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OLEUROPEINA NJË AGJENT I RI TERAPEUTIK NË PARANDALIMIN E DIABETIT TIPI 2. RAPORTIMI I NJË STUDIMI KLINIK

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Abstrakti

Në ditët e sotëshme diabeti tipit 2 jo insulino vartës është shndërruar në një problem global në mbarë botën. Në këtë crregullim metabolik kontrolli i homeostazës së glukozës është qëllimi kryesor si për parandalimin ashtu edhe për trajtimin e patologjisë. Udhëzimet shkencore po rekomandojnë produket natyrale për menazhimin e kësaj faze dhe komplikacioneve që sjell diabeti. Objektvi i këtij studimi ishte realizimi dhe testimi në 50 vullnetarë të rastësishëm, në fazën e prediabetit, me glicemi esëll 115 mg/dcl, i një formulimi në trajtë bustinash që treteshin në ujë, me bazë molekulën e oleuropeinës, një polifenol me veprim antioksidant, ekstaktuar nga gjethet e ullirit, *Olea Ouropea*. Pas administrimit për një periudhë 60 ditore, u vu re përmes testeve laboratorike një përmiresim në total i parametrave metabolik me 23% ± 0.2, glukozës esëll, e pas ngrënies si dhe rritje e ndjeshmërisë ndaj insulinës. Këto rezultate premtuese hedhin dritë për rëndësinë e antioxidantëve si dhe për zhvillimin e testimeve të mëtejshme.

Fjalët çelës: *diabeti tipit 2, produkte natyrale, oleuropein, antioksidant, insulinë rezistencë*

OLEUROPEIN AS A NOVEL ANTI-DIABETIC AGENT AGAINST TYPE2 DIABETES. A CASE STUDY WAS REPORTED

Abstract

Nowadays non-insulin dependent type 2 diabetes has become a global problem worldwide. In this metabolic disorder the control of glucose homeostasis is the main goal for both prevention and treatment of pathology. Scientific guidelines are recommending natural products for managing this stage and the complications that diabetes brings. The aim of this study was to perform and test in 50 random volunteers, in the prediabetes stage, with sober glycemia 115 mg / dcl, a formulation in the form of sacs that dissolved in water, based on the molecule oleuropein, a polyphenol with antioxidant action, extracted from olive leaves, Olea Ouropea. After administration for a period of 60 days, through laboratory tests was observed a total improvement of metabolic parameters by 23% ± 0.2, fasting glucose, postprandial glucose and increased insulin sensitivity. These promising results highlight the importance of antioxidants as well as the development of further research studies.

Key words: *type 2 diabetes, natural products, oleuropein, antioxidant, insulin resistance*

1. Introduction

Among the cardiometabolic disease, type 2 Diabetes Mellitus (T2DM) is greatly diffused in the World, affecting several million people. At the base of the T2DM a etiology there is a reduction in β-cells function and/or the presence of insulin-resistance (IR), causing chronically elevated glycemic levels [1-2]. The last decades the role of dietary active components become an important focus of research and have increased the awareness of consumers about diet and proper nutrition. Currently, a great interest has been focused on the valorization of agro-food byproducts, since these matrices could be a source of essential bioactive substance useful to the nutraceuticaland food supplements industry [3-4]. Supporting the perspective of food as medicine, the aim of this study is to provide an innovative alternative tool through the determination of various phytochemical compounds (e.g. carotenoids, polyphenols, terpenoids, abscisic acid) capable to prevent and/or to control the diabetes. For this purpose a randomized, clinical trials have been realized to verify the efficacy of novel nutraceutical formulations from: olive leaves (EOL), on glycaemic and insulinemic responses, in postprandial glycaemia (PPG), impaired fasting glucose (IFG), and impaired glucose tolerance (IGT) conditions [5]. Evidence available in scientific literature showed the beneficial effects of OLE in management of T2DM. [1]

1.1 *Oleuropein*

Oleuropein (OLE) is the most prevalent polyphenol present in olives leaves, the agro-industrial waste matrices studied in this research thesis.

