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Study report on Placebo controlled study of influence of cream with silver fir wood extract (*Abies alba Mill.*) Belinal® on skin

Abstract

The aim of placebo controlled topical study is the use of a silver fir tree branch water extract - Belinal® and formulations containing such an extract, for preventing, reducing and treating undesired skin changes, enabling an effective use for cosmetic and dermatologic purposes. Ten volunteers used the test cream twice per day for 12 consecutive weeks on one half of the face and gluteal area; for control placebo cream was utilized following the same protocol on the other half of the face and gluteal area. The purpose of the study was to determine effects of Belinal® to the skin, therefore test and placebo cream matrix components had been optimised to minimise their effects. The results show that several skin parameters were significantly improved on test side, compared to placebo side, such as dermis density, wrinkles condition, TEWL (indicating improvement of the skin barrier), elasticity, minimal erythema dose (MED); also decoloration of hyperpigmentations was detected. It thus excel significant anti-ageing effects on the skin.

Introduction

Unwanted skin changes are caused by various intrinsic and extrinsic causes, such as biochemical, physiological and structural changes in the dermis and epidermis, which are reflected in reduced elasticity, humidity, changes in pigmentation, micro-relief, the number and size of pores, increased wrinkles, weakened skin barrier, changed pH of the skin surface, increased sensitivity to UV radiation, as well as the development or worsening of skin diseases such as various types of dermatitis, psoriasis, ulcers, acne, rosacea and other.

Belinal is a dried aqueous extract of silver fir branch (*Abies alba Mill.*). It contains a mixture of different phenolic compounds including flavonoids, phenolic acids and lignans. In this placebo controlled study of cream with the Belinal®, we determined that cream containing 2% of Belinal affects many properties and functions of the skin by significantly improving the negative skin changes, whilst not affecting the normal skin condition.

Materials and Methods

Silver fir branch extract Belinal® was provided by the company Abies Labs.

The following equipment was used for determination of the skin parameters:

- **Cortex DermaLab Combo SkinLab** – Cortex Technology ApS, Smedevaenget 10, 9560 Hadsund, Denmark; probes for the analysis of the dermis (density/intensity and thickness of the dermis), elasticity, transepidermal water loss, moisture, colour/redness/pigmentation (CIE LAB).

- **VisioFace Quick (VFQ)**, round shaped model – Courage + Khazaka electronic GmbH, Mathias Brügggen-Str. 91, 50829 Cologne, Germany (for evaluation of wrinkles)
- **Dr. Hoenle Dermalight 80 MED-Tester** (for the determination of the minimal erythema dose – response to UV rays)
- **DermaScope MEDL4D**, Dino-Lite, Netherlands (visual analyses)

Products: Belinal cream (test cream) and placebo cream

The test cream contained 2 % of the silver fir branch water extract (Belinal®), while there was no silver fir branch extract in placebo cream. Test and placebo cream were prepared using ingredients represented in Table 1 according to the following procedure:

The ingredients of phase a) were weighted in a 400 mL cup, and the ingredients of phase b) in a 200 mL cup. Both phases were heated to 75°C. The heated phases were then combined by adding the water phase into the oil phase while mixing with an electrical mechanic blender. Demineralized water and TEA – phase c) were weighted in a 20 mL cup and stirred well to obtain a clear solution which was added to the phase a) and b) compound. After a few minutes of mixing, phase d) was added. The obtained emulsion was cooled to room temperature while constantly mixing it, and the obtained cream with a 2% extract content was put into a container. The test cream was used for the *in vivo* study of effects.

Placebo cream contained the same ingredients but with no Silver fir branch extract. To achieve same light brown color brown food color was used.

