

Original Article

The effects of *Melissa officinalis* (lemon balm) in chronic stable angina on serum biomarkers of oxidative stress, inflammation and lipid profile

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Background and Objectives: Coronary artery disease (CAD) is a major cause of death worldwide. Chronic stable angina (CSA) is the primary sign of CAD. Oxidative stress and inflammation play a substantial role in pathogenesis and progression of CAD. The aim of this study was to investigate the effects of oral administration of powdered *Melissa officinalis* (MO) on biomarkers of oxidative stress, inflammation, and lipid profile in patients with CSA. **Methods and Study Design:** A randomized, double-blind, placebo-controlled clinical trial was performed in 80 patients with CSA. The subjects were randomly assigned to obtain either oral MO 3 g/d (n=40) or placebo (n=40) for eight weeks. Anthropometric indices, biomarkers of oxidative stress, inflammation, and lipid profile were evaluated at baseline and post-intervention. **Results:** The mean serum concentrations of triglycerides, total-cholesterol, LDL-cholesterol, and malondialdehyde (MDA), and high sensitive C-Reactive Protein (hs-CRP) were lower in the intervention group compared with placebo ($p<0.01$) post intervention. Moreover, the mean serum concentration of paroxonase 1 (PNO1) and HDL-c were higher ($p<0.001$) in the intervention group compared with the control group. **Conclusion:** Oral MO supplementation improves the lipid profile, MDA, hs-CRP, and PNO1 in patients with CSA.

Key Words: *Melissa officinalis*, coronary artery disease, oxidative stress, paroxonase 1, lipid profile

INTRODUCTION

Coronary artery disease (CAD) is a major cause of death worldwide, and the main cause of death in Iran, accounting for approximately 35% of all mortalities.^{1,2} Chronic stable angina (CSA) is the primary feature of CAD in about 50% of patients.³ CSA is an ischemic heart disease characterized by symptoms such as burning sensation, pressure, pain, squeezing, or tightness in the chest area.⁴ CSA affects approximately 8 million people in the U.S,⁵ and is associated with re-hospitalizations, decreased quality of life and leads to increased healthcare costs.⁶ There is evidence to suggest that oxidative stress and inflammation play a substantial role in pathogenesis and progression of CAD.^{7,8} Therefore, it is suggested that administering anti-inflammatory and antioxidant reagents in CAD patients may be beneficial in the management and prevention of CAD recurrence.⁹

There has been a growing interest in herbal medicine as

a complementary therapy for the treatment of multiple conditions in recent years.¹⁰ *Melissa officinalis* (lemon balm) belongs to Lamiaceae family, and is traditionally used for the treatment of several disorders including hypertension, ulcers, hyperthyroidism, amenorrhea, bronchitis, and epilepsy.¹¹ Recent studies have reported that administration of *Melissa officinalis* (MO) is may be beneficial in treating hyperglycemia, dyslipidemia, and neurodegenerative diseases.^{12,13} Moreover, several studies sug-

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gest that MO and its derivatives have potential anti-inflammatory,¹⁴ antioxidative,¹⁵ and antiproliferative¹⁶ properties. It is assumed that the constituents of MO including essential oils, flavonoids, tannins, rosmarinic acid and polyphenolic compounds are involved in delivering these therapeutic effects.^{17,18}

Alijaniha et al¹⁹ study found that oral supplementation with 1g/d MO extract significantly reduced the frequency of palpitation episodes and the number of anxious patients in adults suffering from benign palpitations. Another study suggested that a 3 g/d MO drink may be effective in reducing oxidative stress and DNA damage in radiology staff.²⁰

Considering the anti-inflammatory and anti-oxidative properties of MO and the lack of clinical trials on its therapeutic role in patients with CAD, we aimed to investigate the effects of oral administration of powdered MO on serum biomarkers of oxidative stress, inflammation, and lipid profile in patients with CSA.

PARTICIPANTS AND METHODS

Plant material

The aerial parts of MO were collected in August 2016 from the Botanical Garden of Tabriz University, and identified by a botanist of the Department of Botany, Tabriz University. A voucher specimen (KF1429-1) of the plant was deposited in the Herbarium Centre, Faculty of Pharmacy, Tabriz University of Medical Sciences. MO was dried in the shade at room temperature for 12 days.

