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Chios mastiha essential oil exhibits antihypertensive, hypolipidemic and anti-obesity effects in metabolically unhealthy adults - a randomized controlled trial

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ABSTRACT

The essential oil of the resinous exudate from *Pistacia lentiscus* of Chios namely Chios Mastiha Essential Oil (CMEO), is a natural volatile oil rich in monoterpenes α-pinene, β-myrcene, β-pinene. In the present randomized controlled trial, we investigated the effects of CMEO on individuals with abdominal obesity and metabolic abnormalities i.e., dyslipidemia, hypertension, insulin resistance. Eligible patients (N = 94) were randomly assigned to either the intervention group, receiving capsules containing 200 mg of CMEO daily for 3 months adjunct to current treatment for metabolic disorder(s), or the control group. Anthropometric measurements, blood markers, and quality of life (QoL) were assessed. Statistical analysis was performed on an intention-to-treat basis. A significant improvement in blood lipid profile, namely triglycerides ($p = 0.026$) and low-density lipoprotein ($p = 0.05$) of the CMEO group versus controls was observed. Systolic blood pressure ($p = 0.05$) and alanine aminotransferase ($p = 0.022$) significantly decreased only after CMEO intake. Alike, weight decreased only in CMEO ($p = 0.02$), while mean changes in % body fat ($p = 0.005$) and visceral fat ($p = 0.045$) were significantly different between groups post-intervention. Lower oxidized LDL ($p = 0.044$) and higher adiponectin ($p = 0.007$) were recorded in CMEO with significant different mean changes between groups post-intervention. QoL, as assessed by Short Form-12 questionnaire was improved in the CMEO compared to control ($p = 0.041$ for Physical Composite Score, $p = 0.035$ for Mental Composite Score). No adverse effects were reported. An anti-obesity effect of CMEO, probably attributed to modulation of inflammatory and antioxidant processes, is suggested. Conclusively, CMEO can be safe and effective in regulating metabolic abnormalities, adjunct to treatment. (ClinicalTrials.gov. The effect of Mastiha oil in Metabolic Syndrome, ID Number: NCT04785573)

1. Introduction

Natural products have been used for centuries as a potent source of bioactive molecules. For instance, essential (or volatile) oils, defined as complex mixtures of volatile compounds mainly found in aromatic plants, have been extensively studied as pharmaceutical agents [1]. Being located in the cytoplasm of certain cells (e.g., epidermal cells, internal secretory cells) and secreted by trichomes or secretory hairs, essential oils are present in most parts of the plant such as flowers, leaves, rhizomes, seeds, fruits and bark [1–3]. Essential oils are usually recovered by steam distillation of the raw plant material imparting a unique odor [4]. They are water immiscible and contain more than 100 different chemical substances; most of these substances are odorants of low molecular weight (below 300 Da) and mainly belong to the terpene

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chemical family, such as monoterpenes, sesquiterpenes, terpene alcohols and terpenoids [5]. Volatile terpenes are considered as non-nutrient phytochemicals and have a high structural diversity based on a 5C unit repetition, called isoprene, via condensation of isopentenyl diphosphate and dimethylallyl diphosphate [6]. Monoterpenes are formed by combining the two basic two-isoprene units (C10) and represent the major compounds of essential oils [6].

There is evidence that herbal medicines containing essential oils or natural volatile oils have promising pharmacological activities, e.g. the essential oil from garlic exhibit numerous properties, such as antioxidant and immunomodulatory [7]. While for natural products, phenolic compounds are mostly attributed with exhibited health benefits [8], the reported beneficial effects of essential oils, microbial infection [9], inflammation [10], and central nervous system (e.g. pain, stress and anxiety, depression, mood disorders, insomnia, and Alzheimer's) [11–13], have been attributed to the high biological activities of monoterpenes. More recently, monoterpenes have exhibited favorable effects on cardio-metabolic disorders, like atherosclerosis and hypertension, by modulating serum lipid profile, endothelial inflammation and oxidative stress (OS), as well as controlling vasomotor tone through modulation of the nitric oxide (NO) pathway [14,15]. Moreover, the ability of monoterpenes to regulate energy metabolism towards body weight reduction can improve blood lipid profile and hormone release control (e.g., leptin, adiponectin), and can lower the production of inflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) [16–18].

The Chios Mastiha Essential Oil (CMEO) has recently gained remarkable research attention. CMEO is obtained by steam distillation of Chios Mastiha, a dried resinous exudate from the stems and branches of *Pistacia lentiscus var. Chia* [19]. Chios Mastiha is a concentrated source of terpenes, mainly triterpenes such as oleanonic, mastihadienonic, isomastihadienonic and moronic acid with well-documented antioxidant and anti-inflammatory activities [20]. An intake of ≈ 2 g/day of Chios Mastiha has been reported to exert beneficial outcomes in patients with immune-mediated inflammatory diseases including obesity related metabolic disorders such as non-alcoholic fatty liver disease (NAFLD) [21–23]. According to the European Pharmacopoeia monograph (01/2008:1876), Chios Mastiha contains a minimum of 10 ml/kg of CMEO. Monoterpenes of CMEO are comprised by monoterpene hydrocarbons (50%), oxygenated monoterpenes (20%) and sesquiterpenes (25%) [19]. Nevertheless, clinical studies on the potential health effects of CMEO are lacking. Based on a recent bioavailability study in apparently healthy young men, plasma concentrations of major CMEO monoterpenes, namely alpha-pinene, beta-pinene and myrcene were significantly increased 30 min after oral CMEO load, and remained elevated even at 24 h post consumption [19]. At the same time, monoterpene bioavailability was accompanied by greater serum oxidative resistance [19]. This observation could imply a potential CMEO pharmacological activity in OS-related conditions such as obesity and consequential metabolic abnormalities.

Therefore, in the present 3-month randomized controlled trial (RCT) we aimed at investigating the effects of CMEO on individuals with abdominal obesity and at least one metabolic abnormality, namely dyslipidemia, hypertension and insulin resistance/diabetes. This is the first reported study of CMEO in humans with obesity and different related metabolic abnormalities within a frame of three months and adjunct to current medication. Study outcomes included: (a) anthropometric indices i.e., body mass index (BMI), body composition; (b) cardiometabolic parameters i.e. arterial blood pressure, glucose, lipid levels; (c) quality of life indices and (d) markers of systemic inflammation and OS.

2. Methods

2.1. Ethics and patient population

The trial was conducted in two sites; Harokopio University of Athens, Greece (Department of Nutrition and Dietetics) and General Hospital G. Gennimatas, Thessaloniki, Greece (Diabetes Outpatient Department). The Ethics Committee of Harokopio University (ID: 1799–13/06/2019) and General Hospital G. Gennimatas reviewed and approved the trial protocol (ID: 16015–11/11/2020). Throughout the study, the Helsinki Declaration (1964) and the Data Protection Act 1998 were adhered, while all terms of Good Clinical Practice were applied. Registration on *clinicaltrials.gov* was acquired (ClinicalTrials.gov. The effect of Mastiha oil in Metabolic Syndrome, ID Number: NCT04785573).