Table 1: Ingredients and their quantities used for the preparation of test and placebo cream

	Ingredient	Quantity of the ingredient in product (g/100 g)	
		Test cream	Placebo cream
Phase a	Lanette N	5.00	5.00
	Stearic acid	3.50	3.50
	Cetanol	0.50	0.50
	Tegosoft CT	3.00	3.00
	Apricot oil	8.00	8.00
	Cetiol SN	2.00	2.00
	TiO ₂	0.20	0.20
	Paraffin oil	1.00	1.00
	Phase b	Water, demineralized	68.10
Glycerol		3.00	3.00
Urea		1.00	1.00
Phenonip		0.50	0.50
Belinal® silver fir		2.00	/
Phase c	Water, demineralized	2.00	2.00
	TEA	0.20	0.20
Phase d	Fragrance	0.20	0.20
	1 % brown color	/	2.00



Study design

Study was designed as split face and split body, placebo controlled single blind study. 10 volunteers, women aged between 45 and 65, Fitzpatrick's skin phototype II or III, used the study creams (test and placebo) twice per day for 12 consecutive weeks. The test cream contained 2 % of the silver fir branch water extract (Belinal®) and was applied on the one half of the face and additionally to the one half of the gluteal part. Contrary, for the other half of the face, and the other half of the gluteal part the placebo cream was used. Volunteers had to stop using other skin care products on the test areas (such as creams, lotions and serums) and replaced it with study creams. However, they were allowed to continue with use of cleansing products (eg. micellar water, tonic, cleansing gel...), that were part of their daily routine before the study

Results

The results obtained in placebo controlled dermocosmetical study on 10 women demonstrate that regular daily use of cream with 2% of Belinal® is able to improve variety of skin parameters and is very effective anti-aging ingredient.

Silver fir branch - Belinal® has many positive effects on changes in the dermis and epidermis of the skin. The results of the study show that Belinal® is able to improve density of the dermis, as it increased for 17,33% increase in comparison to baseline after 12 weeks ($p < 0.05$) and the change was significant also in comparison to placebo side ($p < 0.05$). Improvement was noticeable already after 6 weeks (9%), but within group change was statistically not significant, while it was significant in comparison to placebo ($p < 0.05$). Improvement of the dermis density indicates the higher amount of collagen in the dermis, which as a consequence of regenerative and protective effects of silver fir extract.

The test cream also increased the thickness of the dermis for 6.2% after 6 weeks and 4.5% after 12 weeks of treatment, but result was significant in comparison to placebo only after 6 weeks.

Improved structure of the dermis can be detected also in reduced expression of wrinkles and better skin elasticity.

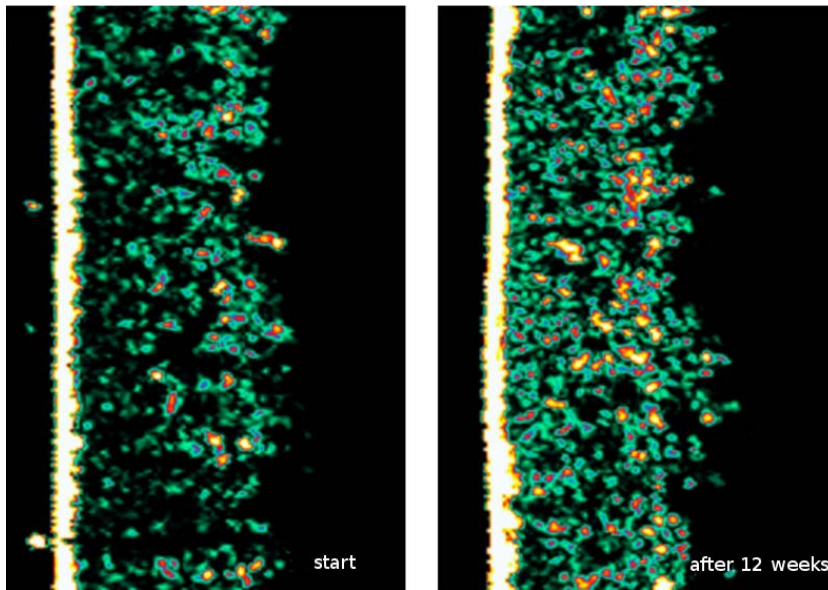


Image 1-2: Ultrasound images of the dermis before and after 12 week of use of Belinal cream: Belinal® significantly improves dermal density of collagen

