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Olea Europaea Effects on Glyco-Metabolic Parameters and on Glycemic Status in Patients with Impaired Fasting Glucose

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Abstract **Aim**: the aim of this study was to evaluate the effects of a food supplement based on an infusion of olive leaves and marigold (Olife®) on glyco-metabolic parameters and glycemic status in patients with impaired fasting glucose (IFG). **Material and methods**: 148 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, 70 ml/die (about half water glass), during breakfast in addition to diet. **Results**: in the group treated with Olife® plus diet there was a significant decrease of fasting and post-prandial plasma glucose after 3 months, both compared to baseline, and to diet group. There was a significant decrease of HOMA index in the group treated with Olife® plus diet, both compared to baseline and to diet. At baseline, all patients were affected by IFG. At the end of the study, 21 of 74 patients (28.3 %) returned to a condition of euglycemia according to OGTT in the Olife® plus diet vs 3 of 74 patients (4.1 %) in the diet group. The group treated with Olife® plus diet had an improvement of LDLox and Hs-CRP levels after 3 months, both compared to baseline, and to diet. **Conclusions:** the study showed the effects of a food supplement based on an infusion of olive leaves and marigold (Olife®) on glyco-metabolic parameters and glycemic status in patients with IFG.

Keywords: *olea europaea, dysglycemia, impaired fasting glucose, lipid profile, oxidatively modified LDL*

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1. Introduction

The olive tree (Olea europaea L.) belongs to the Oleaceae family and Olea genus, and it has been used since ancient times for food. Olives, its fruits, are used for the extraction of olive oil and, to a lesser extent, for direct use in food. Because of the bitter taste of freshly picked olives, due to the polyphenol content, their use as fruits in the diet requires, however, specific treatments performed with various methods aimed at olives debittering and reduction of bitter principles. Also the olive pomace, a byproduct of the olive oil extraction process made up of husks, pulp residues and pits, deserves a mention. The pomace is recycled in the agrifood industry for the extraction of an edible oil thanks to the lipid fraction contained in the olive seeds. The pomace, however, is not to be considered only a waste product, being an interesting source of phenolic compounds, in particular oleuropein and ligstroside derivatives, flavonoids, phenolic acids and lignans with a powerful anti-oxidant activity [1]. The olive tree is rich in polyphenols, and more than 8000 structures are known, including several hundred isolated from edible plants with different biological actions. Olive oil is mainly composed of triacylglycerols (TGA) (98-99%), a diversified group of glycerol esters with different fatty acids. The predominant one present in the TGA of olive oil is monounsaturated oleic acid (up to 83%). There are also palmitic, linoleic, stearic and palmitoleic acid which make up the rest of the TGA of olive oil. There are also several lipophilic or amphiphilic microconstituents in virgin olive oil, including phytosterols, squalene, tocopherols, phenolic compounds and terpenic acid derivatives. Phenolic compounds, in turn, are present as phenolic acids or alcohols, oleuropein derivatives, lignans and flavonoids [2,3]. Polyphenols appear to reduce morbidity and/or slow down the progression of cardiovascular, neurodegenerative and cancer diseases. The mechanism of action of polyphenols is strongly related to their antioxidant activity. It is known that polyphenols reduce the level of reactive oxygen species in the human body. In addition, the health properties of vegetable polyphenols comprise anti-inflammatory, anti-

allergic, anti-atherogenic, anti-thrombotic and anti-mutagenic actions [4]. There is also evidence about their ability to modulate the human immune system by affecting the proliferation and activity of white blood cells, as well as the production of cytokines or other factors that participate in immunological defense [5]. Oleuropein, on the other hand, belongs to the secoiridoids, a group of coumarin derivatives [6]. It has been shown to be effective against various strains of bacteria, viruses, fungi, molds and parasites. It also inhibits platelet aggregation [7]. Oral treatment with oleuropein results in a decreased number of blood vessels demonstrating strong anti-angiogenic properties [8]. Phenolic compounds (oleuropein, protocatechuic acid) of virgin olive oil have also been shown to inhibit the oxidation of low-density lipoprotein (LDL) mediated by macrophages [9]. Olive leaf and fruit extracts containing oleuropein protect the insulin-producing cell line (INS-1) against the deleterious effects of cytokines [10].

