

Soyselect®

Soy seeds

A patented* soy extract specifically developed for the management of menopause-related symptoms
.....
Double standardization in isoflavones and B-group saponins
.....
Clinical and Pharmacological data support its efficacy
.....
A safe product, devoid of any side effect

■ **Epidemiological** data indicate that less than 25% of Japanese climacteric women,¹ 18% of Chinese and 14% in Singapore^{2,3} complain of hot flushes compared with 85% of North American women⁴ and with 70-80% of European.⁵ In addition, when compared with the Western women, Asian women have a lower incidence of cardiovascular disease, osteoporosis and estrogen dependent cancers.^{6,7} These data can be partially explained by the fact that Asian women follow a traditional soy-based diet rich in phytoestrogens.

Phytoestrogens are a broad group of non-steroidal compounds of different structure that have been shown to bind to estrogen receptors (Ers). There are three main classes of phytoestrogens: **isoflavones**, coumestans and lignans. Among the isoflavones, **genistein** and **daidzein** are the most investigated. Functionally, phytoestrogens can exert **both estrogenic and antiestrogenic effects** depending on many factors including their concentration, the level of endogenous sex hormones, the relative levels of estrogen receptors and , and the nature of the response elements with which the receptors interact on the estrogen related genes; phytoestrogens have been also shown to interact with pathway of cellular activity that do not involve Ers.⁸

Soy contains also **saponins**, reported to be biologically active molecules. There is a renewed interest in these compounds for their **anticarcinogenic**, **immunostimulatory** and **hypocholesterolemic** properties.^{9,10}

A natural aid for the management of menopause-related symptoms

Extracts containing phytoestrogens are all on the market as food supplements and, not being subjected to strict regulatory controls, they vary in quality and quantitative composition.¹¹

Soyselect® is a standardized extract from soy featuring a **double standardization** in the active ingredients (13-17% of isoflavone glycosides **genistin** and **daidzin**, not less than 18% of **B-group saponins** by HPLC). The **ratio** between isoflavones and saponins significantly improves the bioavailability of isoflavones in comparison with a saponin-deprived soy extract.¹²

Soyselect® has been extensively tested in pre-clinical studies, particularly with respect to **menopause management** and **breast cancer chemoprevention** areas.

Soyselect® is supported by **three clinical studies** aimed at evaluating its efficacy for the relief of **vasomotor symptoms** and improvement of **cognitive function**, demonstrating a good tolerability and a significant effect.¹³⁻¹⁵

*Patent No.: US 6,280,777 - US 6,607,757

Clinical studies

The efficacy of Soyselect® was verified at dosages ranging from 400 to 640 mg/day (corresponding to a dosage of isoflavones between 50 and 110 mg/day) in three clinical trials for the improvement of menopause-related symptoms. The overall figure of the effects of Soyselect® can be summarized as follow:

■ Vasomotor symptoms relief in post-menopausal women (50 mg/day of isoflavones)^{13,14}

- a double-blind, randomized, pilot study has been performed to comparatively evaluate, on 39 postmenopausal women, the activity of Soyselect® and placebo on early climacteric symptoms. A significative reduction of the number of hot flushes (-45% after 5 weeks of treatment with Soyselect®, chart 1) has been observed.
- a double-blind, randomized, parallel group, out-patient, multicenter (15 sites) study has been performed on 177 postmenopausal women. A significative reduction of incidence and severity of hot flushes (-32% at week 10) and number of awakenings due to night sweats (-62% at week 12, chart 2) has been recorded.