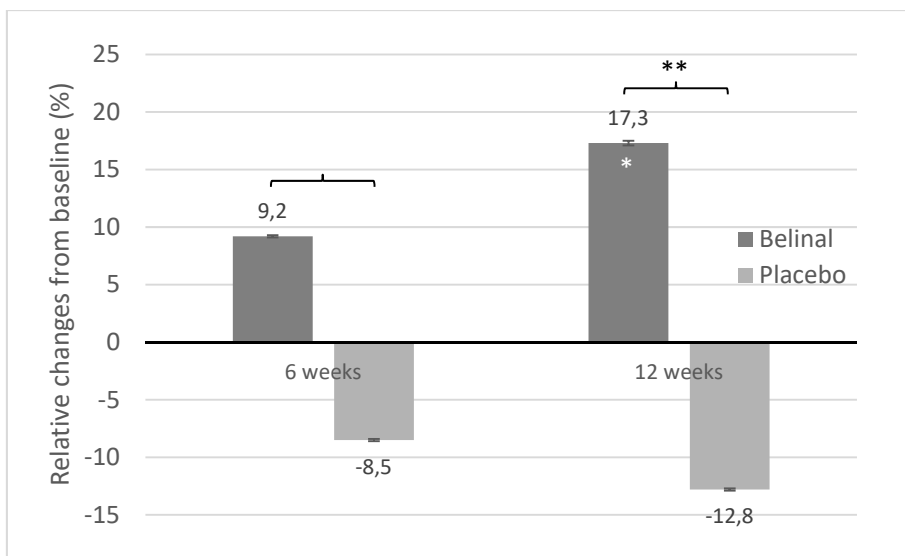


Figure 1: Relative changes of dermis density after 6 and 12 weeks of treatment with belinal and test cream. * $P < 0.5$ significant difference within side between week 0 and 6 or week 0 and 12. ** $P < 0.05$ significant difference between sides.

After 12 weeks of topical treatment compared to placebo Belinal® in a cosmetic product significantly reduced visibility of the wrinkles as periorbital wrinkle area fraction was reduced for 11,2% after 6 weeks ($p < 0.05$ for comparison to placebo) and 37,5% after 12 weeks ($p < 0.05$ integrouop and between group comparison).

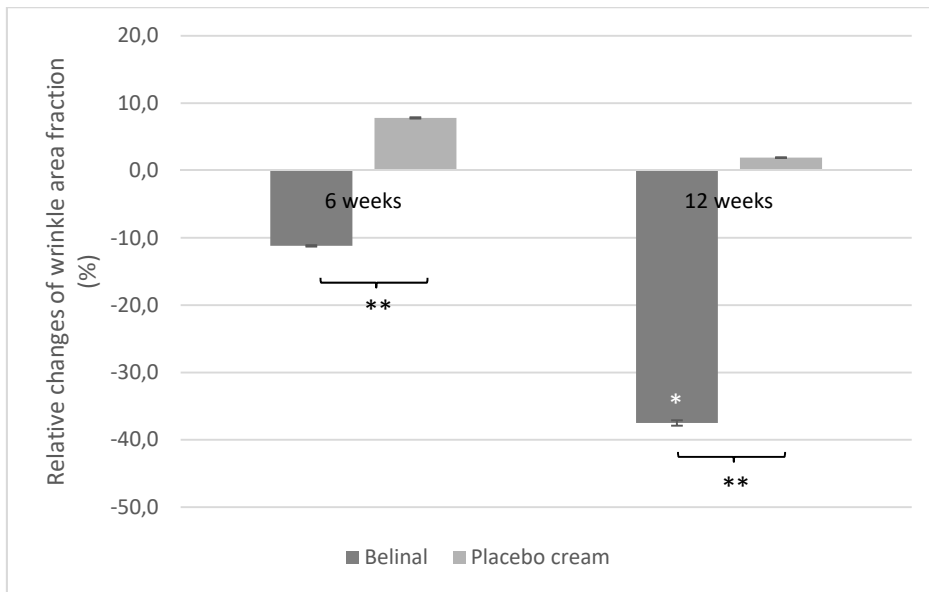


Figure 2. Relative changes of periorbital wrinkle area fraction from baseline. Belinal® cream significantly reduces visibility of periorbital wrinkles* P<0.5 significant difference within group between week 0 and 6 or week 0 and 12.** P<0.05 significant difference between sides.

