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Effects of A Food Supplement Containing Oleuropein, Elenolic Acid, Rutin, Hydroxytyrosol and Tyrosol in Patients with Hepatic Steatosis

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Abstract Non-alcoholic fatty liver disease (NAFLD) represents the leading cause of liver disease and is characterized by obesity, hyperglycemia, insulin-resistance, dyslipidemia and hypertension. The aim of this study was to evaluate if Olife® consumption in patients with NAFLD could improve fatty liver grade and related metabolic and inflammatory parameters. Eighty-two Caucasian patients aged ≥ 18 of both sexes diagnosed with NAFLD, according to practice guidelines, were enrolled in a single-center, double-blind, open label, randomized, controlled study. Patients were randomized to follow a standardized Diet or a standardized Diet + Olife® for 3 months. No changes in BMI, circumference, FPG, TC, LDL-C, HDL-C and Tg were observed with either treatment. In the group of patients receiving Diet + Olife®, a significant decrease in hs-CRP and TNF-α as well as an increase in ADN ($p < 0.05$ compared to Baseline and Diet) was observed, respectively. Transaminases and γ-GT were significantly reduced ($p < 0.05$ vs Baseline and Diet) in Diet + Olife® group compared to Diet group, respectively. In addition, AST/ALT ratio and HIS were significantly lower ($p < 0.05$ vs Baseline and Diet) in the Diet + Olife® group compared to Diet group, respectively. All patients showed signs of hepatic steatosis improvement or disappearance. In conclusion Olife® added to Diet ameliorates hepatic parameters and echography grading, and manages to reduce inflammatory parameters during the 3 months of study.

Keywords: *food supplement, oleuropein, elenolic acid, rutin, hydroxytyrosol, tyrosol, hepatic steatosis*

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1. Introduction

Globally, non-alcoholic fatty liver disease (NAFLD) represents the leading cause of liver disease and is characterized by obesity, hyperglycemia, insulin-resistance, dyslipidemia and hypertension. Several studies have supported the link of NAFLD to type 2 diabetes mellitus and metabolic syndrome suggesting that NAFLD may be a condition that precedes the development of these two diseases rather than being the hepatic manifestation of metabolic syndrome according the conventional paradigm [1].

To date, the recommended therapeutic approach for the treatment of NAFLD is based on lifestyle interventions that include changes in diet and physical exercise [2,3]. Some clinical studies conducted on patients with NAFLD have shown that a greater adherence to the Mediterranean diet seems to be related to an improvement in the severity of the disease in terms of reduction of hepatic steatosis, amelioration of insulin-resistance and reduction of the probability of developing non-alcoholic steatohepatitis (NASH) [4,5,6].

However, due to scarce adherence to this treatment, other therapeutic strategies are being sought for NAFLD also in order to prevent its complications [7].

Current investigations propose the use of some nutraceuticals with antihyperlipidemic and hepatoprotective activity for the treatment of NAFLD that comprise some amino acids (tryptophan, glutamine and l-carnitine), n-3 polynsaturated fatty acids (n-3 PUFAs), some vitamins (niacin and vitamin E), polyphenols (resveratrol, cathechin, quercetin and anthocyanins), prebiotics, probiotics and symbiotics as well as intervention with medicinal herbs [7]. At regard probiotic therapy, it has observed that VSL#3, a mixture of live lactic acid bacteria and bifidobacteria including *Streptococcus thermophilus* BT01, *Bifidobacterium brevis* BB02, *Bifidobacterium animalis* subspecies [subsp.] *lactis*

BL03, *Bifidobacterium animalis* subsp *lactis* BI04, *Lactobacillus acidophilus* BA05, *Lactobacillus plantarum* BP06, *Lactobacillus paracasei* BP07, *Lactobacillus helveticus* BD08, improved liver parameters and hepatic steatosis index after 3 months of treatment in patients with NAFLD [8].