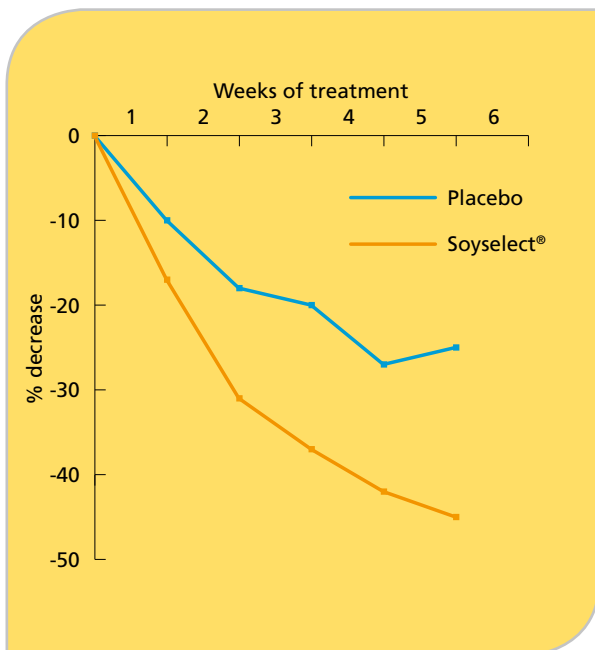


Chart 1: weekly percentage decrease of hot flushes in participants receiving placebo and Soyselect® during the first 6 weeks of the study.

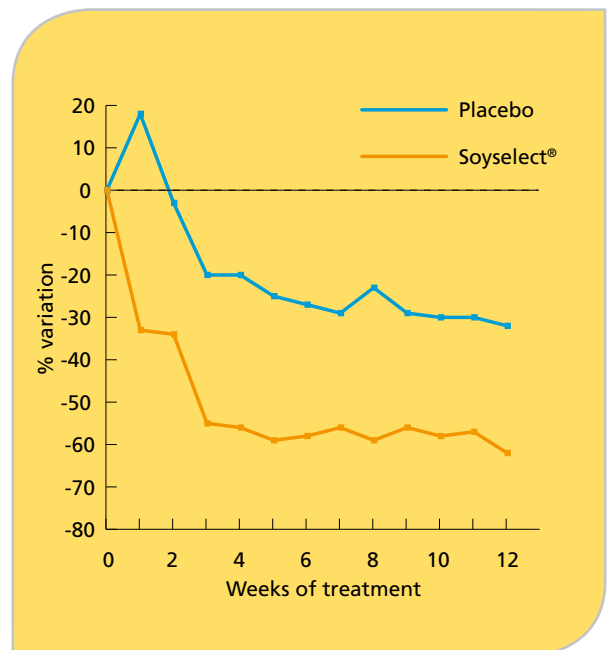


Chart 2: weekly percentage variation of awakenings due to night sweats.

■ Improvement of cognitive function in older women (110 mg/day of isoflavones)¹⁵

- a 6-month, double-blind, randomized, placebo-controlled clinical trial has been performed on 56 postmenopausal women. The postmenopausal women treated with Soyselect® did consistently better, particularly on verbal memory, both in comparison with their own baseline scores and with the placebo group responses.



Pharmacology

Extensive *in vivo* and *in vitro* experimental studies have been carried out on Soyselect® to provide a complete pharmacological profile.

■ *In vivo*

• *bone-targeted estrogenic activity in animal models of menopause*¹⁶⁻¹⁹

Soyselect® in the immature rat uterotrophic assay has shown no effect, leaving **unchanged uterine weight** when compared to controls.

Soyselect® in the ovariectomized rat (OVX) model of menopause produced a **bone-sparing effect** associated with a slowing down in the increased **bone turnover** observed after ovariectomy (as indicated in table 1 by measurements of serum osteocalcin levels and urinary excretion ratio of deoxypyridinoline, DPD). Organ weight data and histopathological analysis did not show any stimulatory activity of Soyselect® on the uterus, thus suggesting a tissue specific response.

Treatment	Bone density (g/cm ³)	Serum osteocalcin (ng/mL)	Urinary DPD (nM DPD/mM creatinine)
Control (non OVX)	1.531±0.006***	6.6±0.4**	133.9±18.5
Control (OVX)	1.462±0.010	38.9±7.7	157.2±25.0
Soyselect® 50 mg/kg/day	1.484±0.008	41.3±8.8	150.8±31.8
Soyselect® 100 mg/kg/day	1.496±0.005*	16.6±1.5*	126.2±13.3
17β-estradiol 0.5 mg/kg/day	1.508±0.010***	11.0±0.8**	80.0±17.6*

* p<0.05, ** p<0.01, *** p<0.001 vs OVX

Values are expressed as Mean±SE

Table 1: bone-related parameters in ovariectomized (OVX) rats after treatment with Soyselect® or 17β-estradiol.

