

TOPICAL APPLICATION OF LIGNANS AND PHYTOSTEROLS IN SEBORRHOIC SKIN

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Summary

Topical application of cosmetic products can be helpful in improving seborrhoeic skin condition. The aim of this work is to evaluate the sebum regulation efficacy of a cosmetic formulation containing REGU®-SEB a compound rich in lignans and phytosterols showing strong 5 α -reductase inhibition "in vitro" activity. Thirty healthy female volunteers with seborrhoea disease in their skin face were involved in the study. Clinical evaluation as well as biophysical non-invasive measurements were taken in order to monitor product effects. The parameters considered were sebum skin content (Sebumeter® SM 815) and skin hydration (Corneometer® CM 825). The results showed improvement of skin conditions with statistically significant reduction of clinical score ($p < 0.001$) and sebum skin content ($p < 0.05$) and with increase of skin hydration ($p < 0.01$).

The formulation was in general well accepted and tolerated even if some subjects judged the formulation greasy and difficult to be absorbed by the skin. Further studies could be carried out on formulations containing lower amounts of lipophilic phase (gels for example) more suitable to seborrhoeic skins.

Riassunto

L'applicazione topica di prodotti cosmetici può rappresentare un valido aiuto nel migliorare le condizioni della cute affetta da seborrea.

Lo scopo di questo lavoro è la valutazione dell'efficacia seboregolatrice di una formulazione contenente REGU®-SEB un composto ricco in lignani e fitosteroli che presenta "in vitro" elevata attività di inibizione nei confronti della 5 α -reduttasi.

Lo valutazione è stata condotta su trenta volontari sani di sesso femminile con problemi di seborrea cutanea al viso. Al fine di studiare l'efficacia della formulazione sono state utilizzate sia la valutazione clinica che l'applicazione di misure biofisiche non invasive. I parametri indagati sono il contenuto cutaneo di sebo (Sebumeter® SM 815) e l'idratazione cutanea (Corneometer® CM 825). I risultati hanno mostrato un miglioramento delle condizioni cutanee con significativa riduzione dello score clinico ($p < 0,001$) e del contenuto di sebo ($p < 0,05$) e con aumento dell'idratazione cutanea

($p < 0,01$).

La formulazione è stata in generale ben accettata e tollerata anche se alcuni soggetti in studio hanno giudicato la formulazione grassa e di difficile assorbimento. Ulteriori studi dovrebbero essere condotti su formulazioni contenenti minori quantità di fase liofila (per esempio gel), maggiormente adatti alle pelli affette da seborrea.

INTRODUCTION

Seborrheic skin is perceived by many people to be a serious cosmetic problem and it often provokes much concern for people who suffer from it. The skin appears greasy and shiny and is often accompanied by large pores on the cheeks, nose, chin and forehead. Oily skin results from large quantities of sebum being produced by the sebaceous glands, filling the follicular reservoir, and excretion onto the skin surface (1).

Sebum is an oily mixture of lipids, keratin and cellular membrane structures excreted by the sebaceous glands (2). These glands form part of the pilosebaceous unit, i.e. they are always found in connection with a hair follicle. They are found mainly on the face and on the back, and they are the anatomical substrate of *acne vulgaris*. Moreover increased sebum production stimulated by androgens is nearly always the first listed pathogenic factor promoting acne (3).

Sebaceous gland activity depends largely on endocrine stimulation by the androgen hormones. Testosterone is transformed to the active metabolite dihydrotestosterone (DHT) by the enzyme 5α -reductase, which is present in sebaceous glands.

Acne and its variants from comedones to the cystic nodules of acne conglobata, is the most important and an extremely frequent disease of the pilosebaceous unit. The development of acne is closely correlated to seborrhea and the suppression of sebum production is a powerful therapeutic principle for acne management (4).

The aim of this work is the "*in vivo*" evaluation of the sebum regulation efficacy of a cosmetic formulation containing as active a solution of Argania Spinosa Kernel Oil, Serenoa Serrulata Fruit Extract and Sesamum Indicum Seed Extract (REGU®-SEB). This solution shows "*in vitro*" strong 5α -reductase inhibition as determined by decreasing DHT levels in reconstructed human epidermis SkinEthic® (5,6) due to its

excellent balance of natural polyphenols and lignans.

MATERIALS AND METHODS

Materials

Argania Spinosa Kernel Oil/Serenoa Serrulata Fruit Extract/Sesamum Indicum (Sesame) Seed Extract (REGU®-SEB) was a gift by Pentapharm Ltd (Switzerland). Sweet Almond Oil was purchased by Balestrini Chimica (Italy), Cetearyl alcohol/Cetearyl/Glucoside, Polyacrylamide/C13-14 Isoparaffin/Laureth-7 were supplied by Seppic Inc (Italy). Methyl/Ethyl/Butyl-parabens, tocopherol, lecyrin, ascorbic acid and citric acid were purchased by Biochim Srl (Italy). Finally Glycerine, Imidazolidinyl urea, Disodium EDTA were supplied by Acef (Italy).

