Journal of Functional Foods 42 (2018) 38–46

Contents lists available at ScienceDirect

Journal of Functional Foods

journal homepage: www.elsevier.com/locate/jff

Plasma lipid lowering effect by a novel chia seed based nutraceutical formulation

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ARTICLE INFO

Keywords:
Nutraceutical
Chia seeds
PUFA
Dyslipidemia
Plasma triglycerides

ABSTRACT

Atherosclerotic cardiovascular diseases (ASCVD) are preferential targets of preventive medicine partially through therapies to improve atherogenic lipid profiles. The aim of the present work was to test a novel nutraceutical formulation (CSN) for its potential effects on plasma triglyceride levels of healthy subjects with moderate dyslipidemia. A cohort of 52 individuals were administered daily, for 8 weeks, with four gastro-resistant capsules of CSN, each one containing 500 mg of cryo-micronized chia seeds and 15 mg of vitamin E, according to a single centre, randomized, placebo controlled, 16 weeks trial. Data showed the following mean lipid changes: triglycerides, −27.5% ($P = .0095$); total cholesterol, −8.0% ($P = .0019$); High Density Lipoprotein cholesterol, +5.7% ($P = .0042$); Low Density Lipoprotein cholesterol, −10.2% ($P = .0021$). CSN may be regarded as a novel complementary and/or alternative safe remedy with clinical relevance in the primary cardiovascular disease prevention.

1. Introduction

The importance of dietary polyunsaturated ω-3 fatty acids (FA) is well recognised, as concerns, particularly, their positive influence on the cardiovascular system. It has been reported that ω-3 FA intake can reduce triglyceride plasma levels and platelet aggregation, and stabilizes the cardiac rhythm (Harris, 2009). Fish and seafood products are the main dietary source of ω-3 FA, mainly represented by docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). The protective effects are still appreciable even in case of low consumption levels: at least 30 g fish/day can support a significant prevention against cardiovascular diseases; interestingly, an increase of 20 g/day in fish consumption would favour a decrease by 7% of death risk incidence for cardiovascular disorders in individuals who occasionally consume fish (Mozaffarian et al., 2003). A ω-3/ω-6 FA ratio of 1:6 is considered to be appropriate for the nutritional requirements of most healthy subjects. Such ratio would exert an important influence on plasma lipids and serve cardiac and endothelial functions to impact the prevention and treatment of coronary heart diseases (CHD) (Wijendran & Hayes, 2004; Makni et al., 2010). Today, this balance in the current Western diet has been strongly shifted in favour of ω-6 FA (from 1:10 to even 1:30), due to a very low consumption of seafood products, and a large use of vegetable oils (the main source of ω-6 FA). Over the last few years, this imbalance has been considered as one of the main causes of the dramatic increase of cardiovascular disorders (Wijendran & Hayes, 2004).

Apart sea products, the vegetable world represents an important alternative source of ω-3 FA, mainly under the form of alpha-linolenic acid (ALA; 18:3 ω-3). The estimated average ALA intake in the United States and most European countries is 1.3–1.7 g/day (Gebauer, Psota, Harris, & Kris-Etherton, 2006; Zatónski, Campos, & Willett, 2008; Hulshof et al., 1999). The Institute of Medicine (IOM) of the National Academies established for ALA an Adequate Intake (AI) of 1.6 g/day for men and 1.1 g/day for women (IOM, 2002). The IOM noted that intakes of ALA above the AI may confer additional health benefits, especially with respect to cardiovascular health. Many advisory boards consider ALA intakes greater than 1.5 g/day important for human health (Gebauer et al., 2006). The most important vegetable sources of ALA are the seeds of *Dracocephalum moldavica*, *Perilla frutescens*, and *Aleurites moluccana* (Stuchlík & Žák, 2002). However, among more common vegetable sources, flax (*Linum usitatissimum*) seeds and chia (*Salvia hispanica*) seeds are of major interest, not only as regards their high ALA contents, but also for their ω-3/ω-6 FA ratio of about 4:1 (Ciftci, Przybylski, & Rudzińska, 2012). Nevertheless, chia seeds are more valuable than flax seeds in terms of nutritional values, specifically

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https://doi.org/10.1016/j.jff.2018.01.007
Received 26 October 2017; Received in revised form 5 January 2018; Accepted 5 January 2018
1756-4646/

regarding their higher amounts (for 100 g seeds) of calcium (631 mg *vs* 255 mg), fiber (34.4 mg *vs* 27.3 mg), phosphorus (860 mg *vs* 642 mg), their superior levels of antioxidants (mainly polyphenols) and proteins characterised by a high biological value (https://ndb.nal.usda.gov), and, of no secondary importance, their much lower contents in compounds of toxicological concern (Choi, Kim, Pyo, Jo, & Han, 2007).

Although many types of oily seeds are indicated as a good source of ω-3 FA, and strongly suggested to be consumed daily for a proper intake of such precious cardioprotective nutrients, some of them, such as flax seeds and chia seeds, are characterised by a very small size, so can be quite difficult, or almost impossible, to be properly chewed and, thus, crushed. This aspect could be at the basis of a very low bioaccessibility of seed ω-3 FA. What is more, some oily seeds are characterised by a very high capacity of hydration: chia seeds, for example, can absorb up to 27 times their weight in water, forming a gelatinous mass which, on one side, usefully contributes to stimulate the intestinal peristalsis, but, on the other hand, provides a very low, or even no, bioaccessibility of ω-3 FA (Tha Goh et al., 2016). A preventive grinding before the consumption of such oily seeds could be expected to favour the bioaccessibility of ω-3 FA. Actually, previous authors have clearly demonstrated that the administration of ground chia seeds both to animals and humans favours a significant increase of ALA plasma levels and exerts a positive influence on some cardiovascular risk factors, respect to what can be achieved with whole chia seeds (Ayerza & Coates, 2007; Nieman et al., 2012; Nieman et al., 2009; Ayerza & Coates, 2005; Pereira da Silva, Morais Dias, de Castro Moreira, Toledo, & Pinheiro-Sant'Ana, 2016; Jin et al., 2012). Nevertheless, it is extensively reported that the effects of gastrointestinal digestion on vegetable and animal oils, not only lead to a significant degradation of ω-3 FA, thus, much decreasing their actual intestinal bioaccessibility (Nieva-Echevarría, Goicoechea, & Guillén, 2017; Domoto, Koenen, Havenaar, Mikajiri, & Chu, 2013; Cofrades et al., 2017), but, more importantly, also favour the development of oxidation products which would be absorbed along the duodenal tract (Maestre, Douglass, Kodukula, Medina, & Storch, 2013). Thus, the aim of the present work was to develop a novel nutraceutical formulation, based on gastro-resistant (GR) micronized chia seeds and antioxidative co-formulants. Specifically, this formulation was tested for its potential effects on human plasma triglyceride levels through a randomised clinical trial.

2. Materials and methods

2.1. Reagents and standards

All chemicals and reagents used were analytical-reagent. The water was treated in a Milli-Q water purification system (Millipore, Bedford, MA, USA) before use. Chemicals and reagents used to simulate the gastrointestinal digestion were: potassium chloride (KCl), potassium thiocyanate (KSCN), monosodium phosphate (NaH_2PO_4), sodium sulphate (Na_2SO_4), sodium chloride (NaCl), sodium bicarbonate ($NaHCO_3$), urea, α-amylase, hydrochloric acid (HCl), pepsin, pancreatin, bile salts (Sigma Chemical Co., St. Louis, MO, USA). Vitamin E (DL-alpha-tocopheryl acetate) was purchased from Farmalabor Srl (Canosa di Puglia, Italy).

