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Anti-hyperlipidaemic Effects of an Essential Oil of *Melissa officinalis*. L in Cholesterol-fed Rabbits

Isaac KARIMI[*1] Hossein HAYATGHEYBI[2] Mostafa RAZMJO[1] Másume YOUSEFI[3]
Amir DADYAN[4] Mohammadmehdi HADIPOUR[1]

[1]Department of Biochemistry, Physiology and Pharmacology, College of Veterinary Medicine, Razi University, Kermanshah, IRAN

[2]Department of Physiology, Islamic Azad University, Urmia branch, Urmia, IRAN

[3]Division of Geographical information system (GIS), Veterinary Organization, Kermanshah, IRAN

[4]Department of Genetics and Animal breeding, Agriculture College, Zabol University, Sistan-Balochestan, IRAN

[*]**Corresponding Author**
e-mail: isaac_karimi2000@yahoo.com

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Abstract

The aim of this study was to investigate the effects of an essential oil (EO) of a traditional herb; *Melissa officinalis* (MO), on the lipid profile of rabbits fed a cholesterol-rich diet (HC). Twenty rabbits were divided into four groups (n=5 for each): Normal control (NC) which fed a standard diet and three cholesterol-fed groups: HC, HC + 1% MO, and HC + 3% MO groups which received 0%, 1% , and 3% EO, respectively for four weeks. Blood samples were collected on day 0 and then at weeks 1, 2, 3, and 4 to determine the levels of total serum cholesterol (TC), high density lipoprotein-cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C), very low density lipoprotein-cholesterol (VLDL-C), triglycerides (TGs) and atherogenic index (AI). There were no significant differences in body weight gain and food intake in all groups. The HC apparently raised the TC, LDL-C, VLDL-C, HDL-C and TGs without significant accrual effect on AI. In comparison to NC group, EO in both HC + 1% MO and HC + 3% MO groups significantly decreased serum lipid and lipoprotein level whereas no significant effect on AI was observed. It can be concluded that EO extracted from MO leaves contributes to a lipid-lowering action in cholesterol-fed rabbits.

Keywords: Lemon balm; Hyperlipidaemia; Essential oil; Cholesterol; Triglycerides.

INTRODUCTION

Metabolic syndrome, the cluster of ailments including obesity, type 2 diabetes, hypertension, dyslipidemia, and atherosclerosis, is the most rapidly growing health concern of Iranian people [1-4]. The metabolic syndrome is increasingly recognized as a strong predictor of patient risk for developing coronary artery disease (CAD). It is associated with an atherogenic dyslipidemia characterized by elevated levels of triglycerides (TGs), reduced levels of high-density lipoprotein cholesterol (HDL-C) and a preponderance of small dense low-density lipoprotein (LDL) particles [5]. An atherogenic dyslipidemia is an integral component of metabolic syndrome, and a major contributor to the cardiovascular risks in patients [5]. Lowering plasma low-density lipoprotein cholesterol (LDL-C) through nondrug strategies, such as ingesting nutraceutical components, would therefore be most desirable.

Lemon Balm, *Melissa officinalis* L., a perennial herb in both wild and cultivated states has been reported from the Mediterranean and central Asian areas. The plant grows erect and reaches a height of 0.5 to 1.0 m. The highest levels of essential oil (EO) have been extracted in late summer from the lower parts of the plants [6]. Oil of balm has also been shown to have antiviral, antibacterial, antitumoral, antioxidant, antispasmodic and nootropic activity [7-10]. Balm has been reported to be an insect repellent [11]. Lemon balm leaves contain 0.2% to 0.3% of a lemon-scented EO similar to that of lemon grass. It's major mono- and sesquiterpenes include geranial, neral, nerol, beta-caryophyllene, beta-caryophyllene oxide, linalool, citronellal, eugenol acetate and geraniol [8, 12, 13]. R (+)-methyl citronellate is characteristic of *Melissa* oil and distinguishes it from lemon grass oil [14]. Flavonoids, oleanane, and triterpenes have also been isolated from the plant [9, 12]. Major nonvolatile constituents are caffeic acid and its di- and trimeric derivatives, including rosmarinic acid and melitric acids A and B [15]. Several studies have reported high content of total phenolics, l-ascorbic acid, and carotenoids of *M. officinalis* [8, 9, 12, 15, 16, 14-17].