Olea europaea, commonly called olive tree, is often utilized in conventional herbal remedies. The most used parts of this plant are the leaves and the fruits, the olives, due the presence of several biologically active compounds such as polyphenols, oleuropein (Figure 1A), hydroxytyrosol (Figure 1B), tyrosol, ligstroside, lignans and flavonoids known for their hypoglycemic and hypocholesterolemic as well as antioxidant, antihypertensive, antiatherogenic, and antinflammatory activities [9,10]. The main active component is oleuropein, a natural product belonging to the secoiridoids that has been associated with improved glycemic parameters, as reported in vitro and in vivo studies [11,12]. *Olea europaea* can exert its hypoglycemic action by suppressing α-glucosidase and α-amylase activity, rising glucose absorption in peripheral tissues, promoting insulin and glucagon-like peptide-1 (GLP-1) release, improving insulin-resistance and blocking protein glycation thereby lowering the production of advance glycation end products (AGEs) [13].

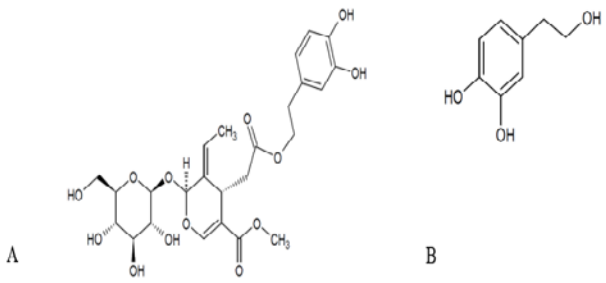

Figure 1. Chemical structure of bioactive compounds. A: Oleuropein. B: Hydroxytyrosol

Olive oil is one of the key components of the Mediterranean diet and its protective role against cardiometabolic diseases has been extensively documented. As regard NAFLD, it has been showed that olive oil supplementation significantly ameliorated fatty liver grade and span, insulin-resistance, hepatic enzymes [aspartate aminotransferase (AST) and alanine aminotransferase (ALT)], and lipid parameters [14,15,16,17]. In addition, a 6-year follow-up study examined the effect of the Mediterranean diet with extra virgin olive oil on NAFLD using the fatty liver index (FLI) that presented a lower change over time respect that produced by a low-fat diet [18].

Recently it has been examined the effects of Olife®, a food supplement commercialized in Italy consisting of an infusion of olive leaves and marigold, on glucose metabolism in subjects with impaired fasting glucose (IFG). After 3 months of treatment the supplement ameliorated both glycemic profile and status [19].

Based on the above, a study was planned to assess if Olife® consumption in patients with NAFLD could improve fatty liver grade and related metabolic and inflammatory parameters.

2. Materials and Methods

Study design

This 3-months, monocenter, double-blind, randomized, clinical study was conducted at the Centre of Diabetes and Metabolic Diseases, University of Pavia, and IRCCS Policlinico San Matteo Foundation, PAVIA, Italy. The study protocol was conducted in accordance with the 1994 Declaration of Helsinki [20], and its amendments and the Code of Good Clinical Practice. All patients provided written informed consent to participate in this study after a full explanation of the study was given.

Material and methods

We enrolled 82 Caucasian patients aged ≥ 18 of either sex, with diagnosis of NAFLD, according to practice guidance [21] and in particular with these characteristics:

-transaminases (AST and ALT) increase

-ratio inversion AST/ALT

-absence of virus markers of hepatitis

-absence of alcohol consumption or consumption less than 20 g/day in women and 30 g/day in men

For further and additional confirmation of the diagnosis of NAFLD, the Hepatic Steatosis Index (HSI) has been calculated using the formula:

8 x (AST/ALT ratio) + BMI (+ 2 if woman; + 2 if diabetic) and the result of the formula could be < 30.0 or > 36.0 to furtherly classify these patients as having NAFLD [22].

An additional criterion was hepatomegaly and/or the presence of hepatic echography with a framework of NAFLD such as the hyper-reflective (bright) surface of the liver.