Participants were invited via written announcements (posters) and social media posts. They were informed in detail about the aims, methods, benefits, and potential harms of the study. A signed informed consent was obtained from all subjects involved, and every subject kept a copy of the signed document. The study took place in 2021 and 2022.

Metabolically unhealthy individuals were recruited according to predetermined inclusion and exclusion criteria. In short, the cardiometabolic parameters given in Table 1 were applied to determine eligibility along with a stable body weight for $>$ 3 months and unchanged treatment regimen for $\geq$ 6 months. Eligible subjects were randomly assigned to either the control group or the intervention (CMEO) group. Researchers and patients were aware of the treatment allocation, except for the appointed statistician, who was blinded. The statistician applied simple randomization through a computer-generated randomization sequence, and the randomization list was available only to the principal investigator.

2.2. Preparation of CMEO capsules

CMEO soft gel capsules were provided by Chios Mastiha Growers Association and were manufactured in Italy by an Authorized manufacturer (According to Dir 89/389/CE license number no. 2011/DIET. Off 67 issued on 11/03/2011). The notification number for food supplements from the National Organization for Medicines (EOF) is 14489–05/02/2020.

Soft gel capsules were filled with 200 mg CMEO, 100 mg medium chain triglycerides as excipients, bovine gelatin and glycerol in the cell and brown iron oxide as shell dye. Capsules were packed in a bottle with safety seal (30 pieces per item) that was delivered to patients once per month for a total of 3 months. All CMEO capsules had the same batch origin. The quality was tested for uniformity of content, chemical stability during storage, microbiological properties and heavy metals. No

Table 1
Inclusion and exclusion criteria for participation in the study.

Inclusion Criteria	Exclusion Criteria
Adult men and women 18–75 years of age with abdominal obesity (waist circumference $>$ 94 cm for males and $>$ 80 cm for females), and the presence of at least one metabolic abnormality: 1. triglyceride level $\geq$ 150 mg/dL or HDL cholesterol $\leq$ 40 mg/dL in men and $\leq$ 50 mg/dL in women, 2. increased blood pressure $\geq$ 130/85 mm Hg, 3. elevated fasting blood sugar $\geq$ 100 mg/dL. A stable weight for $\geq$ 3 months pre-intervention An unchanged treatment regimen for $\geq$ 6 months pre-intervention Provided consent for participation	Hepatotoxic Medication Untreated Diabetes Mellitus Dysthyroidism, hypopituitarism, Cushing syndrome / disease Pregnancy, lactation Psychiatric or mental disorder Any use of antioxidant-phytochemical rich supplement, vitamin D supplement, anti-, pre- or pro-biotics within 3 months pre-intervention Those who did not consent or were unable to provide consent

pathogenic organisms or their metabolic products in amounts which are harmful to the health of the final consumer were detected and heavy metals levels were under the detection limits. CMEO was obtained with distillation process with steam from the resin (100%) of the plant. For standardization, the composition of the extracted essential oil was analyzed by gas chromatography- with flame-ionization detection (GC-FID). According to certification analysis each capsule contained the following monoterpenes: α-pinene (82.5%), β-myrcene (10.5%), β-pinene (2.52%), limonene (0.67%), β-caryophyllene (0.55%), camphene (0.41%), α-thujene (0.77%), o-methyl anisol (0.22%).

2.3. Study design

At baseline, printed information on lifestyle based on the National Dietary Guidelines for Adults was given out to all participants for ethical reasons [24]. In addition, every patient of the intervention arm had to consume one CMEO capsule 30 min before meal with water or juice, on a daily basis on top of their standard medication for three months. Compliance to CMEO intake was monitored biweekly through phone calls by the appointed researchers.

2.4. Endpoints

The primary endpoint of the trial was the changes in blood lipids i.e., triglycerides (TG), total cholesterol (TC) and low-density lipoprotein (LDL), and in insulin sensitivity (glucose, insulin). Secondary endpoints included changes in inflammatory and OS status indices (such as C-reactive protein (CRP), myeloperoxidase (MPO) and interleukin-6 (IL-6)).

2.5. Clinical assessments and anthropometric measurements

The appointed physician obtained a detailed medical record including personal and family medical history, drug treatments, as well as arterial blood pressure measurements expressed as systolic blood pressure (SBP) and diastolic blood pressure (DBP). Components of anthropometry were measured by experienced dieticians; body weight was measured to the nearest kilogram (kg) with a flat scale, height to the nearest centimeter (cm) with a stadiometer (Seca Mode 220, Hamburg, Germany) and BMI was calculated using weight in kg divided by the square of height in meters (m^2). Waist circumference (WC) and hip circumference (HC) were measured with a non-stretch but flexible tape on minimal clothing. Additionally, body composition was analyzed with bioelectrical impedance analysis (Tanita BC-418, Tokyo, Japan) and included body fat, fat-free mass (FFM), total body water (TBW) and visceral fat level (VFL). All measurements were performed at the start and the end of the trial.

2.6. Biochemical and inflammatory markers

Twenty ml of blood was collected after an overnight fast. Serum was isolated by whole blood centrifugation (3000 rpm, 10 min, 20∘ C) and was used for quantification of biochemical indices and biomarkers. All measurements were performed in duplicate at baseline and the endpoint of the trial.

2.6.1. Biochemical indices

Fasting glucose, insulin, urea, uric acid, creatinine, TC, high-density lipoprotein (HDL) cholesterol, LDL cholesterol, TG, alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-glutamyl transferase (γ-GT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and albumin were measured in serum with an automatic biochemical analyzer (Cobas 8000 analyzer, Roche Diagnostics GmbH, Mannheim, Germany).

2.6.2. Biomarkers

CRP was measured in serum with an automatic biochemical analyzer (Cobas 8000 analyzer, Roche Diagnostics GmbH, Mannheim, Germany). Levels of IL-6, TNF-α, leptin, adiponectin (R&D Systems, Inc., Minneapolis, MN, USA), MPO (Thermo Fisher Scientific Inc., Waltham, MA, USA) and oxidized low-density lipoprotein (oxLDL) (Mercodia, AB, Uppsala, Sweden) were measured applying Enzyme-Linked Immunosorbent Assay (ELISA) as indicators of chronic inflammatory grade and OS. All measurements were performed in duplicate.

2.7. Assessment of quality of life (QoL)

Elements of health-related QoL were assessed using self-reported validated questionnaires at baseline and the endpoint of the trial. The Athens Insomnia Scale (AIS) was used for the evaluation of sleep quality. It consists of eight items (rated on a 4-point numerical rating scale) that evaluate sleep induction, awakenings, sleep duration and quality, well-being, functioning capacity and sleepiness during the day [25]. The AIS score ranges from 0 to 28 with higher scores indicating worse symptoms of insomnia. The Center for Epidemiologic Studies Depression Scale Revised (CESD-R) is a widely used self-reported tool for depression screening. It consists of 20 items (a final score from 0 to 60, scores equal to or above 16 represent a risk for clinical depression) regarding mood, somatic complaints, interactions with others, and motor functioning [26]. Self-esteem was assessed by applying the 10-item Revised Rosenberg Self-Esteem Scale (RSES), a highly reliable measure of global self-esteem, with a 4-point scale format ranging from strongly agree to strongly disagree and a final score from 0 to 30. Scores below 15 indicate a low self-esteem [27]. Finally, the self-reported Short Form-12 (SF-12) questionnaire evaluated the impact of health on participants' everyday life with its two summary scores reporting on a mental (MCS-12) and a physical component (PCS-12) [28].

