Randomized study to assess the efficacy of a facial cosmetic product with nanoencapsulated cysteamine in women presenting melasma

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Introduction.

This protocol was conducted after submission and approval by the local Ethics commission and under regulatory rules from ANVISA (1)

Melasma is a common and persistent pigmentary disease that particularly affects women, during their reproductive period (2,3) It has a higher prevalence in Orientals and Hispanic individuals, as well as in women with higher phototypes such as IV to VI (according to the Fitzpatrick classification), especially those who live in areas with intense ultraviolet radiation.

It is characterized by symmetrical hyperpigmented macules or spots with irregular edges, more often distributed on the face (4). Areas of hyperpigmentation can be further categorized by area of distribution: centrofacial (involving the forehead, cheeks, nose, upper lip and chin), malar (affecting the cheeks and nose) or mandibular (along the jaw line) (5)

The diagnosis of melasma is essentially clinical, and its management is challenging because despite the use of broad-spectrum sunscreen and topical bleaching agents, the disease can be recalcitrant to treatments, and relapses are common, making treatment often difficult (6,7)

The chronicity of the disease and the involvement of visible areas during working age generates a great negative impact on the quality of life of affected individuals, causing

significant emotional and psychological effects, including frustration, embarrassment and depression.(8)

There are several therapeutic options for the treatment of melasma that act on different stages of melanogenesis. The vast majority of effective depigmenting agents are tyrosinase inhibitors, of which hydroquinone is the most studied and most effective drug. However, there is concern about its tolerability and its prolonged use due to the risk of adverse events such as exogenous ochronosis. Therefore, there is an interest in seeking other depigmenting agents in the treatment of melasma (6,7,8)

The present study aimed to use nano encapsulated cysteamine, a promising and more tolerable option when compared to other treatments in the treatment of melasma. L-cysteamine (b-mercaptoethylamine hydrochloride) is an aminothiol compound with antioxidants and depigmenting agents. The exact mechanism by which cysteamine inhibits melanogenesis is not fully understood, but studies show that this compound is able to increase intracellular glutathione, thereby altering the synthesis of eumelanin to pheomelanin. Cysteamine has a strong sulfur odor, characteristic of its composition, but an alternative pharmacotechnical encapsulation of the compound brought a significant reduction of this odor, making the use of cysteamine a promising way in the treatment of melasma.(6,7,8)

The present study aimed to evaluate the whitening efficacy of the investigational product when applied under normal conditions of use for 84 days by participants with melasma. The study was divided into two groups: one of them used the investigational product associated with sunscreen (investigational group) while the other used only sunscreen (control group). Thus, the results obtained by the investigational group were compared with the results of the control group.

To demonstrate the effectiveness of the investigational product the following evaluations were carried out: clinical evaluation through standardized scales, evaluation of perceived efficacy by subjective evaluation questionnaires, evaluation of hyperpigmentation through measurements with Chromameter®, illustration through images captured by Color Face® and Skincam® equipment, evaluation by digital MASI and evaluation by Confocal Microscopy reflective in vivo.

In addition, as secondary objectives this study also evaluated cosmetic tolerance through spontaneous reports of adverse events and feelings of local intolerance.

Materials and Methods. This single-blind, randomized, and comparative study of a cosmetic product with nano encapsulated cysteamine and sunscreen versus sunscreen alone was conducted to investigate the efficacy of a cosmetic product with nano encapsulated cysteamine in improving melasma hyperpigmentation and improving the quality of life of subjects with melasma through MelasQoL in adult women. The study included 43 adult women, aged 33 - 55 years, phototype III to V (Fitzpatrick), having melasma in their face and chronic non-smokers.

These subjects were randomized into two groups: 24 subjects used the IP daily plus sunscreen SPF 60 for 84 days, while 19 subjects used only the sunscreen SPF 60 for 84 days. Evaluations were performed on day 0, day 56, and day 84 by a dermatologist. Melasma hyperpigmentation intensity, the area affected by melasma, skin tone uniformity, skin hydration, softness, luminosity and oiliness were assessed. Assessment of skin hyperpigmentation with Chromameter ®, Dermatoscopy evaluation with Skincam®, Assessment with the MelasQoL questionnaire, Standard photo registration with Color Face®, Assessment by MASI Digital, Moreover, any possible local intolerance and adverse event were investigated and collected by the dermatologist. The study was randomly divided into two groups:

- Treatment group (investigational product + sunscreen):25 participants were included in this group in order to finish the study with at least 20 valid cases.
- Control group (sunscreen only):26 participants were included in this group in order to end the study with at least 20 valid cases

Instrumental evaluations were performed to investigate the efficacy of a cosmetic product with nano encapsulated cysteamine in the characteristics of the epidermis, dermo-epidermal junction, and dermis – through Confocal Reflecting Microscopy. In addition, Color Face and Skincam photography equipment were also used to illustrate the efficacy of the investigational product. In 28, 56, 84 days after products use, subjects answered

questionnaires regarding the subjective perceived efficacy and the impact of melasma on their quality of life through MelasQoL.

