



ORIGINAL ARTICLE

# The effect of aqueous extract of *Lavandula officinalis* to reduce cholesterol, triglyceride and other lipid metabolites on female BALB/c mice



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## KEYWORDS

*Lavandula officinalis*;  
Cholesterol;  
LDL;  
Lavender;  
Triglyceride

## Abstract

**Background:** Hyperlipidemia is a prevalent disorder and a main component of the metabolic syndrome resulting from various factors. The aerial parts and flowers of *Lavandula officinalis* possesses antioxidant activity, therefore, in this study; the effects of *L. officinalis* extract were investigated on serum lipid levels of mice.

**Methods:** Experimental mature female BALB/c mice were treated with 100, 300 or 500 mg/kg/day of lavender aqueous extract or distilled water for 15 days via intraperitoneally injections. At the end of 15th day, the serum biochemical parameters include cholesterol, triglyceride, HDL and LDL levels as well as the liver cell function test were determined.

**Results:** The aqueous extract of lavender significantly decreased serum cholesterol and LDL levels. Serum cholesterol level was lower in the 300 and 500 mg/kg/day experimental groups when compared with the control group. In liver histology evaluation, fat accumulation was not observed in the experimental group, which treated with high-fat foods and receiving high doses of extract.

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**PALABRAS CLAVE**

*Lavandula officinalis*;  
 Colesterol;  
 LDL;  
 Lavanda;  
 Triglicéridos

**Conclusion:** *L. officinalis* extract exerts a hypolipidemic effect in studied groups, however, further phytochemical and biological tests are suggested to determine the active chemical constituent responsible for these activities.

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### Efecto del extracto acuoso de *Lavandula officinalis* en la reducción de colesterol, triglicéridos y otros metabolitos lipídicos en los ratones BALB/c hembra

**Resumen**

**Antecedentes:** La hiperlipidemia es un trastorno prevalente y un componente principal del síndrome metabólico originada por diversos factores. Las partes aéreas y flores de la *Lavandula officinalis* tienen una actividad antioxidante y, por tanto, investigamos en este estudio los efectos de su extracto en los niveles lipídicos séricos en ratones.

**Métodos:** Se trataron ratones BALB/c hembra maduras experimentales con 100, 300 o 500 mg/kg/día de extracto acuoso de lavanda o agua destilada durante 15 días, mediante inyecciones intraperitoneales. Al finalizar el 15.º día se investigaron los parámetros bioquímicos séricos incluyendo colesterol, triglicéridos, niveles de HDL y LDL, así como la prueba de la función hepática.

**Resultados:** El extracto acuoso de lavanda disminuyó significativamente los niveles séricos de colesterol y LDL. El nivel de colesterol sérico fue inferior en los grupos experimentales de 300 y 500 mg/kg/día, en comparación con el grupo control. En la evaluación de la histología hepática no se observó acumulación de grasa en el grupo experimental, que fue tratado con alimentos de alto contenido en grasa y recibió altas dosis de extracto.

**Conclusión:** El extracto de *Lavandula officinalis* ejerce un efecto hipolipidémico en el grupo estudiado, aunque son precisas más pruebas fitoquímicas y biológicas para determinar el constituyente químico activo responsable de estas actividades.

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**Introduction**

Cardiovascular complications are considered as the main factors of morbidity and mortality. Actually, fatty acid composition of dietary influences on blood lipids and lipoproteins that are associated with the development of atherosclerosis and ischemic heart disease.<sup>1</sup> The formation of atherosclerotic plaque involves accumulation of low-density lipoprotein (LDL) in the intima, LDL oxidation, uptake of oxidized LDL by macrophage scavenger receptors, influence of macrophages on foam cells, and stabilization of plaque. When the blockage of the coronary arteries reaches more than 75%, usually the symptoms of angina will gradually appear.<sup>2</sup> Otherwise, the best treatment of diseases such as atherosclerosis is prevention. Therefore, conventional medical approaches generally focus on lifestyle changes, such as a reduction in the consumption of saturated fats, quitting smoking, and aerobic exercise. Drugs are also used to lower cholesterol levels or blood pressure; however, most of them possess considerable side effects.<sup>3</sup> Using alternative treatments, especially medicinal plants and their complements, to treat different diseases such as hyperlipidemia,<sup>4</sup> diabetes,<sup>5</sup> and cardiovascular diseases<sup>6,7</sup> has increased over the recent decades in the majority of countries worldwide. In fact, herbal medicines are preferred