Belinal® cream improves also skin hydration. In a placebo controlled clinical study on 10 women, hydration increased by **12.6% after 6 weeks and 26.2% after 12 weeks** of use of test cream in comparison to baseline value, (p<0.05 for both). Both results were significant also in comparison to placebo.

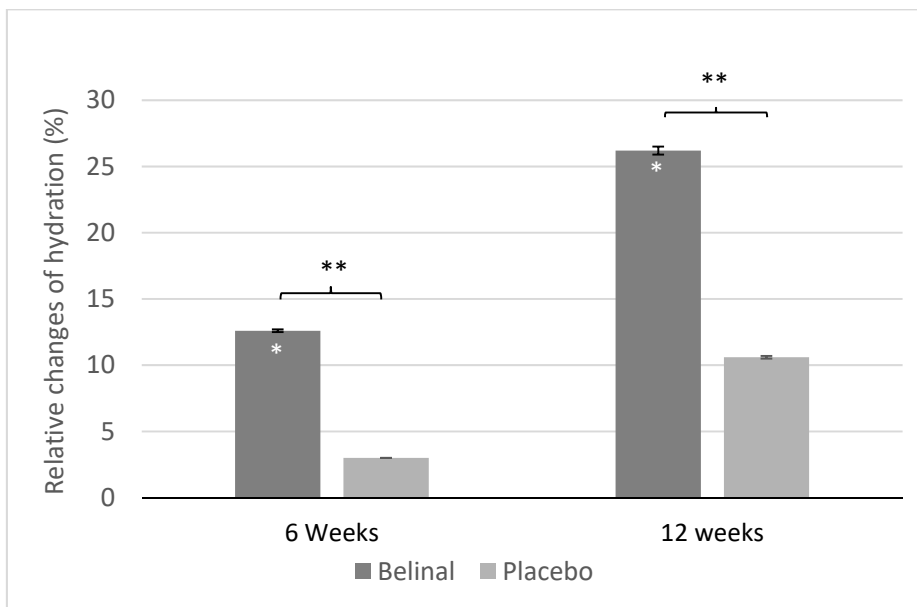


Figure 3: Relative change of skin hydration from baseline. Belinal® significantly improves skin hydration. * P<0.5 significant difference within group between week 0 and 6 or week 0 and 12. ** P<0.05 significant difference between sides.

Transepidermal water loss (TEWL) is the amount of water that passively evaporates through skin to the external environment due to water vapor pressure gradient on both sides of the skin barrier and is used to characterize skin barrier function. The measurement of TEWL is a good indicator of the integrity of the skin barrier function which inherently refers to the skin's ability to retain moisture. An increase in the TEWL indicates an impaired barrier function. TEWL increases due to disruption of the skin barrier including wounds, burns and dry skin, irritation... It is also affected by external factors including wind, sun and extreme temperatures.

In the study the 12 week of use of Belinal® cream resulted in decreased TEWL (13,1% after 6 weeks and 20,5% after 12 weeks in comparison to baseline values; $p < 0.05$), indicating improvement in skin barrier function. Both results were significant also in comparison to placebo.

