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The Effect of *Cumin cyminum* L. Plus Lime Administration on Weight Loss and Metabolic Status in Overweight Subjects: A Randomized Double-Blind Placebo-Controlled Clinical Trial

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Abstract

Background: Limited data are available regarding the effects of combined administration of *Cumin cyminum* L. and lime on weight loss and metabolic profiles among subjects with overweight subjects.
Objectives: The current study aimed to assess the effects of combined administration of *Cumin cyminum* L. and lime on weight loss and metabolic profiles among subjects with overweight.
Patients and Methods: This randomized double-blind placebo-controlled clinical trial was conducted on 72 subjects with overweight, aged 18 - 50 years old. Participants were randomly divided into three groups: Group A received high-dose *Cumin cyminum* L. and lime capsules (75 mg each, n = 24), group B low-dose *Cumin cyminum* L. and lime capsules (25 mg each, n = 24) and group C placebos (n = 24) twice daily for eight weeks.
Results: After eight weeks of intervention, compared with low-dose *C. cyminum* L. plus lime and placebo, taking high-dose *C. cyminum* L. plus lime resulted in significant weight loss (in the high-dose group: -2.1 ± 1.7 vs. in the low-dose group: -1.2 ± 1.5 and in the placebo group: + 0.2 ± 1.3 kg, respectively; P < 0.001) and body mass index (-0.8 ± 0.6 vs. -0.5 ± 0.5 and +0.1 ± 0.5 kg/m^2, respectively; P < 0.001). In addition, administration of high-dose *C. cyminum* L. plus lime compared with low-dose *C. cyminum* L. plus lime and placebo, led to a significant reduction in fasting plasma glucose (FPG) (P < 0.001) and a significant rise in quantitative insulin sensitivity check index (QUICKI) (+ 0.02 ± 0.02 vs. + 0.01 ± 0.02 and 0.01 ± 0.01, respectively; P = 0.01). Moreover, a significant decrease in serum triglycerides (-14.1 ± 56.2 vs. +13.9 ± 36.8 and + 10.6 ± 25.1 mg/dL; respectively; P = 0.03), total-cholesterol (-18.4 ± 28.6 vs. +8.6 ± 28.5 and -1.0 ± 24.8 mg/dL; respectively; P = 0.004) and low density lipoproteins- (LDL)-cholesterol levels (-11.8 ± 20.7 vs. +6.5 ± 23.2 and -2.9 ± 20.4 mg/dL, respectively; P = 0.01) was observed following the consumption of high-dose *C. cyminum* L. plus lime compared with low-dose *C. cyminum* L. plus lime and placebo.
Conclusions: Results of the current study indicated that taking high-dose *C. cyminum* L. plus lime for eight weeks among subjects with overweight had beneficial effects on weight, BMI, FPG, QUICKI, triglycerides, total-cholesterol and LDL-cholesterol levels.

Keywords: Lim, Overweight, Insulin Resistance, *Cuminum cyminum* L

1. Background

Obesity is an important worldwide public health problem resulted from the accumulation of excessive food energy intake into visceral fat (1). In addition, a link between obesity and insulin resistance, increased lipid profiles and oxidative stress is documented (2, 3). The prevalence of obesity was reported 18.5% among Iranian adults (4). Previous studies reported that obesity is associated with a number of co-morbidities including type 2 diabetes mellitus (T2DM), hypertension, cardiovascular diseases such as heart failure and coronary heart disease (5), osteoarthritis and liver steatosis (6).

Nowadays there is an increased demand for herbal drugs to treat obesity instead of using synthetic ones, which may have adverse effects and are more expensive. *Cuminum cyminum* L. (cumin) from Apiaceae family is native to the Mediterranean region and is used in traditional medicine including stomach disorders and diarrhea (7). Lime is a tree from the citrus family (Rutaceae) with diverse phytochemicals, including polyphenols and terpenes (8). Authors previous study on subjects with overweight revealed that taking 300 mg *C. cyminum* L. for eight weeks results in significant decreases in weight, body mass index (BMI), insulin and homeostatic model assessment-Beta cell function (HOMA-B), but did not influence lipid pro-

files and biomarkers of oxidative stress (9). In addition, another study by Asnaashari et al. (10) reported a significant reduction in body weight following the consumption of lime essential oil in mice after 45 days. D-limonene administration also resulted in significant decreases in fasting blood glucose (FPG), serum triglycerides, low density lipoproteins- (LDL)-cholesterol levels and a significant increase in high density lipoproteins- (HDL)-cholesterol in obese mice (11).

