Gold in Olive Oil

Common Name
Hydroxytyrosol

Chemical Name
3,4-Dihydroxyphenylethanol

CAS Number
10597-60-1

Molecular Formula
C_8H_{10}O_3

Molecular weight
154.164 Daltons
Hydroxytyrosol may be generated endogenously from dihydroxyphenylacetic acid through dihydroxyphenylacetic reductase of the brain [2]. It can also be generated from dopamine [3]. De la Torre et al. [4] have suggested that the understanding of the function of Hydroxytyrosol is hampered by the fact that Hydroxytyrosol is a metabolite of dopamine and that Hydroxytyrosol concentration in body fluids is a combination of the exogenous and endogenous sources. Exogenous sources are olives and olive oil. The fact that hydroxytyrosol occurs naturally in human body and the history consumption of exogenous sources of hydroxytyrosol states that hydroxytyrosol is a safe substance.

A recent analysis of more than 1.5 million healthy adults demonstrated that: “Greater adherence to a Mediterranean diet is associated with a significant improvement in health status, as seen by a significant reduction in overall mortality (9%), mortality from cardiovascular diseases (9%), incidence of or mortality from cancer (6%), and incidence of Parkinson’s disease and Alzheimer’s disease (13%). These results seem to be clinically relevant for public health, in particular for encouraging a Mediterranean-like dietary pattern for primary prevention of major chronic diseases” [1].

In the mediterranean diet daily consumption of olive oil, with its biophenol contents, is a key for a better health; more than 20 clinical trials attested it. The secret in olive oil is its most active biophenol, hydroxytyrosol.

**Health advantages of mediterranean diet**

**Hydroxytyrosol** is the health secret of olive oil daily intake

An expert panel of EFSA (European Food Safety Authority) declared its positive scientific opinion in which hydroxytyrosol enriched and standardized olive oil is helpful in preventing Lipoprotein (LDL) particles oxidative damages; so this healthfull effect can prevent one of the reason of cardiovascular diseases.

Where to find Hydroxytyrosol in nature

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TIROSOIL HT®
A patented process
Lachifarma had been investing in R&D activities on hydroxytyrosol since 1995.
Lachifarma manufactures TIROSOIL HT® with its patented technology EU patent n° EP 1623960 A1, US patent n° 7,427,358 and others.

TIROSOIL HT® Characterization
TIROSOIL HT® is obtained at high purity (> 98%) of Hydroxytyrosol.
Related substances specification:
- Tyrosol (NMT 0.5%);
- Gallic Acid (NMT 0.1%).

![Figure 1 - TIROSOIL HT® typical chromatogram UV-Vis at 280 nm](image)
Hydroxytyrosol chromatographic purity 99.6% in analysis of batch n. 10-007

![Figure 2 - Mass spectra of chromatographic peak at 16.793 min](image)

HYDROXYTYROSOL (HT) is one of the major phenolic compounds present in olives and olive oil. Evidence generated by Lachifarma R&D Department shows that TIROSOIL HT® (Hydroxytyrosol > 98% purity) possesses the following properties.

TIROSOIL HT® anti-oxidant activity
The anti-oxidant activity of Hydroxytyrosol measured with ORAC(1) test compared with other well known substances shows that Hydroxytyrosol has a strong anti-oxidant activity. For example the parent molecule of Hydroxytyrosol, Oleuropein, shows a modest anti-oxidant activity, significantly inferior to Hydroxytyrosol.

![Figure 3 - ORAC (oxygen radical absorbance capacity) of hydroxytyrosol vs other powerfull antioxidants](image)

![Figure 5 - TIROSOIL HT® reduces UV inflammation](image)
It has been demonstrated that TIROSOIL HT® (Hydroxytyrosol >98% purity) protects the monolayer epithelial cells against damage caused by hyper-oxidation that consists of:
- reduction of the cellular vitality;
- abnormal increase of the permeability of the membrane and therefore inflammation and oxidation of polyunsaturated acids of cellular membranes.