Soyselect® has a tissue-specific effect and is efficacious in preventing experimental osteoporosis

• *anticancer activity*²⁰⁻²²

the chemopreventive effect of Soyselect® has been evaluated on 7,12-dimethylbenz[a]anthracene (DMBA) induced mammary tumors in rats. Only tumors classified as adenocarcinomas were considered for data evaluation. In treated animals, mammary tumors took a longer period of time to develop, with a dose-dependent percentage reduction of poorly differentiated tumors.

• *antiangiogenic activity*²³

Soyselect® has been proven effective in inducing a significant inhibition of angiogenesis caused by means of basic Fibroblast Growth Factor (bFGF) in athymic nude mice.

■ *In vitro*

• *antioxidant activity*²⁴⁻²⁶

Soyselect® and its isoflavone fraction have been characterized for their antioxidant effects by means of different experimental models (cells free, membrane and cell systems).

• *cardiovascular protective activity*^{27,28}

Soyselect® isoflavone fraction has been evaluated for the inhibition of polymorphonuclear leukocytes (PMN) adhesion to activated platelets or endothelial cell lining on the injured vascular wall. This mechanism is involved in the inflammatory process and tissue damage that characterize pathological situations such as reperfusion injury, atherosclerosis and thrombosis.

Bioavailability

Pharmacokinetic studies performed in animals²⁹⁻³² and humans^{33,34} demonstrate for isoflavone plasma levels an overlapping micromolar range at pharmacological effective dosages (table 2).

From a comparative pharmacokinetic study¹² performed in rats after single oral administrations of Soyselect® in comparison with a saponin-deprived soy extract, it was proven that isoflavones from Soyselect® are **1.7 fold more bioavailable** (table 3). The results obtained in this study indicate that the bioavailability of isoflavones is markedly improved by the presence of **B-group saponins** in Soyselect® in a patented ratio between these two classes of compounds.

Study	Genistein (µM)	Daidzein (µM)	Equol (µM)
Epidemiology (diet)	0.5	0.2	0.06
Clinical trial in post-menopausal women (capsules)	0.7	0.5	Not analyzed
Rat model of menopause (gavage)	0.9-2.7	1.5-3.3	2.2-2.3
DMBA-induced mammary cancer (diet)	0.5-0.8	0.4-0.6	0.9-2.0
Reproductive toxicity (diet)	1.5-2.8	2.3-4.6	7.3-10.7
Human breast cancer xenograft MCF-7/MDA-MB-231 (gavage)	0.8-1.8	1.1-2.9	Not detectable

Table 2: total plasma levels obtained in preclinical studies, clinical trials and epidemiology of genistein, daidzein and its metabolite equol.

The presence of B-group saponins makes Soyselect® isoflavones 1.7 fold more bioavailable

	AUC 0-8 h (ng·h/mL)	Ratio*
Soyselect® 200 mg/kg 30 mg/kg ISOFLAVONES	749.09	53.13
Saponin-deprived extract 200 mg/kg 80 mg/kg ISOFLAVONES	1057.86	31.34

*Ratio between AUC and percent of isoflavone concentrations in the extracts

Table 3: AUCs of total isoflavones after oral administration of 200 mg/kg of Soyselect® (corresponding to 30 mg/kg of isoflavones) and a saponin-deprived extract (corresponding to 80 mg/kg of isoflavones).

