

Efficacy of Tri-active Brightening and Anti-aging Complex in Treatment of Facial Skin Hyperpigmentation

Malwina Zasada¹, Renata Dębowska², Monika Pasikowska², Beata Ostrowska³ and Elżbieta Budzisz¹

1. Department of Cosmetic Raw Materials Chemistry, Faculty of Pharmacy, Medical University of Lodz, Lodz 91-151, Poland

2. Dr. Irena Eris Cosmetic Laboratories, Dr. Irena Eris Centre for Science and Research, Warsaw 02-595, Poland

3. Dr. Irena Eris Cosmetic Laboratories, Technological and Implementation Division, Piaseczno 02-595, Poland

Abstract: Hyperpigmentation can be caused by long-term UV (ultraviolet) exposure, hormonal imbalances, skin ageing processes, as well as skin inflammation, skin injuries and accumulation of hemosiderin. A brightening complex consisting of niacin, *Rumex spp.* and biomimetic peptide is supposed to be an efficient alternative for commonly used brightening agents. *In-vivo* research of night cream (1474) was conducted in order to confirm the safety and efficiency of tri-active brightening cream in treatment of facial skin hyperpigmentation. The research was conducted on a group of 30 female patients, and the night cream was applied once a day for six weeks. The research was done by the use of VISIA[®] system, multifunctional MPA and PRIMOS[®] projection system, which was applied with VISIOSCAN camera. Besides, the research also included a questionnaire. A decrease in melanin by 16% and 25% at 93% and 96% of patients, respectively, was observed after three and six weeks of regular application of the cream. Furthermore, we also noticed reduction of erythema which was accompanied by an increase in the skin moisture. Brightening of changes on hyperpigmented facial skin proved to be efficient after an application of tri-active complex which was a component of the night cream.

Key words: Hyperpigmentation, brightening, tri-active complex.

1. Introduction

Skin changes, including changes in the epidermis structure, are effects of day-to-day functioning of human skin cells. Factors determining the skin condition include both endogenous factors that change over one's life and exogenous factors which affect us differently each day. The skin affected by the process of chronological ageing and photo-ageing manifests with progressive, inaccurate and irregular distribution of melanocytes, which are responsible for the skin color. The skin also has significant hormonal receptors: estrogen, androgen and progesterone. Androgen receptors are located in keratinocytes' nuclei, in hair follicles and sebaceous glands. Progesterone receptors are present in melanocyte cells. In the etiopathogenesis of skin hyperpigmentation, the most important are estrogen receptors located in vast numbers in

melanocytes' cell nuclei. Proper functioning of hypothalamic-pituitary axis is crucial for accurate control of steroid hormones, thyroid and pituitary gland that significantly influence the melanogenesis process.

Tyrosinase is the main enzyme for melanine synthesis. The enzyme acts as a catalyst for a change of L-tyrosine into L-DOPA (3,4-dihydroxyphenylalanine) and, afterwards, acts as catalyst for its further oxidation into L-Dopaquinone. Processes modifying dopaquinone lead to formation of eumelanin or pheomelanin. Presence of amino acids containing the sulpho group, such as cysteine or/and glutathione, results in binding of dopaquinone to SH groups and formation of cysteinyl-DOPA—the precursor for pheomelanin. Lack of amino acids containing sulphur results in oxidation of dopaquinone to dopachrome, an intermediate reaction, and next, to formation of eumelanin [1].

The activation of the melanogenesis process is triggered by a hormone stimulating

Corresponding author: Malwina Zasada, Ph.D. candidate, research fields: dermatology and cosmetology.

melanocytes- α MSH (melanocytes stimulating hormone alpha), as well as factors triggering the synthesis of cAMP (adenosine 3',5'-monophosphate). α MSH is secreted by the central lobe of the pituitary gland. The hormone remains active during pregnancy and during foetal life. Afterwards, its functions are transferred to hormones from proopiomelanocortin containing the amino acid MSH sequence.

Skin exposure to UV (ultraviolet) radiation results in the occurrence of free radical reactions in a human body. Free radicals react with DNA of human skin cells, proteins and carbohydrates, which results in their oxidation and formation of oxidation products. These in turn, by releasing inflammation mediators, generate inflammatory reactions, lead to oedema and skin irritation. UV radiation has negative effects as it causes disturbances and inhibits mechanisms responsible for proper functioning of skin control [2]. This results in photoaging of the skin, and as a consequence, hyperpigmentation, wrinkles, loss of skin firmness and flexibility [3, 4]. Application of formulas combining protective functions with therapeutic ones becomes necessary and use of brightening-stimulating creams becomes part of everyday skin treatment. Furthermore, in order to reduce negative effects of hyperpigmentation spots, the formulas should contain ingredients with potential anti-inflammatory and anti-oxidant characteristics. The tested night cream (1474) contained niacin, biomimetic peptide, extract of *Rumex spp.*, Vitamin C, Vitamin E and papain.