Suitable patients, identified from review of case notes and/or computerized clinic registers, were contacted by the investigators in person or by telephone.

Patients were excluded if they have chronic liver disease; impaired renal function (defined as serum creatinine level higher than the ULN for age and sex); endocrine disorders, or gastrointestinal disorders; current or previous evidence of ischemic heart disease, heart failure, or stroke; malignancy; and significant neurological or psychiatric disturbances, including alcohol or drug abuse. Excluded medications (within the previous 3 months) were anorectic agents, laxatives, β-agonists (other than inhalers), diuretics, cyproheptadine, anti-depressants, anti-serotoninergics, phenothiazines, barbiturates, oral corticosteroids, and anti-psychotics. Women who were pregnant or breastfeeding or of childbearing potential and not taking adequate contraceptive precautions were also excluded.

Diet and physical activity

At baseline, all patients were already following an adequate diet. The controlled-energy diet (~600 kcal daily deficit) was based on NCEP-ATP III recommendations [23], that contained 50% of calories from carbohydrates, 30% from fat (< 7% saturated, up to 10% polyunsaturated, and up to 20% monounsaturated), and 20% from proteins, with a maximum cholesterol content of 300 mg/d, and 35 g/d of fiber. Standard diet advice was given by a dietitian and/or specialist physician. Individuals were also encouraged to maintain their usual physical activity.

Treatment

Patients were randomized to follow a standardized diet or a standardized diet plus Olife® for 3 months, in an open label, randomized, controlled study design. Olife® was self-administered, once a day, 35 ml/die (orange flavored sachets, each box containing 30 sachets), during breakfast in addition to diet. Olife® was marketed in Italy by Evergreen Life Products [San Giovanni al Natisone (Udine), Italy] as a food supplement based on an infusion of olive leaves and marigold. In particular, the beverage contains: concentrated aqueous extract of olive leaves and marigold flowers (Olivum®) 93%, Fructose, Glycerin as stabilizer, Potassium sorbate as preservative, Citric acid as acidifier, natural flavours (Table 1).

Table 1. Olife® GEL composition

Components	%
Aqueous extract of olive leaves and marigold (Olea europeae L., Calendula officinalis L.)	80-95
Fructose	10-20
Glicerine	1-2
Xanthan gum	0.5-1
Potassium sorbate	0.1-0.2
Natural orange aroma	0.2–0.3
Natural mandarin aroma	0.2-0.3
Elderberry alcohol	0.1-0.2
Caramel	0.4-0.5
Vanilla aroma	0.05-0.1

Randomization was done using a drawing of envelopes containing randomization codes prepared by a statistician. Medication compliance was assessed by counting the number of boxes containing the sachets returned at the time of specified clinic visits. Throughout the study, we will instruct patients to take their first dose of new medication on the day after they were given the study medication. At the same time, all unused medication will be retrieved for inventory. All medications will be provided free of charge (Figure 2).

