The effect of oleoylethanolamide supplementation on pyroptotic cell death in obese patients with non-alcoholic fatty liver disease: A double-blinded randomized controlled clinical trial

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Abstract

Background

Non-alcoholic fatty liver disease (NAFLD) is now the main contributor to the worldwide chronic liver diseases. Pyroptosis has recently gained interest as a pathway for hepatocyte death under liver injuries. Blocking nucleotide-binding oligomerization domain-like receptor family pyrin domain containing 3 (NLRP3)-Inflammasome, a prominent player in the pyroptosis pathway, has been indicated to positively affect the NAFLD pathophysiology. Animal studies show that oleoylethanolamide (OEA) supplementation can reduce nuclear factor-kappa B and some other inflammatory cytokines, which play pivotal roles in the pyroptosis pathway.

Materials and methods

The current doubleblinded randomized controlled clinical trial aimed to study the effects of OEA supplementation on the pyroptosis pathway in 65 adults with obesity and NAFLD. The intervention lasted for 12 weeks, and included 250 mg/day of OEA or placebo (starch). Subjects were also administered a personalized calorie-restricted diet based on the results of indirect calorimetry.

Results

The OEA group showed significant improvements in their anthropometric measures. All study genes indicated significant differences compared to their baseline in both groups; the expression of cysteinedependent aspartate-specific protease (Caspase) 1, NLRP3, and interleukin (IL)-18 decreased, while the expression of toll-like receptor 4 (TLR4) increased. However, no significant intergroup differences were witnessed for none of the study genes.

Conclusions

The current study indicates that OEA supplementation has positive effects on anthropometric measures (weight, BMI, WC, WHR, and WHtR); however, significant intergroup differences were not witnessed in the pyroptosis pathway. More studies are recommended to evaluate the current findings.

Trial registration

The protocol of this clinical trial is registered with the Iranian Registry of Clinical Trials (https://www.irct.ir, identifier: IRCT20110530006652N2).

1. Background

Non-alcoholic fatty liver disease (NAFLD), a spectrum of chronic liver conditions, is described as an excessive fat accumulation of at least 5% of liver weight in the absence of other etiologies, such as alcohol over-consumption (1). This spectrum comprises a range from simple steatosis (Non-alcoholic fatty liver (NAFL)), which lacks hepatocellular injury, to steatohepatitis (NASH), which is concurrent to inflammation and hepatocyte injury with or without fibrosis (2). NAFLD is the main contributor to worldwide chronic liver diseases, in parallel with the growing availability of drugs to cure chronic HCV and treat chronic HBV (3, 4). The results of a meta-analysis of studies from 1989 to 2015 estimated the global prevalence of NAFLD to be 25.2%, and 59.1% of the biopsied NAFLD patients were indicated to have NASH (5). NAFLD prevalence is highly correlated with obesity, metabolic syndrome, and type 2 diabetes (7). It is estimated that NAFLD and obesity will follow a similar growth pattern in the upcoming years (6). In a study in 2019, NAFLD prevalence was estimated to be 15–30% among the general population, while it was 50–90% prevalent among the obese (7).

As NAFLD is closely linked to metabolic syndrome, lifestyle modification is considered an essential management approach (8, 9). Some other helpful therapeutic approaches include insulin sensitizers (e.g., thiazolidinediones/peroxisome proliferator-activated receptor (PPAR)-γ ligands), anti-TNF-α agents (e.g., Pentoxifylline), anti-hypertensive Drugs (e.g., Valsartan), endocannabinoid receptor antagonists (e.g., Rimonabant), antioxidants (e.g., vitamin E), weight-loss medications (e.g., Orlistat), lipid-lowering agents (e.g., statins, fibrates, Probucol, ω-3 FAs), ursodeoxycholic acid (UDCA), Silymarin (milk thistle), betaine (choline metabolite), acetylsalicylic acid, and L-carnitine. Bariatric surgery for weight loss and liver transplantation for cirrhotic patients are also among other approaches (1, 10–16).

Several mechanisms are proposed to be involved in excessive intrahepatic fat accumulation, including increased hepatic influx of fatty acids (FAs), greater *de novo* lipogenesis (DNL), and/or lower clearance of FA through very-low-density lipoprotein (VLDL) secretion or β-oxidation (17). The exact physiopathology for NAFLD is not completely clarified yet. In particular, there remain questions why some may progress towards inflammation, fibrosis, and cirrhosis.