The aim of this study was to evaluate the efficiency and functional advantages of brightening, anti-aging night cream (1474), applied in patients with hyperpigmented skin with different etiology, with the use of instrumental evaluation of skin condition and a questionnaire.

2. Materials and Methods

A group of 30 patients ($n = 30$) consisted of people suffering from facial skin hyperpigmentation with different etiology. The selection of patients in term of

age was random and the age of the patients ranged between 18 and 80 years. The mean age was 49 years (49.40). The study was completed by 27 participants ($n = 27$). The participation was voluntary and each patient had their patient's chart with their personal profile created in software tools used for the research. The study was approved by the Bioethics Commission and given No. RNN/58/15/KE/M.

Study tools used for the purpose of the research before D(0), after three weeks D(21) and after six weeks of treatment D(42) included:

(1) System VISIA[®], allowing to make a visual and nominal analysis of skin condition;

(2) Multifunctional MPA tool with the use of probes:

- Mexameter[®] to measure skin color, i.e., melanin and erythema;

- Corneometer[®] to measure moisture;

- Sebumeter[®] to measure oiling;

- Cutometer[®] to measure firmness and flexibility;

(3) PRIMOS[®], system of tri-dimensional projection used in order to measure anti-aging effects;

(4) VISIOSCAN (Video Digitizer[®] VD 300) camera.

Skin analysis was made in a measurement room at stabilized temperature ($T = 19\sim 21\text{ }^{\circ}\text{C}$) and humidity ($H = 30\sim 50\%$). The study was preceded by a minimum 20 min acclimatization in the measurement room. The criteria excluding from the study group were: use of tanning beds, application of other brightening therapies, inflammatory states, as well as infections of the facial skin, pregnancy and breast feeding. It evaluates skin condition with the use of nominal values by making three pictures: from the left and right profiles and en face. The pictures go through multi-spectrum image processing which analyzes the skin structure, pigmentation, size of pores, porphyrin (traces of bacteria), UV stains, sun damage and wrinkles—factors which influence the skin condition. The system allows to take pictures with UV lighting (visualization of hyperpigmentation and porphyrin),

white light (visualization of hyperpigmentation, porphyrin, wrinkles and skin structure), as well as cross-polarization light in the RBX[®] technology (visualization of hyperpigmentation and blood vessels).

A Video Digitizer[®] VD 300 camera enables to visualize the skin look with the use of UV rays. Photographic documentation allowed us to picture the external level of the epidermis which changed along with changing skin conditions and external factors. The parameters that we measured included skin smoothness with special consideration given to the level of epidermis hydration—Smoothness (*SEsm*) and without consideration given to the level of epidermis hydration—Surface (*S*), skin roughness—Roughness (*SEr*), level of skin variance—Variance (*Var*), overall skin condition—Energy (*NRJ*), depth and number of wrinkles—Volume (*V*), relation between horizontal and vertical wrinkles—Wrinkles (*SEw*), level of dryness in the stratum corneum (skin scaliness)—Scaliness (*SEsc*).

Questionnaires conducted after three and six weeks of treatment with the brightening cream allowed us to make a subjective evaluation of functional advantages and efficiency of the product. Before testing the product, a questionnaire on skin fragility was prepared in order to receive information on possible skin hypersensitivity and/or allergies as well as their causes.

The study included a control group consisting of 12 patients. Measurements with the application of Mexameter[®] were made three times over a period of three months (January, February and March). The Mexameter[®] probe was used to evaluate changes in the amount of melanin with an application of standard treatment, without any use of specialist brightening cosmetics.

The composition of the tested formula included 5% niacin, 0.000395% biomimetic peptide, 1% extract from *Rumex spp.* (*Rumex occidentalis*) and additional ingredients, such as 0.5% Vitamin C, 0.5% Vitamin E and 2% papain.

Obtained results were statistically analyzed with the

use of Statistica software (StatSoft 12.0, Poland). Value $p < 0.05$ was adopted as significant. The normality of data distribution was checked by the Shapiro-Wilk test. The non-parametric ANOVA Friedman test was also used in order to compare parameters measured in three time period points D(0), D(21) and D(42).

3. Results

A complete documentation was obtained for 27 participants. The data enabled a reliable and thorough analysis of changes in facial skin color. Results obtained due to an application of the VISIA[®] system were calculated in a way which allowed to obtain average results from three measurements made for each participant. Furthermore, we presented results of measurements made in patients who demonstrated 50% improvement between the first and subsequent measurement points.