2.8. Sample size calculation and statistical analysis

Continuous variables are presented with mean and standard deviation (SD). Qualitative variables are presented with absolute and relative frequencies. Concerning baseline characteristics, for the comparison of proportions chi-square and Fisher's exact tests were used, while for the comparison of means between the two treatment groups Student's t-test was computed. All analyses were conducted on an intention-to-treat (ITT) basis. To reduce the bias implicit in utilizing only complete cases, multiple imputation procedures in case of missing data were implemented. Differences in changes of study variables during the follow up period between the two treatment groups were evaluated using general linear models and were presented as mean difference plus 95% confidence interval (CI). Statistical significance at follow up was assessed by looking at the interaction effect of time with study group. Log-transformations were applied in case of not normal distributed data. The study had only one repeat assessment, so no correction for multiple comparisons were made. All p-values reported are two-tailed. Statistical significance was set at 0.05 and analyses were conducted using SPSS statistical software (version 26.0).

Regarding sample size calculation, a minimum sample size of 78 patients (39 per arm) was sufficient to result in a clinically important decrease by 25% (or −35 mg/dL) in TG levels [standard deviation of mean (SD) = 50] using a two-tailed t test with 80% power and a 5% level of significance. Considering a possible 15–20% dropout rate, eventually a total of 94 patients were recruited.

3. Results

3.1. Participants and baseline characteristics

As shown in Fig. 1, a total of 176 potential participants were initially assessed for recruitment. After excluding 28 people due to COVID-19

CONSORT
TRANSPARENT REPORTING of TRIALS

CONSORT 2010 Flow Diagram

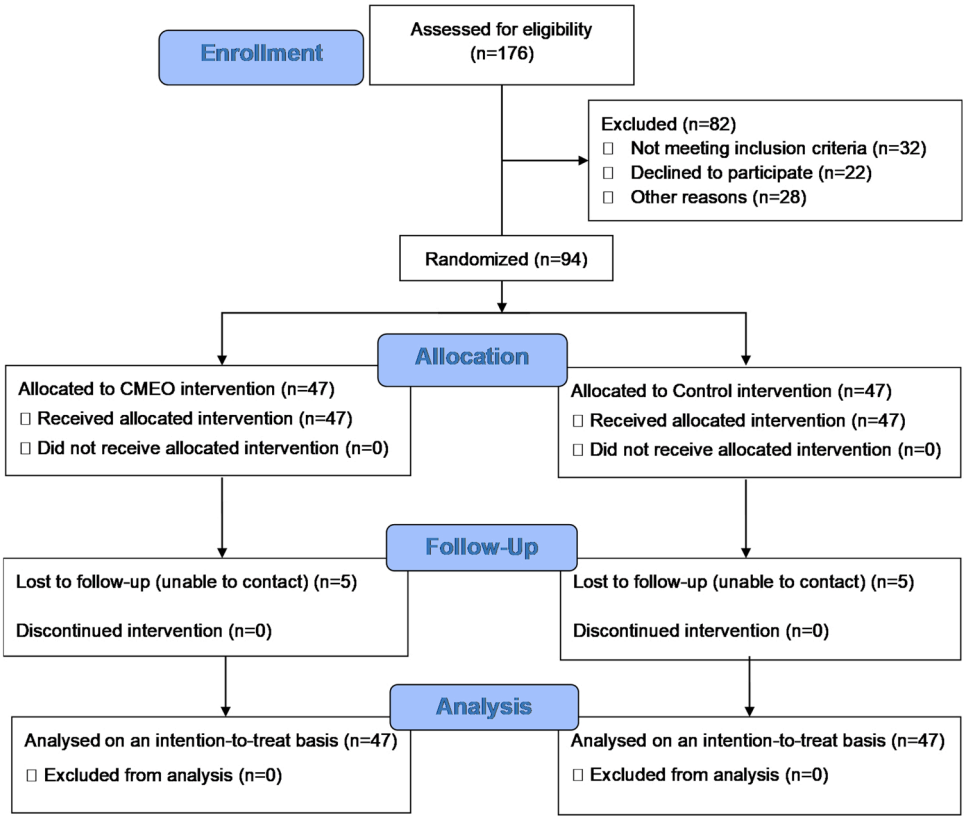

Fig. 1. Consort 2010 Flow Diagram.

restrictions (i.e. positive test, long-COVID-19 diagnosis), and 54 either not meeting the inclusion criteria (n = 32) or declining to participate (n = 22), 94 participants met the inclusion criteria and took part in the study. During the follow up period, 10 patients i.e., 5 in the CMEO group and 5 in the control group did not respond to our communication efforts (by email or phone) and did not finish the trial. All 94 participants, 47 in the CMEO group and 47 in the control group, were included in the ITT analysis.

Participants' baseline characteristics by group are presented in Table 2. No significant differences were found between the two study groups, except for the presence of hypertension, which was significantly lower in the CMEO group. Also, Table 2 includes details on the standard medication that was used for metabolic abnormalities, which was similar in the two groups at baseline and constant throughout the intervention.

3.2. Anthropometric measurements

Changes after the intervention in patients' anthropometric measurements by group are presented in Table 3. In fact, body weight ($p = 0.008$), % body fat ($p < 0.001$), VFL ($p < 0.001$), BMI ($p = 0.008$), and SBP ($p = 0.005$) decreased significantly after the intervention only in the CMEO group. Mean changes in % body fat ($p = 0.005$) and VFL ($p = 0.045$) differed significantly between the CMEO group and controls. Regarding all other characteristics, no significant changes were found.

3.3. Biochemical indices

Changes after the intervention in biochemical measurements by group are presented in Tables 4. More specifically, TG ($p = 0.011$), LDL ($p = 0.006$) and ALT ($p = 0.022$) levels decreased significantly only in the CMEO group, with the mean changes in the cases of TG ($p = 0.026$) and LDL ($p = 0.050$) being significantly different between the two groups. Regarding all other characteristics, no significant changes were found. Interestingly no significant alterations were presented in markers commonly used to monitor adverse health effects (such as creatinine and albumin for nephrotoxicity or liver enzymes for hepatotoxicity). The lack of significant changes in these markers coincide with the zero reporting of serious adverse side effects.

3.4. Inflammatory and OS markers

Changes after the intervention in patients' inflammatory and OS markers by group are presented in Table 5. Decrease in oxLDL ($p = 0.026$) and increase in adiponectin ($p = 0.002$) levels occurred only in the CMEO group with a significant difference in the mean changes between the two groups ($p = 0.044$ for oxLDL and $p = 0.007$ for

Table 2
Participants' baseline characteristics by group.

