A Comparative Study of Hypolipidemic Activities of the Extracts of *Melissa officinalis* and *Berberis vulgaris* in Rats

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Abstract

Background: Hyperlipidemia is a heterogeneous group of disorders characterized by an excess of lipids in the bloodstream

Objective: Given previous studies on barberry and *Melissa officinalis* extracts, this study aims at comparing hypolipidemic activities of *Melissa officinalis* extract and *Berberis vulgaris.*

Methods: For the purpose of this study, 64 Wistar rats were selected and divided into 8 groups (n=8). The control group was administered with ordinary diet; the sham group was administered with high-fat diet and intraperitoneally 0.2 ml/dl of the extract solvent (normal saline); and similarly, experimental groups received minimal, moderate and maximum dosages of barberry and *Melissa officinalis* extracts. The treatment group's was given high-fat diet for 21 days. After this period, blood samples were taken and the gathered data were analyzed using SPSS software.

Results: The amount of cholesterol, triglyceride and LDL were increased in the model group compared to the control group, whereas the same substances were decreased significantly in the group receiving the extract compared to the model group ($p<0.05$).

Conclusion: Hypolipidemic properties of alcohol extracts of *Melissa officinalis* are more effective than those of *Berberis vulgaris*. Moreover, it should be noted that it is rather the antioxidant properties of *Melissa officinalis* and their effects on the increase in thyroid hormones as well as the presence of alkaloid compounds, such as berberine in *Berberis vulgaris*, that inhibits cholesterol synthesis and enables its excretion.

Keywords: ***Berberis vulgaris*, *Melissa officinalis*, Cholesterol, Hypolipidemic, Rat**

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Introduction

A high-cholesterol diet affects the liver so that it reduces the number of LDL receptors in its cells due to elevated LDL levels in blood. Brown and Goldestin showed that LDL receptors appear under low-fat diet and that a high-cholesterol diet represses formation of LDL receptors, thus allowing for cholesterol increase to alerting levels and cardiovascular diseases [1-3].

The mechanism of mobilizing energy from lipids requires numerous enzymes and neurohormonal factors. Lipid intake and breaking it down are done by pancreatic and gastric lipase and the absorption of long-chain triglycerides (LCTs) and free fatty acids take place in the small intestine. Chylomicrons enter venous system through lymphatic channels and are removed by liver parenchymal cells where lipoproteins (enzymes that are derived from lipid cells) occur. Released fatty acids are combined with glycirophosphat and make triglyceride. Formation and availability of glycerophosphate is limited in lipogeter and this process depends on the presence of glucose [1, 4].

Given the expansion of fat diseases, complications of chemical medicines as well as the costs incurred to patients, researchers try to find a desirable treatment method which can control blood lipid while sustaining minimum complications. Therefore, it has been for years that drugs from natural sources, particularly herbal medicine, have been subject of interest. *Melissa officinalis* and Berberis vulgarisare among the recommended herbs of this type [5, 6].

Melissa officinalis is an aromatic plant the medicinal properties of which were known to the people of some lands from a long time ago and it had been used by people for treatment of some diseases. The leaves and vegetative body of this plant have been mentioned in authentic pharmacopoeias [7]. For the first time, the extract of *Melissa officinalis* was used as a heart tonic in the treatment of diseases in middle ages. Avicenna maintained that the plant entailed vitality strengthening and also stated that intake of it could relieve irritability and impatience [8]. *Melissa officinalis* extract is almost green and its smell is similar to that of lemon, hence it was also called lemon balm [9]. It probably can increase bile efficiency through the liver. The herb is used for treatment of gastrointestinal disorders of neural origin. On the other hand, through increasing blood fat, it also results in elevated urine and sweat levels. *Melissa officinalis* is also used for improving memory and learning as well as treatment of Alzheimer and epilepsy. Moreover, it has anti-spasmodic, analgesic and hypnotic properties as well [7-11]. *Berberis vulgaris* root possesses antioxidant properties and contains several types of alkaloids which control cholesterol synthesis. In addition, its extract can inhibit bacterial growth, reduces the contraction of smooth muscles, reduces inflammation, stimulates the secretion of bile, and decreases blood fat and pressure [5, 12 - 15] Therefore, regarding the previous studies on the antioxidant properties of *Berberis vulgaris* and *Melissa officinalis*, in the present study, the hypolipidemic activity of *Melissa officinalis* and *Berberis vulgaris* extracts are investigated in rats.

