

# PHYTOSOME®

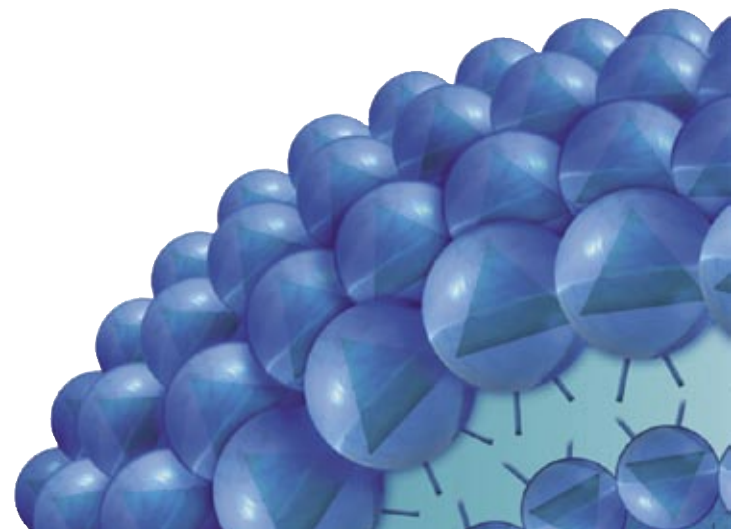
---

## a technical revolution in phytomedicine

**Bioavailability:** a major concern

Flavonoids: a brief **overview**

Background to the  
**Phytosome® technology**



**Indena S.p.A.**  
Viale Ortles, 12  
20139 Milan - Italy  
tel.+39.02.574961  
fax+39.02.57404620

[www.indena.com](http://www.indena.com)

**Indena USA Inc.**  
811 First Avenue  
Suite 218  
Seattle, WA 98104 - USA  
tel. +1.206.340.6140  
fax +1.206.340.0863  
[botanicals@indena.com](mailto:botanicals@indena.com)

**Indena France S.A.S.**  
23, Rue de Madrid  
75008 Paris - France  
tel. +33.1.45229128  
fax +33.1.45220291

**Indena S.A.**  
Gran Via de Carles III 94, 10, 1º  
08028 Barcelona - Spain  
tel. +34.93.330.35.66  
+34.93.330.38.16  
fax +34.93.4110246

**Indena Japan Co., Ltd.**  
KDDI Otemachi Building  
21F. 8-1 Otemachi 1-Chome  
Chiyoda-ku  
Tokyo 100-0004 - Japan  
tel. +81.3.3243.9924  
fax +81.3.3243.9925

© 2005 Indena S.p.A.

## 2 COVER STORY

### Phytosome®: an independent report to Indena USA Inc. by Parris M. Kidd, Ph.D

Preparation of plants or parts of them have been widely used in popular medicine since ancient times and till today the use of phytomedicines is widespread in most of the world's population. During the last century chemical and pharmacological studies have been performed on a lot of plant extracts in order to know their chemical composition and confirm the indications of traditional medicine. It has often been observed that the separation and purification of the various components of an extract may lead to a partial loss of specific activity for the purified component. Very often the chemical complexity of the extract seems to be essential for the bioavailability of the active components. The PHYTOSOME® technology, developed by the Italian company Indena S.p.A., markedly enhances the bioavailability of selected phytomedicines.



- ## 4 FEATURES
- Greenselect® Phytosome®**  
from green tea leaves, is characterized by the presence of epigallocatechin and its derivatives. These compounds are demonstrated to be strong *in vitro* modulators of several biochemical processes mainly involved in the pathogenesis of major chronic degenerative diseases such as cancer and atherosclerosis.
- Ginkgoselect® Phytosome®**  
an easy absorbable form of the standardized extract of Ginkgo biloba leaves. Its major indications are cerebral insufficiency and peripheral vascular disorders.
- Leucoselect® Phytosome®**  
composed of oligomeric polyphenols (grape procyanidins) complexed with soy phospholipids. This results in a markedly improved oral bioavailability of procyanidins, which are widely recognized to exert a protective activity on the cardiovascular system.
- Siliphos®**  
the most absorbable form of silybin known up to now, allowing silybin to reach the target organ, the liver, in concentrations which are reported to be effective as antihepatotoxic.

