

The most innovative
Green Tea extract

.....
Caffeine free,
fully bioavailable

.....
Clinical and
pharmacological data
support its efficacy

.....
A safe product, devoid of
any side effect




Greenselect[®]
Phytosome[®]

■ Tea is second only to water as the most popular beverage in the world; it is prepared by infusion of the leaves of *Camellia sinensis* L., originally grown in the South of China where it has been cultivated since the ancient times. According to the Chinese tradition, tea drinking was introduced in China in the third millennium BC, during the reign of the legendary emperor Shen Nong. At first tea was regarded as a **medicine**, and its **stimulant** and **invigorating properties** were much appreciated by the Buddhist monks, who contributed to its diffusion throughout China and Japan by encouraging the habit of tea drinking as a **remedy for intemperance**. The development of a flourishing trade led to the introduction of tea cultivations in Japan, and since the 19th century in India, Ceylon, Java, Sumatra, Georgia and Kenya.



Types of tea

There are three main groups of teas: black tea, oolong tea and green tea.

- **Black tea** is obtained from the leaves allowed to ferment (enzymatic fermentation) and then dried. During fermentation there is an extensive enzymatic oxidation of the catechin polyphenols.
- **Oolong (or Wulong) tea** is obtained from partially fermented leaves; heating at a predetermined stage stops the fermentation process when required.
- **Green tea** is produced by steaming or drying fresh leaves at elevated temperatures, and this process leaves the polyphenols intact.

Green tea is widely used in China, Japan and other Eastern countries, while black tea is more diffuse in the Western countries. Nevertheless, green tea is also known and used in North America and Europe. The average consumption of tea catechins in the Eastern countries rises to 700-900 mg of tea catechins (by HPLC), whereas one "Western" cup of tea* only provides 50 mg of tea catechins (by HPLC), a much lower quantity compared to the Asian average consumption. In many Eastern cultures, tea is in fact often served at fancy social events as afternoon tea and tea parties

Green tea infusion contains **flavanols** (catechin polyphenols), **flavonols** and **phenolic acids**.¹ Several flavanols have been isolated and identified from green tea, and two of these, **(-)-epigallocatechin 3-O-gallate (EGCG)** and **(-)-epigallocatechin (EGC)** exhibited a **marked antioxidant activity**.

*2 g dry leaves in 200 ml hot water, infused for 5 minutes

Clinical Use

The **antioxidant activity** of green tea has been extensively studied; however, the results obtained from dietary intervention studies are controversial probably due to the differences in the considered population and in experimental protocols.

Consumption of green tea has been correlated with **low incidence of chronic pathologies** in which oxidative stress seems to be involved, such as cancer or cardiovascular diseases: a higher dietary intake of antioxidant compounds might be considered **preventive** in the **onset of ROS (Reactive Oxygen Species)-related diseases** in humans by increasing the antioxidant potential of subjects.²

The effectiveness of a moderate intake of **Greenselect®** (green tea extract) on the antioxidative status, on lipid profile in plasma, the resistance to oxidative damage of lipids and DNA structures as well as the activity of antioxidant enzymes on lymphocytes have been investigated.^{3,4} The importance of demonstrating the antioxidant effects of tea in human beings is emphasized by the evidence that catechins are extensively metabolized *in vivo* and that the antioxidant and biological activities of their metabolites may differ from those of their original compound.

Epidemiological data also indicate that green tea consumption may help weight management by **improving energy expenditure**. The weight loss promotion appears to be sustained by the thermogenic effect exerted by galloyl catechinic derivatives.⁵ Greenselect® Phytosome® has been tested on a multicentric trial versus low calories diet only⁶ to assess its capability to **promote weight loss**.

Clinical studies on Greenselect® Phytosome®

■ **Weight loss promotion of Greenselect® Phytosome® on obese and overweight patients**⁵

One hundred patients affected by overweight and obesity have been treated with 150 mg of Greenselect® Phytosome® twice daily (300 mg/day). Along the study all patients (treated and placebo) have been subjected to a low calories diet (1250-1350 Kcal for women and 1650-1750 Kcal for men) distributed in at least 4 meals per day.

Parameters such as body weight, body mass index, waistline, total cholesterol, basal glycemia and total triglycerides have been measured at the beginning, after 45 days and after 90 days (end of the study).

The average weight loss was of 6 kg in the diet only group and 14 kg in the treated group. Accordingly, relevant results have been reported in terms of body mass index, waistline and blood parameters (Chart 1). Greenselect® Phytosome® may be a valid support in **weight management**.

