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Camelina oil in the context of a weight loss programs improves glucose homeostasis, inflammation and oxidative stress in NAFLD patients: A randomized, triple -blind, placebo-controlled clinical trial

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Abstract

Background: Over the past few years, the benefits of co-administration of omega-3 and antioxidants have been reported in the management of non-alcoholic fatty liver disease (NAFLD) complications.This study evaluated the effects of Camelina sativa oil (CSO) supplementation as one of the richest dietary sources of omega-3 fatty acids on glucose homeostasis,inflammation, metabolic endotoxemia, and oxidative stress in NAFLD patients. Methods: In all, 43 subjects with NAFLD were allocated to either an intervention (20g/d CSO) or placebo (20g/d sunflower oil) group receiving a calorie-restricted diet for 12 weeks. Fasting serum levels of glycemic indices, hs-CRP, endotoxin, antioxidant enzymes activity, total antioxidant capacity (TAC), malondialdehyde (MDA), 8-iso-prostaglandin F2α (8-iso-PGF2α), and uric acid were measured at baseline and post-intervention. Results: CSO intake led to a significant decrease in insulin concentration (-17.49%), homeostasis model assessment of insulin resistance (HOMA-IR) (-20%), high-sensitive C-reactive protein (hs-CRP), (-12.94%), lipopolysaccharide endotoxin (-32.55%), malondialdehyde (MDA) (-18.75%), 8-iso-prostaglandin F2α (8-iso-PGF2α) (-19.55%) and a significant increase in the levels of total antioxidant capacity (TAC) (31.82%) and superoxide dismutase activity (10.22%) in the CSO group compared with the placebo group. Also, there was no significant difference between the two groups in fasting plasma glucose, quantitative insulin sensitivity check index (QUICKI), catalase, glutathione peroxidase activity, and uric acid level. In addition, within-group analyses showed a significant reduction of insulin, QUICKI, hs-CRP, and endotoxin levels in the intervention group. Conclusion: These findings indicate that CSO may improve glycemic, inflammation, metabolic endotoxima, oxidative stress status, and mental health in patients with NAFLD.

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Results: CSO intake led to a significant decrease in insulin concentration (-17.49%), homeostasis model assessment of insulin resistance (HOMA-IR) (-20%), high-sensitive C-reactive protein (hs-CRP), (-12.94%), lipopolysaccharide endotoxin (-32.55%), malondialdehyde (MDA) (-18.75%), 8-iso-prostaglandin F2α (8-iso-PGF2α) (-19.55%) and a significant increase in the levels of total antioxidant capacity (TAC) (31.82%) and superoxide dismutase activity (10.22%) in the CSO group compared with the placebo group. Also, there was no significant difference between the two groups in fasting plasma glucose, quantitative insulin sensitivity check index (QUICKI), catalase, glutathione peroxidase activity, and uric acid level. In addition, within-group analyses showed a significant reduction of insulin, QUICKI, hs-CRP, and endotoxin levels in the intervention group.

Conclusion: These findings indicate that CSO may improve glycemic, inflammation, metabolic endotoxima, oxidative stress status, and mental health in patients with NAFLD.

Keywords: Camelina; glycemic; metabolic endotoxima, oxidative stress, inflammation, NAFLD

What is already known about this topic?

Some previous studies have shown the beneficial effects of camelina oil in patients with hypercholesterolemia, and impaired glucose metabolism. There have been no human studies investigating the effect of Camelina oil supplementation on glucose homeostasis, inflammation, and oxidative stress in NAFLD patients.

What does this article add?

This study is the first investigation in humans designed to evaluate the effect of Camelina oil in the context of a weight loss programs improves glucose homeostasis, inflammation and oxidative stress in NAFLD patients. The present study was indicated that Camelina oil improved glucose homeostasis, inflammation, and oxidative stress in patients with NAFLD.

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) is a chronic liver disease that characterized by the lipid droplets accumulation in more than 5% of the hepatocytes. NAFLD encompasses a wide range from simple steatosis to non-alcoholic steatohepatitis (NASH), advanced fibrosis, cirrhosis, and hepatocellular carcinoma.[1] The global prevalence of NAFLD is estimated at nearly 25% [2], and in Iran was reported 33.9%.[3] The occurrence of NAFLD is closely linked with an increased prevalence of obesity, insulin resistance (IR), oxidative stress (OS), and cardiovascular diseases, metabolic syndrome and Type 2 diabetes diseases.[4]According the "two-it" model and "multi-parallel hit" hypothesis, IR, increased free radical oxidation products and decreased total antioxidant capacity result in NAFLD progression. Furthermore, dysbiotic has been proposed as a critical risk factor for NAFLD development .[1] It has been shown that altering the gut microbiota (GM) profile to phyla Bacteroidetes and Firmicutes, and decreasing Akkermansia muciniphila [5] in NAFLD patients lead to metabolic endotoxemia (ME) that exacerbates obesity, IR, OS and inflammation in these patients.