2.2. Chia seed based nutraceutical formulation

The nutraceutical formulation used in this study consisted of GR capsules containing cryo-micronized chia seeds (500 mg/cps) and vitamin E (15 mg/cps). Cryo-micronized chia seeds were purchased by MB-Med Company (Turin, Italy). The product was formulated by the Department of Pharmacy, University of Naples "Federico II" (Naples, Italy) and indicted as CSN.

2.3. In vitro gastrointestinal (GI) digestion

The assay was performed according to the procedure described by Raiola, Meca, Mañes, and Ritieni (2012), with slight modification. GI digestion was divided into salivary, gastric and duodenal digestive steps. The following samples were submitted to GI digestion: sample 1, 500 mg chia seeds; sample 2, 500 mg cryo-micronized chia seeds; sample 3, one GR capsule containing cryo-micronized chia seeds (500 mg); sample 4, one GR capsule containing cryo-micronized chia seeds (500 mg) and vitamin E (15 mg). For the salivary digestion, the samples were mixed with 6 mL of artificial saliva composed of: KCl (89.6 g/L), KSCN (20 g/L), NaH_2PO4 (88.8 g/L), Na_2SO_4 (57.0 g/L), NaCl (175.3 g/L), $NaHCO_3$ (84.7 g/L), urea (25.0 g/L) and 290 mg of α-amylase. The pH of the solution was adjusted to 6.8 with HCl 0.1 N. The mixture was introduced in a plastic bag containing 40 mL of water and homogenised in a Stomacher 80 Microbiomaster (Seward, Worthing, UK) for 3 min. Immediately, 0.5 g of pepsin (14,800 U) dissolved in HCl 0.1 N was added, the pH was adjusted to 2.0 with HCl 6 N, and then incubated at 37 °C in a Polymax 1040 orbital shaker (250 rpm) (Heidolph, Schwabach, Germany) for 2 h. After the gastric digestion, the pancreatic digestion was simulated as follows: the pH was increased to 6.5 with $NaHCO_3$ 0.5 N and then 5 mL of a mixture pancreatin (8.0 mg/mL) and bile salts (50.0 mg/mL) (1:1; v/v), dissolved in 20 mL of water, were added and incubated at 37 °C in an orbital shaker (250 rpm) for 2 h. After each step of digestion, 10 mL of the obtained extract were centrifuged at 4000 rpm and 4 °C for 1 h: before each following step, the digestion procedure was started over again. To determine the peroxide values and the polyunsaturated fatty acids (PUFA) quali-quantitative profile, the intestinal digestive solution was freeze-dried and, then, subjected to lipid extraction according to AOAC (1995) method 948.16, by using a 6-place units Extraction Unit E-816 Soxhlet (Buchi, Flawil, Switzerland). After centrifugation at 3000*g* for 5 min, supernatants were transferred into a pre-weighed scintillation vial, and dried under nitrogen.

2.4. Peroxide value determination

Peroxide values were measured by treating each lipid extract sample (5 ± 0.05 g) with 30 mL acetic acid-chloroform (3:2, v:v) and 0.5 mL of saturated potassium iodide solution, followed by titration with 0.1 N sodium thiosulphate (AOCS 1998).

2.5. Analysis of fatty acid composition

Lipid extracts were dissolved in 2 mL of *n*-eptane and treated with 0.2 mL of 2 N potassium hydroxide methanolic solution (11.2 g of potassium hydroxide in 100 mL methanol). The mixture was shaken energetically for 1 min at room temperature and then centrifuged (3000*g* for 5 min). Supernatants were collected and analysis of fatty acid methyl esters was performed by gas chromatography using a DANI GC instrument (DANI Instruments, Milan, Italy) coupled to a flame ionization detector (FID) and equipped with a HP-5 capillary column (Agilent, Milan, Italy). The temperature program started at 150 °C (10 min), increased by 2 °C/min to 180 °C and then increased again by 3 °C/min to 240 °C (20 min).

2.6. Study population and protocol

Study participants were recruited by the Samnium Medical Cooperative (Benevento, Italy). Patients were enrolled in February 2017. Patients aged 18–83 years were eligible for enrolment if they had the following values of serum parameters at baseline: TC, 200–260 mg/dL; HDL-C, 31–45 mg/dL; LDL-C, 179–205 mg/dL; glucose, 90–125 mg/dL; TG, 170–280 mg/dL. The subjects were asked to keep their dietary habits unchanged throughout the entire study: to this regard, they were provided with a food diary on which annotate their daily dietary habits.

Exclusion criteria were: smoking, obesity (BMI > 30 kg/m^2), diabetes, hepatic disease, renal disease, heart disease, family history of chronic diseases, drug therapy or supplement intake for hypercholesterolemia, drug therapy or supplement intake containing apple polyphenols, heavy physical exercise (> 10 h/week), pregnant women, women suspected of being pregnant, women who hoped to become pregnant, breastfeeding, birch pollen allergy, use of vitamin/mineral supplements 2 weeks prior to entry into the study and donation of blood less than 3 months before the study.

The subjects received oral and written information concerning the study before they gave their written consent. Protocol, letter of intent of volunteers, and synoptic document about the study were submitted to the Scientific Ethics Committee of AO Rummo Hospital (Benevento, Italy). The study was approved by the committee (protocol 26,875 of 22/02/2016), and carried out in accordance with the Helsinki declaration of 1964 (as revised in 2000). The subjects were asked to make records in an intake-checking table for the intervention study and side effects in daily reports. The study was a randomised, single centre, placebo-controlled trial conducted at the Samnium Medical Cooperative (Benevento, Italy).

The study duration was 16 weeks: the group underwent 4 weeks of placebo treatment, consisting in the administration of identically appearing capsules containing only maltodextrin, followed by 8 weeks of nutraceutical treatment, and 4 weeks of follow-up. Both the examinations and the study treatment were performed in an outpatient setting. Clinic visits and blood sampling were performed after 12 h of fasting at weeks 0, 4, 8, 12, and 16. Subjects were informed not to drink alcohol or perform hard physical activity 48 h prior to blood sampling. All blood samples were taken in the morning and immediately after measurement of heart rate and blood pressure. Blood samples were collected in 10-mL EDTA-coated tubes (Becton–Dickinson, Plymouth, UK) and plasma was isolated by centrifugation (20 min, 2.200g, 4 °C). All samples were stored at −80 °C until analysis. Plasma TC, HDL-C, LDL-C, glucose, and TG levels were determined using commercially available kits from Diacron International (Grosseto, Italy). Analyses were performed on a Diacron International Free Carpe Diem, and intra- and inter-day variations were 1.4 and 1.6% for TC, 1.6 and 2.2% for LDL-C, 2.0 and 2.3% for HDL-C, 1.1 and 1.7% for glucose, and 1.3 and 1.8% for TG, respectively. In addition to these five clinic visits, six standardised telephone interviews were performed every 14 days starting from the first clinic visit, to verify compliance and increase protocol adherence. In particular, these interviews reminded patients to complete their intake-checking table for the intervention study and to record any treatment discontinuation, or adverse events they might have experienced in the meantime (which were also documented regularly on the case report forms during each telephone and clinic visit).