Pyroptosis is a highly inflammatory type of programmed cell death (PCD) and is dependent on inflammatory caspases, mainly caspase-1 (Canonical pathway) (18–20). Pyroptosis has more recently been indicated to play a role in hepatocyte death under liver injuries (21). Blocking nucleotide-binding oligomerization domain-like receptor family pyrin domain containing 3 (NLRP3)-inflammasome, a prominent player in the pyroptosis pathway, has been indicated to positively affect NAFLD pathophysiology (22, 23). NLRP3-Inflammasome activation is suggested to be undertaken by a primary signal (signal I) in advance of or simultaneous with a secondary signal (signal II). Signal I is carried out by nuclear factor-kappa B (NF-κB)-activating receptors, such as tumor necrosis factor (TNF) receptor, toll-like receptors (TLRs), interleukin (IL)-1 receptor, and IL-18 receptor (24–26). Subsequently, signal II is accomplished by the agonists that induce activation of NLRP3 and assembling of the inflammasome complex, ultimately resulting in Caspase-1 activation, production of mature IL-1β and IL-18, and also drives cleavage of gasdermin D (GSDMD), which generates an N-terminal fragment that oligomerizes to

develop pores on the host cell membrane and causes rupture, lytic cell death, and release of the intracellular pro-inflammatory contents (27).

The endocannabinoid system (ECS) is a homeostatic system that has roles in energy homeostasis (28–30). Endocannabinoid-like compounds are bioactive lipids that share the same biosynthesis and degradation pathway, although not binding to the cannabinoid receptors (31). Oleoylethanolamide (OEA) is such a compound, which unlike the ECS, has been shown to suppress appetite (32, 33). It has been shown that OEA supplementation could positively affect NAFLD and related factors, such as steatosis, glycemic profile, lipid profile, the level of liver enzymes, and redox status (34–38). OEA has also been indicated to ameliorate body inflammatory status (37–42). As mentioned before, the pyroptosis pathway has recently been shown to play a role in hepatocyte death under liver injuries. These bring to mind the question of whether OEA could also affect the highly inflammatory pyroptosis pathway and thereafter, ameliorate the NAFLD pathophysiology.

The development of novel adjuvant therapies for the prevention or treatment of NAFLD patients seems necessary, especially due to its growing epidemy and burden. Here, we intended to examine whether OEA could ameliorate the NAFLD status by affecting the highly inflammatory pyroptosis pathway in human subjects with NAFLD. It has been suggested that peripheral blood mononuclear cells (PBMCs) are reflective for metabolic responses of hepatocytes, and also they can be used to explore the response of dietary interventions in relation to inflammation (43, 44). Therefore, we decided to study the effects of our intervention on PBMCs of obese adults with NAFLD. To the best of our knowledge, the current research was the first human randomized controlled clinical trial (RCT) to examine the effects of OEA supplementation on the transcription of the pyroptosis pathway genes in PBMCs of obese adults with NAFLD.

It should be mentioned that this study was conducted as part of a relevant mega project, in which we had previously shown that OEA could significantly improve the glycemic profile, lipid profile, and the level of liver enzymes, as well as inducing the expression of PPAR-α, uncoupling protein (UCP)-1, and UCP-2 (35). Here, a mechanistic hypothesis regarding the effect of OEA on the pyroptosis pathway in obese adults with NAFLD is examined.

2. Materials And Methods

The current double-blinded randomized controlled clinical trial included newly-diagnosed NAFLD adults aged 20–50 years old and body mass index (BMI) of 30–50 kg/m^2. The diagnosis of NAFLD was performed by a radiologist through ultra-sonography (SonoAce X4 ultrasound system, South Korea) in a fasting state. The exclusion criteria were (1) routine use of non-steroidal anti-inflammatory drugs (NSAIDs), antibiotics, and corticosteroids, (2) use of herbal medicines, hormonal drugs, hepatotoxic drugs (such as Amiodarone, Phenytoin, and Tamoxifen), anti-hypertensive drugs, weight-loss drugs, and lipid-lowering agents, (3) use of prebiotic and probiotic supplements, vitamins, minerals, antioxidants, and ω-3 supplements, (4) diagnosed pathological conditions affecting liver (e.g., liver transplantation, viral

hepatitis, cystic fibrosis, hemochromatosis, Wilson's disease, alpha-1 antitrypsin deficiency, and acute systemic disease), (5) pregnancy and lactation, (6) diagnosed thyroid disorders, kidney diseases, gastrointestinal diseases (e.g., Celiac disease), diabetes, heart failure, autoimmune diseases, malignancies, and severe psychological disorders. The study took place at Tabriz University of Medical Sciences, Iran from February to June of 2019.

2.1. Sample size and randomization

As this research was the first human trial to study the effects of OEA supplementation on the pyroptosis pathway genes, the sample size was estimated based on the expression of NF-κB (34), a closely relevant factor in the pyroptosis pathway. According to the formula, and considering 95% confidence interval (CI), 90% power, as well as 10% potential loss to follow-up, 70 volunteers were included in the study. Subjects were randomly assigned to one of the study groups using Random Allocation Software by an independent colleague out of the team. Stratification was performed based on gender and fatty liver grading.