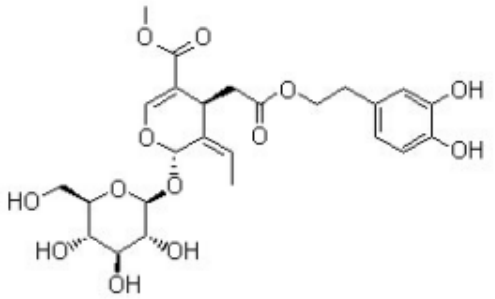

Figure 1. Chemical structure of oleuropein.

1. 1 *American Diabetes Association. 2017. Classification and diagnosis of diabetes. Diabetes Care.40. S11-S24.*

2. *Prabhakar, P.K., Doble, M., 2011. Mechanism of Action of Natural Products Used in the Treatment of Diabetes Mellitus. Chin. J. Integr. Med.17. 563–574. doi: 10.1007/s11655-011-0810-3*

3. *Rudra, S.G., Nishad, J, Jakhar, N., Kaur, C. 2015. Food industry waste: mine of nutraceuticals. Intl J Sci Environ Techno. 4. 205– 29.*

4. *Gordon M.H, , Paiva-Martins F., , Almeida M,. 2001. Antioxidant activity of hydroxytyrosol acetate compared with that of other olive oil polyphenols. Journal of Agricultural and Food Chemistry. 49. 2480-2485.*

5. *Gestuvo, M.K, Hung, W.W. Common dietary supplements for cognitivehealth. 2012. Aging health.8. 89–97.*

Few studies have been conducted to elucidate the mechanisms of action of OLE in management of T2DM as shown in Figure 2. One of the first proposed mechanisms is the inhibition of several enzymes involved in glucose metabolism. Oleuropein has been reported as a good

antioxidant *in vitro* through chelation of Cu^{2+} and Fe^{3+} metallic ions which then catalyse free radical formation. Studies *in vivo* indicated the inhibition of enzymatic oxidation e.g. lipoxygenases [6-8]. Besides this, interestingly in healthy subjects, the administration of 20 mg OLE significantly increased the levels of glucagon like peptide (GLP-1) compared to placebo, contributing to attenuate the postprandial glycaemic levels [9]2

Particularly, it acts through different mechanisms of action, as shoën in Figure 2, including:

a. Inhibition of digestive enzymes activities,

b. Inhibition of glucose transport,

c. Increase of the incretin release,

d. AMPK activation

e. antioxidant activity

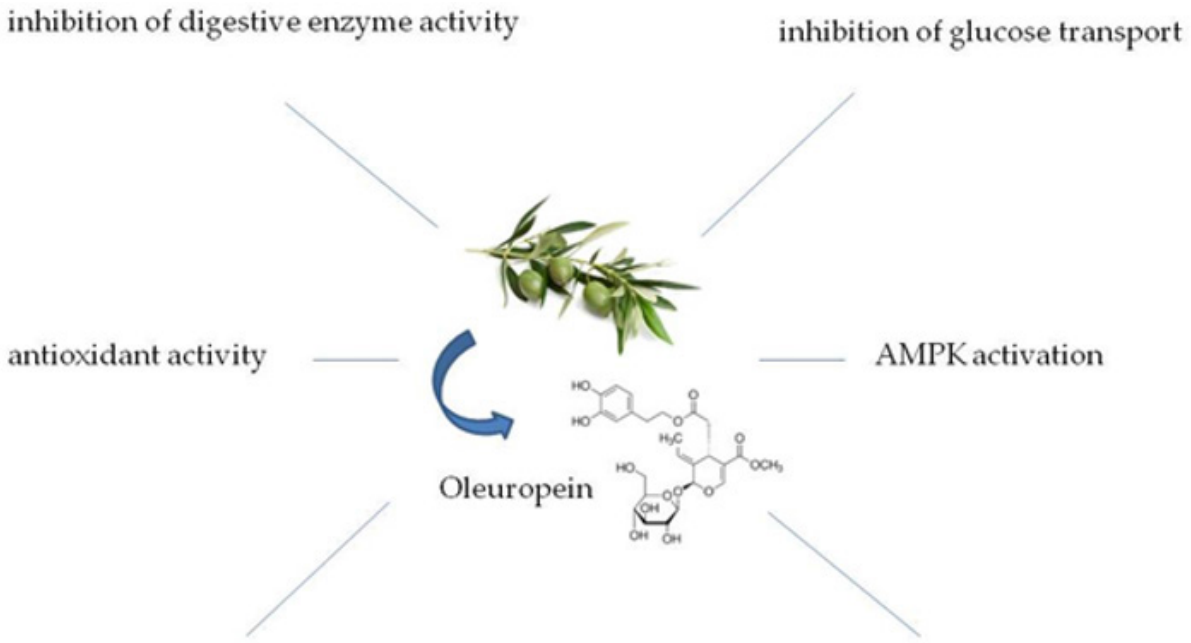

Figure 2. Health benefits of Oleuropeine

1. 2 *Santini, A., Cammarata, S.M., Capone, A., et al. 2018. Nutraceuticals: opening the debate for a regulatory framework. Br J Clin Pharmacol. 84. 659–672.*

2. Prabhakar, P.K., Doble, M., 2011. Mechanism of Action of Natural Products Used in the Treatment of Diabetes Mellitus. Chin. J. Integr. Med.17. 563–574. doi: 10.1007/s11655-011-0810-3

3. Leiherer A., Mündlein A., Drexel, H. 2012. Phytochemicals and their impact on adipose tissue inflammation and diabetes. VasculPharmacol. 58. 3-20.

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Methodology

2.1 Sample Collection

Olive leaves (cultivar Ravece) were provided in September 2018 by the Agriturismo Petrilli Company (Flumeri, Avellino, Italy). Samples were immersed in liquid nitrogen (N_2), and maintained at -80 °C until analysis. Then, samples were weighed and ground in liquid N_2.

Polyphenols were extracted in darkness, according to the procedure described byVioli et al.2017 with slight modifications. In particular, an aliquot (1.0 g) of olive leaves powder was extracted with 20 mL ethanol (70%), stirred well by piping for homogenization for one minute, then placed in the ultrasonic bath at (75 ± 2) °C, for 30 min, filtered with 0.45 μm filters and kept at -20 °C until use.

Oleuropein determination in olive leaves extracts was carried out as previously described Barclay et al 2019 [(10)] with minor modifications. A Kinetex® C18 column 100A (250 mm x 4.6 mm, i.d. 5 μm) (Phenomenex, Torrance, CA, United States) was used with the following gradient mobile phase program: A, 2.5% acetic acid; B, acetonitrile; flow rate, 1.0 mL/min. The temperature was set at 25 °C. The injection volume was 20 μL.The chromatograms were monitored at a wavelength of 254 nm, for the oleuropein determination.[3]

2.2 Study treatment

The reference glucose solutions and the treatment beverages all contained 75 g glucose. The treatment beverage (TB) was prepared mixing glucose solutions with the following samples: 400 mg *EOL* (60.0 mg oleuropein) → TB *EOL.*

2.3 Preliminary test in *vivo*

According to the safety of natural product in dietary supplements and novel foods, as indicated by the updated version (January 2015) of Regulation (EC) No. 258/1997 of the European Commission, a preliminary test *in vivo* to evaluate in glucose metabolism the efficacy of oleuropen as un water solubile nutraceuticals. Participants maintained their usual dietary and lifestyle patterns throughout the study. Both TB and reference glucose solutions were served into dark jars, in order to blind subjects and researchers of the study to the different colors of the solutions mixed with the nutraceutical tested. The study was conducted in accordance with the 1964 Helsinki Declaration (revised in 2000) and approved by the Scientific Ethics Committee of AO Rummo Hospital (Benevento, Italy) with protocol no. 28 of 15 May 2017.

2.4 Study procedures

Participants arrived at the research centre in the morning after 12 h of fasting. All blood samples were taken in the morning and immediately after measurement of heart rate and blood pressure.