Participants

Eighty CSA patients aged 40-75 years old, in cardio logical care, were recruited from Golestan Hospital, Ahvaz Jundishapur University of Medical Sciences (AJUMS), Iran. The inclusion criteria were a diagnosis of CSA, BMI between 18.5 -40, and an Ahvaz city inhabitant. Patients were excluded if they had a history of hypertension, diabetes mellitus, arrhythmia, renal, hepatic, gastrointestinal, endocrine logical or hemato logical disease, musculoskeletal disease or rheumatoid arthritis, were a dietary vegan, substance abuser, smoker, had a history of alcoholism, supplement use in the last 2 months, or any allergy to MO. The study was conducted in accordance with the Declaration of Helsinki and written consent was obtained from all patients prior to the study. The trial was approved by Ahvaz Jundishapur University of Medical Sciences (AJUMS) Research Ethics Committee and was registered in the Iranian website for clinical trials (www.irct.ir IRCT code: IRCT2016052928152N1).

Sample size calculation

Sample size was calculated based on type one (α) and type two errors (β) as 0.05 and 0.10 (power=90%) respectively. according to the previous study²¹ considering 8.6 and 9.9 as standard deviations (SD) and 7.2 as the difference in mean or effect size (d) of serum HDL concentration, the main outcome. Therefore, a sample size of 35 patients in each group was calculated. To account for probable dropouts during the study, 40 patients were recruited in each group.

Study design

This study was a randomized, placebo-controlled, double-blind parallel-group clinical trial. Participants were randomly allocated to two groups of the intervention group (n=40), receiving 3 g per day of MO (3 capsules) for 8 weeks, and the control group (n=40), receiving 3 capsules (3 g) per day of placebo for 8 weeks. The capsules containing MO were prepared by an automatic filler, each filled with 1 g MO. The placebo capsules were similarly filled with 1 g corn starch each. The appearance, colour, shape, size, and packaging of MO and placebo capsules were similar. All patients and researchers were unaware of randomization until the main analysis at the end of the study. Participants were asked not to change the dosage and type of medication they were using during this study. Patient compliance to MO was checked weekly through phone calls to patients and they were asked to return their medication containers to the facility monthly.

Anthropometry, physical activity and dietary intake assessments

Body weight was measured without shoes with minimal clothing using a digital scale (Seca, Hamburg, Germany) to the nearest of 0.1 kg. Height was measured to the nearest of 0.1 cm using a tape measure (Seca, Hamburg, Germany). Body mass index (BMI) was calculated as body weight in kg divided by the square of the body height in meters. Waist circumference (WC) was measured using an inelastic tape measure (Seca, Germany) at the mid-point between the costal margin and the iliac crest. Patients' medical and medication history were recorded. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) was measured using a sphygmomanometer in the sitting position and after 5-minutes rest.

The quantitative international physical activity questionnaire (IPAQ) was used to evaluate physical activity level before and after the intervention. Data expressed as met-min/week. A 3-day dietary record (including 2 working days and 1 weekend day) was used to evaluate the dietary intake one week before and at the end of the intervention. The dietary records were analyzed using Nutritionist IV software (First Databank, San Bruno, CA, USA) adjusted for Iranian foods.

Biochemical assessment

Fasting blood samples (7 ml) were collected at baseline and at the end of the intervention. Blood samples were immediately centrifuged at 3000 rpm for 10 min to separate serum and stored at -80 °C until analysis. Malondialdehyde (MDA), Paraxonase 1 (PNO1) and serum high sensitive C - reactive protein (hs-CRP) were measured using ELISA kits (Eastbiopharm, China). Serum concentrations of total cholesterol (TC), triglycerides (TG), LDL, and HDL-cholesterol were measured by enzymatic methods using auto-analyzer system and commercial kits (Pars Azmoon, Tehran, Iran).

