

Effects of Topical Boswellic Acid on Photo and Age-Damaged Skin: Clinical, Biophysical, and Echographic Evaluations in a Double-Blind, Randomized, Split-Face Study

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Key words

- boswellic acids
- *Boswellia serrata* Roxb.
- Burseraceae
- skin aging
- 20 MHz B-mode echography

Abstract

Boswellic acids (BAs) are pentacyclic triterpenes with strong anti-inflammatory activity; their most important source is the extract of the gum resin of *Boswellia serrata*, a tropical tree that grows in India and Africa. In the present randomized, double-blind, split-face, comparative study we have assessed efficacy, tolerability, and safety of a base cream containing 0.5% BAs as compared to the same cream without these active ingredients in the treatment of clinical manifestations of photoaging of facial skin. Fifteen female volunteers were enrolled; they applied creams once daily for 30 days. At baseline, at the end of the treatment, and after a 2-month follow-up, clinical

findings were assessed according to the Dover classification scale for photoaging and by biophysical and echographic measurements. We registered a significant improvement of tactile roughness and fine lines in the half side of the face treated with BAs; noninvasive instrumental diagnostic investigations showed an improvement of elasticity, a decrease of sebum excretion, and a change of echographic parameters suggesting a reshaping of dermal tissue. The treatment was always well tolerated without adverse effects. The present findings seem to indicate that the topical application of BAs may represent a suitable treatment option for selected features of skin photoaging.

Introduction

Boswellia serrata Roxb. (Burseraceae) is a large, branching, deciduous tree which grows in India, Ethiopia, Somalia, and the Arabic peninsula. The lipophilic fraction of its fragrant gum is used in ayurvedic medicine for the treatment of various inflammatory disorders and tumors. The active fraction, the boswellic acids (BAs), are pentacyclic triterpenes, with alpha or beta structure (depending on the position of the methyl groups), with a carboxyl group and at least another functional (hydroxyl-, acetyl-, or keto-) group; they have been isolated from the gum resin where they represent up to 30% of the weight [1].

Experimental investigations of the molecular mechanisms underlying the biological effects of BAs have identified IkappaB kinases as the molecular target, with consequent inhibition of NF-kappa B activation and TNF- α release from activated monocytes [2, 3]. Another well-known biological activity of BAs is the inhibition of matrix metalloproteinase protein (MMP) transcription in fibroblasts and endothelial cells [4]. MMP, in particular

MMP-1, plays a pivotal role in the cleavage of dermal fibrillar collagen resulting in dermal damage [5].

These biological properties are the rationale of a possible use of BAs in the treatment of skin lesions due to chronic ultraviolet (UV) exposure. In fact, UV causes dermal inflammation via a cascade of biological and molecular events that involves chronic perivenular infiltration of blood leucocytes (macrophages and neutrophils), increased production of prostaglandins (PGs) as the consequence of increased lipid peroxidation, and increased synthesis (via activation of NF-kappa B) and release of TNF- α and other inflammatory cytokines (IL1, IL6) that increase the oxidative stress [6, 7]. There are also elastosis, breakdown, and replacement of mature collagen fibers by collagen (with increased transcription of MMP-1 in fibroblasts and endothelial cells), large increase in deposition of glycosaminoglycans, and deposition of elastotic material [8–10].

The aim of the present randomized, double-blind, split-face comparative study was to evaluate the efficacy and tolerability of a cream containing a

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low (0.5%) concentration of BAs in the treatment of photoaged skin of the face.

Clinical results were assessed visually by blinded investigators according to the Dover classification scale for photoaging [11] and with noninvasive diagnostic techniques.

In addition, high-resolution 20 MHz B-Mode echography was employed to investigate the UV-related dermal changes [12].

Materials and Methods

Patients

Fifteen women, mean age 44.4 years (range 31–68) with various clinical features of photodamaged skin of the face were enrolled in the study. The study was approved by the local ethics committee and all patients gave their written informed consent.

Exclusion criteria were: pregnancy and lactation, inflammatory or neoplastic skin diseases of the treated area, subjects who had undergone physical treatments or any other cosmetic treatment for photoaging, as well as subjects who had used any systemic drug (including oral contraceptives) in the past 3 months.

Plant material, extraction, and isolation

The commercially available plant product, 5-Loxin® (batch number I 508392), produced by P.L. Thomas, wherefrom it was purchased, was used in this study.