Preparation and characterization of the cosmetic formulation

A formulation containing 5% of REGU®-SEB was prepared mixing lipophilic and aqueous phases warmed at 70°C using a turboemulsifier Silverson SL2T (Silverson machine Ltd, England) at 3300 rpm for 40 minutes to obtain a O/W emulsion. Thermosensitive components were added after slowly cooling of the emulsion at 40°C.

The formulation was characterized for density weighing exact volumes (measured with a syringe) of cream collected in different parts of the container and for viscosity using a Brookfield apparatus RVT 230V (Brookfield Engineering Labs. Inc., USA). Tests were performed to evaluate the stability of the cosmetic preparation to light conserving a portion of the formulation for three months in a transparent glass box and to temperature increase submitting a portion of the formulation to a thermic treatment at 45°C for a period of two weeks.

Sebum regulation efficacy

Thirty healthy volunteers (female subjects, age range 20 to 30) participated in this study. Each woman had at least a moderate degree of seborrhoea in her skin face. The subjects were observed before the treatment and after two weeks always by the same investigator. All were instructed to apply the test cream on their face (T zone) twice a day (morning and evening) for two weeks and they were not allowed to use any other skin care product during the study period. Volunteers were selected according to the following inclusion criteria:

- ◆ female subject aged between 20 to 30 with seborrheic skin as defined by an overall score between 2 and 2.5 on a visual analogue scale 0 (none) to 3 (severe);
- ◆ absence of hypersensitivity against any ingredient of the test cream;
- ◆ discontinuation of systemic/topical treatments (cosmetics, drug products, nutraceuticals) which could interfere with the results of the study at least three weeks before;
- ◆ absence of lesions in investigation anatomical sites;
- ◆ signing of an informed consent.

Pregnant women and nursing-mothers were excluded from the study.

Clinical evaluation

Clinical evaluations were performed on the day one (baseline) and after two weeks (end of the treatment). Control of seborrhoea was performed by a trained investigator using a visual scoring system based on a scale ranging from 0 to 3 according to the following ranks:

- 0 = no clinically relevant seborrheic skin;
- 1 = slightly seborrheic skin;
- 2 = moderate seborrheic skin;
- 3 = obviously seborrheic skin.

Biophysical measurement

The skin surface sebum was controlled by

means of the Sebumeter® SM 815 (CK electronic GmbH, Germany) on the 1st day (baseline) and after two weeks. Determination is based on grease-spot photometric measurement of light transmission through a special tape that becomes transparent in contact with the sebum of the skin surface. A microprocessor calculates the result, which is shown on the display in units from 0 to 350.

Skin hydration of the horny layer was assessed by measuring electrical capacitance of the skin surface by the Corneometer® CM 825 (CK electronic GmbH, Germany). When the probe is applied to the skin (recording time 1 s) the capacitance is displayed in arbitrary units (0-130).

The parametric t-test was used to analyze differences between pre- and post-treatment values. A p-value of less than 0.05 was taken as a significant difference, a p-value less of 0.001 was taken as a very significant difference.

RESULTS

Emulsion preparation and characterization

The optical microscopy analysis of the formulation by using methylene blue as colorant highlights that we are in presence of a O/W homogeneous emulsion. The density of the formulation is 1.0052 ± 0.02 mg/ml, close to the density value of water as expected because of the large amount of water present in it. Rheologic analysis shows that the formulation has a pseudo plastic behaviour, with a decrease of viscosity as the applied force increases.

Stability tests show that the formulation is stable to light and to temperature increase, in fact observing the emulsion with optical microscope there are no differences in samples before and after treatment.

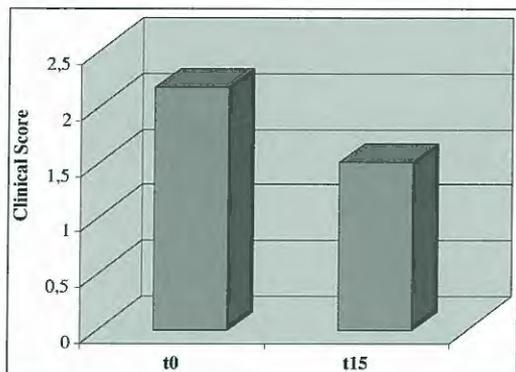


Fig. 1 Results of clinical evaluation. Highly statistically significant ($p= 2.41 \times 10^{-7}$) decrease of seborrhoea clinical score comparing basal (t_0) and after treatment (t_{15}) data.

