore, in the current study hypercholesterolemia was induced in rats, as was evidenced by the total cholesterol level in plasma after feeding the animals a high-cholesterol diet. After the model was established, the possible hypercholesterolemic effects of chronic administration of *Urtica dioica* extract were examined in rats. In this context, the plasma lipid profile, liver enzymes, and tissues were investigated.

According to the results, the effects of *Urtica dioica* extract at 100 and 300 mg/kg, and lovastatin at 10 mg/kg in reducing plasma TC and LDL-C were statistically significant as compared with the untreated group. However, it seems that the effects of *Urtica dioica* extract at 100 mg/kg were greater than administration of *Urtica dioica* extract at 300 mg/kg.

This result is similar to previous finding by Avci et al[18]. They found that ethanol extract of *Urtica dioica* at a dose of 100 mg/kg greatly reduced TC and LDL-C as compared with aqueous extract. In addition, Daher et al[19] found that aqueous extract of *Urtica dioica* (150 mg/kg) could reduce TC and LDL/HDL cholesterol ratio. It was suggested that the extract may have a direct role in lipoprotein synthesis and metabolism.

A hypercholesterolemic diet caused an increase in oxidative stress in the liver, resulted in an increase in AST and ALT activities, and induced fatty liver[20, 21]. Therefore, in the present study, ALT and AST, apart from cholesterol, were also evaluated in order to reveal the protective effects of *Urtica dioica* extract on hepatic enzymes. As a result, it seems that supplementing high diet cholesterol with 100 and 300 mg/kg *Urtica dioica* extract or lovastatin could decrease AST and ALT activities. As the LW/BW ratio indicates, it is possible that animals that received *Urtica dioica* extract at 100 mg/kg or 300 mg/kg with their hypercholesterolemic diets have less fat accumulation in the liver.
Meguro et al. has explained several mechanisms about the cholesterol-lowering activity of plant sterols. It was reported that plant sterols which are structurally similar to cholesterol could displace cholesterol from mixed micelles, since they are more hydrophobic than cholesterol. This replacement causes a reduction in micellar cholesterol concentration and consequently lowers cholesterol absorption. Thus, it is possible that Urtica dioica extract could decrease the storage of cholesterol through this mechanism.

Furthermore, the other possibility for the hepatoprotective effects of Urtica dioica extract may be related to antioxidant activity to prevent liver injury. Another possible explanation for the decreased liver damage and lower liver enzymes for the Urtica dioica extract (100 mg/kg) with a high cholesterol diet in animals depends on the anti-inflammator effects of it, as anti-inflammatory effects of Urtica dioica extract have been reported in several studies. However, further studies are needed to establish the exact mechanism.

Statins also have an anti-inflammatory effect that is independent of a change in cholesterol level.

In conclusion, our findings indicate that Urtica dioica extract at doses of 100 and 300 mg/kg lowered liver weight as well as plasma TC and LDL contents and also liver enzymes activities in animals with a high cholesterol diet. The hepatic histopathological results reflect a correlation of Urtica dioica extract (100 and 300 mg/kg) with both liver weight and the levels of plasma TC and LDL-C. However, histopathological results showed that liver injury in rats given 100 mg/kg Urtica dioica extract was less than in rats given 300 mg/kg Urtica dioica extract. Our data clearly indicate the anti-hypercholesterolemic effects of Urtica dioica extract in an animal model.

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