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The effects of a *Melissa officinalis* L. based product on metabolic parameters in patients with type 2 diabetes mellitus: A randomized double-blinded controlled clinical trial

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Abstract:
Background: Diabetic patients are at increased risk for coronary artery disease. Since phytotherapy has been greatly common, finding safe and effective treatments is of importance. This study aimed to evaluate the effects of a *Melissa officinalis* L. based product (MO) in patients with type 2 diabetes.
Methods: A randomized double-blinded controlled study was conducted with 37 dyslipidemic diabetic patients, assigned to either MO or placebo (P) groups receiving two 500 mg capsules daily for 3 months. Finally, 32 cases completed the study and were included in the analysis; MO (n=16) and P (n=16).
Results: Safe and significant effects in terms of decreasing the serum level of triglyceride (TG) in all patients after 2 months (p-value=0.02) and in patients with higher baseline serum levels of TG (TG≥200 mg/dl) after 3 months (p-value=0.04) were shown in the MO group. However, no metabolic significant changes were seen compared to the control group. Significant decrease in both systolic and diastolic blood pressure from baseline values were also found in patients with higher systolic blood pressure (SBP≥130 mmHg) (p-value=0.02) and those with higher diastolic blood pressure (DBP≥85 mmHg) (p-value=0.02) in the MO group.
Conclusion: This study showed that MO might be safe and beneficial in decreasing the serum TG level in dyslipidemic diabetic patients. Although, larger long-term studies are required.

Keywords: Diabetes mellitus, Dyslipidemia, Lemon balm, *Melissa officinalis* L., *Rosa damascena* Mill., Traditional Persian Medicine

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Introduction

Diabetes and Cardiovascular diseases are the most growing health challenges worldwide with greatest mortalities and morbidities [1–3]. Furthermore, patients with type 2 diabetes Mellitus (T2DM) are at increased risk of coronary artery disease due to the presence of risk factors described as diabetic dyslipidemia; a combination of elevated serum levels of triglycerides (TGs) and decreased high-density lipoprotein (HDL) cholesterol levels

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associated with almost normal level of low-density lipoprotein (LDL) cholesterol. Since the insulin resistance and the oxidative stress has been found to be the major pathophysiologic factors causing diabetic dyslipidemia [4, 5], exploring newer and safer herbal treatments to manage these abnormalities has become more important.

Moreover, diabetic patients are using complementary and alternative medicine therapies such as herbal supplements without disclosing their physicians [6]. The effectiveness and safety of herbalism along with the knowledge of drug and herb interactions is controversial. Thus, conducting high quality clinical trials would be the first steps to find appropriate evidence as many clinical trials have been performed to investigate the effectiveness and safety of these interventions in T2DM [6–10].

Mellissa officinalis L. (M. officinalis), known as lemon balm, is a famous traditional herbal medicine with a lemon like fragrance. It is a perennial plant of the Lamiaceae family, native of the eastern Mediterranean region and west Asia, which is widely cultivated all over the world [10, 11]. Lemon balm has been traditionally used as a sedative, anti-spasmodic, brain and cardiac tonic for centuries [12] as Rhazes (865–925 AD) [13], Avicenna (980–1037 AD) [14], and *Aghili Shirazi* (1670–1747 AD) [15] described its various medical effects indeed on the gastrointestinal (GI) tract and the cardiovascular system [16, 17].

Apart from a variety of documented remarkable pharmacologic properties [18] such as sedation, anti-anxiety, anti-depression [19–21], anti-Alzheimer [22], anti-viral [12] and beneficial GI and cardiac effects [23, 24], M. officinalis has shown potent anti-oxidative effects [25, 26]. It contains abundant phenolic compounds [18] which would explain its potent metabolic effects in the literature [27, 28]. Nevertheless, few relevant clinical studies have been found in the literature [10, 29, 30].

Furthermore, like other traditional systems in the world, famous herbs are mostly prescribed in combined formulations with different medicinal plants for specific purposes. Indeed, the combination enhances the biologic effect and reduces or eliminates the probable side effects of the natural products [31, 32]. According to Traditional Persian Medicine (TPM) textbooks, the formulations of M. officinalis for GI and cardiac tonic purposes are frequently combined with various herbs, mostly *Rosa damascena* Mill. (*R. damascena*) or Damask rose [33]. This herb is a famous astringent with cardiac, brain, and GI tonic effects which improves the M. officinalis efficacy and protects the GI tract, alleviating probable adverse reactions [34–36]. In addition, it has shown beneficial anti-hyperlipidemic [37], anti-oxidative [38] and hepatoprotective effects [39] with reducing effects of postprandial hyperglycemia in diabetes [40].

