BIOLOGICAL ACTIVITIES OF OLIVE OIL POLYPHENOLS

PROJECT INTERREG MED

"ARISTOIL" & "ARISTOIL PLUS"

Bibliography Review until 1-3-2022

Athens, May 2022

Interreg Mediterranean EUROPEAN UNION

ARISTOIL PLUS

Project co-financed by the European Regional Development Fund

One of the main objectives of Interreg MED project "Aristoil" **is consumers' awareness on high quality olive oil benefits**.

This edition is the result of 3 Faculties of pharmacy efforts and includes the registration of researches published in scientific magazines. These researches prove that phenols in olive oil contribute to health claim of human body.

Olive oil, rich in phenols, is a valuable food with health claim protection.

According to the 432/2012 EU regulation, the daily consumption of 20gr of olive oil that contains at least 5mg tyrosol and hydroxytyrosol derivatives, is enough for blood lipids oxidative protection. This olive oil can be given the characterization of HEALTH CLAIM.

We express our warmest thanks to the Associate. Professor from the Pharmacy School of EKPA and Scientific Supervisor of the AristOil Project, Mr. Prokopis Magiatis, and to his collaborators Dr. Eleni Melliou and Dr. Panagiotis Diamantakos for the selection and presentation of the scientific articles included in this edition.

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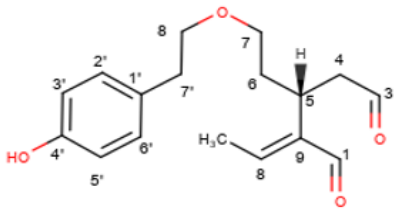
"Aristoil" & "AristOil Plus" Project Coordinator

Dr E. Melliou, Assoc. Prof P. Magiatis, Dr N. Krimnianiotis

1. OLEOCANTHAL

Oleocanthal is one of the most important and well-studied constituents among the olive oil phenolic fraction. Its presence in olive oil was firstly mentioned by researcher Montedoro in 1993[1] and it appears in varying levels in olive oil. According to extended and well documented studies oleocanthal's concentration may reach 2275 mg/Kg in olive oil and the concentration is affected by different genetic and pedoclimatic factors[2]. The biological activities of oleocanthal started studying many years later by Beauchamp in 2006[3] in research which the anti-inflammatory activity was highlighted and also revealed that oleocanthal is the constituent that gives olive oil the pungent taste. Since then, many researchers have published various data, confirming numerous of biological activities for this constituent.



[1] GianFrancesco Montedoro, 'Simple and Hydrolyzable Phenolic Compounds in Virgin Olive Oil. 1. Their Extraction, Separation, and Quantitative and Semiquantitative Evaluation by HPLC | J', *Ournal of Agricultural and Food Chemistry*, 40.9 (1992), 1571–76.

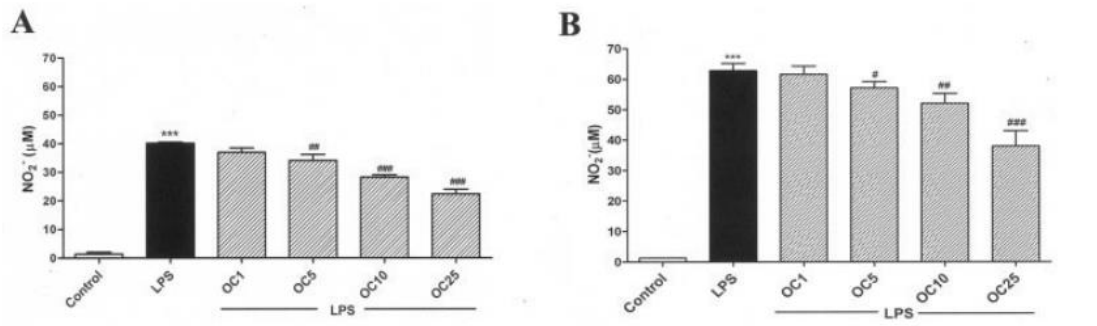
[2] Panagiotis Diamantakos and others, 'A New Definition of the Term "High-Phenolic Olive Oil" Based on Large Scale Statistical Data of Greek Olive Oils Analyzed by QNMR', Molecules (Basel, Switzerland), 26.4 (2021), 1115 <https://doi.org/10.3390/molecules26041115>.

[3] Gary K. Beauchamp and others, 'Phytochemistry: Ibuprofen-like Activity in Extra-Virgin Olive Oil', Nature, 437.7055 (2005), 45–46 <https://doi.org/10.1038/437045a>.

1.1 Anti-inflammatory activity

In vitro studies

The first biological activity that was reported for oleocanthal was the anti-inflammatory activity by Beauchamp in 2006[3]. In his research Beauchamp and his partners based on the fact that the bitterness of certain compounds is correlated with
their pharmacological activity, they tested whether oleocanthal might mimic the pharmacological effects of ibuprofen. Ibuprofen is a strong anti-inflammatory drag which also cause a similar taste perception. Ibuprofen is a non-selective inhibitor of the cyclooxygenase enzymes COX-1 and COX-2 which are strongly related with the mechanism of inflammation. The *in vitro* studies showed that oleocanthal causes a dose-dependent inhibition of COX-1 and COX-2 activities, similar to ibuprofen but in lower doses which finally leads in the reduction of inflammation.
The above evidence is very important because inflammation is related with many diseases. In 2010, Iacono and her co-authors studied the effect of oleocanthal on the modulation of NO production in chondrocytes[4]. The increase of NO which has been associated with cartilage degradation, causes progress of joint diseases like osteoarthritis. Cultured ATDC-5 chondrocytes were tested with different doses of oleocanthal, and the results showed that decreased lipopolysaccharide-induced NOS2 synthesis in chondrocytes without significantly affecting cell viability at lower concentrations.



Oleocanthal (OC) suppresses lipopolysaccharide (LPS)–induced nitric oxide (NO) production. ATDC-5 cells (8 104) were pretreated with 1–25 M oleocanthal for 12 hours and then exposed to 250 ng/ml LPS for 24 hours (A) or 48 hours (B)4

[4] Anna Iacono and others, 'Effect of Oleocanthal and Its Derivatives on Inflammatory Response Induced by Lipopolysaccharide in a Murine Chondrocyte Cell Line', Arthritis and Rheumatism, 62.6 (2010), 1675–82 <https://doi.org/10.1002/art.27437>.

Expanding this research field, the same research team tried to evaluate the anti-inflammatory activity of oleocanthal in murine macrophages J774 and murine chondrocytes ATDC5 with a particular focus on the inhibition of gene expression of pro-inflammatory factors such as MIP-1α and IL-6[5]. They found that oleocanthal inhibits LPS-induced NO production in J774 macrophages, without affecting cell viability. Moreover, it inhibits MIP-1α and IL-6 mRNA expression, as well as protein synthesis, in both ATDC5 chondrocytes and J774 macrophages.

The inflammation genesis via COX1 mechanism is responsible for many serious chronic diseases such as cardiovascular diseases and cancer. Rosignoli and her team studied the effect of oleocanthal on the release of superoxide anions (O2−), prostaglandin E2 (PGE2) and tumor necrosis factor α (TNFα) and the expression of COX2[6]. All these mediators are normally produced by neutrophils and monocytes in order to heal an inflammation. In pathogenic condition, the abovementioned processes are unregulated and amplified with the consequent overproduction of the inflammatory mediators which leads in serious diseases. The researchers showed that oleocanthal inhibited the production of $O2^-$ at 36% and highlighted the ability of olive oil phenols to modulate the production of pro-inflammatory molecules, a property common to non-steroidal anti-inflammatory drugs.

Similar results were reported some years later in two researchs by Scotese and by Montoya[7] in different pro-inflammatory factors such as IL-6, IL-8, CCL3, LCN2 and TNF-a induced by LPS in human primary osteoarthritis chondrocytes without having any cytotoxic effect[8].

[5] Morena Scotece, Rodolfo Gómez, and others, 'Further Evidence for the Anti-Inflammatory Activity of Oleocanthal: Inhibition of MIP-1α and IL-6 in J774 Macrophages and in ATDC5 Chondrocytes', Life Sciences, Contribution of Intraneuronal Amyloid β Accumulation to Alzheimer's Disease, 91.23 (2012), 1229–35 <https://doi.org/10.1016/j.lfs.2012.09.012>.

[6] Patrizia Rosignoli and others, 'Effect of Olive Oil Phenols on the Production of Inflammatory Mediators in Freshly Isolated Human Monocytes', The Journal of Nutritional Biochemistry, 24.8 (2013), 1513–19 <https://doi.org/10.1016/j.jnutbio.2012.12.011>.

[7] Tatiana Montoya, Maria L. Castejón, and others, 'Oleocanthal Modulates LPS-Induced Murine Peritoneal Macrophages Activation via Regulation of Inflammasome, Nrf-2/HO-1, and MAPKs Signaling Pathways', Journal of Agricultural and Food Chemistry, 67.19 (2019), 5552–59 <https://doi.org/10.1021/acs.jafc.9b00771>.

[8] Morena Scotece, Javier Conde, and others, 'Oleocanthal Inhibits Catabolic and Inflammatory Mediators in LPS-Activated Human Primary Osteoarthritis (OA) Chondrocytes Through MAPKs/NF-KB Pathways', Cellular Physiology and Biochemistry: International Journal of Experimental Cellular Physiology, Biochemistry, and Pharmacology, 49.6 (2018), 2414–26 <https://doi.org/10.1159/000493840>.

In vivo

The anti-inflammatory and ibuprofen – like activity of oleocanthal in the above-mentioned evidence showed that oleocanthal may prevent several serious diseases like osteoarthritis. In order to document this hypothesis, more complex studies were conducted.

1.1.1. Dietary Oleocanthal Supplementation Prevents Inflammation and Oxidative Stress in Collagen-Induced Arthritis in Mice[9]

Summary:

In this research Montoya et al designed to evaluate the preventive role of dietary oleocanthal-supplemented effects in collagen-induced arthritis (CIA) murine model. Since the pathogenesis of rheumatoid arthritis (RA) involves inflammatory and oxidative components, they tested whether oleocanthal, which has well documented antioxidant, anti-inflammatory, antimicrobial, anticancer and neuroprotective effects could be used for rheumatoid arthritis prevention.

Animals were fed with a preventive OLE-enriched dietary for 6 weeks previous to CIA induction and until the end of experiment time. Dietary OLE prevented bone, joint and cartilage rheumatic affections induced by collagen. Levels of circulatory matrix metalloproteinase (MMP)-3 and pro-inflammatory cytokines (IL-6, IL-1β, TNF-α, IL-17, IFN-γ) were significantly decreased in secoiridoid fed animals. Besides, dietary OLE was able to diminish COX-2, mPGES-1 and iNOS protein expressions and, also, PGE2 levels. Overall, our results exhibit preliminary evidence about OLE, as a novel dietary tool for the prevention of autoimmune and inflammatory disorders, such as RA.

[9] Tatiana Montoya, Marina Sánchez-Hidalgo, and others, 'Dietary Oleocanthal Supplementation Prevents Inflammation and Oxidative Stress in Collagen-Induced Arthritis in Mice', Antioxidants, 10.5 (2021), 650 <https://doi.org/10.3390/antiox10050650>.

1.1.2. Neuroprotective Effects of Oleocanthal, a Compound in Virgin Olive Oil, in a Rat Model of Traumatic Brain Injury[10]

Summary:

In this research Mete et al studied the neuroprotective effects of oleocanthal in traumatic brain injury (TBI). This pathogenic condition has two distinct phases: primary and secondary injury. The researchers focused in the second phase in which many agents have been used to prevent in the past. Based on Oleocanthal's anti-inflammatory and antioxidant properties similar to non-steroidal anti-inflammatory drugs, they evaluated the neuroprotective effects in a rat model of TBI. Twenty-six adult male, Wistar rats were used and were divided into 4 groups which treated with different doses of oleocanthal via saline intraperitoneally. Group 1 was the sham group. Group 2 was the trauma group in which rats were treated with 10 mg/kg twice a day. Groups 3 and 4, rats were treated with 10 or 30 mg/kg OC IP twice a day. For each group, brain samples were collected 72 hours after injury. Brain samples and blood were evaluated with histopathological and biochemical methods. Histopathological evaluation revealed a significant difference between Group 2 and Group 4. Biochemical findings demonstrated that the oxidative stress index was highest in Group 2 and lowest in Group 4. Researchers demonstrated that oleocanthal has a protective effect on neural cells after TBI. This effect is achieved by reducing oxidative stress and apoptosis.

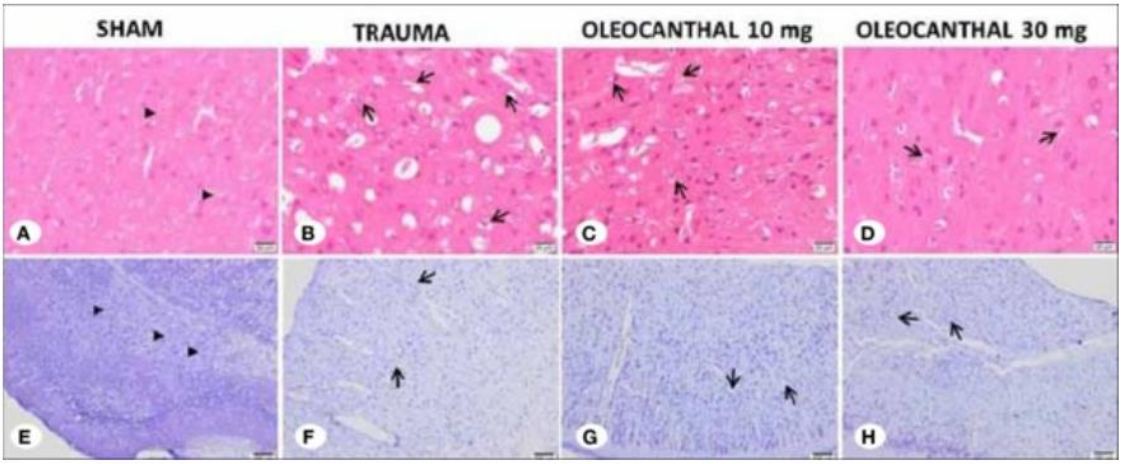


Fig. In the OC-treated samples (C, D, g, h), traumatic changes were less intense than in the trauma group (B and F)

[10] Mesut Mete and others, 'Neuroprotective Effects of Oleocanthal, a Compound in Virgin Olive Oil, in a Rat Model of Traumatic Brain Injury', *Turkish Neurosurgery*, 28.6 (2018), 858–65 <https://doi.org/10.5137/1019-5149.JTN.21417-17.2>.

1.1.3. Oleocanthal protects against neuronal inflammation and cardiopulmonary bypass surgery-induced brain injury in rats by regulating the NLRP3 pathway[11]

Summary:

In this study Liu et al investigated the potential protective effect of oleocanthal pre-treatment against cardiopulmonary bypass (CPB)-induced cerebral injury. These neuronal injuries are very common after open heart surgery with the aid of cardiopulmonary bypass (CPB) techniques. Researchers treated with 30 mg/kg via saline intraperitoneally and was administered 3 h before CPB induction in the treated group. Behavioral neurological scores and cerebral injury were assessed to determine the effects of oleocanthal, based on oxidative stress and serum mediators of inflammation by enzyme-linked immunosorbent assay (ELISA). The findings suggest that pre-treatment with oleocanthal reduced neurological dysfunction and cerebral injury. Parameters of oxidative stress and cytokine levels were reduced in the serum of the oleocanthal treated group compared with the CPB-only group. Pre-treatment with oleocanthal ameliorated the expression of TLR-4, IRAK4, and Zonula occludens-1 (ZO-1) proteins in the cerebral tissue of the CPB-injured rats. The results revealed that treatment with oleocanthal protected against cerebral damage by controlling microglia inflammation through the TLR-4 pathway.

[11] Xiuye Liu and others, 'Oleocanthal Protects against Neuronal Inflammation and Cardiopulmonary Bypass Surgery-Induced Brain Injury in Rats by Regulating the NLRP3 Pathway', *Restorative Neurology and Neuroscience*, 39.1 (2021), 39–44 <https://doi.org/10.3233/RNN-201073>.