Cuminum cyminum L. and lime intake may decrease weight through inhibiting serotonin re-uptake in synaptic clefts (12), increased lipolysis and reduced appetite (13). However, these findings might in turn suggest the importance of cumin plus lime co-supplementation to treat patients with obesity, the current study hypothesized that cumin plus lime co-administration may contribute to management of obesity and metabolic status. Authors are aware of no reports evaluating the effects of *C. cyminum* L. and lime co-administration on markers of insulin resistance, lipid concentrations and biomarkers of oxidative stress in subjects with overweight and obesity.

2. Objectives

The present study aimed to investigate the effects of *C. cyminum* L. and lime co-administration on weight loss, markers of insulin resistance, lipid concentrations and biomarkers of oxidative stress in subjects with overweight and obesity.

3. Patients and Methods

3.1. Participants

The current randomized double-blind clinical trial included 72 subjects aged 18 - 50 years with overweight or obesity (BMI $\geq$ 25 kg/m^2) from March 2015 to May 2015 in Kashan, Iran. The exclusion criteria were as follows: individuals aged 18 - 50 years, BMI $<$ 25 kg/m^2, subjects with hypertension, thyroid, diabetes or cardiovascular disorders, pregnancy and lactation and the use of hormonal or anti-obesity medications. Informed consent form was taken from all subjects and the protocol was approved by the ethics committee of Kashan University of Medical Sciences (KUMS). The present study was carried out according to the Helsinki declaration guidelines (9). The current trial is recorded in the Iranian registry of clinical trial (http://www.irct.ir: IRCT2015030812438N10) (9).

3.2. Study Design

After stratification for BMI ($\geq$ 30 kg/m^2) and age ($\geq$ 40 y), subjects were randomly assigned to three groups receiving: 1) high-dose *C. cyminum* L. and lime capsules (75 mg each, n = 24); 2) low-dose *C. cyminum* L. and lime capsules (25 mg each, n = 24) and 3) placebos (n = 24) twice daily for eight weeks. Individuals were requested to consume lime before, and cumin after lunch and dinner. *C. cyminum* L., lime and placebo capsules were produced by department of the formulation of Barij medicinal plants research center (Barij Essence Pharmaceutical, Kashan, Iran) (9). To produce cumin and lime hard capsules, at first cumin and lime essential oil was solved in poly ethylene glycol 4000 in 25°C, and then it was en-coated into a capsule (9). *Cumin cyminum* L. and lime capsules were analyzed in Barij Essence laboratory, Kashan, Iran by Gas chromatography spectrometry method (9). The appearance of the placebo capsules, such as color, shape, size, and packaging were identical to cumin and lime (9). The randomized allocation sequence and participants' assignment to the groups were done by a trained nutritionist in the diet therapy clinic. Compliance to the consumption of cumin, lime and placebo capsules throughout the study was monitored through asking participants to bring the medication containers and receiving short message on cell phone by the subjects (9). All participants completed three-day dietary records and three physical activity records during intervention (9). Both dietary and physical activity records were completed in weeks two, four and six of the intervention. Physical activity was described as metabolic equivalents (METs) in hour/day. To calculate the METs for each subject, the times (hour/day) reported for each physical activity was multiplied by its related METs coefficient by standard tables (14). The current study used Nutritionist 4 software (First Data Bank, San Bruno, CA, USA) to determine the macro-and micro-nutrient intakes based on dietary records (9).

3.3. Endpoints and Sample Size Estimation

The primary endpoint of the current study was weight and BMI. The secondary endpoints of the current study included glucose homeostasis parameters, lipid profiles and biomarkers of oxidative stress.

3.4. Assessment of Anthropometric and Clinical Measures

Weight was determined by a digital scale (Seca, Hamburg, Germany) to the nearest 0.1 kg (9). Height was measured by a non-stretched tape measure (Seca, Hamburg, Germany) to the nearest 0.1 cm (9). Weight and height were quantified according to standard protocols at the onset and end of the study in the diet therapy clinic by a

trained nutritionist in an overnight fasting status without shoes in a minimal clothing state (9). BMI was determined as weight in kilogram divided by height in meters squared. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were quantified via a sphygmomanometer (ALPK2, Zhejiang, China). All anthropometric and clinical measures were performed by a trained nutritionist. Furthermore, the nutritionist was blinded to the randomization assignments.