The aim of this study was to determine the efficacy and safety of a M. officinalis based product (in combination with *R. damascena*) on metabolic parameters in patients with T2DM in a randomized double-blinded controlled study.

Material and methods

Plant material

The aerial parts of M. officinalis were collected from Semnan province located in the north and center of Iran, in spring 2016. The flowers of *R. damascena* were also collected from Azarshahr, Azarbayejan province of Iran at the same time. Taxonomic identification was confirmed by M. Kamalinejad. Voucher specimens of the plants have been recorded respectively as 8022-SBUM and 8013-SBUM in the Central Herbarium, Faculty of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Plant extraction and drug preparation

Leaves of M. officinalis were washed and dried in the room temperature and then the aqueous extract was obtained by the infusion method. One hundred grams of the leaves was placed into a beaker and one liter of boiling water was added. The mixture was left for around 6 h. Later, the contents of the beaker were filtered and condensed on bain-marie. Finally, 10 g of the dry extract was obtained. The aqueous extract of *R. damascena* flowers was obtained by the same method. Twenty grams of the dry extract was obtained from 100 grams of flowers.

Capsules of the intervention were prepared by adding 150 mg of *R. damascena* extract to 350 mg of M. officinalis dried extract. The placebo (P) capsules were filled by 500 mg of maize starch. The appearance, color and size of the drug and P capsules were identical.

Standardization of the product

The Folin-ciocalteu method was utilized to determine the total phenolic content of the product. Distilled water was added to one mg/ml of the crude extract to make up to 3 mL, and then mixed with 0.5 mL of Folin-ciocalteu reagent for 10 min. Then, 4 mL sodium carbonate was added. The final mixture was put in darkness for a further 30 min. Absorbance was measured to be 765 nm. The total phenolic content was calculated from the calibration curve. The total phenol percentage of gallic acid per mg dry weight was the result [41]. Aluminium chloride reagent was separately used for determining the total flavonoid content of the product. The mixture of 2.5 mL (20 mg/ml) of the ethanolic solution of the the reagent with 2.5 mL of the diluted product (400 μg/ml) was incubated in room temperature. After 40 min, the absorbance was measured to be 415 nm and the total flavonoid content was calculated from the calibration curve. The total flavonoid percentage of rutin per mg dry weight was the result.

Study design

A pilot study with two parallel arm, as a double-blinded randomized controlled clinical trial was conducted in the Diabetes Clinic of the Valiasr hospital of Tehran University of Medical Sciences. Eligible diabetic patients meeting the inclusion criteria were recruited from January 2016 till March 2017.

Ethical issues

Written informed consent was obtained from all participants before enrollment. The study was approved by Shahid Sadoughi University of Medical Sciences (SSUMS) Medical Ethics Committee (IR.SSU.REC.1394.61). Also, the study protocol was registered at the Iranian Registry of Clinical Trials (registration No.: IRCT2015112625251N1). The implementation protocol was in accordance with the declaration of Helsinki (October 2013).

Participants

Consecutive patients with T2DM who met the inclusion criteria were recruited. The inclusion criteria were: Serum TG levels between 150 and 500 mg/dl; Serum HbA1c $\leq$ 8.5%; age between 30 and 65 years; not taking anti-lipid medications or supplements during the past 3 weeks; no change in their current anti-glycemic treatment during the last 3 months; and not being diagnosed with cardiac, renal or hepatic chronic diseases. The exclusion criteria were severe systemic, renal or hepatic diseases; uncontrolled hypertension; malignancies; psychological disorders; pregnancy or lactation; smoking or addiction; use of any new drug affecting blood glucose or lipid levels.

Intervention

At the first step, consecutive patients were assessed and eligible ones were randomly assigned to either the P group or the M. officinalis based product group by the random allocator software with blocking method (size of blocks: 4). The study was double blinded and allocation concealment was performed.