Due to lack of special pharmacologic treatments to control or improve NAFLD, lifestyle modifications known a first-line approach for NAFLD management.[6] It is well established that having a healthy diet and physical activity can reduce the risk of occurrence or progression of this disease. Recently, omega-3 fatty acids (OM3FA) and antioxidants co-administration is considered to treatment and prevention of NAFLD due to low OM3FA dietary intake, the high hepatic n-6: n-3 ratio and the low antioxdant levels in plasma and liver of NAFLD patients.[7] Thus, it seems that modification of dietary fats can affect hepatic fat deposition. A meta-analysis found that OM3FA interventions can improve liver functions and steatosis scores in NAFLD patients.[8] OM3FA sources might be useful in improvement of the complications of NAFLD by modify the GM and controlling IR, OS, inflammation, lipid metabolism, and hepatic fat deposition.[9] However, due to

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recent concerns regarding fish oil supplements contaminated with heavy metals and consequent side effects, and avoiding vegetarianism and veganism for consuming animal-derived products, modifying OM3FA sources from animal to plant sources has been considered .[10]

Camelina sativa (L.), known as false flax, is one of the richest dietary sources of OM3FA, with PUFA amounts over 50%, alpha-linolenic acid (ALA) content of 40% to 45%, and linoleic acid (LA) of about 15%, n-3/n-6 PUFA ratio of 1.79–2.17, low SFA content (about 6%), high contents of phytosterols (331–442 mg/100 g), carotenoids (103–198 mg of carotene/kg) and tocopherols (55.8–76.1 mg/100g).[11] Inhibited autoxidation of extracted oil by high levels of antioxidants in Camelina sativa oil (CSO) has led to the superiority of this oil than other richest dietary sources of OM3FA, such as flaxseed oil (FSO).[11] Furthermore, it has been reported that CSO has less fertilizer contamination compared to other oils .[12]

According to previous data, plant sources of OM3FA can improve glycemic status [13], ME [14], inflamation[15] and OS indices.[16]However, such effects have mostly been showed in preclinical studies.[17,18] Recently, limited clinical trials have reported favorable impacts of CSO on modulation of the lipid profile, OS, and immune system in subjects with impaired glucose metabolism (IGM).[19-22] To our knowledge, the effects of CSO intake on glycemic, inflammation, ME, and OS status in NAFLD patients have not been examined. Therefore, we aimed to investigat combined effects of caloric restriction and CSO on glycemic, inflammation, ME, and OS status in NAFLD patients.

2. Materials and methods

2.1 Study design and patients

This triple-blind, placebo-controlled, randomized clinical trial was conducted between 2019 October and 2020 March. Seventy NAFLD patients (Body mass index (BMI): 25-35 kg/m^2) aged 20-50 years old were engaged from Valiasr hospital of Tabriz, Iran and clinics via advertisements and posters. The subjects were diagnosed by a physician after an ultrasound examination on the basis of steatosis. Adherence to a stable diet, constant physical activity level (PAL) and willingness for participation were inclusion criteria of the study. The exclusion criteria were a history of biliary disease, hepatitis B and C, copper and iron storage disease; cardiovascular, gastrointestinal, and renal disease, pancreatic, thyroid disorders, cancer, smoking; pregnancy or lactation; post-menopausal; using antilipidemic and fatty liver inducing medications; currently antibiotics consumption, taking antacids, antidiarrheal, anti-inflammatory or laxative medicines and patients with special diets or dietary limitations. Also, patients were asked to consume minimum amount of nuts and fish and not to take antioxidants and omega-3 supplements. At baseline, patients were given a full information of the study and asked to sign a written informed consent. Also, they fulfilled a demographic questionnaire containing variables including the sex, age, PAL and the current medications of the subjects. The patients were requested to keep their usual PALs and follow the designed diet until the end of the intervention.

After a 2-week run-in period, patients were randomly allocated to either intervention (CSO, jahan, Tabriz, Iran) or placebo (sunflower oil (SFO), oila, Tehran, Iran) group based on age, sex, and BMI by block randomization method with a block size of 4. Random allocation software was performed to generate allocation sequence. The participants were assigned to the study groups by a third person (entirely unrelated to the trial) to achieve the blinding in the assessment process. The participants, principal researcher and statistical advisor were blind until the end of the analysis. The participants in the study groups were instructed to consume 15% of the daily total fat intake (˜20g) from the provided oil during the intervention period. Measuring cups with the volume of 10 mL were provided for the subjects to add two cups of oil daily to rice or salad at the time of consumption. The rest of the required oil was provided from meats and low-fat dairy, as well as cooking oil. A calorie restricted diet was designed for participants in both groups (50-55% from carbohydrates, 30-35% from fat and 10-15% of energy from protein). After a full explanation of the diet, the patients received an exchange list to facilitate the adherence to the designed diet during the intervention. Also, patients in both groups were asked to consume the least amount of oil during cooking, low-fat dairy and meat and avoid fried foods. The half of the oil bottles was provided at baseline

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for participants and the remaining packages at 45 days of the study. Patients were followed up biweekly to remind the recommendations on oils, diet and PAL, in addition to explore any possible side effects. Also, a checklist was provided for participants to mark after each consumption of the prescribed oil to evaluate for cases of non-compliance.

The sample size was calculated regarding the changes in superoxide dismutase (SOD) level as one of the primary outcomes. A power study of 90% and a confidence interval of 95% [23] were assumed in the Pocock formula and calculated at least 21 subjects in each group. Also, considering a 10% attrition rate, the sample size increased to 23 per group.