3.5. Biochemical Assessment

At the beginning of the study and after the eight-week intervention, blood samples (10 mL) were took at Kashan reference laboratory in an early morning after an overnight fast (9). The current study used available enzymatic kits (Pars Azmun, Tehran, Iran) to quantify FPG, serum triglycerides, total-cholesterol, LDL-cholesterol and HDL-cholesterol (9). All inter- and intra-assay coefficients of variations (CVs) for FPG and lipid concentrations were less than 5%. Serum insulin was determined using the enzyme-linked immunosorbent assay (ELISA) kit (DiaMetra, Milano, Italy) with intra- and inter-assay CVs 2.3% and 5.2%, respectively. The homeostatic model of assessment for insulin resistance (HOMA-IR), HOMA-B and the quantitative insulin sensitivity check index (QUICKI) were determined based on the suggested formulas (15). Plasma total antioxidant capacity (TAC) and total glutathione (GSH) were quantified using the ferric reducing ability of plasma (FRAP) method developed by Benzie and Strain (16) and that of Beutler et al. (17), respectively. All inter- and intra-assay CVs for oxidative stress biomarkers were less than 5%. Measurements of markers of insulin resistance, lipid concentrations and oxidative stress biomarkers were done in a blinded fashion.

3.6. Statistical Methods

To evaluate the normal distribution of variables, Kolmogrov-Smirnov test was applied. One-way analysis of variance (ANOVA) was used to detect differences in general characteristics and dietary intakes among the three groups (9). Pearson Chi-square test was used to compare the categorical variables. One-way repeated measures analysis of variance was employed to compare changes after eight weeks of intervention among the three groups. In this analysis, the treatment (high-dose *C. cyminum* L. plus lime vs. low-dose *C. cyminum* L. plus lime and placebo) was regarded as between-subject factor and time with two time-points (study baseline and after eight-week intervention) was considered as within-subject factor. To identify between-group differences for non-normally distributed variables, Kruskal-Wallis test was used. Results of the normally distributed variables (all variables except FPG) and non-normally distributed variables (FPG) were presented as mean $\pm$ standard deviations (SDs) and median interquartile range (IQR), respectively. To control the confounding markers (baseline values, age and BMI at baseline), analysis of covariance (ANCOVA) was used. The SPSS version 18 (SPSS Inc., Chicago, Illinois, USA) was employed for statistical analyses. Statistical analyses were blinded. The standard formula suggested for clinical trials by considering type one error (α) of 0.05 and type two error (β) of 0.20 (power = 80%) (9) was used to calculate the sample size. Based on a previous randomized placebo-controlled trial (9), 1.2 kg was used as SD and 1.1 kg as the difference in mean (d) of weight loss as primary variable. Therefore, the study needed20 subjects in each group. Assuming dropouts of four subjects in each group, 24 subjects were selected for each group.

4. Results

At the screening visit, 250 subjects were screened in the diet therapy clinic. One hundred-twenty-five subjects out of 250 screened persons were excluded from the first visit. Then, among the remaining 125 subjects assessed for eligibility, 53 were excluded due to not living in Kashan (n = 23) and unable to commit to the study (n = 30) (Figure 1). Totally, 72 subjects were randomized into three groups receiving: 1) high-dose *C. cyminum* L. and lime capsules (n=24); 2) low-dose *C. cyminum* L. and lime capsules (n = 24) and 3) placebos (n=24); all subjects completed the trial. On average, the rate of compliance in the current study was high; more than 90% of the capsules were taken throughout the trial in the three groups.

Mean age and height of the study subjects were not statistically different among the three groups (Table 1). Baseline weight and BMI, METs, SBP and DBP were not significantly different among the three groups. After eight weeks of intervention, compared with low-dose *C. cyminum* L. plus lime and placebo, taking high-dose *C. cyminum* L. plus lime resulted in a significant weight loss (changes from baseline in the high-dose group: -2.1 $\pm$ 1.7 vs. in the low-dose group: -1.2 $\pm$ 1.5 and in the placebo group: + 0.2 $\pm$ 1.3 kg, respectively; $P < 0.001$) and BMI (-0.8 $\pm$ 0.6 vs. -0.5 $\pm$ 0.5 and +0.1 $\pm$ 0.5 kg/m^2, respectively; $P < 0.001$).