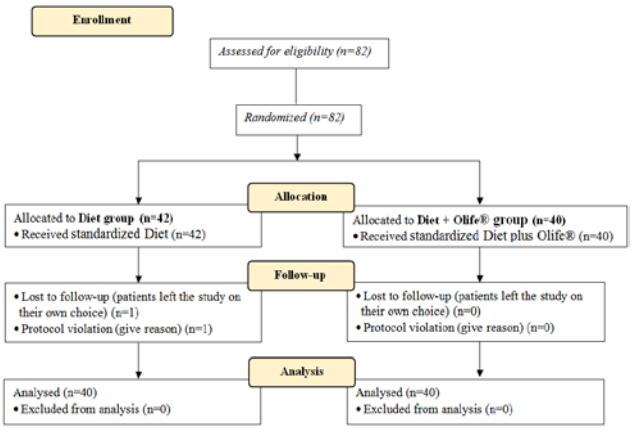

Figure 2. Flow Chart of the study

Assessments

Before starting the study, all patients underwent an initial screening assessment that include a medical history, physical examination, vital signs (blood pressure and heart rate), a 12-lead electrocardiogram, measurements of height and body weight, calculation of BMI, abdominal circumference (Abd. Cir.), waist circumference (Waist Cir.), and hip circumference (Hip Cir.), fasting plasma glucose (FPG), total cholesterol (TC), low density lipoprotein-cholesterol (LDL-C), high density lipoprotein-cholesterol (HDL-C), triglycerides (Tg), transaminases (AST and ALT), gamma-glutamyltransferase (γ-GT), creatinine (Cr), high-sensitivity C-reactive protein (Hs-CRP), adiponectin (ADN), and tumor necrosis factor–α (TNF-α).

All parameters were assessed at baseline, and after 3 months since the study start.

All parameters were determined in fasting state, after a 12-h overnight fast, in the plasma. Venous blood samples were taken for all patients between 8 and 9 a.m. and were drawn from an antecubital vein with a 19-gauge needle without venous stasis.

We used plasma obtained by addition of Na_2-EDTA, 1 mg/ml, and centrifuged at 3000 g for 15 minutes at 4°C. Immediately after centrifugation, the plasma samples were frozen and stored at -80°C for no more than 3 months. All measurements were performed in a central laboratory.

BMI was calculated by the investigators as weight in kilograms divided by the square of height in meters. Plasma glucose was assayed using a glucose-oxidase method (GOD/PAP, Roche Diagnostics, Mannheim, Germany) with intra- and inter-assay coefficients of variation (CsV) <2% [24].

Total cholesterol and Tg levels were determined using fully enzymatic techniques [25,26] on a clinical chemistry analyzer (HITACHI 737; Hitachi, Tokyo, Japan); intra- and inter-assay CsV were 1.0 and 2.1 for TC measurement, and 0.9 and 2.4 for Tg measurement, respectively. High density lipoprotein-cholesterol level was measured after precipitation of plasma apo B-containing lipoproteins with phosphotungstic acid [27] intra- and inter-assay CsV were 1.0 and 1.9, respectively; LDL-C level was calculated by the Friedewald formula [28].

Transaminases, Cr, and γ-GT were evaluated in central laboratory according to standard methods. High sensitivity C-reactive protein was measured with use of latex-enhanced immunonephelometric assays on a BN II analyser (Dade Behring, Newark, Delaware, USA). The intra- and inter-assay CsV were 5.7% and 1.3%, respectively [29]. Adiponectin level was determined using ELISA kits (B-bridge International, Sunnyvale, CA). Intra-assay CsV were 3.6% for low- and 3.3% for high-control samples, whereas inter-assay CsV were 3.2% for low- and 7.3% for high-control samples, respectively [30]. Tumor necrosis factor–α level was assessed using commercially available ELISA kits according to manufacturer's instructions (Titer-Zyme EIA kit; Assay Designs, Ann Arbor, MI). Intra-assay CsV were 4.5% for low- and 3.6% for high-concentration samples whereas the inter-assay CsV were 6.0% for low and 11.8% for high-concentration samples, respectively [31].

Hepatic echography

Each patient underwent abdominal ultrasonography using a 3.0 MHz curved array transducer and a standard Acuson Sequoia 512 system (Acuson, Mountain View, California, USA). Grading of severity of fatty liver disease in patients with NAFLD was determined as described previously [32,33]. Level 0 was defined as a normal hepatic echo pattern, level 1 as a slight increase in echo pattern with normal visualization of vessels and diaphragm, level 2 as a moderate increase in echogenicity

with reduced visibility of portal veins and diaphragm, and level 3 as a pronounced increase in hepatic echo pattern with poor visibility of intrahepatic vessels and posterior right lobe of the liver.

Safety measurements

Treatment tolerability was assessed at each study visit using an accurate interview of patients by the investigators, and comparisons of clinical and laboratory values with baseline levels. Safety monitoring included physical examination, vital sign assessment, weight, adverse events, and laboratory tests. All adverse events were recorded.