## An Independent Report to Indena USA Inc.

by Parris M. Kidd, Ph.D

December 2004

Phytomedicines, complex chemical mixtures prepared from plants, have been used for health maintenance since ancient times. But many phytomedicines are limited in their effectiveness because they are poorly absorbed when taken by mouth. The PHYTOSOME® technology, developed by Indena S.p.A. of Italy, markedly enhances the bioavailability of select phytomedicines. Most of the bioactive constituents of phytomedicines are flavonoids. However, a majority of the flavonoids are poorly bioavailable when taken by mouth - only a small fraction of a given dose reaches the blood. This report presents the evidence that by converting certain flavonoid nutrients to their phytosome equivalents, Indena's proprietary process increases their bioavailability.

## Background to the PHYTOSOME® Technology

The poor absorption of flavonoid nutrients is likely due to two main factors. First, these are multiple-ring molecules not quite small enough to be absorbed from the intestine into the blood by simple diffusion. Nor does the intestinal

lining actively absorb them, as occurs with some vitamins and minerals. Second, flavonoid molecules typically have poor miscibility with oils and other lipids. This severely limits their ability to pass across the lipid-rich outer membranes of the enterocytes, the cells that line the small intestine. The PHYTOSOME® technology meets this challenge. Certain of the water - phase flavonoid molecules can be converted into lipid - compatible molecular complexes, aptly called phytosomes. These are better able to transition from the water phase external to the enterocyte, into the lipid phase of its outer cell membrane and from there into the cell, finally reaching the blood.

The lipid - phase substances that Indena successfully employed to make flavonoids lipid - compatible are phospholipids from soy, mainly phosphatidylcholine (PC). PC is miscible both in the water phase and in oil/lipid phases, and is excellently absorbed when taken by mouth. PC is the principal molecular building block for cell membranes (Fig. 2), and the molecular



Fig.1 - Organization of the PHYTOSOME® molecular complex. A flavonoid molecule (lower right) is enveloped by a phospholipid molecule.

properties that suit PC for this role also render it close to ideal for its PHYTOSOME® role. Precise chemical analysis indicates the unit phytosome is usually a flavonoid molecule linked with at least one PC molecule. A bond is formed between the two molecules to create a hybrid molecule (Fig. 1). This hybrid is highly lipid - miscible, better suited to merge into the lipid phase of the enterocyte's outer cell membrane (Fig. 2).

Once there, it can cross the enterocyte and reach the circulating blood.

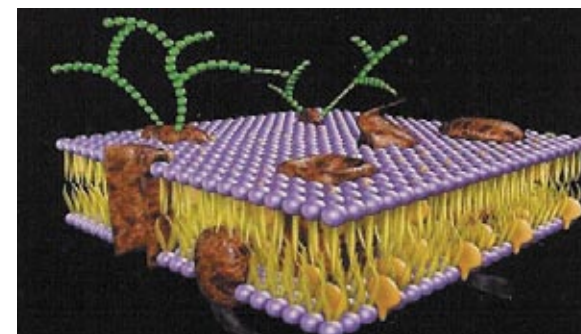


Fig.2 - Cell membranes are largely lipid - phase. A double molecular layer consisting of PC and other phospholipids provides a continuous matrix into which the proteins insert. From Singer and Nicolson.