Comparison of the dietary intakes of the study subjects throughout the study showed no significant changes in macro-nutrient intakes including energy, carbohydrates, proteins, fats, saturated fatty acids (SFA), polyunsaturated fatty acid (PUFA), monounsaturated fatty acid (MUFA), cholesterol and total dietary fiber (TDF) among the three groups (Table 2).

After eight weeks of intervention, compared with low-dose *C. cyminum* L. plus lime and placebo, high-dose *C.*

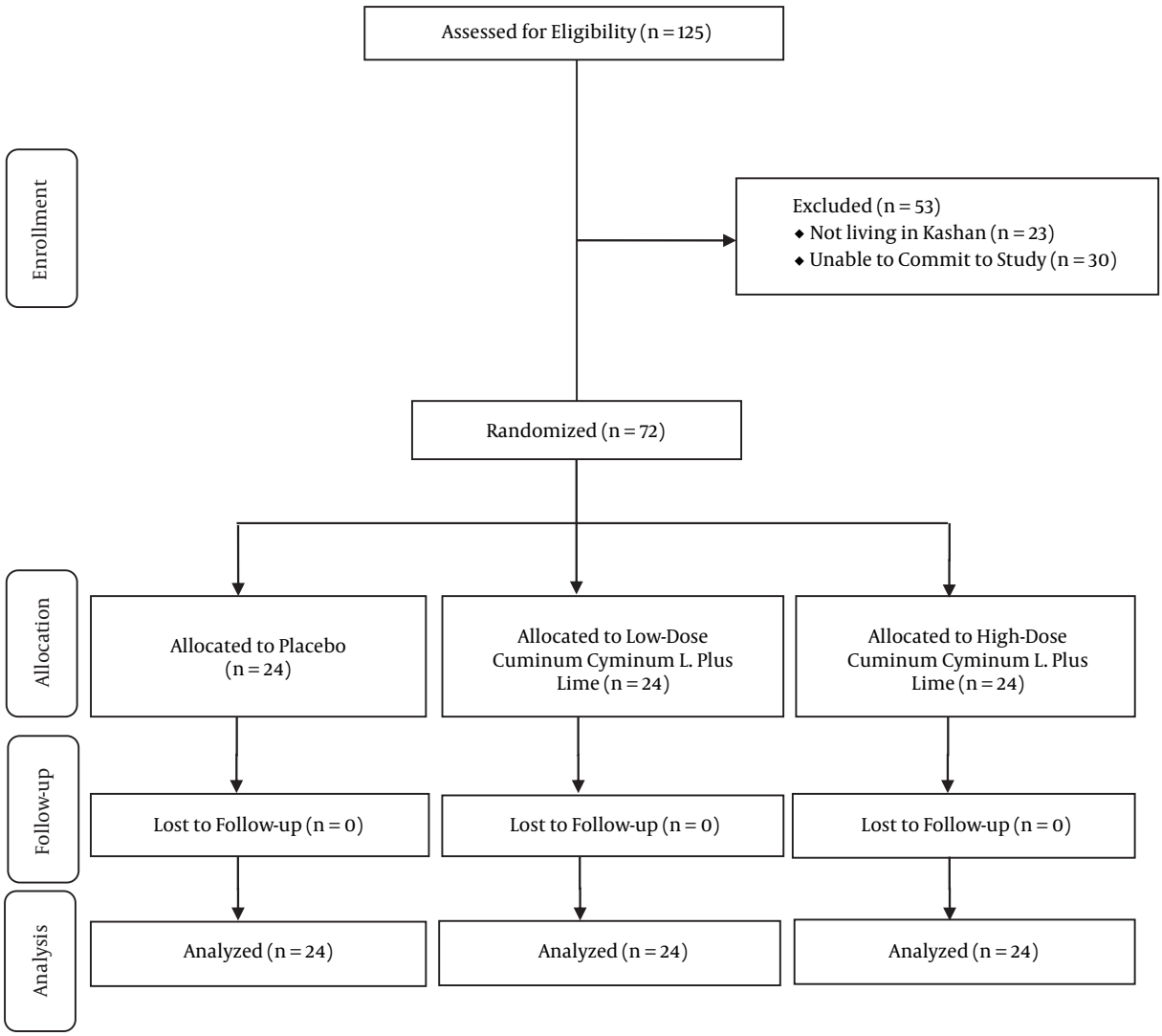

Figure 1. A Summary of Patients' Flow Diagram

cyminum L. plus lime administration led to significant reductionin FPG ($P < 0.001$) and a significant elevation in QUICKI (changes from baseline in the high-dose group: $+0.02 \pm 0.02$ vs. in the low-dose group: $+ 0.01 \pm 0.02$ and in the placebo group: -0.01 ± 0.01, respectively; $P = 0.01$) (Table 3). Moreover, a significant decrease in serum triglycerides (-14.1 ± 56.2 vs. $+13.9 \pm 36.8$ and $+10.6 \pm 25.1$ mg/dL, respectively; $P = 0.03$), total-cholesterol (-18.4 ± 28.6 vs. $+8.6 \pm 28.5$ and -1.0 ± 24.8 mg/dL, respectively; $P = 0.004$) and LDL-cholesterol levels (-11.8 ± 20.7 vs. $+6.5 \pm 23.2$ and -2.9 ± 20.4 mg/dL, respectively; $P = 0.01$) was observed following the consumption of high-dose *C. cyminum* L. plus lime compared with low-dose *C. cyminum* L. plus lime and placebo. High-dose *C. cyminum* L. plus lime intake had no significant effects on serum insulin, HOMA-IR, HOMA-B, HDL-cholesterol and biomarkers of oxidative stress compared with those of low-dose *C. cyminum* L. plus lime and placebo.