over modern medicine due to their safety, efficacy, cultural acceptability, and lesser side effects.<sup>8,9</sup> Reducing the levels of cholesterol, triglyceride and other lipid metabolites with herbal remedies has proven effective at lowering the risk of heart disease. This success has inspired the development of new therapeutics that target other lipid fractions and further reduce cardiovascular risk.<sup>10</sup>

Lavender (*Lavandula officinalis*) is one of these plants which has been traditionally used for different diseases.<sup>11</sup> Lavender is indigenous to Southern Europe and is sometimes found growing wild in the Mediterranean area between the coast and the lower mountain slopes. Iran also is one of the richest countries of the world in terms of having a substantial number of different medicinal plant species include lavender grown in various ecological conditions. Leaves and flower of *L. officinalis* have the highest amount of essential oils.<sup>12</sup> Phytochemical studies revealed that linalool, linalyl acetate and some other monoterpene and sesquiterpenes, flavonoids like luteolin, triterpenoids like ursolic acid and coumarins like umbelliferone and coumarin were the main components of the aerial parts and flowers of *L. officinalis*, which might be effective on serum lipid levels.<sup>13</sup>

The aim of this experiment is to evaluate the effect of *L. officinalis* on the reduction of cholesterol and triglyceride and lipid metabolism in female mouse BALB/c.

## Materials and methods

This experimental study was conducted in the Karaj Branch of Islamic Azad University, Alborz, during 2016 and 2018. This research was approved by the ethical committee of regional Medical Research of Islamic Azad University. In addition, the study was done in accordance with good clinical practice and declaration of Helsinki guidelines. Throughout the experiments, the authors tried to follow all ethical principles of working on laboratory animals to impose the lowest possible stress on them.

### Extract preparation

Fresh leaves and flower of the *L. officinalis* cultivated in standard conditions were obtained from the center of planting ornamental plants, Alborz, Iran. For aqueous extraction, 10 g of air-dried and powdered plant was added to 100 ml of sterilized distilled water in a conical flask, plugged with cotton wool and kept for 24 hrs. Then the extract was filtered using Whatman filter paper No. 4 and centrifuged at  $10\,000 \times g$  for 20 min, the supernatant was collected. The procedure was repeated twice and the supernatant was evaporated till dryness using an oven at  $50^\circ\text{C}$  then stored at  $4^\circ\text{C}$  which used for further testing.

### Animals

A total of 72 female BALB/c mice weighing 18–21 g from Razi Institute of Iran, were housed in a temperature and light-controlled room ( $23 \pm 2^\circ\text{C}$ , a 12-h cycle starting at 08:00 a.m.) and were fed and allowed to drink water ad libitum. The mice were divided into 6 groups as follows: Group I was consisted of six control mice received normally water and food. Group II (control II) was consisted of six mice received high-fat diet (HFD) and water. Group III includes six mice was Sham-operated group which were managed according to the group II, but distilled water was injected intraperitoneally. Group IV includes 18 mice was the first experimental group received low-dose lavender extract with a dose of 100 mg/kg and fed with HFD and water (Group A). Group V include 18 mice was second experimental group received lavender extract with a dose of 300 mg/kg and fed with HFD and water (Group B). Group VI also include 18 mice was third experimental group received lavender extract with a dose of 500 mg/kg and fed with HFD and water (Group C).