Descriptive Statistics

We leave Yt represent the values observed at time t. The quantitative variables, or those that can reasonably be treated as such have been summarized using minimum, maximum, measures of central tendency such as the mean and median & measures of dispersion such as the standard deviation (SD). Qualitative variables were summarized as counts and percentages.

Statistical Methodology

For each parameter and for each area (melasma and normal skin, where applicable), a graphical representation of ±95% CI means was produced to visually assess evolution over time by group. The asterisks (*) in the graphs represent the significance obtained for group comparison.
The percentage variation of each group in Kinetics t (after baseline, D0) was calculated on the average value observed for each parameter

Evolution over time

For each parameter and for each area (melasma and normal skin) when applicable, the evolution to over time (relative to baseline) for each group was investigated by comparing Ytand YD0using Student's t test for paired data or the Wilcoxon test, depending on the normality of the difference data. The latter was tested using a Shapiro Wilk test with 1% significance. The null and alternative hypotheses are defined below:
H0: There is no difference between the two investigated time points
H1: There is a difference between the two time points investigated

Comparison between groups for parameter derived from Chromameter® readings (deltas), dermatological assessment of skin parameters and MelasQoL questionnaire

For each parameter, the comparison between groups was performed at each time point, comparing the difference data (Yt– YD0) for both groups. The investigation was performed using the test of independent samples or the Mann Whitney-U test, depending on the normality of the difference data. The latter was verified with the Shapiro Wilk test with 1% significance for each group separately. The null and alternative hypotheses are as follows:

H0: There is no difference between the two groups compared

H1: There is a difference between the two groups compared

Note: When more than one measurement was taken in an identified zone, the average of these measurements was used for statistical analysis.

Significance level

The null hypothesis was rejected when ap valueless than or equal to 0.05 (significance level of 5%) was revealed by the statistical procedure.

Software

- SPSS 19.0
- Microsoft Excel 2010 or higher

Results.

Clinical results showed that for the treatment group, which used the investigational product and sunscreen FPS60, after 84 studies there was a significant improvement ($p < 0.001$) of 44.7% in the melasma hyperpigmentation intensity. The affected area by melasma also showed a significant improvement ($p < 0.001$) of 27.5% after 84 days of treatment. Furthermore, for the treatment group, after 84 days, there was a significant ($p < 0.001$)

improvement of 41.5% in skin tone uniformity, significant improvement ($p < 0.001$) of 60.5% in skin hydration, significant ($p<0.001$) improvement of 68.9% in skin smoothness, significant improvement ($p<0.001$) of 76.9% in skin luminosity and significant improvement ($p<0.034$) of 15.8% in skin oiliness. For the control group, after 84 days of study, there was a significant improvement (p 0.003) of 29.0% in the melasma hyperpigmentation intensity, a significant improvement (p 0.025) of 15.6% in skin hydration, a significant improvement ($p < 0.001$) of 44.3% in skin smoothness, significant improvement (p 0.003) of 31.0% in skin luminosity and significant improvement (p 0.014) of 20.7% in skin oiliness.

MelasQol results revealed a significant difference (p 0.003) in the impact of melasma on quality of life after 84 days after IP use when compared to the control group. Reflective confocal microscopy results showed pigment reduction in keratinocytes in the epidermis, around adnexal structures and at the dermal-epidermal junction. General characteristics of the subjects are depicted in table 1. Results are depicted in table 2, 3 and Graph 1. Picture 1 shows Pigment reduction in keratinocytes in the epidermis on Confocal analysis.