Phytosomes are not liposomes - structurally, the two are distinctly different as shown in Fig.3. The phytosome is a unit of a few molecules bonded together, while the liposome is an aggregate of many phospholipid molecules that can enclose other phytoactive molecules but without specifically bonding to them. Liposomes are touted delivery vehicles, but for dietary supplements their promise has not been fulfilled. But for Indena's phytosome products numerous studies prove they are markedly better absorbed and have substantially greater clinical efficacy. Indena has successfully applied this technology to a number of standardized flavonoid preparations. The PHYTOSOME® technology is a breakthrough model for:

- Marked enhancement of bioavailability
- Significantly greater clinical benefit
- Assured delivery to the tissues
- No compromise of nutrient safety

## The Flavonoids - A Brief Overview

Plants are endowed with myriad healthgiving substances, prominent among these being the flavonoids. First recognized for their antioxidant properties, flavonoids are widely distributed in food and medicinal plants. To

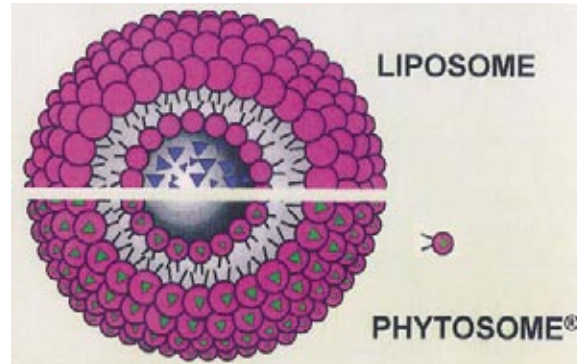


Fig.3 - The molecular organization of the liposome (upper segment) versus many individual phytosomes (lower segment). The unit phytosome is PC linked to the flavonoid molecule (lower right). From Bombardelli et al.

date, more than 4,000 naturally occurring flavonoids have been identified, each with its own distinctive molecular structure and 3-D shape. Flavonoids are part of a broader class of dietary antioxidants called polyphenols (literally, having more than one phenolic ring). The flavonoids are distinctive for their triple ring structures. Subclasses of the flavonoids exist, classified mostly on the degree of oxidation of the oxygen heterocycle or C-ring (Fig. 4). Molecular 3-D shape or "configuration" is an important aspect of flavonoid biology. Individual flavonoids have been found to specifically protect vulnerable molecular sites on cells, to stimulate or inhibit the active sites

of enzymes and receptors, or to exert other shape - specific molecular actions. Among foodstuffs, the flavonoids are most abundant in berries and other fruits, a few vegetables, and in cocoa and tea beverages. As a rule, the flavonoids are poorly absorbed from foods - for greater than 10% of the administered dose to reach the blood is the rare exception. Epidemiological evidence suggests that the lower intakes of flavonoids are associated with heightened risk of cardiovascular disease, but is not yet conclusive.

A very active area of current research is focused on flavonoids that down-regulate receptors for prostaglandins, cytokines, or hormones on cancerous or other abnormal cells. Molecular configuration may prove to be just as important as antioxidant action in the diverse anti - inflammatory, anti-allergic, antiviral, anticancer, and immune-stimulant applications of flavonoids.

Drawing on their seasoned experience with nature's riches, Indena discovered phytosomes. Then by taking flavonoid preparations with the greatest healthgiving potential and making them into phytosomal preparations, Indena scientists achieved a breakthrough in phytomedicine. ■

# Greenselect® Phytosome®

Green tea extract with superior bioavailability



The tea plant *Camellia sinensis* (Family Theaceae) is native to either China or India. Its leaves contain a variety of polyphenolic substances that provide many health benefits.

The most active of these are flavonoids of the flavan-3-ol class catechins and their various gallate derivatives, of which (-)-Epigallocatechin 3-O-gallate (EGCG) and (-)-Epicatechin 3-O-gallate (ECG) are the most abundant, both endowed with marked antioxidant activity.

## Green tea activities

According to the folk Chinese medicine, tea possesses stimulant, digestive, diuretic, analgesic and antitoxic properties. The benefits of green tea flavonoids stem from much more than their strictly antioxidant action. Since the second half of 1980, the health effects of green tea and of green tea polyphenols have been the subject of numerous scientific studies. The results of these investigations highlighted interesting pharmacological activities such as cancer-preventing, antimutagenic, antiatherosclerotic, hypocholesterolemic, cardioprotective, antibacterial and anticariogenic effects.