Blood samples were collected from each participant before administration of the reference glucose solutions and the treatment beverages, in 3-mL EDTA-coated tubes (Becton–Dickinson, Plymouth, UK).

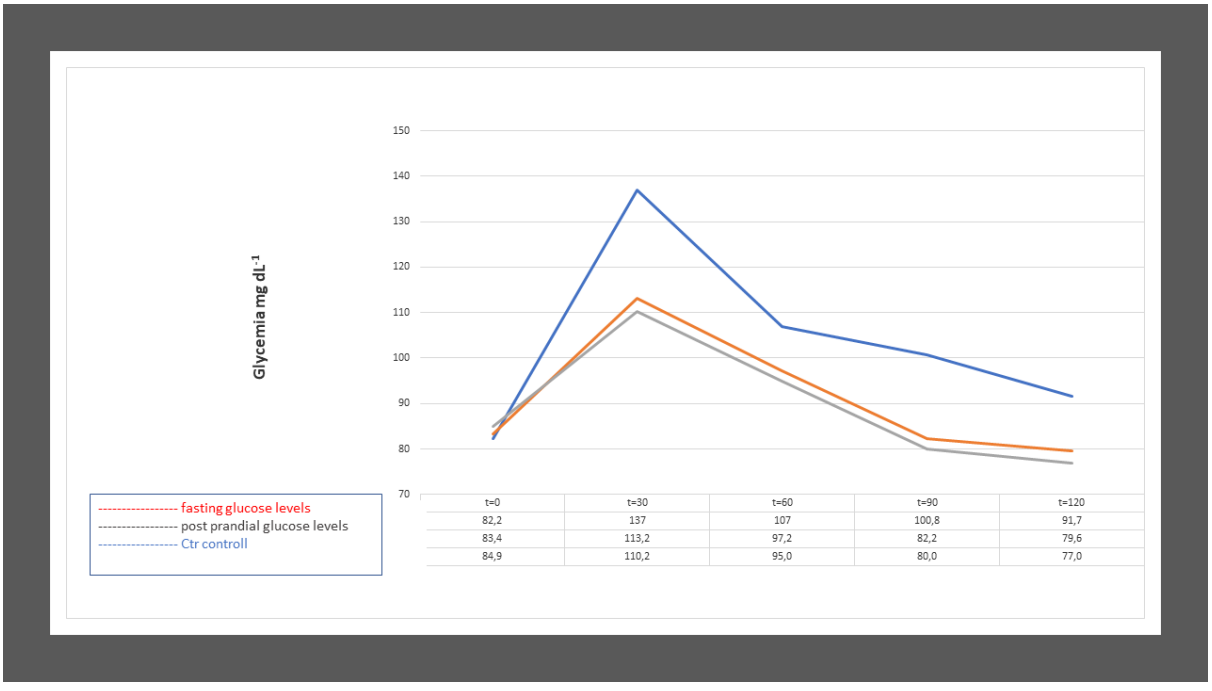
1. 3 *Fraga, C.G., Galleano, M., Verstraeten, S.V., Oteiza, I. 2010. Basic biochemical mechanisms behind the health benefits of polyphenols. Mol Aspects Med. 31. 435–450.*

Plasma was immediately isolated by centrifugation (20 min, 2.200 g, 4 °C). Additional fingerprick blood samples were collected at 30, 60, 90, 120 and 150 min after starting consumption of the reference glucose solutions and the TB. All seven test sessions for each participant were analysed within the same assay. All samples were stored at -80 °C until analysis. Plasma glucose levels were determined using commercially available kits from Diacron International (Grosseto, Italy). Analyses were performed on a Diacron International Free Carpe Diem. The assay sensitivity for the individual analytical determination was determined as follows: glucose, 4 mg/dL. Intra- and inter-day variations were 1.1 and 1.7% for glucose. Plasma insulin concentrations were measured using an enzyme linked immunosorbent (ELISA) assay commercial kit (InterMedicalsrl, Italy). The reference glucose solutions and the treatment beverages were consumed within 5 min.