Material and Methods

Extract preparation: In order to obtain extracts of *Melissa officinalis* (A) and *Berberis*

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vulgaris (B) (Figure 1), standard extraction methods were used. After obtaining aerial parts of *Melissa officinalis* and *Berberis vulgaris root* and cleansing and drying them, the herbs were ground into powder and were put in capped glass containers and medicinal alcohol 96% was added. The compounds were left for 72 hours to soak thoroughly. Then, the compounds were filtered and centrifuged and evaporated to dryness under reduced pressure at a maximum of 40ºC using a rotary evaporator instrument.

Preparation of high-cholesterol diet: In order to prepare the high-cholesterol diet 2%, an amount of 20g of pure cholesterol powder (Fluke Chemika) was solved in 5 ml of warmed olive oil and was thoroughly mixed with 1kg of rat food.

Laboratory species: For the purpose of this study, male Wistar rats Within the weight range of 180 ± 10 g were used. All the rats were obtained from the reproduction and breeding center of Razi Institute, Fars Province. After one week, in order for the rats to adapt to the environment, injections began, and over a period of 21 days, they were provided with 12 hours of light, temperature of 20±5°C, and sufficient moisture. The rats were fed the prepared food without any limit concerning water and food.

Treatment of rats: There were 64 rats which were divided randomly into 8 groups of 8, as follows:

The first group was the control group and received no solvent or drugs during the experiment and was under ordinary diet. Of the remaining seven groups which were given high-fat food, one group was taken as the model group and the remaining six groups were categorized as the experimental groups. Three of the experimental groups received respectively minimal, moderate and maximum doses of 25, 50, and 75 mg/kg of *Melissa officinalis* extract and the other three groups were given respectively minimal, moderate and maximum doses of 75, 150, and 300 mg/kg of *Berberis vulgaris*. The extracts were given to the rats intraperitoneally.

Biochemical tests: 48 hours after the last injection, the rats were anesthetized with chloroform and blood samples were taken. Then, blood samples were centrifuged at 3000rpm for 20 minutes and serum was separated. Serum cholesterol and triglyceride levels were determined using Darman Kav Co. Kit (made in Iran) through colorimetric analysis. Lipoproteins were measured using a combination of precipitation technique and ultracentrifugation via Darman Kav Co. kits. HDL-cholesterol was measured using precipitation technique. In the first step, precipitation reagent was added to the serum so that non-HDL compounds are aggregated. Then, the compounds were precipitated for 10 minutes using centrifugation. Then HDL-cholesterol was measured using enzymatic method. LDL-cholesterol was measured using Friedewald formula [2].

Statistical analyses: All obtained values are expressed as mean±SD and data analyses were done using SPSS ver.11.5. The group's data means were compared by one-way analysis of variance and Tukey's post hoc test. The level of significance was set at 0.05.

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Figure 1- ***Melissa officinalis*** **(A),** ***Berberis vulgaris*** **(B)**

Results

The results of the statistical tests reveal that:

The increase in the amount of cholesterol in the model group compared to the control group was significant, whereas all the groups receiving *Melissa officinalis* and *Berberis vulgaris* extracts showed a significant decrease in cholesterol compared to the model group. Moreover, the experimental groups receiving moderate and maximum *Melissa officinalis* dosages also showed a significant decrease in cholesterol compared to the group receiving the minimum and moderate dosages of *Berberis vulgaris* ($p<0.001$). That is, *Melissa officinalis* extract has been effective in reducing cholesterol levels. There was no significant difference between the groups receiving *Melissa officinalis* extract (Table 1).

In terms of triglyceride (TG), its amount in the model group was increased significantly, whereas all the groups receiving *Melissa officinalis* and *Berberis vulgaris* extracts showed a significant decrease in TG compared to the model group. Moreover, the amount of TG in all the groups receiving *Melissa officinalis* extract also showed a significant decrease compared to the all the groups that received dosages of *Berberis vulgaris* extract ($p<0.001$). This in fact indicates that *Melissa officinalis* extract is more effective in reducing TG levels. There was also no intra group significant difference in the groups receiving *Melissa officinalis* extract and also *Berberis vulgaris* extract (Table 1).