The primary outcomes of the current study were high sensitivity- C reactive protein (hs-CRP) malondialdehde (MDA), 8-iso-prostaglandin F2α (8-iso-PGF2α), uric acid, SOD, total antioxidant capacity (TAC), glutathione peroxidase (GSH-Px), catalase and LPS. The secondary outcomes were insulin, and fasting plasma glucose (FPG).

2.2 Ethics

The study protocol was approved by the ethics committee of the Tabriz University of Medical Sciences (TBZMED) and has been registered in the "Iranian Registry of Clinical Trials" website with the number of IRCT20150205020965N5.

2.3 Measurements

2.3.1 Chemical analysis of oils

The fatty acid compositions of CSO and SFO were determined using gas chromatography.[24] The main difference between the fatty acid composition of used CSO and SFO in this study was the percentage of LA (15.2% in CSO versus 62.1% in SFO), oleic acid (14.20% in CSO versus 27.80% in SFO) and ALA (38.4% in CSO versus 0.16% in SFO). The percentage of other fatty acids has been compared in Table 1.

2.3.2 Questionnaires

The participants' height and weight were measured. BMI was computed as the body weight (kg) divided by the square of the height (m). The dietary intake was assessed using a 3-day food diary (2 weekdays and 1 weekend) before starting the low calorie diet and supplements and also at the end of the study during last week. Dietary intake composition data were analyzed by "Nutritionist 4" (First Databank Inc., Hearst Corp., San Bruno, CA, USA). The subjects' PAL was evaluated using the International Physical Activity Questionnaire [25] pre and post intervention.

2.3.3 Biochemical measurements

A venous blood sample (10 ml) was collected from every patient after overnight fasting. Plasma were applied to determine insulin, FPG, MDA, TAC, uric acid, hs-CRP, LPS, and 8-iso-PGF2α. FPG and uric acid were measured via the enzymatic method by an autoanalyzer using kits (Pars-Azmoon Co., Tehran, Iran). Insulin concentration was determined using a chemiluminescent immunoassay method. 8-iso-PGF2 (Abcam, Cambridge, UK), and LPS levels (LAL kit endpoint-QCL1000; Cambrex BioScience, Walkersville, Maryland, USA) were determined using an enzyme-linked immunosorbent assay (ELISA) kit. hs-CRP serum concentrations were measured using an immunoturbidimetric assay (Pars Azmoon Co., Tehran, Iran). The activity levels of TAC, GSH-Px, and SOD were measured through a colorimetric method (TAC: RANDOX kits, GSH-Px: RANSEL kits and SOD: RANSOD kits; RANDOX Laboratory, UK) by an automatic analyzer. MDA levels were determined by spectrofluorimeter (Kontron, model SFM 25A, Italy).[26] The method of Aebi was applied to determine catalase activity.[27]

2.3.4 Glucose homeostasis

Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) and quantitative insulin sensitivity check index (QUICKI) were employed to assess the IR via the following formula:

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HOMA-IR = [fasting insulin (mU/L) × fasting blood glucose (mg/dL)] / 405

QUICKI = 1/(log (insulin, U/ml) + log (FPG, mg/dl)) [28]

2.3.5 Statistical analyses

SPSS version 24.0 was used to analyze the data. The results were showed as mean (SD) and frequency (percent) for quantitative and qualitative data, respectively. Intention to treat approach was used to include data of subjects who discontinued the trial. The Kolmogorov– Smirnov test was used to evaluate the distribution normality of data. Un-paired sample student T-test and Chi-square test were applied to assess between group differences of quantitative and qualitative data, respectively. Analysis of covariance (ANCOVA) were applied to compare the quantitative variables between groups post intervention. Also, paired sample student T-test was used to compare the differences within groups between baseline and post-intervention. Percent change was calculated as [100 × (Intervention values -placebo values)/placebo values] to determine the differences between groups. P <0.05 was considered statistically significant.

3. Results

3.1 Patients

Of the 70 patients that were recruited for trial eligibility, 43 patients (n=21 in the CSO group and n=22 in the placebo group) completed the study. Fig. 1 shows the study flow chart and a description of the causes for the loss to follow up. Overall, 90.5% of participants were compliant with supplements, by the end of the trial. Any adverse effects were reported by patients with SFO and CSO consumption. There was no difference between groups in any of the demographic variables including gender, age, employment, education level, marital status and PAL.

3.2 Anthropometric indices and nutrients intake

Between group analysis were non-significant for the body weight, BMI (Table 2), energy, macro-and micronutrients intake at baseline (Table 3). Weight and BMI showed a statistically significant reduction in intervention group at the end of the trial, compared to placebo group (Table 2, P< 0·05) after adjusting for confounding factors (sex, PAL, energy intake and baseline). Within group analysis showed that there was a non-significant reduction of body weight and BMI in the placebo group, whereas it was significant in the intervention group (paired T-test, P< 0·05). The mean of antioxidant micronutrients intake was not different between placebo and intervention groups. The dietary intake of macronutrients and energy were significantly changed at the end of the intervention (except for protein and fiber intake) in the intervention group compared to placebo group (ANCOVA, P<0·05). Also, the intake of energy and macronutrients (carbohydrate, protein, total fat and fiber) was significantly different in the intervention and placebo groups after 12 weeks intervention compared with baseline (paired Student t-test, P < 0.05).