After adjusting the analysis for baseline values, age and BMI at baseline, the above-mentioned findings remained significant except for QUICKI ($P = 0.06$) and serum triglycerides levels ($P = 0.13$) (Table 4).

5. Discussion

Results of the current study demonstrated that high-dose *C. cyminum* L. plus lime administration for eight weeks among subjects with overweight had beneficial

Table 1. Demographic Data of Study Participants[a]

Value	Placebo Group, n = 24	Low-Dose *Cuminum cyminum* L. Plus Lime Group, n = 24	High-Dose *Cuminum cyminum* L. Plus Lime Group, n = 24	P Value[b]
Gender				1.00[c]
Male	2 (8.3)	2 (8.3)	2 (8.3)	
Female	22 (91.7)	22 (91.7)	22 (91.7)	
Age, y	40.1±11.3	33.2 ± 11.8	34.5 ± 10.2	0.08
Height, cm	160.8 ± 7.0	164.2 ± 5.6	162.9 ± 6.1	0.18
Weight at study baseline, kg	83.4 ± 15.4	89.5 ± 16.1	82.3 ± 13.6	0.21
Weight at end-of-trial, kg	83.6 ± 15.8	88.3 ± 16.6	80.2 ± 13.5	0.20
Weight change, kg	0.2 ± 1.3	-1.2 ± 1.5[d]	-2.1 ± 1.7[d]	< 0.001
BMI at study baseline, kg/m^2	32.4 ± 6.3	33.2 ± 5.6	31.1 ± 5.0	0.41
BMI at end-of-trial, kg/m^2	32.5 ± 6.5	32.7 ± 5.8	30.3 ± 5.0	0.27
BMI change, kg/m^2	0.1 ± 0.5	-0.5 ± 0.5[d]	-0.8 ± 0.6[d]	< 0.001
MET-h/d	28.5 ± 2.3	28.7 ± 2.1	28.9 ± 2.0	0.85
SBP, mmHg	118.7 ± 4.5	119.1 ± 4.2	119.2 ± 4.1	0.90
DBP, mmHg	79.5 ± 3.1	79.1 ± 2.8	79.4 ± 2.9	0.90

[a]Values are expressed as means ± SD or No. (%).
[b]Obtained from ANOVA test.
[c]Obtained from Pearson Chi-square test.
[d]Significant difference with the placebo group.
Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; METs, metabolic equivalents; SBP, systolic blood pressure.

