Silymarin effect on human skin and its enhancement by other active ingredients

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Introduction

Silymarin, flavonoid extracted from the seeds and fruit of *Silybum marianum (Compositae)*, has hepatoprotective, anti-inflammatory and anticarcinogenic properties. The data in the literature indicate that silymarin acts in four different ways: as an antioxidant, a scavenger and regulator of intracellular glutathione content, as a cell membrane stabilizer and permeability regulator, and also as a promoter of ribosomal RNA synthesis. In cosmetic manufacturing, silymarin presents a great interest mainly in: anti-aging, anti-free radicals products and anti-cellulite products. In *Dr Irena Eris Centre for Science and Research* we are conducting research on creating different complexes of silymarin and other actives enhancing effects of silymarin.

Material and methods

Cell culture

The tests were preformed on keratinocytes (KB line, ATCC) cultured in RPMI medium containing 10% FCS and 1% glutamine and primary human fibroblasts (generated from separated dermis) cultured in standard MEM medium with 10% FCS, 10 mM HEPES, 2 mM L-glutamine and antibiotics (100 U/ml penicillin, 0,25 g/ml streptomycin sulphate). Passages between 2 to 8 were used. Cell viability was determined by MTT assay.

Microscopic analysis

For cytoskeleton observation cells were cultured on coverslips coated with 1 % gelatin for 10 days. After that cells fixed in 4 % paraformaldehyde were incubated with anti--tubulin monoclonal antibody. Cells were analyzed in a confocal microscope (LEICA TSC SP2 Spectral & Confocal Microscope).

In vivo study

In vivo study was conducted in the population of 30 patients: 26 women and 4 men, aged 24 to 64 years with visible face redness and couperoses. The study was a double blind, placebo controlled study. The cream has been used for 6 weeks, twice a day.

The following measurements were recorded:

- Skin moisturization (using MPA 5 probe Corneometer CM 825, Courage-Khazaka Electronic GmbH, Germany)
- Skin greasiness (using MPA 5 probe Sebumeter CM 825, Courage-Khazaka Electronic GmbH, Germany)
- Face redness (using MPA 5 probe Mexameter SM 815, Courage-Khazaka Electronic GmbH, Germany)

At the end of study all subjects filled the special questionnaires in which they can assess the cosmetic features of the products and give self-assessment of proposed therapy. Scale: 1-2 strongly negative (bad); 3-4 poor (average); 5-6 appropriate; 7-8 good; 9-10 very good (positive)

UV-B radiation

UV-B irradiation was carried out using fluorescent bulb TL12 (Philips) which emits most of its energy within range 290-320 nm (emission peak at 313 nm). Confluent cells were exposed (250 J / m2) through phosphate buffered saline (Sigma). Thereafter the cells were trypsinized and suspended in MEM medium. Cell viability was determined by MTT assay.

DPPH radical scavenging method

The antioxidant activity of the tested compounds was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH·) radical scavenging method [1]. The examination of reactivity with DPPH is very useful for providing basic information on the scavenging ability and structure-activity relationships of antioxidants. The reaction of compounds with DPPH was measured by Electron Paramagnetic Spectroscopy. A 1ml of 1mg/ml solution of DPPH was mixed with solutions of S(10-6%), TP (10-6%) and S+TP. EPR spectra of reaction mixture were measured after 15min. A DPPH-scavenging ability unit was recalculated as the amount of scavenged DPPH [mg] per 1ml added substance.

Synergy Measurement

One way to measure synergism was published in 1961 by Kull F.C. et al1 who studied the effect of antifungal compounds. We have used the Kull's equation to measure synergy of actives combined in a complex which stimulates skin cells' proliferation.

References
1. Kull FC, Eisman PC, Sylwestrowicz HD, Mayer RL Mixtures of quaternary amonium compounds and long-chain fatty acids as antifungal agents. Appl Microbiol 1961, 9, 538-541.
2. Steinberg DC Measuring synergy. Cosmet Toil 2000, 115; 59-62.

Results

Proliferation of skin cells measured by MTT assay. Synergy was calculated according to the Kull's equation.
Active ingredients tested in combination with silymarin: folic acid, sucralfate, copper gluconate, FitoDHEA (Wild Yam extract), *Clintonia borealis extract*, Lutein, Lana Blue®, wit PP, amino-acid derivative (TP compound). Complexes which showed synergistic effect was listed below:

Proliferation of skin cells measured by MTT assay	Keratinocytes	Fibroblasts
silymarin + folic acid	synergy	synergy
silymarin + FitoDHEA	synergy	synergy
silymarin + vit PP	synergy	no synergy
silymarin + TP compound	synergy	no synergy

For further research we chose the following combinations: **silymarin + vitPP and silymarin + TP compound (named as Antioxidant Complex)**

Silymarin+vit PP *in vitro*

Fig.1. Proliferation of keratinocytes measured by MTT assay

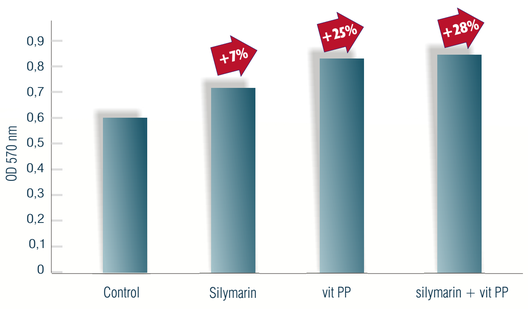
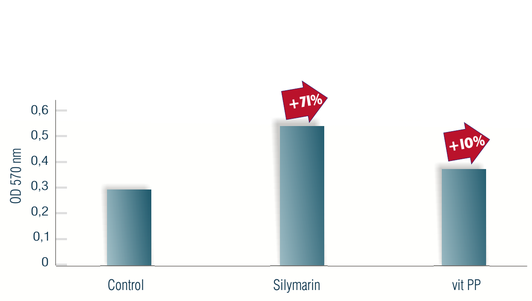