As a medicinal plant, lemon balm has traditionally been employed against catarrh, fever [18], flatulence [19] and headaches [20]. In Iranian traditional medicine, *Melissa officinalis* (Per: Baderanjboyeh) has been used for different ailments as single or as a part of herbal formulation. *M. officinalis* as chief component of herbal formulation has been used prescribed for hiccup, colitis, headaches, insomnia and gastroenteritis. Also it has

been reported that *M. officinallis* has cardioprotective, antispasmodic, cholagogic and calming properties. Hence, in this study, we investigate the effect of balm EO on lipid profile in cholesterol fed rabbits.

MATERIALS and METHODS

Plant collection and authentication

The leaves of *M. officinalis* (MO) were purchased from the Ghamsar botanical garden at Kashan, and air dried in the shade. It was authenticated by botanists in Department of biology, College of Science, Urmia University.

Preparation of essential oil (EO)

The EO (3% v/w) was obtained from dried powdered leaves of MO by steam distillation for 3 hrs, using a Clevenger type apparatus. The resulting EO was diluted with distilled water to prepare 1% v/w.

Diet preparation

25g cholesterol plus 175ml ethanol were added to 800ml sunflower oil. The resulting solution vigorously has been homogenized and immediately has been mixed with standard pellet (Niro-Sahand Co. Tabriz, Iran) in the ratio of 15% v/w and again has been pelleted under UV-illuminated and air-ventilated room. The resulting "high-cholesterol (HC) diet" contained in 0.47% cholesterol.

Animals

Adult weight- and age-matched healthy rabbits (n=20), were obtained from the Animals House, College of Veterinary Medicine, Urmia University , Western Azerbaijan, Iran. They were maintained in an air-conditioned room (26° ± 1°C) and were divided into groups of 5 each.

Group NC served as negative controls that received only standard pellet and orally gavaged by 10ml distilled water. Group HC served as positive control that received cholesterol enriched pellet and daily gavaged by 10ml distilled water. Group HC + 1%MO received cholesterol enriched pellet and daily gavaged by 10ml EO 1% v/w of lemon balm. Group HC + 3%MO received cholesterol enriched pellet and daily gavaged by 10ml EO 3% v/w of lemon balm. Blood samples were collected through auricular vein on day 0 and then at weeks 1, 2, 3, and 4. Sera were separated by centrifugation at 1400 × g at 4 °C for 15 min, and stored at –20 °C until analysis.

Analytical procedures

The concentrations of total cholesterol (TC), LDL-C and TGs, in the serum were enzymatically determined with a commercial diagnostic kit (ELI TECH Diagnostic, French). Plasma lipoprotein fractions HDL-C were determined by immunoinhibition method (ELI TECH Diagnostic, French). Very low density lipoprotein-cholesterol (VLDL-C) was calculated by Friedewald's formula: VLDL-C = TGs/5 [21]. Atherogenic index (AI) was calculated as (TC-HDL-C)/HDL-C.

Statistical analysis

All data are reported as mean ± SEM. All parameters were analyzed using ANOVA for a split-plot in time design, with rabbit diet as the whole-plot factor and time (weeks) sampled as the subplot factor. This procedure allowed testing for the effects of diet and week and their interaction. When significant differences were found, *post hoc* comparisons were made between control and all other (treated) groups with a Duncan's multiple range tests. All data were analyzed using the General Linear Models Procedure of SAS (SAS Institute, Inc., Cary, NC). Statements of significance were based on $P<0.05$ unless otherwise noted.

RESULTS

In this experiment, mean (±SEM) calories consumed and mean body weight gains were statistically similar throughout study in all groups (results not shown). Serum TC, LDL-C, VLDL-C, HDL-C and TGs levels increased significantly after 28 days of cholesterol feeding while atherogenic index was increased non-significantly ($P>0.05$; Table 1). Concurrent administration of *M. officinalis* essential oil (1% and 3%) with cholesterol caused a significant decrease in the levels of serum TC, LDL-C, HDL-C, VLDL-C and TGs when compared with cholesterol fed control rabbits (Table 1).