3.1 Questionnaire Results

Prior to commencement of the study, all the participants were examined for presence of hyperpigmentation and after a personal interview the type of facial skin hyperpigmentation was identified (Fig. 1).

Allergies were confirmed in 28% of participants in medical examination. A temporary erythema occurred in most cases after exposure to frost (38%), sunshine (28%), as well as after hot baths and saunas (28%). Skin and/or conjunctiva irritation in patients was mainly a result of sun exposure (38%) and application of cosmetics (31%).

The brightening effect and improvement of skin color was noted in 92% of participants both after three and six weeks of treatment. 92% of them demonstrated an increase in skin moisture, whereas 88% noted higher skin smoothness, natural brightening, and levelness of skin color after six weeks of treatment (Fig. 2). Part of subjective assessment of product workability is presented in Fig. 3.

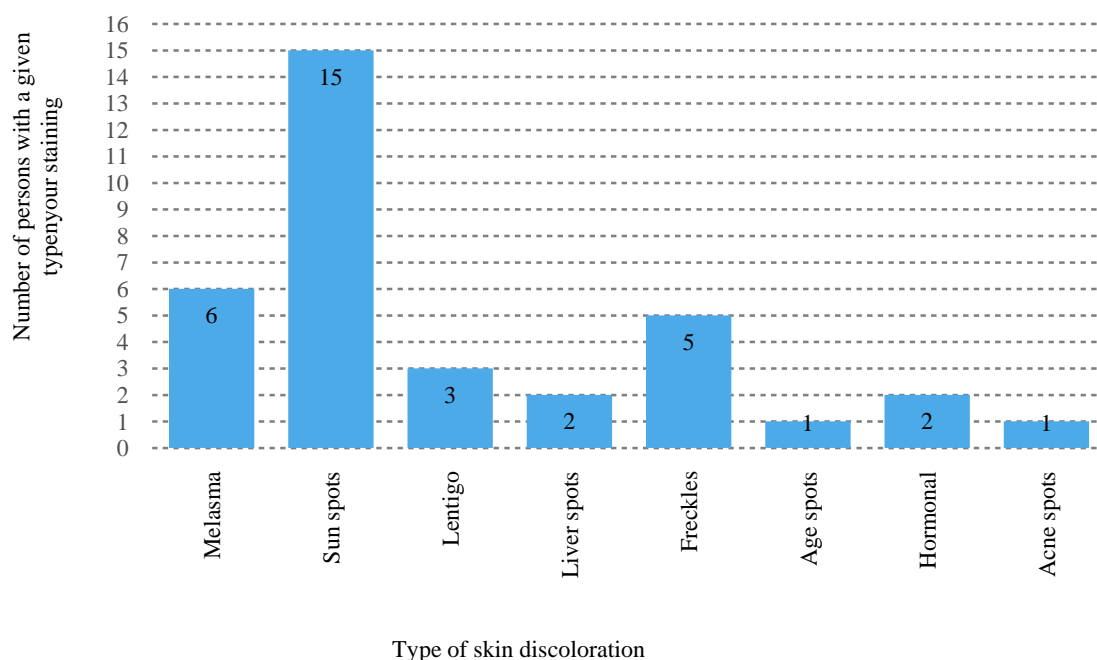


Fig. 1 Types of hyperpigmentation diagnosed in people taking part in the study night cream (1474).

3.2 Instrumental Results

An analysis of results of the study after six weeks of treatment on the base of results obtained with the use of multifunctional MPA apparatus revealed (Table 1):

- Increase in moisture by 14% in 59% of participants ($p = 0.19819$, the result was not statistically significant);
- Increase in flexibility by 7% in 59% of participants ($p = 0.23588$, the result was not statistically significant);
- Brightening of skin color by 25% in 96% of participants ($p < 0.0001$);
- Reduction of erythema by 10% in 75% of participants ($p = 0.00689$) in comparison to measurements obtained during the first visit D(0). In the control group, the average value of melanin reached 99 in January, 99 in February and 100 in March.

Effects of six weeks of treatment measured with the

use of the VISIA® (Table 2) system (i.e., overall size, area and intensity) were the following:

- Stains decreased by 11% ($p = 0.00004$);
- Skin irregularities decreased by 47% ($p = 0.00061$);
- UV stains decreased by 2% ($p = 0.04784$, the result was not statistically significant);
- Brown stains decreased by 3% ($p > 0.05$, the result was not statistically significant);
- Erythema decreased by 4% ($p = 0.01193$).