On this basis, the aim of this study was to evaluate the effects of a food supplement based on an infusion of olive leaves and marigold (Olife®) on glyco-metabolic parameters and glycemic status in patients with impaired fasting glucose (IFG).

2. Materials and Methods

2.1. Study Design

This 3-months, open label, randomized, controlled, clinical trial was conducted at the Centre of Diabetes and Metabolic Diseases, Department of Internal Medicine and Therapeutics, University of Pavia and Fondazione IRCCS Policlinico San Matteo, PAVIA, Italy.

The study protocol was approved by institutional review board and was conducted in accordance with the 1994 Declaration of Helsinki [11], 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.

2.2. Patients

We enrolled adult patients with IFG diagnosed after an oral glucose tolerance test (OGTT), not taking hypoglycemic agents (both pharmaceuticals or nutraceutical agents). 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 had type 1 or type 2 diabetes mellitus or impaired glucose tolerance (IGT), impaired hepatic function (defined as plasma aminotransferase and/or gamma-glutamil transpeptidase (γ-GT) level higher than the three times the upper limit of normal [ULN] for age and sex), impaired renal function (defined as serum creatinine level higher than the ULN for age and sex), or gastrointestinal disorders; current or previous evidence of ischemic heart disease, heart failure, or stroke; weight change of > 3 Kg during the preceding 3 months; malignancy, and significant neurological or psychiatric disturbances, including alcohol or drug abuse. Excluded medications (within the previous 3 months) included hypoglycemic agents, laxatives, β-agonists (other than inhalers), 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.

2.3. Treatments

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, 70 ml/die (about half water glass), 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: 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® composition

Components	%
Aqueous extract of olive leaves (Olea europaea L.)	80-95
Aqueous extract of marigold (Calendula officinalis)	5-15
Fructose	5-15
Citric acid	0.1-0.2
Glicerine	0.1-0.9
Natural flavours	0.2-0.5
Potassium sorbate	0.02

Randomisation was done using a drawing of envelopes containing randomisation codes prepared by a statistician. Medication compliance was assessed by counting the number of bottles returned at the time of specified clinic visits. Throughout the study, we instructed 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 was retrieved for inventory. All medications were provided free of charge.

2.4. Diet and Exercise

Patients began a controlled-energy diet (near 600 Kcal daily deficit) based on American Heart Association (AHA) recommendations [12] that included 50% of calories from carbohydrates, 30% from fat (6% saturated), and 20% from proteins, with a maximum cholesterol content of 300 mg/day and 35 g/day of fiber. Patients were not treated with vitamins or mineral preparations during the study.

Standard diet advice was given by a dietician and/or specialist doctor. Dietician and/or specialist doctor periodically provided instructions on dietary intake recording as part of a behaviour modification program and then later used the subject's food diaries for counselling. Individuals were also encouraged to increase their physical activity by walking briskly for 20 to 30 minutes, 3 to 5 times per week, or by cycling. The recommended changes in physical activity throughout the study were not assessed.

2.5. Assessments

Before starting the study, all patients underwent an initial screening assessment that included a medical history, physical examination, vital signs (blood pressure and heart rate), a 12-lead electrocardiogram, measurements of height and body weight, calculation of body mass index (BMI), assessment of fasting plasma glucose (FPG), post-prandial plasma glucose (PPG), glycated hemoglobin (HbA_{1c}), fasting plasma insulin (FPI), HOMA index, total cholesterol (TC), low density lipoprotein-cholesterol (LDL-C), high density lipoprotein-cholesterol (HDL-C), triglycerides (Tg), oxidatively modified LDL (LDLox), aspartate aminotransferase (AST), alanine aminotransferase (ALT), high-sensitivity C-reactive protein (Hs-CRP).

All parameters were assessed at baseline and after 3 months since the study start. Moreover, at baseline, and after 3 months, patients underwent an OGTT.

All plasmatic variables were determined after a 12-hour overnight fast, with the exception of PPG. Venous blood samples were drawn by a research nurse for all patients between 8:00 am and 9:00 am. We used plasma obtained by addition of Na2-EDTA, 1 mg/mL, and centrifuged at 3000g for 15 minutes at 4°C. Immediately after centrifugation, the plasma samples were frozen and stored at -80°C for ≤3 months. Laboratory technicians drew blood samples and the biologist responsible for the laboratory performed the assays. All measurements were performed in a central laboratory.