Table 2. Dietary Intakes of Participants Throughout the Study[a]

Value	Placebo Group, n = 24	Low-Dose *Cuminum cyminum* L. Plus Lime Group, n = 24	High-Dose *Cuminum cyminum* L. Plus Lime Group, n = 24	P Value[b]
Energy, kcal/d	2497 ± 161	2518 ± 169	2529 ± 168	0.80
Carbohydrates, g/d	340.4 ± 42.1	344.1 ± 43.4	343.9 ± 33.8	0.93
Protein, g/d	89.5 ± 10.3	87.9 ± 14.1	92.5 ± 13.3	0.44
Fat, g/d	91.0 ± 12.0	91.8 ± 13.1	90.6 ± 12.4	0.94
SFA, g/d	26.3 ± 5.5	26.2 ± 5.6	27.4 ± 5.2	0.72
PUFA, g/d	30.2 ± 8.3	27.8 ± 7.6	28.1 ± 6.5	0.46
MUFA, g/d	24.4 ± 4.4	25.2 ± 6.6	26.5 ± 6.7	0.45
Cholesterol, mg/d	201.2 ± 101.2	211.6 ± 123.1	226.3 ± 127.3	0.76
TDF, g/d	20.2 ± 4.2	18.8 ± 5.4	20.2 ± 4.6	0.50

[a]Values are expressed as means ± SD.
[b]Obtained from ANOVA test.
Abbreviations: MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; TDF, total dietary fiber.

effects on weight, BMI, FPG, QUICKI, triglycerides, total-cholesterol and LDL-cholesterol levels. No significant effect of *C. cyminum* L. plus lime intake was observed on insulin, HOMA-IR, HOMA-B, HDL-cholesterol and biomarkers of oxidative stress. To the authors' best knowledge, the current study was the first that evaluated the beneficial effects of *C. cyminum* L. plus lime administration on weight and metabolic status in subjects with overweight.

Subjects with overweight are susceptible to several diseases including coronary heart disease, T2DM, certain types of cancer and osteoarthritis (18). The current study revealed that taking high-dose *C. cyminum* L. plus lime for eight weeks among subjects with overweight led to significant decrease in weight and BMI compared with low-dose

Table 3. The Effects of Cumin Plus Lime Intake on Markers of Insulin Resistance, Lipid Profiles and Biomarkers of Oxidative Stress (n = 24)[a]

Value	Placebo Group			Low-Dose *C. cyminum* L. Plus Lime Group			High-Dose *C. cyminum* L. Plus Lime Group			P Value[b]		
	Wk 0	Wk 8	Change	Wk 0	Wk 8	Change	Wk 0	Wk 8	Change	Time	Group	Time x Group
FPG,mg/dL	87.0 (22.2)	90.0 (25.2)	0 (12.3)	97.5 (12.2)	87.0 (17.5)	-9.0 (16.0)	101.5 (12.0)	82.5 (20.2)	-22.0 (9.0)	-	-	< 0.001[c]
Insulin, μIU/mL	8.0 ± 3.2	7.6 ± 3.0	-0.4 ± 2.0	8.4 ± 2.7	7.8 ± 3.1	-0.6 ± 3.2	8.7 ± 4.2	7.4 ± 3.3	-1.3 ± 3.5	0.03	0.92	0.57
HOMA-IR	1.8 ± 0.9	1.8 ± 0.7	-0.04 ± 0.6	2.1 ± 0.7	1.9 ± 0.9	-0.2 ± 1.0	2.4 ± 1.1	1.8 ± 1.2	-0.6 ± 1.0	0.004	0.57	0.06
HOMA-B	29.0 ± 15.1	26.6 ± 15.5	-2.4 ± 9.5	27.1 ± 12.1	27.7 ± 15.9	0.6 ± 13.8	25.9 ± 17.9	27.1 ± 15.6	1.2 ± 12.9	0.87	0.94	0.53
QUICKI	0.35 ± 0.02	0.34 ± 0.02	-0.01 ± 0.01	0.34 ± 0.01	0.35 ± 0.02	0.01 ± 0.02	0.34 ± 0.02	0.36 ± 0.03	0.02 ± 0.02[d]	< 0.001	0.70	0.01
TG, mg/dL	124.5 ± 51.4	135.1 ± 56.2	10.6 ± 25.1	116.7 ± 57.1	130.5 ± 54.9	13.9 ± 36.8	138.7 ± 81.3	123.6 ± 52.8	-14.1 ± 56.2[e]	0.52	0.88	0.03
TC, mg/dL	187.3 ± 30.1	186.3 ± 37.4	-1.0 ± 24.8	174.8 ± 37.0	183.4 ± 43.2	8.6 ± 28.5	176.2 ± 34.7	157.8 ± 34.0	-18.4 ± 28.6[e]	0.26	0.12	0.004
LDL-C, mg/dL	108.0 ± 23.3	105.1 ± 32.3	-2.9 ± 20.4	96.5 ± 30.1	103.0 ± 35.5	6.5 ± 23.2	93.7 ± 25.4	81.9 ± 27.6	-11.8 ± 20.7[e]	0.29	0.06	0.01
HDL-C, mg/dL	54.4 ± 14.2	54.1 ± 15.2	-0.3 ± 7.7	55.0 ± 9.5	54.3 ± 10.8	-0.7 ± 5.4	54.8 ± 5.6	51.2 ± 6.4	-3.6 ± 6.3	0.05	0.84	0.16
TAC, mM/	748.6 ± 155.4	777.6 ± 156.5	29.0 ± 107.0	789.9 ± 186.3	844.9 ± 118.1	54.9 ± 162.0	741.3 ± 89.4	783.1 ± 74.3	41.7 ± 53.3	0.003	0.21	0.74
GSH,(μM/L)	593.9 ± 229.0	570.1 ± 194.8	-23.8 ± 204.7	579.6 ± 112.2	555.9 ± 90.1	-23.8 ± 98.0	501.6 ± 113.5	489.0 ± 132.9	-12.6 ± 148.1	0.28	0.05	0.96