1.1.4. Topical treatment with oleocanthal extract in reducing inflammatory reactions after photodynamic therapy: a prospective quasi-experimental pilot study[12]

Summary:

Palacios et al studied another case of inflammation which is a side effect of a therapy, the photodynamic therapy. Photodynamic therapy (PDT) is an effective treatment against skin field cancerization. The researchers evaluated the topical efficacy of an oily fluid enriched with oleocanthal (OC) extract, in comparison with a conventional oily fluid, in reducing the degree of inflammatory reaction after conventional PDT. This pilot study, before-after with a control group, performed with a cohort of consecutive patients diagnosed with actinic keratosis/field cancerization (AK/FC) in the forehead and/or scalp, treated by PDT. A group of 24 consecutive patients received the topical application, three times daily for one week, of an emollient oily fluid in the area treated with PDT. Subsequently, another group, of 23 consecutive patients, received the same treatment pattern with an oily fluid enriched with oleocanthal extract. The post-PDT inflammatory reaction was measured using visual scale of erythema (from 0 to 4). The assessment was conducted at 30 min and at 48 h post-PDT. In the assessment at 48 h after treatment, the inflammation had improved more among the patients treated with oleocanthal (median: 25%, 95%CI: -5.3 to 28.5) than in the non-oleocanthal group (median: 0%; 95%CI: -45.2 to -6.2). The difference was statistically significant ($p < 0.01$), and the Cohen's d value was 0.89 (large effect). At three months after PDT, a complete response had been obtained by 60.9% of the patients treated with OC compared to 29.2% of the non-OC group, and the difference was close to statistical significance ($p=0.059$). The topical application of an oily fluid enriched with oleocanthal extract achieved a greater reduction in post-PDT cutaneous inflammation and a better treatment response, in comparison with the application of a conventional oily fluid.

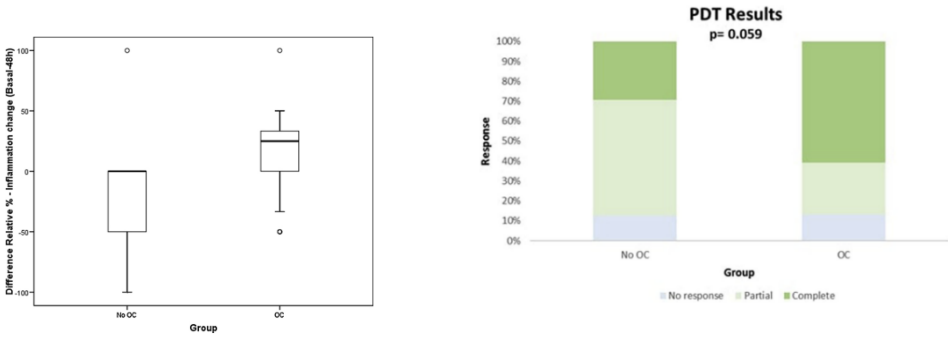


Fig. Box-plot of the relative percentage distribution of inflammation change (Baseline – After 48 h)/ No OC: group without oleocanthal. OC: group with oleocanthal. Bar chart of the distribution of clinical responses at three months after starting PDT treatment.

[12] Juan Manuel Segura Palacios and others, 'Topical Treatment with Oleocanthal Extract in Reducing Inflammatory Reactions after Photodynamic Therapy: A Prospective Quasi-Experimental Pilot Study', *Complementary Therapies in Medicine*, 42 (2019), 298–301 <https://doi.org/10.1016/j.ctim.2018.12.003>.

1.2 Anti-cancer activity

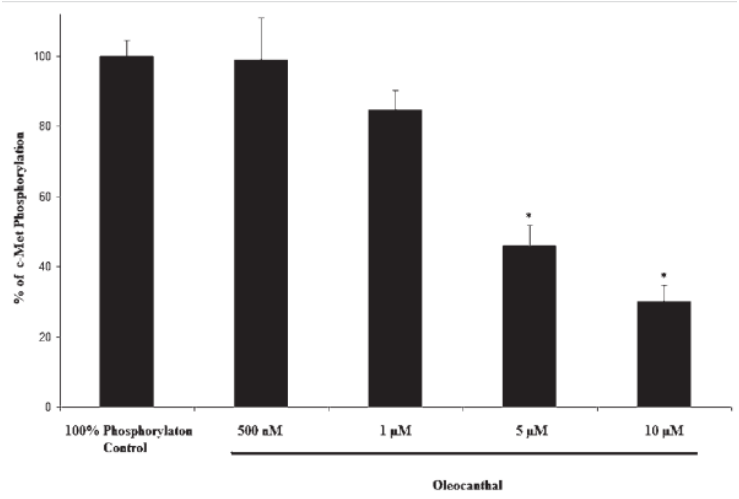
In vitro studies

Another biological activity that has been attributed to oleocanthal is the anti-cancer activity. Cancer is a group of diseases involving abnormal cell growth with the potential to invade or spread to other parts of the body.

The last decade many *in vitro* studies have been applied to investigate the anticancer activity of oleocanthal. Primary investigations by LeGendre[13] in 2015 showed that OC induced cell death in all cancer cells examined as rapidly as 30 minutes after treatment in the absence of serum. Treatment of non-transformed cells suppressed their proliferation but did not cause cell death and also induced both primary necrotic and apoptotic cell death via induction of lysosomal membrane permeabilization.

There are many possible pathways for the genesis of cancer including over expressions of proteins like mTOR and c-Met. In 2011 Elnagar[14] tested whether oleocanthal could inhibit the overexpression of c-Met which has an important oncogenic role in many tumors. Oleocanthal inhibited the proliferation, migration, and invasion of the epithelial human breast and prostate cancer cell lines, it demonstrated anti-angiogenic activity and it inhibited the phosphorylation of c-Met kinase.

Fig. 8 c-Met phosphorylation inhibition by various doses of 1 using Z'-LYTE™ assay kit



[13] Onica LeGendre, Paul As Breslin, and David A. Foster, '(-)-Oleocanthal Rapidly and Selectively Induces Cancer Cell Death via Lysosomal Membrane Permeabilization', Molecular & Cellular Oncology, 2.4 (2015), e1006077 <https://doi.org/10.1080/23723556.2015.1006077>.

[14] Ahmed Y. Elnagar, Paul W. Sylvester, and Khalid A. El Sayed, '(-)-Oleocanthal as a c-Met Inhibitor for the Control of Metastatic Breast and Prostate Cancers', Planta Medica, 77.10 (2011), 1013–19 <https://doi.org/10.1055/s-0030-1270724>.

The same research team[15] a few years later expanded their research, testing whether oleocanthal could inhibit Dysregulation of the Hepatocyte growth factor (HGF)/c-Met signaling. Results showed that oleocanthal inhibits the growth of human breast cancer cells and also caused a dose-dependent inhibition of HGF-induced cell migration, invasion and G1/S cell cycle progression.

The anticancer activity of oleocanthal in breast cancer cells without affecting non-tumoral breast cells was also highlighted by Diez-Bello and his partners in 2019[16].

There is also well documented evidence that the anticancer activity of oleocanthal is related to the inhibition of the kinase mTor. Khanfar and his partners[17] examined the possibility that oleocanthal inhibits mTOR which has reported that it has an important role in cancer and Alzheimer's disease. The authors showed that oleocanthal shared nine out of ten critical binding interactions with a potent dual PIK3-γ/mTOR natural inhibitor which leads in the inhibition of the enzymatic activity of mTOR. Oleocanthal treatment caused a marked downregulation of phosphorylated mTOR in metastatic breast cancer cell line (MDA-MB-231).

Apart from breast cancer, researchers have also investigated the anti-cancer activity in different types of cancer which have similar pathways. In 2016 Fogli and his partners[18], tested oleocanthal treatment on *in cutaneous* malignant melanoma. The researchers investigated the selective *in vitro* antiproliferative activity of oleocanthal against human malignant melanoma cells. The results showed that oleocanthal had a remarkable and selective activity for human melanoma cells versus normal dermal fibroblasts in low concentration.

A few years later, in 2018 Polini and her research team[19] evaluated the *in vitro* chemopreventive and anticancer action of EVOO extracts and oil-derived compounds in non-melanoma skin cancer models. Both oleocanthal and oleacein reduced non-melanoma skin cancer cell viability and migration, prevented colony and spheroid formation, and inhibited proliferation of atypical keratinocytes stimulated with

[15] Mohamed R. Akl and others, 'Olive Phenolics as C-Met Inhibitors: (-)-Oleocanthal Attenuates Cell Proliferation, Invasiveness, and Tumor Growth in Breast Cancer Models', PloS One, 9.5 (2014), e97622 <https://doi.org/10.1371/journal.pone.0097622>.

[16] R. Diez-Bello and others, '(−)-Oleocanthal Inhibits Proliferation and Migration by Modulating Ca2+ Entry through TRPC6 in Breast Cancer Cells', Biochimica et Biophysica Acta (BBA) - Molecular Cell Research, 1866.3 (2019), 474–85 <https://doi.org/10.1016/j.bbamcr.2018.10.010>.

[17] Mohammad A. Khanfar and others, 'Olive Oil-Derived Oleocanthal as Potent Inhibitor of Mammalian Target of Rapamycin: Biological Evaluation and Molecular Modeling Studies', Phytotherapy Research: PTR, 29.11 (2015), 1776–82 <https://doi.org/10.1002/ptr.5434>.

[18] Stefano Fogli and others, 'Cytotoxic Activity of Oleocanthal Isolated from Virgin Olive Oil on Human Melanoma Cells', *Nutrition and Cancer*, 68.5 (2016), 873–77 <https://doi.org/10.1080/01635581.2016.1180407>.

[19] Beatrice Polini and others, 'Oleocanthal and Oleacein Contribute to the in Vitro Therapeutic Potential of Extra Virgin Oil-Derived Extracts in Non-Melanoma Skin Cancer', *Toxicology in Vitro*, 52 (2018), 243–50 <https://doi.org/10.1016/j.tiv.2018.06.021>.

epidermal growth factor. The mechanism beyond this activity was the inhibition of two protein- kinase, Erk and Akt, which are strongly related with melanoma.

Back in 2013, Scotece[20] demonstrated that oleocanthal has a remarkable *in vitro* activity by inhibiting MIP-1 expression and secretion in multiple myeloma cells. The researcher showed that oleocanthal inhibits myeloma cells proliferation by inducing the activation of apoptosis mechanisms and by down-regulating the same kinases that was mentioned before, ERK1/2 and AKT.

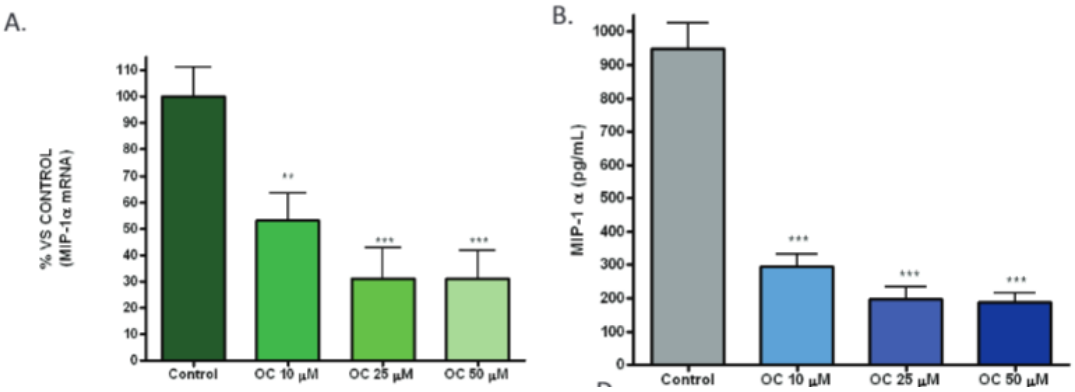


Fig. Oleocanthal inhibits MIP1- mRNA expression and protein secretion in ARH-77 cells. (A). Human MIP-1 mRNA expression in ARH77 cells after treatment with OC (10, 25 and 50 M) for 3 hours (B). ARH-77 cells were treated with OC (10, 25 and 50 M) for 3 hours.

The same period Cusimano and her partners[21] evaluated the potential anticancer effects of oleocanthal in hepatocellular carcinoma (HCC) and colorectal carcinoma (CRC) models. They showed that oleocanthal induced cell growth inhibition in HCC and CRC cells, inhibited colony formation and induced apoptosis in a dose dependent-manner. At the same time oleocanthal was not toxic in primary normal human hepatocytes.

[20] M. Scotece, R. Gómez, and others, 'Oleocanthal Inhibits Proliferation and MIP-1α Expression in Human Multiple Myeloma Cells', *Current Medicinal Chemistry*, 20.19 (2013), 2467–75 <https://doi.org/10.2174/0929867311320190006>.

[21] Antonella Cusimano and others, 'Oleocanthal Exerts Antitumor Effects on Human Liver and Colon Cancer Cells through ROS Generation', *International Journal of Oncology*, 51.2 (2017), 533–44 <https://doi.org/10.3892/ijo.2017.4049>.

In 2020, Ünsal and his research team[22] published their work on the anti-cancer activity of oleocanthal in neuroblastoma. Their analysis demonstrated that cells were significantly less viable after oleocanthal treatment, inhibited neurite growth in neuroblastoma and prevented growth and proliferation of neuroblastoma cells in culture by increasing oxidative stress and apoptosis.

In another research Pei[23] showed that oleocanthal inhibits proliferation and cell cycle progression and induced apoptosis in human hepatocellular carcinoma cells in vitro.

It also suppressed tumor growth in an orthotopic hepatocellular carcinoma cells model by inhibiting STAT3, a protein which has a crucial role in the genesis of the hepatocellular cancer.

[22] Ülkün Ünlü Ünsal and others, 'Inhibiting Effect of Oleocanthal on Neuroblastoma Cancer Cell Proliferation in Culture', *Biotechnic & Histochemistry: Official Publication of the Biological Stain Commission*, 95.3 (2020), 233–41 <https://doi.org/10.1080/10520295.2019.1674919>.

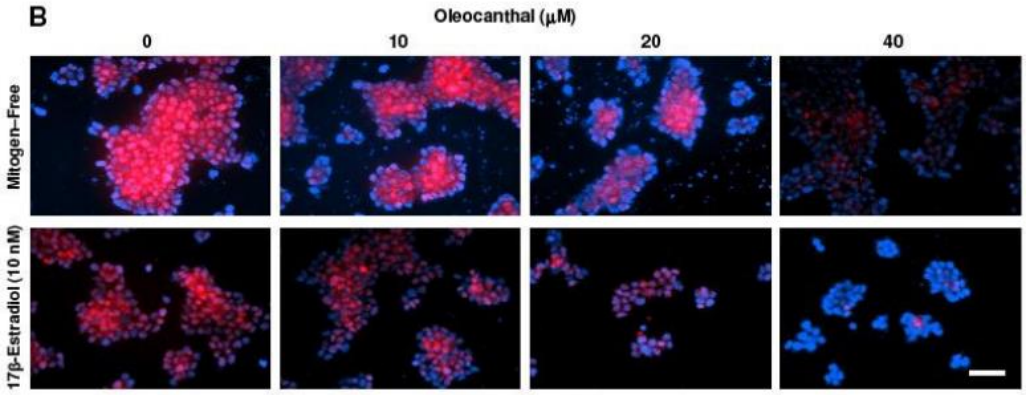
[23] Tiemin Pei and others, '(-)-Oleocanthal Inhibits Growth and Metastasis by Blocking Activation of STAT3 in Human Hepatocellular Carcinoma', *Oncotarget*, 7.28 (2016), 43475–91 <https://doi.org/10.18632/oncotarget.9782>.

In vivo studies and clinical trials

1.2.1 The olive oil phenolic oleocanthal modulates estrogen receptor expression in luminal breast cancer in vitro and in vivo and synergizes with tamoxifen treatment[24]

Summary:

The aim of this study was to explore the effect of oleocanthal treatment on growth of luminal breast cancer cells and to examine the effect of combination of oleocanthal with tamoxifen. Results showed that oleocanthal inhibited growth of various human breast cancer cells in mitogen-free media with IC_{50} values of 32.7 to 80.93µM. Similarly, oleocanthal suppressed growth of these cells in 17β-estradiol-supplemented media with IC_{50} values of 22.28 to 83.91µM. Combined oleocanthal and tamoxifen treatments resulted in a synergistic growth inhibition of the cells with combination index values of 0.65 to 0.53 for each cell line. Studies indicated high degree of overlapping for the binding of oleocanthal and 17β-estradiol to estrogen receptors, while oleocanthal and tamoxifen have distinguished binding modes. Treatment with 5mg/kg or 10mg/kg (-)-oleocanthal resulted in 97% inhibition of tumor growth in mice. (-)-Oleocanthal treatment reduced total levels of estrogen receptors in cells both in vitro and in vivo. Collectively, (-)-oleocanthal showed a potential beneficial effect in suppressing growth of hormone-dependent breast cancer and improving sensitivity to tamoxifen treatment. These findings provide rational for evaluating the effect of (-)-oleocanthal in combination with endocrine treatments in luminal breast cancer.



[24] Nehad M. Ayoub and others, 'The Olive Oil Phenolic (-)-Oleocanthal Modulates Estrogen Receptor Expression in Luminal Breast Cancer in Vitro and in Vivo and Synergizes with Tamoxifen Treatment', *European Journal of Pharmacology*, 810 (2017), 100–111 <https://doi.org/10.1016/j.ejphar.2017.06.019>.