All patients underwent a standardised physical examination, assessment of medical history (for up to five years before enrolment), laboratory examination, measurement of blood pressure and heart rate, and evaluation of BMI. At each clinic visit, patients had to complete three self-administered questionnaires on quality of life aspects, and their diaries were checked for data completeness and quality of documentation to ensure patient comprehension of the diary items.

2.7. Randomisation, concealment, and blinding

A total of 300 eligible patients were randomly assigned to five subgroups. If a patient dropped out before the intervention period, he or she was replaced by the next eligible patient enrolled at the same centre. The concealed allocation was performed by an internet based randomisation schedule, stratified by study site. The random number list was generated by an investigator with no clinical involvement in the trial. Patients, clinicians, core laboratories, and trial staff (data analysts, statisticians) were blind to treatment allocation.

2.8. Study treatments

The group of 300 patients (160 men and 140 women, 18–83 years of age) was randomly divided into five subgroups (each one of 60 subjects). The volunteers enrolled in this study had the following values of plasma parameters at baseline: TC, 200–260 mg/dL; HDL-C, 31–45 mg/dL; LDL-C, 179–205 mg/dL; glucose, 90–125 mg/dL; TG, 170–280 mg/dL. Each sub-group was assigned to a different intervention, as follows: sub-group 1, 5 g chia seeds/day; sub-group 2, 5 g cryo-micronized chia seeds/day; sub-group 3, 4 GR capsules containing cryo-micronized chia seeds (500 mg each)/day; sub-group 4, 4 GR capsules containing cryo-micronized chia seeds (500 mg each) and vitamin E (15 mg each)/day; sub-group 5, 4 GR capsules containing vitamin E (15 mg each)/day. All subjects assuming capsules were instructed to take two capsules at lunch, and two capsules at dinner.

2.9. Study outcomes and data collection

2.9.1. Primary and secondary efficacy outcomes

Primary endpoints measured were the variations of TC, HDL-C, LDL-C, glucose, and TG, while key secondary outcomes collected during clinic visits were measurements of blood pressure and heart rate, and evaluation of BMI.

All raw patient ratings were evaluated in a blinded manner at the site of the principal investigator. The decision process was performed according to a consensus document (unpublished standard operating procedure) before unblinding in order to define conclusive primary and secondary efficacy data from a clinical perspective.

2.9.2. Safety

We assessed safety from reports of adverse events as well as laboratory parameters concerning the hepatic and renal function, vital signs (blood pressure, pulse, height, weight, and body mass index), and physical or neurological examinations. Safety was assessed over the entire treatment period at weeks 0, 4, 8, and 12, including adverse events occurring in the first three weeks after cessation of treatments.

2.10. Statistics

2.10.1. Methodology

During the trial, it became apparent that dropouts and incomplete diary documentation created missing data that could not be adequately handled by the intended robust comparison. To deal with the missing data structure, we used a negative binomial, generalised linear mixed effects model (NB GLMM) that not only yields unbiased parameter estimates when missing observations are missing at random (MAR) (Little & Rubin, 2002), but also provides reasonably stable results even when the assumption of MAR is violated (Molenberghs et al., 2004; O'Kelly & Ratitch, 2014). Patients who did not provide any diary data (leading to zero evaluable days) were excluded from the MAR based primary efficacy analysis, according to an "all observed data approach" as proposed by White and colleagues (White, Carpenter, & Horton, 2012). This approach is statistically efficient without using multiple imputation techniques (Carpenter & Kenward, 2007). Data retrieved after withdrawal of randomised study treatment were also included in the analysis.

Unless otherwise stated, all of the experimental results were expressed as mean ± standard deviation (SD) of at least five replications. Statistical analysis of data was performed by the Student's *t* test or two-way ANOVA followed by the Tukey-Kramer multiple comparison test to evaluate significant differences between a pair of means. The statistic heterogeneity was assessed by using Cochran's test ($p < .1$). The I^2 statistic was also calculated, and $I^2 > 50\%$ was considered as significant heterogeneity across studies. A random-effects model was used if significant heterogeneity was shown among the trials. Otherwise, results were obtained from a fixed-effects model. Percent change in mean and

SD values were excluded when extracting SD values for an outcome. SD values were calculated from standard errors, 95% CIs, p-values, or t if they were not available directly. Previously defined subgroup analyses were performed to examine the possible sources of heterogeneity within these studies and included health status, study design, type of intervention, duration, total polyphenols dose, and Jadad score. Treatment effects were analysed using PROC MIXED with treatment and period as fixed factors, subject as random factor and baseline measurements as covariates, and defined as weighted mean difference and 95% CIs calculated for net changes in serum parameters and blood pressure values. Data that could not meet the criteria of variance homogeneity (Levenes test) and normal distribution (determined by residual plot examination and Shapiro–Wilks test) even after log transformation were analysed by a nonparametric test (Friedman). The level of significance (α-value) was 95% in all cases ($P < .05$).

2.10.2. Analysis sets

The full analysis set population included all randomised patients, and patients who did not fail to satisfy a major entry criterion. We excluded patients who provided neither primary nor secondary efficacy data from efficacy analyses. The per protocol set consisted of all patients who did not substantially deviate from the protocol; they had two characteristics. Firstly, this group included patients for whom no major protocol violations were detected (for example, poor compliance, errors in treatment assignment). Secondly, they had to have been on treatment for at least 50 days counting from day of first intake (completion of a certain pre-specified minimal exposure to the treatment regimen). Hence, patients who prematurely discontinued the study or treatment before day 44 were excluded from the per protocol sample.

2.10.3. Determination of sample size

Sample size calculation was based on an anticipated 30% (0.45 mmol/L) change in plasma triglycerides (primary outcome) with triglyceride standard deviation 0.387 mmol/L, level of significance 0.05 and 80% power (Chan et al., 2016). Thus, a minimum of thirteen subjects had to be recruited in each group.

2.11. Patient involvement

No patients were involved in setting the research question or the outcome measures, nor were they involved in developing plans for participant recruitment, or the design and implementation of the study. There are no plans to explicitly involve patients in dissemination. Final results will be sent to all participating sites.

3. Results

3.1. In vitro GI digestion of chia seeds and chia seed based formulations: Fatty acid bioaccessibility and peroxide values in the intestinal solutions

Table 1 shows results regarding the effects of the simulated GI digestion on the intestinal bioaccessibility of fatty acids from chia seeds (sample 1), cryo-micronized chia seeds (sample 2), GR capsules containing cryo-micronized chia seeds (sample 3), GR capsules containing cryo-micronized chia seeds and vitamin E (sample 4). On average, the following recoveries of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA), were detected in the intestinal solutions: sample 1, SFA 1.5%, MUFA and PUFA 0.06%,respectively; sample 2, SFA 50%, MUFA and PUFA 7%, respectively; sample 3, SFA 50%, MUFA and PUFA 20%, respectively; sample 4, SFA 50%, MUFA and PUFA 40%, respectively. Peroxide values measured in the intestinal solutions after GI digestion of samples 1–4 (Table 2) indicated that the highest oxidative degradation of unsaturated FA regarded sample 2, followed by sample 1 and sample 3, while the lowest value was shown by sample 4.

3.2. Enrolment and subject attrition

Patients were enrolled in February 2017. A total of 408 patients were screened for eligibility; 108 patients (26.5%) did not pass the screening stage; 300 patients were randomised. The most common reason was that patients did not meet the inclusion criteria regarding values of serum parameters at baseline (n = 46), followed by general refusal to participate for no specific reasons (n = 16), and concerns about the protocol, especially fear of placebo (n = 6). Some fulfilled exclusion criteria (n = 40).