Statistical Analysis

Patients were included in the tolerability analysis if they received ≥ 1 dose of trial medication after randomization and underwent a subsequent tolerability observation. Continuous variables were tested using a two-way repeated measures analysis of variance (ANOVA). Intervention effects were adjusted for additional potential confounders using analysis of covariance. Analysis of variance was also used to assess the significance within and between groups. Statistical analysis of data was performed using the Statistical Package for Social Sciences software version 14.0 (SPSS Inc., Chicago, Illinois, USA). Data were presented as mean (SD). For all statistical analyses, $p < 0.05$ were considered statistically significant [34].

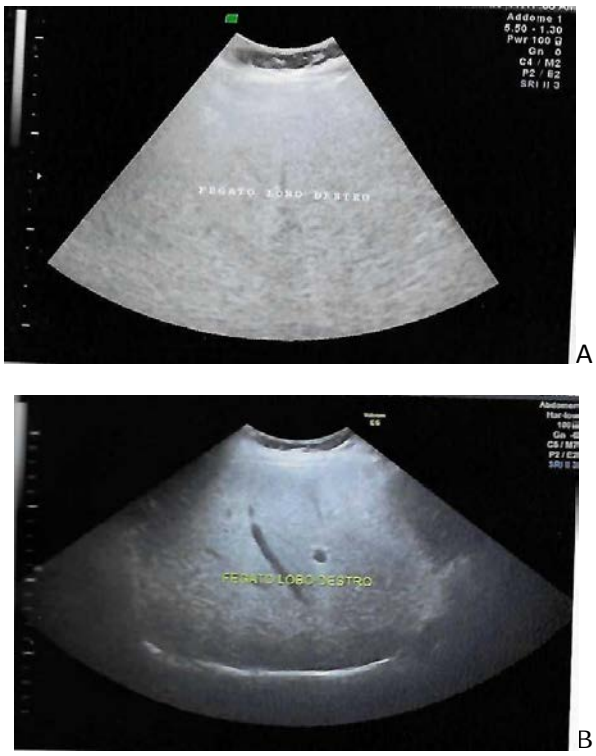

Figure 3. A) Ultrasound image of the liver before Olife® treatment; B) Ultrasound image of the liver after Olife® treatment. In Figure 2A, there is an increased echogenicity of the liver, a classic sonographic finding of hepatic steatosis. In Figure 2B, ultrasound image shows normal echogenicity of the liver parenchyma

3. Results

Study sample

Eighty-two patients began the study and 80 patients completed the study. Forty-two (51.2%) were randomized to Diet and 40 (48.8%) to Diet + Olife®. There were 2 patients (1 male and 1 female) who did not complete the study and the reasons for premature withdrawal included lost to follow-up, and protocol violation in Diet group after 3 months. The characteristics of the study and the division into the categories found at study entry are shown in Table 4.

Table 4. Grade of fatty liver during Olife® treatment at Baseline and after 3 months

	Diet		Diet + Olife®	
	Baseline	3 months	Baseline	3 months
N	42	40	40	40
Grade 0, n (%)	0	0	0	4 (10)**§
Grade 1, n (%)	28 (66.7)	28 (70.0)	23 (57.5)	22 (55.0)§
Grade 2, n (%)	8 (19.0)	8 (20.0)	10 (25.0)	8 (20.0)*
Grade 3, n (%)	6 (14.3)	4 (10.0)	7 (17.5)	6 (15.0)

Data are expressed as number or percentage
*p < 0.05 vs Baseline; **p < 0.01 vs Baseline; §p < 0.01 vs Diet

3 months Diet + Olife®
1 pt from Grade 3 to Grade 2
3 pts from Grade 2 to Grade 1
4 pts from Grade 1 to Grade 0

Anthropometric parameters

We did not observe any change in BMI or circumferences with neither treatment (Table 2).