2.2. Supplements

OEA supplements were produced by the Nutrition Research Center of Tabriz University of Medical Sciences (Tabriz, Iran) through gas chromatography-mass spectrometry (GC-MS) under patent number 101756. The detailed process is explained elsewhere (33). All of the capsules were similar in shape and color.

2.3. Intervention protocol

Participants in the OEA and the placebo groups were asked to take one 125 mg OEA capsule or similar amounts of placebo (starch) one hour before their lunch and dinner (two capsules per day) for 12 weeks. Both capsules were identical in appearance and patients were provided with 90 capsules twice during the study. The corresponding intervention dose was decided based on a similar trial by Payahoo *et al.* (33), in which no side effects were reported. All subjects were also administered an individually-designed calorie-restricted diet with approximately 500 kcal/day less than their estimated energy expenditure and based on their previous dietary habits. Indirect calorimetry (COSMED, Italy) was used to estimate daily energy expenditure. Confounding variables were statistically controlled and included baseline values, age, changes in physical activity, energy intake, and BMI.

2.4. Demographics questionnaires and anthropometric measurements

Baseline characteristics were collected from the participants, including demographic data (age, gender, marital status, occupation, and level of education), disease history, and medications. A digital scale (Seca, Germany) and a stadiometer (Seca, Germany) were used to measure weight and height. Weight was measured with minimum clothing without shoes and in a fasting state. Measures were rounded to the nearest 0.5 cm and 0.1 kg. BMI was calculated as weight/height2 (kg/m2). Circumferences were

measured by a non-stretching measuring tape. WC was the smallest horizontal girth between the costal and iliac crest, and hip circumference (HC) was measured over the widest part of the great trochanters. Waist to hip ratio (WHR) and waist to height ratio (WHtR) were calculated accordingly.

2.5. Gene transcription study

Blood samples (10 mL) were obtained in a fasting condition (10–12 hours, water permitted) at the baseline and endpoint of the study using Ethylenediaminetetraacetic acid (EDTA)-containing vacuum collection tubes. Samples were transferred from the sampling site to the laboratory, while preserving the cold chain, without freezing.

PBMCs were isolated using Lymphodex solution (Ficoll and sodium diatrizoate, Inno-train, Germany) and density gradient centrifugation. Total RNA was purified using TRIzol™ solution (Invitrogen™, Thermo Fisher Scientific, USA), according to the manufacturer's instructions. The extracted RNA was assessed for quality and quantity by a NanoDrop™ spectrophotometer (Thermo Fisher Scientific, USA). Later, total RNA was converted to complementary DNA (cDNA) using oligo (dT), random primer, and reverse transcriptase, according to the cDNA synthesis Kit (BioFact™ RTase, South Korea) protocol.

The real-time polymerase chain reaction technique (Roche LightCycler®, Switzerland) was utilized to assess the mRNA expression levels of TLR4, NLRP3, Caspase1, and IL-18, using SYBR® Green Master mix (BioFACT™ 2X Real-Time PCR Master Mix, South Korea). Primer Bank was the reference for designing primer sequences (Table 1). Threshold cycle (CT) values were obtained for each sample and fold changes were calculated by the $2^{-\Delta\Delta CT}$ formula. β-actin was considered as the housekeeping gene.

Table 1
Primer sequences of the study genes

TLR4	**Forward: TATTAAATGCTGCCACATGTC**
	Reverse: GTTGGTTGAAATGCCCAC
NLRP3	Forward: AGCATCGGGTGTTGTTGTCA
	Reverse: AAGATAGCGGGAATGATGATATGAG
Caspase1	Forward: GTCAAGCCGCACACGTCT
	Reverse: TTTCACATCTACGCTGTACCCCA
IL-18	Forward: GACTGTAGAGATAATGCACCC
	Reverse: TTTCTCACACTTCACAGAGAT

2.6. Ethics

The study protocol was approved by the Ethics Committee of Tabriz University of Medical Sciences (Tabriz, Iran), based on the Declaration of Helsinki standards (ethics code: IR.TBZMED.REC.1398.1131).

This work is listed in the Iranian Registry of Clinical Trials (IRCT) (registration number: IRCT20110530006652N2).