Overall, 300 patients were assigned to the group of intervention study: they were divided into five subgroups (each one made of 60 patients). Patients of subgroups underwent a placebo period during the first 4 weeks before the treatment period of 8 weeks. Follow-up period last other 4 weeks. Fig. 1 shows the flow of participants through the trials together with the completeness of diary information over the entire treatment period.

No patient prematurely terminated study participation before allocation to treatment. Fig. 1 follows the CONSORT PRO reporting guideline (Calvert et al., 2013) and reveals that within the assessment period, the following percentage of patients for each subgroup provided data for the primary endpoint: subgroup 1, 76.9% (40 of 52 patients); subgroup 2, 76.0% (38 of 50 patients); subgroup 3, 70.6% (36 of 51 patients); subgroup 4, 75.0% (39 of 52 patients); subgroup 5, 73.6% (39 of 53 patients). In each group, a few patients did not submit any diaries, giving no specific reason for this. Completeness of the patient diaries did not differ between the treatment groups.

3.3. Participants' baseline characteristics

Table 3 shows the demographic and clinical characteristics assessed at the baseline visit of all 300 patients randomised. Overall, about half of the randomised patients were female; the total age range was 18–83 years. The groups were well balanced for demographics and clinical factors.

3.4. Primary efficacy outcome measures

No significant variations of plasma TC, LDL-C, HDL-C, glucose, and TG levels, with respect to the baseline values, were registered in subjects of all subgroups, at the end of the placebo period (Table 3). Analyzing results regarding the variation of plasma lipid values at the end of the intervention period (Table 4), we can assert that the most significant achievement was registered as regards TG levels in all subgroups. Specifically, sample 4 exerted the highest TG lowering effect, decreasing TG levels by 27.5% ($P = .0095$), followed by sample 3 and sample 2, which led the TG levels to −17.5% ($P = .0042$) and −10.2% ($P = .0032$), respectively, while sample 1 and sample 5 showed the lowest effects (−6.3%, $P = .0036$, and −3.1%, $P = .0029$, respectively). Analyzing TC level variations, we can assert that sample 4 was the most effective in reducing the TC levels (−8.0%, $P = .0019$), followed by sample 3 (−5.6%, $P = .0012$). The same trend was observed as regards the LDL-C levels. Interestingly, HDL-C levels significantly raised in patients belonging to subgroups 3 and 4. Specifically, sample 4 showed the greatest effect (+5.7%, $P = .0042$), followed by sample 3 (+3.4%, $P = .0027$), while sample 2 (+1.8%, $P = .0095$), sample 1 (+1.4%, $P = .0042$), and sample 5 (+1.3%, ($P = .0032$) led to scarcely significant variations of HDL-C levels. A negligible hypoglycemic effect was revealed in all subjects belonging to all subgroups (Table 4). Noteworthy, all of the experimental results were achieved already after one month of intervention study and were confirmed at the end of the second month. Comparing the *in vitro* results, regarding the effects of simulated GI digestion on the bioaccessibility of fatty acids in chia seeds and chia seed based formulations (Table 1), with the clinical values, concerning the triglyceride lowering effects of samples 1–5 (Table 4), a significant correlation was found. Specifically, sample 4, exhibiting the

Table 1
Lipid content and fatty acid composition of chia seeds and gastrointestinal digested chia seed based samples.

	Control	Intestinal digesta			
Fatty acids	Chia seeds	Sample 1	Sample 2	Sample 3	Sample 4
14:0	0.71 ± 0.01^{a}	0.01 ± 0.007^{b}	0.34 ± 0.08^{c}	0.29 ± 0.02^{c}	0.30 ± 0.06^{c}
15:0	0.62 ± 0.03^{a}	0.01 ± 0.005^{b}	0.29 ± 0.04^{c}	0.32 ± 0.02^{c}	0.31 ± 0.04^{c}
16:0	28.6 ± 1.5^{a}	0.42 ± 0.03^{b}	16.2 ± 1.6^{c}	15.9 ± 1.4^{c}	16.4 ± 1.8^{c}
16:1	0.53 ± 0.04^{a}	nd	0.04 ± 0.007^{b}	0.16 ± 0.02^{c}	0.25 ± 0.05^{d}
17:0	0.94 ± 0.02^{a}	0.01 ± 0.007^{b}	0.50 ± 0.01^{c}	0.56 ± 0.01^{d}	0.60 ± 0.09^{e}
17:1	0.54 ± 0.01^{a}	nd	0.04 ± 0.005^{c}	0.18 ± 0.01^{c}	0.24 ± 0.07^{c}
18:0	8.71 ± 0.8^{a}	0.12 ± 0.04^{b}	5.02 ± 0.7^{c}	4.68 ± 0.2^{d}	4.97 ± 0.10^{d}
18:1	30.1 ± 1.7^{a}	0.02 ± 0.006^{b}	2.06 ± 0.1^{c}	6.34 ± 0.9^{d}	11.9 ± 1.2^{e}
18:2 ω-6	60.2 ± 1.9^{a}	0.06 ± 0.01^{b}	4.45 ± 0.2^{c}	11.8 ± 1.1^{d}	25.7 ± 2.1^{e}
20:0	1.72 ± 0.2^{a}	0.02 ± 0.007^{b}	0.92 ± 0.01^{c}	0.86 ± 0.06^{c}	0.77 ± 0.06^{c}
18:3 ω-6	1.33 ± 0.2^{a}	0.002 ± 0.0004^{e}	0.07 ± 0.001^{c}	0.32 ± 0.01^{a}	0.59 ± 0.02^{d}
18:3 ω-3	232.1 ± 5.9^{a}	0.26 ± 0.09^{b}	15.8 ± 2.5^{c}	44.1 ± 3.1^{d}	94.8 ± 4.2^{e}
20:1	0.60 ± 0.03^{a}	nd	0.05 ± 0.008^{c}	0.12 ± 0.01^{c}	0.31 ± 0.06^{a}
20:2 ω-6	0.42 ± 0.02^{a}	nd	0.02 ± 0.007^{b}	0.13 ± 0.02^{c}	0.34 ± 0.04^{d}
22:0	0.51 ± 0.03^{a}	0.01 ± 0.004^{b}	0.22 ± 0.06^{c}	0.19 ± 0.02^{c}	0.23 ± 0.02^{c}
24:0	0.30 ± 0.01^{a}	0.007 ± 0.001^{b}	0.17 ± 0.07^{c}	0.20 ± 0.01^{d}	0.22 ± 0.03^{d}
Groups					
SFA	42.0 ± 1.9^{a}	0.06 ± 0.001^{b}	23.7 ± 1.8^{c}	23.0 ± 2.1^{c}	23.8 ± 2.4^{c}
MUFA	31.7 ± 1.8^{a}	0.04 ± 0.005^{b}	2.19 ± 0.6^{c}	6.80 ± 0.9^{d}	12.7 ± 2.1^{e}
PUFA	244.0 ± 6.9^{a}	0.44 ± 0.08^{b}	20.3 ± 2.2^{c}	56.3 ± 5.2^{d}	121.4 ± 4.8^{e}
Ratio ω-3/ω-6	3.74	4.33	3.48	3.60	3.56
Lipids	317.7 ± 8.4^{a}	0.54 ± 0.11^{b}	46.2 ± 3.1^{c}	86.1 ± 1.31^{d}	157.9 ± 4.6^{e}

Values are expressed as mg fatty acids/g chia seeds, and are reported as mean ± SD of five replicate analyses (n = 5).
^{abcde}Mean values in rows with different superscript letters are significantly different by the Tukey-Kramer multiple comparison test (P < .05).
SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.
Sample 1: 500 mg chia seeds; Sample 2, 500 mg cryo-micronized chia seeds; Sample 3, 1 gastro-resistant capsule containing cryo-micronized chia seeds (500 mg); Sample 4, 1 gastro-resistant capsule containing cryo-micronized chia seeds (500 mg) and vitamin E (15 mg).
nd: not detected.