Sebum regulation efficacy

Results of sebum regulation efficacy are showed in Figures 1 and 2.

The results of clinical evaluation, reported as mean of scores evaluated for each subject before and after treatment, show a significant decrease of the score from 2.167 to 1.500. This reduction suggests an improvement in skin condition. A reduction in sebum skin content is also supported by biophysical non-invasive measurements carried out with Sebumeter® SM 815. Results obtained comparing the average of basal results with those obtained after 2 weeks treatment show a reduction in sebum content from 224.625 to 196.438.

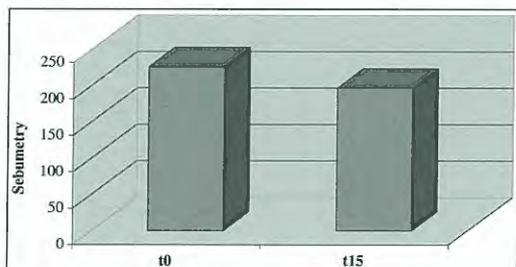


Fig. 2 Results of Sebumeter. Statistically significant ($p= 0.049126$) reduction in sebum skin content comparing basal (t_0) and after treatment (t_{15}) data.

Finally the evaluation of the moisture content of epidermis (Fig. 3) measured by the Corneometer® CM 825 show that the application of the emulsion test cause an improvement in skin hydration.

Hydration increase is probably due not only to the application of an emollient formulation (pseudo-occlusive effect) but also to the humectant function of glycerol that is able to retain the water of the formulation in the horny layer.

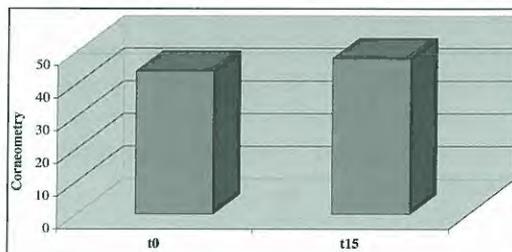


Fig. 3 Results of Corneometer. Statistically significant ($p= 0.002551$) improvement in skin hydration comparing basal (t_0) and after treatment (t_{15}) capacitance values.

DISCUSSION

REGU®-SEB is a compound showing strong “in vitro” activity on 5 α -reductase inhibition. An “in vivo” protocol was carried out joining clinical evaluation and biophysical non-invasive measurements to evaluate its effective sebum regulation efficacy.

The physician’s visual assessment performed highlight a significant decrease in severity of seborrhoea (30.77% decrease in mean score) after two week of application.

Sebumeter data support this result showing a sebum secretion decrease in the application sites after the treatment. It’s also important that the application of the formulation doesn’t cause cutaneous dryness or other skin disease and Corneometer data show that after the treatment the skin hydration degree results increased.

The product is generally well accepted by the

majority of the subjects in study, even if some subjects judge the formulation greasy and difficult to be absorbed by the skin. There are no adverse events related to the test product during this study.

Results obtained in this pilot study demonstrate the efficacy of the tested formulation in improving skin condition. In conclusion REGU®-SEB show good efficacy in decreasing the severity of seborrhoea and can be considered a helpful compound in the treatment of seborrheic skins.

Further studies could be carried out to optimize the vehiculation of the compound by studying formulations containing less quantities of lipophilic phase (gels for example), more suitable to seborrheic skins.

References

- 1) **Rizer RL (1999)** Oily skin: Claim Support Strategies. In: *Cosmetics: Controlled efficacy studies and regulation*, Elsner P., editor, p.81-91
- 2) **Caputo R., Monti M. (1995)**, La deodorazione. In: *Manuale di Dermocosmetologia Medica*, Raffaello Cortina, editor, p.121-133
- 3) **Youn SW, Park ES, Lee DH, Huh CH, Park KC (2005)**, Does facial sebum excretion really affect the development of acne?, *Brit. J. Dermatol*, **153**:919-924
- 4) **Elsner P (1995)** Sebum. In: *Bioengineering of the skin: Method and Instrumentation*. Berardesca E, Elsner P, Whielm KP, Mailhach HI editors, CRC Press, p.81-89
- 5) **Bernard FX, Barrault C, Deguercy A (2000)** Expression of type I 5 α -reductase and metabolism of testosterone in reconstructed human epidermis (SkinEthic®): a new model for screening skin-targeted androgen modulators, *Int. J. Cosm. Sci.*, **22**:397-407
- 6) **Pentapharm Ltd. (2001)** Bioalternatives study n° AD010818, Effect of the compound "REGU-SEB" on testosterone metabolism in reconstructed human epidermis

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