2.7. Statistical analysis

Statistical analyses were performed using IBM Statistical Package for the Social Sciences (SPSS) 23 (IBM, USA). Shapiro-Wilk test, along with the analysis of skewness, kurtosis, and histograms were used to determine the normality of the values. Data were presented as mean (standard deviation (SD)) for parametric numerical data, median (interquartile range) for non-parametric numerical data, and frequency (percent) for categorical variables. To compare the baseline differences between the study groups, independent samples T-test or Mann-Whitney U test were used, as appropriate. Paired samples T-test and Wilcoxon matched-pair signed-rank test were used for intragroup comparison, as appropriate. To assess between- and within-group differences of qualitative variables, Fisher's exact test and Sign test were applied, respectively. Confounding factors (baseline values, age, and changes in physical activity, energy intake, and BMI) were adjusted by analysis of covariance (ANCOVA) test via three separate models: baseline values (model 1); baseline values, age, and changes of physical activity and energy intake (model 2); and, baseline values, age, changes of physical activity and energy intake, and BMI (model 3). For values with abnormal distribution, quantile regression was applied to compare intergroup changes, using STATA software version 16 (StataCorp, USA), while adjusting for the above potential confounders. In addition, mean difference (95% CI) was presented for parametric values using SPSS, while median difference (95% CI) was reported for non-parametric values, using STATA. Percent changes were calculated as follows: ((end value – baseline value) / baseline value) * 100. Figures for gene transcriptions are rendered using GraphPad Prism 8 (GraphPad Software, USA). P-values below 0.05 were considered statistically significant.

3. Results

Figure 1 represents the flow of the study. The results for 65 individuals were analyzed at the endpoint of the study.

3.1. Baseline characteristics of the study groups

Baseline characteristics of the study population are represented in Table 2. There were 35 males (53.8%) and 30 females (46.2%) in the study. No significant intergroup differences existed in terms of age, gender, marital status, education level, occupation, physical activity level, as well as fatty liver severity.

Table 2
Baseline characteristics of the study groups

Parameters		OEA (n = 34)	Placebo (n = 31)	P
Age (years)		40.32 (7.4)	41.81 (7.7)	0.435[a]
Gender	Male	18 (52.9)	17 (54.8)	1.00[b]
	Female	16 (47.1)	14 (45.2)	
Marital status	Single	5 (14.7)	3 (9.7)	0.814[b]
	Married	26 (76.5)	26 (83.9)	
	Divorced or widow	3 (8.8)	2 (6.5)	
Education level	Illiterate	2 (5.9)	4 (12.9)	0.628[b]
	Diploma or lower	23 (67.6)	21 (67.7)	
	Bachelors or higher	9 (26.5)	6 (19.4)	
Occupation	Housewife	13 (38.2)	11 (35.5)	0.854[b]
	Employee	13 (38.2)	14 (45.2)	
	Self-employed	8 (23.5)	6 (19.4)	
Physical activity level	Low	25 (73.5)	26 (83.9)	0.668[b]
	Moderate	8 (23.5)	4 (12.9)	
	High	1 (2.9)	1 (3.2)	
Fatty liver severity	Mild	30 (88.2)	22 (71)	0.054[b]
	Moderate	3 (8.8)	9 (29)	
	Severe	1 (2.9)	0 (0)	

OEA, Oleoylethanolamide

Age is presented as mean (SD); other variables are presented as frequency (%).

[a] Independent Samples T-Test. [b] Fisher's exact test.

3.2. Effects of OEA on the pyroptosis pathway

Effects of the intervention on the PBMC level expression of TLR4, Caspase1, NLRP3, and IL-18 are shown in Fig. 2. Data are presented as logarithms of fold changes (median (95% CI)). All of the study genes had significant differences compared to their baseline in both study groups; i.e. TLR4 expression increased in both study groups, while the expression of the other study genes decreased. When compared between the study groups, none of the genes showed a significant difference.

3.3. Effects of OEA on the anthropometric measures

As indicated in Table 3, baseline anthropometric measures (weight, BMI, WC, HC, WHR, and WHtR) did not reveal significant intergroup differences. However, all of the indices were decreased significantly in both groups at the endpoint of the study, except for weight in the placebo group, which indicated a non-significant reduction. The OEA group had significantly lower anthropometric measures, compared to the placebo group after adjusting based on both models 1 (baseline values) and 2 (baseline values, age, and changes in physical activity and energy intake). Model 3 (baseline values, age, changes of physical activity and energy intake, and BMI) was not applied in analyzing the anthropometric measures, as BMI in this model was one of the target anthropometric measures.

4. Discussion

NAFLD has now emerged as the most frequent form of chronic liver disease. Currently, the FDA is not suggesting any definite treatment, and lifestyle modification still comes as the first-line treatment approach. Pyroptosis is a highly inflammatory form of PCD, which has just come to an interest as a pathway for hepatocyte death under liver injuries. The present study was an RCT aiming to examine the effects of OEA supplementation on the transcription of the pyroptosis pathway genes in obese adults with NAFLD. In the present study, 250 mg/day OEA supplementation for twelve weeks significantly decreased the expression of Caspase1, NLRP3, and IL-18, and increased the expression of TLR4 within each of the study groups, while no meaningful intergroup differences were witnessed.

