

LEUCOSELECT™ PHYTOSOME®

BIOAVAILABLE GRAPE SEED PROCYANIDINS
FOR THE PROTECTION OF THE CARDIOVASCULAR SYSTEM





FROM VITAMIN P TO THE “FRENCH PARADOX”

In the thirties, Hungarian investigators discovered that flavonoids, commonly present in the human diet, had relevant properties in respect to the cardiovascular system.¹ Flavonoids and other polyphenols were described as exerting a vitamin-like action, which the investigators named vitamin P activity, primarily associated with the capacity to improve capillary resistance and to normalize an altered capillary permeability. In the following years, a controversial debate took place in the scientific community about the validity of the term vitamin P, and finally this denomination was rejected since the role of polyphenols was recognized to be not comparable to that of vitamins. Nevertheless what remained well demonstrated by a number of pharmacological tests, pharmacokinetic evidences and clinical trials was the tropism of some polyphenols for the cardiovascular system. Within this huge family of natural products, some specific compounds, such as grape procyanidins, demonstrated an elevated specificity in targeting the cardiovascular system. This constituted the basis for the development (in some European countries) of prescription drugs

containing standardized extracts from grape seeds (a part of the plant particularly rich in procyanidins) and devoted to the relief of disturbances of microcirculation.

Recently, another interesting finding originating from epidemiology, suggested that red wine (very rich in polyphenols) could exert a preventive action in respect of the development of chronic diseases of the cardiovascular system, such as atherosclerosis.² This evidence was revealed mostly from the evaluation that the low incidence of cardiovascular diseases in France is paralleled by a very high dietary intake of lipids, but concomitantly by an elevated consumption of red wine. Scientists agree that this last event well explains the apparent “French Paradox” and they suggest that the “antioxidant properties” of red wine polyphenols could be good candidates for the preventive effect.^{3,4}

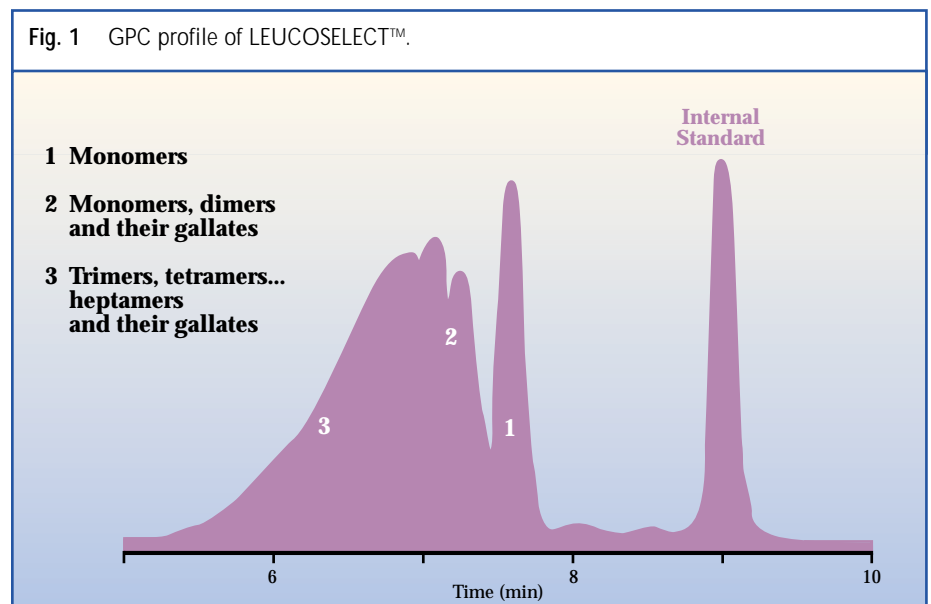
Currently, a number of papers are available dealing with the biological properties of natural polyphenols, most of the studies having been conducted *in vitro* and focused on monomeric products.

In this respect, the studies on grape procyanidins constitute an unique example, since they deal with **oligomeric polyphenols** and include both *in vitro* and *in vivo* investigations.

Most of the studies performed on grape procyanidins were made possible by the availability of standardized extracts which could guarantee the constancy of composition of such a complex chemical mixture. Recently, the composition of the industrially produced grape seed extract LEUCOSELECT™ (in Fig. 1 the GPC profile) has been completely elucidated after fractionation with Sephadex®. HPLC-UV and HPLC-TSP MS allowed the identification of (+)-catechin, (-)-epicatechin, their dimers and (-)-epicatechin gallate. ES-MS allowed the detection of trimers, tetramers, pentamers, hexamers, heptamers and their mono-, di- and trigallates.⁵ The composition of LEUCOSELECT™ is as follows:

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

Fig. 1 GPC profile of LEUCOSELECT™.