This improvement was visualized with the VISIA® system (Figs. 4-6).

Measurements with the use of PRIMOS® (Table 3) were made in five randomly selected female participants. An average levelness of the nasolabial groove by 42 μm and average decrease in the volume of the groove by 4% were noted in 4 out of 5 patients. The depth of wrinkles in the eye area (the so-called

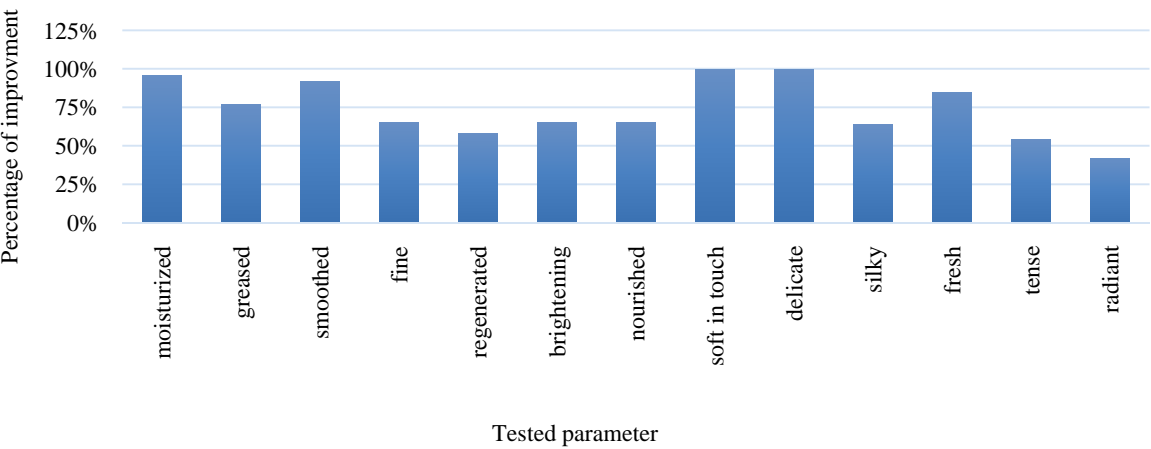


Fig. 2 Assessment of the application of the product overnight, $n = 26$.

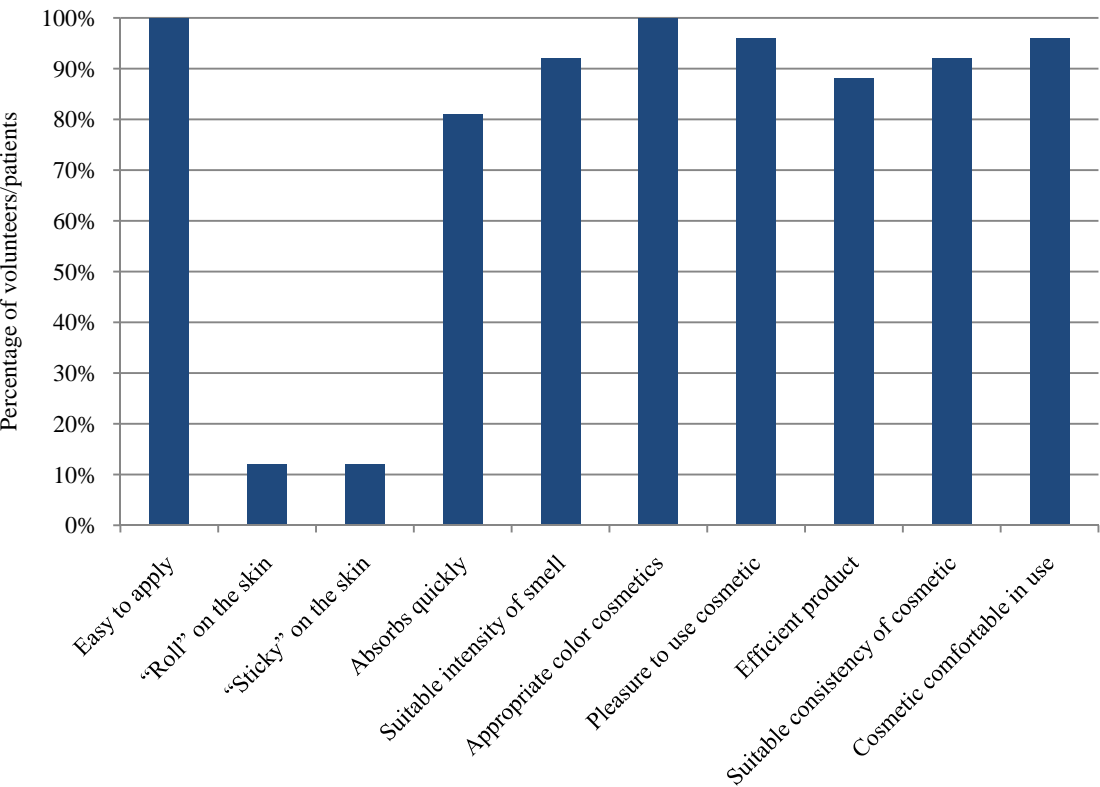


Fig. 3 Subjective assessment of product workability, $n = 26$.