In the M. officinalis group, the subjects received two capsules (each of them containing 350 mg M. officinalis and 150 mg *R. damascena* aqueous extracts) after breakfast, while the P group took two capsules (each of them containing 500 mg of maize starch) at the same time.

Both groups received identical capsules in appearance, color and odor which were monthly delivered. The participants were requested to have monthly interviews for 3 months and were also advised to use their current diabetic medications without any change in their usual diet and physical activities.

Data collection and measurements

A trained physician recorded all the measures in predesigned questionnaires of demographic data, anthropometric measures and clinical parameters of patients at the baseline and follow up interviews. Patients were

interviewed after each month of intervention to check their compliance, subjective symptoms, systolic blood pressure (SBP), diastolic blood pressure (DBP) and fasting lipid profile.

Anthropometric measures were recorded before and after the intervention period. For example, the weight (by light clothing) via a weighting scale (d~0.1 kg), the height (standing position without shoes) to the nearest 0.5 cm by a stadiometer (Seca, Hamburg, Germany), and the waist circumference (WC) to the nearest 0.1 cm by an inelastic tape measure (Seca, Hamburg, Germany) in the mid-way of the inferior margin of the rib cage and iliac crest were measured. Hip circumference was also measured in the iliac crest margin.

After an overnight 12 h of fasting, venous blood samples were collected from each patient at the initial and follow-up visits. All biochemical parameters were measured before and after 3 months. The lipid profile was checked monthly. The samples were centrifuged at the room temperature at 3000 rpm for 10 min to separate the serum. Serum total cholesterol (TC), TG, low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) levels were measured by enzymatic colorimetric analysis (Pars Azmun commercial kits, Karaj, Iran). Moreover, the percentage of glycated hemoglobin (HbA1c) was determined by high performance liquid chromatography (HPLC). Fasting blood sugar (FBS), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALK-P), Urea, Creatinine (Cr) and Uric acid levels were measured by kinetic UV and Jaffe colorimetric analysis. All the tests used standard enzymatic kits produced by BIONIC Company, Tehran, Iran with Hitachi Auto-analyzer device, Japan.

To survey the patients' diet in terms of daily intake of energy and macronutrients, two 24 h dietary-recall questionnaires were filled out at each interview during the study. By Nutritionist IV software (First Databank, San Bruno, CA, USA) adjusted for Iranian foods nutrition, nutrient analysis was implemented.

Statistical analysis

Statistical analysis was performed using Statistical Package for the Social Sciences software for Windows, version 16 (Armonk, NY: IBM Corp.). Kolmogorov–Smirnov test was used to determine the normal distribution of data. For demographic data and within and between groups comparison, Chi-square tests, Independent Student's t-test and Paired sample t-test analysis were performed. p Value <0.05 was considered significant statistically.

Safety measures

All participants were asked about possible allergic or adverse reactions at follow-up interviews. Questionnaires of adverse effects such as GI, dermatologic, neurologic and respiratory complains were filled with open-ended questions.

Results

The total phenolic content in each capsule of the product (containing 350 mg of M. officinalis aqueous extract and 150 mg of *R. damascena* aqueous extract) was equivalent to 75.45 mg according to gallic acid and the total flavonoid content was 9.55 mg, according to rutin.

Of 92 assessed patients for eligibility, 37 patients were enrolled and allocated to M. officinalis and P groups. Finally, 32 cases completed the study and were included in the analysis. The study flow-chart is presented in Figure 1.