## Greenselect® and Greenselect® Phytosome®

The Indena GREENSELECT® green tea extract is a decaffeinated, standardized polyphenolic fraction (not less than 66.5 percent) obtained from green tea leaves. For this extract, Indena took advantage of its sophisticated phospholipid technology to complex the green tea polyphenols with phospholipids and markedly improve their limited oral bioavailability. This preparation is the GREENSELECT® PHYTOSOME®.

A study on absorption of GREENSELECT® preparations was performed in healthy volunteers (Fig. 1). Each volunteer received a single dose of 400 mg tea flavanols, either as Indena's standardized, non-complexed GREENSELECT® ex-

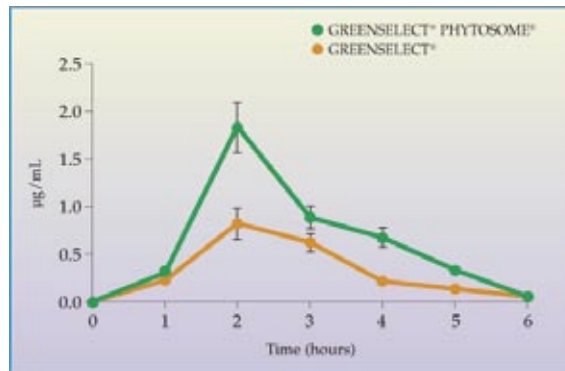


Fig. 1 - Time course of plasma EGCG after ingestion of GREENSELECT® and GREENSELECT® PHYTOSOME®.

tract or as the GREENSELECT® PHYTOSOME®. Absorption was measured as EGCG blood levels. Over the study period of 6 hours the blood concentration of EGCG was more than doubled when coming from the phytosomal versus the non-phytosomal extract.

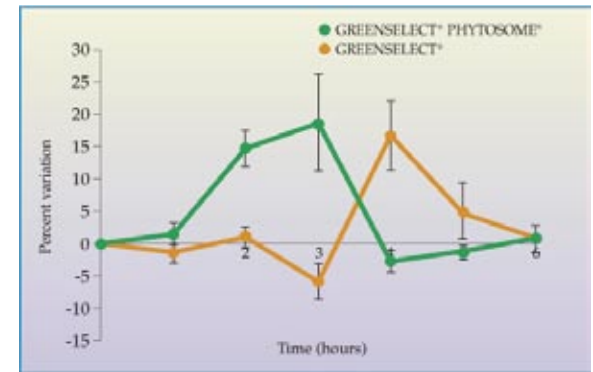


Fig. 2 - TRAP percent variation (means  $\pm$ SE,  $\mu$ mol/L TROLOX) after intake of GREENSELECT® and GREENSELECT® PHYTOSOME®.

Antioxidant capacity was measured as TRAP (Total Radical-trapping Antioxidant Parameter). The peak antioxidant effect was a 20% enhancement and the AUCs (Areas Under the Curves) showed that the phytosome formulation had about double the total antioxidant effect. Indena's phytosome preparation made from GREENSELECT® was demonstrated to be safe and well tolerated, with the ability to amplify all the known benefits of green tea. ■

# Ginkgoselect® Phytosome®

*Ginkgo biloba* extract with superior bioavailability



*Ginkgo biloba* (Family Ginkgoaceae), also known as maidenhair tree, is believed native to China. Its generic origin dates back as much as 200 million years, and the evolutionary staying power of this plant species has been attributed to its content of protective substances.

## Ginkgo biloba activities

Modern *Ginkgo biloba* extracts (GBE) are prepared from the leaves and should contain around 24% flavonoids, 6% terpenes, and less than 5 parts per million of the potentially toxic ginkgolic acids. The major dietary supplement indications for GBE are cognitive decline, especially when linked to circulatory insufficiency, sharpening of mental processing in healthy subjects, mood problems, including premenstrual syndrome, peripheral vascular disorders stemming from atherosclerotic damage, protection of the microcirculation (capillary beds throughout the tissues, including those of the retinal macula and the inner ear), potent antioxidant and anti-hypoxia activity, especially proven for the brain.