Fig. 4 - Cell without damage and cell under free radicals attack

Positive effect of TIROSOIL HT®  
Negative effect without TIROSOIL HT®

Proteic degradation
Oxidation of membrane lipids
DNA damage
Oxidative enzyme activation (i.e. kinase)
Oxidized LDL accumulation (foam cells)

Thanks to its double hydrophilic and lipophilic nature and its small dimension Hydroxytyrosol passes through, more than other molecules, cell membranes and acts as effective protective of the cells from the oxidative injuries provoked by free radicals. The protecting effect of Hydroxytyrosol against the oxidations has turned out to be really powerful.

TIROSOIL HT® anti-inflammatory activity
Through proprietary controlled human trial it was demonstrated that TIROSOIL HT® reduces inflammation produced by UV exposition and produces also anti-aging effects.

![Figure 5 - TIROSOIL HT® reduces UV inflammation](image)

(1) An Oxygen Radical Absorbance Capacity (ORAC) score is a test tube analysis that measures the antioxidant levels of chemical substances.
TOXICOLOGICAL EVALUATION

The toxicological evaluation showed that TIROSOIL HT® is a safe product:

- Acute oral toxicity: non toxic [48]
- Subchronic toxicity on rats: non toxic
- Cytotoxicity: non cytotoxic
- Skin irritation: non irritant
- Skin sensitization: non sensitizing
- Embryo-fetal toxicity/reproductive toxicity: non toxic [49]
- Mutagenic test: non mutagenic [49]
- Genotoxicity test: non genotoxic [49]

CONCLUSIVE REMARKS

TIROSOIL HT® is a highly purified hydroxytyrosol extract obtained from a selected Italian variety of Olea Europea, particularly rich in polyphenols. From Mediterranean tradition a highly purified and standardized extract is particularly rich in polyphenols. From Mediterranean tradition a highly purified and standardized extract is effective in preventing oxidative stress and radical damage with well established health benefits. In particular TIROSOIL HT® Oleosan is enriched hydroxytyrosol and standardized olive oil able to preserve lipoprotein (LDL particles) against oxidative damage, avoiding in this way atherosclerotic plaques, protecting against cardiovascular risk diseases.

Bibliography

The following is evidence taken from the literature supporting the health benefits of TIROSOIL HT®.