[a] Values are expressed as means ± SD or No. (%) for normally distributed variables and median (IQR) for non-normally distributed variables.
[b] Obtained from repeated measures ANOVA test.
[c] Obtained from Kruskal-Wallis test.
[d] Significant difference with the placebo group.
[e] Significant difference with low-dose *Cuminum cyminum* L. plus lime group.
Abbreviations: FPG, fasting plasma glucose; HOMA-IR, homeostasis model of assessment-insulin resistance; HOMA-B, homeostatic model assessment-Beta cell function; QUICKI, quantitative insulin sensitivity check index; LDL-C, low density lipoprotein-cholesterol; HDL-C, high density lipoprotein-cholesterol; TC, total cholesterol; TG, triglycerides; TAC, total antioxidant capacity; GSH, total glutathione.

Table 4. Adjusted Changes in Metabolic Variables of Patients With Overweight[a]

Value	Placebo, n = 24	Low-Dose *Cuminum cyminum* L. Plus Lime, n = 24	High-Dose *Cuminum cyminum* L. Plus Lime, n = 24	P Value[b]
FPG,mg/dL	1.8 ± 4.3	-6.6 ± 4.1	-19.2 ± 4.1	0.004
Insulin, μIU/mL	-0.4 ± 0.5	-0.7 ± 0.5	-1.0 ± 0.5	0.75
HOMA-IR	-0.2 ± 0.2	-0.2 ± 0.2	-0.5 ± 0.2	0.47
HOMA-B	-0.7 ± 2.4	-0.6 ± 2.3	0.6 ± 2.3	0.89
QUICKI	0.003 ± 0.005	0.009 ± 0.005	0.02 ± 0.005	0.06
TG, mg/dL	9.3 ± 7.4	9.3 ± 7.3	-9.2 ± 7.3	0.13
TC, mg/dL	0.4 ± 5.7	8.1 ± 5.7	-19.3 ± 5.6	0.003
LDL-C, mg/dL	-2.1 ± 4.6	6.9 ± 4.5	-12.8 ± 4.5	0.01
HDL-C, mg/dL	-0.4 ± 1.4	-0.8 ± 1.4	-3.4 ± 1.3	0.22
TAC, mM/L	21.2 ± 19.7	69.7 ± 19.7	34.7 ± 19.3	0.21
GSH,μM/L	3.9 ± 26.9	-20.9 ± 26.8	-43.0 ± 26.8	0.48

[a] All values are expressed as means± SE. Adjusted for baseline values, age and baseline BMI.
[b] Obtained from ANCOVA.
Abbreviations: FPG, fasting plasma glucose; HOMA-IR, homeostasis model of assessment-insulin resistance; HOMA-B, homeostatic model assessment-Beta cell function; QUICKI, quantitative insulin sensitivity check index; LDL-C, low density lipoprotein-cholesterol; HDL-C, high density lipoprotein-cholesterol; TC, total cholesterol; TG, triglycerides; TAC, total antioxidant capacity; GSH, total glutathione.

C. cyminum L. plus lime and placebo. Supporting the current study, authors' previous study showed that taking 300 mg *C. cyminum* L. among subjects with overweight for eight weeks resulted in significant decrease in weight and BMI (9). In addition, a reduction in body weight and food consumption was observed following the intake of lime essential oil in mice after 45 days (10). The same findings were reported among males with obesity following the consumption of 1.5 g black cumin daily for three months (19) and in rats after intake of 5 mg/kg per day thymoquinone for five days (20). The exact mechanism by which *C. cyminum* L. and lime intake might affect weight and BMI are unknown.