Body mass index 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 interassay coefficients of variation (CsV) <2% [13].

Plasma insulin was assayed with Phadiaseph insulin radio immuno assay (RIA) (Pharmacia, Uppsala, Sweden) by using a second antibody to separate the free and antibody-bound 125 I-insulin (intra- and interassay CsV 4.6 and 7.3%, respectively) [14].

The HOMA-IR index was calculated as the product of basal glucose (mmol/l) and insulin levels (μU/ml) divided by 22.5 [15,16].

Total cholesterol and Tg levels were determined using fully enzymatic techniques [17,18] on a clinical chemistry analyzer (Hitachi 737; Hitachi, Tokyo, Japan); intra- and interassay CsV were 1.0% and 2.1% for TC measurement, and 0.9% and 2.4% for Tg measurement, respectively. HDL-C level was measured after precipitation of plasma apo B-containing lipoproteins with phosphotungstic acid [19]; intra- and interassay CsV were 1.0% and 1.9%, respectively. LDL-C level was calculated using the Friedewald formula [20].

The LDLox dosage was obtained using a standardized enzyme-linked immunosorbent assay kit (Mercodia, Uppsala, Sweden), including monoclonal antibodies against specific antigenic determinants on the oxidized apolipoprotein B100 (ApoB) [21].

Transaminases and creatinine were evaluated in central lab 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 interassay CsV were 5.7% and 1.3%, respectively [22].

2.6. 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, electrocardiogram, adverse events, and laboratory tests including liver and kidney function.

2.7. Oral Glucose Tolerance Test

All patients drank a glass of water (200 ml), in which 75g of glucose had been dissolved over a period of 5 min in the morning, between 8 and 9 a.m. after a 12-h fast, and after dietary assessment to ensure a carbohydrate intake > 150 g/day over the previous 3 days [23]. Normal physical activity was allowed over the previous 3 days. No smoking was allowed during the test. Blood samples were collected in EDTA-containing tubes (Becton Dickinson, Meylan Cedex, France) through a venous catheter from an antecubital vein immediately before and at 120 min after the glucose load for the measurement of the considered parameters of the study. On the basis of the results recorded 2 hours after the OGTT, we diagnosed patients as being affected by IFG defined by glycemia at 120 minutes from OGTT < 140 mg/dl.

2.8. Statistical Analysis

An intention-to-treat (ITT) analysis was conducted in patients who had received ≥1 dose of study medication and had a subsequent efficacy observation. Patients were included in the tolerability analysis if they had received ≥1 dose of trial medication after randomization and had undergone a subsequent tolerability observation. The statistical significance of the independent effects of treatments on the other variables was determined using ANCOVA. A 1-sample t test was used to compare values obtained before and after treatment administration; 2-sample t tests were used for between-group comparisons. Analysis of data was performed using the Statistical Package for Social Sciences software version 11.0 (SPSS Inc., Chicago, Illinois, USA) [24]. Data are presented as mean (SD). For all statistical analyses, $p < 0.05$ was considered statistically significant.

3. Results

3.1. Study Sample

The study sample included 148 patients with IFG: 74 were enrolled in the Olife® plus diet group, and 74 in the diet group. All patients completed the study.