## Ginkgoselect® Phytosome®: proven efficacy

Both the flavonoids and the terpenes of GBE can become degraded while in the intestinal lumen awaiting absorption. The phytosome preparation seems to help protect against intestinal breakdown, conserving the bioactive constituents and improving the overall bioactivity of the preparation. In a bioavailability study conducted with healthy human volunteers, the GINKGOSELECT® PHYTOSOME® was given by mouth at levels sufficient to provide 160 mg per day of the GBE constituents (flavonoids plus terpenes, 24:6). For comparison, non complexed GBE was given also at 160 mg per day.

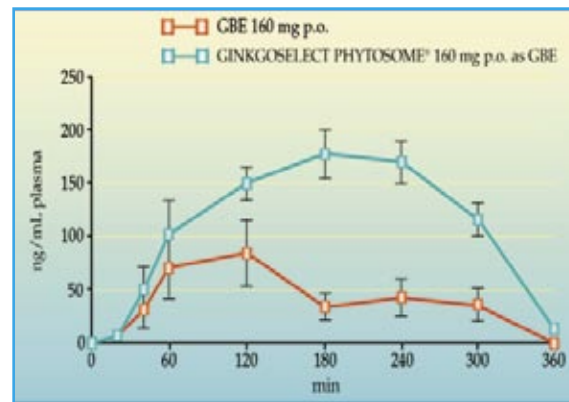


Fig.1 - Plasma levels (LC-MS) of total ginkgolides (A and B) and bilobalide in healthy volunteers.

As shown in Fig.1, the plasma levels reached by the three major Ginkgoterpenes - total ginkgolides (A and B) and bilobalide - were markedly higher from the phytosomal preparation than from the plain GBE. The levels from the phytosomal form peaked after 3 hours and persisted higher for at least 5 hours. By comparing the areas under the uptake curves (AUCs), it was found that the phytosomal GBE produced a 2-4 times greater blood content of terpenes than did the non-phytosomal GBE. The most proven applications to date are for cerebral insufficiency and circulatory problems (such as Raynaud's Phenomenon and Peripheral Arterial Occlusive Disease), but longer term the most effective applications are likely to be for conditions that specifically involve derangement of the capillary microcirculation. Among these are tinnitus, vertigo, equilibrium disorders, macular degeneration, venous insufficiency.

GINKGOSELECT® PHYTOSOME® is endowed with superior and more constant pharmacological activity in comparison with GBE. Besides the complexation of GBE with phospholipids enhances the absorption of the flavonoidic components as well as the bioavailability of terpene trilactones and the complex is well tolerated.

# Leucoselect® Phytosome®

Grape seed extract with superior bioavailability



Within the family of natural polyphenols, procyanidins demonstrated an elevated specificity in targeting the cardiovascular system. Procyanidins are oligomeric polyphenols, a class of polymers (linked molecules) made from relatively few molecules. LEUCOSELECT® PHYTOSOME® is a phytosomal dietary supplement with a fully characterized composition. Prepared from the grape seeds (*Vitis vinifera*), a part of the plant particularly rich in procyanidins, LEUCOSELECT® PHYTOSOME® is one of the most efficient cardiovascular protectant.

## Leucoselect® Phytosome® activities

More than ten mechanisms of action have been demonstrated in pharmacological studies. The main properties of LEUCOSELECT® PHYTOSOME® are an increase in total antioxidant capacity and stimulation of physiological antioxidant defences of plasma, protection against ischemia/reperfusion induced damages in the heart, protective effects against atherosclerosis. The product has also proven effective in preventing oxidative damage in smokers and diabetic subjects.