LEUCOSELECT™ demonstrated *in vitro* the following properties:⁶⁻⁸

- DPPH scavenging activity
- Hydroxyl radical entrapping capacity after water sonolysis (ESR study)
- Superoxide anion quenching action in a non-enzymatic generating system
- Peroxyl radical scavenging activity in phosphatidylcholine liposomes and methyl-linoleate micelles stimulated by ultrasounds and UV
- Fe²⁺/Cu²⁺ chelating properties
- Sparing effect on α-tocopherol
- Regenerating effect on α-tocopherol
- Inhibition of proteases
- Inhibition of xanthine oxidase
- Protection of rabbit heart against ischemia/reperfusion injury
- Stimulation of prostacyclin release from isolated rabbit heart

All these properties make LEUCOSELECT™, at least *in vitro*, a complete natural antioxidant and from a biochemical standpoint one of the most efficient among botanical derivatives.

This was a strong basis for investigating the activity *in vivo*. For this purpose, LEUCOSELECT™ has been complexed with soy phospholipids to obtain LEUCOSELECT™ PHYTOSOME® (EP 0 275 224; US Patent 4, 963, 527). Complexation of polyphenols with phospholipids, in fact, improves their oral bioavailability which is generally low and erratic.^{9,10}

LEUCOSELECT™ PHYTOSOME®

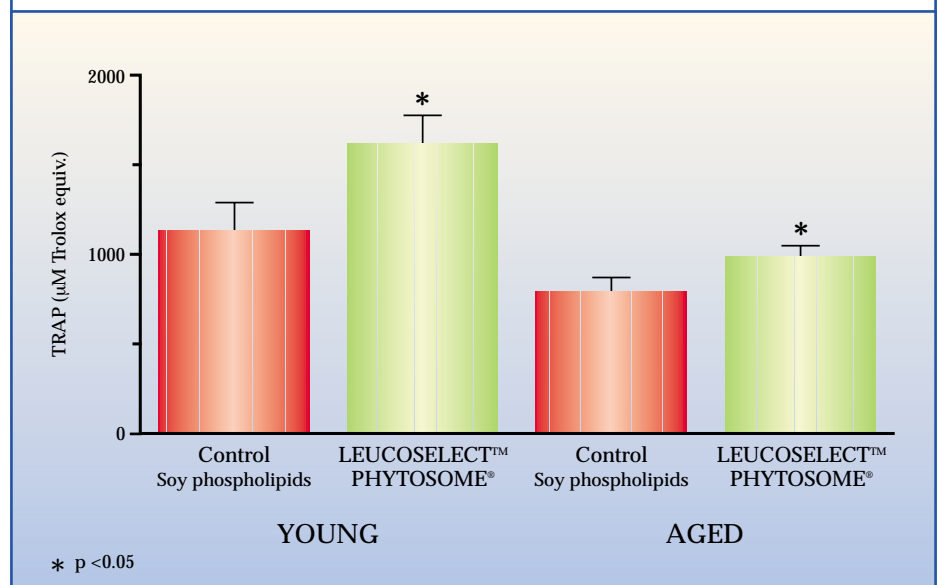
Bioavailable standardized grape procyanidins for the protection of the cardiovascular system.

Several *in vivo* investigations have been performed in order to establish the capacity of the product to exert its properties following oral administration in both experimental animals and human volunteers.

The main pharmacological properties of LEUCOSELECT™ PHYTOSOME®, highlighted in these studies, are:

- increase in total antioxidant capacity and stimulation of physiological antioxidant defences of plasma in young and aged rats fed on a diet containing LEUCOSELECT™ PHYTOSOME® at the concentration of 2.4% for 30 days (Fig. 2).¹¹
- protection against ischemia/reperfusion

Fig. 2 Effect of LEUCOSELECT™ PHYTOSOME® diet (2.4%) on total plasma antioxidant capacity (TRAP) in young and aged rats.



induced damages in the heart of young and aged rats fed on a diet containing LEUCOSELECT™ PHYTOSOME® at the concentration of 2.4% for 30 days (Fig. 3).¹¹