Table 2. Study results

Parameters	Olife® + Diet			Diet		
	Baseline	1 month	3 months	Baseline	1 month	3 months
Patients (n)	74	74	74	74	74	74
M/F (n)	35/39	35/39	35/39	36/38	36/38	36/38
Height (m)	1.68±0.05	-	-	1.69 ± 0.06	-	-
Weight (Kg)	77.1±6.0	77.1±6.0	76.6±5.9	77.6±6.2	77.6±6.2	77.2±6.1
BMI (Kg/m^2)	27.3±1.5	27.3±1.5	27.1±1.4	27.2±1.4	27.2±1.4	27.0±1.3
WC (cm)	87.1±5.3	87.1±5.3	86.9±5.2	86.7±5.0	86.7±5.0	86.6±4.9
HC (cm)	89.6±5.7	89.6±5.7	89.3±5.5	89.2±5.3	89.2±5.3	89.1±5.2
AC (cm)	97.9±5.8	97.9±5.8	97.4±5.4	97.6±5.5	97.6±5.5	97.5±5.4
FPG (mg/dl)	107.3±6.1	106.4±5.9	101.3±5.4*^	106.9±6.0	104.8±5.7	103.9±5.5
PPG (mg/dl)	144.6±12.9	140.3±11.9	136.2±11.2*^	146.1±14.5	144.9±13.1	141.2±12.0
HbA_{1c} (%)	5.8±0.5	5.8±0.5	5.7±0.4	5.7±0.4	5.7±0.4	5.6±0.3
FPI (µU/ml)	9.8±7.2	9.7±7.0	9.4±6.8	10.3±7.5	10.1±7.4	9.9±7.1
Homa index	2.52±0.6	2.48±0.5	2.29±0.4*	2.64±0.9	2.54±0.7	2.47±0.6
TC (mg/dl)	210.3±14.2	209.1±14.0	203.4±13.2	213.2±14.6	211.5±14.4	209.6±14.1
LDL-C (mg/dl)	143.7±16.9	142.5±16.4	137.8±15.4	148.0±18.1	146.7±17.7	144.9±17.1
HDL-C (mg/dl)	44.3±6.4	44.9±6.5	45.6±6.9	43.9±6.0	44.0±6.1	44.3±6.2
Tg (mg/dl)	111.5±28.9	108.3±26.7	100.2±22.7	106.6±25.4	104.2±25.0	101.8±22.9
LDLox (ng/ml)	35.7±20.5	31.6±19.2	22.3±12.8*^	36.7±22.7	35.1±21.9	34.3±21.2
Hs-CRP (mg/l)	1.2±0.4	1.1±0.3	0.8±0.2*^	1.3±0.5	1.3±0.5	1.2±0.4
AST (UI/l)	27.2±9.3	27.1±9.2	26.8±8.8	27.0±9.1	26.9±9.0	26.5±8.8
ALT (UI/l)	24.3±8.5	24.2±8.3	24.1±8.1	25.9±8.9	25.5±8.7	25.0±8.6
Creatinine (mg/dl)	0.8±0.2	0.7±0.3	0.8±0.2	0.7±0.3	0.7±0.3	0.8±0.2

*p < 0.05 vs baseline; ^p < 0.05 vs diet.
M: males; F: females; BMI: body mass index; WC: waist circumference; HC: hip circumference; AC: abdominal circumference; FPG: fasting plasma glucose; PPG: postprandial glucose; HbA_{1c}: glycated hemoglobin; FPI: fasting plasma insulin; TC: total cholesterol; LDL-C: low density lipoprotein-cholesterol; HDL-C: high density lipoprotein-cholesterol; Tg: triglycerides; LDLox: oxidatively modified LDL; Hs-CRP: high sensitivity C-reactive protein, AST: aspartate aminotransferase; ALT: alanine aminotransferase.

3.2. Anthropometric Parameters

No variations of body weight or abdominal, waist or hip circumference were recorded in neither group (Table 2).

3.3. Metabolic Parameters

In the group treated with Olife® plus diet there was a significant decrease of FPG, and PPG after 3 months, both compared to baseline, and to diet group ($p < 0.05$ for both). No variation of HbA_{1c}, or FPI were recorded; however, there was a significant decrease of HOMA index in the group treated with Olife® plud diet, both compared to baseline and to diet ($p < 0.05$ for both) (Table 2). No differences between males and females were recorded.

3.4. OGTT Results and Glycemic Status

At baseline, all patients were affected by IFG. At the end of the study, 21 of 74 patients (28.3 %) returned to a condition of euglycemia according to OGTT in the Olife® plus diet vs 3 of 74 patients (4.1 %) in the diet group (Table 3, and Table 4). No differences between males and females were recorded.

3.5. Lipid Profile and Inflammatory Parameters

No variation of TC, Tg, LDL-C or HDL-C was observed in neither group; however, the group treated with Olife® plus diet had an improvement of LDLox and Hs-CRP levels after 3 months, both compared to baseline, and to diet (Table 2). No differences between males and females were recorded.

3.6. Safety and Treatment Acceptance

Renal and hepatic function did not change during the study (Table 2). Olife® was well accepted and tolerated by patients.