Table 1 Assessment of the condition of the skin in the research unit of apparatus MPA.

Parameter		After three weeks		After six weeks	
		Results (%)	Percentage of patients with enhancement (%)	Results (%)	Percentage of patients with enhancement (%)
Color of the skin	Melanin	-13	93	-25	96
	Erythema	-10	50	-10	75
Moisturizing		+14	52	+14	59
Elasticity (R2)		+8	68	+7	59

Table 2 Test results of cream on the night—a system VISIA®.

Parameter	After three weeks		After six weeks	
	Number of features	Result (intensity & size)	Number of features	Result (intensity & size)
Spots (brown or red skin lesions: hyperpigmentation, acne scars, vascular lesions)	Reduction at 5%	No enhancement	Reduction at 11%	Reduction at 6%
UV spots (sun damage)	Reduction at 2%	Reduction at 1%	Reduction at 2%	Reduction at 2%
Brown spots (hyperpigmentation, liver spots)	Reduction at 3%	Reduction at 4%	Reduction at 5%	Reduction at 3%
Ruggedness (texture of the skin)	Reduction at 1%	No enhancement	Reduction at 11%	Reduction at 47%
Redness (erythema and telangiectasia)	No enhancement	No enhancement	Reduction at 6%	Reduction at 4%
Wrinkles	No enhancement	Reduction at 10%	No enhancement	No enhancement

**Fig. 4** Reduction in the number of irregularities: front view and left profile. Patient TW (551) prior to and after six weeks of treatment with night cream. Image in natural daylight.