1.2.2. Oleocanthal Prevents Breast Cancer Locoregional Recurrence After Primary Tumor Surgical Excision and Neoadjuvant Targeted Therapy in Orthotopic Nude Mouse Models[25]

Summary:

In this study Siddique and his partners studied another aspect of Breast cancer (BC). Researchers focused on recurrence of Breast Cancer which represents a challenge for survivors who have had their primary tumors surgically excised, and/or have completed radiation, neoadjuvant, or adjuvant therapeutic regimens. Current BC treatments mostly lack the ability to reduce the risk of disease recurrence. About 70% of BC patients will subsequently suffer disease relapse, manifesting as local, regional, or distant tumor recurrence. Based on oleocanthals anti-cancer and anti- inflammatory activity, researchers tested whether oleocanthal could act as a novel recurrence inhibitor.

The researchers reported the novel activity of daily oral treatment with OC (10 mg/kg) in preventing BC locoregional recurrence in a nude mouse xenograft model generated by orthotopic inoculation with BT-474 cells as a luminal type B model. They reported inhibition of tumor recurrence. However, in a recurrence model of triple-negative breast cancer (TNBC), OC treatment (10 mg/kg) did not effectively prevent tumor recurrence, but rather, was seen to significantly reduce the growth of recurrent tumors as compared to vehicle control-treated animals. Inhibition of tumor recurrence was associated with significant serum level reductions of the human BC recurrence marker CA 15-3 at the study end in animals treated with OC. OC treatment upregulated the expression of the epithelial marker E-cadherin and downregulated the levels of the mesenchymal marker vimentin in recurrent tumors vs. untreated control animals. OC treatment also reduced the activation of MET and HER2 receptors, as indicated by reduced phosphorylation levels of these proteins in recurrent tumors vs. controls. Collectively, the results of the study provide the first evidence for suppression of BC tumor recurrence by oral OC treatment in an animal model for such recurrence.

[25] Abu Bakar Siddique, Nehad M. Ayoub, and others, '(-)-Oleocanthal Prevents Breast Cancer Locoregional Recurrence After Primary Tumor Surgical Excision and Neoadjuvant Targeted Therapy in Orthotopic Nude Mouse Models', *Cancers*, 11.5 (2019), E637 <https://doi.org/10.3390/cancers11050637>.

1.2.3. Oleocanthal and oleocanthal-rich olive oils induce lysosomal membrane permeabilization in cancer cells[26]

Summary:

In this research Goren et al, demonstrated that oleocanthal, as well as naturally oleocanthal-rich extra virgin olive oils, induce damage to cancer cells' lysosomes leading to cellular toxicity in vitro and in vivo. The current study Lysosomal membrane permeabilization following oleocanthal treatment in various cell lines was assayed via three complementary methods. Additionally, they found that oleocanthal treatment reduced tumor burden and extended lifespan of mice engineered to develop pancreatic neuroendocrine tumors. Finally, they observed that extra virgin olive oils naturally rich in oleocanthal sharply reduced cancer cell viability and induced lysosomal membrane permeabilization while oleocanthal-poor oils did not.

1.2.4. Oleocanthal as a Dual c-MET-COX2 Inhibitor for the Control of Lung Cancer[27]

Summary:

Siddique and his research team have also studied the effect of oleocanthal treatment in different types of cancer like lung cancer (LC). This type represents the topmost mortality-causing cancer in the U.S. and patients have overall poor survival rate with limited available treatment options. Dysregulation of the mesenchymal epithelial transition factor (c-MET) and cyclooxygenase 2 (COX2) initiates aggressive LC profile in a subset of patients. In their study they showed the ability of oleocanthal to suppress LC progression and metastasis through dual targeting of c-MET and COX-2. They

[26] Limor Goren and others, '(-)-Oleocanthal and (-)-Oleocanthal-Rich Olive Oils Induce Lysosomal Membrane Permeabilization in Cancer Cells', *PloS One*, 14.8 (2019), e0216024 <https://doi.org/10.1371/journal.pone.0216024>.
[27] Abu Bakar Siddique, Phillip C. S. R. Kilgore, and others, '(-)-Oleocanthal as a Dual c-MET-COX2 Inhibitor for the Control of Lung Cancer', *Nutrients*, 12.6 (2020), E1749 <https://doi.org/10.3390/nu12061749>.

highlighted significant reduction in the total and activated c-MET levels and inhibition of COX1/2 activity in the lung adenocarcinoma cells A549 and NCI-H322M, in vitro. In addition, OC treatment caused a dose-dependent inhibition of the HGF-induced LC cells migration. Daily oral treatment with 10 mg/kg OC for 8 weeks significantly suppressed the LC A549-Luc progression and prevented metastasis to brain and other organs in a nude mouse tail vein injection model by dual targeting of c-MET and COX2. Thus, the EVOO-based OC is an effective lead with translational potential for use as a prospective nutraceutical to control LC progression and metastasis.

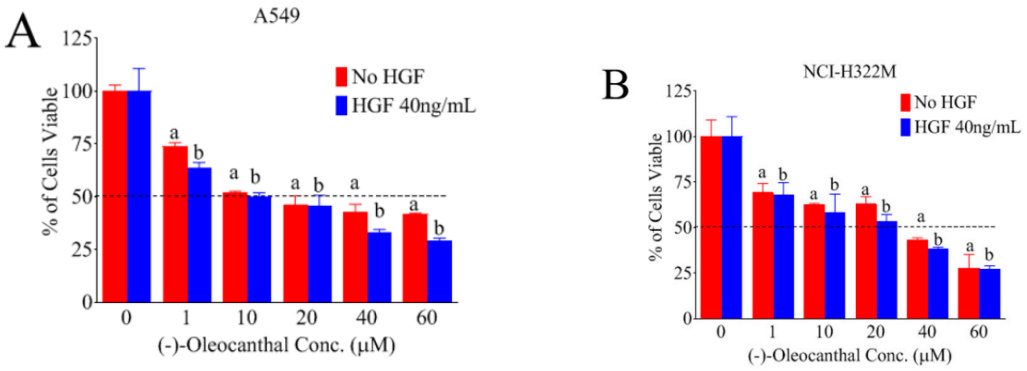


Figure Oleocanthal selectively inhibited the viability of A549 and NCI-H322M LC cell lines and minimally affected the non-tumorigenic human microvascular endothelial cells viability. Effects of Oleocanthal (OC) treatment against the growth of A549 (A) and NCI-H322M (B) LC cells in the presence or absence of 40 ng/mL mitogenic HGF over 48 h treatment period

1.2.5. The Effect of Dietary Intervention with High-Oleocanthal and Oleacein Olive Oil in Patients with Early-Stage Chronic Lymphocytic Leukemia: A Pilot Randomized Trial[28]

Summary:

Recently the first clinical trial with Early-Stage Chronic Lymphocytic Leukemia patients and oleocanthal-rich olive oil was presented by Rojas Gil et al. The aim of this pilot study was to test if patients at early stage of chronic lymphocytic leukemia (CLL) could adhere and tolerate an intervention with high OC/OL extra virgin olive oil (EVOO) and if this intervention could lead to any changes in markers related to the disease. A pilot dietary intervention (DI) was made in patients with CLL in Rai stages 0-II who did not follow any treatment. In the first intervention (DI1), 20 CLL patients were included in a blind randomized study and were separated into two groups. One group (A) of 10 patients consumed 40 ml/day of high OC/OL-EVOO (416 mg/Kg OC and 284 mg/kg OL) for 3 months. A second group (B) of 10 patients consumed 40 ml/day of low OC/OL (82 mg/kg OC and 33 mg/kg OL) for 3 months. After a washout period of 9-12 months, a second intervention (DI2) only with High OC/OL-EVOO for 6 months was performed with 22 randomly selected patients (16 from the DI1 (8 from each group) and 6 new). Hematological, biochemical, and apoptotic markers were analyzed in the serum of the patients. In addition, cellular proliferation and apoptosis markers were studied in isolated proteins from peripheral blood mononuclear cells.

The results of the DI1 showed beneficial effects on hematological and apoptotic markers only with High OC/OL-EVOO. During the DI2, a decrease in the white blood cell and lymphocyte count was observed ($p \leq 0.05$), comparing 3 months before the intervention and 6 months after it. After 3 and 6 months of DI2, an increase ($p \leq 0.05$) was observed in the apoptotic markers ccK18 and Apo1-Fas, and also in the cell cycle negative regulator p21, and also a decrease in the antiapoptotic protein Survivin, and in the cellular proliferation marker Cyclin D.

[28] Andrea Paola Rojas Gil and others, 'The Effect of Dietary Intervention With High-Oleocanthal and Oleacein Olive Oil in Patients With Early-Stage Chronic Lymphocytic Leukemia: A Pilot Randomized Trial', *Frontiers in Oncology*, 11 (2021), 810249 <https://doi.org/10.3389/fonc.2021.810249>.

1.3 Oleocanthal activity against Alzheimer / Δράση της ελαιοκανθάλης κατά της νόσου Alzheimer

In vitro studies

Alzheimer's disease is a dementia that generally manifests spontaneously in later adulthood as a result of synaptic and neuronal loss and there is no established treatment yet. It is proposed that neurodegeneration and dementia observed in AD, is caused by the neurotoxin ‘’Aβ plaques’’. Subsequent experiments have implicated soluble Aβ oligomers (also referred to as ADDLs) as the toxic species responsible for the development of Aβ plaques. As the interaction of Aβ oligomers with neurons appears to be a critical step in the initiation of AD pathology, considerable research has focused on preventing the formation of toxic oligomeric species.
In 2009 Pitt et al[29] focused on oleocanthal (OC), as a compound capable of altering the assembly state of soluble oligomers of amyloid-β1-42 peptide (ADDL), which peptide is a neurotoxin that causes Alzheimer’s disease (AD). OC increased the immunoreactivity of soluble Aβ species, indicating changes in oligomer structure. Analysis of oligomers in the presence of OC showed an upward shift in molecular weight and a ladder-like distribution of SDS-stable ADDL subspecies. Decreased binding to synapses was accompanied by significantly less synaptic deterioration assayed by drebrin loss.

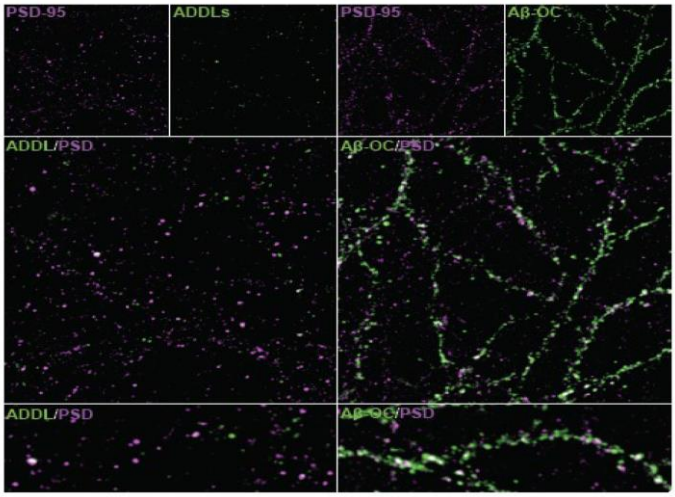


Figure. Specific binding of Aβ-OC to synapses shows greatly enhanced immunoreactivity compared to ADDLs PSD-95 (magenta) was used as a synaptic marker to determine if the pattern of binding was similar between ADDLs or Aβ-OC (both in green).

[29] Jason Pitt and others, ‘Alzheimer’s-Associated Aβ Oligomers Show Altered Structure, Immunoreactivity and Synaptotoxicity with Low Doses of Oleocanthal’, *Toxicology and Applied Pharmacology*, Toxicants and Neurodegenerative Diseases, 240.2 (2009), 189–97 <https://doi.org/10.1016/j.taap.2009.07.018>.

Further explanations for this activity were demonstrated the same year and in 2011 by Monti[3031] and his partners, who showed that oleocanthal is capable of altering the fibrillization of tau protein, which is one of the key factors at the basis of neurodegenerative diseases, and of covalently reacting with lysine amino groups of the tau fragment K18 in an unspecific fashion. Oleocanthal found to interact with tau441, inducing stable conformational modifications of the protein secondary structure and also interfering with tau aggregation.

The same mechanism was also studied the same year by Li et al[32] . In this study reserchers demonstrated that oleocanthal abrogates fibrillization of tau by locking tau into the naturally unfolded state. Using PHF6 consisting of the amino acid residues VQIVYK, a hexapeptide within the third repeat of tau that is essential for fibrillization, they showed that oleocanthal forms an adduct with the lysine via initial Schiff base formation. Structure and function studies demonstrate that the two aldehyde groups of oleocanthal are required for the inhibitory activity.

As a result of the above activity some years later, Batarseh et al[33] investigated the ability of oleocanthal to alter the toxic effect of Ab on brain parenchymal cells. The researchers investigated oleocanthal effect on modulating Ab oligomers (Abo) pathological events in neurons and astrocytes. Their findings demonstrated oleocanthal prevented Abo-induced synaptic proteins, SNAP-25 and PSD-95, down-regulation in neurons, and attenuated Abo-induced inflammation, glutamine transporter (GLT1) and glucose transporter (GLUT1) down-regulation in astrocytes. Abo-induced inflammation was characterized by interleukin-6 (IL-6) increase and glial fibrillary acidic protein (GFAP) upregulation that were reduced by oleocanthal.

[30] Maria Chiara Monti, Luigi Margarucci, Raffaele Riccio, and others, 'Modulation of Tau Protein Fibrillization by Oleocanthal', *Journal of Natural Products*, 75.9 (2012), 1584–88 <https://doi.org/10.1021/np300384h>.

[31] Maria Chiara Monti, Luigi Margarucci, Alessandra Tosco, and others, 'New Insights on the Interaction Mechanism between Tau Protein and Oleocanthal, an Extra-Virgin Olive-Oil Bioactive Component', *Food & Function*, 2.7 (2011), 423–28 <https://doi.org/10.1039/c1fo10064e>.

[32] Wenkai Li and others, 'Inhibition of Tau Fibrillization by Oleocanthal via Reaction with the Amino Groups of Tau', *Journal of Neurochemistry*, 110.4 (2009), 1339–51 <https://doi.org/10.1111/j.1471-4159.2009.06224.x>.

[33] Yazan S. Batarseh and others, 'Oleocanthal Ameliorates Amyloid-β Oligomers' Toxicity on Astrocytes and Neuronal Cells: In Vitro Studies', *Neuroscience*, 352 (2017), 204–15 <https://doi.org/10.1016/j.neuroscience.2017.03.059>.

In vivo studies and clinical trials

1.3.1 Olive-Oil-Derived Oleocanthal Enhances β-Amyloid Clearance as a Potential Neuroprotective Mechanism against Alzheimer's Disease: In Vitro and in Vivo Studies[34]

Summary:

The mechanism by which oleocanthal exerts its neuroprotective effect was investigated by Abuznait et al whose study provide in vitro and in vivo evidence for the potential of oleocanthal to enhance Aβ clearance from the brain via up-regulation of P-glycoprotein (P-gp) and LDL lipoprotein receptor related protein-1 (LRP1), major Aβ transport proteins, at the blood-brain barrier (BBB). Results from *in vitro* and *in vivo* studies demonstrated similar and consistent pattern of oleocanthal in controlling Aβ levels. In cultured mice brain endothelial cells, oleocanthal treatment increased and LRP1 protein expression and activity. Studies showed that administration of oleocanthal to C57BL/6 wild-type mice resulted in Aβ clearance from the brain and increased the brain efflux index from 62.0 % for control mice to 79.9% for oleocanthal treated mice. Increased P-gp and LRP1 protein expression in the brain microvessels and inhibition studies confirmed the role of up-regulation of these proteins in enhancing Aβ clearance after oleocanthal treatment, which leads to Aβ degradation. In conclusion, these findings provide experimental support that potential reduced risk of AD associated with extra-virgin olive oil could be mediated by enhancement of Aβ clearance from the brain.

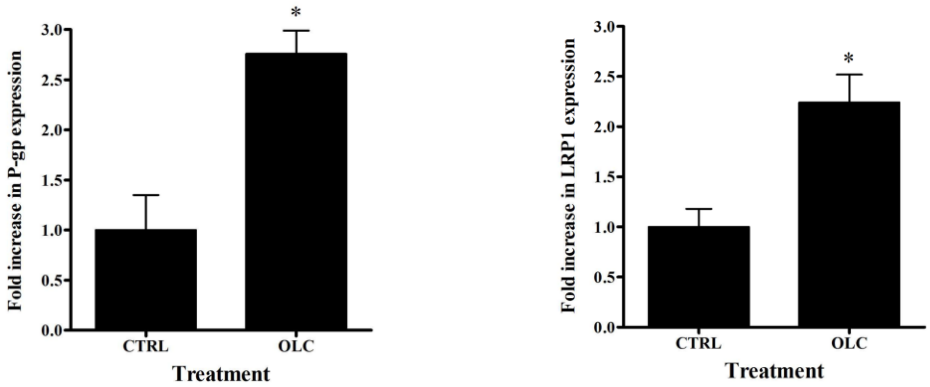


Figure: Quantitative folds change in P-gp and LRP1 expression were measured using ImageJ version 1.44.