5-Loxin® is obtained from a *Boswellia serrata* gum resin extract (collected in India by Laila Impex Research Center), and is enriched with 3-O-acetyl-11-keto- β -boswellic acid (AKBA; 30% vs. 1–10% of the natural gum resin content; ● Fig. 1). AKBA is known to be the most pharmacologically potent of all the naturally occurring boswellic acids isolated from *Boswellia serrata*.

Besides 30% AKBA, 5-Loxin® also contains 20%, cumulatively, of all other natural boswellic acids, namely: β -boswellic acid, 3-acetyl- β -boswellic acid, 11-keto- β -boswellic acid, α -boswellic acid, and 3-acetyl- α -boswellic acid. The remaining 50% is unknown plant material.

5-Loxin® is prepared by oxidation of the gum resin extract followed by acetylation. All details of the chemical processes for producing it are described in the US patent that is freely available in the web [13]. In brief: the natural gum resin is subjected to di-

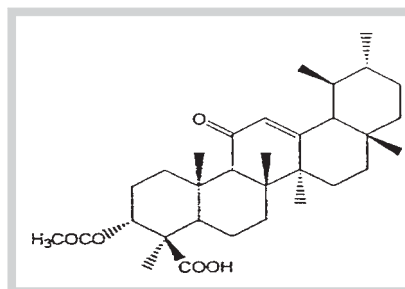


Fig. 1 Chemical structure of acetyl-11-keto- β -boswellic acid.

oxane extraction, and the extract is then oxidized with N-bromosuccinimide and calcium carbonate, and dried under vacuum. The dry powder is then acetylated using dichloroethane, acetyl chloride, and pyridine. AKBA content is determined by an HPLC method and confirmed to be 30% in the batch of 5-Loxin® used in this study.

Treatment protocol

The face of each patient was split into two parts along the vertical axis. One side was randomly assigned to the treatment with a base cream containing 0.5% BAs (list of ingredients reported in ● Table 2); the other half side was treated with an identical cream without BAs, and served as control. Both preparations were provided by Scharper Healthcare Srl in identical tubes identified by blinded coding. Both patients and physicians were not aware of the side of the face where the cream containing BAs was applied.

Application was repeated once a day for 30 days. At baseline (T0), at the end of the therapy (T1), and at the 2-month follow-up visit (T2) digital photographs were taken under standardized conditions of illumination, and the severity of the clinical manifestations of photoaging was scored independently by two blinded observers according to the 0–4 visual-analogue scale of the modified Dover photodamage classification system [11] (● Table 1).

For the purposes of the present investigation, unlike the original scoring system, the scores of each side of the face were summed separately and the score of the dorsa of the hands, which were not treated, was ignored.

Table 1 Scores on an 8-point scale (0–4) for skin manifestations of photodamage (modified Dover score).

Fine surface lines	0, no evidence; 1, rare, widely spaced; 2, several, discrete; 3, moderate, in close proximity; 4, many, densely packed
Mottled pigmentation	0, evenly pigmented skin; 1, small areas of light hypo- or hyper-pigmentation; 2, small areas of moderate hypo- or hyper-pigmentation or moderate areas of light hypo- or hyper-pigmentation; 3, moderate areas of moderate hypo- or hyper-pigmentation, large areas of light hypo- or hyper-pigmentation, or small areas of heavy hypo- or hyper-pigmentation; 4, severe hypo- or hyper-pigmentation
Sallowness	0, pink skin; 1, skin with a slight suggestion of yellowness; 2, skin is pale with a moderate suggestion of yellowness; 3, skin is pale with a distinct suggestion of yellowness; 4, pale skin with severe yellowness
Tactile roughness	0, skin is smooth; 1, skin is smooth, with occasional rough areas; 2, mild roughness; 3, moderate roughness; 4, severe roughness
Coarse wrinkling	0, no evidence; 1, superficial on one area (forehead, glabella, chin, nasolabial and periorbital area); 2, superficial on more than one area or moderate in one; 3, moderate on more than one area or deep in one; 4, deep on more than one area
Facial erythema	0, no evidence; 1, small areas of light erythema; 2, small areas of moderate erythema or moderate areas of light erythema; 3, moderate areas of erythema, large areas of light erythema, or small areas of erythema; 4, large areas of severe erythema
Teleangiectasia	0, no evidence; 1, rare, widely spaced; 2, several, discrete; 3, moderate, in close proximity; 4, many, densely packed
Sebaceous gland hypertrophy	0, no evidence; 1, rare comedos and/or milia on one skin area (cheeks, forehead, chin); 2, rare comedos and/or milia on more than one skin area or several, discrete on one skin area; 3, several, discrete on more than one skin area or many, densely packed on one skin area; 4, many, densely packed on more than one skin area

Table 2 Composition of the cream (Sharper Healthcare Srl).