- protective effects of 8-week treatment with a diet containing 2% LEUCOSELECT™ PHYTOSOME® against mild atherosclerosis induced

in rabbits by using a balanced 0.2% cholesterol-rich diet (Fig. 4).¹²

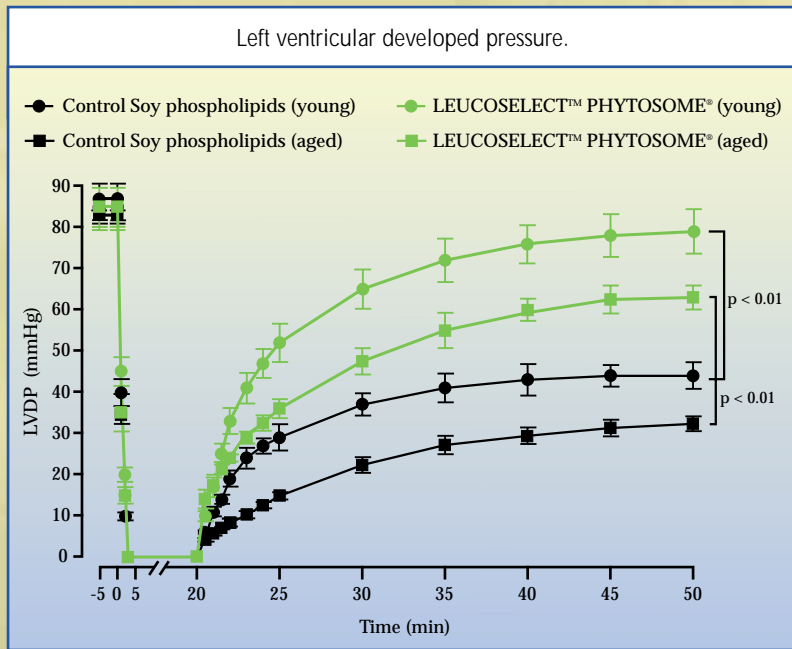


Fig. 3 - Effect of LEUCOSELECT™ PHYTOSOME® diet (2.4%) against ischemia/reperfusion induced damages in the heart of young and aged rats.

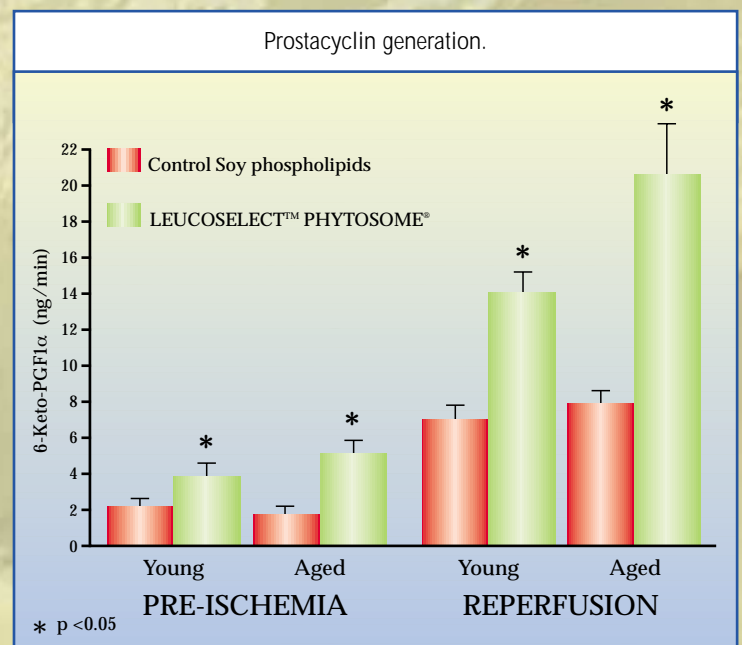
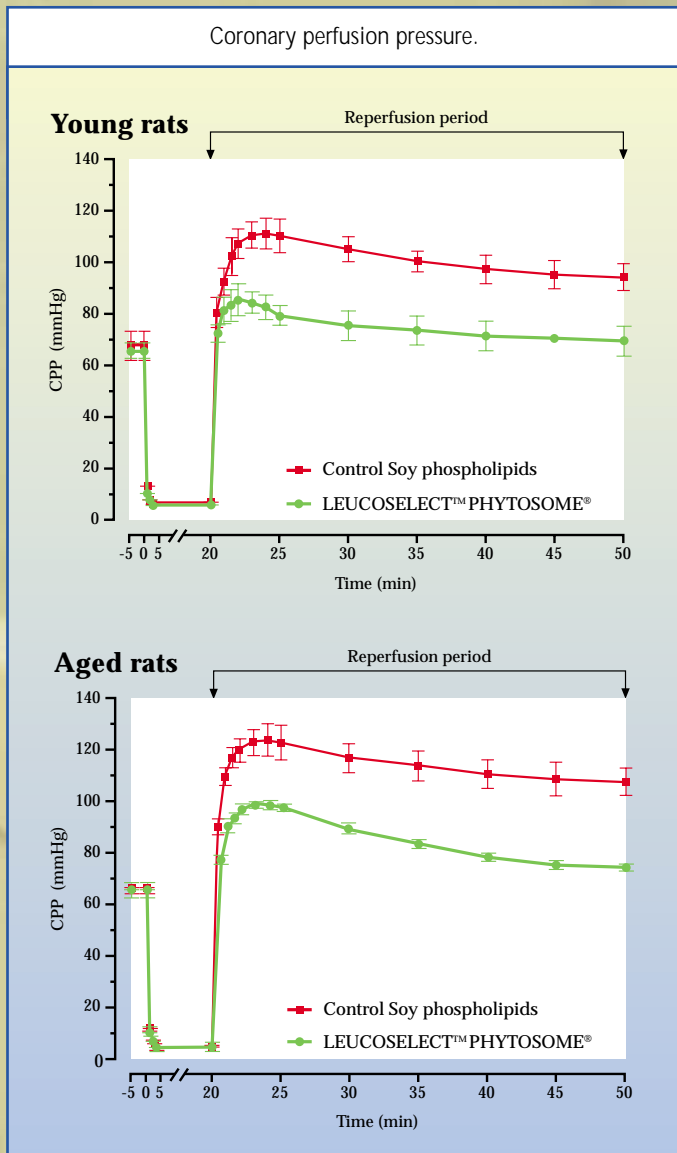