[34] Alaa H. Abuznait and others, 'Olive-Oil-Derived Oleocanthal Enhances β-Amyloid Clearance as a Potential Neuroprotective Mechanism against Alzheimer's Disease: In Vitro and in Vivo Studies', *ACS Chemical Neuroscience*, 4.6 (2013), 973–82 <https://doi.org/10.1021/cn400024q>.

1.3.2. Extra-virgin olive oil attenuates amyloid-β and tau pathologies in the brains of TgSwDI mice[35]

Summary:

In this study Qosa et al investigated the effect of EVOO-enriched diet on amyloid- and tau-related pathological alterations that are associated with the progression of AD and cerebral amyloid angiopathy (CAA) in TgSwDI mice. Feeding mice with EVOO-enriched diet for 6months, beginning at an age before amyloid-β (Aβ) accumulation starts, has significantly reduced total Aβ and tau brain levels with a significant improvement in mouse cognitive behavior. This reduction in brain Aβ was explained by the enhanced Aβ clearance pathways and reduced brain production of Aβ via modulation of amyloid-β precursor protein processing. On the other hand, although feeding mice with EVOO-enriched diet for 3months, beginning at an age after Aβ accumulation starts, showed improved clearance across the blood-brain barrier and significant reduction in Aβ levels, it did not affect tau levels or improve cognitive functions of TgSwDI mouse. Collectively, results of this study suggest that the long-term consumption of EVOO-containing diet starting at early age provides a protective effect against AD and its related disorder CAA.

[35] Hisham Qosa and others, 'Extra-Virgin Olive Oil Attenuates Amyloid-β and Tau Pathologies in the Brains of TgSwDI Mice', *The Journal of Nutritional Biochemistry*, 26.12 (2015), 1479–90 <https://doi.org/10.1016/j.jnutbio.2015.07.022>.

1.3.3. Oleocanthal-rich extra-virgin olive oil enhances donepezil effect by reducing amyloid-β load and related toxicity in a mouse model of Alzheimer's disease[36]

Summary:

In 2018 Batarseh and Kaddoumi based on their previous study that was mentioned above, investigated whether the consumption of EVOO rich with oleocanthal could act as a medical food on enhancing the effect of donepezil on attenuating Aβ load and related toxicity in 5xFAD mouse model of Alzheimer's disease (AD).

Donepezil is an acetylcholine esterase inhibitor approved for use for all AD stages and has been reported to have limited Aβ-targeting mechanisms beside its acetylcholine esterase inhibition. The researchers showed that EVOO consumption in combination with donepezil significantly reduced Aβ load and related pathological changes. Reduced Aβ load could be explained, at least in part, by enhancing Aβ clearance pathways including blood-brain barrier (BBB) clearance and enzymatic degradation, and shifting amyloid precursor protein processing toward the nonamyloidogenic pathway. Furthermore, EVOO combination with donepezil up-regulated synaptic proteins, enhanced BBB tightness and reduced neuroinflammation associated with Aβ pathology. In conclusion, EVOO consumption as a medical food combined with donepezil offers an effective therapeutic approach by enhancing the noncholinergic mechanisms of donepezil and by providing additional mechanisms to attenuate Aβ-related pathology in AD patients.

[36] Yazan S. Batarseh and Amal Kaddoumi, 'Oleocanthal-Rich Extra-Virgin Olive Oil Enhances Donepezil Effect by Reducing Amyloid-β Load and Related Toxicity in a Mouse Model of Alzheimer's Disease', *The Journal of Nutritional Biochemistry*, 55 (2018), 113–23 <https://doi.org/10.1016/j.jnutbio.2017.12.006>.

1.3.4. Oleocanthal Nutraceuticals for Alzheimer's Disease Amyloid Pathology: Novel Oral Formulations, Therapeutic, and Molecular Insights in 5xFAD Transgenic Mice Model[37]

Summary:

In this research Talmim and his research team based on the anti-inflammatory activity of oleocanthal through COX system inhibition with potency comparable to the standard non-steroidal anti-inflammatory drug (NSAID) like ibuprofen, tested different oleocanthal formulations in mice model of Alzheimer's Disease. As far as oleocanthal has a pungent, astringent, and irritant taste, researchers demonstrated that it should be formulated in acceptable dosage form before its oral use as a potential nutraceutical. Oleocanthal was formulated in powder formulation (OC-PF) and solid dispersion formulation with erythritol (OC-SD). Both formulations showed an improved OC dissolution profile. OC-PF and OC-SD improved memory deficits of 5xFAD mice in behavioral studies. OC-PF and OC-SD exhibited significant attenuation of the accumulation of Aβ plaques and tau phosphorylation in the brain of 5xFAD female mice. Both formulations markedly suppressed C3AR1 (complement component 3a receptor 1) activity by targeting the downstream marker STAT3. Collectively, these results demonstrate the potential for the application of OC-PF as a prospective nutraceutical or dietary supplement to control the progression of amyloid pathogenesis associated with AD

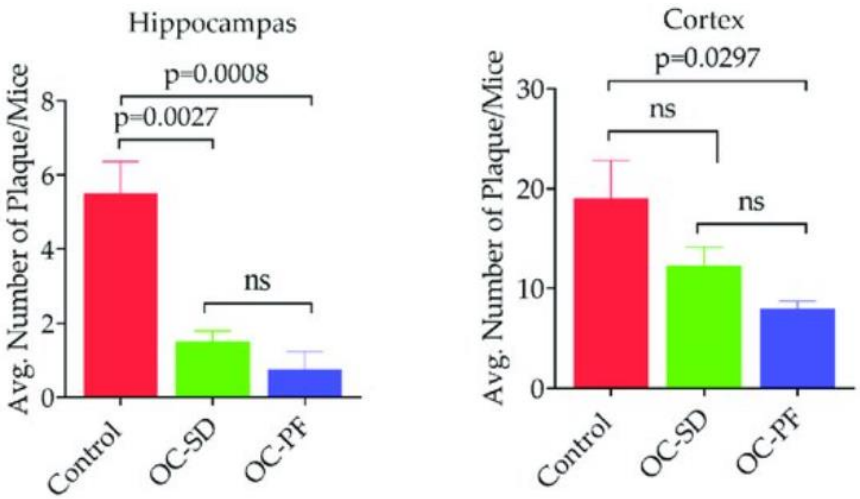
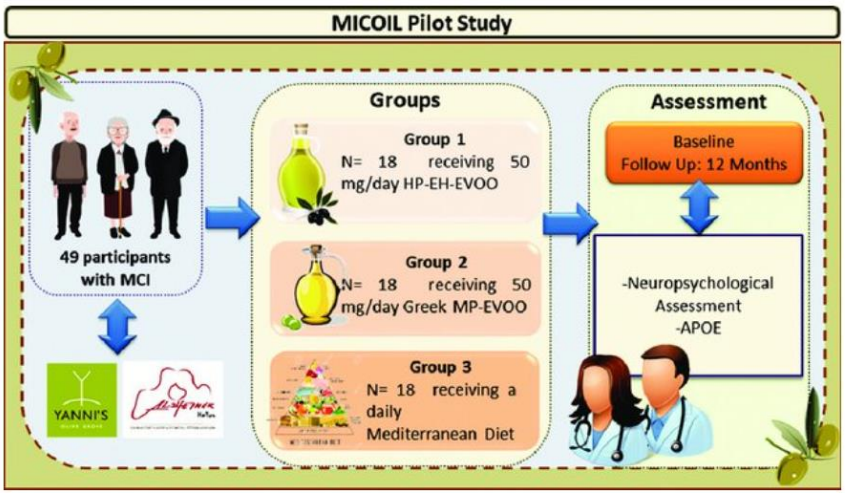


Fig. Quantitative analysis of average Aϐ plaque count in tested mice brain hippocampus sections

[37] Afsana Tajmim and others, '(-)-Oleocanthal Nutraceuticals for Alzheimer's Disease Amyloid Pathology: Novel Oral Formulations, Therapeutic, and Molecular Insights in 5xFAD Transgenic Mice Model', *Nutrients*, 13.5 (2021), 1702 <https://doi.org/10.3390/nu13051702>.

1.3.5. A Randomized Clinical Trial of Greek High Phenolic Early Harvest Extra Virgin Olive Oil in Mild Cognitive Impairment: The MICOIL Pilot Study[38]

Summary:
Recently the first randomized clinical study with high phenolic extra virgin olive oil against Mild Cognitive Impairment was published by Tsolaki et al. The researchers conducted a prospective study so as to examine the high phenolic olive oil and medium phenolic olive oil versus simple Mediterranean died in Mild Cognitive Impairment. Genetic predisposition (APOEε4) to Alzheimer's disease (AD) was tested and an extensive neuropsychological battery was administered at baseline and after 12 months. Each participant was randomized and assigned one of three groups: 1) Group 1 received the HP-EH-EVOO (50 mL/day); 2) Group 2 received the MP-EVOO (50 mL/day), and 3) Group 3 received only the MeDi instructions. The researchers showed that better follow-up performance was found in Group 1 compared to Group 2 and Group 3 in the almost all cognitive domains. Moreover, Group 2 showed also significant improvement compared to Group 3 in ADAS-cog (p = 0.001) and MMSE (p = 0.05), whereas Group 3 exhibited worse or similar to baseline performance in almost all domains. In particular, Group 1 and Group 2 had better outcomes with regards to ADAS-cog (p = 0.003), Digit Span (p = 0.006), and Letter fluency (p = 0.003). Moreover, there was a significant difference (p = 0.001) in the presence of APOEε4 between the Groups 1 and 2 versus Group 3. Conclusion: Long-term intervention with HP-EH-EVOO or MP-EVOO was associated with significant improvement in cognitive function compared to MeDi, independent of the presence of APOEε4.



[38] Magda Tsolaki and others, 'A Randomized Clinical Trial of Greek High Phenolic Early Harvest Extra Virgin Olive Oil in Mild Cognitive Impairment: The MICOIL Pilot Study', *Journal of Alzheimer's Disease: JAD*, 78.2 (2020), 801–17 <https://doi.org/10.3233/JAD-200405>.

1.4 Antiplatelet activity Αντιαιμοπεταλιακή δράση

In vitro studies

Type 2 diabetes is an impairment in the way the body regulates and uses sugar (glucose) as a fuel. This long-term (chronic) condition results in too much sugar circulating in the bloodstream. Eventually, high blood sugar levels can lead to disorders of the circulatory, nervous and immune systems. Post-prandial glucose has been associated with a higher incidence of cardiovascular events in patients with and without diabetes.

Recently, Lammi et al[39] studied the potentially beneficial effect of EVOO consumption on the atherosclerotic process and diabetes. Their results showed that oleocanthal inhibited the expressin of two hormons (GLP-1 and GIP) , two incretins playing essential roles in controlling post-prandial glycemia s eliciting insulin secretion and lowering post-prandial blood glucose.

[39] Carmen Lammi and others, 'Phenolic Extracts from Extra Virgin Olive Oils Inhibit Dipeptidyl Peptidase IV Activity: In Vitro, Cellular, and In Silico Molecular Modeling Investigations', *Antioxidants*, 10.7 (2021), 1133 <https://doi.org/10.3390/antiox10071133>.

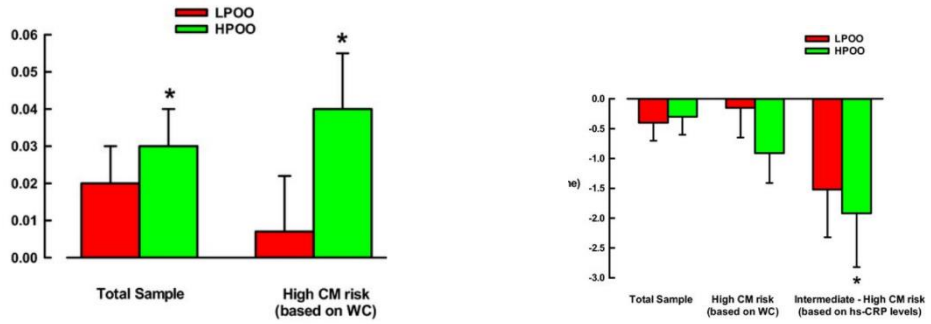
In vivo studies and clinical trials

1.4.1. Post-prandial effects of high-polyphenolic extra virgin olive oil on endothelial function in adults at risk for type 2 diabetes: A randomized controlled crossover trial[40]

Summary:

Recently Njike and her research team compared the effects of high phenolic extra virgin olive oil (EVOO) and refined olive oil without polyphenols on endothelial function (EF) in adults at risk for Type 2 diabetes mellitus (T2DM). In this trial, randomized, controlled, double-blind, crossover trial of 20 adults (mean age 56.1 years; 10 women, 10 men) at risk for T2DM (i.e., as defined by either prediabetes or metabolic syndrome) assigned to one of two possible sequence permutations of two different single dose treatments (50 mL of high-polyphenolic EVOO or 50 mL of refined olive oil without polyphenols), with 1-week washout. Participants received their olive oils in a smoothie consisting of ½ cup frozen blueberries and 1 cup (8 oz) low-fat vanilla yogurt blended together. Primary outcome measure was EF measured as flow-mediated dilatation. Participants were evaluated before and 2 h after ingestion of their assigned olive oil treatment. In their results, the researchers showed that EVOO acutely improved EF as compared to refined olive oil (1.2 ± 6.5% versus −3.6 ± 3.8%; p = 0.0086). No significant effects on systolic or diastolic blood pressure were observed. The vascular effects of olive oil ingestion should specify the characteristics of the oil.

Fig. Effect of weeks daily consumption of extra virgin high polyphenol olive oil (HPOO) and low polyphenol olive oil (LPOO) on total antioxidant capacity (TAC) and on on high-sensitivity C-reactive protein (hs-CRP)



[40] Valentine Y. Njike and others, 'Post-Prandial Effects of High-Polyphenolic Extra Virgin Olive Oil on Endothelial Function in Adults at Risk for Type 2 Diabetes: A Randomized Controlled Crossover Trial', *International Journal of Cardiology*, 330 (2021), 171–76 <https://doi.org/10.1016/j.ijcard.2021.01.062>.

1.4.2. Oleocanthal-rich extra virgin olive oil demonstrates acute anti-platelet effects in healthy men in a randomized trial[41]

Summary:

In this trial Agrawal and his partners studied the acute EVOO intake on platelet function. The researchers investigated whether the phenolic profiles of extra virgin olive oils (EVOOs) influence their cardiovascular benefits. Participants (n = 9) consumed 40 mL of EVOO weekly. EVOOs were matched for total phenolic content and were either tyrosol-poor with 1:2 oleacein/oleocanthal (D2i0.5), or 2:1 oleacein/oleocanthal (D2i2), or predominantly tyrosol (D2i0). Ibuprofen provided a platelet inhibition control. Blood was collected pre- and 2 h post-EVOO intake. D2i0.5 and D2i2 reduced 1 mg/mL collagen-stimulated maximum platelet aggregation (Pmax), with effects best correlated to oleocanthal intake (R = 0.56, P = 0.002). Total phenolic intake was independently correlated to eicosanoid production inhibition, suggesting that cyclooxygenase blockade was not responsible for the Pmax inhibition. Five participants exhibited >25% DPmax declines with D2i0.5 and D2i2 intake and plasma metabolomic profiles discriminated subjects by oil responsivity. Platelet responses to acute EVOO intake are associated with oil phenolic composition and may be influenced by diet.

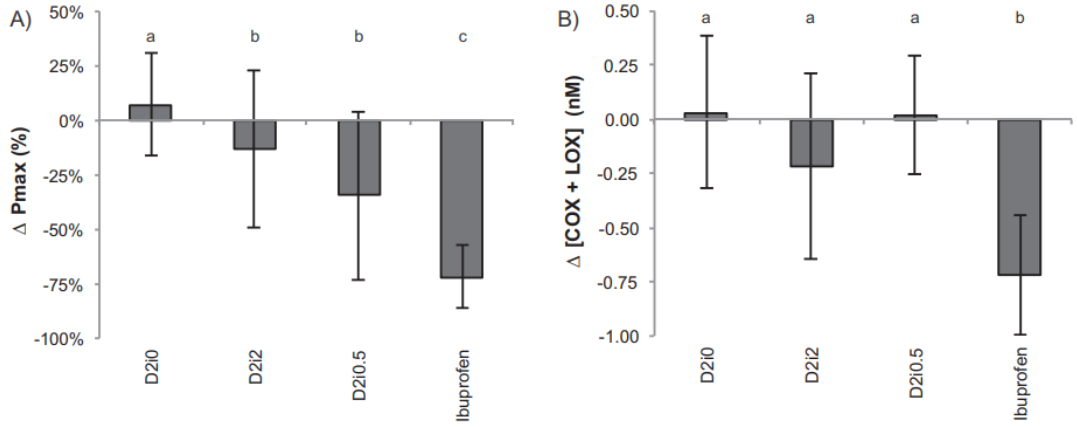


Fig. Effects of tested EVOOs on (A) maximum platelet aggregation and (B) oxylipins associated with platelet function in healthy male subjects. D2i2, D2i0.5 and Ibuprofen all decreased maximum platelet aggregation compared to D2i0, and Ibuprofen decreased oxylipin concentrations compared to all oils

[41] Karan Agrawal and others, 'Oleocanthal-Rich Extra Virgin Olive Oil Demonstrates Acute Anti-Platelet Effects in Healthy Men in a Randomized Trial', *Journal of Functional Foods*, 36 (2017), 84–93 <https://doi.org/10.1016/j.jff.2017.06.046>.