Table 3. OGTT results

	Olife® + Diet		Diet	
Time (minutes)	Glycemia (mg/dl)		Glycemia (mg/dl)	
	Baseline	3 months	Baseline	3 months
0	107.3 ± 6.1	101.3 ± 5.4	106.9 ± 6.0	103.9 ± 5.5
120	127.2 ± 8.3	122.8 ± 7.9	129.4 ± 9.1	124.2 ± 8.3

OGTT: oral glucose tolerance test.

Table 4. Glycemic status at the end of the study

	Olife® + Diet		Diet	
OGTT results	**Baseline**	**3 months**	**Baseline**	**3 months**
Patients (n)	74	74	74	74
IFG (n)	74	53	74	71
Euglycemia (n)	-	21	-	3

OGTT: oral glucose tolerance test; IFG: impaired fasting glucose.

4. Discussion

The current study showed beneficial effects of the food supplement on FPG, and PPG, with 21 of 74 patients returning to a condition of euglycemia. Even if the nutraceutical contain Oleuropein and marigold, the main role in obtaining this effect is surely Oleuropein: Oleuropein gains considerable attention for its positive roles in regulating diabetes and diabetes complications on the basis of preclinical and clinical research [25]. Marigold, instead, has been claimed to have emollient and soothing action, it has an action on the function of the oropharyngeal mucosa, it contrasts menstrual cycle disorders, it has an action of the digestive system, and on skin trophism. It is recommended individuals consume oleuropein, and oleuropein-enriched foods, such as olive fruit, olive oil, and olive leaf extracts, continuously and regularly, though the current intake guidance for oleuropein in humans is still lacking. Oleuropein has long been reported to exhibit several pharmacological benefits: several in vitro and in vivo studies support the anti-oxidant, anti-inflammatory, anti-atherogenic, cardioprotective, anti-hypertensive, hypoglycemic, anti-microbial, anti-viral, cytostatic, molluscicidal, and endocrinal activities of Oleuropein [26,27,28].