At 4 weeks, the 1% and 3% *M. officinalis*-treated groups displayed the 12.5 and 16.5 percent of significant decrement in VLDL-C level compared to cholesterol fed control: HC rabbits, respectively (Table 1). Also, significant decreases in circulating VLDL-C were detected with increasing levels of *M. officinalis*. A significant difference in the serum VLDL-C levels of five groups on 1, 2, 3 and 4 weeks was observed ($P_{ANOVA}<0.001$; Figure 1). At 4 weeks, the 1% and 3% *M. officinalis*-treated groups displayed the 5.2 and 11.0 percent of decrement in LDL-C level compared to HC rabbits, respectively ($P<0.001$;Table 1). Also, non-significant decreases in circulating LDL-C were detected with increasing levels of *M. officinalis* (Table 1). A significant difference in the serum LDL-C levels of five groups on 1, 2, 3 and 4 weeks was observed ($P_{ANOVA}<0.001$; Figure 1). Administration of *M. officinalis* caused a significant ($P<0.001$) decrease (~7.0%) in the HDL-C level compared to HC group (Table 1). Also, non-significant decreases in circulating HDL-C were detected with increasing levels of *M. officinalis* (Table 1). A significant difference in the serum HDL-C levels of five groups on 1, 2, 3 and 4 weeks was observed ($P_{ANOVA}<0.001$; Figure 1).

In comparison to HC group, the TGs also decreased to a significant level ($p<0.001$) by 11.5 and 15.7% in HC + 1%MO and HC + 3%MO groups, respectively. A significant difference in the serum TGs levels of five groups on 1, 2, 3 and 4 weeks was observed ($P_{ANOVA}<0.001$; Figure 1). No significant differences were observed in plasma LDL-C, HDL-C, TGs as well as AI between HC + 1%MO and HC + 3%MO groups. *M. officinalis* significantly decreased ($P<0.001$) plasma TC

Table 1. Effect of essential oil of lemon balm on lipid profile and atherogenic index (AI) in cholesterol fed rabbits compared to normal control.

Group	TC	LDL-C	VLDL-C	HDL-C	TGs	AI
NC	59.04±1.02	25.84±0.76	13.12±0.51	17.64±0.63	65.2±2.58	2.40±0.11
HC	92.32±4.00*	39.28±1.53*	21.44±1.38*	24.44±1.64*	106.08±7.09*	2.88±0.15
HC+1%MO	83.16±2.87†	37.24±1.33†	18.72±0.73†	22.68±1.37†	93.88±3.70†	2.83±0.13
HC+3%MO	78.56±2.39†	34.96±1.20†	17.85±0.63†	21.68±1.18†	89.36±3.09†	2.85±0.14

Values are mean±SEM (n=5);*Data in columns were significantly different at P<0.05 compare to NC: Normal control; † Data in columns were significantly different at P<0.05 compare to HC: Hypercholesterolemic control. HC+1% MO and HC+3%MO groups received 1 and 3% essential oil of lemon balm plus hypercholesterolemic diet, respectively.

by the level of 10 and 15 percent in HC + 1%MO and HC + 3%MO groups as compared to HC group, respectively. Also, significant (P<0.001) decrease in circulating TC was detected with increasing levels of *M. officinalis* (Table 1). A significant difference in the serum TC levels of five groups on 1, 2, 3 and 4 weeks was observed (P_{ANOVA}<0.001; Figure 1). With respect to HC group, AI also showed a decrease of 3.7 and 3.0% in HC + 1%MO and HC + 3%MO groups, respectively (P>0.05;Table 1).

DISCUSSION

Numerous studies have indicated that diet regulation and drug therapy to control blood cholesterol can subsequently reduce coronary heart disease morbidity and mortality. Though synthetic lipid-lowering drugs are useful in treating hyperlipidaemia, there are a number of adverse effects. However, because of the side effects of cholesterol-lowering pharmacological agents, patients look for alternatives [22-24].