Ingredients
Demineralized water
Allantoine
Tetrasodium glutamate diacetate
Betaine monohydrate 99%
Carbomer
Panthenol
Hydrogenated polydecene
Limnanthes alba seed oil
Butyrospermum parkii butter
Ppg-15 stearyl ether
Steareth-2
Steareth-21
Cetyl alcohol
Ethylhexyl methoxycinnamate
Butyl methoxydibenzoylmethane
Triclosan
Phenoxyethanol
Sodium hydroxide
Citric acid
Olea europaea leaf extract
Dimethyl isosorbide
Tocopheryl acetate
5-Loxin®
Sodium silicoaluminate
Diazolidinyl urea
Glycerin
Ectoin
Sodium hyaluronate
Silk amino acids
Glycoproteins
Polymethyl methacrylate

During the study period, patients were asked to not change the frequency of washing or their cleansing agent. Use of other cosmetic creams was not allowed. The study was performed during winter months in order to minimize the influence of exposure to natural sunlight. Positive controls were not included in the present investigation, because studies evaluating skin echographic modifications after topical application of anti-aging preparations were not previously reported.

Biophysical measurements

At T0, T1, and T2 stratum Trans Epidermal Water Loss (TEWL) was measured in grams per square meter and per hour ($\text{g/m}^2 \times \text{h}$) with the Tewameter TM 210; the water content of the horny layer with the Corneometer CM 820 and the skin surface lipids with the Sebumeter SM 810 PC (all from Courage and Khazaka GmbH). Skin distensibility (Uf), expressed in mm, was measured with the MicroCAMERA, (Dermotricos s.r.l). Measurements were taken according to established methods [14–16]. The reported values represent the average of two measurements taken exactly on the same skin area at each visit in order to avoid possible intra-individual variations of biophysical parameters.

Echographic investigations

At T0, T1, and T2 a high frequency echographic evaluation with a 201–255 amplitude band was done in two areas (forehead and temples) to obtain data of epidermal and dermal changes. We used a Dermascan C (Cortex Technology). In this device, a 20 MHz transducer ($22.4 \text{ mm} \times 2.4 \text{ mm}$) is mounted in a water

chamber and placed perpendicularly to the skin that is lubricated with a conductive gel.

Ultrasonic waves penetrate into the skin and are reflected backwards at the boundary between adjacent structures of different density generating echoes of various amplitudes. The reflected waves are collected by a detector and analyzed by a computer that visualize on the screen a two-dimensional image with colors that are arbitrarily related to the growing intensity of the echoes (black, green, red, yellow, and white) [17].

Two-dimensional B-mode color images were taken at each measurement and 4 digital parameters were evaluated by an image analysis program (Dermavision 2D, Technology).

1. Skin thickness (mm): the distance from the epidermal surface to the interface between reticular dermis and subcutaneous fat was measured directly in millimeters on the photograph.
2. The number of echogenic pixels composing the image: pixels are the smallest objects in a visual display with controllable color and brightness and their number raises with growing photodamage in the upper dermis [17].
3. The area in which the echoes' amplitude is included within the values of the selected band (201–255) and whose extension can be calculated in mm^2 .
4. The thickness of the subepidermal low-echogenic band (SLEB; mm); this band is defined as the clearly seen low-echogenic band in the upper dermis immediately below the epidermal entrance echo. Its thickness is related to skin aging and higher in photoexposed areas [12, 18].

Statistical analysis

Data are given as mean $\pm$ standard deviation ($m \pm \text{SD}$). Clinical scores and instrumental measurements were compared with a parametric test, the paired Student's t-test. Significance was defined as $p < 0.05$.

Results

▼ All patients completed the study. Treatment was well tolerated and adverse effects were not registered. At baseline, half sides of the face showed similar values for clinical and instrumental measurements.