Fig. 4 Effect of LEUCOSELECT™ PHYTOSOME® diet (2%) on fatty lesions in aortic arch of rabbits fed on 0.2% cholesterol diet.

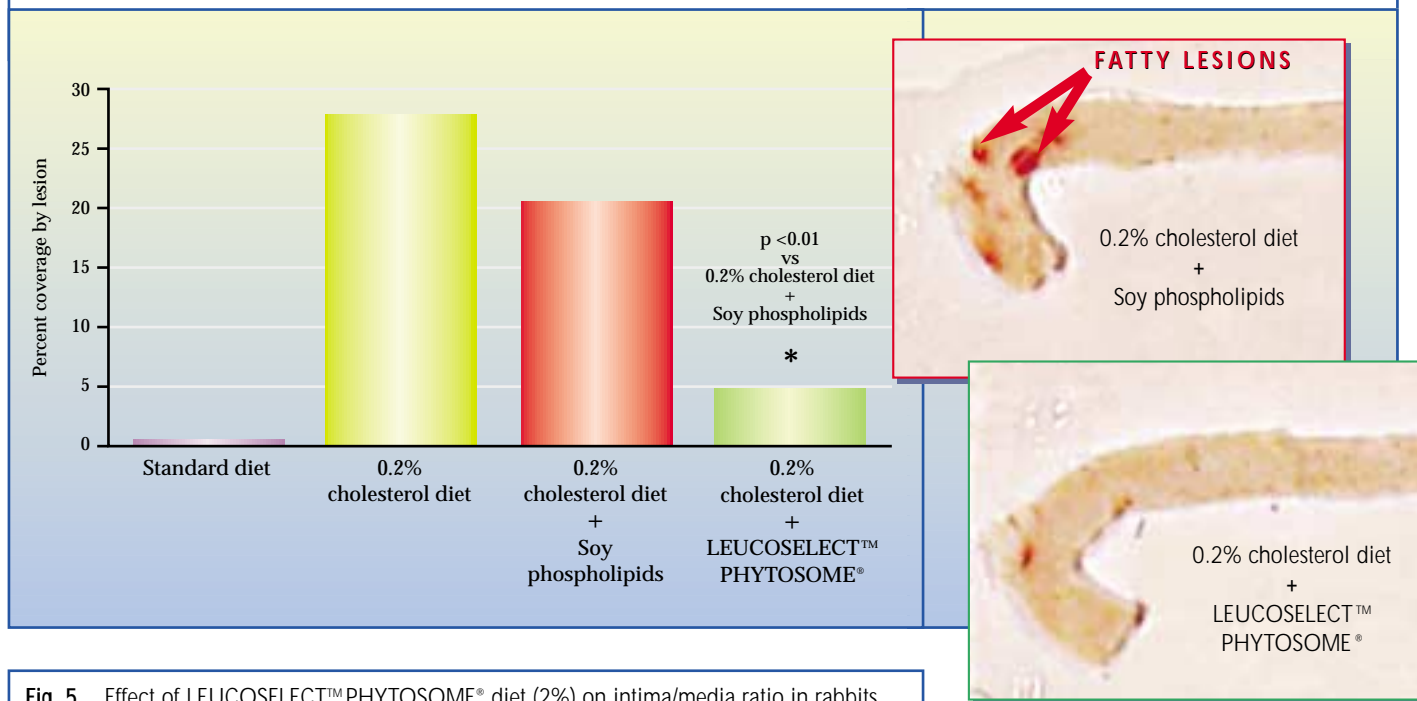
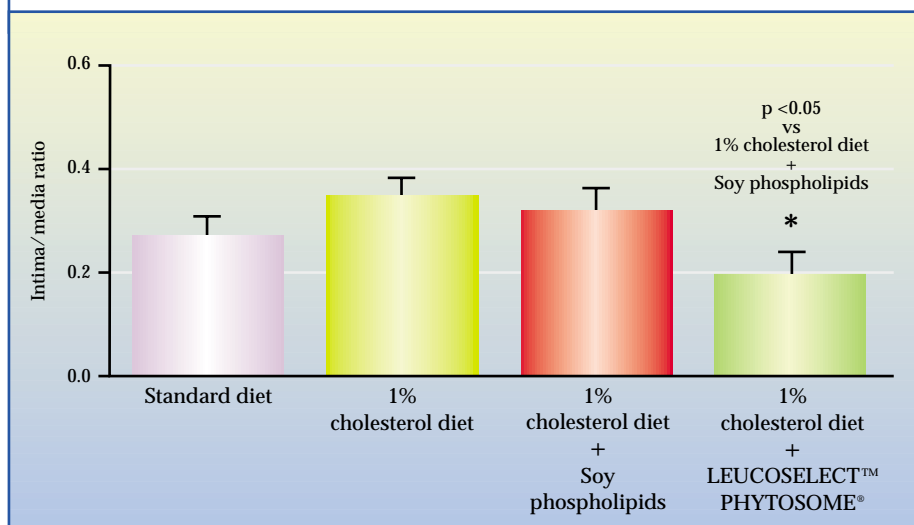
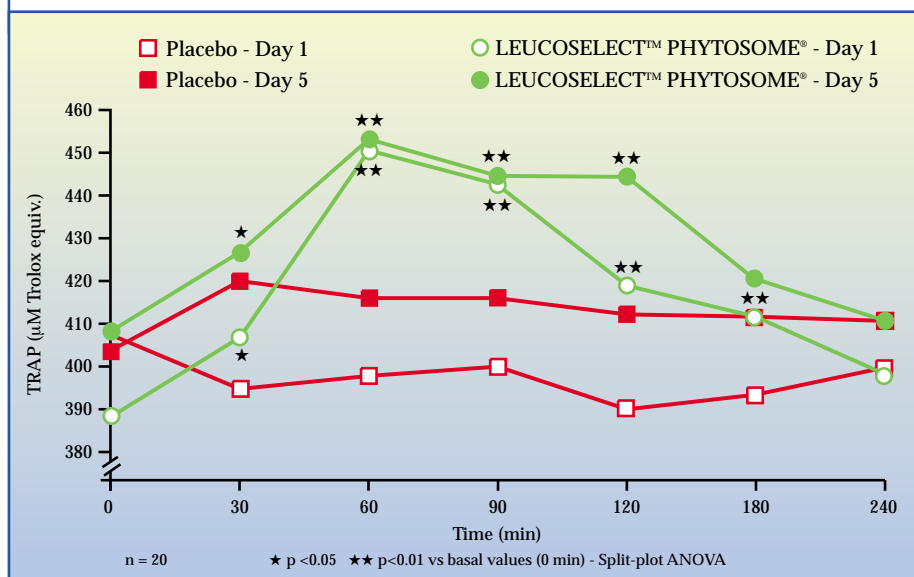


Fig. 5 Effect of LEUCOSELECT™ PHYTOSOME® diet (2%) on intima/media ratio in rabbits fed on 1% cholesterol diet.



LEUCOSELECT™ PHYTOSOME® improves the bioavailability of grape procyanidins, which are widely recognized to exert a protective activity on the cardiovascular system.

Fig. 6 Effect of LEUCOSELECT™ PHYTOSOME® (300 mg/daily as LEUCOSELECT™) on total antioxidant capacity (TRAP) in healthy volunteers.



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14. Study performed at The University of Birmingham, Clinical Pharmacology Section, Department of Medicine, Prof. M.J. Kendall. (Indena S.p.A. - Data on file)