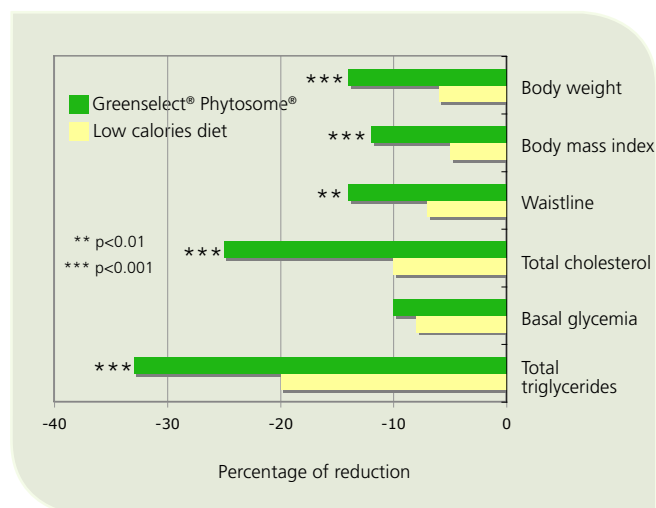


Chart 1: Effect of Greenselect® Phytosome® on weight loss promotion

Clinical studies on Greenselect®

■ **Effectiveness of Greenselect® on improvement of antioxidant defenses in plasma**⁴

Greenselect® was administered at a dosage of 160 mg/b.i.d. (320 mg/day) to 24 healthy female volunteers held on a controlled diet, for six weeks' treatment. At the end of the treatment, the subjects taking Greenselect® had a significantly lower LDL cholesterol level, while the Total Antioxidant Activity (TAA) was significantly increased compared to control subjects (Chart 2 and 3).

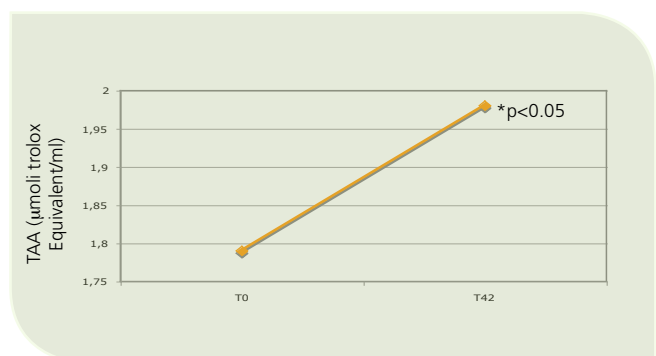


Chart 2: Effect of Greenselect® on total antioxidant activity in plasma



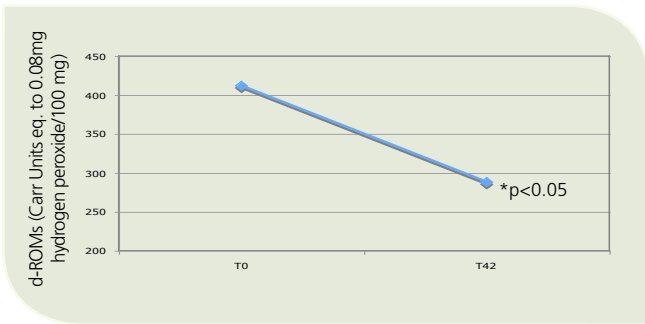


Chart 3: Effect of Greenselect® on peroxide level measured by Carr Units

It is noticeable that blood samples were obtained 12 hours after the last consumption, indicating that the effect on the antioxidative status of plasma persists at least for that period of time. When tea consumption is regular, in fact, the mean plasma concentration of catechins reaches a steady state significantly higher than the baseline levels, and remains for at least 12 hours after the last ingestion. The results of this study suggest that a moderate intake of Greenselect® in healthy subjects **improves antioxidant defenses** in plasma and protects lymphocytes from DNA oxidative damage.