Table 2
Peroxide values of intestinal solutions after gastrointestinal digestion of chia seeds and chia seed based samples.

	Intestinal digesta			
	Sample 1	Sample 2	Sample 3	Sample 4
Peroxide value (meq O_2/kg)	0.18 ± 0.02^{a}	0.48 ± 0.05^{b}	0.16 ± 0.03^{a}	0.035 ± 0.002^{c}

Values are reported as mean ± SD of five replicate analyses (n = 5).
^{abc}Mean values in raw with different superscript letters are significantly different by the Tukey-Kramer multiple comparison test (P < .05).
Sample 1:500 mg chia seeds; Sample 2, 500 mg cryo-micronized chia seeds; Sample 3, 1 gastro-resistant capsule containing cryo-micronized chia seeds (500 mg); Sample 4, 1 gastro-resistant capsule containing cryo-micronized chia seeds (500 mg) and vitamin E (15 mg).

highest *in vitro* bioaccessibility of ω-3 FA fraction (Table 1), was the most effective in decreasing plasma TG levels (Table 4), while samples 1 and 2, characterised by the lowest bioaccessibility *in vitro* of ω-3 FA fraction, showed the poorest TG level lowering effects.

3.5. Safety issue, study strength and limitations

Table 5 shows data regarding blood indicators of hepatic and renal functions. Clinical results clearly indicated that no toxicity was registered during and at the end of the intervention period, with the exception of subjects assuming cryo-micronized chia seeds (sample 2) who revealed an average slight increase of all measured parameters. Other safety assessments, such as vital signs, blood pressure, or electrocardiographic findings, were all periodically monitored and baseline values did not change substantially during and at the end of the trial.

The major strengths of the clinical trial herein presented reside in the originality of the study and in the evaluation of the treatment effects in real-world settings. Conversely, the main limitations of our study include the short-term assessment for the treatment of a chronic condition, the choice of exclusively white race, and the wide age range due to the availability of such individuals at the stage of the recruitment.

4. Discussion

Our *in vitro* experimental results (Table 1) clearly indicated that chia seeds, although their high content of PUFA (24.4%) and, specifically, of ω-3 FA (23.2%), are characterised by a quite low intestinal bioaccessibility of such bioactive compounds. Thus, the extensively reported healthy effects of chia seeds, referred to their important PUFA content, seem to remain just a potential beneficial property, not realistically achievable by their regular consumption. In agreement with these data, our results from the clinical study would confirm that, after two months of regular consumption, chia seeds are responsible for a modest beneficial influence on blood triglycerides (−6.3%) (Table 4). It could be hypothesised that, due to their very small size, and their resistance to mastication, chia seeds would be very difficult, or almost impossible, to be properly chewed and, thus, crushed, so that their PUFA content would be characterised by a quite low or no intestinal bioaccessibility.

Interestingly, micronized chia seeds revealed a higher *in vitro* intestinal bioaccessibility of their SFA content (about +50%) than intact seeds, while a modest increase of MUFA and PUFA intestinal levels (about +7%, respectively) was detected (Table 1). Such *in vitro* observations are consistent with our clinical results which highlighted a slight increase of plasma triglyceride levels in subjects assuming micronized chia seeds than subjects assuming intact seeds (Table 4). Our results would confirm what already reported by previous authors as regards the enhancement of ALA and EPA concentrations in both animal and human plasma when administered with milled chia seeds (Ayerza & Coates, 2007; Nieman et al., 2012; Nieman et al., 2009; Ayerza & Coates, 2005; Pereira da Silva et al., 2016; Jin et al., 2012).

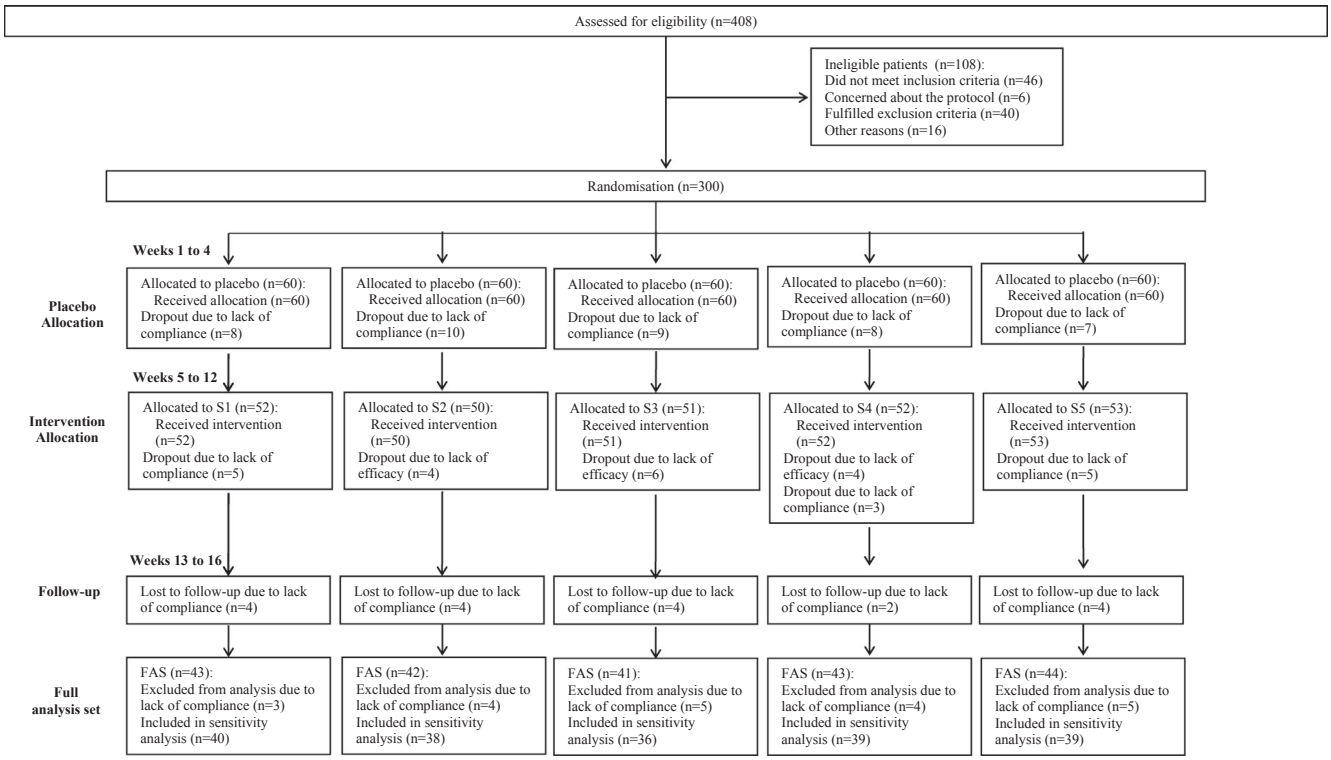

Fig. 1. Study flowchart, according to the consolidated standards of reporting trials (CONSORT). The diagram shows enrolment and primary efficacy endpoints based on patient diaries, from prescreening to data collection; and the extent of exclusions, loss to follow-up, and completeness of diary documentation available across the entire trial period. S1 (subgroup 1) = Sample 1 (chia seeds); S2 (subgroup 2) = Sample 2 (cryo-micronized chia seeds); S3 (subgroup 3) = Sample 3 (gastro-resistant capsules containing cryo-micronized chia seeds); S4 (subgroup 4) = Sample 4 (gastro-resistant capsules containing cryo-micronized chia seeds and vitamin E); S5 (subgroup 5) = Sample 5 (gastro-resistant capsules containing vitamin E); FAS = full analysis set.