3.3 Glycemic index, inflammatory biomarker, and endotoxin

Any significant differences were observed in terms of glycaemia, LPS, and inflammatory biomarkers between the two groups at baseline. There was a remarkable reduction in levels of fasting insulin concentration (-1.76 μU/ml, -17.49%), HOMA-IR (-0.50, -20%), hs-CRP (-1.25 pg/ml, -12.94%), and LPS (-5.59 EU/mL, -32.55%) between the two groups (Table 4) (P < 0.05, adjusted for baseline values, sex, energy intake, and weight changes). Decrease in FPG (-3.63 mg/dL, 3.57%), and QUICKI (-0.15, -4.33%) were not statistically significant between groups. Within group differences of insulin, QUICKI, hs-CRP and endotoxin in the intervention group were significant (P <0.05, paired Student t-test). All mentioned measurements, with the exception of hs-CRP, remained unchanged in the placebo group when compared with the baseline.

3.4 Antioxidant enzymes activity and oxidative stress status

No significant differences were observed in antioxidant enzymes activity and OS status between groups at baseline (Table 5). Mean levels of MDA (-0.70 nmol/mL, -18.75%), 8-iso-PGF2α (-6.65 pg/ml, -19.55%) reduced significantly in the intervention group but not in the placebo group. A significant elevation was

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observed in the mean levels of TAC (0.35 mmol/L, 31.82%), and SOD (165.50 U/g Hb, 10.22%) in the intervention group compared with the placebo group. Changes in the levels of uric acid (0.40 mg/dL, 6.66%), GSH-Px (2.15 U/g Hb, 6.42 %), and catalase (6.81 U/g Hb, 10.20%), were not statistically significant in the intervention group compared with the placebo group ($P<0.05$, adjusted for baseline values, sex, weight changes and energy intake). TAC, SOD, GSH-Px, 8-iso-PGF2α and MDA levels were significantly altered in the intervention group compared with baseline, but catalase and uric acid remained unchanged ($P<0.05$, paired T-test). No significant within group differences were observed for the oxidative stress/antioxidant parameters in the SFO group ($P > 0.05$, paired Student t -test).

4. Discussion

To the best of our knowledge, our study was the first randomized clinical trial to assess the combined effects of caloric restriction and CSO on NAFLD penitents. Our findings showed that CSO, as a rich plant source of OM3FA may exert beneficial effects on glycemic indices, inflammation, ME and oxidative stress/antioxidant status in NAFLD patients. Dietary intake of CSO significantly decreased energy, carbohydrate, and fat intake, body weight, BMI, insulin, HOMA-IR, hs-CRP, LPS, 8-iso-PGF2α, MDA, and significantly increased TAC and SOD in our study. However, changes of FPG, QUICKI, catalase, GSH-Px, and uric acid were not significant in the intervention group

Weight management has been suggested as one of the most applicable strategies for NAFLD treatment. It has been reported that a 5% reduction in BMI results in a 25% decrease in the lipid content of the liver.[29] Our results on body weight and BMI are in agreement with Rezaei et al. [4] and with a systematic review and meta-analysis of FSO supplementation on body weight in overweight and obese adults.[30] However, some trials did not report the beneficial impacts of the CSO on anthropometric indices. In a study which investigated the effect of CSO and canola oil on anthropometric indices in postmenopausal women for six weeks, a significant changes in WC observed in both groups and a significant reduction was found in waist-to-hip ratio only in the CSO group. While, weight and BMI changes were not significant.[31] The weight-decreasing effect of CSO may be attributed to a modulation of the GM, ME, leptin, ghrelin, adiponectin, peroxisome proliferator-activated receptors (PPARs) gene expression, gut hormones, including PYY and GLP-1 and short chain fatty acid (SCFA) production, fatty acid synthesis and oxidation through up-regulating β-oxidation gene expression, like carnitine palmitoyltransferase-1 (CPT-1) and peroxisome proliferator-activated receptor α (PPARα) and repressing lipogenic genes expression such as sterol regulatory element binding proteins (SREBPs), carbohydrate-responsive element-binding protein (ChREBP) and PPA[32] which regulate food intake and energy expenditure.[31]

The results of our study showed a significant improvement in insulin levels and HOMA-IR after 12 weeks of supplementation with CSO. While, no significant reduction was observed in FPG and QUICKI levels at the end of the intervention. The effects of dietary intervention with CSO on glycemic indices has been reported in subjects with IGM.[19] A systematic review reported a 0.2 reduction in IR after OM3FA supplementation.[33] In agreement with the present study, Hutchins et al reported that the FSO supplementation as another OM3FA rich plant oil reverses IR.[13]However, Schwab et al reported that CSO oil did not affect fasting, post load plasma glucose or serum insulin concentrations in IGM.[19] Furthermore, in a study by Hajiahmadi et al. significant improvements in FPG and insulin concentrations were found in pre-diabetic patients who were supplemented with 2000 mg FSO for 14 weeks.[34] These conflicting findings may be attributed to designed

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diet, omega-6 to omega-3 ratio in diet, placebo group, basal levels of glycemic indices, trial duration, type and dosage of supplementation, as well as medical condition of patients.