Fig.2. Keratinocytes viability after UVB measured by MTT assay



Cell shape and cytoskeleton organization

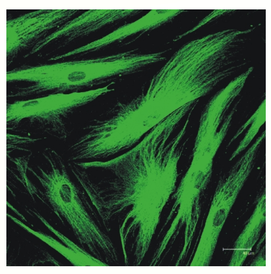


Fig. 3.Control fibroblasts, died with anti-α-tubulin antibody, confocal microscopy, x40

Fig. 4. Silymarin-treated fibroblasts, died with anti-α-tubulin antibody, confocal microscopy, x40

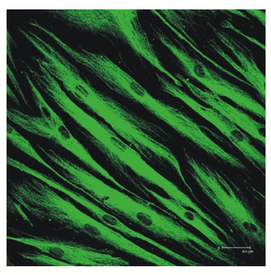


Fig. 5. Vit PP-treated fibroblasts, died with anti-α-tubulin antibody, confocal microscopy, x40

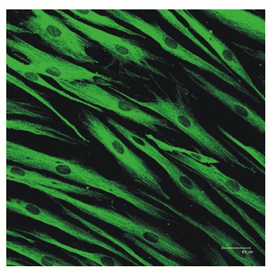


Fig. 6. Complex-treated fibroblasts, died with anti-α-tubulin antibody, confocal microscopy, x40

Our in vitro study showed that:
- the combination of silymarin and vitamin PP enhance keratinocytes proliferation,
- the complex-treated cells, in contrast to the control cells, were very regular in shape (spindle-shaped),
- silymarin-treated or vitamin PP-treated cells have higher viability after UVB exposure.

Silymarin+vit PP *in vivo*

Fig. 7. Intensity of redness (cheeks)

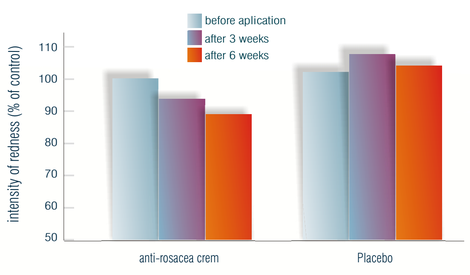
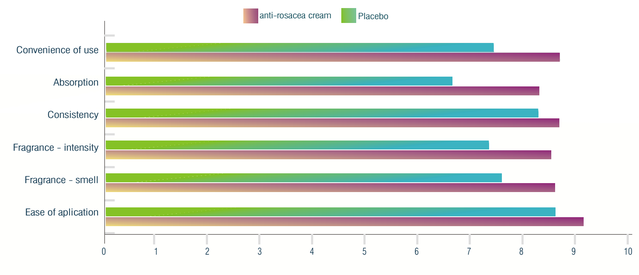


Fig. 8. Evaluation of properties of anti-rosacea and placebo cream



Clinical evaluation and subject response demonstrated gradual decreasing in erythema. In addition, the cream was well tolerated and has good cosmetic features.

Silymarin+TP *in vitro*

Fig.1. Keratinocytes viability after UVB measured by MTT assay

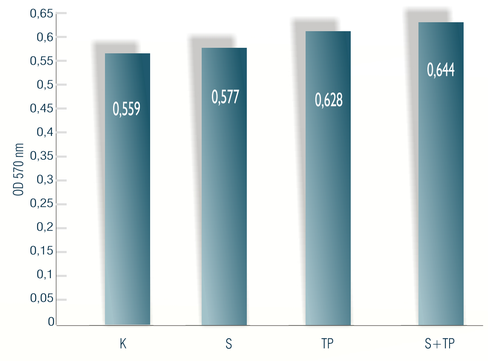


Fig.2. Fibroblasts viability after UVB measured by MTT assay

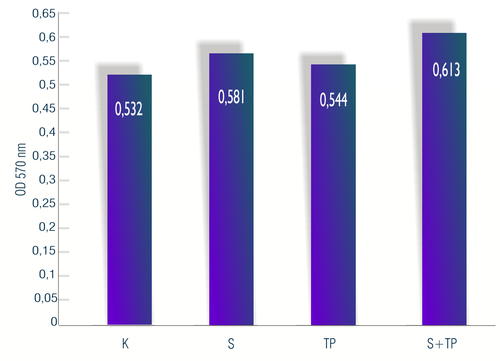
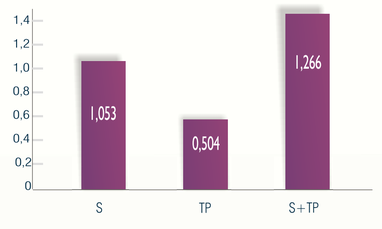


Fig.3. DPPH - scavenging ability [mg DPPH/ml substance]



Our in vitro study showed that:
- Antioxidant Complex (Sylimarin + TP) enhanced both keratinocytes and fibroblasts viability after UVB exposure (Fig. 1, Fig. 2),
- Antioxidant Complex (silymarin+TP) seems to be promising for creating modern anti-aging cosmetics and skin care products. (Fig.3)

Conclusions

Silymarin is a great asset in modern cosmetology. Our research suggests that even better results can be achieved by exploiting the effects of this compound's synergistic activity with other ingredients.

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