1.4.3. Daily Use of Extra Virgin Olive Oil with High Oleocanthal Concentration Reduced Body Weight, Waist Circumference, Alanine Transaminase, Inflammatory Cytokines and Hepatic Steatosis in Subjects with the Metabolic Syndrome: A 2-Month Intervention Study[42]

Summary:

In 2020, Patti et al studied the cardiometabolic effects of extra virgin olive oil with high oleocanthal concentration. The researchers administered EVOO with a high oleocanthal concentration daily to 23 subjects with the metabolic syndrome (MetS) and hepatic steatosis (15 men and 8 women, age: 60 ± 11 years) for 2 months. Anthropometric data, metabolic parameters, hepatic steatosis (by fatty liver index, FLI), abdominal fat distribution (by ultrasound), and pro- and anti-inflammatory cytokines were assessed before and after the intervention. EVOO supplementation was associated with a reduction in body weight, waist circumference, body mass index (BMI), alanine transaminase and FLI, as well as interleukin (IL)-6, IL-17A, tumor necrosis factor-α and IL-1B, while IL-10 increased. Maximum subcutaneous fat thickness (SFT max) also increased, with a concomitant decrease in the ratio of visceral fat layer thickness/SFT max. Correlation analysis revealed positive associations between changes in body weight and BMI and those in SFT max, along with an inverse association between changes in IL-6 and those in SFT max. In conclusion, ingestion of EVOO with a high OC concentration had beneficial effects on metabolic parameters, inflammatory cytokines and abdominal fat distribution in MetS subjects with hepatic steatosis, a category of patients at high cardiometabolic risk.

[42] Angelo M. Patti and others, 'Daily Use of Extra Virgin Olive Oil with High Oleocanthal Concentration Reduced Body Weight, Waist Circumference, Alanine Transaminase, Inflammatory Cytokines and Hepatic Steatosis in Subjects with the Metabolic Syndrome: A 2-Month Intervention Study', *Metabolites*, 10.10 (2020), E392 <https://doi.org/10.3390/metabo10100392>.

SNAPSHOT & ACTIVITIES FROM THE

KickOff Meeting KALAMATA

KickOff Meeting KALAMATA

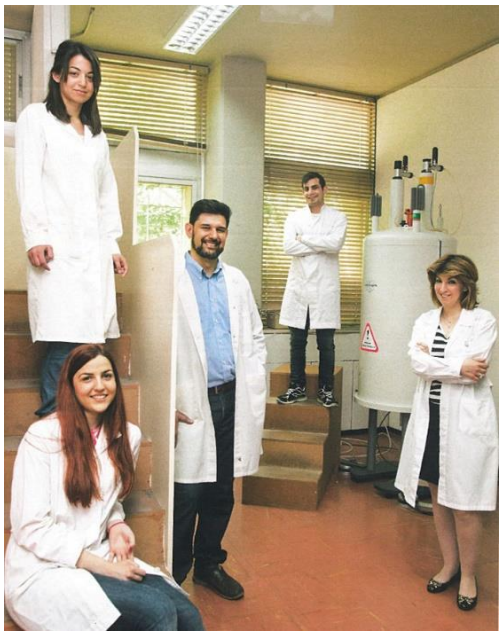
Presentation of AristOil project in Old Pareliament (Greece)

Associate Professor Prokopis Magiatis

Dr Eleni Melliou

“AristOil” & “AristOil Plus” PROJECTS

ARISTOMETRO



Laboratory of Associate Prof. Magiatis

Portable olive mill of the AristOil project

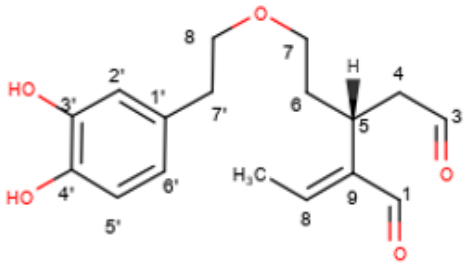
SIAL International Food Fair in Paris

Award for candidate researchers with a scholarship

Adoption of an olive tree in Pnyx (Akropolis))

2. OLEACEIN – ΕΛΑΙΑΣΙΝΗ

Oleacein is the second most important phenolic constituent of olive oil and, similar to oleocanthal, its concentration is varying in deferent olive oils depending in genetic and pedoclimatic factors. Oleacein is formed after the enzymatic transformation of oleuropein, which is the most abundant phenolic of the olive fruit, and which has numerous of biological activities. Oleacein, due to the dihydroxy phenolic ring in its structure, was initially considered as a simple antioxidant. After well documented studies though, which are presented below, many biological activities have been reveal, which partly explain the health protective properties of olive oil.



In vitro studies

2.1. Effects of olive oil polyphenols on erythrocyte oxidative damage[43]

Summary:

In this study Paiva-Martins investigated the capacity of oleacein, to protect red blood cells (RBCs) from oxidative injury. The red blood cell (RBC), has poor repair and biosynthetic mechanisms, suffering and accumulating oxidative lesions whenever oxidative stress develops and are particularly exposed to endogenous oxidative damage because of their specific role as oxygen carriers. Power antioxidants like oleacein needed to prevent oxidative damage.

The in vitro oxidative stress of RBCs was induced by the water-soluble radical initiator 2,2'azobis (2amidinopropane) dihydrochloride and changes were evaluated either by optical microscopy or by the amount of hemolysis. The researchers showed that oleacein protected RBCs from oxidative damage in a dose-dependent manner. Oleacein had the most powerful effect at 20mM, within the other polyphenols. Even at 3mM, oleacein still had an important protective activity. For the first time it was demonstrated that oleacein may play a noteworthy protective role against ROS-induced oxidative injury in human cells since lower doses of this compound were needed to protect RBCs in vitro from oxidative mediated hemolysis.

[43] Fátima c and others, 'Effects of Olive Oil Polyphenols on Erythrocyte Oxidative Damage', *Molecular Nutrition & Food Research*, 53.5 (2009), 609–16 <https://doi.org/10.1002/mnfr.200800276>.

2.2. Oleacein enhances anti-inflammatory activity of human macrophages by increasing CD163 receptor expression.[44]

Summary:

In 2015, Filipek et al, based on its antioxidant and anti-inflammatory activity examined whether oleacein could increase CD163 and IL10 receptor expression as well as intracellular secretion of protein heme oxygenase 1 (HO1) in human macrophages. The effect of oleacein (10 and 20 µmol/l) or oleacein together with complexes of haemoglobin (Hb) and haptoglobin 11 (Hp11) or haptoglobin 22 (Hp22) on expression of IL10 and CD163 receptors was determined by Flow Cytometry. HO1 intracellular secretion in macrophages was investigated by ELISA. Oleacein together with complexes HbHp11 or HbHp22 stimulated the expression of CD163 (30-100 fold), IL10 (170-300 fold) and HO1 secretion (60-130 fold) after 5 days of co-incubation.
Their results suggested that oleacein enhances anti-inflammatory activity of complexes haemoglobin with haptoglobin 11 and 22 and could play a potential role in the prevention of inflammatory disease related to atherosclerosis.

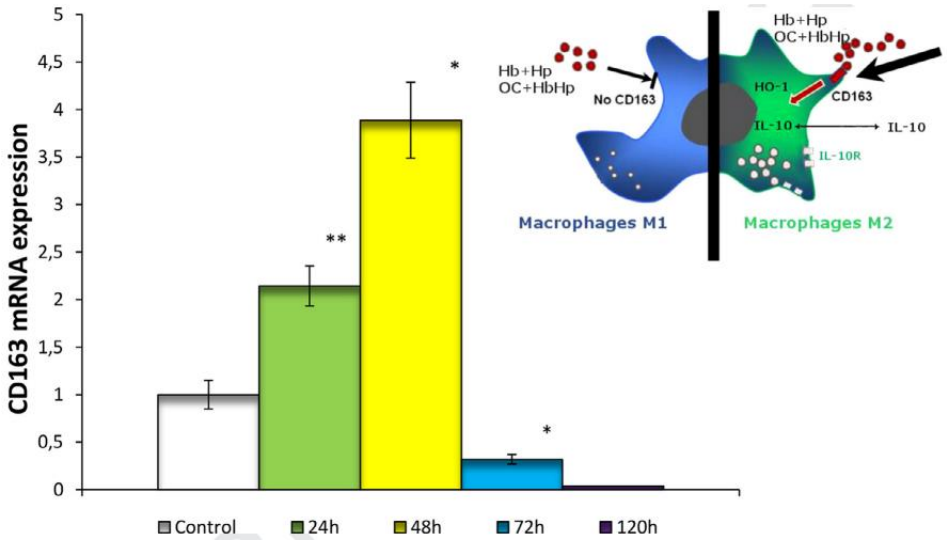


Fig. Influence oleacein together with complexes of haemoglobin and haptoglobin on increases CD163 mRNA transcription.

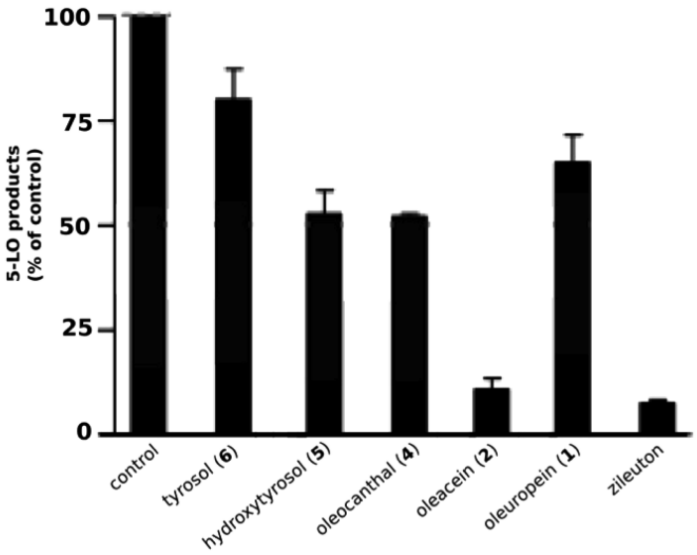
[44] Agnieszka Filipek, Monika E. Czerwińska, Anna K. Kiss, Małgorzata Wrzosek, and others, 'Oleacein Enhances Anti-Inflammatory Activity of Human Macrophages by Increasing CD163 Receptor Expression', *Phytomedicine: International Journal of Phytotherapy and Phytopharmacology*, 22.14 (2015), 1255–61 <https://doi.org/10.1016/j.phymed.2015.10.005>.

2.3. One-Step Semisynthesis of Oleacein and the Determination as a 5-Lipoxygenase Inhibitor.[45]

Summary:

In 2014, Vougogiannopoulou et al proceeded in the one-Step Semisynthesis of Oleacein and examined oleacein as a 5-Lipoxygenase Inhibitor. The researchers showed that 5-lipoxygenase is a direct target for oleacein with an inhibitory potential (IC50: 2 µM) more potent than oleocanthal and oleuropein. This enzyme catalyzes the initial steps in the biosynthesis of pro-inflammatory leukotrienes. This investigation presented here an alternative solution to isolation or total synthesis for the procurement of oleacein, thus facilitating the further development as a potential anti-inflammatory agent.

Figure 4. Activity of isolated human recombinant 5-LO after preincubation for 10 min at 4 °C with vehicle (0.1%



DMSO; control) or with the indicated compounds (at 10 µM)

[45] Konstantina Vougogiannopoulou and others, 'One-Step Semisynthesis of Oleacein and the Determination as a 5-Lipoxygenase Inhibitor', *Journal of Natural Products*, 77.3 (2014), 441–45 <https://doi.org/10.1021/np401010x>.

2.4. Inhibition of human neutrophils activity, CD11b/CD18 expression and elastase release by 3,4-dihydroxyphenylethanol-elenolic acid dialdehyde, oleacein[46]

Summary:

In this research, Czerwinska and her team studied the possible Inhibition of human neutrophils NEP activity by oleacein. Neutrophils(PMN), stand in the first line of defence of the innate immune system. Early inflammatory events attract neutrophils to the injured tissues, where damage is extended by proinflammatory mediators, released from neutrophils. However, during the chronic inflammatory process, neutrophils are still activated by a great variety of stimuli and an increase in neutrophil mediators can be observed. Overexpression of NEP activity is involved in the pathogenesis of atherosclerosis.

According to authors, oleacein with a concentration of 100 lM inhibited NEP activity, elastase, MMP-9 and IL-8 release from neutrophils. Oleacein with a concentration of 50 lM suppressed CD11b/CD18 expression by 63.6 ± 3.1% and to a lesser extent, increased CD62L expression by 27.3 ± 8.3% on the surface of neutrophils, in comparison with stimulated cells. Oleacein by inhibiting NEP activity, adhesion molecules expression and elastase release might play a role in the protective effects of olive oil against endothelial injuries.

[46] Monika E. Czerwińska, Anna K. Kiss, and Marek Naruszewicz, 'Inhibition of Human Neutrophils NEP Activity, CD11b/CD18 Expression and Elastase Release by 3,4-Dihydroxyphenylethanol-Elenolic Acid Dialdehyde, Oleacein', *Food Chemistry*, 153 (2014), 1–8 <https://doi.org/10.1016/j.foodchem.2013.12.019>.

2.5. Oleacein may intensify the efflux of oxLDL from human macrophages by increasing the expression of the SRB1 receptor, as well as ABCA1 and ABCG1 transporters[47]

Summary:
The aim of the present study, which was conducted by Filipek and Gierlikowska, was to investigate whether oleacein could increase efflux cholesterol from macrophages. The therapeutic strategies for the treatment of atherosclerosis primarily consist of reducing the transport of cholesterol to the arterial wall by lowering the LDL fraction. Atherosclerotic regression of early lesions may involve a reduction of atherosclerotic plaque by decreasing foam cells, inhibiting inflammation in arterial walls, and regenerating the endothelium strongly associated with reverse cholesterol transport.
Researchers showed that oleacein in dose-dependent manner (OLEA20, OLEA50) significantly reduced lipid deposits in macrophages. Oleacein significantly up-regulated protein expression of ABCA1 and ABCG1 transporter. Moreover, oleacein increased of HO-1 intracellular secretion, as well as increased activation of Nrf2. Whereas the level of NF-κB protein was almost completely inhibited by oleacein at the same concentrations. For OLEA10, no significant changes were observed for the parameters tested. This study demonstrated that oleacein may reduce foam cell formation. Therefore, the researchers proposed a novel explanation for the oleacein, that can be used to develop new therapeutic strategies for the treatment of atherosclerosis.

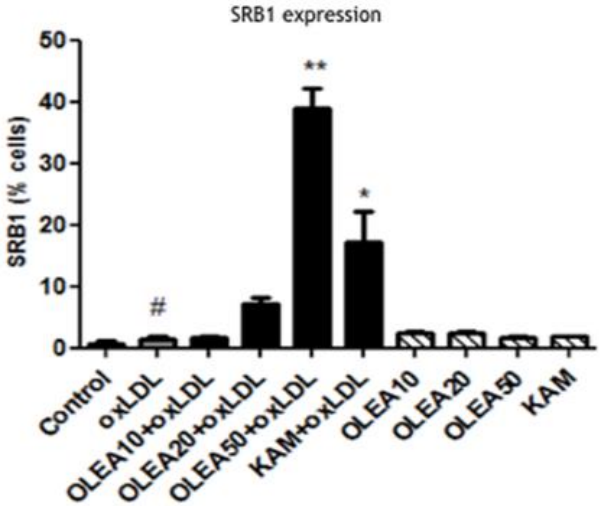


Fig.. Influence of oleacein on SRB1 expression in oxLDL-induced macrophages. (A) The results are presented as the percent of cells with SRB1 expression ± SEM (n = 9)

[47] A. Filipek and B. Gierlikowska, 'Oleacein May Intensify the Efflux of OxLDL from Human Macrophages by Increasing the Expression of the SRB1 Receptor, as Well as ABCA1 and ABCG1 Transporters', *Journal of Functional Foods*, 78 (2021), 104373 <https://doi.org/10.1016/j.jff.2021.104373>.