		Control (n = 47)	CMEO (n = 47)	p
Sex	Males, N (%)	14 (29.8)	20 (42.6)	0.198 +
	Females, N (%)	33 (70.2)	27 (57.4)	
Age, mean (SD)		53.7 (11.7)	53 (11.5)	0.756‡
BMI, mean (SD)		35.1 (7.1)	32.7 (5.5)	0.080
Hypertension, N (%)		39 (83)	26 (57.8)	**0.008**+
Diabetes, N (%)		21 (44.7)	17 (36.2)	0.401 +
Dyslipidemia, N (%)		42 (89.4)	41 (89.1)	> 0.999 +
Family status	Married, N (%)	37 (80.4)	32 (68.1)	0.707 + +
	Divorced, N (%)	1 (2.2)	3 (6.4)	
	Single, N (%)	6 (13)	9 (19.1)	
	In a relationship, N (%)	1 (2.2)	2 (4.3)	
	Widowed, N (%)	1 (2.2)	1 (2.1)	
Education	None, N (%)	0 (0)	0 (0)	0.694 + +
	Primary, N (%)	2 (4.3)	1 (2.1)	
	Secondary, N (%)	16 (34.8)	12 (25.5)	
	Tertiary, N (%)	22 (47.8)	26 (55.3)	
	Other, N (%)	6 (13)	8 (17)	
Medication for metabolic disorders				
Statins, (YES/NO)		13/34	10/37	0.472 +
Antihypertensive treatment, (YES/NO)		19/28	12/35	0.125 +
Antidiabetic agents, (YES/NO)		11/36	12/35	0.810 +
Smoking status (%)				
Smoking, (YES/NO)		11/36	12/35	0.810 +

Continuous variables are presented as mean and standard deviation (SD). Qualitative variables are presented with absolute (N) and relative frequencies (%). For the comparison of proportions chi-square and Fisher's exact tests were used, while for the comparison of means between the two treatment groups Student's t-test was computed. Statistical significance was set at $p < 0.05$. +Pearson's chi-square test; + +Fisher's exact test; ‡Student's t-test. CMEO, Chios Mastiha Essential Oil

adiponectin). No other significant differences were observed.

3.5. Health-related QoL

With regard to patients' QoL (Table 6), PCS-12 ($p = 0.002$) and MCS-12 ($p = 0.004$) increased significantly only in the CMEO group and mean changes were significantly different ($p = 0.041$ for PCS-12 and $p = 0.035$ for MCS-12) in the two groups as well, indicating a significant improvement of mental and physical health only in CMEO patients. Regarding all other characteristics, no significant changes were evident.

4. Discussion

To our knowledge, this is the first RCT investigating the potential anti-obesity effects of CMEO. Individuals with abdominal obesity and related metabolic abnormalities consumed a capsule of CMEO on a daily basis and on top of standard medication. At the end of the 3-month period, patients of the CMEO arm exhibited significant reductions in blood lipids compared to controls. Additionally, SBP decreased only in the CMEO group. These changes were accompanied by significant improvements in anthropometric indices and markers of inflammation and OS, contributing to better health-related QoL scores (Fig. 2).

It is common knowledge that the epidemic of overweight and obesity threatens to overwhelm both developed and developing countries [29]. In 2019, obesity prevalence across the EU reached 17% of the total adult population [30] and by 2025, it is expected to surpass 20% [31]. Abdominal obesity, the main element of metabolic syndrome (MetS), is associated with less disease-free years [32] and higher risk for all-cause mortality and cardiovascular disease-specific mortality [33]. Individuals with overweight/obesity but without metabolic disorders (i.e., dyslipidemia, hypertension, diabetes) show lower risk of disease development, but gradually a transition towards health complications has been reported [34]. The European Society of Cardiology (ESC) and the European Atherosclerosis Society (EAS) recommend a modest body weight loss (5–10%) in an effort to modulate metabolic risk factors i.e., blood lipids, blood pressure, and fasting glucose levels. To this end, the ESC/EAS suggests lifestyle modification with or without medication depending on patient's risk level [35]. Currently, different dietary patterns [36], and natural products [37,38] are studied for the management of obesity and related comorbidities, and their synergy may be potentially interesting i.e. due to the increase of human antioxidant capacity.

To support metabolic regulation, herbal medicines containing essential oils and their bioactive compounds (i.e., monoterpenes) have gained much research attention. Studies conducted in rodents fed on high-fat diets showed that the intake of monoterpenes (such as D-

Table 3
Anthropometric measurements after the intervention.

		Baseline	Follow up			Control vs CMEO	
	Group	Mean (SD)	Mean (SD)	Mean change from baseline (95% CI)	p	Mean difference (95% CI)	p
Weight (kg)	Control	96.92 (20.75)	96.79 (21.36)	-0.13 (−1.25; 1)	0.852	-1.71 (−3.63; 0.21)	0.080
	CMEO	92.44 (18.21)	90.6 (18.19)	-1.84 (−3.42; −0.26)	**0.008**		
Fat (%)	Control	41.07 (7.54)	41.08 (7.73)	0.01 (−1.2; 1.23)	0.986	-2.73 (−4.64; −0.83)	**0.005**
	CMEO	38.1 (7.52)	35.37 (7.66)	-2.72 (−4.23; −1.22)	**< 0.001**		
FFM (kg)	Control	55.08 (12.23)	54.89 (13.15)	-0.19 (−1.71; 1.32)	0.762	0.84 (−0.94; 2.62)	0.353
	CMEO	56.23 (12.75)	56.88 (12.4)	0.65 (−0.35; 1.64)	0.312		
TBW (kg)	Control	41.72 (10.65)	39.4 (10.33)	-2.31 (−5.34; 0.72)	0.073	1.84 (−1.74; 5.42)	0.310
	CMEO	42.9 (8.43)	42.43 (8.74)	-0.47 (−2.47; 1.52)	0.712		
VFL	Control	13.82 (4.73)	13.46 (4.99)	-0.36 (−0.81; 0.09)	0.167	-0.75 (−1.48; −0.02)	**0.045**
	CMEO	12.86 (4.23)	11.75 (4.27)	-1.11 (−1.7; −0.52)	**< 0.001**		
BMI (kg/m^2)	Control	35.1 (7.15)	34.4 (8)	-0.71 (−2.01; 0.6)	0.704	-0.56 (−1.23; 0.11)	0.102
	CMEO	32.71 (5.51)	32.06 (5.60)	-0.65 (−1.20; −0.09)	**0.008**		
WC (cm)	Control	111.47 (15.08)	111.64 (15.74)	0.18 (−1.59; 1.94)	0.858	-1.5 (−4.26; 1.27)	0.285
	CMEO	107.92 (13.25)	106.6 (14.04)	-1.32 (−3.50; 0.85)	0.183		
HC (cm)	Control	123.34 (20.99)	119.51 (14.08)	-3.83 (−8.36; 0.70)	0.125	3.77 (−0.96; 8.49)	0.117
	CMEO	116.51 (10.25)	116.45 (10.81)	-0.06 (−1.61; 1.50)	0.973		
SBP (mm/Hg)	Control	141.24 (18.95)	139.26 (17.98)	-1.98 (−6.52; 2.56)	0.374	-4.38 (−10.62; 1.86)	0.166
	CMEO	136.28 (18.27)	129.91 (12.98)	-6.37 (−10.77; −1.96)	**0.005**		
DBP (mm/Hg)	Control	84.89 (13.78)	89.87 (33.6)	4.98 (−4.56; 14.51)	0.171	-6.49 (−16.63; 3.65)	0.207
	CMEO	77.93 (12.27)	76.42 (16.08)	-1.51 (−5.34; 2.32)	0.676		

Data are presented as mean and standard deviation (SD). Differences in changes of variables between the two treatment groups were evaluated using general linear models. Statistical significance was set at $p < 0.05$. CI, confidence interval; CMEO, Chios Mastiha Essential Oil; FFM, free fat mass; TBW, total body water; VFL, visceral fat level; BMI, body mass index; WC, waist circumference; HC, hip circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure

Table 4
Biochemical indices after the intervention.