In terms of LDL, its amount in the model group and also the groups receiving minimum and moderate doses of *Berberis vulgaris* extract exhibited a significant increase compared to the model group, whereas its amount in all the groups that were given *Melissa officinalis* extract showed a significant decrease when compared to the model group as well as all the groups that received *Melissa officinalis* extract. Here, the impacts of *Melissa officinalis* are stronger. The intra

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Table 1- Comparison of hypolipidemic activities of various doses of *Berberis vulgaris* stem and *Melissa officinalis* extracts

Group / Parameters	Control	Sham	Min Ber (75 mg/kg)	Mod Ber (150 mg/kg)	Max Ber (300 mg/kg)	Min Mel (25 mg/kg)	Mod Mel (50 mg/kg)	Max Mel (75 mg/kg)
Cholesterol	85±0.1	97.3±3.1 *	86±5.4 #	86±4 #	76.1±5.1 #	79.3±3.3	68.4±1.9 α#Ŧ	70.5±2.9 Ŧ
TG	86±3.5	113±8.7 *	150.8±10 #	150.1±14.1 #	144.7±9 #	77.6±3 Ŧ	65±1.5 Ŧ	67.6±6.8 Ŧ
LDL	31.3±2.6	41.6±3.7 *	62.8±6 #	56.1±5.1 #	46.5±6.4	30.1±0.6µ Ŧ	28.4±0.7 Ŧ	33.6±1.3 Ŧ
HDL	24.6±0.1	28±1.4	33.5±1 #	33.5±1.9 #	28.3±1.5 α	22.7±0.9Ŧ	23.5±1.1 αŦ	26.5±0.9 Ŧ

* is indicative of significant changes in compared with the control group;

is indicative of significant changes of each group compared with the model group;

Ŧ is indicative of significant changes of *Melissa officinalis* extract compared to *Berberis vulgaris* stem at $p \leq 0.05$.

There was no significant difference when comparing the groups that received various doses of *Berberis vulgaris* stem extract with each other.

The only exception was related to LDL level, where the group receiving maximum dosage of *Berberis vulgaris* stem showed a significant decrease in LDL compared to the group that received minimum dosage (α).

group comparison of groups receiving *Melissa officinalis* extract as well as the groups that were given *Berberis vulgaris* extract, the amount of LDL showed a significant decrease in the group receiving maximum dosage of *Berberis vulgaris* extract compared to minimum dosage group ($p<0.001$) (Table 1).

In terms of HDL, its amount in the model group was not significant compared to its amount in the control group. The groups receiving minimum and moderate doses of *Berberis vulgaris* extract exhibited a significant increase compared to the model group; however, the group that was given *Melissa officinalis* extract showed a significant decrease when compared to the model group. The group receiving maximum dosage of *Berberis vulgaris* extract showed a significant increase compared to the group receiving moderate dosage of *Melissa officinalis* extract ($p = 0.00$). Here, the impact of *Berberis vulgaris* on HDL increase is stronger. In the intra group comparison of groups receiving *Melissa officinalis* extract as well as the groups that were given *Berberis vulgaris* extract, the amount of LDL did not show any significant difference.

Generally, it can be said that treatment of the model group with high-fat food leads to increase blood fat; however, in the experimental groups that received *Melissa officinalis* or *Berberis vulgaris* extract together with the high-fat food, blood lipid decreased. Moreover, the hypolipidemic impacts of *Melissa officinalis* is stronger than those of *Berberis vulgaris.*

Discussion

The results of this study show that lipid profile of the model group under cholesterol treatment shows increase. In the group receiving *Melissa officinalis* extract, cholesterol, triglyceride, and LDL levels were decreased and in the group treated with

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Berberis vulgaris extract, cholesterol and triglyceride were declined, but there was an increase in HDL level. Moreover, findings indicate that hypolipidemic properties of *Melissa officinalis* extract are more effective than those of *Berberis vulgaris* extract. The results of this study show that *Berberis vulgaris* extract elevates HDL level and decreases cholesterol level. HDL can remove cholesterol and LDLs released from cells or other lipoproteins found in blood stream. Another way by which HDL removes cholesterol focuses on absorbing free cholesterol from cell membranes. Free cholesterol is esterified and is moved toward the HDL core. Therefore, HDL can extract cholesterol through carrying it to some areas to be used (by steroid-producing cells) or to be metabolized and to be secreted (by the liver) [1, 16]. That is, according to the results of this study, it is likely that *Melissa officinalis* extract decrease cholesterol and LDL through increasing HDL.

A high-cholesterol diet affects the liver in such a way that it reduces the number of LDL receptors in its cells due to increased levels of blood LDL. Brown and Goldestin showed that LDL receptors appear under low-fat diet and that high levels of fat and a high-cholesterol diet repress formation of LDL receptors, thus allowing for cholesterol increase upto alerting levels and cardiovascular diseases [1-3]. As it was seen in the model group of this research, intake of high-cholesterol food by the rats increased their LDL level. Moreover, concerning the group that received *Berberis vulgaris* extract, increase in HDL was accompanied with decreased cholesterol level, which is in line with the findings of previous research.