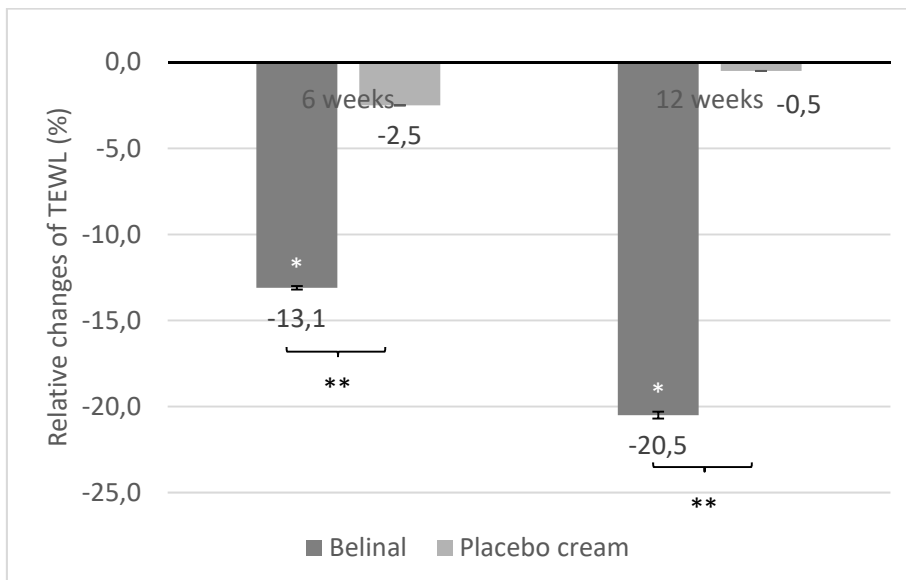


Figure 4: Relative change of TEWL from baseline. Belinal® significantly improves skin hydration. * $P < 0.5$ significant difference within group between week 0 and 6 or week 0 and 12. ** $P < 0.05$ significant difference between sides.

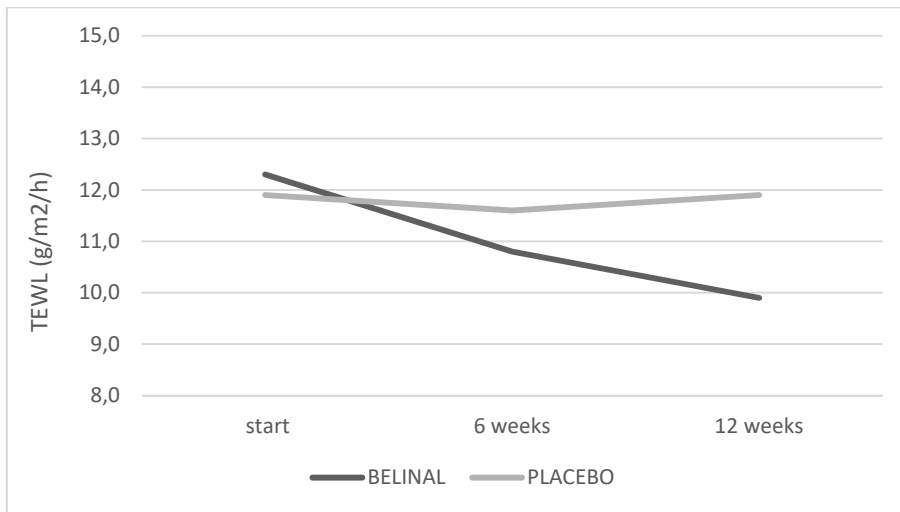


Figure 5: Belinal® significantly reduces TEWL, indicating improvements in skin barrier function

Silver fir branch extract is able to improve skin retraction, time needed for skin to return to initial state after suction application. Retraction is elasticity parameter measured simultaneously with viscoelasticity. Shorter retraction time (lower retraction value) indicates better skin elasticity. In the study the retraction improved by 42,7% in comparison to baseline value after 12 weeks of test cream use ($p < 0.05$). There was no significant change for neither of the products regarding viscoelasticity or Young's modulus. Similar trend was observed for viscoelasticity, but the result was not statistically significant.