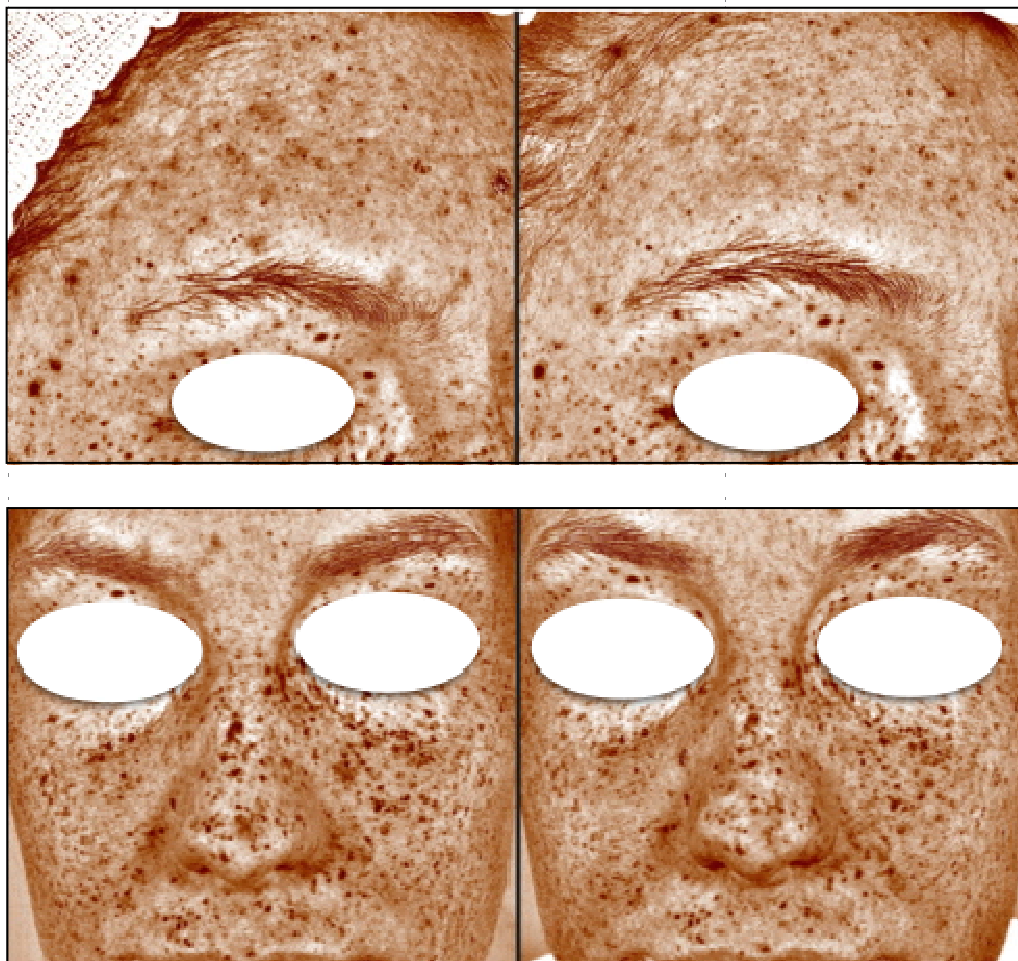


Fig. 5 Patient IS (211) before and after six weeks of using the cream for the night. Reducing the amount of brown spots view from the right profile and en face. Images in the light of the cross-polarization in RBX[®]. Photo without founded the mask.

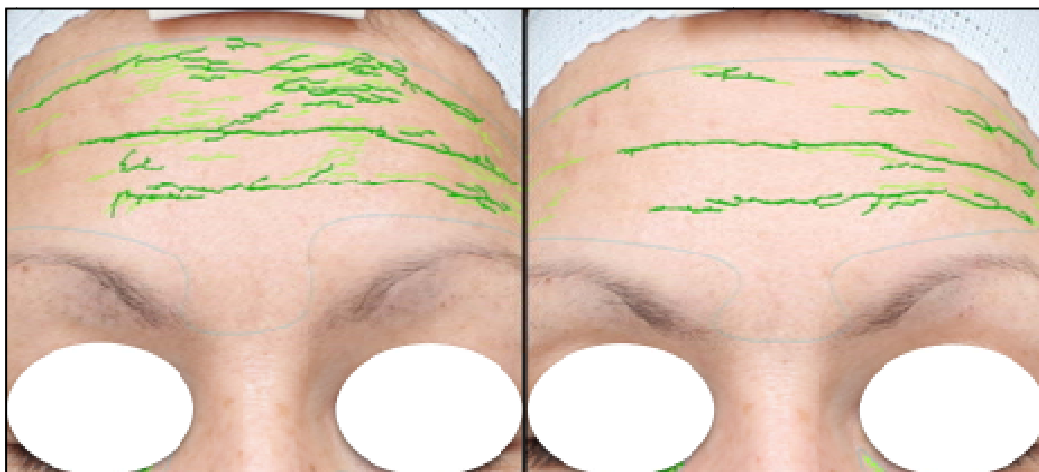


Fig. 6 Reducing the amount of wrinkles: en face. Patient SN (391) prior to and after six weeks of treatment with night cream. Image in natural daylight.

Table 3 The measurement results night cream PRIMOS[®], *n* = 5.

PRIMOS [®]	Designated parameters	
	Depth	Volume
Measured area of the face		
Nasolabial fold	Average of minimizing about 42 μm on 100% The biggest improvement—76 μm	An average decrease of 4% in 80% of patients The biggest improvement—0.32271 mm^3
The area around the eyes, “Crow’s feet”	Average of minimizing about 41 μm in 60% patients Największa poprawa—54 μm	An average decrease of 13% in 60 % of patients The biggest improvement—0.97149 mm^3

Table 4 Changes in skin smoothness after six weeks of night cream study, *n* = 24.

Parameter describing skin smoothness	Percentage of enhancement	Percentage of patients
<i>NRJ</i> (energy) —overall skin condition	Lack of enhancement in min. 50% of patients	Less than 50%
<i>SER</i> —skin roughness	34	At 63
<i>SEsm</i> —smoothness including moisturizing	Lack of enhancement in min. 50% of patients	Less than 50%
<i>SEw</i> —the number of wrinkles in different layers	18	At 63
<i>S</i> (surface)—level of skin smoothness	Lack of enhancement in min. 50% of patients	Less than 50%
<i>V</i> (volume)—depth, volume and number of grooves and wrinkles	Lack of enhancement in min. 50% of patients	Less than 50%

“crow’s feet”) decreased on average by 41 μm in 60% of participants while their volume decreased by 13% in 60% of patients.

Results of visualization made with the use of the VISIOSCAN camera (Table 4).

4. Discussion

The aim of this study was to check the efficiency of night cream meant for people suffering from facial skin hyperpigmentation. Knowledge on properties of brightening products allows us to apply ingredients which act selectively in different phases of melanogenesis. The evaluated product contained experimentally prepared, innovative complex of ingredients with a synergic, three-step stimulating-brightening effects:

(1) Biomimetic peptide, an antagonist competing with natural ligand αMSH on its specific receptor. It prevents from further activation of tyrosine by blocking melanin synthesis [5];

(2) Extract from *Rumex spp.* (*Rumex occidentalis*) inhibiting tyrosinase enzyme by preventing from further activation of melanogenesis track [5];

(3) Niacinamide (Vitamin PP) blocking transportation of melanin to the epidermis and effectively preventing from creation of new pigmented

lesions, as well as intensification of already existing ones [6, 7].