In the half side treated with the cream containing BAs, the total scores for photoaging showed a significant difference after the therapy and after follow-up 2 months later (T2) in comparison to the baseline (8.69 ± 5.15 at T0 to 7.45 ± 4.31 at T1; ● Table 3). Statistically significant reductions ($p < 0.05$) of tactile roughness (1.46 ± 1.20 to 1.08 ± 0.95) and fine surface lines (2.23 ± 0.83 to 1.69 ± 0.18) were observed after 30 days (T1) and remained statistically unchanged at the follow-up (● Table 3). Other clinical features of photoaging did not show any significant change at T1 and T2 (● Table 3).

In the half side treated with BAs, skin sebum ($63.80 \pm 33.70 \mu\text{g/cm}^2$ at T0 to $45.00 \pm 24.20 \mu\text{g/cm}^2$ at T1) and skin distensibility ($2.21 \pm 0.46 \text{ mm}$ at T0 to $1.87 \pm 0.47 \text{ mm}$ at T1) significantly ($p < 0.05$) decreased; at the follow-up these decreases remained statistically significant in comparison to the baseline ($40.38 \pm 23.68 \mu\text{g/cm}^2$ and $1.89 \pm 0.40 \text{ mm}$, respectively; ● Table 4). There were no statistical differences of TEWL or corneometry after the therapy (T1) or at the follow-up (T2) in comparison to the baseline (● Table 4).

Echographic measurements on the side of the face treated with BAs showed a statistical increase of skin thickness: $1.36 \pm$

Table 3 Variation of parameters (mean ± SD) of the Dover score measured on the face at baseline (T0), after 4 weeks (T1), and after 3 months (T2).

	Half side treated with the cream containing boswellic acids			Control half side treated with the cream without boswellic acids		
	T0	T1	T2	T0	T1	T2
Fine surface lines	2.23 ± 0.83	1.69 ± 0.18*	1.77 ± 0.60*	2.23 ± 0.83	2.23 ± 0.83	2.17 ± 0.80
Mottled pigmentation	0.92 ± 0.86	0.92 ± 0.86	0.85 ± 0.90	0.69 ± 0.85	0.92 ± 0.86	1.07 ± 0.64
Sallowness	0.69 ± 0.85	0.69 ± 0.85	0.61 ± 0.76	0.69 ± 0.85	0.69 ± 0.85	0.77 ± 0.72
Tactile roughness	1.46 ± 1.20	1.08 ± 0.95*	1.08 ± 0.95*	1.46 ± 1.20	1.15 ± 1.06	1.30 ± 1.11
Coarse wrinkling	1.92 ± 0.76	1.77 ± 0.83	1.69 ± 0.94	1.92 ± 0.76	1.85 ± 0.80	1.92 ± 0.64
Facial erythema	0.46 ± 0.52	0.38 ± 0.51	0.54 ± 0.66	0.46 ± 0.52	0.38 ± 0.51	0.52 ± 0.52
Teleangiectasia	0.46 ± 0.52	0.38 ± 0.51	0.30 ± 0.48	0.46 ± 0.52	0.54 ± 0.52	0.38 ± 0.50
Sebaceous gland hypertrophy	0.54 ± 0.66	0.54 ± 0.66	0.38 ± 0.65	0.54 ± 0.66	0.54 ± 0.66	0.69 ± 0.63
Total score	8.69 ± 5.15	7.45 ± 4.31*	7.23 ± 3.8*	8.46 ± 4.99	8.30 ± 5.05	8.85 ± 3.67

* p < 0.05 in comparison to the baseline

Table 4 Variation of biophysical parameters (mean ± SD) measured on the face at baseline (T0), after 4 weeks (T1), and after 3 months (T2).