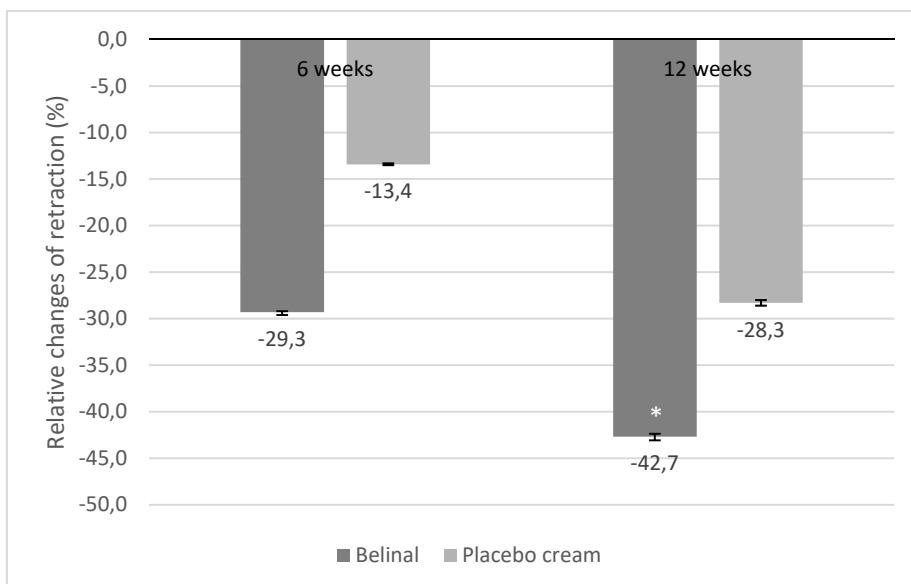


Figure 6: The improvement of retraction, one of the elasticity parameters, was statistically significant in comparison to baseline on side where Belinal cream was used after 12 weeks of treatment

In the study minimal erythema dose (MED) was determined on gluteal part of the body before and after 12 weeks of using test and placebo cream, each on one side of the gluteal part. MED is defined as the threshold dose that may produce sunburn and is measure for skin sensitivity towards UV rays. Daily application of topical cream containing silver fir branch extract - Belinal® for 12 weeks resulted in increase of the minimal erythema dose (MED) - it was increased for 8,6% in comparison to baseline value; rise of MED on test cream side in comparison to placebo was statistically significant ($p < 0.05$) after 12 weeks. Those results indicate that extract is able to contribute to UV protection of the skin, as it reduces the erythema response to UVB. Therefore, it has the potential for use in the sun protection cosmetic as UV filter complement.

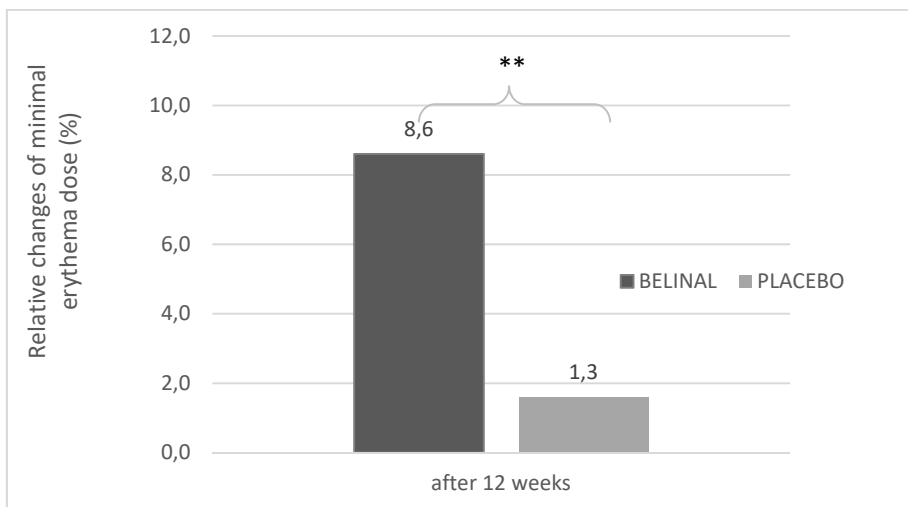


Figure 7: Belinal® cream is able to contribute to UV protection of the skin, as it reduces the erythema response to UVB. ** $P < 0.05$ significant difference between sides.

During the study we noticed lightening of solar lentigines in four volunteers, where on average colour difference between selected lentigines and surrounding skin reduced for 15% after 6 weeks and for 41% after 12 weeks on the test side, but, due to a small number of volunteers, the results were not further statistically analyzed. In order to study influence of belinal on pigmentations study with greater number of subjects with hyperpigmentations should be done.



Figure 8: Belinal® has potential to reduce hyperpigmentation for examplesolar lentigo in one of the volunteers.

Conclusion

Placebo controlled study of influence of cream with 2% of silver fir wood extract on skin shows that it is able to improve various skin parameters and has significant anti-ageing effects. Its use resulted in improvement of TEWL, skin hydration, retraction (elasticity parameter) and dermis density already after 6 weeks of its use, while results were additionally improved after 12 weeks of test cream use.

It was shown that cream with 2% of Belinal® contributes also to UVB protection of the skin, as it reduced the erythema response to UV after 12 weeks of twice daily use. It has also promising whitening effects on solar/senile lentigos.

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