## Leucoselect® Phytosome®: proven efficacy

Complexation of polyphenols with phospholipids in phytosomal formulation improves their oral bioavailability, which is generally low and erratic. LEUCOSELECT® PHYTOSOME® markedly elevates blood total antioxidant capacity. In a randomized human trial, young healthy volunteers received LEUCOSELECT® PHYTOSOME® (containing 300 mg of LEUCOSELECT® every day), or a placebo. The blood TRAP (Total Radical-trapping Antioxidant Parameter) was measured at several time points during day 1, then also on day 5. Already by 30 mins after administration on day 1, blood TRAP levels were significantly elevated over placebo (Fig.1).

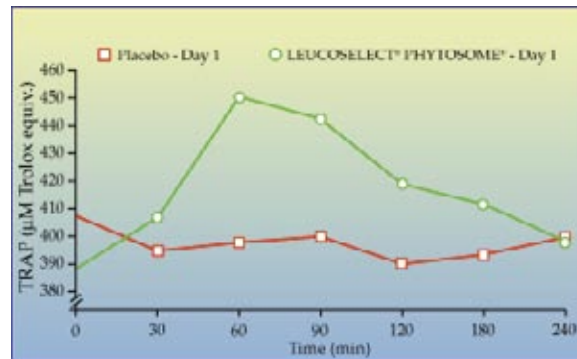


Fig.1 - Effect of LEUCOSELECT® PHYTOSOME® (300 mg/daily as LEUCOSELECT®) on total antioxidant capacity (TRAP) in healthy volunteers.

In an animal model, rabbits were fed a high-cholesterol diet for 6 weeks, to markedly elevate their blood cholesterol and induce atherosclerotic lesions in their aortas and carotid arteries. One group of rabbits got LEUCOSELECT® PHYTOSOME® in their feed for the first 6 weeks, then 4 weeks of the high-cholesterol diet. These developed significantly less aortic plaque than did the control groups (Fig.2).

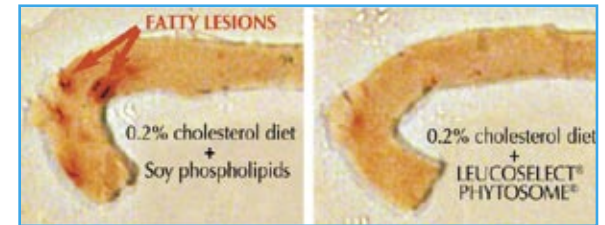


Fig.2 - Effect of LEUCOSELECT® PHYTOSOME® diet (2%) on fatty lesions in aortic arch of rabbits fed on 0.2% cholesterol diet.

As shown by clinical data, in humans LEUCOSELECT® PHYTOSOME® provides an effective antioxidant boost for the blood, lowers oxidative breakdown products in the blood and urine, and raises LDL resistance to oxidation. This preparation is able to raise the blood antioxidant status of diabetic subjects and smokers, who live under the continual huge burden of the chemical oxidative stressors so abundant in cigarette smoke. ■

# Siliphos®

## Milk thistle extract with superior bioavailability



The fruit of the milk thistle plant (*Silybum marianum*, Family Asteraceae) contains flavonoids that are proven liver protectants. The standardized extract known as silymarin contains mainly three flavonoids of the flavonol subclass. Silybin predominates, followed by silydianin and silychristin. The liver, due to the vital role it plays in metabolism, is particularly exposed to the harmful action of endogenous and exogenous toxic substances (alcohol, drugs, hormones, etc.). Unfortunately, milk thistle extract have poor intestinal absorption, thus limiting its benefits.

### Milk thistle extract activities

The modern milk thistle standardized extract has many proven benefits, including amelioration of toxic liver damage, normalization of enzymic damage indicators in alcoholic liver disease, lowering of markers of fibrotic progression in the liver parenchyma, potent antioxidant effect in liver cells, improvement of hepatitis B-related inflammation, upregulation of systemic antioxidant activity and stimulation of liver cell renewal.