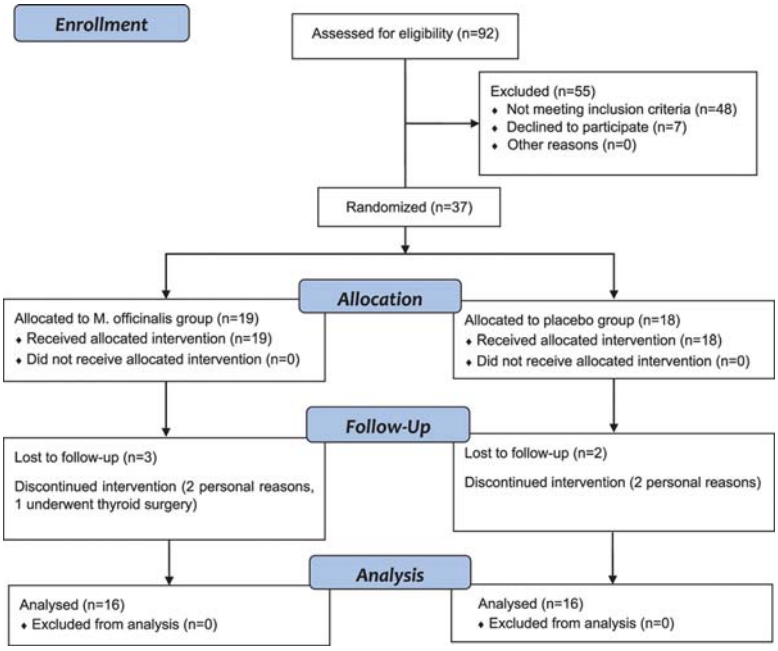

Figure 1: Flow chart of patients' participation.

There were no significant differences regarding baseline characteristics of the two groups, except for urea serum levels (Table 1). Except for baseline total energy and carbohydrate intake, there were no other significant differences between the groups, regarding dietary nutrient and anti-oxidant vitamins intake. Furthermore, no confounding change in dietary intake was detected during the trial in two groups. It is notable that during the intervention, the total dietary fat intake was reduced within each group. However, the total energy and carbohydrate intake did not change (Table 2).

Table 1: Baseline characteristics of patients in Melissa and placebo groups.

Variables	Groups		p-Value
	MO	Placebo	
Sex:			0.48[a]
-Men	7(43.8%)	9(56.2%)	-
-Women	9(56.2%)	7(43.8%)	-
Disease duration, year	7.3 (5.4)	6.9 (3.8)	0.82
Age, year	54.9 (5.8)	53.7 (6.6)	0.61
Weight, kg	80.5 (13.2)	73.7(8.8)	0.10
WC, cm	101.5(8.9)	97.9 (4.8)	0.17
SBP, mmHg	137.2 (18.6)	131 (14.6)	0.31
DBP, mmHg	86.2 (12)	81.1(8.8)	0.18
FBS, mg/dl	140.1(42)	141.3(33.2)	0.93
HbA1C, %	7.5 (1.6)	7.1 (0.8)	0.49
TG, mg/dl	258.8(101.6)	229.2 (62.9)	0.33
LDL-C, mg/dl	119.2(30.7)	112.8(25.6)	0.53
HDL-C, mg/dl	45.6(11.5)	42(7.2)	0.30
AST, IU/L	21.7(9.3)	24.6(8.3)	0.37
ALT, IU/L	26.9(12.6)	32.3(16.4)	0.30
Men	35.6(12.7)	34.4(18.5)	0.89
Women	20.1(7.7)	29.6(14.3)	0.11
Creatinine, mg/dl	1.04(0.15)	1.01(0.16)	0.66
Urea, mg/dl	33.2(7.6)	27.4(6.7)	0.03
Uric acid, mg/dl	5.2(0.9)	5.2(1.2)	0.82

[a]p-Value reported based on Chi-Square test
Quantitative data represented as Mean (SD), p-value reported based on Independent -Samples T test, Qualitative Data reported as frequency (Percentage).
MO, Melissa Officinalis; WC, Waist Circumference; SBP, Systolic blood pressure; DBP, Diastolic blood pressure; TG, triglyceride; HbA1C, hemoglobin of A1C; TC, total cholesterol; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALK-P, alkaline phosphatase, FBS, Fasting Blood Sugar.

Table 2: Patients dietary intake, before and after the intervention.

Variables		Groups MO Mean ± SD	p-Value[a]	Placebo Mean ± SD	p-Value[a]	p-Value[b]
Energy, Kcal/day	Before	1827 ± 115.16	0.795	1985 ± 147.42	0.053	0.005
	After	1836 ± 169.11		1913 ± 108.02		0.162
Protein, g/day	Before	47.88 ± 15.42	0.185	53.05 ± 11.32	0.214	0.362
	After	22.81 ± 5.87		25.00 ± 4.93		0.105
Fat, g/day	Before	86.84 ± 8.33	0.026	91.10 ± 8.40	0.010	0.208
	After	74.25 ± 15.02		80.28 ± 16.17		0.316
Carbohydrates, g/day	Before	216.21 ± 33.50	0.068	245.47 ± 32.63	0.922	0.034
	After	244.31 ± 42.86		245.81 ± 27.41		0.913
Beta-carotene, µg/d	Before	878.91 ± 577.12	0.733	581.9 ± 483.05	0.206	0.184
	After	1064.1 ± 1969.9		1024 ± 692.72		0.944
Vitamin E, mg/-day	Before	25.94 ± 5.53	0.533	28.36 ± 5.89	0.607	0.303
	After	24.15 ± 6.81		25.96 ± 6.54		0.481
Vitamin C, mg/-day	Before	75.69 ± 31.86	0.467	70.10 ± 26.48	0.840	0.644
	After	67.03 ± 32.62		69.63 ± 51.07		0.873