Furthermore, in its formula, the product also contains additional ingredients which are characterized with enhancing antiaging effects:

(1) Papain, being a proteolytic enzyme, contributes to flaking of eternal structures of hyperpigmented epidermis, accelerates depigmentation and balances the skin color visually. It also facilitates deeper pigmentation of Vitamin C into the skin, intensifying its re-vitalization and brightening effects;

(2) Vitamin E, a natural antioxidant is an inhibitor in ageing processes [8, 9].

A decrease in the number of features (by 11%), as well as overall size and intensity (by 6%), was the highest for the stain parameter measured with the VISIA[®] (after six weeks of treatment with the night cream (1474) the number of changes including stains, UV stains and brown stains decreased when compared to prior-study results measured with VISIA[®]). After six weeks of the application of the agent, UV stains and brown stains decreased as well.

Studies conducted by the manufacturer revealed higher efficiency of the *Rumex* extract compared to other active ingredients characterized with brightening properties and available on the market, such as

hydroquinone, arbutin or cumarin. The study conducted with the use of Mexameter® probe showed a brightening effect on the skin by 25% in 96% of patients applying the night cream (1474). A decrease in the number of wrinkles in different levels by 18% in 63% of patients was confirmed in a study with the application of the VISIOSCAN camera.

In-vitro studies indicated that the biomimetic peptide (Melanostatine®), antagonizing the transfer of signal induced by α -MSH, contributes to a decrease in melanin synthesis by 33% [5].

Furthermore, analyses conducted by the manufacturer confirmed that synergic and additional effects of biomimetic peptide and *Rumex spp.* extract leads to a decrease in post-sun stains, freckles and irregular skin pigmentation, which contributes to a general brightening effect and smoothing of the external parts of facial skin [5].

Sabancilar et al. [10] tested the efficacy of a brightening cream, including mainly *Rumex occidentalis* and glycolic acid, by colorimetry. The clinical study conducted in 27 patient, during six months (for the first three months the cream was applied twice a day, and for the other three months twice a week). The facial area with melasma was analyzed before the study, in Weeks 12 and 24 with the application of colorimetry. The cream containing *Rumex spp.* was tolerated, effective and can be an alternative in treatment of melasma [10].

A study, conducted by Lee et al. [11], tested the effectiveness of a combination of niacinamide and TXA (tranexamic acid). Topical brightening and moisturizing formulation was applied by 42 Korean women and the therapy lasted eight weeks. The cream contained 2% niacinamide + 2% TXA also the vehicle cream with control group was applied twice a day. Pigmentation was measured by mexameter, chromameter and using clinical photographs. Niacinamide combined with TXA was significantly ($p < 0.05$) more effective than the vehicle control [11].

A study conducted by Hakozaki et al. [12] proved

that niacinamide decreased skin hyperpigmentation and transfer of melanin. *In-vivo* study conducted on 120 Asian women with confirmed skin hyperpigmentation indicated that an application of niacinamide results in 35~68% inhibition of melanosome transfer in a co-culture model and reduction of pigmentation in the PREP (pigmented reconstructed epidermis) skin model. After four weeks of treatment with the use of products containing niacinamide, the study confirmed brightening effects and decreased hyperpigmentation compared to a trial (conducted with a moisturizing carrier) not containing 2% niacinamide [12].

Bazela et al. [13] conducted a study on a product containing niacin, extract of *Rumex occidentalis* and the biomimetic peptide. The research conducted on a group of 10 people covered both instrument measurements and an analysis of surveys. Regular use of the cream during a 4-week treatment period resulted in brightening of the skin pigment by 21.5%. The effect of a 6-week applied whitening hyperpigmentation and planishing the color of the skin products in questionnaire analyses [13].

The study confirmed an improvement of moisture and skin elasticity after a 3-week preparation application of the preparation.

5. Conclusions

A combination of niacinamide, biomimetic-peptid, extract from *Rumex spp.*, as well as papain enzyme, Vitamin C and Vitamin E, provides a high-efficient brightening and re-vitalizing effects on the skin. The cream gradually brightens hyperpigmentation and does not cause any discoloration in the healthy skin.

The cream does not cause allergies; It is a hypo-allergic product. It is highly efficient and its components are well tolerated by the skin. The brightening effect and first symptoms of enhancement in skin condition are visible after at least three weeks of its application.