IR as a main cause of NAFLD, augments lipid aggregation, and stimulates inflammation in hepatocytes [29]. On the other hand, it was reported that development of IR and consequently NAFLD are associated with dysbiosis. In the presence of dysbiosis, there is an increased production of LPS from Gram-negative bacteria or ME[1] that exacerbates IR via enhancement of the expression of pro-inflammatory cytokines such as tumor necrosis factor (TNF-α), interleukin (IL)-1, and IL-6 [35], reducing insulin action through inhibiting phosphorylation of the insulin receptor substrate (IRS)-1 and IRS-2 and reducing or even suppressing the IRS-1 and IRS-2 expression.[36] It was reported that OM3FA improve ME [37] via SCFAs production.[38] SCFAs as byproduct of OM3FA may alleviate IR via inducing GLP-1 secretion from L cells in the colon[39], activating intestinal gluconeogenesis, and exerting beneficial effects on host glucose and energy homeostasis. Thus, CSO supplementation may promote hypoglycaemic effects via reducing energy intake, weight, LPS levels, and SCFAs production.

Another outcome of CSO supplementation in patients with NAFLD was inflammation reduction via modulation of ME and inflammatory biomarkers. ME proposed as a triggering factor for the systemic inflammation.[40] Limited animal studies reported beneficial effects of OM3FA on endotoxin levels. It has been shown that post-prandial ME caused by coconut oil decreased in pigs following fish oil supplementation.[41] In another animal study, feeding high levels of omega-6 in mice led to increased levels of ME, which were dramatically reduced in transgenic mice with the ability to convert omega-6 to OM3FA.[14] In a recent animal study, dietary FSO in diabetic rats improved ME via modulating GM as well as SCFAs. Additionally, a negative relationship was reported between ME with Bacteroidetes and Alistipes and a positive relationship with Blautia and Firmicutes.[38] It is believed that exposure to LPS are related to activating the NF-ϰB pathway leading to inflammation in NAFLD patients.[1,5] Probably, LPS binding to TLR4 resulted in production of inflammatory cytokines such as NFkB, IL-6 and interferon gamma (IFNγ) and ME.[42]Possibly CSO, as a source of OM3FA decreases systemic inflammation and subsequently ME [43] via inhibiting the growth of Bilophila wadsworthia and increasing the growth of A. muciniphila and bifidobacteria, decreasing postprandial lipaemia involved in chylomicron-LPS complex transport, influencing lipids transportation through the intestinal barrier cells phospholipid membranes, increasing endogenous activity of intestinal ALP involved in LPS production and intestinal permeability improvement, and inhibiting the TLR4-induced signaling pathway through modulation of the G protein-coupled receptor (GPR)-120.[37] Furthermore, anti-inflammatory effects of OM3FA may be related to the suppression of the formation of omega-6-derived inflammatory lipid derivatives, production of omega-3-derived endocannabinoids, ethanolamides and oxylipins, as well as endocannabinoid system modulation.[5,44]

In the current study, another effect of CSO in patients with NAFLD was the modulation of OS biomarkers. Our study is in agreement with a recent preclinical study investigating the CSO effect on OS parameters in mice with irritable bowel syndrome (IBS). The authors found that CSO intervention caused a significant reduction in MDA levels, as well as increases in SOD and GHP-x levels.[45] The effect of CSO on OS has been examined only in one clinical trial and reported no remarkable changes in urinary prostanoids in patients with IGM.[22] Han et al. found that FSO consumption led to a significant reduction in MDA levels and a significant increase of GSH and SOD in mice.[17] The underlying mechanisms of the effect of CSO on OS is not fully explored. Several studies indicated that ROS contributes to development of NAFLD via increasing IR, lipid peroxidaition, inflammation and ME.[46] OM3FA may reduce OS by suppressing the IϰB kinase (IKK) responsible for dissociation of nuclear factor-kappa B (NF-ϰB) from IKB-α as a modulator of pro-inflammatory cytokine production.[47] Furthermore, ALA is a precursor of long-chain docosahexaenoic acid. It has been shown that docosahexaenoic acid increases the activity of glutathione reductase, GSH-Px, and SOD and decreases MDA concentration in mice with nonalcoholic steatohepatitis.[48] Additionally, favorable effects of intervention on OS biomarkers can be attributed to the presence of high levels of natural antioxidants such as tocopherols, carotenoids and phytosterols in CSO [11] that may exert inhibitory effects on lipid peroxidation and ROS production. Other possible underlying mechanism associated with changes in the GM. Costantini et al. reported that OM3FA intake stimulated the growth of A. muciniphila,

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Bifidobacteria, Lactobacilli, Faecalibacterium, Roseburia, and inhibited some pathogenic bacteria, such as the Enterobacteriaceae, Clostridium, and Streptococcus.[49] It was reported that A. muciniphila alleviated OS in diabetic rats [50] due to having thiol specific antioxidant proteins such as typical 2-Cys Peroxiredoxins as a family of thioredoxin (Trx)-scaffold enzymes and ubiquitous.[51] Bifidobacterium longum and Lactobacillus acidophilus inhibited lipids peroxidation by scavenging ROS[52], reducing MDA levels and increasing SOD and TAC levels.[5] Also, Clostridium perfringens, probably, triggers OS by α-toxin production, and activation of mitogen-activated protein kinase/ERK kinase/extracellular-signal-regulated kinase (MEK/ERK), protein kinase C, and NF-κB pathways.[53]Short-chain fatty acids such as butyrate reduces ROS production via suppression of IKK [54], increases in plasma antioxidant enzymes production, modulation of FOXO3A and MT2 transcription through histone deacetylases inhibition [55], reduction of colonic myeloperoxidase activity [56] and the MAPK/ERK, p38/MAPK and c-Jun N-terminal kinase activation.[57]Finally, CSO may protect against IR, OS damage, ME, and inflammation by improving gut microbial dysbiosis (Fig 2).