Safety

Soyselect® has been proven safe in studies performed in rats aimed at evaluating 30-day oral toxicity and effects on reproductive functions.^{35,36}

No effects have been shown on the growth of estrogen-dependent human breast cancer xenograft (MCF-7, chart 3) in ovariectomized athymic mice and on the growth of estrogen-unresponsive human breast cancer xenograft (MDA-MB-231) in athymic mice.³⁷

No adverse effects have been observed during clinical trials.

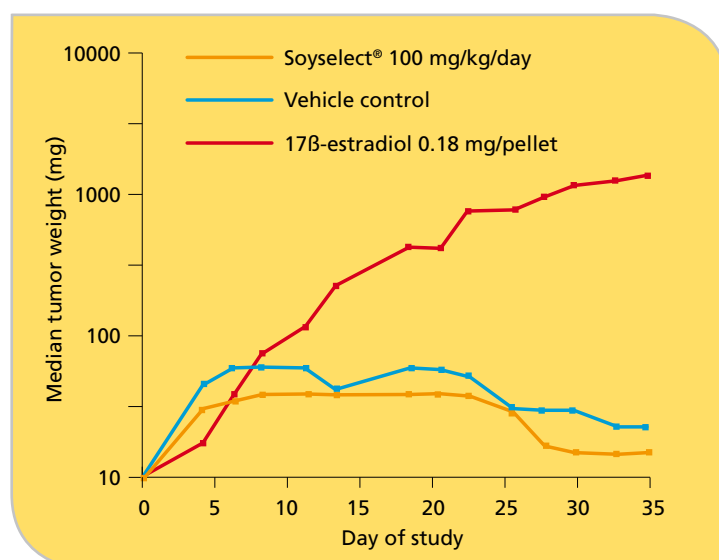


Chart 3: effect of Soyselect® on the growth of estrogen-dependent human breast cancer xenograft in ovariectomized athymic mice.