2.6. Extra Virgin Olive Oil Contains a Phenolic Inhibitor of the Histone Demethylase LSD1/KDM1A[48]

Summary:
In this study, Cuyàs and her research team, evaluated the ability of oleacein to target LSD1. The lysine-specific histone demethylase 1A (LSD1) also known as lysine (K)-specific demethylase 1A (KDM1A) is a central epigenetic regulator of metabolic reprogramming in obesity-associated diseases, neurological disorders, and cancer.. Molecular docking and dynamic simulation approaches revealed that oleacein could target the binding site of the LSD1 cofactor flavin adenosine dinucleotide with high affinity and at low concentrations. At higher concentrations, oleacein was predicted to target the interaction of LSD1 with histone H3 and the LSD1 co-repressor (RCOR1/CoREST), likely disturbing the anchorage of LSD1 to chromatin. AlphaScreen-based in vitro assays confirmed the ability of oleacein to act as a direct inhibitor of recombinant LSD1, with an IC50 as low as 2.5 µmol/L. Further, oleacein fully suppressed the expression of the transcription factor SOX2 in cancer stem-like and induced pluripotent stem (iPS) cells, which specifically occurs under the control of an LSD1-targeted distal enhancer. Conversely, oleacein failed to modify ectopic SOX2 overexpression driven by a constitutive promoter.

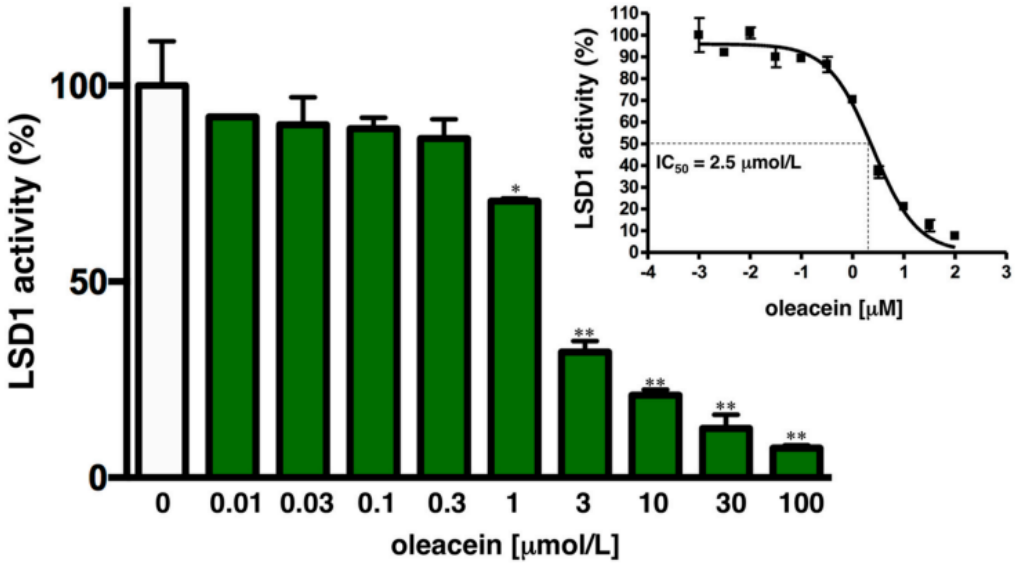


Fig.. Oleacein inhibits LSD1 activity. Dose–response curves of LSD1 demethylation activity were created by plotting AlphaScreen signals as the function of oleacein concentration.

[48] Elisabet Cuyàs and others, 'Extra Virgin Olive Oil Contains a Phenolic Inhibitor of the Histone Demethylase LSD1/KDM1A', *Nutrients*, 11.7 (2019), E1656 <https://doi.org/10.3390/nu11071656>.

2.7. Oleacein inhibits STAT3, activates the apoptotic machinery, and exerts anti-metastatic effects in the SH-SY5Y human neuroblastoma cells[49]

Summary:

The aim of this research was to study the molecular mechanisms underlying the anti-proliferative and anti-metastatic capacity of oleacein in the SH-SY5Y human neuroblastoma cell line. Cirmi and his partners, demonstrated that oleacein is able to reduce the proliferation of the SH-SY5Y cells by blocking the cell cycle in the S phase and inducing apoptotic cell death through the increase in both Bax and p53 as well as a reduction in the Bcl-2 expression and STAT3 phosphorylation. Moreover, oleacein caused reduction in the SH-SY5Y cell adhesion and migration. Overall, these findings indicate that oleacein exerts anti-cancer effects against neuroblastoma cells, suggesting a promising role as a candidate against this type of cancer.

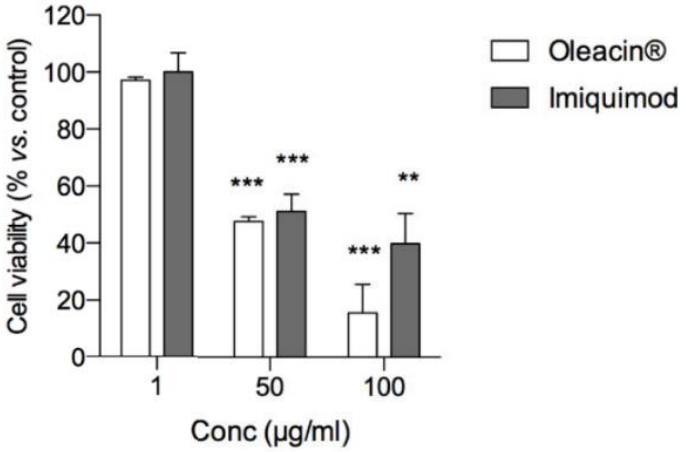
2.8. miRNA Modulation and Antitumor Activity by the Extra-Virgin Olive Oil Polyphenol Oleacein in Human Melanoma Cells[50]

Summary:

In this study, Carpi et al aimed at determining *in vitro* the antimelanoma activity of oleacein and the relative mechanism of action. Oleacein induced cell growth inhibition in 501Mel melanoma cells with an IC50 in the low micromolar range of concentrations. Moreover, an oleacein concentration approximating the IC50 induced G1/S phase arrest, DNA fragmentation, and downregulation of genes encoding antiapoptotic (BCL2 and MCL1) and proproliferative (c-KIT, K-RAS, PIK3R3, mTOR) proteins, while increased transcription levels of the proapoptotic protein BAX. Concordantly, oleacein increased the levels of miR-193a-3p, miR-34a-5p and miR-16-5p, while decreased miR-214-3p

[49] Santa Cirmi, Marilena Celano, and others, 'Oleacein Inhibits STAT3, Activates the Apoptotic Machinery, and Exerts Anti-Metastatic Effects in the SH-SY5Y Human Neuroblastoma Cells', *Food & Function*, 11.4 (2020), 3271–79 <https://doi.org/10.1039/D0FO00089B>.
[50] Sara Carpi and others, 'MiRNA Modulation and Antitumor Activity by the Extra-Virgin Olive Oil Polyphenol Oleacein in Human Melanoma Cells', *Frontiers in Pharmacology*, 11 (2020), 574317 <https://doi.org/10.3389/fphar.2020.574317>.

(targeting BAX). These modulatory effects might contribute to the inhibition of 501Mel melanoma cell growth observed after treatment with an olive leaves-derived formulation rich in oleacein, with potential application against in situ cutaneous melanoma. Altogether, these results demonstrate the ability of OA to contrast the proliferation of cutaneous melanoma cells through the transcriptional modulation of relevant genes and microRNAs, confirming the anticancer potential of EVOO and suggesting OA as a chemopreventive agent for cancer disease therapy.



Fig| Oleacin® and Imiquimod decrease 501Mel cell viability, 501Mel cells were treated with 1, 50, and 100 µg/ml of either Oleacin® or Imiquimod. Growth inhibition was measured at 72 h

2.9. Anti-tumor Activity and Epigenetic Impact of thePolyphenol Oleacein in Multiple Myeloma[51]

Summary:

In 2019, Juli and her partners By, investigated the anti-tumor potential of oleacein and the underlying bio-molecular sequelae using in vitro models of human multiple myeloma (MM). Within a low micromolar range, oleacein reduced the viability of MM primary samples and cell lines even in the presence of bone marrow stromal cells

[51] Giada Juli and others, 'Anti-Tumor Activity and Epigenetic Impact of the Polyphenol Oleacein in Multiple Myeloma', *Cancers*, 11.7 (2019), 990 <https://doi.org/10.3390/cancers11070990>.

(BMSCs), while sparing healthy peripheral blood mononuclear cells. Oleacein also inhibited MM cell clonogenicity, prompted cell cycle blockade and triggered apoptosis. They evaluated the epigenetic impact of oleacein on MM cells, and observed dose-dependent accumulation of both acetylated histones and α-tubulin, along with down-regulation of several class I/II histone deacetylases (HDACs) both at the mRNA and protein level, providing evidence of the HDAC inhibitory activity of this compound; Of potential translational significance, oleacein synergistically enhanced the in vitro anti-MM activity of the proteasome inhibitor carfilzomib. Altogether, these results indicate that oleacein is endowed with HDAC inhibitory properties, which associate with significant anti-MM activity both as single agent or in combination with carfilzomib.

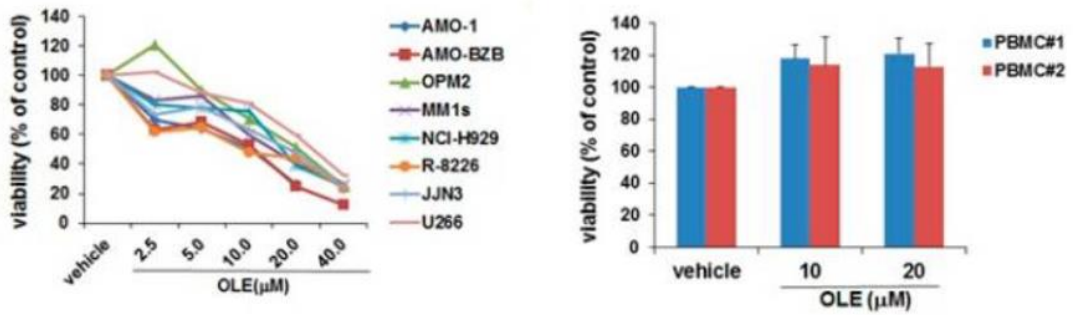


Fig. Effects of oleacein on multiple myeloma (MM) cell survival.. Cell viability of MM cell lines as determined by Cell Titer Glo (CTG) assay 48 h after treatment with increasing doses of oleacein or vehicle (DMSO). CTG assay performed on peripheral blood mononuclear cells (PBMCs) from three different healthy donors treated with oleacein for 48 h

2.10. Oleacein Attenuates Lipopolysaccharide-Induced Inflammation in THP-1-Derived Macrophages by the Inhibition of TLR4/MyD88/NF-κB Pathway[52]

Summary:

Recently, Cirmi and his partners investigated the potential antioxidant activity of oleacein, as well as its anti-inflammatory effect in lipopolysaccharide (LPS)-stimulated THP-1-derived macrophages.
LPS brought a dramatic increase of both release and gene expression of pro-inflammatory cytokines (IL-6,IL-1βand TNF-α), as well as a decrease of anti-inflammatory ones (IL-10), the effects of which arereverted by OLC. Moreover, it reduced the levels of COX-2, NO and PGE2 elicited by LPS exposurein THP-1 macrophages. Interestingly, OLC modulated inflammatory signaling pathways throughthe inhibition of CD14/TLR4/CD14/MyD88 axis and the activation of NF-κB. Finally, OLC showedrelevant anti-oxidant capability, assessed by abiotic assays, and reduced the intracellular amountof ROS generated by LPS exposure in THP-1 macrophages.

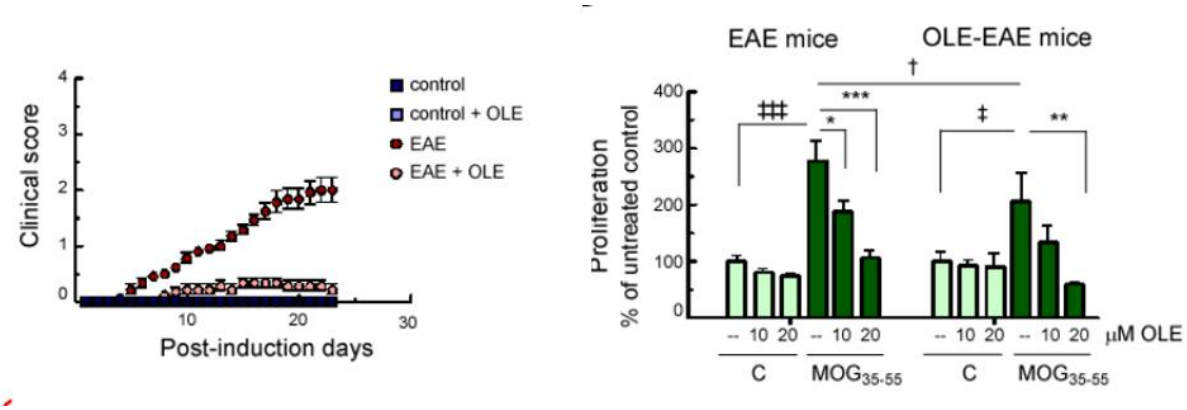


Fig 1. Oleacein (OLE) treatment protects mice from experimental autoimmune encephalomyelitis (EAE). Clinical scores of EAE in mice treated with 10 mg/kg/day of OLE Effect of OLE on splenocyte cell functions

[52] Santa Cirmi, Alessandro Maugeri, and others, 'Oleacein Attenuates Lipopolysaccharide-Induced Inflammation in THP-1-Derived Macrophages by the Inhibition of TLR4/MyD88/NF-KB Pathway', *International Journal of Molecular Sciences*, 23.3 (2022), 1206 <https://doi.org/10.3390/ijms23031206>.

Ex vivo and in vivo studies/ Μελέτες ex vivo και in vivo

2.11. Oleacein may inhibit destabilization of carotid plaques from hypertensive patients. Impact on high mobility group protein-1[53]

Summary:

In 2017 Filipek and his research team, expanded their research on the anti-inflammatory field investigating whether oleacein could have a potential role in attenuation of carotid plaque destabilization ex vivo. Plaque destabilization and rupture is a high risk in patients with hypertension the haemorrhage into carotid atherosclerotic plaque.

Samples of atherosclerotic plaque were harvested from 20 patients with hypertension /11 women and 9 men/, who underwent carotid endarterectomy after transient ischemic attacks. Matching pieces of each plaque were incubated with increased concentration of pure oleacein /range 0-20 μM/ for 24 h. In their results showed that Oleacein at the concentrations of 10 and 20 μM significantly decreased secretion of HMGB1 (up 90%), MMP-9 (up to 80%), MMP-9/NGAL complex (up to 80%) and TF (more than 90%) from the treated plaque, as compared to control. At the same time IL-10 and HO-1 release increased by more than 80%. Those results indicate that oleacein possess ability to attenuate the destabilization of carotid plaque and could be potentially useful in the reduction of ischemic stroke risk.

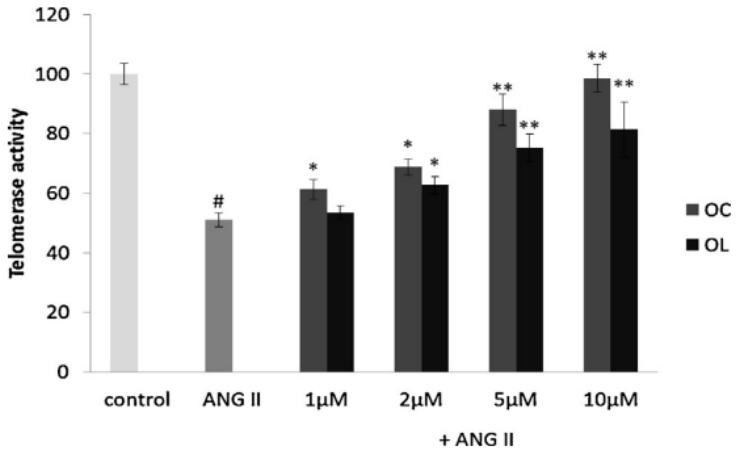
[53] Agnieszka Filipek, Monika E. Czerwińska, Anna K. Kiss, Jerzy A. Polański, and others, 'Oleacein May Inhibit Destabilization of Carotid Plaques from Hypertensive Patients. Impact on High Mobility Group Protein-1', *Phytomedicine: International Journal of Phytotherapy and Phytopharmacology*, 32 (2017), 68–73 <https://doi.org/10.1016/j.phymed.2017.06.004>.

2.12. Oleuropein and oleacein may restore biological functions of endothelial progenitor cells impaired by angiotensin II via activation of Nrf2/heme oxygenase-1 pathway[54]

Summary:

In this research Parzonko et al examined whether oleoeuropein and oleacein could protect endothelial progenitor cells against impairment of their functions due to angiotensin-induced cell senescence. These cells are responsible for neovascularization of ischaemic tissue and may participate in re-endothelization of an injured arterial wall. Angiotensin II, one of the pathological factors of hypertension, has the capacity to damage vascular endothelium by inducing oxidative stress. The researchers examined oleacein as a natural antioxidant which can protect endothelial cells.
CD31+/VEGFR-2+ cells were isolated from young healthy volunteer's blood samples and cultured on fibronectin-coated plates with angiotensin (1.0 M) in presence or absence of increasing concentrations (from 1.0 to 10.0 M) of oleacein. As compared to angiotensin II-treated cells, EPCs exposed to oleacein prior to angiotensin II showed a significant increase of proliferation and telomerase activity, and a decrease in the percentage of senescent cells and intracellular ROS formation. Oleacein restored migration, adhesion and tube formation of EPCs diminished by angiotensin II in a concentration-dependent manner.