Limitations in this study included lack of assessment of gut and fecal microbial composition, serum fatty acids, glucose clamp, serum SCFA, and other inflammatory/oxidative stress biomarkers. However, this study is the triple-blind, placebo-controlled, randomized clinical design and stratification by BMI, gender and age factors, which eliminates inter-individual variation. Also, it was the first study to evaluate the impact of CSO on glycemic indices, inflammatory and OS parameters, and ME in NAFLD patients.

5. Conclusion

The current study found that CSO might improve NAFLD via glycemic indices, inflammation, ME, and oxidant/antioxidant biomarkers modulation. CSO as one of the richest dietary sources of OM3FA can be recommended as a complementary therapy in NAFLD patients. Further trials are required to confirm the positive results of CSO in NAFLD patients.

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Author Contribution:

VM: Drafting of the manuscript, Acquisition of data, Approval of the article

PD: Contributions to concept/design, Data analysis/interpretation, Critical revision of the manuscript, Approval of the article

SS: Acquisition of data, Critical revision of the manuscript, Approval of the article

MA: Critical revision of the manuscript, Approval of the article

DATA AVA I L A B I LIT Y S TATEMENT

The data are available from the corresponding author on reasonable request.

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Table 1. Composition of fatty acids present in Camelina oil and Sunflower oil

Fatty acid	Name	Name	Camelina oil (%)	Sunflower oil (%)
14:0	14:0	Myristic acid	0.10	0.09
16:0	16:0	Palmitic acid	5.70	6.2
18:0	18:0	Stearic acid	2.50	1.74
20:0	20:0	Arachidic acid	1.55	0.21
22:0	22:0	Behenic acid	0.30	0.85
24:0	24:0	Lignoceric acid	0.10	0.31
Saturated fatty acids	Saturated fatty acids		10.20	9.4
16:1n-7	16:1n-7	Palmitoleic acid	0.13	0.06
18:1n-9	18:1n-9	Oleic acid	14.20	27.80
18:1n-7	18:1n-7	Cis-Vaccenic acid	0.70	0.06
20:1n-9	20:1n-9	Eicosenoic acid	14.40	0.16
22:1n-9	22:1n-9	Erucic acid	3.21	nd
24:1n-9	24:1n-9	Nervonic acid	0.60	0.19
Monounsaturated fatty acids	Monounsaturated fatty acids		33.20	28.69
18:2n-6	18:2n-6	Linoleic acid	15.20	62.1
18:3n-3	18:3n-3	Alpha-linolenic acid	38.40	0.16
20:2n-6	20:2n-6	Eicosadienoic acid	3.50	0.04
Polyunsaturated fatty acids	Polyunsaturated fatty acids		57.10	62.3

Table 2 Baseline characteristics of the study participants

Variables	Placebo group (n=21)	Placebo group (n=21)	Intervention group (n=22)	P	P	P
Age(y) *(range)	43.86 (6.07) (32-45)	43.86 (6.07) (32-45)	44.30 (4.38) (35-47)	44.30 (4.38) (35-47)	0.798^{+}	0.798^{+}
Gender, n (%)					0.608^{++}	0.608^{++}
Male	11 (52.4)	11 (52.4)	10 (45.5)	10 (45.5)		
Female	10 (47.6)	10 (47.6)	12 (54.5)	12 (54.5)		
Education level						0.548^{++}
Illiterate	8 (38.10)	6 (27.30)	6 (27.30)	6 (27.30)	6 (27.30)	
Diploma	8 (38.10)	8 (36.40)	8 (36.40)	8 (36.40)	8 (36.40)	
Bachelor degree	5 (23.80)	8 (36.40)	8 (36.40)	8 (36.40)	8 (36.40)	
Employment, n (%)						0.269^{++}
Employed	11 (52.40)	9 (40.90)	9 (40.90)	9 (40.90)	9 (40.90)	
Unemployed	10 (47.60)	13 (59.10)	13 (59.10)	13 (59.10)	13 (59.10)	
PAL, n (%)						0.301^{++}

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Variables	Placebo group (n=21)	Placebo group (n=21)	Intervention group (n=22)	P	P	P
Light	7 (33.30)	8 (36.40)	8 (36.40)	8 (36.40)	8 (36.40)	
Moderate	11 (52.40)	12 (54.50)	12 (54.50)	12 (54.50)	12 (54.50)	
Vigorous	3 (14.30)	2 (9.10)	2 (9.10)	2 (9.10)	2 (9.10)	
Marital status, n (%)						0.421^{++}
Single	4 (19.00)	6 (27.30)	6 (27.30)	6 (27.30)	6 (27.30)	
Married	17 (81.00)	16 (72.70)	16 (72.70)	16 (72.70)	16 (72.70)	
Anthropometric indices						
Height (cm)	156.19 (3.70)	154.65 (6.90)	154.65 (6.90)	154.65 (6.90)	154.65 (6.90)	0.374^{+}
Weight (kg) at baseline	82.70 (5.15)	81.29 (3.80)	81.29 (3.80)	81.29 (3.80)	81.29 (3.80)	0.316^{+}
Weight (kg) at end of trial	80.30 (4.75)	74.15 (3.05)$^{\&}$	74.15 (3.05)$^{\&}$	74.15 (3.05)$^{\&}$	74.15 (3.05)$^{\&}$	$0.004^{¥}$
BMI at baseline (kg/m^2)	33.90 (2.65)	34.19 (3.55)	34.19 (3.55)	34.19 (3.55)	34.19 (3.55)	0.797^{+}
BMI at end of trial (kg/m^2)	32.97 (2.40)	31.19 (3.22)$^{\&}$	31.19 (3.22)$^{\&}$	31.19 (3.22)$^{\&}$	31.19 (3.22)$^{\&}$	$0.046^{¥}$