It is possible that phytoestrogens of *C. cyminum* L. inhibit serotonin re-uptake and thereby increase the levels of serotonin in synaptic clefts (12), which in turn would result in enhanced satiety. In addition, limonene may reduce body weight through increased lipolysis by a histaminergic response and reduced appetite (13).

The current study indicated that high-dose *C. cyminum* L. plus lime administration for eight weeks among subjects with overweight was associated with a significant reduction in FPG and a significant rise in QUICKI compared with low-dose *C. cyminum* L. and lime and placebo, but did not influence serum insulin, HOMA-IR and HOMA-B. In agreement with the current study, a significant decrease of serum glucose levels was observed in obese rats treated with extracts of herbal mixture formulation for four weeks (12). Moreover, d-limonene consumption lowered FPG levels in obese mice (11). Improved insulin sensitivity was also observed following the administration of various combinations of essential oils such as fenugreek, cinnamon, cumin and oregano in Zucker fatty rats (21). In addition, treatment with antioxidant supplementation, including d-limonene and vitamin E, improved insulin sensitivity in obese rabbits after two months (22). Cumin intake may result in improved insulin sensitivity via preserving pancreatic beta-cell integrity (23). In addition, anti-inflammatory properties of d-limonene may lead to improved insulin function (24).

The current study showed that taking high-dose *C. cyminum* L. plus lime for eight weeks among subjects with overweight resulted in significant decrease in triglycerides, total-cholesterol and LDL-cholesterol concentrations compared with low-dose *C. cyminum* L. plus lime and placebo, but did not affect HDL-cholesterol and oxidative stress biomarkers. In line with the current study, significant decrease in serum total-cholesterol and LDL-cholesterol levels were observed in the birds fed with black seed (4 g/kg) for 28 days (25). A 20-week administration of Nigella sativa also led to a significant decrease in LDL-cholesterol concentrations in rats. Furthermore, d-limonene intake in obese mice resulted in a significant decrease in triglycerides and LDL-cholesterol levels (11). Administration of limonene in diabetic rats for 45 days led to a significant increase in the activities of antioxidant enzymes (26). However, such beneficial effects observed in few studies. For instance, taking cumin extract three times a day in patients with hypercholesterolemia for six weeks did not influence lipid profiles (27). Similar findings were observed following the treatment of *C. cyminum* methanol extract at dose levels of 100 and 200 mg/day in male albino rats after 60 days (28). Previous studies showed that phytosterols of *C. cyminum* have the ability to reduce the intestinal absorption of diet biliary cholesterol (12, 29). Lime intake may result in improved lipid profiles through activating peroxisome proliferator-activated receptor-α signaling, and inhibited liver X receptor-β signaling (11). Absence of significant effect on HDL-cholesterol and oxidative stress biomarkers following the administration of *C. cyminum* L. plus lime in the current study might be mediated by the different study designs, discrepancy in subjects, different doses of *C. cyminum* and duration of the study.

Some of the main strengths of the current study were the assessment of insulin resistance markers, lipid profiles, oxidative biomarkers and the randomized design. Some limitations should be taken in account in the interpretation of the results. Beneficial effects of *C. cyminum* L. plus lime intake on leptin, thyroid hormones and inflammatory cytokines were not evaluated. Furthermore, the sample size was small and duration of the intervention was short in the current study. Further studies with bigger sample sizes, and longer duration of the intervention are needed to confirm the findings.

Taken together, the current study indicated that taking high-dose *C. cyminum* L. plus lime for eight-week among subjects with overweight had beneficial effects on weight, BMI, FPG, QUICKI, triglycerides, total-cholesterol and LDL-cholesterol levels; however, it did not affect insulin, HOMA-IR, HOMA-B, HDL-cholesterol and oxidative stress biomarkers.

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Footnotes

Authors' Contribution: Zatollah Asemi: conception, design, statistical analysis and drafting of the manuscript; Mohsen Taghizadeh, Mohammad Reza Memarzadeh, Fatemeh Abedi, Nasrin Sharifi, Fatemeh Karamali and Zohreh Fakhrieh Kashan: data collection and manuscript drafting; Zatollah Asemi: supervision of the study. All authors approved the final version of the manuscript.

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