Leucoselect® is a grape seed extract with a well-defined chemical composition, which was completely elucidated by HPLC-UV, GPC and HPLC-TSP-MS as follows: 9

- 15% (+)-catechin, (-)-epicatechin
- 80% (-)-epicatechin gallate, dimers, trimers, tetramers and their gallates
- 5% pentamers, hexamers, heptamers and their gallates

This high content of smaller size OPCs is crucial for the biological activity being the absorption of procyanidins affected by molecular weight. 10 Hence procyanidins of higher molecular weight are discarded in the production of Leucoselect®, allowing for the concentration of the smaller interesting molecules dimers, trimers, tetramers and their gallates.

To further improve their bioavailability, Leucoselect® has been formulated with soy phospholipids (about 1:2.6 w/w), thus obtaining Leucoselect® Phytosome®.

Its cardiovascular protecting activity is supported by four clinical trials, 11-14 and by extensive pharmacological data. 16-25
Clinical studies

The efficacy of Leucoselect® Phytosome® was verified at a dosage corresponding to 300 mg/day of procyanidins in five clinical trials. It was proven effective in improving antioxidant defenses and in countering the oxidative stress, both in normal and in circumstances characterized by an increase in oxidant generation and a decrease in antioxidant protection.

**Improvement of the total antioxidant capacity of plasma in healthy volunteers**

Leucoselect® Phytosome® was administered for 5 days to 20 young subjects in a single-blind randomized placebo-controlled crossover trial. The product induced a significant increase of serum total antioxidant capacity (TRAP) assessed on day 1 and day 5, starting already from 30 min postdose with a further increase at 60 min postdose, in comparison with baseline values (chart 1).

Antioxidant defenses protect low-density lipoproteins (LDLs) from oxidation by free radicals

**Improvement of plasma oxidative status in healthy volunteers after a fatty meal**

The capacity of Leucoselect® Phytosome® to prevent the plasma oxidative stress after a fatty meal, rich in lipidic peroxides (“Milanese” steak and French fries), has been evaluated in 8 healthy volunteers. At the beginning of the trial the subjects received the lipidic peroxides rich meal and after a week the same meal and Leucoselect® Phytosome®. The product was proven able to reduce the oxidative stress induced by the meal, providing a significant reduction of plasma postprandial lipid hydroperoxide concentration (chart 2) with an increase of TRAP and resistance of LDLs to oxidative modification.

Leucoselect® Phytosome® is able to prevent plasma postprandial oxidative stress. It decreases the oxidants, increases the antioxidant levels in plasma and enhances the resistance of LDLs to oxidative modification.
**Reduction of LDL susceptibility to oxidative stress in heavy smokers**

Leucosect® Phytosome® was administered for 4 weeks to 24 healthy male heavy smokers, aged 50 or more, in a randomized double-blind crossover trial. The product induced a significant improvement of LDL resistance to oxidation, as shown by lipid peroxidation parameters: thiobarbituric acid reactive substances concentration (TBARS, an index of lipid peroxidation and oxidative stress) was significantly reduced while the lag phase (an index of LDL resistance to oxidation) was prolonged, both in comparison with placebo and basal values (table 1).

Leucosect® Phytosome® is endowed with a significant efficacy in a common model of oxidative stress as smoking.

**Cigarette smoke contains carbon and oxygen-centered free radicals, which can directly initiate and propagate the process of lipid peroxidation**

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Leucosect® Phytosome®</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>basal</td>
<td>4 weeks Δ%</td>
</tr>
<tr>
<td>TBARS (nmol/mg proteins)</td>
<td>0.56±0.10</td>
<td>0.57±0.08 +5.0</td>
</tr>
<tr>
<td>Lag phase (min)</td>
<td>59.0±13.0</td>
<td>57.7±9.8 -0.13</td>
</tr>
</tbody>
</table>

*p<0.005 vs baseline  *p<0.05, *p<0.005 vs placebo

**Improvement of oxidative stress in humans with impaired glucose homeostasis**

Leucosect® Phytosome® was administered for 4 weeks to 24 subjects with sugar metabolism challenges in a double blind crossover parallel study, significantly reducing urinary excretion of 8-epi-PGF$_2$α in comparison with placebo. Enhanced urinary excretion of 8-epi-PGF$_2$α is a marker of oxidative stress linked with increased formation of F$_2$ isoprostanes, non enzymatic products of arachidonic acid peroxidation.