		Baseline	Follow up			Control vs CMEO	
	Group	Mean (SD)	Mean (SD)	Mean change from baseline (95% CI)	p	Mean difference (95% CI)	p
Urea (mg/dl)	Control	29.28 (6.86)	29.09 (9.34)	-0.19 (−3.58; 3.19)	0.898	1.26 (−3.00; 5.53)	0.557
	CMEO	29.57 (6.57)	28.11 (8.23)	-1.46 (−4.15; 1.23)	0.339		
Uric acid (mg/dl)	Control	5.37 (1.32)	5.21 (1.06)	-0.15 (−0.42; 0.11)	0.259	0.28 (−0.09; 0.66)	0.138
	CMEO	5.25 (1.28)	5.38 (1.15)	0.13 (−0.15; 0.42)	0.330		
Creatinine[1] (mg/dl)	Control	0.75 (0.12)	0.75 (0.12)	0 (−0.03; 0.03)	0.836	-0.02 (−0.06; 0.02)	0.307
	CMEO	0.77 (0.13)	0.75 (0.11)	-0.02 (−0.05; 0)	0.100		
Glucose[1] (mg/dl)	Control	99.47 (21.7)	99.96 (23.74)	0.49 (−7.12; 8.1)	0.887	-2.78 (−12.45; 6.9)	0.570
	CMEO	100.57 (30.38)	98.29 (30.19)	-2.28 (−8.47; 3.9)	0.509		
Insulin (μIU/ml)	Control	18.79 (13.23)	17.5 (12.08)	-1.29 (−5.13; 2.55)	0.429	-1.53 (−6.10; 3.04)	0.508
	CMEO	14.08 (9.29)	14.31 (9.72)	0.24 (−2.35; 2.82)	0.885		
TC (mg/dl)	Control	208.56 (53.16)	203.78 (48.55)	-4.78 (−16.27; 6.70)	0.391	-9.88 (−25.46; 5.71)	0.211
	CMEO	203.26 (39.21)	208.35 (40.6)	5.09 (−5.75; 15.94)	0.361		
TG (mg/dl)	Control	149.28 (75.19)	148.03 (70.55)	-1.25 (−18.49; 15.99)	0.871	-24.55 (−46.12; −2.98)	**0.026**
	CMEO	139.69 (53.58)	113.89 (45.28)	-25.8 (−39.24; −12.35)	**0.011**		
HDL (mg/dl)	Control	50.89 (10.14)	49.79 (13.72)	-1.10 (−3.99; 1.79)	0.394	-2.33 (−5.94; 1.28)	0.203
	CMEO	51.31 (9.87)	52.54 (11.77)	1.23 (−1.01; 3.47)	0.342		
LDL (mg/dl)	Control	137.91 (46.22)	137.79 (42)	-0.12 (−9.75; 9.52)	0.980	-13.22 (−26.46; −0.02)	**0.050**
	CMEO	135.26 (34.29)	121.92 (35.94)	-13.34 (−22.68; −4)	**0.006**		
AST[1] (iu/l)	Control	19.05 (7.28)	18.77 (6.86)	-0.29 (−1.82; 1.25)	0.722	-0.79 (−3.04; 1.45)	0.485
	CMEO	18.35 (5.42)	17.27 (6.01)	-1.08 (−2.76; 0.6)	0.181		
ALT[1] (iu/l)	Control	23.63 (12.89)	21.61 (11.3)	-2.02 (−4.79; 0.76)	0.136	-1.12 (−4.88; 2.65)	0.556
	CMEO	23.48 (12.44)	20.34 (12.36)	-3.13 (−5.75; −0.52)	**0.022**		
γ-GT[1] (iu/l)	Control	30.74 (26.03)	28.6 (19.22)	-2.13 (−5.88; 1.61)	0.174	0.64 (−3.74; 5.01)	0.773
	CMEO	21.82 (9.61)	20.32 (8.19)	-1.5 (−3.87; 0.88)	0.339		
ALP (U/L)	Control	70.38 (22.6)	74.16 (23.67)	3.78 (−0.38; 7.94)	0.065	3.94 (−1.75; 9.64)	0.172
	CMEO	68.17 (17.08)	68 (18.75)	-0.17 (−4.16; 3.83)	0.935		
Albumin (g/dl)	Control	4.61 (0.27)	4.6 (0.32)	0.01 (−0.09; 0.09)	0.967	-0.04 (−0.16; 0.09)	0.559
	CMEO	4.5 (0.26)	4.53 (0.27)	0.03 (−0.05; 0.12)	0.433		
LDH (U/l)	Control	160.82 (46.6)	163.01 (42.81)	2.19 (−10.45; 14.82)	0.708	-8.42 (−24.78; 7.94)	0.309
	CMEO	147.41 (38.77)	141.17 (39.86)	-6.24 (−16.97; 4.5)	0.287		

Data are presented as mean and standard deviation (SD). Differences in changes of variables between the two treatment groups were evaluated using general linear models. [1]Analysis was based on logarithmic transformation. Statistical significance was set at $p < 0.05$. CI, confidence interval; CMEO, Chios Mastiha Essential Oil; TC, total cholesterol; TG, triglycerides; HDL, high-density lipoprotein; LDL, low-density lipoprotein; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γ-GT, γ-glutamyl transferase; ALP, alkaline phosphatase; LDH, lactate dehydrogenase

Table 5
Inflammatory and oxidative stress markers after the intervention.