BMI: Body mass index, PAL: physical activity level. *Data are presented as mean (SD), $^{+}$Independent sample t-test,$^{++}$ Fisher's exact test,$^{\&}$P<0.05, paired student t test for comparison of data within groups $^{Y=}$ P<0.05, Analysis of covariance for comparison of data between groups after adjusting for sex, PAL, energy intake and baseline values.

Table 3. Dietary intakes of patients at baseline and at the end of the study

Variables	Period	Placebo group (n=21)	Intervention group (n=22)
Energy (kcal/d)	Initial	2372.30 (209.70)	2304.95 (244.80)
	End	2099.80 (199.0) [a]	1927.30 (208.80)[a,b]
Carbohydrate (g/d)	Initial	322.87 (60.20)	302.60 (45.25)
	End	267.99 (44.80) [a]	240.20 (34.40)[a, b]
Protein (g/d)	Initial	88.49 (14.20)	85.98 (12.87)
	End	98.70 (14.60) [a]	100.99 (10.40) [a]
Total fat (g/d)	Initial	77.30 (10.60)	80.04 (12.80)
	End	69.01 (13.85) [a]	62.94 (10.50)[a,b]
Dietary fiber (g/d)	Initial	12.19 (3.7)	12.60 (2.28)
	End	13.86 (3.30) [a]	15.30 (3.82) [a]
Vitamin C (mg/d)	Initial	102.7 (21.8)	108.9 (31.45)
	End	107.3 (25.6)	127.4 (42.40)
Vitamin E (mg/d)	Initial	5.92 (1.46)	6.41 (1.45)
	End	6.23 (1.83)	5.79 (1.90)
Selenium (µg/d)	Initial	63.95 (15.35)	65.50 (14.23)

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Variables	Period	Placebo group (n=21)	Intervention group (n=22)
	End	59.40 (15.42)	57.65 (18.73)
Cu (mg/d)	Initial	2.3 (0.15)	3.01 (0.25)
	End	2.7 (0.12)	2.70 (0.23)
Zn (mg/d)	Initial	5.6 (1.14)	6.35 (1.03)
	End	6.13 (2.12)	5.43 (1.75)
β-Carotene (mg/d)	Initial	3.75) 1.18)	4.73 (1.04)
	End	4.35 (1.75)	4.45 (1.15)
Mn (mg/d)	Initial	3.99 (1.08)	4.01 (1.02)
	End	4.35 (2.08)	5.03 (1.09)

Data are presented as mean (SD), [a] *P<0.05* , paired student *t* test for comparison of data within groups. [b] *P<0.05* , Analysis of covariance for comparison of data between groups after adjusting for sex and baseline values.

Table 4. Changes in glycemic indices, endotoxemia, and inflammatory biomarkers of patients at baseline and the end of the study[1]

Variables	Period	Placebo group (n=21)	Intervention group (n=22)	MD (95% CI$^{++,}$) b
FPG (mg/dL)	Initial	99.70 (5.30)	98.32 (4.06)	-1.40 (- 1.50 to 4.30
	End	101.22 (5.15)	97.60 (7.30)	-3.63 (-0.30 to 7.50)
	MD (95% CI) within groups	1.50 (-3.50 to 0.50)	-0.73 (-2.30 to 3.70)	
Insulin (μU/ml)	Initial	10.70 (2.60)	10.50 (4.20)	-0.17 (-2.00 to 2.36)
	End	10.06 (1.99)	8.30 (2.66) [a,b]	-1.76 (-3.22 to -0.31
	MD (95% CI) within groups	-0.62 (-0.04 to 1.21)	-2.22 (-0.55 to 3.90)	
HOMA-IR	Initial	2.64 (0.70)	2.55 (1.05)	-0.09 (-0.46 to 0.64)
	End	2.50 (0.50)	2.00 (0.70)[b]	-0.50 (-0..90 to -0.1
	MD (95% CI) within groups	-0.13 (-0. 04 to 0.30)	-0.55 (-0.13 to -0.96)	
QUICK	Initial	3.01 (0.12)	2.98 (0.17)	-0.03 (-0.06 to 0.12)
	End	3.00 (0.85)	2.87 (0.17) [a]	-0.15 (-0.03 to 0.21)
	MD (95% CI) within groups	-0.01 (-0.01- to 0.05)	-0.11 (-0.03 to -0.17)	
hs-CRP (ng/ml)	Initial	11.40 (3.15)	10.88 (3.50)	-0.50 (-1.49 to 2.55)
	End	9.50 (2.49)	8.27 (1.65) [a, b]	-1.25 (-2.5 to -0.47)
	MD (95% CI) within groups	-1.90 (-0.65 to 3.25)[a]	-2.60 (-1.30 to -3.93)	
LPS (EU/ml)	Initial	19.59 (6.25)	16.60 (6.60)	-2.99 (-1.60 to 10.36
	End	18.46 (7.90)	12.45 (6.25)[a,b]	-5.59 (-1.61 to -10.3
	MD (95% CI) within groups	-1.15 (-0.52 to 2.75)	-4.10 (-2.35 to -5.80)	