As this study was the first human trial to examine the effects of OEA supplementation on the pyroptosis pathway, no direct study was found in this regard. However, we discuss studies concerning the effects of OEA on essential mediators of the pyroptosis pathway. OEA has been shown to inhibit the TLR4-mediated NF-κB signaling pathway, and interfere with ERK1/2-dependent cascade (TLR4/ERK1/2/AP-1/STAT3) (40, 42, 45–50). Antón *et al.* indicated that OEA pre-treatment (5 mg/kg, i.p.) in advance of intragastric alcohol gavage (3 times/day, 4 days) in rats attenuated the expression of TLR4 and high mobility group box 1 (HMGB1) danger signal, and prevented the NF-kB cascade. OEA diminished the levels of IL-1β, TNF-α, COX-2, inducible nitric oxide synthase (iNOS), and the monocyte chemoattractant protein-1 (MCP-1). OEA also blocked alcohol-induced lipid peroxidation, caspase-8, and pro-apoptotic caspase-3 activation (51). A study by Sayd *et al.* in 2015 revealed that 10 mg/Kg i.p. OEA pretreatment (10 minutes in advance of LPS administration) led to a drop in plasma TNF-α levels, attenuated brain TNF-α, and inhibited the LPS-induced NF-κB/IκB-α upregulation in the nuclear and cytosolic extracts, respectively. OEA also decreased the expression of IL-1β, iNOS, COX-2, PGE2, and microsomal PGE2 synthase levels. Further, it diminished the LPS-induced oxidative/nitrosative stress. OEA also disrupted the LPS-induced anhedonia, as evidenced by the saccharine preference test (42). Consistently, Yang *et al.* utilized LPS (1 μg/ml) to stimulate THP-1 cells, a human monocytic cell line, with or without OEA (10, 20, and 40 μM). OEA acted as a potent anti-inflammatory agent and decreased TLR4 expression and the formation of pro-inflammatory cytokines (TNF-α, IL-1β, and IL-6) and raised PPAR-α expression. The protective role of OEA in the LPS-induced inflammation relied on PPAR-α and TLR4. Moreover, OEA inhibited the LPS-induced

NF-κB activation, IκB-α degradation, and AP-1 expression. OEA also prevented the phosphorylation of ERK1/2 and STAT3. These effects suggest a potential role for OEA in inflammatory diseases (41). Hu *et al.* indicated that OEA administration (10 mg/Kg) could significantly inhibit the LPS/D-galactosamine (D-Gal)-induced hepatocytes injury, diminished the expression of Bax and Bcl-2, and cleaved Caspase-3 to suppress hepatocyte apoptosis. Additionally, significant reductions were observed in the number of activated intrahepatic macrophages, as well as mRNA expression of TNF-α, IL-6, and MCP1 after OEA administration. OEA obviously increased hepatic PPAR-α expression and reduced the expression of IL-1β in the liver and plasma through the inhibition of NLRP3 and Caspase-1, which indicated that OEA could suppress the NLRP3 inflammasome pathway (52).

In 2020, Otagiri *et al.* induced colitis in rats by oral dextran sulfate sodium (DSS) administration. They demonstrated that OEA suppressed the LPS-induced expression of TNF-α and phosphorylation of IκB-α in human embryonic kidney cells. Also, OEA diminished the expression of IL-8 and IL-1β, and prohibited IκB-α and p65 phosphorylation in human enterocytes (Caco-2), all induced by TNF-α. MKK866, a PPAR-α antagonist, prevented the effect of OEA on lowering IL-8 expression. In their *in vivo* studies, they indicated that OEA administration significantly improved the colitis-induced weight loss in the rats, independent of food intake. OEA also tended to decrease the histological score and the expression of inflammatory cytokines in rectum. These data suggest that OEA administration led to a significant amelioration in the colitis model, probably via inhibition of the NF-κB signaling pathway through PPAR-α receptors (40).

In an RCT on obese NAFLD patients in 2018, Payahoo *et al.* showed that 250 mg/day OEA supplementation for eight weeks could significantly reduce serum concentrations of IL-6 and TNF-α; nevertheless, reductions in MDA, hs-CRP, and TAS were not significant (39). In a study in 2020, Tutunchi *et al.* illustrated that hepatic fibrosis did not significantly improve in newly-diagnosed NAFLD patients intervened with 250 mg OEA and calorie restriction; however, it led to substantial reductions in NF-κB and IL-6 levels, while increasing IL-10. Also, the OEA group experienced a significant decline in fat mass and a rise in fat-free mass, compared to the

placebo group (34).