		Baseline	Follow up			Control vs CMEO	
	Group	Mean (SD)	Mean (SD)	Mean change from baseline (95% CI)	p	Mean difference (95% CI)	p
CRP[1] (mg/l)	Control	4.39 (4.02)	4.71 (5.18)	0.32 (−0.73; 1.36)	0.857	-0.21 (−1.89; 1.48)	0.566
	CMEO	4.19 (4.74)	4.72 (5.31)	0.52 (−0.83; 1.87)	0.323		
IL-6[1] (pg/ml)	Control	3.27 (2.01)	3.65 (2.55)	0.38 (−0.20; 0.97)	0.366	0.21 (−0.56; 0.98)	0.903
	CMEO	2.66 (2.31)	2.84 (2.54)	0.17 (−0.34; 0.69)	0.304		
oxLDL (U/L)	Control	94.61 (51.12)	98.18 (37.98)	3.57 (−11.1; 18.25)	0.534	-16.54 (−32.62; −0.45)	**0.044**
	CMEO	90.94 (31.13)	77.98 (24.48)	-12.97 (−20.06; −5.87)	**0.026**		
MPO[1] (ng/ml)	Control	163.01 (104.66)	174.34 (111.94)	11.33 (−4.61; 27.27)	0.251	-8.12 (−35.66; 19.41)	0.559
	CMEO	164.17 (151.81)	167.38 (178.09)	3.21 (−19.7; 26.11)	0.744		
Adiponectin[1] (μg/ml)	Control	6.36 (5.85)	6.18 (5.75)	-0.18 (−0.83; 0.47)	0.474	-1.12 (−2.17; −0.06)	**0.007**
	CMEO	5.41 (3.62)	6.35 (3.76)	0.94 (0.09; 1.79)	**0.002**		
TNF-α[1] (pg/ml)	Control	1.28 (0.57)	1.25 (0.65)	-0.03 (−0.13; 0.07)	0.196	-0.02 (−0.13; 0.09)	0.338
	CMEO	1.14 (0.6)	1.13 (0.55)	-0.01 (−0.07; 0.05)	0.954		
leptin[1] (ng/ml)	Control	79.08 (102.87)	90.32 (109.08)	11.24 (−6.70; 29.18)	0.945	9.54 (−9.4; 28.47)	0.967
	CMEO	37.71 (25.12)	39.41 (31.63)	1.71 (−5.11; 8.52)	0.898		

Data are presented as mean and standard deviation (SD). Differences in changes of variables between the two treatment groups were evaluated using general linear models. [1]Analysis was based on logarithmic transformation. Statistical significance was set at $p < 0.05$. CI, confidence interval; CMEO, Chios Mastiha Essential Oil; CRP, C-reactive protein; IL-6, interleukin-6; oxLDL, oxidized low-density lipoprotein; MPO, myeloperoxidase; TNF-α, tumor necrosis factor-alpha

limonene, thymol, cuminaldehyde, carvacrol) attenuated increases in body weight [39–42]. Similar results were evident for essential oils derived from orange peel and clove [43,44].

However, the anti-obesity effects of essential oils on humans are rather scarce (Table 7). According to a recent RCT by Jafari and co-workers in pre-diabetic patients, a daily intake of 75 mg cumin essential oil (rich in cumin aldehyde, β-pinene) for 10 weeks was associated with improved BMI and WC [45]. In the present study, the body weight of patients receiving CMEO decreased by 2% within 12 weeks.

Upon increased energy availability, the adipose tissue initiates lipogenesis by re-esterification of free fatty acids (FFA) (derived from TG hydrolysis in very low-density lipoproteins and chylomicrons) and *de novo* lipogenesis. As a result, TG deposition leads to adipose tissue hypertrophy, obesity and finally dysfunction of adipocytes, affecting tissue's endocrine and immunological responses [46,47]. Therefore, another important finding of the present study was the significant

Table 6
QoL indices after the intervention.

Scoring		Baseline	Follow up			Control vs CMEO	
	Group	Mean (SD)	Mean (SD)	Mean change from baseline (95% CI)	p	Mean difference (95% CI)	p
AIS	Control	6 (3.63)	6.06 (3.49)	0.06 (−0.71; 0.82)	0.876	0.5 (−0.5; 1.51)	0.322
	CMO	4.84 (3.35)	4.39 (2.61)	-0.45 (−1.12; 0.22)	0.214		
CESD-R	Control	15.42 (8.74)	17.03 (9.81)	1.61 (−1.14; 4.36)	0.211	1.5 (−2.1; 5.09)	0.410
	CMO	14.65 (9.96)	14.77 (9.61)	0.11 (−2.27; 2.5)	0.929		
RSES	Control	31.26 (4.72)	31.06 (4.72)	-0.2 (−1.36; 0.96)	0.787	0.19 (−1.85; 2.23)	0.855
	CMO	31.64 (5.77)	31.26 (5.91)	-0.39 (−2.1; 1.33)	0.597		
PCS-12	Control	43.57 (9.56)	44.4 (9.36)	0.83 (−1.33; 2.98)	0.400	-2.3 (−5.04; −0.45)	**0.041**
	CMO	44.91 (10.17)	48.03 (9.24)	3.12 (1.36; 4.88)	**0.002**		
MCS-12	Control	48.49 (9.03)	48.33 (8.87)	-0.15 (−2.48; 2.18)	0.910	4 06 (0.30; 7.82)	**0.035**
	CMO	47.3 (11.19)	51.22 (9.76)	3.91 (0.89; 6.93)	**0.004**		

Data are presented as mean and standard deviation (SD). Differences in changes of variables between the two treatment groups were evaluated using general linear models. [1]Analysis was based on logarithmic transformation. Statistical significance was set at $p < 0.05$. CI, confidence interval; CMEO, Chios Mastiha Essential Oil; AIS, Athens Insomnia Scale; CESD-R, Center for Epidemiologic Studies Depression Scale Revised; RSES, Rosenberg Self-esteem Scale; PCS-12, Physical Composite Score; MCS-12, Mental Composite Score.

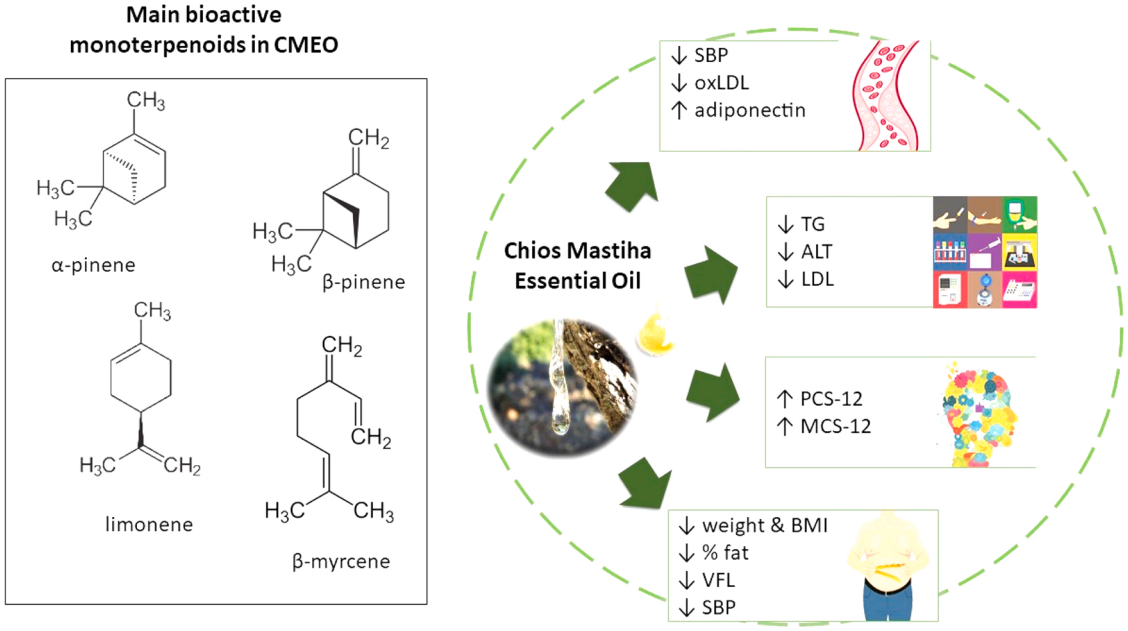

Fig. 2. Overview of the study results. Metabolically unhealthy patients who received CMEO, a monoterpene rich essential oil, exhibited regulation in SBP, lipid profile, anthropometric measurements, inflammatory status and quality of life. CMEO, Chios Mastiha Essential Oil; TG, triglycerides; LDL, low density lipoprotein; oxLDL, oxidized low-density lipoprotein; ALT, alanine aminotransferase; SBP, systolic blood pressure; VFL, visceral fat level; PCS-12, physical component score; MCS-12, mental component score.

improvement in % body fat and visceral fat level, key elements of MetS [48], in the CMEO group compared to controls.