Statistical analysis

Normal distribution of all variables was tested by Kolmogorov-Smirnov test. All variables were reported as mean±standard deviation (SD). Demographic variables were analysed using Chi-square, Mann-Whitney U test or

independent sample t-test, as appropriate. Within group comparisons were done by paired-sample t-test. Independent t-test was performed to detect differences between two groups independently. Analysis of covariance (ANCOVA) was applied to identify any differences between two groups at the end of the study, adjusting for baseline value and dietary MUFA intake. P value <0.05 was considered as statistically significant. Statistical analysis was performed using the Statistical Package for Social Science version 20 (SPSS Inc., Chicago, Illinois, USA).

RESULTS

A total of 73 patients completed the trial. The flowchart of this study is presented in Figure 1. Forty-eight percent (n=17) and 50% (n=19) of the study population were men in the MO and placebo groups respectively. The mean

age of participants in MO and placebo groups was 58.8 ± 8.3 and 56.5 ± 8.9 y respectively. The mean BMI in MO and placebo groups was 28 ± 3.7 and 29 ± 3.7 kg/m² respectively. There were no significant differences in gender, age, BMI, WC, SBP, DBP, physical activity, duration of disease, and medication history ($p>0.05$ for all) between two groups (Table 1). No significant differences were seen in dietary intakes of total energy, carbohydrate, protein, fat, PUFA, SFA, cholesterol, beta-carotene, vitamin C and E except for MUFA between two groups at the end of the intervention (Table 2).

Between groups analysis showed that the mean concentration of MDA was significantly ($p<0.001$) lower in the intervention group compared with the control group (11.42 ± 10.22 vs 15.78 ± 9.81 nmol/mL) post intervention. The mean concentration of hs-CRP was also significantly ($p=0.016$) lower in the intervention group compared with

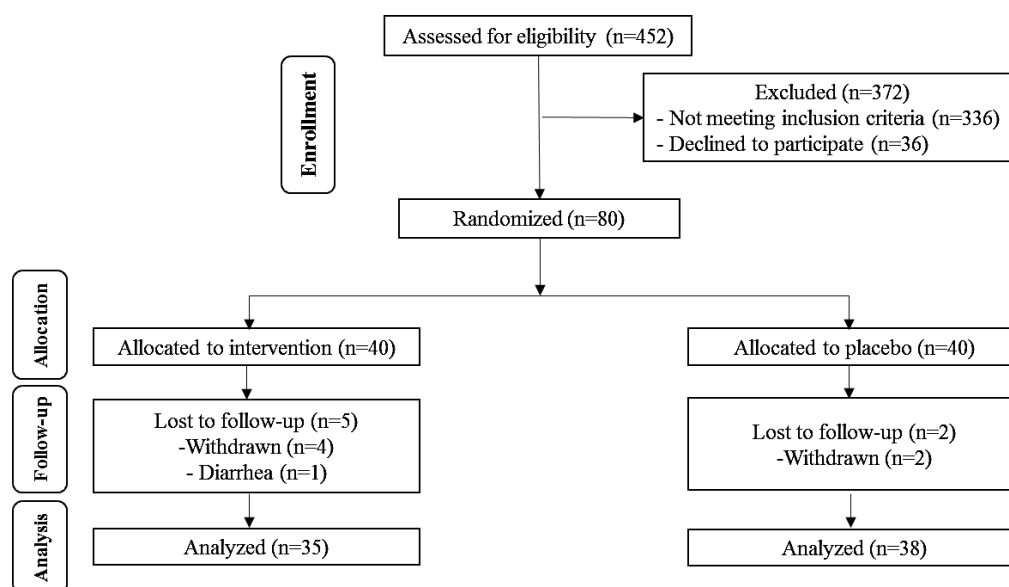


Figure 1. Patient flow diagram.

Table 1. Demographic characteristics of the study participants.