**A glucose disregulation is associated with enhanced lipid peroxidation and cardiovascular challenges**

**Improvement life conditions in elderly subjects**

In a randomized, double blind, placebo-controlled study, Leucosect® Phytosome® was administrated for 4 weeks at a dosage of 300 mg/die to 20 elderly subjects challenged by various conditions linked to the aging processes. The results demonstrated a link between these epidemiological observations and the intake of procyanidins, showing that Leucosect® Phytosome® could change the profile of plasma cytokines in terms of a potentiation of the immune response and a moderation of the inflammatory response markers. In particular, responses mediated by T-helper cells of type 1 were upregulated (IL-2, interferon-gamma), while those mediated by T-helper cells of type 2 were attenuated (production of TNF-alfa).
Pharmacology

The pharmacological profile of Leucoselect® Phytosome® has been defined by extensive *in vitro* and *in vivo* experimental studies. For the *in vitro* studies the unformulated form has been used in.

- **in vitro**

  - antioxidant activity
    
    Leucoselect® demonstrated its effective antioxidant capacity through different mechanisms: free radical scavenging activity, chelation of transition metals, inhibition of enzymes, quenching of singlet oxygen, sparing and regenerating effect on α-tocopherols.

- **in vivo**

  - antioxidant activity in rats
    
    Leucoselect® Phytosome®, administered for 3 weeks at 2.4% concentration in a standard diet, increased TRAP in young and aged rats (Δ% = +40 and +30) and physiological antioxidant defences of plasma.

  - cardiovascular protective activity
    
    Leucoselect® reduced ischemia/reperfusion injury concurrently stimulating prostacyclin release in the isolated rabbit heart, protected endothelial cells from peroxynitrite-induced damage and modulated the endothelium-dependent NO release in human artery. Furthermore, Leucoselect® was proven effective on several enzymes involved in the degradation of the extravascular matrix.

- arterial health in rabbits

  Leucoselect® Phytosome®, administered at 2% concentration in a standard diet, was proven effective for arterial health.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% coverage by lesion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard chow-fed diet</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>0.25% cholesterol-rich diet</td>
<td>18.2±7.6</td>
</tr>
<tr>
<td>0.25% cholesterol-rich diet + 2% Leucoselect® Phytosome®</td>
<td>3.0±1.9</td>
</tr>
</tbody>
</table>

Table 2: percentage coverage of aortic arch by fatty lesions in mild experimental arterial challenge in rabbits.
Leucoselect® Phytosome®, administered for 3 weeks at 2.4% concentration in a standard diet, supported heart health in young and aged rats.

The recovery of myocardial function, expressed by left ventricular developed pressure (LVDP), at the end of reperfusion was 93% and 74% of the preischemic values, respectively (chart 4).

The protective effect on heart contractility was also strictly associated with a preserved coronary blood flow, expressed by the reduction of coronary perfusion pressure (CPP) close to the preischemic value both in young and aged rats (chart 5).

**Conclusive remarks**

Leucoselect® Phytosome® was proven able to reduce oxidative stress and to improve plasma antioxidant defences.

The efficacy of Leucoselect® Phytosome® is guaranteed by its fully elucidated chemical composition and its standardized content of smaller size OPCs.

The patented Phytosome® formulation further enhances OPCs bioavailability; hence it represents an effective and safe aid supporting the cardiovascular system.
References

25. Study performed at the University of Milan, Institute of Pharmacological Sciences, School of Pharmacy, Prof. C. Galli. (Indena S.p.A., data on file).