### Siliphos®: proven efficacy

To overcome the poor bioavailability of silybin, the most potent of milk thistle flavonoid, Indena complexed this molecule with soy phospholipids exploiting Phytosome® technology. As demonstrated by pharmacokinetic studies in comparison with free silybin and silymarin, SILIPHOS® represents the most absorbable form of silybin known until now.

In rats, after oral administration of 200 mg/kg of silybin, the plasma levels of this drug and its conjugated metabolites were below the analytical detection limit, while, after oral administration of SILIPHOS® (200 mg/kg as silybin) the plasma levels of silybin (free and total) were easily measurable (Fig. 1), being well absorbed within minutes when in phytosomal form.

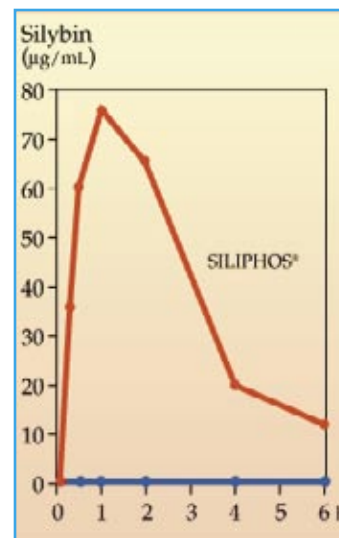


Fig.1 - Mean plasma levels of total silybin after treatment with SILIPHOS® and silybin in rats.

Silybin in phytosome form is also extremely well absorbed in humans. Pharmacokinetic studies, in fact, conducted with human subjects showed a similar pattern. Clinical studies have been performed to evaluate the properties of this new complex in subjects with pathological impairment of liver function (Fig.2).

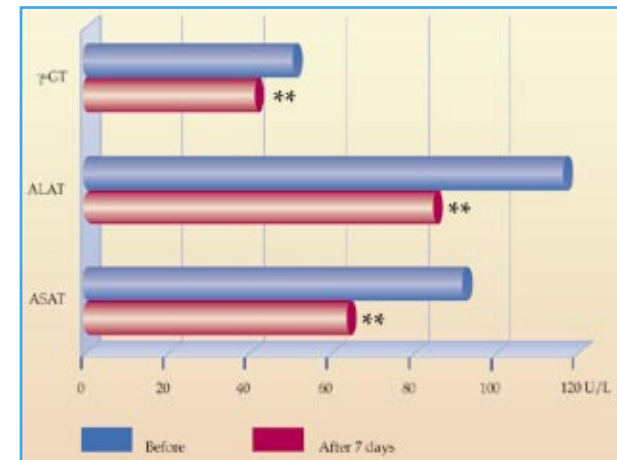


Fig.2 - Effect of SILIPHOS® on hepatocellular damage measured as serum enzyme activity in patients with chronic active hepatitis.

The results, including the optimal tolerability obtained in these “extreme” clinical situations, give strong support for the use of SILIPHOS® in those non pathological conditions mostly associated with the action of damaging agents on liver cells. ■

# Know more about PHYTOSOME®

## Indena USA Inc.

811 First Avenue  
Suite 218  
Seattle, WA 98104 - USA  
tel. +1.206.340.6140  
fax +1.206.340.0863

[botanicals@indena.com](mailto:botanicals@indena.com)

## Indena France S.A.S.

23, Rue de Madrid  
75008 Paris - France  
tel. +33.1.45229128  
fax +33.1.45220291

## Indena S.A.

Gran Via de Carles III 94, 10, 1º  
08028 Barcelona - Spain  
tel. +34.93.330.35.66  
+34.93.330.38.16  
fax +34.93.4110246

## Indena Japan Co., Ltd.

KDDI Otemachi Building  
21F. 8-1 Otemachi 1  
Chome Chiyoda-ku  
Tokyo 100-0004 - Japan  
tel. +81.3.3243.9924  
fax +81.3.3243.9925

## Indena S.p.A.

Viale Ortles, 12  
20139 Milan - Italy  
tel.+39.02.574961  
fax+39.02.57404620

[www.indena.com](http://www.indena.com)