Pharmacokinetics

Bioavailability of flavonoids, both in aglyconic or glycosidic form, is reported to be low and erratic due to limited absorption, elevated presystemic metabolism and rapid elimination. Flavonoid aglycons and glycosides, once ingested, reach the small intestine intact and then are metabolized to the methylated, glucuronidated or sulfated metabolites. Flavonoids or flavonoids metabolites that reach the colon are usually further metabolized by local enzymes and then absorbed. This figure is at the base of a **non-linear pharmacokinetics** and has to be taken into account when evaluating the relationship among *in vitro* biochemical effects, oral dosages and plasma levels.⁶

Previous experiences indicate that the complexation of polyphenolic compounds with phospholipids improves the bioavailability of the natural molecules. In order to **increase the bioavailability** of green tea polyphenols, **Greenselect® Phytosome®** was produced from the complexation of Greenselect® (a standardized caffeine free green tea extract) with soy phospholipids in a 1:2 ratio.

■ Improvement of EGCG plasma concentrations with Greenselect® Phytosome®⁷

Twelve healthy male volunteers were randomly divided in two groups. One received a single dose of Greenselect® (containing 240 mg of tea catechins by HPLC). The second group received 1,200 mg of Greenselect® Phytosome® (containing 240 mg of tea catechins by HPLC). EGCG was chosen as the biomarker for absorption. The peak concentration at 2 hours is more than doubled with Greenselect® Phytosome® in comparison with to the non complexed Greenselect®. Further the plasma levels of EGCG remain considerably higher with Greenselect® Phytosome® (Chart 4).⁷

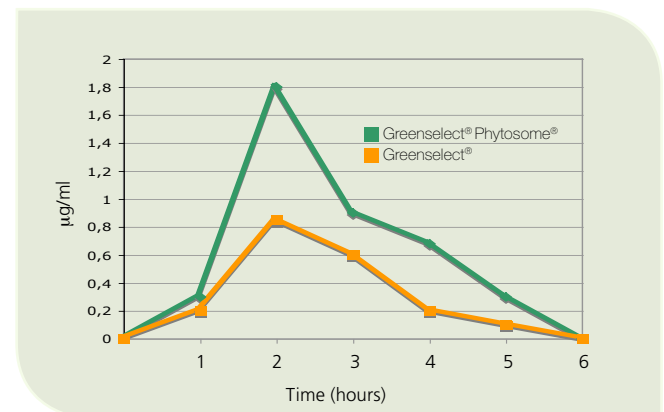


Chart 4: Time course of EGCG after ingestion of Greenselect® and Greenselect® Phytosome®

■ Improvement of radical antioxidant parameter (TRAP) with Greenselect® Phytosome®⁷

In the same trial, with Greenselect® and Greenselect® Phytosome® the antioxidant parameter TRAP, measured as Trolox equivalents, has been evaluated. A 20% increase of the antioxidative capacity was observed in the volunteers treated with Greenselect® Phytosome® compared to the ones treated with Greenselect® (Chart 5).

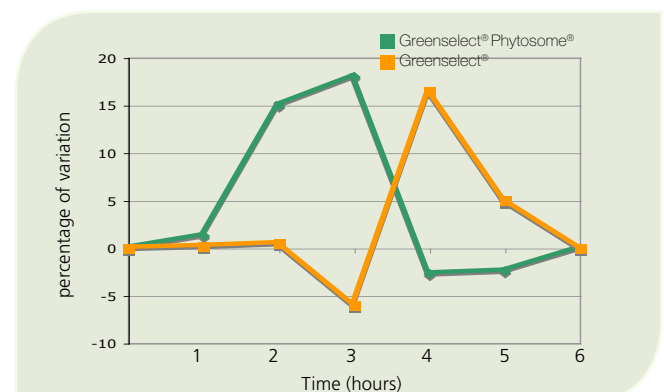
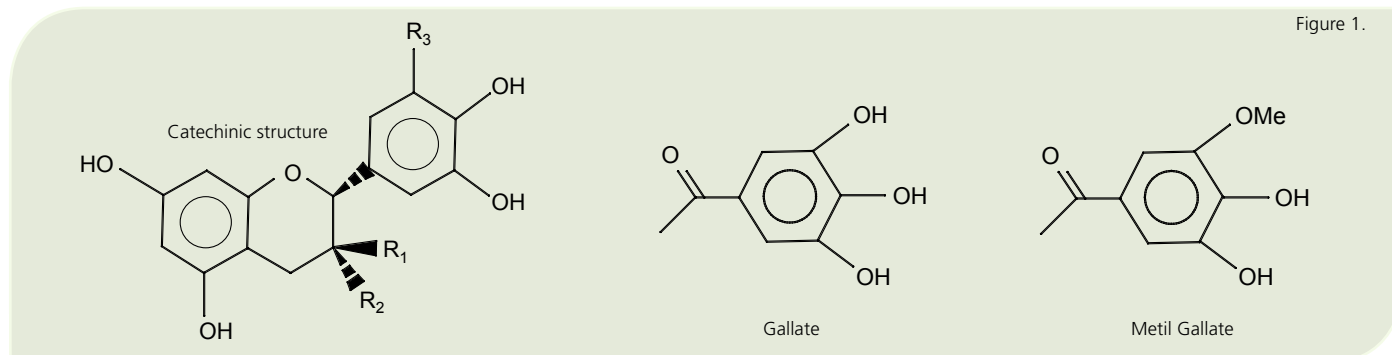