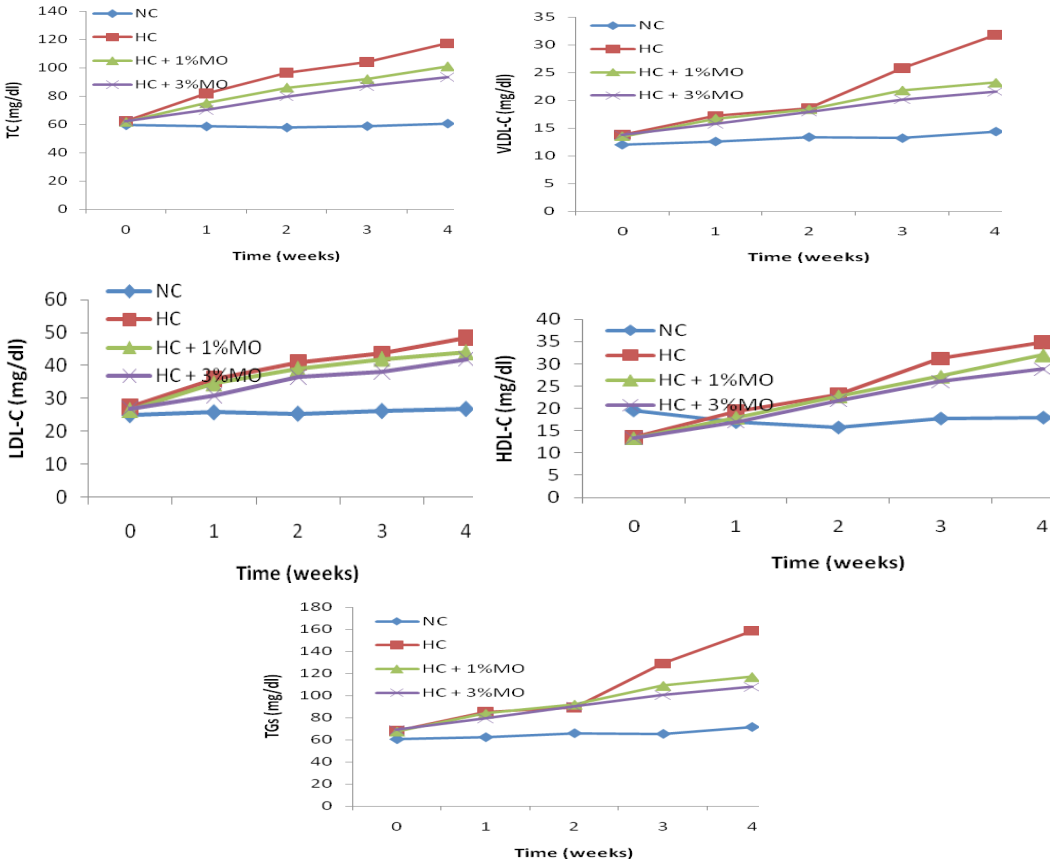

Figure 1. Lipid profiles at 0, 1, 2, 3 and 4 week of treatment with 1.0 and 3.0% essential oil of *Melissa officinalis* in HC + 1%MO and HC + 3%MO, respectively compare with Normal Control (NC) and Hypercholesterolemic Control (HC).

MO is widely used in Iran, not only as a vegetable but also a medicine to treat various kinds of diseases [13]. In hyperlipidaemic rats and mice, lemon balm extracts improved the lipid profile as well as liver enzyme markers such as aspartate transaminase, alanine transaminase, alkaline phosphatase and increased glutathione levels in the tissue [25, 26]. Though MO leaves expressed the hypolipidaemic effect in normal and hyperlipedemic animals, it is not known which compounds contribute to this action. Since MO leaves are rich in EO, it is possible that EO in MO leaves is responsible for the hypolipidaemic action.