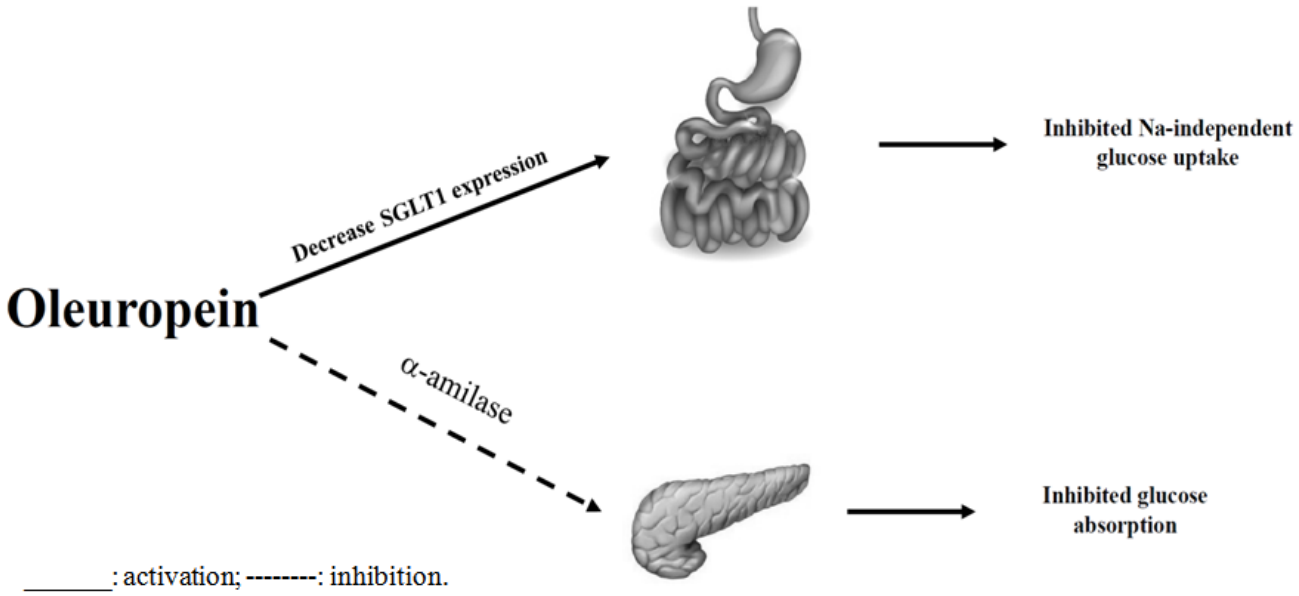

Figure 1. Hypoglycemic effects of olive leaves

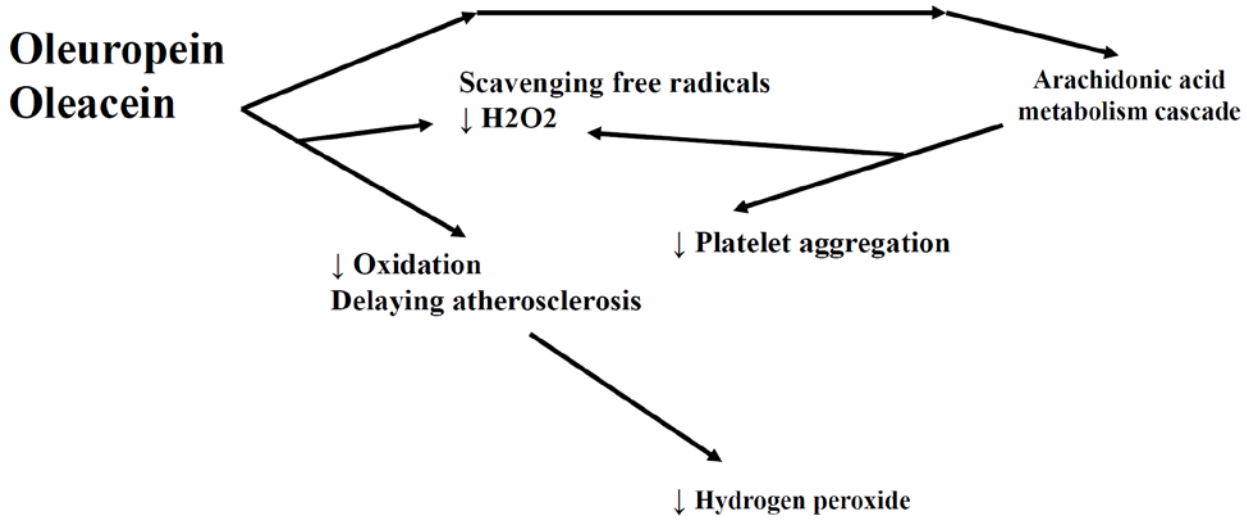

Figure 2. Anti-atherosclerotic and anti-oxidant effects of olive leaves

Regarding glycemia, Oleuropein may compete with glucose for the transport through the SGLT-1, due to its peculiar structure which include a glucose residue, Oleuropein decrease SGLT-1 expression, inhibiting Na-independent glucose uptake [29] (Figure 1). In healthy subjects the administration of 20 mg Oleuropein significantly increased the levels of glucagon like peptide (GLP-1) compared to placebo [30], contributing to attenuate PPG. Oleuropein also inhibits α-amilase expression, leading to an inhibition of glucose absorption in the bowel (Figure 1). Oleuropein also increase glucose-induced insulin release and increase peripheral uptake of glucose (Figure 1). Regarding anti-atherosclerotic and anti-oxidant activity, our study showed a decrease of Hs-CRP and LDLox, also in this case Oleuropein plays a pivotal role: Oleuropein reduces lipoxygenases, so decreasing reactive oxygen species (ROS) and oxidative damage (Figure 2). These actions lead to anti-atherosclerotic effect of olive leaves, scavenging free radicals reduces hydrogen peroxide. Our results are in line with data reported by Qabaha et al. [31], where they showed a downregulation of TNF-α secretion in polymorphonuclear cells culture in response to Oleuropein treatment. This shows that this polyphenol-rich extract has an anti-inflammatory effect, and Oleuropein is the major responsible for this effect [31].

5. Conclusions

The study showed the effects of a food supplement based on an infusion of olive leaves and marigold (Olife®) on glyco-metabolic parameters and glycemic status in patients with IFG.

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.

Contributions Section

Design and conduction of the study: Giuseppe Derosa, Pamela Maffioli; data collection: all Authors; data interpretation and manuscript writing: Giuseppe Derosa, Pamela Maffioli. All authors read and approved the final version of the manuscript.

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