Chart 5: TRAP modification with Greenselect® and Greenselect® Phytosome®

Chemical profile

Greenselect® Phytosome® is a complex of a standardized caffeine free green tea extract (Greenselect®) and soy phospholipids. Its active constituents are mainly represented by a group of compounds having a flavan-3-olic structure and generally defined as **green tea catechins**. There are seven main catechins in green tea extract, whose structures are represented below (Figure 1, Table 1).



The active constituents of green tea leaves are this group of polyphenols belonging to the flavanol class.

Greenselect® contains not less than 60% of catechins, [the main constituent (-)-epigallocatechin 3-O-gallate (EGCG) accounting for at least 40% of the extract].

Greenselect® Phytosome® results from the **complexation** of Greenselect® with soy phospholipids in a 1:2 ratio.

Greenselect® Phytosome® thus contains 19% to 25% of catechins, the main constituent (-)-epigallocatechin 3-O-gallate accounting for at least 13% of Greenselect® Phytosome® (see Table 2).

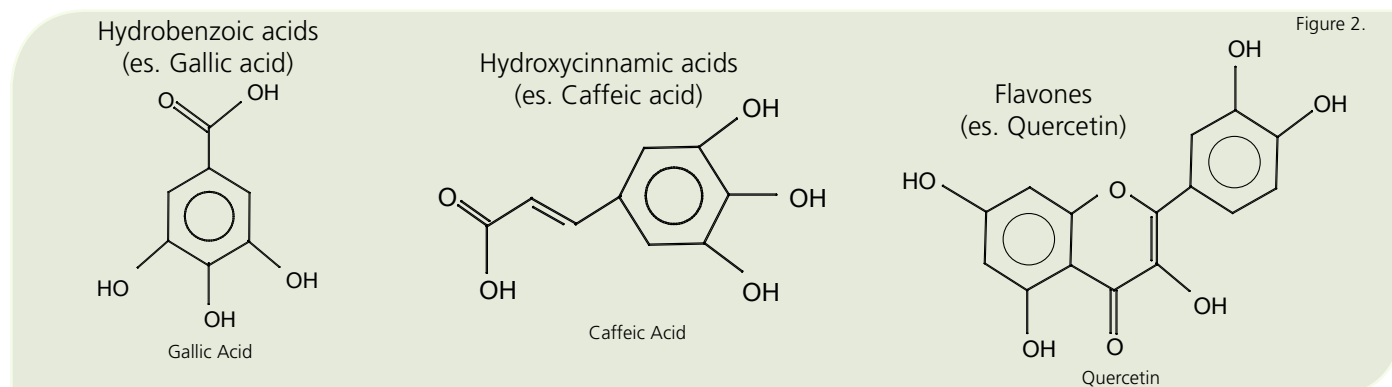
Other polyphenols are actually present in green tea leaves and its extracts Greenselect® and Greenselect® Phytosome®. The most representative are shown in Figure 2.

Catechins	R ₁	R ₂	R ₃
(-)-Epigallocatechin	H	OH	OH
(+)-Catechin	OH	H	H
(-)-Epicatechin	H	OH	H
(-)-Epigallocatechin 3-O-gallate	H	OG	OH
(+)-Gallocatechin 3-O-gallate	OG	H	OH
(-)-Epigallocatechin 3-O-(3'-O-methyl)-gallate	H	OGMe	OH
(-)-Epicatechin 3-O-gallate	H	OG	H

HPLC content of catechins expressed as (-)-epigallocatechin 3-O-gallate	19.0-25.0%
HPLC content of (-)-epigallocatechin 3-O-gallate	≥ 13.0%

A common method of analysis for green tea extracts is Folin-Ciocalteu method, where polyphenols are determined as gallic acid. This spectrophotometric assay is simple but unspecific, including compounds different from catechins, as hydrobenzoic acids, hydroxycinnamic acids, flavones or catechin dimers, trimers and polymers (see Figure 2).