Table 3
Baseline characteristics of intention to treat sample according to study treatment.

Placebo					
Characteristics	S1 (n = 60)	S2 (n = 60)	S3 (n = 60)	S4 (n = 60)	S5 (n = 60)
Demographics					
Age (years)	43.8 ± 10.5	42.7 ± 11.2	42.4 ± 10.6	44.8 ± 11.2	41.8 ± 10.8
Male sex (No (%))	32 (53.3%)	32 (53.3%)	34 (56.7%)	30 (50.0%)	32 (53.3%)
White ethnicity (No (%))	60 (100%)	60 (100%)	60 (100%)	60 (100%)	60 (100%)
Clinical parameters					
TC (mg/dL)	239.1 ± 13.4	233.5 ± 12.6	240.2 ± 11.5	236.3 ± 12.4	236.1 ± 13.8
LDL-C (mg/dL)	191.0 ± 11.3	184.1 ± 12.3	187.9 ± 12.1	185.2 ± 11.9	187.8 ± 13.1
HDL-C (mg/dL)	36.8 ± 6.1	36.3 ± 7.2	39.9 ± 6.8	36.2 ± 7.7	32.9 ± 7.3
Glucose (mg/dL)	99.5 ± 8.1	91.4 ± 9.4	111.2 ± 9.2	100.8 ± 2.2	100.1 ± 7.3
Triglycerides (mg/dL)	187.1 ± 9.2	222.8 ± 12.1	193.6 ± 11.8	202.4 ± 10.2	212.3 ± 12.1
Treatment					
Characteristics	S1 (n = 52)	S2 (n = 50)	S3 (n = 51)	S4 (n = 52)	S5 (n = 53)
Demographics					
Age (years)	44.2 ± 10.6	45.5 ± 10.7	43.5 ± 10.2	42.1 ± 11.1	43.8 ± 12.2
Male sex (No (%))	28 (53.8%)	26 (52.0%)	27 (52.9%)	24 (46.0%)	25 (47.2%)
White ethnicity (No (%))	52 (100%)	52 (100%)	51 (100%)	52 (100%)	53 (100%)
Clinical parameters					
TC (mg/dL)	235.5 ± 13.3	237.6 ± 14.3	239.1 ± 11.9	235.9 ± 13.7	238.4 ± 15.3
LDL-C (mg/dL)	185.7 ± 11.1	189.1 ± 11.6	189.6 ± 11.7	183.7 ± 12.3	189.1 ± 11.6
HDL-C (mg/dL)	36.7 ± 7.5	35.4 ± 6.7	38.5 ± 7.2	37.9 ± 8.1	34.2 ± 6.9
Glucose (mg/dL)	92.8 ± 2.7	102.1 ± 8.9	108.2 ± 8.5	101.9 ± 10.0	99.5 ± 9.3
Triglycerides (mg/dL)	220.0 ± 16.8	185.4 ± 8.3	195.2 ± 9.0	206.1 ± 10.4	210.5 ± 11.8

Values are means ± SD (n = 5).
Results were significantly different at a level of P = .001.
S1 (subgroup 1) = Sample 1 (chia seeds); S2 (subgroup 2) = Sample 2 (cryo-micronized chia seeds); S3 (subgroup 3) = Sample 3 (gastro-resistant capsules containing cryo-micronized chia seeds); S4 (subgroup 4) = Sample 4 (gastro-resistant capsules containing cryo-micronized chia seeds and vitamin E); S5 (subgroup 5) = Sample 5 (gastro-resistant capsules containing vitamin E).

Table 4
Effects of chia seeds and chia seed based formulations on plasma cholesterol, glucose and triglyceride metabolism.

		S1	Δ (%)	S2	Δ (%)	S3	Δ (%)	S4	Δ (%)	S5	Δ (%)
TC (mg/dL)	*t 0*	235.5 ± 13.3		237.6 ± 14.3		239.1 ± 11.9		235.9 ± 13.7		238.4 ± 15.3	
	t 30	231.3 ± 14.2	*−1.8*	229.7 ± 12.8	*−3.3*	226.2 ± 13.5*	*−5.4*#	216.3 ± 14.1*	*−8.3*#	235.3 ± 11.9	*−1.3*
	t 60	230.3 ± 13.6	*−2.2*	229.5 ± 12.1	*−3.4*	225.7 ± 13.8*	*−5.6*#	217.0 ± 13.4*	*−8.0*#	235.8 ± 12.0	*−1.1*
LDL−C (mg/dL)	*t 0*	185.7 ± 11.1		189.1 ± 11.6		189.6 ± 11.7		183.7 ± 12.3		189.1 ± 11.6	
	t 30	181.8 ± 11.3	*−2.1*	182.3 ± 10.9	*−3.6*	177.6 ± 0.8*	*−6.3*#	166.1 ± 11.7*	*−9.6*#	184.1 ± 12.0	*−2.6*
	t 60	182.2 ± 13.6	*−1.9*	181.5 ± 11.2	*−4.0*	178.2 ± 1.2*	*−6.0*#	165.0 ± 12.1*	*−10.2*#	184.1 ± 12.3	*−2.6*
HDL-C (mg/dL)	*t 0*	36.7 ± 7.5		35.4 ± 6.7		38.5 ± 7.2		37.9 ± 8.1		34.2 ± 6.9	
	t 30	37.3 ± 7.5	*+1.6*	36.2 ± 7.9	*+2.2*	40.0 ± 8.0	*+3.8*	40.1 ± 6.8*	*+5.9*#	34.7 ± 6.4	*+1.5*
	t 60	37.2 ± 7.0	*+1.4*	36.0 ± 8.1	*+1.8*	39.8 ± 8.3	*+3.4*	40.1 ± 6.0*	*+5.7*#	34.6 ± 5.6	*+1.3*
Glucose (mg/dL)	*t 0*	92.8 ± 12.7		102.1 ± 8.9		108.2 ± 8.5		101.9 ± 10.0		99.5 ± 9.3	
	t 30	92.1 ± 10.8	*−0.7*	101.0 ± 12.0	*−1.1*	107.5 ± 12.4	*−2.1*	98.8 ± 8.3	*−3.0*	98.4 ± 10.1	*−1.1*
	t 60	91.9 ± 11.6	*−1.0*	100.3 ± 13.1	*−1.8*	105.5 ± 10.3	*−2.5*	98.6 ± 10.1	*−3.2*	98.2 ± 19.8	*−1.3*
Triglycerides (mg/dL)	*t 0*	220.0 ± 16.8		185.4 ± 8.3		195.2 ± 19.0		206.1 ± 10.4		210.5 ± 11.8	
	t 30	187.5 ± 19.3*	*−5.7*#	167.4 ± 16.4*	*−9.7*#	160.4 ± 21.1*	*−17.8*#	152.9 ± 16.7*	*−25.8*#	203.1 ± 13.4*	*−3.5*#
	t 60	186.1 ± 14.7*	*−6.3*#	166.5 ± 17.3*	*−10.2*#	161.0 ± 15.2*	*−17.5*#	149.4 ± 14.2*	*−27.5*#	204.0 ± 16.5*	*−3.1*#