MO: Melissa Officinalis.
[a]p-Value reported based on Paired- samples T test.
[b]p-Value reported based on Independent-Samples T test.

Anthropometric changes after 12 weeks of intervention were not significant; nevertheless, mean values of hip circumferences decreased in the M. officinalis group, while it had no change in the P group (mean differences: 5.30 cm versus 0.009 cm). The systolic and DBPs had also no significant changes whereas considering the cut-off points of the ATP III guideline [43] significant decreases in both systolic and diastolic pressures from baseline values were found in patients with higher systolic blood pressures (SBP≥130 mmHg)(p-value=0.02) and in those with higher diastolic pressures (DBP≥85 mmHg)(p-value=0.02) in the M. officinalis group (Table 3).

Table 3: Comparison of effects on anthropometric parameters and blood pressures between groups.

Variables	Week	Groups MO Mean(SD)	Placebo Mean(SD)	[a]p-Value
WC, cm	0 week.	101.5(8.9)	97.9(4.8)	0.17
	12 week.	101.9(8)	98(4.8)	0.12
Mean (SD) of differences		0.28(2.3)	−0.15(0.9)	0.50
HC, cm	0 week.	107.3(17.2)	101.1(7.2)	0.29
	12 week.	102(5.4)	101.1(7.2)	0.76
Mean (SD) of differences		5.3(16.1)	−0.009(0.4)	0.32
weight, kg	0 week.	80.5(13.2)	73.7(8.8)	0.10
	12 week.	79.7(13.4)	73.7(9)	0.16
Mean (SD) of differences		0.7(1.5)	−0.01(1.1)	0.11
SBP, mmHg	0 week.	137.2(18.6)	131(14.6)	0.31
	12 week.	130(14.2)	127.5(16.4)	0.64
Mean (SD) of differences		5.5(16.7)	3.5(14.4)	0.72
DBP, mmHg	0 week.	86.2(12)	81.1(8.8)	0.18
	12 week.	81.3(8.2)	82.9(9.8)	0.63
Mean (SD) of differences		3.3(10.0)	−1.8(9.6)	0.15
SBP≥130 mmHg	0 week.	145.5(13)	142(8.1)	0.34
	12 week.	133.7(12.3)	137.2(14.4)	0.57
	[b]p-value	0.02	0.44	-
Mean (SD) of differences		11.8(13.8)	4.7(17.7)	0.34

DBP≥85 mmHg	0 week.	93.6(8)	87.9(2.3)	0.03
	12 week.	84.3(9.1)	87.4(9.3)	0.50
	[b]p-value	0.02	0.89	-
Mean (SD) of differences		9.2(8.1)	0.44(9.6)	0.07

[a] p-Value reported based on Independent -Samples T test.
[b] p-Value reported based on Paired - Samples T test.
WC, Waist Circumference; MO, Melissa officinalis extract; SD, Standard Deviation; HC, Hip Circumference; SBP, systolic blood pressure; DBP, Diastolic Blood Pressure; Mean (SD) of differences, mean of (baseline values – values after 12 weeks of intervention)

After 12 weeks of intervention, in the M. officinalis group the serum mean values of TG decreased more than the P group (mean differences: 44.27 vs. 10.93 mg/dl) and in patients with higher baseline serum levels of triglyceride (TG≥200 mg/dl) decreased significantly (p-value=0.04). Moreover, the M. officinalis intake decreased the baseline serum TG levels significantly even after 8 weeks (p-value=0.02). Nevertheless, there were no significant changes in the mean values of all biochemical parameters between the M. officinalis and P groups.

Furthermore, the mean values of the FBS and HbA1c in the M. officinalis group decreased rather more than the P group indicating a slightly decrease of the overall serum glucose levels during the 3 months of intervention (Table 4 and Table 5).