References

1. Lock M., Encounters with aging. Mythologies of menopause in Japan and North America. Berkley and Los Angeles, University of California Press, 1993.
2. Boulet M.J., Oddens B.J., Leher P., Vemer H.M., Visser A., *Maturitas* 19, 157 (1994).
3. Tang G.W.K., *Maturitas* 19, 177 (1994).
4. Notelovitz M., *Am. J. Obstet. Gynecol.* 161, 1832 (1989).
5. Rekers H., Mastering the menopause, in: A portrait of the menopause, H. Burger, M. Boulet (eds), The Parthenon Group, Park Ridge, New Jersey (1991).
6. Aldercreutz H., *Environ. Health Perspect.* 103, 103 (1995).
7. Grady D., Rubin S.M., Petitti D.B., Fox C.S., Black D., Ettinger B. et al., *Ann. Intern. Med.* 117, 1016 (1992).
8. Benassayag C., Perrot-Applanat M., Ferre F., *J. Chromatogr. B* 777, 233 (2002).
9. Rao A.V., Sung M. K., *J. Nutr.* 125, 717 S (1995).
10. Kerwin S.M., *Curr. Med. Chem. Anti-Cancer Agents* 4, 263 (2004).
11. Setchell K.D.R., Brown N.M., Desai P., Zimmer-Nechemias L., Wolfe B.E., Brashear W.T., Kirschner A.S., Cassidy A., Heubi J.E., *J. Nutr.* 131, 1362S (2001).
12. Report Pharm/01/02, Università Cattolica del Sacro Cuore - Indena S.p.A., Internal File.
13. Scambia G., Mango D., Signorile P.G., Anselmi Angeli R., Palena C., Gallo D., Bombardelli E., Morazzoni P., Riva A., Mancuso S., *Menopause* 7, 105 (2000).
14. Upmalis D.H., Lobo R., Bradley L., Warren M., Cone F.L., Lamia C.A., *Menopause* 7, 236 (2000).
15. Kritz-Silverstein D., Von Mühlen D., Barrett-Connor E., Bressel M.A.B., *Menopause* 10, 196 (2003).
16. Report Pharm/04/03, Università Cattolica del Sacro Cuore - Indena S.p.A., Internal File.
17. Report Pharm/05/03, Università Cattolica del Sacro Cuore - Indena S.p.A., Internal File.
18. Gallo D., Zannoni G.F., Apollonio P., Martinelli E., Ferlini C., Passetti G., Riva A., Morazzoni P., Bombardelli E., Scambia G., *Menopause* 12, 589 (2005); Report Pharm/01/03, Università Cattolica del Sacro Cuore - Indena S.p.A., Internal File.
19. Report Pharm/08/03, Università Cattolica del Sacro Cuore - Indena S.p.A., Internal File.
20. Report Pharm/03/98, Università Cattolica del Sacro Cuore - Indena S.p.A., Internal File.
21. Gallo D., Giacomelli S., Cantelmo F., Zannoni G.F., Ferrandina G., Fruscella E., Riva A., Morazzoni P., Bombardelli E., Mancuso S., Scambia G., *Breast Cancer Res. Treat.* 69, 153 (2001).
22. Gallo D., Ferrandina G., Giacomelli S., Fruscella E., Zannoni G.F., Morazzoni P., Riva A., Bombardelli E., Mancuso S., Scambia G., *Cancer Lett.* 186, 43 (2002).
23. Report Pharm/20/02, Università Cattolica del Sacro Cuore - Indena S.p.A., Internal File.
24. Report Istituto di Chimica Farmaceutica e Tossicologica dell' Università degli Studi di Milano, 24 September 1999 - Indena S.p.A., Internal File.
25. Report Istituto di Chimica Farmaceutica e Tossicologica dell' Università degli Studi di Milano, 7 December 2000 - Indena S.p.A., Internal File.
26. Maffei Facino R., Carini M., Aldini G., Piccone M., Morazzoni P., Bombardelli E., Joint Meeting of the ASP, AFERP, GA and PSE 1999, 2000 Years of Nat. Prod. Res. "Past, Present and Future", July 26-30, Amsterdam, 1999.
27. Report Consorzio Mario Negri Sud, 22 June 2001 - Indena S.p.A., Internal File.
28. Rotondo S., Totani L., Manarini R., Piccoli A., Martelli N., Pecce R., Riva A., Morazzoni P., Evangelista V., Food & Nutrition for Better Health, 13-15 June 2001, Santa Maria Imbaro - Lanciano, Italy.
29. Report Pharm/01/99, Università Cattolica del Sacro Cuore - Indena S.p.A., Internal File.
30. Report Pharm/01/00, Università Cattolica del Sacro Cuore - Indena S.p.A., Internal File.
31. Report Pharm/02/98, Università Cattolica del Sacro Cuore - Indena S.p.A., Internal File.
32. Gallo D., Cantelmo F., Giacomelli S., Riva A., Morazzoni P., Bombardelli E., Mancuso S., Scambia G., Eurotox' 99 - XXXVII European Congress of Toxicology, June 27-30, 1999 - Oslo, Norway.
33. Report B.T. Biotecnica 01/97, March 28, 1997 - Indena S.p.A., Internal File.
34. Report B.T. Biotecnica 04/97/CL, April 28, 1997 - Indena S.p.A., Internal File; Report B.T. Biotecnica 04/97/AN, April 28, 1997 - Indena S.p.A., Internal File.; Report B.T. Biotecnica 04/97/PK, May 5, 1997 - Indena S.p.A., Internal File.
35. Report Dipartimento di Scienze Biomediche dell' Università degli Studi di Trieste, November 4, 1997 - Indena S.p.A., Internal File.
36. Gallo D., Cantelmo F., Distefano M., Ferlini C., Zannoni G.F., Riva A., Morazzoni P., Bombardelli E., Mancuso S., Scambia G., *Food Chem. Toxicol.* 37, 493 (1999); Report Sta/03/97 - Indena S.p.A., Internal File.
37. Gallo D., Ferlini C., Fabrizi M., Prislei S., Riva A., Morazzoni P., Bombardelli E., Scambia G., 6th International Symposium on the Role of Soy, 30 October - 2 November 2005, Chicago, USA.



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