Several essential oils have been documented to exert in vivo hypolipidemic effect, such as those derived from thyme, oregano, ginger, cumin, sage, lemon balm, peppermint, lavender. Treatment with essential oils has been linked to lower FFAs, TC, LDL and TG blood levels in rodents fed on a high-fat diet [1,39–44]. Modulation of hepatic function by lowering serum AST, ALT, and LDH has also been reported [49]. In the study by Jafari and co-workers significant ameliorations in TG and LDL levels were reported in pre-diabetic patients consuming cumin essential oil vs controls [45]. Treatment of diabetic patients with sage essential oil also resulted in decreased blood lipids [50,51]. In the present study, CMEO intake was associated with improved TG and LDL blood concentrations by almost 20% and 10% respectively, confirming a hypolipidemic effect. A significant decrease of ALT was also evident.

Two possible mechanisms of action of essential oils' lipid lowering activity have been suggested through in silico studies. The first one is agonism to peroxisome proliferator-activated receptors and the second one involves direct interaction with sterol-sensing domains, which lead to decreased transcription and accelerated degradation of HMG-CoA reductase [52]. The same study suggests a potential synergy of terpene derivatives with statins which enhances the potential use of CMEO as adjunct and complementary therapy in hyperlipidemia. Our results support that CMEO supplementation along with standard medication for metabolic disorders has promising results in the regulation of parameters related to metabolic health and can be used as adjunct to already prescribed medication. Although the synergistic interactions between statins, antihypertensive and antidiabetic agents and essential oils are not fully explored, our study offers some first evidence that this parallel administration might be safe for metabolically unhealthy patients. This is totally new insight into the drug-nutraceutical interaction that however should be viewed with caution.

Treatment with CMEO in the present trial was also linked with an anti-hypertensive activity, since SBP dropped by 6.5% in the CMEO group compared with baseline. Previous clinical studies confirm the anti-hypertensive effects of essential oils in MetS [53]. With regard to a

Table 7
Clinical trials investigating the effects of essential oils on obesity related metabolic abnormalities.

No	Bioactive compound	Study design	Effect	Ref.
1	*Cuminum cyminum* capsule at 75 mg/day vs. placebo	1. double blind RCT; patients with T2DM were divided into two groups receiving 2. *C. cyminum* capsule, 75 mg/day (n = 32) 3. placebo (n = 32) 4. duration: 10 weeks	body weight, BMI, WC ↓ blood lipids (TG, LDL, HDL) ↓ (no effect in males) (no adverse effects)	[45]
2	*Salvia officinalis* at 500 mg/day vs. placebo	1. double blind RCT; patients with T2DM were divided into two groups receiving 2. *S. officinalis* capsule, 500 mg/day (n = 40) 3. placebo (n = 40) 4. duration: 12 weeks	fasting glucose, glycosylated hemoglobin, TC, TG, LDL ↓ HDL↑ (no adverse effects)	[50]
3	*Salvia officinalis* at 500 mg/day vs. placebo	1. double blind RCT; patients with T2DM were divided into two groups receiving 2. *S. officinalis* capsule, 500 mg/day (n = 50) 3. placebo (n = 50) 4. add-on: statin treatment 5. duration: 12 weeks	fasting glucose, post-prandial glucose, glycosylated hemoglobin, TC, TG, LDL ↓ HDL ↑ (no adverse effects)	[51]
4	*Cuminum cyminum* capsule at 75 mg/day vs. placebo	1. double blind RCT; patients with MetS were divided into three groups receiving 2. C. cyminum capsule, 75 mg/day (n = 22) 3. placebo (n = 22) 4. duration: 8 weeks	body weight, BMI, WC, WHR↔ SBP ↔ DBP↓ (two volunteers with mild abdominal pain)	[53]
5	*Cuminum cyminum* capsule at doses 50 or 100 mg/day vs. placebo	1. double blind RCT; patients with T2DM were divided into three groups receiving 2. C. cyminum capsule, 100 mg/day (n = 33) 3. C. cyminum capsule, 50 mg/day (n = 33) 4. placebo (n = 33) 5. duration: 8 weeks	fasting glucose, glycosylated hemoglobin, insulin ↓ insulin sensitivity (HOMA-IR) ↑ TNF-α, CRP ↓ Adiponectin ↑ (no adverse effects)	[68]

WC, waist circumference; WHR, waist to hip ratio; TC, total cholesterol; TG, triglycerides; LDL, low-density lipoprotein; HDL, high-density lipoprotein; TNF-α, tumor necrosis factor-alpha; CRP, C-reactive protein; SBP, systolic blood pressure; DBP, diastolic blood pressure. ↓, decrease; ↑, increase; ↔, no change.

possible underlying mechanism, it has been proposed that α-pinene and β-pinene, both major monoterpenes of CMEO, induce hypotension associated with tachycardia, which could be suggestive of an effect on the peripheral vascular resistance with consequent baroreflex response [54].

In obesity, the adipose tissue is characterized by disrupted adipokine production involving increments of TNF-α, IL-6 and leptin, and depletion of adiponectin, that in turn stimulate inflammation and the development of cardiometabolic lesions [55]. At the same time, mitochondria of adipocytes produce large amounts of reactive oxygen species (ROS) that trigger OS, processes that further aggravate inflammation [56]. Upon LDL oxidation, the oxLDL molecules are uptaken by visceral adipocytes, and their increase stimulates the pro-inflammatory profile in the adipose tissue. Furthermore, oxLDL is not recognized by the LDL receptors and is accumulated by macrophages leading to foam cell formation [57].