### Acute toxicity

Median lethal dose (LD50) values were determined as described by Litchfield and Wilcoxon.<sup>14</sup> Three groups of female mice received intraperitoneally doses at different concentrations (100, 300 and 500 mg/kg). The control group received only the water or saline solution. After a single dose administration, mice were placed in individual clear plastic boxes and continuously observed for 6 h and at 24 h time interval to detect any eventual side effects. The number of animals, which died during this period, was expressed as a percentile.<sup>15,16</sup>

## Serum biochemical parameters

At the end of the 15th day, all animals were anesthetized with chloral hydrate (400 mg/kg i.p.) and a venous blood sample was drawn and centrifuged at  $7000 \times g$  for 10 min. The supernatant (serum) was frozen at  $-20^\circ\text{C}$  until assay for HDL, LDL, triglyceride, and cholesterol content (Pars Azmun, Iran, according to manufacturer's instructions) using auto-analyzer (Liasys, Roma, Italy).<sup>17</sup>

### Liver cell function test

In order to evaluate the liver of the mice and determine the fat content of the liver cells, the liver was removed after dissecting the mice and prepared for further evaluation. Tissue was prepared by high-pressure freezing EM-PACT (Leica, Vienna, Austria), the method of choice for specimens in the 100- to 300-nm thickness range. Frozen hydrated sections are cut with a UCT ultra microtome with a CM-1850 cryo kit (Leica, Vienna, Austria).<sup>18</sup> Oil Red staining was used for evaluation of the liver tissue and of deposits. In this staining, fats and liver cells become red and blue, respectively.

### Statistical analysis

Cholesterol, triglyceride, LDL, and HDL were analyzed using one-way ANOVA test (post hoc LSD). SPSS Version 23 (IBM SPSS Statistics, New York, USA) was used for statistical analysis. Data were expressed as means  $\pm$  SEM.  $p < 0.05$  was considered statistically significant.

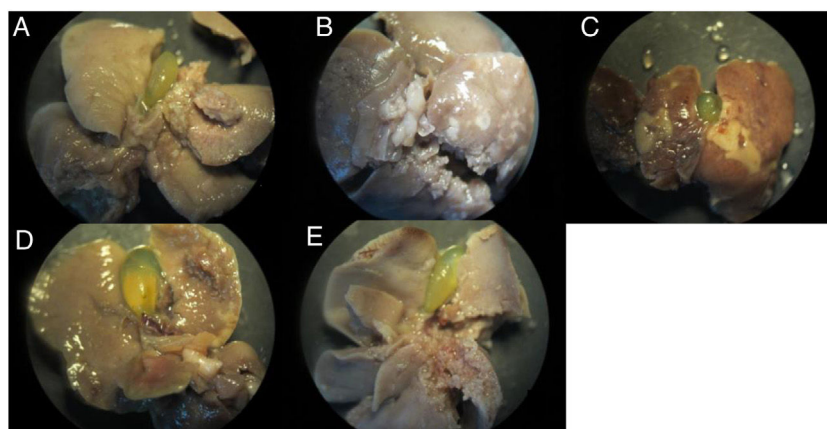
## Results

### Macroscopic examination

In the macroscopic examination, mouse liver was evaluated qualitatively. The few changes were seen in the control II and sham groups, which showed a little fat accumulation and this change were not seen in other groups (Fig. 1). The first day of experiment and after the end of last day of the injections, the mice were weighed, which there was not a significant association for weight gain in mice groups (Table 1).

### Microscopic examination

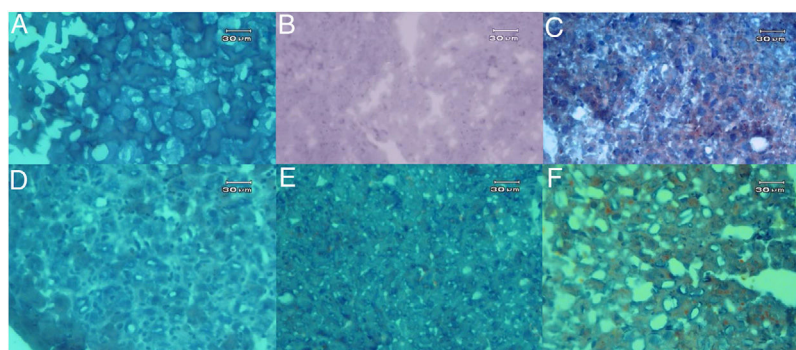
Microscopic observations of the liver tissue showed that no fat storage was present in the control group, as the group used ordinary non-fat meals. In the group II, poorly fat deposits were observed, however, the highest fat stores were observed in the sham group, which fatty deposits were present everywhere in the liver. This group was fed with HFD and received distilled water injections. On the other hand, in experimental group C, fat stores were not observed, since this group was treated with high-fat foods and receiving the highest dose of extract. Very little fat deposits were seen in the experimental group B, which were treated with fatty foods and the middle dose of the extract. In the experimental group A, more fatty deposits were seen than Group B, as this group was treated by HFD and low dose of the extract, which this amount of the extract led to reduced fat but not



**Figure 1** Photomicrograph of mice liver in different groups (G22). A: group I; B: group II; C: group III; D: group B; E: group C.