Indena HPLC method provides a simple identification and quantification of the catechin derivatives monomeric catechins and their galloyl derivatives (see Table 1). The specificity of this method has been extensively studied by HPLC-MS.



Pharmacology

Several epidemiological studies have suggested the association between green tea consumption and a reduced risk of different kind of human diseases. In fact, the antioxidant activity of green tea flavonoids has been related to a protecting effect on human health by the prevention of degenerative diseases. Pharmacological activities as cancer prevention, cardioprotection, antimutagenic activity, hypocholesteremic activity, glucose management, antibacterial and anti-inflammatory have been reported. Ongoing evaluations⁵ also suggest a potential aid of **Greenselect[®] Phytosome[®]** on **weight management**. Following are some of the most up to date references on pharmacological data on Greenselect[®] and Greenselect[®]Phytosome[®].

■ Cardiovascular protection

- Epigallocatechin 3-O-gallate contained in Greenselect[®] protects cardiac myocytes from ischemia-reperfusion induced apoptosis. EGCG has been demonstrated to reduce STAT-1 phosphorylation and protects cardiac myocytes from I/R induced apoptic cell death.⁸ The cell death reduction is associated with improved haemodynamic recovery and ventricular function, being able to mediate cardioprotection and enhance cardiac function.

- Greenselect[®] protects from hypoxia/reoxygenation injury in cultured cardiac cells.⁹ The experimental models of hypoxia/reperfusion have shown the generation of a magnitude of free radicals. The antioxidant capacity of Greenselect[®] might therefore be important in the prevention of myocardial ischemia, but might also be useful in counteracting extensive tissue damage in the luckless case of ischemia onset.

- Greenselect[®] polyphenol extracts attenuates ischemia/reperfusion injury in the gut.¹⁰ The treatment provided a

substantial reduction of the nitration of proteins, the formation of pro-inflammatory cytokines and may be useful in treating conditions associated with I/R of the gut as well as other organs.

- Greenselect[®] has an antioxidant capacity ranging from 7800-8700 $\mu\text{mol TE/g}$ evaluated by ORAC_{FL} test and expressed as Trolox Equivalents/g according to the methodology described by Ou *et al.*¹¹

■ Cancer-preventing activity

The cancer preventive effects of green tea are widely supported by results from epidemiological, cell culture, *in vivo* and human studies. They show that tea polyphenols potently induce apoptic cell death in tumor cells but not in their normal counterparts. Green tea polyphenols were shown to affect multiple pathways.

- Greenselect[®] Phytosome[®] provides antineoplastic activity by reducing the appearance of aberrant crypt foci, polyps and tumors in the rat colon *in vivo*.¹²

- Greenselect[®] polyphenols induced apoptosis in human osteosarcoma SAOS-2 cells involving a complex mechanism of downregulation of nuclear factor K-B.¹³

■ Glucose management

- Greenselect[®] modulates α_1 adrenergic stimulated glucose transport in cultured rat cardiomyocytes,¹⁴ by interrupting the PCK signaling pathways.

■ Anti-inflammatory activity

- Greenselect[®] exerts anti-inflammatory properties due to its inhibitory effects of several pro-inflammatory genes.¹⁵

Conclusive remarks

The complexed form of green tea catechins (Greenselect[®] Phytosome[®]) proved to be **more bioavailable** compared to the uncomplexed form. The results obtained in the *in vitro*, *in vivo* and in human trials suggest that Greenselect[®] Phytosome[®] is **effective and safe** for the prevention of some of the most common diseases. The free radical scavenging capacity of the extract accounts for most of the biological activities, Greenselect[®] Phytosome[®] is also reported to trigger **other mechanisms of action**.

- Enhancement of the antioxidant defence systems^{8,9}
- Inhibition of enzymatic activities involved in tumor promotion¹²
- Inhibition of enzymatic pathways by downregulation of gene expression involved in cancer initiation¹³
- Stimulation of α_1 adrenergic stimulated glucose transport¹⁴
- Interference with the formation of pro-inflammatory cytokines¹⁵
- Prevention of fatty acids alteration¹⁶

Greenselect[®] Phytosome[®] is a caffeine free extract from green tea leaves complexed in the Phytosome[®] form to improve bioavailability and efficacy. It is an important aid in the **prevention of free radicals-mediated tissue damages** and **weight management**.

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