Fig. 4. Effects of oleacein (OC) and oleuropein (OL) on telomerase activity. Cells were treated with angiotensin II (1 M) and oleuropein or oleacein in indicated concentrations



[54] Andrzej Parzonko and others, 'Oleuropein and Oleacein May Restore Biological Functions of Endothelial Progenitor Cells Impaired by Angiotensin II via Activation of Nrf2/Heme Oxygenase-1 Pathway', *Phytomedicine: International Journal of Phytotherapy and Phytopharmacology*, 20.12 (2013), 1088–94 <https://doi.org/10.1016/j.phymed.2013.05.002>.

2.13. Oleacein Attenuates the Pathogenesis of Experimental Autoimmune Encephalomyelitis through Both Antioxidant and Anti-Inflammatory Effects[55]

Summary:

In 2020, Gutiérrez-Miranda, determined the potent antioxidant and anti-inflammatory effects of oleacein in multiple sclerosis. Oxidative stress and proinflammatory cytokines are factors affecting multiple sclerosis (MS) disease progression and oleacein could have a key role in neuroinflammatory disorders treatment. The researchers investigated the impact of oleacein on the main clinic-pathological features of experimental autoimmune encephalomyelitis (EAE), an animal model for MS, including paralysis, demyelination, central nervous system (CNS) inflammation/oxidative stress and blood-brain barrier (BBB) breakdown. Mice were immunized with the myelin oligodendrocyte glycoprotein peptide, MOG35-55, to induce EAE, and OLE was administrated from immunization day. Serum, optic nerve, spinal cord and cerebellum were collected to evaluate immunomodulatory activities at a systemic level, as well as within the CNS.

The results showed that OLE treatment effectively reduced clinical score and histological signs typical of EAE. Histological evaluation confirmed a decrease in leukocyte infiltration, demyelination, BBB disruption and superoxide anion accumulation in CNS tissues of OLE-treated EAE mice compared to untreated ones. OLE significantly decreased expression of proinflammatory cytokines (IL-13, TNFα, GM-CSF, MCP-1 and IL-1β), while it increased the anti-inflammatory cytokine IL-10. Mechanistically, OLE prevented NLRP3 expression, phosphorylation of p65-NF-κB and reduced the synthesis of proinflammatory mediators induced by relevant inflammatory stimuli in BV2 cells.

[55] Beatriz Gutiérrez-Miranda and others, 'Oleacein Attenuates the Pathogenesis of Experimental Autoimmune Encephalomyelitis through Both Antioxidant and Anti-Inflammatory Effects', *Antioxidants (Basel, Switzerland)*, 9.11 (2020), E1161 <https://doi.org/10.3390/antiox9111161>.

2.14. Effects of Oleacein on High-Fat Diet-Dependent Steatosis, Weight Gain, and Insulin Resistance in Mice[56]

Summary:
In 2018, Lombardo and his research team evaluated the protective effects of oleacein, on the damages/metabolic alterations caused by high-fat diet (HFD) in male C57BL/6JolaHsd mice. After 5 weeks of treatment with 20 mg/kg of oleacein, body weight, glycemia, insulinemia, serum lipids, and histologic examination of liver tissue indicated a protective action of oleacein against abdominal fat accumulation, weight gain, and liver steatosis, with improvement of insulin-dependent glucose and lipid metabolism. Both serum parameters and hepatic histologic examination were altered in mice fed with HFD. By contrast, in the animals that received oleacein, plasma glucose, cholesterol and triglyceride serum levels, and liver histology were similar to controls fed with normocaloric diet. In addition, protein levels of FAS, SREBP-1, and phospho-ERK in liver were positively modulated by oleacein, indicating an improvement in liver insulin sensitivity. In a group of obese mice, treatment with oleacein determined a light, but still significant reduction of the increase in body weight, mainly due to lesser liver steatosis enlargement, associated with reduced levels of SREBP-1 and phospho-ERK and lower levels of total serum cholesterol; in these animals, altered plasma glucose and triglyceride serum levels were not reverted by oleacein. These results indicate that HFD-related hepatic insulin resistance may be partially prevented by oral administration of oleacein, suggesting a protective role of this nutraceutical against diet-dependent metabolic alterations.

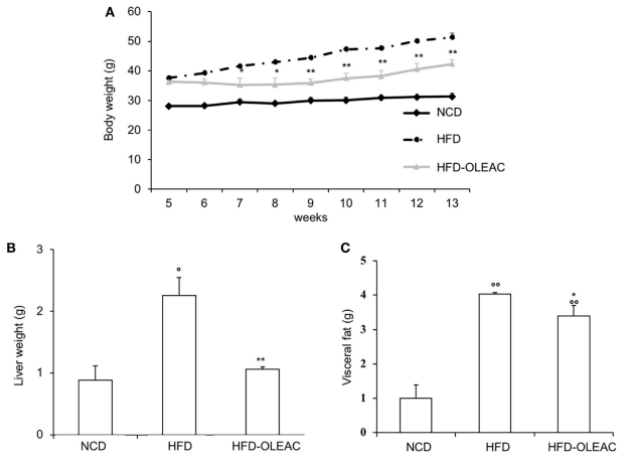


Fig6 | Effects of oleacein on body weight, visceral fat, and liver weight in obese mice fed with high-fat diet (HFD). Body weight (A), liver weight (B), and visceral fat (C) were measured in obese mice fed with HFD.

[56] Giovanni Enrico Lombardo and others, 'Effects of Oleacein on High-Fat Diet-Dependent Steatosis, Weight Gain, and Insulin Resistance in Mice', *Frontiers in Endocrinology*, 9 (2018), 116 <https://doi.org/10.3389/fendo.2018.00116>.

2.15. Oleacein Prevents High Fat Diet-Induced Adiposity and Ameliorates Some Biochemical Parameters of Insulin Sensitivity in Mice[57]

Summary:

In this research, Lepore et al, investigated the effects of oleacein on certain markers of adipogenesis and insulin-resistance in vitro, in 3T3-L1 adipocytes, and in vivo in high-fat diet (HFD)-fed mice. During the differentiation process of 3T3-L1 preadipocytes into adipocytes, oleacein strongly inhibited lipid accumulation, and decreased protein levels of peroxisome proliferator-activated receptor gamma (PPARγ) and fatty acid synthase (FAS), while increasing Adiponectin levels. In vivo, treatment with oleacein of C57BL/6JOlaHsd mice fed with HFD for 5 and 13 weeks prevented the increase in adipocyte size and reduced the inflammatory infiltration of macrophages and lymphocytes in adipose tissue. These effects were accompanied by changes in the expression of adipose tissue-specific regulatory elements such as PPARγ, FAS, sterol regulatory element-binding transcription factor-1 (SREBP-1), and Adiponectin, while the expression of insulin-sensitive muscle/fat glucose transporter Glut-4 was restored in HFD-fed mice treated with oleacein. Collectively, our findings indicate that protection against HFD-induced adiposity by oleacein in mice is mediated by the modulation of regulators of adipogenesis. Protection against HFD-induced obesity is effective in improving peripheral insulin sensitivity.

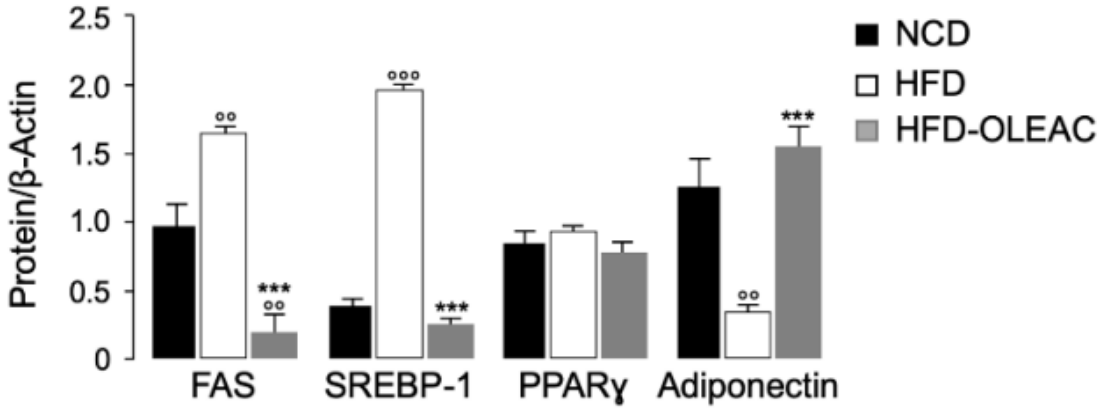
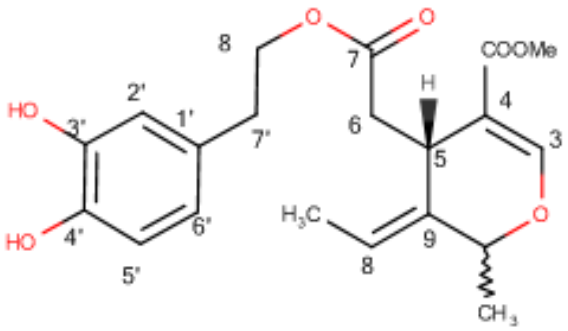


Fig. Effects of oleacein on adipogenesis-related markers. Representative Western blots of abdominal fat protein from mice

[57] Saverio Massimo Lepore and others, 'Oleacein Prevents High Fat Diet-Induced Adiposity and Ameliorates Some Biochemical Parameters of Insulin Sensitivity in Mice', *Nutrients*, 11.8 (2019), E1829 <https://doi.org/10.3390/nu11081829>.

3. OLEUROPEIN AGLYCONE – ΆΓΛΥΚΟ ΤΗΣ ΕΛΕΥΡΩΠΑΪΝΗΣ

The **monoaldehyde form of the oleuropein aglycone** is directly formed after the enzymatic deglycosylation of oleuropein after olive fruit crushing in an olive mill. It was among the first phenolic constituent that was found in olive oil phenolic fraction. The concentration of oleuropein aglycone is lower comparing to the previous constituents, but many studies have also revealed important biological activities which contribute to the health promoting properties of olive oil.



In vitro studies

3.1. Oleuropein aglycone prevents cytotoxic amyloid aggregation of human amylin[58]

Summary:
In this study Rigacci and her research team investigated the effects on amylin aggregation and cytotoxicity of the Secoiridoid oleuropein aglycone. Pancreatic amyloid deposits of amylin are a hallmark of Type II diabetes and considerable evidence indicates that amylin oligomers are cytotoxic to beta-cells. Oleuropein aglycone is a naturally occurring molecules, that could be able to hinder amylin aggregation or to protect cells against aggregate cytotoxicity.
The authors found that oleuropein aglycone, when present during the aggregation of amylin, consistently prevented its cytotoxicity to RIN-5F pancreatic beta-cells. A lack of interaction with the cell membrane of amylin aggregates grown in the presence of oleuropein was shown by fluorescence microscopy and synthetic lipid vesicle permeabilization. Moreover, our ThT assay, circular dichroism analysis and electron microscopy images suggested that oleuropein interferes with amylin aggregation, resulting in a different path skipping the formation of toxic pre-fibrillar aggregates.
These results provide data for the possible pharmacological use of oleuropein aglycone to prevent or to slow down the progression of type II diabetes.

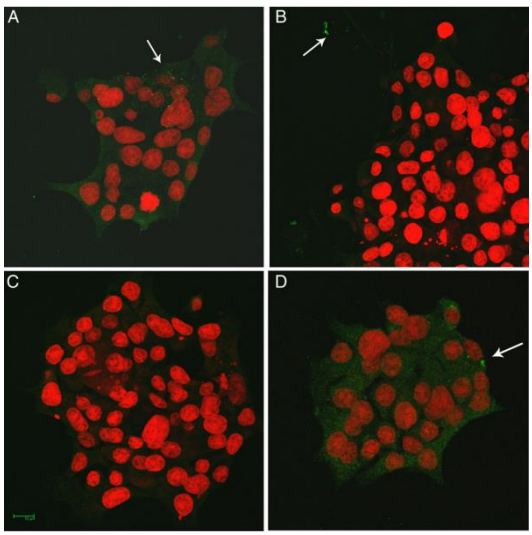


Fig. 2. (A) Cells treated with hIAPP. (B) Cells treated with hIAPP incubated with oleuropein. (C) Control, untreated cells. (D) Cells treated with hIAPP that was aged without oleuropein and given to cells together with oleuropein

[58] Stefania Rigacci and others, 'Oleuropein Aglycone Prevents Cytotoxic Amyloid Aggregation of Human Amylin', *The Journal of Nutritional Biochemistry*, 21.8 (2010), 726–35 <https://doi.org/10.1016/j.jnutbio.2009.04.010>.

3.2. Oleuropein aglycone stabilizes the monomeric α-synuclein and favours the growth of non-toxic aggregates[59]

Summary:

In this study Palazzi and her research team studied whether oleuropein aglycone exhibits anti-amyloidogenic power in vitro by interacting with, and stabilizing, α-synuclein monomers. α-synuclein plays a key role in the pathogenesis of Parkinson's disease (PD); its deposits are found as amyloid fibrils in Lewy bodies and Lewy neurites, the histopathological hallmarks of PD. Amyloid fibrillation is a progressive polymerization path starting from peptide/protein misfolding and proceeding through the transient growth of oligomeric intermediates widely considered as the most toxic species.

The researchers showed that oleuropein aglycone exhibits anti-amyloidogenic power in vitro by interacting with, and stabilizing, α-synuclein monomers thus hampering the growth of on-pathway oligomers and favouring the growth of stable and harmless aggregates with no tendency to evolve into other cytotoxic amyloids. They also found that OleA reduces the cytotoxicity of α-synuclein aggregates by hindering their binding to cell membrane components and preventing the resulting oxidative damage to cells.

[59] Luana Palazzi and others, 'Oleuropein Aglyconee Stabilizes the Monomeric α-Synuclein and Favours the Growth of Non-Toxic Aggregates', *Scientific Reports*, 8.1 (2018), 8337 <https://doi.org/10.1038/s41598-018-26645-5>.

3.3. Extra-virgin olive oil polyphenols inhibit HER2 (erbB-2)-induced malignant transformation in human breast epithelial cells: Relationship between the chemical structures of extra-virgin olive oil secoiridoids and lignans and their inhibitory activities on the tyrosine kinase activity of HER2[60]

Summary:

Menendez et al in their study explored the ability of oleuropein aglycone to modulate HER2 tyrosine kinase receptor-induced in vitro transformed phenotype in human breast epithelial cells. Using MCF10A normal breast epithelial cells it was further determined the relationship between chemical structure of oleuropein aglycone and its inhibitory activities on the tyrosine kinase activity of the HER2 oncoprotein. When compared with untreated cells, MCF10A/HER2 cells, treated with oleuropein aglycone, grew less dense, were significantly bigger in volume and showed a profound reorganization of cell-cell contacts with the appearance of multiple extrusions. Oleuropein aglycone was one of the most active inhibitors of HER2 expression in MCF10A/HER2 cells, with a reduction 63%, and IC50 64µM. HER2 overexpression further promoted an exacerbated sensitivity to the apoptotic effects of oleuropein aglycone.

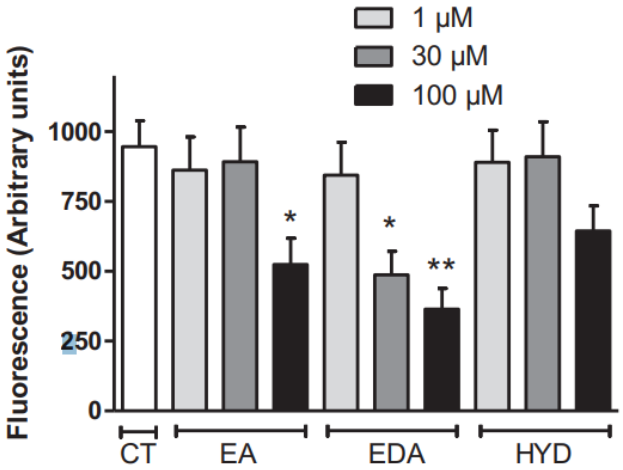
[60] Javier A. Menendez, Alejandro Vazquez-Martin, Cristina Oliveras-Ferraros, and others, 'Extra-Virgin Olive Oil Polyphenols Inhibit HER2 (ErbB-2)-Induced Malignant Transformation in Human Breast Epithelial Cells: Relationship between the Chemical Structures of Extra-Virgin Olive Oil Secoiridoids and Lignans and Their Inhibitory Activities on the Tyrosine Kinase Activity of HER2', *International Journal of Oncology*, 34.1 (2009), 43–51.