Variables	Groups		p-value
	Intervention (n=35)	Placebo (n=38)	
Sex	Men	17 (48.60)	0.903 [‡]
	Women	18 (51.40)	
Age (year)	58.8 ± 8.3	56.5 ± 8.9	0.267 [†]
BMI at study baseline (kg/m ²)	28 ± 3.7	29 ± 3.7	0.248 [†]
BMI at end-of-trial (kg/m ²)	27.9 ± 3.6	29 ± 3.7	0.456 [†]
WC (cm)	95 ± 11.4	100.1 ± 11.8	0.064 [†]
SBP (mmHg)	123.4 ± 5.9	122.3 ± 4.8	0.405 [†]
DBP (mmHg)	81 ± 3.5	80.1 ± 2.1	0.222 [†]
Physical activity (met-min/week)	1578.7 ± 644.1	1739.9 ± 801.2	0.379 [†]
Disease duration (year)	4.3 ± 2.4	4.19 ± 2.50	0.751 [†]
Valsartan	0 (0-1)	0 (0-1)	0.758 [§]
Atorvastatin	0 (0-1)	0 (0-1)	0.026 [§]
Acetylsalicylic acid	1 (0-1)	1 (0-1)	0.641 [§]
Aspirin	1 (0-1)	1 (0-1)	0.745 [§]
Nitroglycerin	0 (0-1)	0 (0-1)	0.462 [§]

BMI: body mass index; WC: waist circumference; SBP: systolic blood pressure; DBP: diastolic blood pressure.

[†]p-value reported based on independent sample t test.

[‡]p-value reported based on chi-square test.

[§]p-value reported based on Mann-Whitney U test.

Quantitative data represented as mean \pm SD or median (min-max). Qualitative data reported as frequency (Percentage).

Table 2. Dietary intake of study participants at baseline and end of trial

Variables		Groups				<i>p</i> -value [‡]
		Intervention (n=35)		Placebo (n=38)		
		Mean± SD	PC	Mean± SD	PC	
Energy (kcal/day)	Before	2279.8±624.1	0.10	2416.2±24	0.06	0.836
	After	2282.2±624.8		2417.8±820.8		
	<i>p</i> -value [†]	0.161		0.831		
Protein (g/day)	Before	103.5±40.2	0.11	107.4±41.6	-0.11	0.731
	After	103.6±40.1		107.3±41.7		
	<i>p</i> -value [†]	0.573		0.832		
Carbohydrates (g/day)	Before	166.5±40.2	-12	140.1±30.3	3.05	0.193
	After	144.8±34.6		143.5±29.4		
	<i>p</i> -value [†]	<0.001		0.135		
FAT (g/day)	Before	111.5±80	-0.20	117.1±65.1	-1.65	0.066
	After	110.8±79.4		115.8±65.7		
	<i>p</i> -value [†]	0.182		0.010		
SFA (g/day)	Before	77.7±37.1	-0.31	78.9±42.8	0.14	0.354
	After	77.4±36.7		78.9±42.4		
	<i>p</i> -value [†]	0.182		0.920		
MUFA (g/day)	Before	14.3±5.3	2.69	15.3±7.6	-3.40	0.016
	After	14.7±5.7		14.8±7.7		
	<i>p</i> -value [†]	0.163		0.038		
PUFA (g/day)	Before	19.5±9	1.7	20.1±10.9	-0.3	0.110
	After	20.2±10.9		19.8±10.5		
	<i>p</i> -value [†]	0.235		0.414		
Cholesterol (mg/day)	Before	29.7±14.7	0.88	33.9±22.7	1.78	0.803
	After	30.1±15.3		34.5±22.9		
	<i>p</i> -value [†]	0.325		0.080		
Beta-carotene (µg/d)	Before	255.3±180	-0.24	250.5±164.8	0.31	0.174
	After	254.9±180.2		260.1±164.9		
	<i>p</i> -value [†]	0.188		0.345		
Vitamin E (mg/day)	Before	25.2±10.5	3.81	29.7±15.1	-0.008	0.424
	After	25.8±10.2		29.7±15.2		
	<i>p</i> -value [†]	0.164		0.997		
Vitamin C (mg/day)	Before	175.2±82.9	0.42	171.4±105.3	0.07	0.312
	After	176.1±83.7		170.8±104.5		
	<i>p</i> -value [†]	0.310		0.677		

SFAs: saturated fatty acids; PUFAs: polyunsaturated fatty acids; MUFAs: monounsaturated fatty acids; PC: percent change.

[†]*p*-value reported based on paired sample t test.