**Table 1** Average weight of mice before and after study.

Average weight (g)	Group I	Group II	Group III	Group A	Group B	Group C
Before the test	18.52	19.37	18.67	19.294	19.676	19.697
After the test	20.74	22.17	21.41	21.465	21.843	21.857
<i>p</i> -value	>0.05					



**Figure 2** The histology evaluation of fat deposits of liver tissue in different groups after Oil Red staining with 100× magnification. A, liver of control group without fat stores; B, liver of group II with low fat stores; C, liver of sham group with very high fat stores; D, liver of group C without fat stores; E, liver of group B with very low fat stores; F, liver of group A with low fat stores (with 400× magnification).

as much as Group B and C. The histology evaluation of fat deposits of liver tissue in different groups is shown in Fig. 2. Regarding the safety of the aqueous extract of *L. officinalis* with injection of the highest dose for 15 days, no mortality was observed in the studied female mice.

In the mice that received extract injection, hair loss was observed around the injection site in the sub-abdomen area. The rate of hair loss in group A was 16 cases (88.88%) from 18 mice receiving the highest dose of the extract, while hair loss was seen in four (22.22%) and one (5.56%) mice from group of B and C, respectively (Fig. 3).

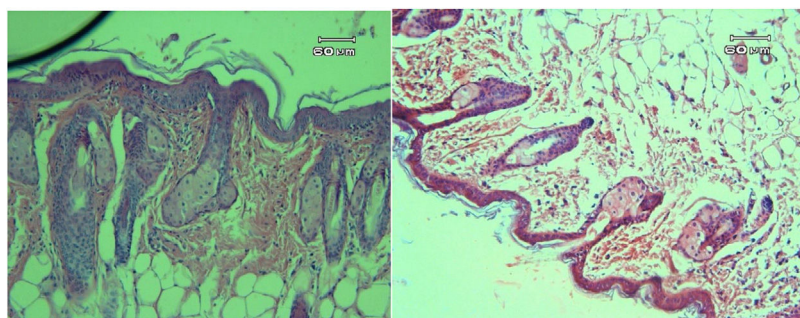
#### Effects of aqueous extract of *L. officinalis* on serum lipid profile

The serum lipid profile in the different studied groups is shown in Table 2. The results obtained from the study show that in the comparison for the homogeneity of

variances, there is a significant difference in Triglyceride and LDL parameters ( $p$ -value <0.05). In addition, the comparison between different parameters with ANOVA analysis showed that there is a statistically significant difference in Cholesterol and LDL parameters ( $p$ -value <0.05). Serum cholesterol level was lower in the 300 and 500 mg/kg/day experimental groups when compared with control group ( $p=0.04$ ,  $p=0.009$ , respectively). *L. officinalis* extract significantly decreased serum LDL level in the doses of 100, 300 and 500 mg/kg/day groups when compared with control group ( $p=0.007$ ,  $p=0.004$ ,  $p=0.04$ , respectively). Serum HDL and Triglyceride level was similar in the controls and different doses of extract groups.

#### Discussion

*L. officinalis* is used in traditional and folk medicines for the treatment of several gastrointestinal, nervous and



**Figure 3** The skin histology of hair loss mice (left) and mice that did not have hair loss (right) with 100× magnification.