Glycemic parameter

Glycemia did not change during the study in both groups (Table 2).

Lipid profile

Total cholesterol, LDL-C, HDL-C, and Tg did not modify during the 3 months of observation, was observed in both groups (Table 2).

Inflammation parameter

A statistically significant Hs-CRP reduction ($p < 0.05$ vs Baseline) was obtained in patients receiving Diet + Olife® compared to the Diet group. This significance ($p < 0.05$ vs Diet) remained even against the group treated with Diet alone (Table 2).

Cytokines

Adiponectin was significantly increased in the Diet + Olife® group compared to the Baseline value ($p < 0.05$) and this value was also significant compared to the group treated with Diet ($p < 0.05$). Tumor necrosis factor–α was also significantly decreased ($p < 0.05$) compared to Baseline in the group treated with Diet + Olife® and also remained significantly reduced ($p < 0.05$) compared to the group treated with Diet (Table 2).

Hepatic values

Transaminases were reduced at the end of the study. Aspartate aminotransferase and ALT were significantly reduced versus Baseline ($p < 0.05$) and versus Diet ($p < 0.05$), respectively. Even the γ-GT was significantly decreased in Diet + Olife® group ($p < 0.05$ vs Baseline and $p < 0.05$ vs Diet) compared to Diet group. Furthermore, AST/ALT ratio was significantly lower in the Diet + Olife® group ($p < 0.05$ vs Baseline and $p < 0.05$ vs Diet) compared to Diet group (Table 3).

We observed a significant decrease in HIS after 3 months of Diet + Olife® treatment ($p < 0.05$ vs Baseline and $p < 0.05$ vs Diet) compared to Diet group (Table 3). All patients reported an improvement or the disappearance

of hepatic steatosis (Table 4) demonstrated by the hepatic echography of the patients subjected to the control after 3 months of Diet + Olife® treatment (Figure 3 A and B). In fact, in Figure 2, we testify that a patient who had hepatic steatosis, also demonstrated by liver ultrasound, was enrolled and took Olife® for 3 months. This ultrasound was done with the same ultrasound machine. At the end of the study the ultrasound was repeated and the performer was the same and who declared the disappearance of the hepatic steatosis.

Table 2. Olife® treatment at Baseline and after 3 months

	Diet		Diet + Olife®	
	Baseline	3 months	Baseline	3 months
N	42	40	40	40
sex (M/F)	22/20	21/19	19/21	19/21
Age (years)	54.2 ± 6.3	-	55.1 ± 6.9	-
Smoking status (M/F)	6/5	5/5	6/6	6/6
Height (m)	1.69 ± 0.07	-	1.68 ± 0.06	-
Weight (Kg)	82.4 ± 9.2	81.2 ± 8.7	80.9 ± 8.3	79.4 ± 7.9
BMI (Kg/m^2)	28.9 ± 0.8	28.4 ± 0.5	28.7 ± 0.6	28.1 ± 0.4
Abd. Cir. (cm)	95.9 ± 3.4	95.4 ± 3.3	95.3 ± 3.2	94.3 ± 3.1
Waist Cir. (cm)	90.3 ± 3.0	89.6 ± 2.8	90.7 ± 3.2	89.8 ± 2.9
Hip Cir. (cm)	101.6 ± 4.3	101.3 ± 4.0	100.8 ± 3.8	100.4 ± 3.7
FPG (mg/dl)	89 ± 7	90 ± 8	88 ± 6	87 ± 5
TC (mg/dl)	209 ± 20	211 ± 22	215 ± 24	208 ± 19
LDL-C (mg/dl)	139 ± 15	142 ± 17	145 ± 18	139 ± 15
HDL-C (mg/dl)	46 ± 9	46 ± 9	45 ± 8	47 ± 11
Tg (mg/dl)	142 ± 36	135 ± 32	146 ± 39	131 ± 30
Hs-CRP (mg/l)	1.3 ± 0.8	1.3 ± 0.8	1.4 ± 0.9	1.1 ± 0.5*^
ADN (μg/ml)	7.9 ± 1.5	8.1 ± 1.7	7.8 ± 1.6	8.4 ± 1.9*^
TNF-α (pg/ml)	1.9 ± 0.5	1.8 ± 0.4	2.1 ± 0.7	1.7 ± 0.3*^