CI$^{++,}$ Confidence interval; FPG; Fasting plasma glucose; HOMA-IR; Homeostatic Model Assessment for Insulin Resistance, QUICK; quantitative insulin sensitivity check index, hs-CRP; high-sensitivity C-reactive protein; LPS; Lipopolysaccharide.[1] Data are presented as mean (SD). [a] P<0.05, paired student t test for comparison of data within groups. [b]P<0.05, analysis of covariance for comparison of data between groups after adjusting for sex, weight, energy intake changes and baseline values.

Variables	Period	Placebo group[1] (n=21)	Intervention group (n=22)	MD (95%CI^{+}
TAC (mmol/L)	Initial	0.95 (0.10)	0.80 (0.15)	-0.15 (-0.56 t
	End	1.10 (0.10)	1.45 (0.35) [a , b]	0. 35 (0. 10 t
	MD (95% CI) within groups	0.15 (-0.31 to 0. 20)	0.65 (-0.82 to -0. 50)	
SOD (U/mg Hb)	Initial	1627.32 (142.90)	1604.75 (97.21)	-22.55(-52.20

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Variables	Period	Placebo group[1] (n=21)	Intervention group (n=22)	MD (95%CI+
	End	1609.65 (154.25)	1774.20 (105.50) [a, b]	165.50 (84.8 t
	MD (95% CI) within groups	-17.98 (-42.57 to 78.55)	169.45 (-218.40 to -121.20)	
GSH-Px (U/g Hb)	Initial	33.53 (3.0)	32.96 (2.31)	0.60 (-1.07 to
	End	33.45 (3.34)	35.60 (3.65) [a]	2.15 (-4.30 to
	MD (95% CI) within groups	-0.08 (-0.70 to 0.85)	2.64 (-3.99 to -1.25)	
Catalase (U/g Hb)	Initial	64.20 (18.17)	59.70 (17.20)	-4. 50 (-3.20 t
	End	66.79 (16.60)	73.60 (26.55)	6.81 (-23.46 t
	MD (95% CI) within groups	2.59 (-7.93 to 13.15)	-13.90 (-23.65 to 4.12)	
Uric acid (mg/dL)	Initial	4.60 (1.26)	4.75 (1.23)	0.15 (-0.91 to
	End	4.50 (1.33)	4.80 (1.20)	0.40 (-1.10 to
	MD (95% CI) within groups	-0.10 (-0.14 to 0..40)	0.15 (-0.39 to 0.26)	
MDA (nmol/mL)	Initial	3.85 (0.84)	3.64 (1.42)	-0.25 (-0.56 to
	End	3.68 (0.68)	2.99 (1.16) [a , b]	-0.70 (-0.25 to
	MD (95% CI) within groups	-0.17 (-0.23 to 0.56)	-0.65 (-0.50 to -1.21)	
8-iso-PGF2α (pg/ml)	Initial	33.60 (7.03)	30.15 (4.50)	-3.30 (-11.80 t
	End	31.20 (5.15)	25.10 (4.15) [a , b]	-6.65 (-8.45 to
	MD (95% CI) within groups	-2.4.0 (-9.15 to 8.20)	-4.90 (-8.05 to -4.60)	

Table 5. Changes in oxidative stress status and antioxidant biomarkers of patients at baseline and the end of the study[1]

CI++: confidence interval; TAC: serum total antioxidant capacity; SOD: superoxide dismutase; GSH-Px: glutathione peroxidase; MDA: malondialdehyde, 8-iso-PGF2α: 8-iso-prostaglandin F2α,[1]Data are presented as mean (SD).[a] P<0.05, paired T-test for comparison of data between within groups. [b] P<0.05, Analysis of covariance for comparison of data between groups after adjusting for sex, weight and energy changes and baseline values.

Figure legends:

Fig 1: Flow chart of the study

Fig 2: Graphical abstract (underlying mechanism of the effect camelina sativa oil on inflammation, oxidative stress, insulin resistance, and metabolic endotoxemia)

ALP= Alkaline phosphatase,DC=Dendritic cell, eCB= Endocannabinoid,IR= insulin resistance, GLP-1=Glucagon-like peptide-1, GLP-2=Glucagon-like peptide-2, LPS= Lipopolysaccharide ME=metabolic endotoxemia,Ros= Reactive Oxygen Species, SCFA=Short chain fatty acid

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`Fig1 (3).doc` available at https://authorea.com/users/421448/articles/527480-camelina-oil-in-the-context-of-a-weight-loss-programs-improves-glucose-homeostasis-inflammation-and-oxidative-stress-in-nafld-patients-a-randomized-triple-blind-placebo-controlled-clinical-trial

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