The anti-inflammatory and cytoprotective effects of OEA are shown to be, at least to an extent, due to PPAR-α, whose activation negatively modulates the transcription of inflammatory response genes by opposing the AP-1 and NF-κB signaling pathways. OEA could also inhibit the TLR4-mediated NF-κB signaling pathway and interfere with the ERK1/2-dependent cascade (TLR4/ERK1/2/AP-1/STAT3) (40, 45–50).

On the other hand, some other studies suggest that OEA or its targets can aggravate cell death. In an *in vitro* study by Chinetti *et al.*, it was shown that PPAR-α ligands could induce apoptosis in macrophages activated with TNF-α/IFN-γ (53). In line with Chinetti *et al.*, Tam *et al.* in 2020 indicated that while OEA could inhibit pyroptosis, it could induce necroptosis in post-influenza superinfection by Staphylococcus aureus in mice (54). In their *in vitro* research in 2011, Lueneberg *et al.* cultured cerebellar granule neurons with URB597 (25, 50, or 100 nM), a FAAH inhibitor, as well as OEA (25 nM) or PEA (100 nM). They

indicated that URB597, OEA, or PEA all promote cellular death (55). While no mechanism of action was proposed, further investigations were recommended by the authors.

Inconsistencies among studies could be rooted in different dosing, sample size, duration, and/or other specific methodologies. Furthermore, as indicated in our previous work (35), as well as other studies mentioned above, OEA seems to affect the NAFLD pathophysiology mainly via the PPAR-α pathway. Therefore, more direct studies are recommended for gaining better insight into the role of OEA in hepatocyte pyroptotic death.

In this trial, OEA supplementation led to significant reductions in the anthropometric measurements of the OEA group. In line with the present study, Payahoo *et al.* demonstrated that 250 mg/day OEA supplementation for eight weeks in obese patients led to significant reductions in weight, BMI, WC, and fat mass, compared to the placebo (56). In our previous study in 2020, it was also illustrated that 250 mg/day OEA supplementation in NAFLD-diagnosed obese adults for 12 weeks could significantly enhance the anthropometric measures (35). The study by Rondanelli *et al.* showed that the administration of an oily complex consisting of 85 mg NOPE and 50 mg epigallocatechin-3-gallate (EGCG) for eight weeks on 138 healthy overweight/obese subjects resulted in a significant increase in the sense of fullness, and also a marked weight loss (57). However, in a work by Mangine *et al.*, supplementing a combination of 120 mg NOPE and 105 mg EGCG for eight weeks on 50 healthy overweight subjects had no significant effects on body mass, body composition, and binge eating behaviors (58).

Consistently, some animal studies suggest positive effects of OEA administration on anthropometric indices (56, 59–63). The etiology behind this is mostly thought to be the anorexic effect of OEA (64–67). There are multiple pathways for the anorexic effect of OEA. One of the most important pathways is increased meal latency (68–72). Lipolysis and β-oxidation are other key mechanisms by which OEA causes weight loss (73, 74). In a study by Thabius *et al.* in 2010, they concluded that up to 58% of the reduced fat gain following OEA supplementation was explained by six pathways: lipid transport, energy intake, energy expenditure regulation, endocannabinoid signaling, lipogenesis, and glucose metabolism (67).

The current research offers some strengths. First of all and to the best of our knowledge, the present study was the first RCT to examine the effects of OEA supplementation on the pyroptosis pathway in NAFLD patients. Moreover, it was conducted on newly-diagnosed NAFLD patients. Finally, both study groups were also administered a personalized calorie-restricted diet.

On the other hand, the present trial had some limitations, as well. In this research, liver ultrasonography, but not liver biopsy, was applied for diagnostic and fatty liver grading purposes. However, note that it was not practical to do liver biopsy on human samples due to its invasive nature Moreover, serum levels of OEA and other pyroptotic mediator genes in the pyroptosis pathway were not measured due to financial shortcomings.

5. Conclusions

In short, the results of the current study indicated that OEA supplementation had favorable effects on anthropometric measures (weight, BMI, WC, WHR, and WHtR); however, significant intergroup differences were not witnessed in the pyroptosis pathway. More interventions with longer durations and higher doses on patients with higher grades of NAFLD are recommended.