Data are expressed as mean ± standard deviations (SD)
*p < 0.05 vs Baseline; ^p < 0.05 vs Diet
Abd. Cir.: abdominal circumference; Waist Cir.: waist circumference; Hip Cir.: hip circumference; BMI: body mass index; FPG: fasting plasma glucose; TC: total cholesterol; LDL-C: low density lipoprotein-cholesterol; HDL-C: high density lipoprotein-cholesterol; Tg: triglycerides; AST: alanine aminotransferase; Hs-CRP: high-sensitivity C-reactive protein; ADN: adiponectin; TNF-α: tumor necrosis factor-α.

Table 3. Hepatic values during Olife® treatment at Baseline and after 3 months

	Diet		Diet + Olife®	
	Baseline	3 months	Baseline	3 months
N	42	40	40	40
AST (IU/l)	54 ± 8	52 ± 6	55 ± 11	42 ± 6*^
ALT (IU/l)	61 ± 13	60 ± 12	63 ± 15	53 ± 12*^
AST/ALT	0.89 ± 0.05	0.87 ± 0.04	0.87 ± 0.04	0.79 ± 0.03*^
γ-GT (IU/l)	39 ± 8	38 ± 7	38 ± 7	31 ± 5*^
HIS	33 ± 3	34 ± 4	35 ± 3	33 ± 2*^

Data are expressed as mean ± standard deviations (SD)
*p < 0.05 vs Baseline; ^p < 0.05 vs Diet
AST: alanine aminotransferase; AST: aspartate aminotransferase; γ-GT: gamma-glutamyltransferase; HIS: hepatic steatosis index.

4. Discussion

Olive oil is likely able to reduce hepatocyte fat deposition independently from metabolic routs, possibly through increased fatty acid oxidation. A part of the metabolic and cytoprotective effects of olive oil is suggested to be exerted by hydroxytyrosol, which is the most abundant phenolic compound in olive oil [35].

Phenols detectable in a virgin olive oil can be grouped as: simple phenols (i.e. tyrosol and hydroxytyrosol), secoiridoids (i.e. oleuropein, oleacein, and oleocanthal), and lignans [36]. Phenols influence oil stability and flavor. Oleuropein derivatives, that is, the dialdehydic form of decarboxymethyl elenolic acid linked to hydroxytyrosol and the isomer of oleuropein aglycone are the main responsible of the resistance to oxidation of a virgin olive oil [37]. These two compounds are secoiridoids linked to elenoic acid or its derivatives. Hydroxytyrosol and secoiridoids were found to have the most effective antioxidant activity between the natural compounds [38].

This is not the first time a study has been carried out with Olife®. Our previous experience [19] had shown that 74 patients with impaired fasting glucose (IFG) treated with Olife® plus diet had a significant decrease of FPG, post-prandial plasma glucose (PPG), HOMA index, oxidatively modified LDL (LDLox), and Hs-CRP levels after 3 months compared to baseline and to diet group, respectively. Moreover, 21 of 74 patients returned to a condition of euglycemia. This study showed that all patients receiving the Olife® added to Diet had an improvement in NAFLD and also the disappearance of the disease in some cases. NAFLD is characterized by the deposition of lipids in hepatocytes and is considered the hepatic manifestation of metabolic syndrome [39]. Our results demonstrated a significant reduction of hepatic parameters as AST (-15 IU/l, -27.3% vs -2 IU/l, -3.7%), ALT (-10 IU/l, -15.9% vs -1 IU/l, -1.6%), AST/ALT (-0.08 IU/l, -9.2% vs -0.02 IU/l, -2.2%), γ-GT (-7 IU/l, -18.4% vs -1 IU/l, -2.6%), and HIS (-2, -5.7% vs 1, -3.0%) in Diet + Olife® compared to Diet group, respectively.