Investigations show that *Berberis vulgaris* extract contains various alkaloids such as berberine [17 & 18]. Berberine enhances theformation of some receptors in the liver which are bonded to cholesterol and enables its excretion [19]. Moreover, alkaloids inhibit cholesterol synthesis [6]. In fact, berberine is a natural product that reduces cholesterol level [12].

Studies also show that extract of *Berberis vulgaris* juice activates the liver and is effective for blood fat decomposition and probably for regulating its level in the blood and also for cardiovascular system, nervous system and treatment of tachycardia, hypertension and neurological disorders such as epilepsy [20] and has hypoglycemic properties [13]. The results of the present study concerning hypolipidemic activity of *Berberis vulgaris* stem are completely in line with those studies previously done on its fruit. Lipase in adipose tissue is sensitive to stimulation by epinephrine and norepinephrine. Other hormones that activate lipase are ACTH, TSH, T_3, T_4, growth hormone, cortisol, glucagon, vasopressin and human placental lactogen. Lipase activity is inhibited by insulin [1].

Ventromedial nucleus of the hypothalamus is the perfect center for appetite and hunger information. Damage to this nucleus decreases satiety signals and causes overeating. Signals entering such CNS centers originate from peripheral tissues. Opioids, substance P and cytokinin pack play a role in enabling sense of taste and as a goalkeeper for feeding when peptides are released in the stomach and serve as satiety messages. Neuropeptides that control appetite include corticotrophin, neurotensin and

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cyclo (hispro) peptides obtained from Proteolysis hormone releasing thyrotropin [1, 21].

Studies by Gorji et al show that *Melissa officinalis* extract have antinociceptive and anti-hormonal (anti-thyrotropic) properties which are not linked to the dosage and that the antinociceptive properties of this plant can be because of its active ingredients and be due to their impact on central and peripheral nervous systems and also on opioid receptors. The antinociceptive properties of this plant are even higher than those of aspirin, but lower than morphine. Moreover, the extract of this plant incurs its effect more through central and peripheral nervous systems and is effective in enhancing memory and learning, treating Alzheimer, epilepsy and sleep disorders [22-24].

Melissa officinalis has potential anti-inflammatory and antioxidant effects and exerts its effects through eliminating free radicals, similar to vitamin C the Rosmarinic acid and benzodioxal present in *Melissa officinalis* extract are 10 times more effective as an antioxidant than vitamins B and C. These elements have high affinity towards nicotinic and muscarinic receptors within the brain [25 - 27].

Zarei et al showed that *Melissa officinalis* extract increases thyroid hormones T_3 and T_4 in hypercholesterolemic rats [28]. In addition, Saeb and Amini stated that there is a reverse and significant relationship between the level of thyroid hormones and lipid profiles [29, 30]. Therefore, taking into account the previous studies on *Melissa officinalis* extract, its hypolipidemic activity can be predicted and the results of this study are in line with those of previous ones.

Concerning the benefits of oral and long-term consumption of barberry, it is already revealed that, because of its high levels of antioxidant, this plant *scavenge* free radicals, protects cells against chemical damages, decreases lipid peroxidation, and protects liver against various stresses. As a result, consumption of this herb has protective effects for body tissues and reduces oxidative stress [31 - 34].

Numerous researchers have shown that some naturally occurring antioxidants, such as berberine, and natural phenols, such as licopen and vitamin E, reduce serum fat [35 - 37]. As a result, herbal remdies and antioxidants with fat-reducing properties can decrease complications such as cardiovascular complications due to hyperlipidemia. In a study on the effects of Berberis integerrima_on blood fat and sugar, Afshari et al (2012) showed that its extract reduces lipid profile [38].

In another study, 500 mg of beberine was fed orally to subjects twice a day for a period of three months. The results showed that total cholesterol and triglyceride levels were reduced by 29% and 3%, respectively [39]. Which are in line with the results of present study.

Conclusion

The results of this study show that hypolipidemic properties of alcohol extract of *Melissa officinalis* are more effective than those of *Berberis vulgaris* and these properties are rather linked to the antioxidant properties of *Melissa officinalis* and its effect on the increase in thyroid hormones. The fat-reducing properties of *Berberis vulgaris* stem from HDL-reducing properties of this herb and the presence of alkaloid compounds such as berberine in this plant, which inhibit cholesterol synthesis and excretion.

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Conflict of interest

The authors declare no conflict of interest.

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