Table 4: Comparison of effects on lipid profile before and after 8 week and 12 week of the intervention in Melissa & placebo groups.

Variable	MO					Placebo					p^d
	0 week	8 week	P^b	12 week	P^c	0 week	8 week	P^b	12 week	P^c	
TC	208(44)	205.7(54.8)	0.41	209.6(56.6)	0.65	193.1(30.9)	217.3(35.9)	0.68	194.4(28)	0.88	0.40
Mean (SD) of differences	_	_	_	−1.35(26.3)	_	_	_	_	−1.62(34.3)	_	0.98
LDL-C	119.2(30.7)	121.4(40.8)	0.71	114(31.7)	0.18	112.8(25.6)	121.7(22.8)	0.40	105.9(29.7)	0.17	0.45
Mean (SD) of differences	_	_	_	7.64(24.6)	_	_	_	_	8.19(22)	_	0.94
HDL-C	45.6(11.5)	41.8(8.9)	0.54	43.9(11.9)	0.38	41,9(7.2)	41.7(9.1)	0.52	41.6(6.3)	0.78	0.49
Mean (SD) of differences	_	_	_	0.65(7.8)	_	_	_	_	0.24(4.5)	_	0.85
TG	258.8(101.6)	211.9(86.3)	0.02[a]	233.1(103.3)	0.07	229.2(62.9)	224.7(75.2)	0.49	214.6(78.2)	0.33	0.57
Mean (SD) of differences	_	_	_	44.2(85.6)	_	_	_	_	10.9(55.6)	_	0.22
TG≥200	339.7(81.5)	269.2(75.1)	0.01[a]	257.8(109.8)	0.04[a]	258.4(52.9)	226(82.3)	0.28	237.3(83.6)	0.29	0.64
Mean (SD) of differences	_	_	_	81.8(93)	_	_	_	_	21.08(63.7)	_	0.10

Quantitative data represented as Mean (SD).
[a] Significant at <0.05
[b]p-Value reported based on within groups Paired- samples T test (before –after 8 weeks of intervention)
[c] p-Value reported based on within groups Paired- samples T test (before –after 12 weeks of intervention)
[d]p-Value reported based on Independent Sample T- test (between groups after 12 weeks of intervention).
Mean (SD) of differences, mean of (baseline values – values after 12 weeks of intervention); TG, triglyceride; TC, total cholesterol; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol;

Table 5: Comparison of effects before and after 12 week of the intervention in Melissa & placebo groups.

Variable	MO			Placebo			MO vs. placebo	Mean (SD) of differences		p-Value[c]
	0 week	12 week	p^b	0week	12 week	p^b		MO	Placebo	
FBS	140.1(50.9)	128.5(38.6)	0.90	141.1(33.2)	137.5(32.8)	0.59	0.51	1.3(43.1)	2.9(29.8)	0.90
HbA1C	7.47(1.6)	7.19(1.3)	0.34	7.15(0.7)	6.99(0.99)	0.22	0.53	0.27(1.1)	0.23(0.7)	0.90
ALT	26.8(12.6)	24.9(13/3)	0.44	32.3(16.4)	32.7(17.9)	0.98	0.30	1.8(9.5)	0.04(8.8)	0.58
Male	35.6(12.7)	39.2(14.6)	0.97	34.4(18.5)	37(20.8)	0.73	0.83	0.6(14.3)	-1.0(7.4)	0.79
Female	20.1(7.7)	17.2(6.4)	0.04[a]	29.6(14.3)	28.7(15)	0.83	0.06	2.8(3.7)	1.0(10.6)	0.64
AST	21.7(9.2)	20.3(6.1)	0.49	23.3(6.4)	22.8(9.3)	0.75	0.39	1.3(7.8)	0.4(5.5)	0.73
Male	23.8(11)	23.7(6.5)	0.98	24.14(6.2)	25.85(8.7)	0.40	0.62	0.10(10.2)	-1.71(5)	0.68
Female	20.11(7.9)	17.77(4.5)	0.26	22.57(6.9)	19.88(9.6)	0.24	0.57	2.33(5.8)	2.68(5.5)	0.90
ALK-P	172.7(37)	164.4(38.5)	0.33	162.1(39.7)	162.6(41.6)	0.34	0.90	6.0(23.8)	3.5(19.4)	0.73