Values are means ± SD ($n = 5$).
t 0: 1st day of treatment; *t 30*: 30th day of treatment; *t 60*: 60th day of treatment.
S1 (subgroup 1) = Sample 1 (chia seeds).
S2 (subgroup 2) = Sample 2 (cryo-micronized chia seeds).
S3 (subgroup 3) = Sample 3 (gastro-resistant capsules containing cryo-micronized chia seeds).
S4 (subgroup 4) = Sample 4 (gastro-resistant capsules containing cryo-micronized chia seeds and vitamin E).
S5 (subgroup 5) = Sample 5 (gastro-resistant capsules containing vitamin E).
* Significantly different from baseline at $P < .01$ (PROC MIXED).
# Significantly different from the other treatments at $P < .05$ (PROC MIXED).

Table 5
Effects of chia seeds and chia seed based formulations on plasma indicators of hepatic and renal function.

		S1	Δ (%)	S2	Δ (%)	S3	Δ (%)	S4	Δ (%)	S5	Δ (%)
AST (GOT) (U/L)	*t 0*	30.2 ± 7.2		25.3 ± 6.7		34.1 ± 7.6		21.6 ± 5.4		38.2 ± 7.5	
	t 30	29.1 ± 5.3	*−3.6*	26.6 ± 4.8*	*+5.2*#	34.9 ± 6.8	*+2.5*	21.5 ± 4.8	*−0.5*	37.7 ± 8.1	*−1.3*
	t 60	29.4 ± 7.9	*−2.6*	26.8 ± 5.9*	*+6.0*#	35.1 ± 8.1	*+3.1*	21.5 ± 5.2	*−0.5*	37.8 ± 6.8	*−1.1*
ALT (GPT) (U/L)	*t 0*	22.6 ± 6.4		34.7 ± 8.2		30.4 ± 5.4		27.9 ± 3.8		25.6 ± 4.7	
	t 30	22.0 ± 5.8	*−2.6*	35.4 ± 7.5	*+2.1*	30.7 ± 5.9	*+1.1*	27.5 ± 4.2	*−1.4*	25.4 ± 5.1	*−2.6*
	t 60	22.1 ± 4.9	*−2.2*	35.7 ± 6.9	*+2.9*	30.9 ± 6.9	*+1.7*	27.6 ± 4.9	*−1.1*	25.4 ± 4.6	*−2.6*
γ-GTP (U/L)	*t 0*	28.9 ± 6.2		32.8 ± 5.5		20.7 ± 3.8		37.2 ± 5.8		30.5 ± 5.8	
	t 30	28.4 ± 8.2	*−1.7*	33.9 ± 5.3	*+3.5*	21.0 ± 4.2	*+1.6*	36.2 ± 6.3	*−2.7*	30.9 ± 5.1	*+1.5*
	t 60	28.3 ± 7.1	*−2.1*	33.8 ± 6.2	*+3.2*	20.9 ± 6.3	*+1.4*	34.4 ± 5.9	*−7.5*	30.8 ± 4.8	*+1.3*
ALP (U/L)	*t 0*	103.2 ± 11.6		89.5 ± 9.2		77.4 ± 8.9		100.2 ± 9.7		80.2 ± 6.3	
	t 30	102.7 ± 10.5	*−0.48*	91.5 ± 8.7	*+2.3*	78.2 ± 9.6	*+1.0*	99.4 ± 10.6	*−0.9*	79.3 ± 7.2	*−1.1*
	t 60	102.5 ± 10.4	*−0.68*	91.1 ± 8.8	*+1.8*	77.9 ± 7.5	*+0.7*	99.1 ± 8.6	*−1.1*	79.1 ± 6.9	*−1.3*
LDH (U/L)	*t 0*	165.9 ± 12.3		189.2 ± 10.1		195.2 ± 13.7		177.0 ± 11.6		200.1 ± 14.7	
	t 30	165.0 ± 11.8	*−0.54*	188.2 ± 11.1	*−0.5*	193.0 ± 12.8	*−1.1*	176.5 ± 12.8	*−0.3*	193.1 ± 13.6	*−3.5*
	t 60	164.8 ± 13.9	*−0.66*	188.1 ± 10.4	*−0.6*	192.5 ± 14.8	*−1.4*	170.2 ± 10.7	*−3.8*	193.9 ± 13.2	*−3.1*
Albumin (g/dL)	*t 0*	4.84 ± 0.97		4.25 ± 0.88		4.06 ± 0.92		4.31 ± 0.83		3.98 ± 0.77	
	t 30	4.62 ± 0.85	*−4.5*	4.38 ± 0.79	*+3.1*	4.12 ± 0.88	*+1.6*	4.32 ± 0.86	*+0.2*	4.03 ± 0.85	*+1.5*
	t 60	4.46 ± 0.79*	*−7.8*#	4.42 ± 0.83	*+4.1*	4.16 ± 0.85	*+2.4*	4.16 ± 0.91	*−3.5*	4.01 ± 0.89	*+1.0*
Total bilirubin (mg/dL)	*t 0*	0.66 ± 0.11		0.82 ± 0.07		0.48 ± 0.05		0.57 ± 0.08		0.78 ± 0.07	
	t 30	0.60 ± 0.10*	*−9.0*#	0.85 ± 0.08	*+3.4*	0.49 ± 0.06	*+1.8*	0.57 ± 0.09	–	0.76 ± 0.07	*−2.4*
	t 60	0.62 ± 0.12*	*−6.0*#	0.86 ± 0.09	*+4.4*	0.50 ± 0.07	*+2.3*	0.54 ± 0.05	*−5.3*	0.75 ± 0.09	*−2.2*
Creatinine (mg/dL)	*t 0*	0.88 ± 0.09		1.04 ± 0.12		0.97 ± 0.09		0.84 ± 0.11		1.12 ± 0.12	
	t 30	0.87 ± 0.10	*−1.1*	1.07 ± 0.13	*+2.7*	0.99 ± 0.08	*+1.7*	0.82 ± 0.09	*−2.4*	1.11 ± 0.11	*−0.9*
	t 60	0.89 ± 0.08	*+1.1*	1.08 ± 0.16	*+3.2*	0.98 ± 0.07	*+1.5*	0.80 ± 0.12	*−4.8*	1.10 ± 0.12	*−0.7*

Values are means ± SD ($n = 5$).
t 0: 1st day of treatment; *t 30*: 30th day of treatment; *t 60*: 60th day of treatment.
S1 (subgroup 1) = Sample 1 (chia seeds); S2 (subgroup 2) = Sample 2 (cryo-micronized chia seeds); S3 (subgroup 3) = Sample 3 (gastro-resistant capsules containing cryo-micronized chia seeds).
S4 (subgroup 4) = Sample 4 (gastro-resistant capsules containing cryo-micronized chia seeds and vitamin E); S5 (subgroup 5) = Sample 5 (gastro-resistant capsules containing vitamin E).
* Significantly different from baseline at $P < .01$ (PROC MIXED).
# Significantly different from the other treatments at $P < .05$ (PROC MIXED).