In vivo studies

3.4. Involvement of endothelium in the vasorelaxant effects of 3,4-DHPEA-EA and 3,4-DHPEA-EDA, two major functional bioactives in olive oil[61]

Summary:

In 2016, Segade and his research team, studied the endothelium-dependent vasorelaxant effect of oleuropein aglycone and oleacein in rat aorta, starting at ~1 µM, and an endothelium-independent vasorelaxant effect, starting at ~10 µM. Oleuropein aglycone and oleacein, also increased NO generation within endothelial cells. At higher concentrations, the two compounds reduced arginine-vasopressin-induced increase of cytosolic Ca2+ concentration ([Ca2+]c) in vascular myocytes. By UV-visible spectroscopy, researchers found that these polyphenols undergo autoxidative processes in organ-bath conditions. Oleuropein aglycone and oleacein have an endothelium-dependent vasorelaxant effect caused by an enhanced NO production, probably through a redox mechanism within endothelial cells and an endothelium-independent vasorelaxant effect mediated by a reduction of agonist-induced [Ca2+]c increase in vascular myocytes. Bearing in mind the plasmatic concentrations of these polyphenols following dietary intake of olive oil, these effects could modulate vascular tone in vivo.



Fig– Effects of a 15-min preincubation with 3,4 DHPEAEA, 3,4 DHPEA-EDA or hydroxytyrosol (1, 30 and 100 M) on the increases in fluo-4 fluorescence (indicative of [Ca2+]c increases) induced by the administration of AVP (control; CT) in A7r5 vascular myocytes52

[61] María Segade and others, 'Involvement of Endothelium in the Vasorelaxant Effects of 3,4-DHPEA-EA and 3,4-DHPEA-EDA, Two Major Functional Bioactives in Olive Oil', *Journal of Functional Foods*, 23 (2016), 637–46 <https://doi.org/10.1016/j.jff.2016.03.024>.

3.5. Effects of a polyphenol present in olive oil, oleuropein aglycone, in a murine model of intestinal ischemia/reperfusion injury[62]

Summary:

The aim of this study which was conducted by Campolo, was to investigate the effects of Oleuropein aglycone on the modulation of the secondary events in mice subjected to intestinal ischemia/reperfusion injury (IRI). This was induced in mice by clamping the superior mesenteric artery and the celiac trunk for 30 min, followed by release of the clamp, allowing reperfusion for 1 h. After 60 min of reperfusion, animals were killed for histological examination of the ileum tissue and immunohistochemical localization of proinflammatory cytokines (TNF-α and IL-1β) and adhesion molecules (ICAM-1 and P-sel). The results obtained by the histological and molecular examinations showed in Oleuropein aglycone-treated mice, a decrease of inflammation and apoptosis pathway versus SAO-shocked mice.

[62] Michela Campolo and others, 'Effects of a Polyphenol Present in Olive Oil, Oleuropein Aglyconee, in a Murine Model of Intestinal Ischemia/Reperfusion Injury', *Journal of Leukocyte Biology*, 93.2 (2013), 277–87 <https://doi.org/10.1189/jlb.0712317>.

3.6. The Polyphenol Oleuropein Aglycone Modulates the PARP1-SIRT1 Interplay: An In Vitro and In Vivo Study[63]

Summary:

In this study Luccarini and his research team, studied the neuroprotective effects of oleuropein aglycone against Alzheimer disease. Poly(ADP-ribose) polymerase-1 (PARP1) activation contributes to the cascade of events initiated by amyloid- (A) peptide eventually leading to cell death in Alzheimer's disease brain. A significant accumulation of PAR polymers and increase of PARP1 expression were detected in the cortex at the early (3.5 months) and intermediate (6 months) stage of A deposition in the TgCRND8 mouse model

The researchers found that 8-week oleuropein aglycone treatment (50 mg/kg of diet) to 6-month-old TgCRND8 mice rescued to control values PARP1 activation. In vitro and in vivo, the OLE-induced reduction of PARP1 activation was paralleled by the overexpression of Sirtuin1 (SIRT1), and, in vivo, by a decrease of NFB and the pro-apoptotic marker p53. In N2a cells, they also found that OLE potentiates the MNNG-induced increase of Beclin1 levels. In conclusion, our data show that OLE treatment counteracts neuronal damage through modulation of the PARP1-SIRT1 interplay.54

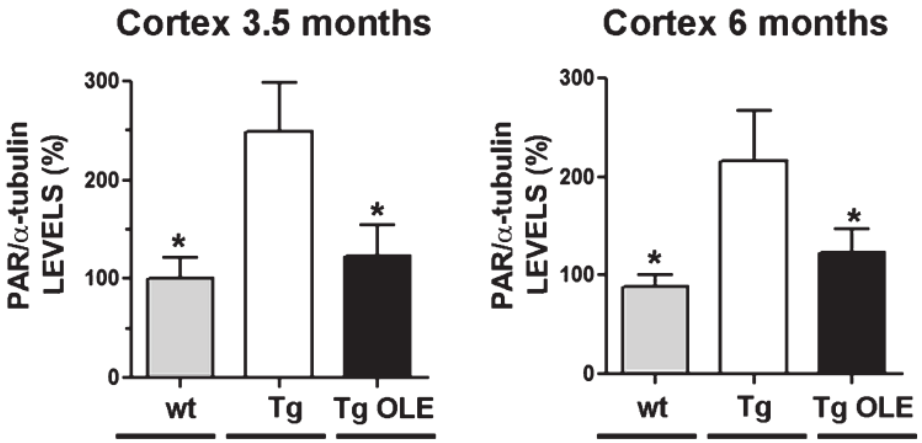
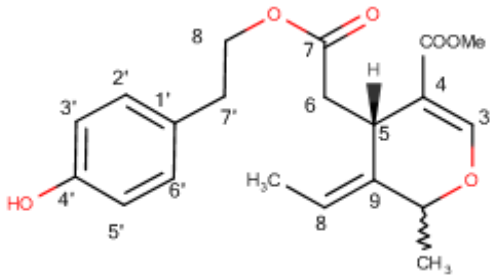


Fig. 1. OLE inhibits PARylation in TgCRND8 mice. Western blotting analysis of PAR protein levels in the cortex of 3.5- and 6-month-old untreated wt and Tg mice and OLE-fed Tg mice

[63] Ilaria Luccarini and others, 'The Polyphenol Oleuropein Aglyconee Modulates the PARP1-SIRT1 Interplay: An In Vitro and In Vivo Study', *Journal of Alzheimer's Disease: JAD*, 54.2 (2016), 737–50 <https://doi.org/10.3233/JAD-160471>.

4. LIGSTROSIDE AGLYCONE – ΆΓΛΥΚΟ ΤΟΥ ΛΙΓΚΣΤΡΟΣΙΔΗ

The **monoaldehyde form of the ligstroside aglycone** is directly formed after the enzymatic deglycosylation of ligstroside after olive fruit crushing in an olive mill. Ligstroside and ligstroside aglycone have similar structure to oleuropein and oleuropein aglycone with the only difference the hydroxy phenolic group in its structure. The concentration of the monoaldehyde form of ligstroside aglycone differs in different olive oils but in general is similar to oleuropein's aglycone and lower than oleocanthal's and oleacein's. The biological activities of ligstroside aglycone are presented through the studies that have been conducted below.



4.1. Olive secoiridoids and semisynthetic bioisostere analogues for the control of metastatic breast cancer[64]

Summary:

In 2013, Belnaser and his research team based on oleocanthal's anti cancer activity, they examined the anticancer activity other phenolics like ligstroside aglycone.
The researchers assessed the c-MET inhibitory activity as well as antiproliferative, antimigratory, and anti-invasive activities against the highly metastatic human breast cancer cell line MDA-MB231 of many olive oil phenolic constituents. Ligstroside aglycone showed the best antimigratory activity. Generally, tyrosol esters showed better activities versus carbamate analogues.. Olive oil secoiridoids are excellent scaffolds for the design of novel c-MET inhibitors.

4.2. Anti-Proliferative Effects of an Extra-Virgin Olive Oil Extract Enriched in Ligstroside Aglycone and Oleocanthal on Human Liver Cancer Cell Lines[65]

Summary:

In this research, De Stefanis and her research team, investigated the anticancer activity of ligstroside aglycon on hepatocellular carcinoma.Hepatocellular carcinoma is a malignant tumor with high mortality rates. Systemic chemotherapy is only marginally effective and is frequently complicated by toxicity. Hepatocellular carcinoma cell lines become more sensitive to taxol when it is combined with Tumor Necrosis Factor α (TNFα). The present work aimed to assess the effects of a polyphenolic extract containing both oleocanthal and ligstroside aglycone on proliferation and/or death in three liver cancer cell lines (HepG2, Huh7 and Hep3B). The possibility to enhance such effect by the addition of TNFα was also investigated. Both cell proliferation and death were enhanced by the exposure to the polyphenolic extract. Such effect was associated with induction of autophagy and could be potentiated by TNFα. The presence of

[64] Belnaser A. Busnena and others, 'Olive Secoiridoids and Semisynthetic Bioisostere Analogues for the Control of Metastatic Breast Cancer', *Bioorganic & Medicinal Chemistry*, 21.7 (2013), 2117–27 <https://doi.org/10.1016/j.bmc.2012.12.050>.
[65] Daniela De Stefanis and others, 'Anti-Proliferative Effects of an Extra-Virgin Olive Oil Extract Enriched in Ligstroside Aglycone and Oleocanthal on Human Liver Cancer Cell Lines', *Cancers*, 11.11 (2019), E1640 <https://doi.org/10.3390/cancers11111640>.

ligstroside aglycone in the extract lowered the oleocanthal concentration required for cytotoxicity. These results show for the first time that the effects of a polyphenol extract can be potentiated by TNFα and that modulation of autophagy likely account for these effects.

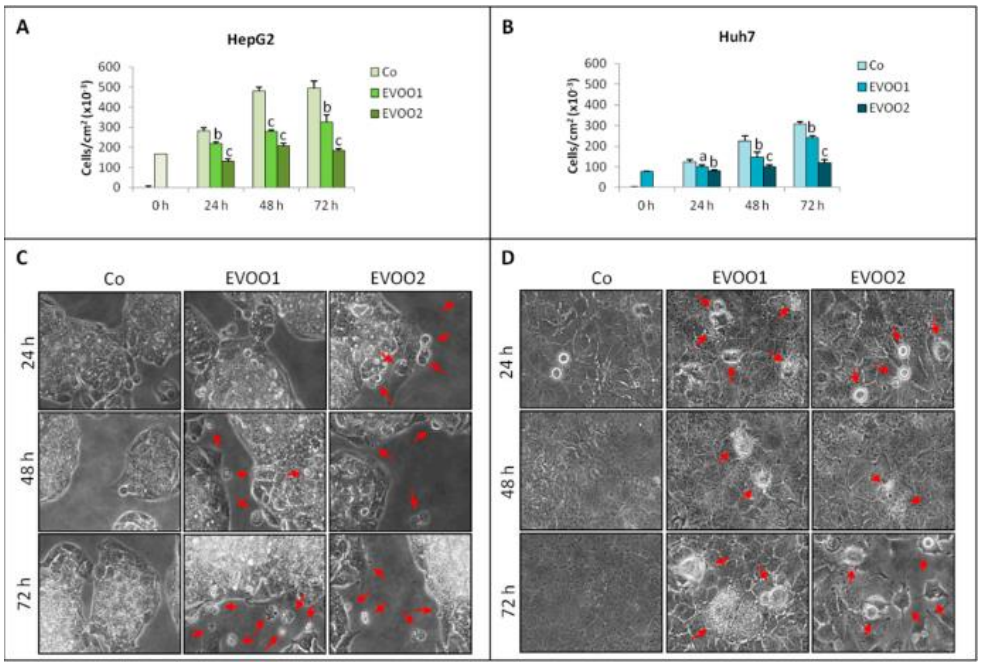


Fig. Phenolic extract reduces liver cancer cell proliferation. (A, C) Growth curve and morphological analysis on HepG2 cell line. (B, D) Growth curve and morphological analysis on Huh7 cell line. The cells were incubated for 24–72 h with two different doses of the extra-virgin olive oil (EVOO) extract.

4.3. tabAnti-HER2 (erbB-2) oncogene effects of phenolic compounds directly isolated from commercial Extra-Virgin Olive Oil (EVOO)[66]

Summary:

Menendez et al in their study explored the ability of ligstroside aglycone to modulate HER2 tyrosine kinase receptor-induced in vitro transformed phenotype in human breast epithelial cells. Using MCF10A normal breast epithelial cells it was further determined the relationship between chemical structure of ligstrocide aglycon and its inhibitory activities on the tyrosine kinase activity of the HER2 oncoprotein. When compared with untreated cells, MCF10A/HER2 cells, treated with ligstroside aglycone, grew less dense, were significantly bigger in volume and showed a profound reorganization of cell-cell contacts with the appearance of multiple extrusions. Ligstroside aglycone was one of the most active inhibitors of HER2 expression in MCF10A/HER2 cells, with a reduction 68%, and IC50 10µM. HER2 overexpression further promoted an exacerbated sensitivity to the apoptotic effects of ligstroside aglycone. These findings molecularly support epidemiological evidence revealing that lingstrocide aglycon anti-breast cancer effects primarily affect the occurrence of breast tumors overexpressing the type I receptor tyrosine kinase HER2 but further suggest that its stereochemistry might provide an excellent and safe platform for the design of new HER2 targeted anti-breast cancer drugs.

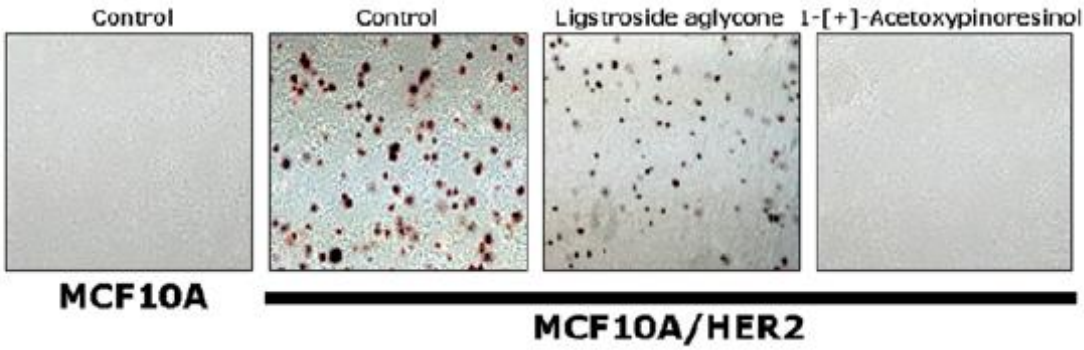


Fig. Effects of the EVOO polyphenols on the transforming ability of HER2.

[66] Javier A. Menendez, Alejandro Vazquez-Martin, Rocio Garcia-Villalba, and others, 'TabAnti-HER2 (ErbB-2) Oncogene Effects of Phenolic Compounds Directly Isolated from Commercial Extra-Virgin Olive Oil (EVOO)', *BMC Cancer*, 8 (2008), 377 <https://doi.org/10.1186/1471-2407-8-377>.

The European Project ARISTOIL - Interreg Med at the top of Europe.

In 8 October 2019, the European Competition Interreg Project Slam took place in Brussels. The competition aimed at promoting the best interregional projects in terms of impact for European Societies for the year 2019. ARISTOIL project took part and was selected as the best euro-mediterranean project among hundreds. The final competition took place among the best 8 projects coming from several european regions and selected among hundreds of european territorial cooperation projects. The ARISTOIL project team presented the innovative production of high phenolic olive oil and its great impact for human health and possibilities for Mediterranean development. The nice performance coupled with its interesting content won the audience's electronic vote!

The ARISTOIL project is coordinated by Dr. Nikos Krimnianiotis, representing the lead partner, EGTC Efxini Poli and its partnership includes partners from Greece (EGTC Efxini Poli, the Region of Peloponnese and the Department of Pharmacognosy and Chemistry of Natural Products, National and Kapodistrian University of Athens), from Italy (Euro Mediterranean Center for the Sustainable Development and the Free Minicipal Consortium of Ragusa), from Spain (the Provincial Government of Malaga and the Department of Analytical Chemistry, University of Cordoba), from Cyprus (ARISTOLEO and Larnaca-Famagusta District Development Agency) and from Croatia (Faculty of Chemistry and Technology, University of Split). The main aim of the project is the reinforcement of Mediterranenan olive oil sector competitiveness through development and application of innovative production and quality control methodologies related to olive oil protecting properties.

The beneficiaries of the project include over 3,000 olive oil producers and olive millers (SMEs), analysis of over 5,000 olive oil samples and several conference on national and international level, as well as networking events, trainings, seminars and info-days on a regional and local level.

After the completion of the project, in January 2020, the work towards the project's aim will continue through the ARISTOIL Cluster, the first cluster for the production of olive oil rich in polyphenols.

Project co-financed by the European Regional Development Fund

ARISTOIL PLUS

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