In the present study, after four weeks of HC diet feeding, TC was markedly enhanced in HC group. These results are consistent with earlier reports [27, 28], which have clearly established a correlation between dietary lipids and serum lipid profile. Supplementation of cholesterol in diet rapidly results in a marked increase in the production of cholesteryl ester rich-VLDL-C by the liver and intestine [29] and a reduced number as well as rate of cholesterol removal by the hepatic LDL receptors [30]. Consequently serum levels of LDL-C and VLDL-C is increased. A significant increased in the ratios of TC: HDL-C and LDL-C: HDL-C indicates increased risk of atherosclerosis and coronary heart disease [31]. We observed that rabbits fed a high-cholesterol diet supplemented with MO for 4 weeks had significantly decreased the levels of serum TGs, TC, VLDL-C, LDL-C, and HDL-C compared to rabbits fed a high-cholesterol diet alone, indicating that MO efficiently regulates triglyceride and cholesterol metabolism in cholesterol-fed rabbits. This implies that the lipid-lowering action of the EO is predominantly due to the suppression of liver lipid synthesis. Lee et al. (2008) [26] have shown that a formulation of herbal extracts from *Morus alba*, *Melissa officinalis*, and *Artemisia capillaries* reduced circulating TC and TGs levels in high-fat diet-induced obese mice through changes in the expression of hepatic peroxisome proliferator-activated receptor (PPARα)- target genes.

The striking feature of the present study is concurrent decrease of HDL-C and LDL-C following intake of MO. The hypocholesterolaemic effect of EO of MO may be attributable to posttranscriptional suppression of 3-hydroxy-3-methylglutaryl-CoA reductase (HMG-CoA reductase) by geraniol, an acyclic dietary monoterpene of MO [32-35]. Also, geraniol induced dose dependent relaxation in rat aorta, which was endothelium-independent [36]. One study has shown that aqueous extract of leaves of *M. officinalis* provoked significant cardiac rate reduction and did not alter the contractile force. The negative cardiac rate effect may have occurred by cardiac muscarinics receptors stimulation [37]. Another two most active monoterpenes of EO of MO, linalool and citronellal, also were shown to inhibit the synthesis *in vitro* of cholesterol from mevalonate. However, these two monoterpenes, when administered orally to rats (0.5 mg. per kg.) for four weeks, had no effect on either the liver or serum cholesterol [38]. Eugenol and eugenol acetate are two main components of EO extracted from MO leaves [13]. A number of biological effects of eugenol have been reported including myorelaxant, antispasmodic and antioxidant effects [39, 40]. Moreover, eugenol has been shown to lower a high serum lipid profile in hyperlipidaemic mice [41]. Therefore eugenol is possibly the significant constituent for the lipid-lowering action of EO extracted from MO leaves in rabbits fed with a HC diet. The oxidative modification of low-density lipoprotein (LDL) plays a pivotal role in the progression of atherosclerosis [42, 43]. Eugenol as an antioxidant could inhibit LDL-C oxidation, thereby preventing atherosclerosis [44, 45]. The attenuation of high levels of serum lipid profile and AI in HC rats treated with EO of MO leaves might be the result of eugenol action. Although EO participates in the anti-hyperlipidaemic action of MO leaves, other components should not be excluded since MO leaves have several kinds of chemical constituents.

The sesquiterpene (*E*)-β-caryophyllene [(*E*)-BCP] is a major plant volatile found in large amounts in the essential oils of many different spice and food plants, such as oregano (*Origanum vulgare* L.), cinnamon (*Cinnamomum* spp.), black pepper (*Piper nigrum* L.) and lemon balm [13, 46-49]. (*E*)-BCP is also a major component (up to 35%) in the essential oil of *Cannabis sativa* L [50]. Recently, Gertsch et al. (2008) [51] have shown that the essential oil component (*E*)-BCP selectively binds to the cannabinoid CB2 receptor, leading to cellular activation and anti-inflammatory effects. Moreover, the CB2 receptor has been described as a potential target for the treatment of atherosclerosis [52]. In the present study, the decrease of atherogenic indices in MO-treated rabbits may be related to the (*E*)-BCP of MO, although further investigation is inevitable.

CONCLUSION

In conclusion, the ability to lower the serum lipid profile suggests that EO in MO leaves could be effective in the treatment of hyperlipidaemic states. Its anti-hyperlipidaemic activity was also great enough to suppress a high level of AI in cholesterol fed rabbits. The lipid- lowering action as well as suppressing a high AI in HC rats implies that the EO of MO may be useful in alleviating atherosclerosis in a hyperlipidaemic state. Further studies are needed to clarify which components of EO of MO are possibly responsible for the lipid-lowering effect.

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