3. Results

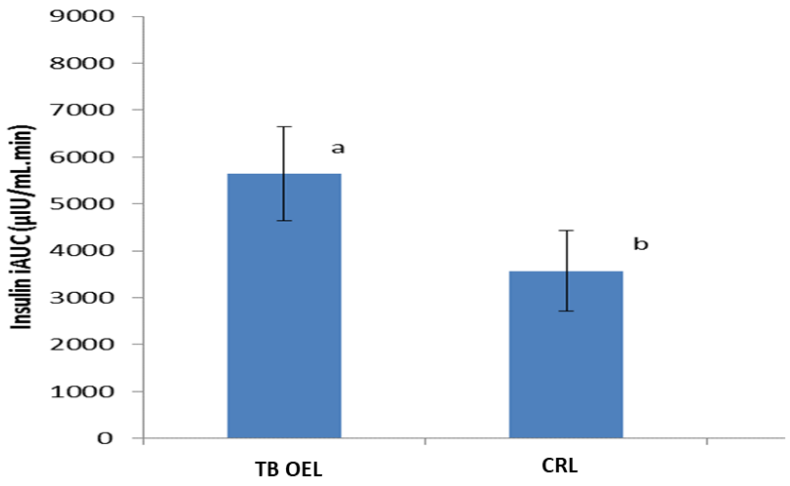
3.1 Glucose Plasmatic Levels

As a follow up of the above mentioned data, a preliminary assessment has been performed on the glycemic response an oral glucose tolerance test (OGTT) *in vivo* after the administration of the oleuropein extract. A glucose reference solution (75% wV^{-1}) was administered and the individual's responses were recorded at at t0, t30, t60, t90, t120. The result are expressed in Graphic 1 as it show bellow. A significant decrease in FPG (fasting plasma glucose), FPP (post prandial glucose) level, were observed in the oleuropein group compared to the baseline value of control group ($p < 0.05$ vs baseline).



3.2 Insuline sensitivity

The present study showed that ABA is effective in improving glyco-metabolic and inflammation parameters. The samples of TB OEL improved insulin sensitivity compared to the reference glucose solution in the treatment subjects. Insulin sensitivity value obtained after oleuropein treatment was higher respect to baseline ($p < 0.001$ vs baseline), as it show bellow in Graphic 2. The results are expressed as ± Standard deviation.



Graphic 2. Insuline sensitivity. TB OEL-Treatment Beverage of Oleuropeine

CRL- Controll samples

3.3 Tolerability of Treatment Beverages

The TB were palatable and well tolerated. No adverse effects were reported.

4. Conclusion

Diabetes is a world wide growing health issue, and nutraceuticals can be a challenge for the future in the area of prevention and/or therapy of this disease The use of natural approaches for the treatment of metabolic diseases, indeed, is currently the most discussed issue, involving public opinion, researcher and physician [(11)]. Among the main points in favor of the diffusion of natural remedies in the clinical practice, surely emerge the almost total absence of side effects, contraindications and/or drug-interactions, that make products safe. On the other hand, the expensive cost of some of them and the lack in documented efficacy represent some of the main reasons for the suspicion that people still have for these products. The major strengths of this studie reside in its originality and in the evaluation*in vitro* and *in vivo* of the beneficial effects of this novel nutraceuticals formulated using olive leaves. [4]

The positive results obtained can be useful to physicians about a novel treatment and or intervention which may represent a valuable support and or alternative in the clinical practice. The main limitations of our study which should be considered are: i) the small sample size of healthy participants with normal glucose tolerance and insulin sensitivity; ii) the short-term assessment for the treatment of a chronic condition which only allowed the investigation of acute postprandial effects of the nutraceutical formulations; iii) the lack of a dose assessment in order to define the range of minimum effective–maximum non-toxic concentrations of therapeutic interest.

1. 4 *Barclay, A.W., Liu, S., Wolever, T.M.S. et al. 2019.Dietary Glycemic Index and Load and the Risk of Type 2 Diabetes: A Systematic Review and Updated Meta-Analyses of Prospective Cohort Studies. Nutrients. 11. 1280, doi:10.3390/nu11061280.*

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