During the past few decades, novel drug approaches targeting obesity and regulation of metabolism have been investigated. For instance, significant increase of adiponectin has been well documented after glucagon-like peptide-1 receptor agonist (GLP-1 RA) treatment in patients with metabolic abnormalities, leading to adipose tissue decrease, alleviation of inflammation and OS, and glucoregulation [58]. According to a recent meta-analysis, there is a greater impact of GLP-1 RA on adiponectin levels than a low-calorie diet [58]. Although the underlying mechanism is not fully understood, it has been shown that GLP-1 RA-induced adiponectin secretion is mediated by the protein kinase A pathway in adipocytes [59], and by the upregulation of adiponectin expression at mRNA and protein levels through Sirt1 and transcriptional factor Foxo-1 signaling pathways [60]. Concomitantly, in cultured endothelial cells, GLP-1 RA ameliorates oxLDL-induced reduction of the transcriptional factor KLF2 and inhibits expression of the vascular adhesion molecules, E-selectin and vascular cell adhesion molecule 1. As a consequence, monocyte adhesion to endothelial cells is inhibited. Alike, the GLP-1 RA attenuates oxLDL-induced reduction of endothelial nitric oxide synthase (eNOS) expression and NO availability [61] and the cardiovascular complications of arterial hypertension by reduction of vascular inflammation [62]. Noteworthy outcomes of the present trial were the lower oxLDL levels along with greater adiponectin production and lower SBP levels in CMEO patients compared to controls. This remark suggests that CMEO, a natural essential oil rich in bioactive monoterpenes, may share common pathway mechanisms with anti-obesity drugs that advocate not only weight loss and body fat reduction, but also vascular inflammation and hypertension. Overall, these observations are of great importance and further future research may result in the synthesis of new drugs with novel molecules, such as from CMEO, to manage the obesity related metabolic abnormalities. This has been highlighted by Vesa and Bungau when investigating the impact of novel molecules introduced in the treatment of diabetes mellitus, dyslipidemia, and CVD [63]. As has been suggested, the numerous beneficial effects of medications, such as GLP-1 agonist, or derivatives of the terpene quinopimaric acid, may have impact in human health and thus they should be considered in clinical practice [63].

The antioxidant and anti-inflammatory activities of monoterpenes are well documented by in vitro and in vivo models, in which monoterpenes inhibited NO release, reduced prostaglandin E2, TNF-α and IL-6 levels in LPS-stimulated RAW264.7 macrophages, and attenuated macrophage migration in monoterpene treated animal models [64]. Reduction of the inflammatory cytokine TNF-α has also been reported after the intake of *Cuminum cyminum* essential oil by diabetic patients for 2 months [65]. According to Maxia and co-workers, essential oil isolated from *P. lentiscus* leaves decreased serum IL-6 and TNF-α in rats with cotton pellet-induced granuloma suggesting a potential role in the treatment of inflammatory conditions [66].

A plethora of data indicates that obesity/overweight is associated with impaired health-related QoL [67]. In this study, mental and physical functioning were significantly improved in patients treated with CMEO versus controls. This improvement can be attributed to the fact that the regulation of cardiometabolic parameters, such as % body fat, VFL, blood lipids, SBP, inflammation and OS may result in a better mental and physical function. Our group recently highlighted the importance of mental health when managing overweight/obesity and related metabolic abnormalities. More specifically, IL-6 was negatively associated with self-esteem (RSES) and TNF-α positively with depression (CESD-R) in a population of 122 metabolically unhealthy obese Greek patients (Amerikanou et al., manuscript submitted for publication).

The dried resinous exudate, Chios Mastiha, has been extensively studied during the last few decades. Its anti-inflammatory and

antioxidant potential in humans is associated with a daily intake of about 2 g [21–23]. This accounts for the 0.2 g dosage of CMEO selected in the present study, considering that the amount of CMEO in the resin is 10% [19]. It is noteworthy that none of the patients reported discomfort or adverse effects after CMEO intake. The long-term safety of CMEO intake has not been sufficiently investigated, while the maximum safe dose remains unknown. However, high doses (up to 1 ml) have been well tolerated in human studies [19]. According to in vitro and in vivo research on CMEO toxicity, no genotoxic, mutagenic or recombinogenic activities were evident [68]. In particular, in vitro experiments showed that lymphocytes treated with CMEO did not increase the frequency of micronuclei. In the same study, in vivo experiments with *Drosophila* fed on CMEO, did not increase total wing spots, indicative of safety as well. Additionally, Zebrafish supplementation with CMEO showed that the essential oil does not interfere with lateral line neuromasts and does not affect gastrointestinal development suggesting its safety at the concentration of 20 ppm [69]. Furthermore, CMEO not only inhibited colon carcinoma tumors growth in mice, but was also proven safe, as no toxicity occurred in the dose of 0.42 + 0.11 g/kg of animal body weight [70]. CMEO is rich in monoterpenes, mainly α-pinene, β-myrcene and β-pinene (Fig. 2) with different pharmacological activities [71]. Interestingly, isolated CMEO compounds have been, investigated as to whether its activities individual or owed to a synergistic effect. Although α-pinene showed the greatest antiproliferative effect in vitro it did not show antitumor effect in vivo, nor in a mixture with myrcene, proving a combined antitumor effect of compounds in the essential oil [70]. This coincides with the literature that suggests that naturally occurring combinations of phytochemicals in essential oils are more effective than individual compounds [71]. Our study supports the safety of CMEO use as the absence of reported advert events was accompanied by a lack of significant changes in biochemical markers of toxicity. Although several essential oils have been documented for their antioxidant properties [72], this is the only human trial with CMEO in participants with obesity and different metabolic abnormalities, including not only antioxidative properties, but also a plethora of anthropometric, clinical, biochemical, inflammatory mental health and overall quality of life variables. Another clinical trial with Mastiha oil is NCT05858372 (clinicaltrials.gov). It has a different design, including only healthy participants and biochemical measurements and published data are anticipated.

5. Conclusion

Patients with metabolic abnormalities co-treated with CMEO, a natural non-toxic natural product rich in monoterpenes, showed significant improvements in blood lipid profile, SBP and anthropometric indices paralleled by improvement in health-related QoL. These favorable regulations were accompanied by modulation of inflammatory and OS processes, and enhancement of adipose tissue function. The present trial had some limitations. The investigation of one CMEO dosage at a single time period (3 months) may also have hindered a more thorough elucidation of the effects of CMEO. The self-reported tools that were used in the present study could also be a source of bias. However, we viewed the sufficient sample size as a significant strength, allowing us to manage the possibility of false-positive or false-negative results. All qualitative assessment methods applied have been previously validated. Finally, the abundance of study variables made data saturation easier. Although these data cannot be generalized and larger studies must be conducted to confirm any possible interactions with common drug treatments, the outcomes of the present RCT suggest the pharmacological potential of CMEO in metabolic disorders.

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CRediT authorship contribution statement

Andriana C. Kaliora: Conceptualization, Supervision, Visualization. **Aristea Gioxari**, **Charalampia Amerikanou**: Methodology, Original draft preparation. **Ioannis Stergiou**: Methodology. **Chara Tzavara**: Statistical analysis. **Aristea Gioxari**, **Charalampia Amerikanou** , **Evdokia Valsamidou**, **Stamatia-Angeliki Kleftaki**, **Aikaterini Kalaitzopoulou**: Investigation, Data curation. All: Writing – review & editing.

Declaration of Competing Interest

Author I.S. owns no shares in Mastiha Research Center. This funding source provided the consumables but did not have any role in study design, collection, and analysis, interpretation of the data or decision to submit this manuscript. The remaining authors declare no conflict of interest.

Data availability

Data will be made available on request.

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