Urea	33.2(7.6)	33.2(6.4)	0.91	27.4(6.7)	27.4(8.5)	0.27	0.04[a]	0.02(6.5)	0.6(6.2)	0.80
Cre	1.04(0.16)	1.05(0.12)	0.75	1.01(0.16)	0.97(0.17)	0.08	0.11	−0.01(0.1)	0.03(0.1)	0.37
Uric acid	5.2(0.9)	5.2(1.2)	0.48	5.3(1.2)	5.6(1.4)	0.83	0.50	−0.08(0.7)	−0.1(1.1)	0.37

Quantitative data represented as Mean (SD).
Mean (SD) of differences: mean of (baseline values – values after 12 weeks of intervention);
[a] Significant at <0.05
[b] p-Value reported based on within groups Paired- samples T test (before –after 12 weeks of intervention)
[c] p-Value reported based on Independent Sample T- test (between groups after 12 weeks of intervention).
FBS, fasting blood sugar; HbA1c, glycosylated hemoglobin; AST, liver enzymes aspartate aminotransferase; ALT, alanine aminotransferase; ALK-P, alkaline phosphatase; Cre, creatinine

There were no differences in the results between the genders, whereas a significant decrease in the serum levels of alanine transaminase (ALT) after 3 months of the intervention was seen in female patients receiving the M. officinalis extract (p-value=0.04). Comparison of the two groups showed that the decrease was also notable (p-value=0.06) (Table 5).

Safety and tolerability

No serious side effects were reported during the study in each of the groups. However, there were some minor reports in the M. officinalis group such as one case of mild transient dizziness, two cases of calmness, two cases of slightly increase in appetite, and one case of decreased appetite. Also, two patients in the P group experienced nausea.

In addition, there were no differences between the groups regarding the mean values of the serum liver enzymes and creatinine after 12 weeks of the intervention.

Discussion

To the best of our knowledge, this is the first clinical study to observe the metabolic effects of M. officinalis on patients with diabetes. In this randomized double-blinded placebo-controlled study, we found that the M. officinalis based product decreased the serum TG levels from baseline after 12 weeks almost significantly in dyslipidemic diabetic patients particularly those with higher TG serum levels (TG≥200 mg/dl); nonetheless, the effect was not significant compared to the placebo group. Although the results are not as significant as previous human studies [10, 29], they do not contradict the results of the experimental studies on animals [27, 45]. Furthermore, human clinical trials were on non-diabetic patients who had received no anti-diabetic medication. Apart from the difference of participants, the M. officinalis based product containing the aqueous extract of the plant would probably have different effects from the powder of leaves used in former human studies [10, 29].

In vitro and *in vivo* studies have shown remarkable hypoglycemic and hypolipidemic effects of M. officinalis [18, 28, 46]. The ethanol extract with a high dosage (200 mg/kg) significantly reduced the insulin resistance and plasma lipids in the obese mice [28]. In addition, the essential oil of this plant in lower doses (0.02, 0.04 mg/day/4 weeks) restored the hyperglycemia in diabetic rats [47]. Various beneficial effects on hyperlipidemia and liver enzymes have also been reported from alcoholic and essential oil extracts [28, 45, 48].

We found few animal studies on the aqueous extract of the plant. Bolkent et al. showed that the oral administration of 2 g/kg of the aqueous extract of M. officinalis for 28 days had significant anti-hyperlipidemic and hepatoprotective results in high fat diet induced rats. They also reported decreased lipid peroxide and increased glutathione levels in the liver tissues of the treated rats, indicating the anti-oxidative effect of the plant due to its flavonoid and phenolic compounds [46].

In Chung's study on diabetic mice (db/db) with the essential oil (0.015 mg/day) for 6 weeks, explicit reduction of blood glucose and TG levels with increased serum insulin levels and insulin sensitivity was shown. They not only described the anti-oxidant effect of lemon balm as a major mechanism of action, but also the beneficial altered gene expression of metabolic key enzymes and mediators in the liver and adipose tissue were detected without any liver toxicity. They documented the increased activity and gene expression of the hepatic key enzyme of glucose hemostasis (named glucokinase), with the reduction of mRNA transcription of gluconeogenic key enzymes (named glucose-6-phosphatase and phosphoenolpyruvate carboxykinase) justifying the anti-diabetic effect of the plant. Simultaneous increased gene expression of sterol regulatory element-binding protein and peroxisome proliferator-activated receptors (PPAR-γ and PPAR-α) which encode fatty acid metabolism and transportation pathways presumably explained the TGs lysis of plasma and adipose tissue [27].