Abbreviations

Non-alcoholic fatty liver disease (NAFLD), non-alcoholic fatty liver (NAFL), non-alcoholic steatohepatitis (NASH), peroxisome proliferator-activated receptor (PPAR), ursodeoxycholic acid (UDCA), fatty acids (FAs), de novo lipogenesis (DNL), very-low-density lipoprotein (VLDL), programmed cell death (PCD), nucleotide-binding oligomerization domain (NOD)-like receptor (NLR), NLR family pyrin domain containing 3 (NLRP3), nuclear factor-kappa B (NF-κB), tumor necrosis factor (TNF), toll-like receptors (TLRs), interleukin (IL), gasdermin D (GSDMD), endocannabinoid system (ECS), oleoylethanolamide (OEA), peripheral blood mononuclear cells (PBMCs), randomized controlled clinical trial (RCT), uncoupling protein (UCP), body mass index (BMI), non-steroidal anti-inflammatory drugs (NSAIDs), confidence interval (CI), gas chromatography-mass spectrometry (GC-MS), waist to hip ratio (WHR), waist to height ratio (WHtR), ethylenediaminetetraacetic acid (EDTA), complementary DNA (cDNA), threshold cycle (CT), Statistical Package for the Social Sciences (SPSS), standard deviation (SD), analysis of covariance (ANCOVA), high mobility group box 1 (HMGB1), inducible nitric oxide synthase (iNOS), and the monocyte chemoattractant protein-1 (MCP-1), human enterocytes (Caco-2), epigallocatechin-3-gallate (EGCG).

Declarations

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Ethics approval and consent to participate

We confirm that any aspect of the work covered in this manuscript that involves human patients has been approved by the ethical committee of Tabriz University of Medical Science, Tabriz, Iran. The protocol of this trial is also registered with the Iranian Registry of Clinical Trials (https://www.irct.ir, identifier: IRCT20110530006652N2).

Consent for publication

Not applicable.

Competing interests

The authors declare no conflict of interest.

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Authors' contributions

MH, MSA, and AO designed the research and contributed to the conception of the project and the development of an overall research plan. MH drafted the manuscript, analyzed the data, and interpreted them. NG was the statistic counselor and NR helped with RT-PCR. MH, HT, and MN were involved in the sampling and data collection. MSA edited the whole manuscript. All authors gave final approval of the version to be published.

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Tables

Table 3 is available in the Supplementary Files section.