	Half side treated with the cream containing boswellic acids			Control half side treated with the cream without boswellic acids		
	T0	T1	T2	T0	T1	T2
Sebum (µg/cm ²)	63.77 ± 33.69	45.00 ± 24.20*	40.38 ± 23.68*	64.00 ± 32.06	57.00 ± 23.42	58.46 ± 20.51
Corneometry (corn.-units)	67.92 ± 15.82	70.92 ± 10.34	70.84 ± 10.00	67.85 ± 15.17	71.69 ± 5.57	71.92 ± 4.85
TEWL (g/m ² · h)	19.38 ± 6.26	19.38 ± 5.23	20.02 ± 5.01	19.17 ± 6.29	19.16 ± 5.22	18.75 ± 5.01
Uf (mm)	2.21 ± 0.46	1.87 ± 0.47*	1.89 ± 0.40*	2.18 ± 0.45	1.99 ± 0.58	2.05 ± 0.39

* p < 0.05 in comparison to the baseline

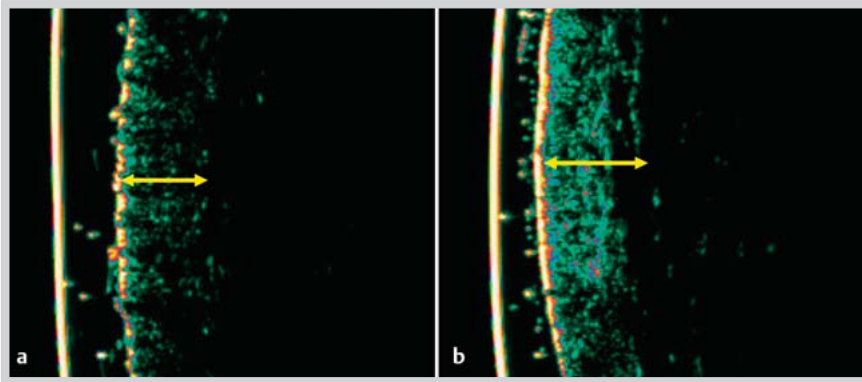


Fig. 2 B mode 20 MHz skin echographic image shows the following structure: 1. the first yellow line on the left represents the epidermal entrance echo; 2. the black space between 2 yellow lines represents gel; 3. the second yellow line represents epidermis; 4. on the right side of the second yellow line we can see the dermis. **a** B mode 20 MHz skin echographic image of skin at T0. **b** B mode 20 MHz skin echographic image of skin at T1: there is an increase of skin thickness (yellow arrows) as reflected by the increased numbers of pixels in comparison to the baseline. Colors are arbitrarily related to the growing intensity of the echoes in the following order: black, green, red, yellow and white.

0.12 mm at T0 to 1.58 ± 0.26 mm at T1 ($p < 0.05$), as well as of the pixel count: 49441.23 ± 7375.70 at T0 to 54504.38 ± 9622.60 at T1 ($p < 0.05$); these values remained statistically significant at T2 in comparison to the baseline (1.60 ± 0.23 mm and 54854.77 ± 9364.17 , respectively; **Fig. 2, Table 5**). Measurements of area and SLEB remained unchanged at T1 and T2 (**Table 5**). The side of the face treated with the emollient cream without BAs did not show any appreciable change in echographic or biophysical values.

Discussion

In the present investigation, the daily application for 30 days of a base cream containing a low (0.5%) concentration of BAs was effective and well tolerated in the treatment of selected clinical features of photodamaged skin. Although the global Dover score for

photodamage improved, the difference was not statistically significant. However significant reductions of the scores of fine lines and tactile roughness were registered and improvements maintained nearly steady at a 2-month follow-up. A skin biopsy of the face skin was not performed before and after the treatment cycle for ethical reasons but echography and noninvasive biophysical measurements may suggest possible explanations of these clinical effects. Through echographic investigation, we found that the reduction of fine lines was accompanied by the increase of the number of pixels and skin thickness, which suggests that a reshaping and a new deposition of collagen and elastic fibers took place. The SLEB thickness decreased as well but the improvement did not reach a statistically significant level. However, the removal of the subepidermal elastotic material is a complex and time-consuming biological process and hence the duration of the treatment cycle, i.e., 30 days, could be too short to significantly clear out the elastotic

Table 5 Variation of echographic measurements (means $\pm$ SD) at baseline (T0), after 4 weeks (T1), and after 3 months (T2).