The tri-active complex has an active brightening

effect on skin hyperpigmentation of different etiology: melasma, freckles, sage spots, liver spots, post-sun spots, hormonal spots, acne spots and post-drug spots.

Results obtained with the use of VISIA® revealed a reduction in the amount, intensity and size of: stains, spots, pigment spots and UV stains. A use of Mexameter® probe confirmed a decrease in intensity of pigment spots and erythema. The above parameters, except for the results of stains obtained after three weeks, improved during the treatment, after 21 days of the cream application, as well as after the completion of the study, i.e., after 42 days.

The participants in their subjective evaluation of the night cream confirmed its efficiency (1474). The night cream (1474) meets all requirements set down for dermo-cosmetics with regards to treatment of hyperpigmented skin. Ingredients of the product guarantee brightening of skin stains, restoring of radiating look and balancing of skin color. To sum up, results obtained by the authors of this study indicate that a treatment with the night cream (1474) during a 6-week application period, results in optimal brightening-stimulating effects in the facial skin.

Unique recipes, patented cosmetics stores and improved commonly used formulas are the necessary complement to the treatment of skin hyperpigmentation. The activity of the ingredients is confirmed by both clinical and *in-vivo* examination. Hence, the formulation night cream (1474) appears to be effective in the treatment of aberration melanogenesis.

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References

- [1] Kobayashi, T., Vieira, W. D., Potterf, B., Sakai, C., Imokawa, G., and Hearing, V. J. 1995. "Modulation of Melanogenic Protein Expression during the Switch from Eu- to Pheomelanogenesis." *J. Cell Sci.* 108: 2301-9.
- [2] Briganti, S., Camera, E., and Picardo, M. 2003. "Chemical and Instrumental Approaches to Treat Hyperpigmentation." *Pigment Cell Res.* 16: 101-10.
- [3] Jabłońska, S., and Majewski, S. 2006. *Skin Diseases and Sexually Transmitted Diseases*. Warszawa: PZWL, 179-85. (in Polish)
- [4] Brenner, M., Hearing, V. J. 2008. "The Protective Role of Melanin Against UV Damage in Human Skin." *Photochem Photobiol.* 84 (3): 539-49.
- [5] Meyer, L. 2012. *Nonapeptide-1: Champlan*. Quebec: Atrium Biotechnologies.
- [6] Ramos-e-Silva, M., Celem, L. R., Ramos-e-Silva, S., and Fucci-da-Costa, A. P. 2013. "Anti-aging Cosmetics: Facts and Controversies." *Clin. Dermatol.* 31 (6): 750-8.
- [7] Zasada, M., Debowska, R., Pasikowska, M., and Budzisz, E. 2016. "The Assessment of the Effect of a Cosmetic Product Brightening the Skin of People with Discolorations of Different Etiology." *Journal of Cosmetic Dermatology* 28: 12249.
- [8] Telang, P. S. 2013. "Vitamin C in Dermatology." *Indian Dermatology Online Journal* 4 (2): 143-6.
- [9] Farbstein, D., Kozak-Blickstein, A., and Levy, A. P. 2010. "Antioxidant Vitamins and Their Use in Preventing Cardiovascular Disease." *Molecules* 15 (11): 8098-110.
- [10] Sabancilar, E., Aydin, F., Bek, Y., Ozden, M. G., Ozcan, M., Senturk, N., et al. 2011. "Treatment of Melasma with a Depigmentation Cream Determined with Colorimetry." *J. Cosmet Laser Ther.* 13 (5): 255-9.
- [11] Lee, D. H., Oh, I. Y., Koo, K. T., Suk, J. M., Jung, S. W., Park, J. O., et al. 2014. "Reduction in Facial Hyperpigmentation after Treatment with a Combination of Topical Niacinamide and Tranexamic Acid: A Randomized, Double-Blind, Vehicle-Controlled Trial." *Skin Res. Technol.* 20 (2): 208-12.
- [12] Hakoziaki, T., Minwalla, L., Zhuang, J., Chhoa, M., Matsubara, A., Miyamoto, K., et al. 2002. "The Effect of Niacinamide on Reducing Cutaneous Pigmentation and Suppression of Melanosome Transfer." *British Journal of Dermatology* 147: 20-31.
- [13] Bazela, K., Debowska, R., Tyszczyk, B., Dzwigalska, A., Rogiewicz, K., and Eris, I. 2010. "Dermocosmetics for the Care of Skin Discoloration Problem—Assessment of the Effectiveness." *Dermat. Estet.* 12 (5): 320-6. (in Polish)