Although reducing the oxidative stress would prevent the diabetic complications [5], more potential metabolic effects of this herb has been explored. In Park et al.'s study, a fraction derived from M. officinalis leaves named ALS: ALS-L1023 with angiogenesis inhibitory effect was administered to high fat diet induced obese mice. They recorded beneficial effects in decreasing the adipose tissue and body weight gain along with reduced serum and liver levels of TG. They also reported elevated mRNA levels of PPARα target enzymes of the liver, suggesting an increase in fatty acid catabolism which reduced hepatic intracellular and plasma levels of TG in addition to anti-angiogenic effects [44]. Additionally, in a recent study, properties of ALS mentioned above were confirmed along with alleviation of non-alcoholic fatty liver in female ovariectomized mice [49].

In the present study, we did not find remarkable antidiabetic and antilipidemic effects compared to controls, but further reduction of serum TG, HbA1c, hip circumference and DPBs in participants who received M. officinalis based product compared to the P group and their significant changes from baseline could be in accordance with the above noted experimental results. The remarkable decrease of ALT (as a more specific hepatic enzyme) in female participants who received the M. officinalis may confirm the mechanistic effect of the plant on gene expression of regulatory enzymes and mediators of glucose and lipid hemostasis.

As previously mentioned in animal studies, the increased gene expression of PPAR-γ and PPAR-α which are found in the liver, adipose tissue and other vital organs (such as the heart) would explain the associated alleviation of fatty liver and relative metabolic effects of natural compounds [49, 50].

Sex differences

Although a significant decrease in the baseline serum levels of ALT enzyme was found in female participants who received the extract, no obvious sex effect has yet been reported [18]. It has been shown that the extracted citral (geranial and neral volatile compounds) of M. officinalis can displace estrogenic receptors in the human cell line indeed without estrogenic activity [42].

Safety

Since there were no case of serious adverse effects either subjectively or objectively (i.e. liver and kidney laboratory tests), the safety of daily oral administration of 700 mg M. officinalis and 300 mg *R. damascena* aqueous extracts for 12 weeks in diabetic patients was shown. Previously, various studies have confirmed the safety of these plants in higher doses in animals [20, 51] and human [18, 34, 52, 53].

Limitations

This study was conducted with a small sample size and a greater number of participants are needed to reach a more definite conclusion. Moreover, the short term study was another limitation and longer term follow-ups are needed. Secondly, the translation of high dosages of plant extracts used in experimental studies on smaller animals with higher metabolic rates to human with slower and more complicated metabolism is a great challenge and requires more attention. Thirdly, although HbA1c may provide an estimation of 3 months of mean values for blood glucose levels, there is no definite marker for estimating the serum lipid mean levels, specifically for TG with tremendous serum levels fluctuations. Fourthly, postprandial Chylomicron metabolism and TG clearance is impaired in insulin resistance and results in a dysregulated fasting hyperlipidemia in diabetic patients compared to healthy individuals [4]. Fifthly, the observed effects cannot be attributed to M. officinalis, merely. According to TPM references, M. officinalis is the main and basic constituent of the used formulation. However, lower doses of *R. damascena* (which has several beneficial biological effects for patients with T2DM) should be added to the product. Therefore, future studies on each constituent of the used formulation will make more knowledge on the efficacy of these plants on T2DM and dyslipidemia. Finally, we did not survey the sonographic liver changes of the participants. It could help us for better understanding about hepatoprotective properties of M. officinalis.

Conclusion

In the present pilot clinical trial on dyslipidemic diabetic patients, prescription of a M. officinalis based product for 12 weeks showed beneficial effects in terms of decreasing TG serum levels from baseline. Although,

changes of other metabolic parameters were not significant. Larger clinical trials on pre-diabetic patients without chronic hyperinsulinemia and its metabolic effects on natural hepatic cellular bio-mechanisms are required. Furthermore, longer term studies of adjuvant therapies with anti-lipid medications is necessary to observe dyslipidemia resolution.

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