Rezaei S et al. conducted a randomized, double-blind, clinical trial on 66 patients with NAFLD. Patients were divided to receive either olive or sunflower oil, each 20 g/d for 12 wk. A hypocaloric diet (-500 kcal/day) was recommended to all participants. They found normal and grades 1, 2, and 3 cases of fatty liver were 0%, 28.1%, 62.5%, and 9.4% at baseline in the olive oil group and 12.5%, 68.8%, 18.8%, and 0% at the end of the intervention, respectively [15]. An intervention study carried out by Nigam P et al. included 93 males with NAFLD followed for 6 months, matched for age and BMI. Patients who were randomized to the olive oil group (n = 30) had a significant post-intervention reduction of the grading of fatty liver (grade I, from 73.3% to 23.3% and from 60.5% to 20%, respectively; grade II, from 20% to 10% and from 33.4% to 3.3%, respectively; and grade III, from 6.7% to none and from 6.1% to none, respectively) [17].

Shidfar F et al. evaluated 50 patients with NAFLD in a clinical trial and these patients were randomly assigned to the olive oil group (receiving the equivalent of 20% of their total daily energy requirement from olive oil) or the control group (with normal consumption of oil) for 12 weeks. At the end of the study, a significant decrease was seen in the ALT and AST levels in the olive oil group, compared to the control group. Moreover, there was a significant difference in both enzymes between the two groups at the end of the study. Although the intragroup liver fat assessment revealed an improvement in both

groups (more in the olive oil), there was no significant difference in steatosis between the two groups at the end of the study [16].

In our study, we observed not only the downgrade of fatty liver in patients with grade 2 and 3 progressing vs grade 1, but also the appearance of 4 patients with a grade 0 of fatty liver in Diet + Olife® vs no patients in Diet group.

In the literature, there are currently no clinical studies on adults on substances taken individually such as elenolic acid, rutin, hydroxytyrosol and tyrosol in NAFLD disease.

Oleuropein has long been reported to exhibit several pharmacological benefits. Several studies support the antioxidant, antinflammatory, antiatherogenic, cardioprotective, antihypertensive, hypoglycemic, antimicrobial, antiviral, cytostatic, and endocrinal activities of oleuropein, in vitro and in vivo [40,41,42].

There are few studies that have evaluated oleuropein in NAFLD and these are in animals. One of these studies demonstrated oleuropein was able to improve the pro-inflammatory and antioxidant defense status in a murine model of NAFLD [43]. Moreover, oral administration of oleuropein in C57BL/6J mice, fed with an unhealthy diet, induced activation of autophagy characterized through AMPK-dependent phosphorylation of ULK1 at Ser555, regardless of the sex [44]. Oleuropein administration has an hepatoprotective and therapeutic effects on carbon tetrachloride-induced liver damage in mice [45]. Furthermore, a diet supplemented with oleuropein reduces induced hepatic steatosis [46] and progression to non-alcoholic steatohepatitis (NASH) [47] in mice fed with a high fat diet. Another study confirmed that oleuropein supplementation is capable to diminish lipid accumulation in the liver of mice fed with a high fat diet [48].

Certainly, our study has some limitations. Firstly, the observation time is short and therefore, we cannot draw definitive conclusions. Then, the comparison, although the study was randomized, did not include a placebo, but Olife® was added to the diet, although this had been standardized for both groups. Further studies will be needed to definitively confirm the action of oleuropein and its association, proposed here in the study, on NAFLD.

Conclusions

Olife® added to Diet ameliorates hepatic parameters and echography grading, and manages to reduce inflammatory parameters during the 3 months of study.

Statement of competing interests

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties. No writing assistance was utilized in the production of this manuscript.

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