### NON HUMAN STUDIES

<table>
<thead>
<tr>
<th>Activities</th>
<th>Effects</th>
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<tbody>
<tr>
<td><strong>Antioxidant and radical scavenging activity</strong></td>
<td>Hydroxytyrosol reduces risk of cardiovascular diseases (CVD) and atherosclerosis because in vitro inhibits copper sulfate-induced oxidation of LDL [5, 6]. Hydroxytyrosol acts as antioxidant and metal chelator in animal models [7, 8, 9]. Hydroxytyrosol effectively scavenges superoxide anion generated by either human polymorphonuclear cells or by xanthine/xanthine oxidase system [10]. Hydroxytyrosol protects against hydrogen peroxide-induced damage in human erythrocytes [11]. Hydroxytyrosol [50 μM] prevents DNA damage and tyrosin nitration induced by peroxynitrite [12-13]. In in vivo studies, low doses of Hydroxytyrosol, [414 μg/rat] are able to inhibit passive smoking-induced oxidative stress [14].</td>
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<td><strong>Anti-Inflammatory activity</strong></td>
<td>Hydroxytyrosol has proven to inhibit chemically induced platelet aggregation, the accumulation of the pro-aggregator agent thromboxane in human serum, the production of pro-inflammatory leukotrienes by activated human leukocytes, and to inhibit arachidonate lipoxygenase [15-18].</td>
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<td><strong>Activity on plasma lipoproteins</strong></td>
<td>Data from a controlled study conducted on 200 healthy male subjects, showed a decrease in TC-to-HDL-C ratio in proportion with increasing intake of hydroxytyrosol in virgin olive oil. An increase in HDL-C was also noted with increasing hydroxytyrosol concentration of olive oil [21]. Consumption of phenol rich (hydroxytyrosol) virgin olive oils resulted in increased circulating HDL-C ranging between 5.1–6.7% in two further human studies [22, 23]. Additionally, an earlier study showed a significant decrease in LDL-C after one week of phenol rich virgin olive oil consumption [24].</td>
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<td><strong>Activity on lipid oxidation</strong></td>
<td>Oxidation of LDL causes damage to the vascular wall, stimulating macrophage uptake and formation of foam cells, which in turn result in the formation of plaque in the arterial wall [26, 27]. Human and animal in vivo studies showed that the level at which LDL oxidizes, decreases linearly with increasing phenolic and hydroxytyrosol concentration [21, 28, 29, 26, 22, 23, 24]. A mechanistic study, demonstrated that hydroxytyrosol and phenolic compounds are able to bind to LDL and the authors suggest that this may account for the increase in LDL resistance to oxidation [30].</td>
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<td><strong>Activity on oxidative DNA damage</strong></td>
<td>A randomized crossover intervention trial showed that intake of hydroxytyrosol and phenol rich virgin oil decreases oxidative DNA damage by up to 30% compared with intake of low phenol virgin olive oil [32].</td>
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<td><strong>Activity on additional markers of oxidation</strong></td>
<td>Goya et al. [34] treated a sample of human hepatoma HepG2 cells with hydroxytyrosol and found that there was a decrease in ROS production [34]. Total plasma antioxidant activity was also reported to increase in humans after intake of olive oil phenolic compounds [35, 36, 23]. Oxidative stress can be indicated by the presence of markers such as F2-isoprostanes, lipid peroxides (LPO), oxidized glutathione (GSSG), reduced glutathione (GSH) and glutathione peroxidase (GSH-Px). F2-isoprostanes are a result of the free radical induced peroxidation of arachidonic acid. LPO is more than likely a by-product of the oxidation of fatty acids [38] and depletion of the protective GSH precedes lipid oxidation and atherogenesis in vivo [39]. Human studies showed beneficial effects of hydroxytyrosol and other phenolic compounds of olive oil on the aforementioned markers of oxidative stress. A randomized cross over study found the intake of olive oil phenolic-enriched breakfast significantly lowered F2-isoprostane levels compared with a low phenolic-enriched breakfast [38]. Visioli and colleagues [40] demonstrated that consumption of hydroxytyrosol and phenolic-rich virgin olive oil was associated with a significant decrease in urinary excretion of F2-isoprostanes. Covas and colleagues [21] found that hydroxytyrosol-rich virgin olive oil beneficially modulated the balance between GSH and GSSG, while Weinbrenner and colleagues [23] found an increase in GSH/Px after hydroxytyrosol-rich virgin olive oil administration in human subjects. Moreover, a decrease in LPO after olive oil rich in hydroxytyrosol and the administration of other phenolic compounds was noted [21, 23].</td>
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<td><strong>Activity on markers of inflammation</strong></td>
<td>Bogani and colleagues [35] found a decrease in TXB2 and LTB4 concentrations with increasing hydroxytyrosol and phenolic concentrations of olive oil. These results were also in accordance with previous investigations [36, 23]. Interleukin-6 (IL-6) IL-6 is a pro-inflammatory agent that stimulates inflammation in response to trauma and C-reactive protein (CR) generally rises when inflammation is present, so Fito and colleagues found that daily consumption of olive oil rich in hydroxytyrosol and phenolic compounds decreased the circulating concentrations of both IL-6 and CRP in 28 stable coronary heart patients.</td>
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<td><strong>Activity on platelet function</strong></td>
<td>Olive oil rich in hydroxytyrosol showed to inhibit the expression of endothelial adhesion molecule upon incubation with human umbilical vein endothelial cells. Virgin olive oil containing a high content (400 mg/kg) of hydroxytyrosol and phenolic compounds was demonstrated to also decrease plasminogen activator inhibitor-1 (PAI-1) and factor VII (FVII). Both PAI-1 and FVII are pro-coagulant factors that have been linked to the development of coronary heart disease (CHD).</td>
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Elevated levels of total cholesterol (TC) and low density lipoprotein cholesterol (LDL-C) are risk factors for atherosclerosis, which is the primary cause of cardiovascular disease (CVD). However elevated high density lipoprotein cholesterol (HDL-C) levels has a protective, anti-inflammatory properties [19,20].

Oxidative stress produced by reactive oxygen species (ROS) has been linked to a number of diseases such as atherosclerosis, certain cancers and neurodegenerative diseases [29, 33].

Atherosclerosis and CVD [25]. LDL oxidation (oxLDL) is considered to be a major risk factor for the development of atherosclerosis and CVD [25].

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