**Table 2** The serum lipids profile in the different studied groups in mg/dl.

Groups	Cholesterol	Triglyceride	HDL	LDL
Group I	106.2 ± 17.3 <sup>a</sup>	72.2 ± 16.9	65.8 ± 22.4	26.6 ± 18.2
Group II	157.3 ± 40.6	79.5 ± 14.4	79.6 ± 13.2	61.6 ± 36.4
Group III	190.5 ± 66.7	110.5 ± 52.1	87.3 ± 28.0	107.7 ± 95.8
Group A	165 ± 36.7	75.1 ± 28.0	78.4 ± 23.5	68.2 ± 43.3
Group B	133.6 ± 41.9	70.7 ± 27.8	67.3 ± 20.8	59.1 ± 37.8
Group C	103.8 ± 33.0	76.9 ± 32.7	69.3 ± 19.5	28.0 ± 25.4

Group I: control group, fed by ordinary food; group II: fed by fatty foods without injections; group III: Sham group, fed by fatty foods and distilled water injection; group A: fatty intake and low-dose injection of extract; group B: fatty intake and injection with medium dose of extract; group C: fatty intake and high-dose injection of extract.

<sup>a</sup> Standard deviation.

rheumatic disorders at different parts of the world.<sup>19</sup> In the study, the aqueous extract of lavender was prepared and injected in doses of 100, 300 and 500 mg/kg intraperitoneally for 15 days to different groups of adult female BALB/c mice. Thereafter, lavender extract decreased the serum cholesterol, and LDL levels in experimental groups, while serum HDL and Triglyceride level was similar in the control and different doses of extract groups. In addition, the photomicrograph images from controls and experimental groups A (low dose injections), group B (medium dose injections) and group C (high dose injections) indicates that there are the fat accumulation in the liver tissue of the control I, sham, experimental groups A and B. This shows that injection of high-dose extracts prevented the accumulation of fat in the liver tissue.

It has been identified that high plasma LDL levels are a major risk factor for vascular diseases (VD), whereas high levels of HDL are considered a negative risk factor for VD.<sup>20</sup> The concentration, the size and the chemical modification of LDL are important for atherogenesis. For more than two decades, it has been known that lipid oxidation plays a central role in atherogenesis. The oxidation hypothesis of atherogenesis has evolved to focus on specific proinflammatory oxidized phospholipids that result from the oxidation of LDL phospholipids containing arachidonic acid. These oxidized phospholipids are largely generated by potent oxidants produced by the lipoxygenase and myeloperoxidase pathways.<sup>21</sup> Flavonoids are polyphenolic antioxidants naturally present in vegetables, fruits, and beverages. Flavonoid intake is inversely associated with mortality from coronary heart disease and show an inverse relation with incidence of myocardial infarction.<sup>22</sup>

It has been proven that these compounds have a multifactorial mechanism interacting with multiple molecular targets related to oxidative stress, inflammation, energy sensing pathways (AMPK/mTOR), mitochondrial viability and biogenesis, etc.<sup>23–25</sup> Antioxidants such as vitamin E can also reduce free-radical formation by modifying LDL.<sup>22</sup>

In a study, the polyphenol-rich extracts of lavender were assessed for their antioxidant, hypocholesterolaemic and hypotriglyceridaemic activities.<sup>26</sup> Their results indicated that, the aqueous extract from *L. multifida*, present a higher antioxidant activity. After 24h treatment, the administration of aqueous *Thymus vulgaris* or *L. multifida* extracts to Triton injected rats did exert any significant effect on all plasma lipid parameters. In a meta-analysis, a 10 mg/dl reduction in plasma cholesterol levels decreased the coronary heart disease (CHD) mortality by up to 9%,<sup>27</sup> which suggests that such a reduction in total cholesterol is possible with linalool consumption from teas and herbs. Therefore, this compound may have significant implications for a reduced rate of CHD in the general population. However, this acyclic monoterpene alcohol is insoluble in water, therefore it does not exist after the aqueous extraction.

In our study the high dose injection of lavender extract decreased the serum cholesterol and LDL levels in experimental groups and does not have a significant effect on the reduction of triglycerides and HDL. Evidence linking dietary antioxidants to atherosclerosis in humans is still circumstantial and although in some studies the association of antioxidant intake and lower risk for atherosclerosis is perceptible, in others this association cannot be established. Also further phytochemical and biological tests are

suggested to determine the active chemical constituent responsible for these activities.

## Conflict of interest

The authors have declared no conflict of interest.

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