Figures

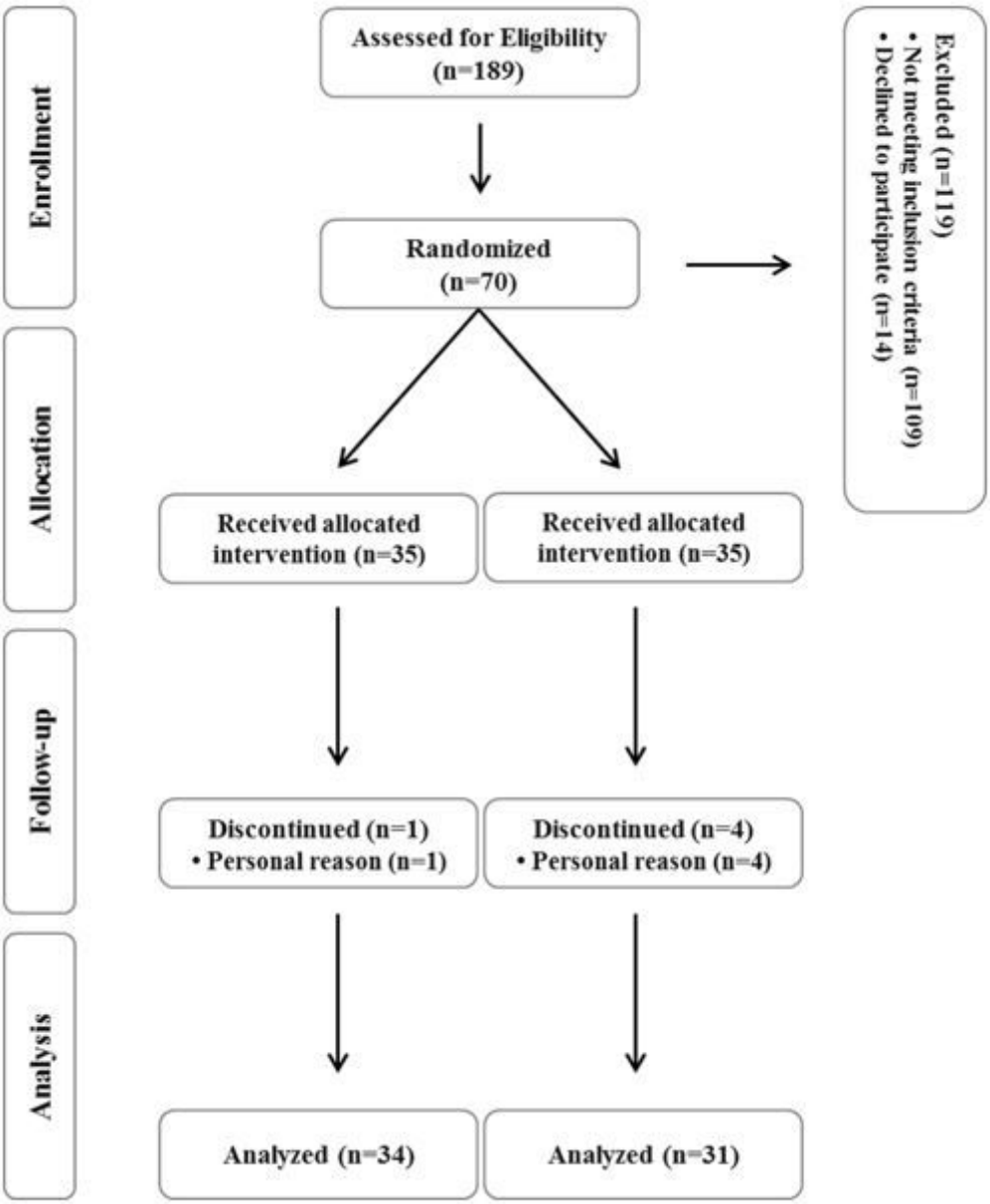

Figure 1

Study flow diagram.

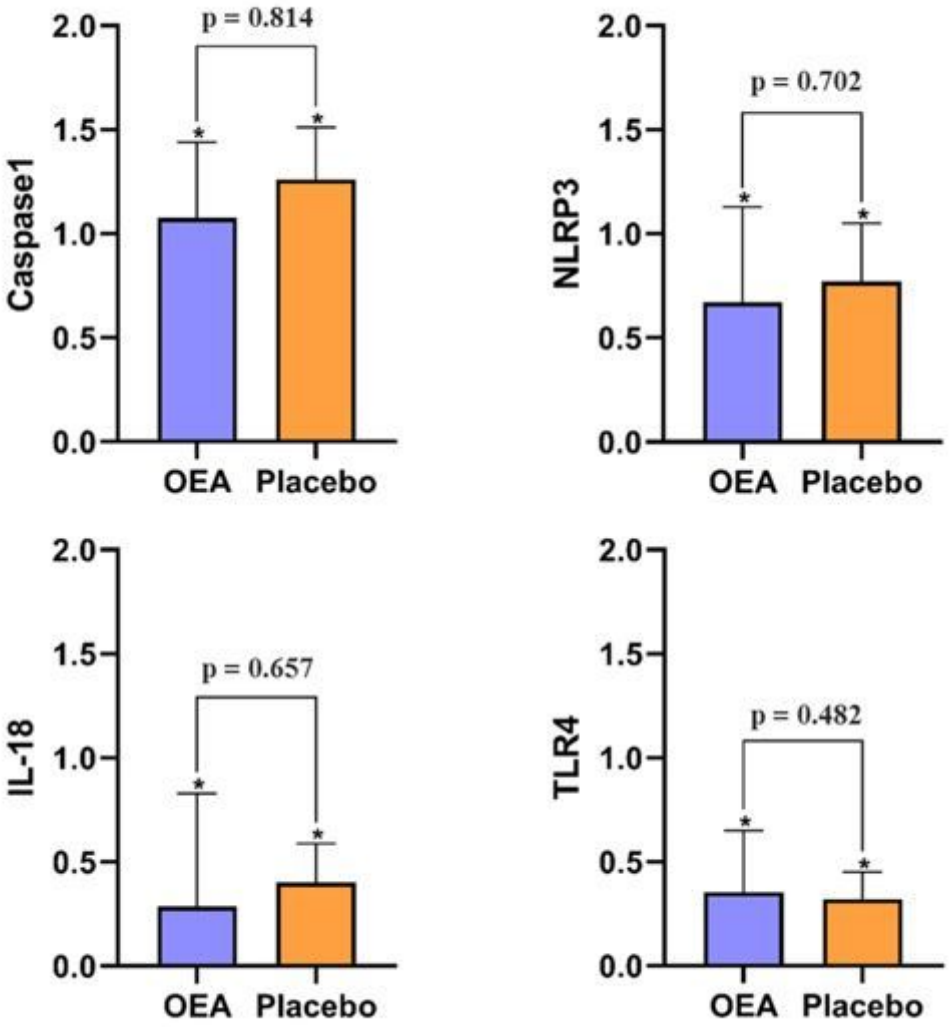

Figure 2

Effects of OEA on TLR4, Caspase1, NLRP3, and IL-18 expression in the study groups. Values are presented as logarithms of fold changes (median (95% CI)). Data analysis was performed using quantile regression (adjusted based on model 3) and Wilcoxon signed-rank test (*P<0.05 vs. baseline). OEA, Oleoylethanolamide; Caspase1, Cysteine-dependent aspartate-specific protease1; IL-18, Interleukin-18; NLRP3, Nucleotide-binding oligomerization domain (NOD)-like receptor (NLR) family pyrin domain containing 3; TLR4, Toll-like receptor 4.

Supplementary Files

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- Table3.jpg