	Half side treated with the cream containing boswellic acid			Half side treated with the cream without boswellic acid		
	T0	T1	T2	T0	T1	T2
Thickness (mm)	1.36 $\pm$ 0.12	1.58 $\pm$ 0.25*	1.60 $\pm$ 0.23*	1.36 $\pm$ 0.12	1.37 $\pm$ 0.12	1.39 $\pm$ 0.12
Number of pixels	49441.23 $\pm$ 7375.68	54504.38 $\pm$ 9622.60*	54854.77 $\pm$ 9364.17*	49346.62 $\pm$ 7362.03	50800.00 $\pm$ 8525.28	50654.31 $\pm$ 8832.93
Area (mm ²)	20.37 $\pm$ 2.54	20.88 $\pm$ 3.08	21.18 $\pm$ 3.24	20.38 $\pm$ 2.55	20.56 $\pm$ 2.48	20.81 $\pm$ 2.58
SLEB (mm)	0.52 $\pm$ 0.17	0.42 $\pm$ 0.14	0.44 $\pm$ 0.16	0.46 $\pm$ 0.14	0.41 $\pm$ 0.14	0.47 $\pm$ 0.14

* $p < 0.05$ in comparison to the baseline

material. It may be therefore hypothesized that a longer treatment period would produce a more pronounced improvement. Also, the beneficial effect on elasticity, i.e., the quantity of functional elastic fibers in the dermis, is demonstrated by the noninvasive measurement of the increased skin distensibility.

These dermal effects of BAs can be related to their activities on dermal inflammation and structure.

BAs have an anti-inflammatory effect that is mediated by the inhibition of TAK/TAB mediated IKappaBKinase (IKK) phosphorylation of IkappaB, that activates NF-kappa B translocation to the nucleus [2,3]. NF-kappa B is an inducible transcription factor with a pivotal role in the innate immune response and chronic inflammation [19,20]; ubiquitously expressed and involved in the activation of a multitude of genes in response to various stress stimuli, it may have a key role in the regulation of target genes that encode for pro-inflammatory cytokines, adhesion molecules, chemokines and growth factors [21]. Among the cytokines induced by NF-kappa B, IL-1 and TNF- α have particular importance because they stimulate the signal transducing pathway to activate NF-kappa B itself, thus activating a vicious cycle [22,23]. BAs also enhance the metabolism of fibroblasts and, at the same time, they inhibit human leukocyte elastase [24] and hyaluronidase [25], as well as TNF- α -induced transcription, expression, and activity of MMP-1, MMP-3, MMP-10, and MMP-12 in human microvascular endothelial cells [5]. All these enzymes have a key importance in the degradation of the extracellular matrix, collagen, and elastic fibers.

The improvement of tactile roughness suggests that an improved and more even keratinization of the epidermis took place along with the disappearance of hyperkeratotic skin areas. Beside the indirect benefits coming from the anti-inflammatory activities and the overall improvement of the underlying dermis, this effect could be related to the anti-proliferative and pro-differentiative effects of BAs that have been demonstrated in several normal and tumoral human cell lines [26–28]. The improvement of tactile roughness was not accompanied by a decrease of the TEWL whereas the water content of the horny layer increased but not at a significant level and was similar to both half sides of the face suggesting that this was due only to the daily application of the base cream. We did not see any significant effect on the sebaceous gland hypertrophy but sebumetry showed a decrease in sebum excretion suggesting an inhibitory effect on the activity of the sebaceous glands that may be related to the above-mentioned anti-proliferative and pro-differentiative activities and is worthy of further investigations. Unlike fine lines, deep wrinkles showed no improvement suggesting that BAs do not modify the contraction of the subcutaneous fat septae. Finally, mottled pigmentation, sallowness, facial erythema, and teleangiectasias did not improve. Further studies with a longer treatment period will

clarify whether BAs presence is simply not effective against these skin damage manifestations or whether the period of application of our present protocol was too short to produce positive results. The present study is the first to investigate the clinical effects of the topical application of BAs, but a number of animal studies and preclinical trials with human subjects have been performed to investigate its therapeutic efficacy after oral administration. In experimental animal models, BAs proved not only anti-inflammatory properties, but also antitumoral effects that can be theoretically useful in the prevention or treatment of skin cancers, which, like photoaging, are most often caused by chronic UV exposure. In conclusion, the present investigation demonstrates that a short, 30-day treatment cycle with a topically applied base cream containing a low concentration of BAs is an effective and well-tolerated treatment option against selected skin manifestations of photoaging, i.e., fine wrinkling and tactile roughness, and provides a sustained efficacy visible after 2 months at follow-up. Further investigations with longer treatment periods will clarify if it is suitable also in treating other skin manifestations of photoaging, i.e., coarse wrinkling, facial erythema, teleangiectasias, and sebaceous gland hypertrophy which improved in the present study but not at a significant level.

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