[‡]*p*-value reported based on ANCOVA after baseline value adjustment.

the control group (2.12±0.95 vs 2.10±0.811 µg/mL) post intervention. For PNO1, there was significant ($p<0.001$) increase in the intervention group compared with the control group (124.31±54.59 vs 123.13±65.78 ng/mL) post intervention (Table 3).

Within-group comparisons showed that the mean concentration of serum MDA (14.96±10.74 vs 11.42±10.22 nmol/mL; $p<0.001$) was significantly reduced and the mean concentration of PNO1 was significantly (110.87±53.34 vs 124.31±54.59 ng/mL; $p<0.001$) increased in the intervention group. The mean concentration of hs-CRP (4.24±2.24 vs 2.12±0.95 µg/mL; $p=0.005$) was also significantly reduced post intervention (Table 3).

The mean concentrations of TG, TC, LDL-C were significantly ($p<0.01$) lower in the intervention group (157.17±67.12, 144.89±64, 75.66±26.13 mg/dL respectively) compared with the control group (177.16±104.48, 143.53±29.46, 77.18±20.62 mg/dL respectively) post intervention (Table 3). In addition, the mean HDL-c was significantly ($p<0.001$) greater in the intervention group (41.51±7.67 mg/dL) compared with the control group (35.61±4.80 mg/dL).

Within-group comparisons showed that the mean concentrations of TG (198.03±95.01 vs 157.17±67.12

mg/dL), TC (166.51±40.1 vs 144.89±64 mg/dL) and LDL-C (90.94±29.03 vs 75.66±26.13 mg/dL) were significantly ($p<0.01$) decreased and the mean concentration of HDL-C (34.80±6.57 vs 41.51±7.67 mg/dL) was significantly ($p<0.001$) increased in the intervention group (Table 3).

DISCUSSION

In this randomized short-term intervention study significant improvements were observed in oxidative status, hs-CRP concentration and lipid profile in patients with CSA receiving oral MO supplementation. To the best of our knowledge, this is the first study to investigate the effects of MO supplementation on serum biomarkers of oxidative stress, inflammation and lipid profile, in CSA patients.

In this study MO supplementation resulted in decreased MDA concentration. Similarly, in one study conducted by Bayat et al it was shown that MO inhibits MDA in ischemic brain injury model.²² In another study on rats, MO extract decreased MDA concentration both in carrageen an-induced paw edema test and formalin-induced paw licking test.²³

PON1 an HDL-associated enzyme carried on apo A-I is critical in detoxifying oxidative stress mediators. In this

Table 3. The effect of *Melissa officinalis* supplementation on metabolic parameters.

Variables		Groups				<i>p</i> -value [‡]	<i>p</i> -value [§]
		Intervention (n=35)		Placebo (n=38)			
		Mean±SD	PC	Mean±SD	PC		
MDA (nmol/mL)	Before	14.9±10.7	-25.93	14±9.8	17.37	<0.001	<0.001
	After	11.4±10.2		15.7±9.8			
	<i>p</i> -value [†]	<0.001		<0.001			
PNO1 (ng/mL)	Before	110.8±53.3	14.07	126.6±66	-2.94	<0.001	<0.001
	After	124.3±54.5		123.1±65.7			
	<i>p</i> -value [†]	<0.001		0.003			
hs-CRP (Ug/mL)	Before	4.2±2.2	-41.95	1.6±0.6	30.64	0.017	0.016
	After	2.1±0.9		2.1±0.81			
	<i>p</i> -value [†]	0.005		<0.001			
TG (mg/dL)	Before	198±95	-16.51	177.4±107.9	2.06	0.001	0.001
	After	157.1±67.1		177.1±104.4			
	<i>p</i> -value [†]	<0.001		0.959			
TC (mg/dL)	Before	166.5±40.1	-12	140.1±30.3	3.05	<0.001	<0.001
	After	144.8±64		143.5±29.4			
	<i>p</i> -value [†]	<0.001		0.135			
HDL (mg/dL)	Before	34.8±6.5	20.37	36.9±5.6	-3.21	<0.001	<0.001
	After	41.5±7.6		35.6±4.8			
	<i>p</i> -value [†]	<0.001		0.001			
LDL (mg/dL)	Before	90.9±29	-15.47	73.5±19.7	-3.21	<0.001	<0.001
	After	75.6±26.1		77.1±20.6			
	<i>p</i> -value [†]	<0.001		0.036			

TG: triglycerides; TC: total cholesterol; LDL-c: low density lipoprotein cholesterol; HDL-c: high density lipoprotein cholesterol; MDA: malondialdehyde; PNO1: paraoxonase1; hs-CRP: high-sensitivity C-reactive protein; PC: percent change.