Nevertheless, no study has evaluated, so far, the possible effects of the direct exposure to GI digestion of PUFAs released from these milled seeds. The quite low *in vitro* intestinal bioaccessibility of MUFA and PUFA content from micronized chia seeds could be ascribed to their massive oxidative degradation, as indicated by our *in vitro* measures of lipid peroxidation index values in the intestinal solution of micronized chia seeds (Table 2). Actually, it is well known that the effects of GI digestion on vegetable and animal oils, not only lead to a significant degradation of unsaturated FA, thus, much decreasing their actual intestinal bioaccessibility (Nieva-Echevarría et al., 2017; Domoto et al., 2013; Cofrades et al., 2017), but, more importantly, also favour the development of oxidation products which would be absorbed along the duodenal tract (Maestre et al., 2013).

Thus, we decided to include micronized chia seeds in GR capsules and to submit them to simulated GI digestion. SFA were available in the intestinal solution at the same level of what detected for non-gastro-protected micronized chia seeds (about +50%) (Table 1). Interestingly, MUFA and PUFA much increased their *in vitro* intestinal bioaccessibility (+20%) (Table 1). Reasonably, this could be due to their protection against the gastric degradation, as confirmed by our peroxide index measures (Table 2), which clearly indicated an important decrease of unsaturated FA oxidative degradation, compared to non-gastro-protected micronized chia seeds. Again, these *in vitro* outcomes seemed to be substantiated by our clinical data which revealed a significant increase of plasma triglyceride levels in subjects assuming gastro-protected micronized chia seeds (Table 4).

Considering the significant decrease of unsaturated FA peroxidation, regarding the gastro-protected micronized chia seeds (Table 2), we hypothesised to further improve the protection against lipoperoxidative degradation of such formulation by adding a specific lipophilic antioxidant compound. The choice of vitamin E revealed successful since it nearly doubled the *in vitro* intestinal bioaccessibility of MUFA and PUFA from gastro-protected micronized chia seeds (Table 1). Probably, this could be due to its important protection of unsaturated FA against oxidative degradation (Raederstorff, Wyss, Calder, Weber, & Eggersdorfer, 2015), as clearly indicated by our results regarding the drastically reduced peroxide values (Table 2). Such fortified formulation, administered to subjects at the dose of four capsules/day for two months, lowered blood triglyceride levels by about 27.5%, which means a +10% efficacy respect to the gastro-protected chia seed based formulation not including vitamin E (Table 4). Interestingly, a peculiar aspect concerning both GR formulations regards the evident synergistic effect of their individual components. In fact, a total dosage of 2 g/day of chia seeds, provided by four capsules of both formulations, allowed a much higher decrease of blood triglyceride levels than what obtained by assuming 5 g/day of either chia seeds or micronized chia seeds. Particularly, results were as follows: GR capsules containing micronized chia seeds, 2.8-fold that of chia seeds, 1.7-fold that of micronized chia seeds; GR capsules containing micronized chia seeds and vitamin E, 4.4-fold that of chia seeds, 2.7-fold that of micronized chia seeds. The very modest effects on plasma triglyceride levels obtained by administering the gastro-protected vitamin E (Table 4) further corroborates the synergistic role of the different components included in the formulation.

Although no specific toxicity studies were performed herein, the type and daily quantity of chia seeds and vitamin E assumed by subjects of each group were expected to be totally compatible with a healthy state. In fact, chia seeds were used at a maximum quantity of 5 g/day, which is lower than the daily amount of common dry fruits suggested for a cardioprotective potential (about 30 g) (Ros, 2010). The dose of vitamin E (60 g/day) is in full accordance with the maximum daily intake allowed for food supplements (Reg. EU 1169/2011). To confirm this, our clinical data regarding the measures of hepatic and renal function indicators (Table 5) clearly denoted no signs of toxicity. An exception was represented by subjects assuming non-gastro-protected micronized chia seeds, who revealed an average slight increase of all measured parameters. Probably, the important *in vitro* peroxidative degradation of unsaturated FA from micronized chia seeds (Table 2) could suggest for subjects assuming micronized chia seeds a significant production of lipoperoxides, which would be absorbed along the duodenal tract, with potential toxicological implications, as previously demonstrated by other authors for vegetable and animal oils (Maestre et al., 2013).

To date, no previous work regarding the effects of chia seeds, or chia seed based nutraceutical formulations, on human plasma lipid profile, are available in the literature. Previous authors evaluated the influence of dietary chia seed oil on vascular function in hypercholesterolemic rabbits (Sierra et al., 2015). Rabbits were fed either regular diet (CD) or 10% chia oil in regular diet or 1% cholesterol diet (HD) or diet containing 1% cholesterol and 10% chia oil (HD-Ch) during 5–6 weeks. HD increased total cholesterol, LDL and triacylglycerol levels, while HD-Ch significantly attenuated the triacylglycerol rise. *In vitro* studies have recently suggested that chia seed polyphenolic fractions may have potential anti-obesity and antidiabetic effects through inhibition of activities against pancreatic lipase, α-glucosidase as well as LDL-cholesterol oxidation (Jiaur Rahman, Costa de Camargo, & Shahidi, 2017). All of these data, together with the undisputable beneficial contribution of fiber, may suggest that the effects on plasma lipidemia by both chia seeds and chia seed based nutraceutical formulations could be due to several bioactive constituents in addition to PUFA. Thus, further research should clarify the multifactorial beneficial properties on plasma lipid profile of such food product.

5. Conclusion

The present study proposes an innovative nutraceutical product which can be regarded as an effective and safe natural remedy for the contribution to a healthy balance of plasma lipid levels, with clinical relevance in the primary cardiovascular disease prevention. Obviously, further *in vitro*, *in vivo*, and clinical trials, are needed to corroborate our preliminary results, specifically, by comparative studies with pharmacological treatments indicated for lipid imbalance. Moreover, specific investigations will be necessary to evaluate the contribution to the described effects by other chia seed constituents, such as the fiber, polyphenols, and protein content.

Contributors

GCT, DC, GB, MDA, and EN were responsible for study concept and design. GCT, DC, GB, MDA, RC, and EN acquired the clinical data. GCT, RC, and EN acquired the *in vitro* data. All authors analysed and interpreted the data and drafted the manuscript. All authors critically revised the manuscript for important intellectual content. EN obtained funding. EN provided administrative, technical, or material support. All authors supervised the study. All authors, external and internal, had full access to all of the data. EN is the guarantor.

Funding

This research was funded by Regione Campania under POR Campania FESR 2007-2013 – O.O. 2.1 (FarmaBioNet).

Competing interests

All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf and declare: EN has received research grants from Regione Campania under POR Campania FESR 2007-2013 – O.O. 2.1 (FarmaBioNet); no other relationships of activities that could appear to have influenced the submitted work.

Ethical approval

The study was approved by the ethics committee at the Hospital AO

Rummo of Benevento, Italy.

Transparency statement

The lead author (the manuscript's guarantor) affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

Data sharing

No additional data available.

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