[†]*p*-value reported based on Paired sample t test.

[‡]*p*-value reported based on ANCOVA after baseline value adjustment.

[§]*p*-value reported based on ANCOVA after baseline and MUFA PC adjustment.

study we have shown that PNO1 serum concentration increases significantly after MO supplementation. One study indicated a potent antioxidant effect against 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical and an inhibitory effect against lipid per oxidation in induction of the Fe(2⁺)/H₂O₂ system.²⁴ Furthermore, another study reported that Glutathione increased in the liver of hyperlipidemic rats after MO supplementation.²⁵ In spite of scarcity of human studies, one study showed that complications of Aluminum intoxication such as oxidative stress alleviated by MO. In that study total antioxidant capacity increased and total thiol molecule decreased significantly consistent with our results.²⁶

The increase in CRP concentration in the blood is known as an indicator of cardiac disease risk, and it has a prognostic value in CAD.²⁷ The results of this study showed that MO has beneficial effects in decreasing hs-CRP concentration and hence decreased systemic inflammation. These results are consistent with other studies published previously. In one study in carrageenan's inflammation model in rat, it was shown that MO oil significantly prevented edema which is an indicator of inflammation in this model.¹⁴ In another study it was reported that MO has important anti-inflammatory effects in ischemic brain injury model.²²

Our findings showed that lipid profile had favorable changes after MO supplementation. After 2 months of MO supplementation HDL-c was significantly increased and LDL-c, TC and TG were significantly decreased in the intervention group. According to the findings of Lee et al study it was shown that the MO supplementation significantly decreased the serum concentrations of TG, LDL/VLDL cholesterol in insulin resistant obese rats

under high-fat diet. This study also showed that MO supplementation could alleviate hyperglycemia, insulin resistance and simultaneously lead to significant reduction in non-esterified fatty acids. PPAR- γ expression revealed that all of these beneficial effects could be partly attributed to this fundamental gene in lipid metabolism.²⁸ In another study MO oil led to reduction in SREBP-1c gene which is ensued with fatty acid synthesis reduction.²⁹ Therefore MO may be beneficial in CSA patients due to its lipid lowering effects.

The exact functional mechanism of MO with respect to oxidative stress, inflammation and lipid profile is unclear. However, rosmarinic acid which is one of the major polyphenolic components in MO has been reported to reduce local inflammation (carrageenan-induced paw edema model in the rat) and systemic inflammation (rat liver ischemia-reperfusion (I/R) and thermal injury models). In the liver I/R model, rosmarinic acid significantly reduced serum concentration of transaminases (AST and ALT) and LDH. In the thermal injury model, rosmarinic acid led to the significant reduction in multi-organ dysfunction markers (liver, kidney, lung) by modulating NF- κ B and metalloproteinase-9.³⁰ Moreover, rosmarinic acid can prevent oxidative damage.³¹ Furthermore, it has been reported that MO is rich in flavonoids¹⁷, and these flavonoids may have anti-inflammatory activity,³² as well as antioxidant and lipid lowering effects.^{33,34} Other components in MO which have antioxidative activity are phenolic acids, terpenes and caffeic acids.³¹ Therefore, it is suggested that the beneficial effect of MO supplementation on biomarkers of oxidative stress, inflammation and lipid profile is related to the presence of such

beneficial components.

Conclusion

The present study showed that MO supplementation has beneficial effects on MDA, PON1, hs-CRP and lipid profiles in patients with CSA. However, further investigations are required to confirm these results.

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AUTHOR DISCLOSURES

No conflict of interests to declare.

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