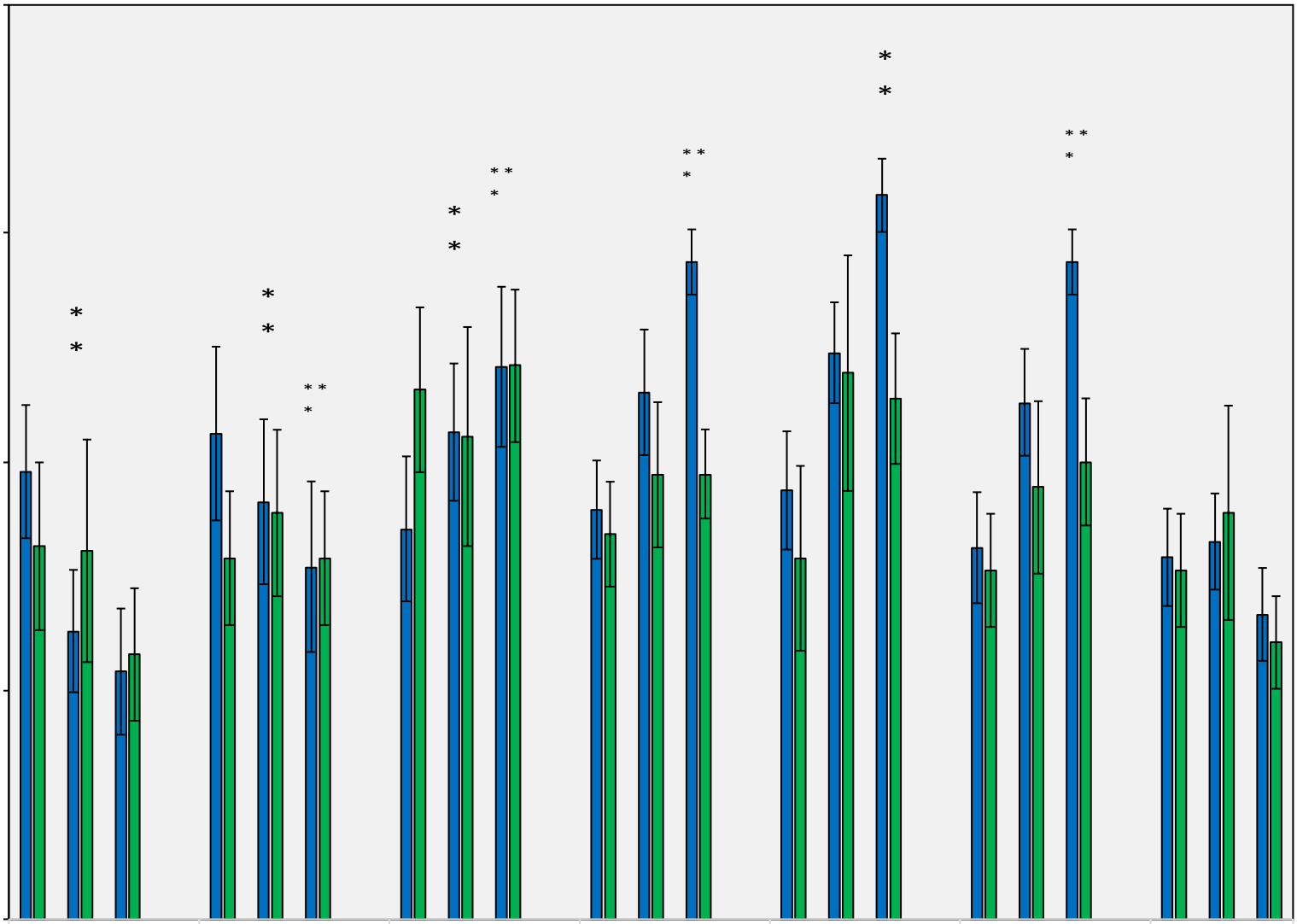
Table 1

		full panel		Investigational Group		Group control	
AGE AT VISIT 01 (YEARS OLD)	Average	**45**		**45**		**44**	
	median	43		43		44	
	Minimum	*33*		*37*		*33*	
	Maximum	*55*		*55*		*55*	
	SD (standard deviation)	**6**		**6**		**6**	
	EPM (Standard Error of Means)	**1**		**1**		**1**	
	95% CI	two		two		3	
TREATMENT GROUP	investigational	24	55.8%				
	Control	19	44.2%				
	Total	43	100.0%				
SEX	Feminine	43	100.0%	24	100.0%	19	100.0%
	Male	0	0.0%	0	0.0%	0	0.0%
	Total	43	100.0%	24	100.0%	19	100.0%
PHOTOTYPE	I	0	0.0%	0	0.0%	0	0.0%
	II	0	0.0%	0	0.0%	0	0.0%
	III	9	20.9%	6	25.0%	3	15.8%
	IV	21	48.8%	13	54.2%	8	42.1%
	V	13	30.2%	5	20.8%	8	42.1%
	SAW	0	0.0%	0	0.0%	0	0.0%
	Total	43	100.0%	24	100.0%	19	100.0%
USING METHOD CONTRACEPTIVE?	Yea	37	86.0%	21	87.5%	16	84.2%
	Not	0	0.0%	0	0.0%	0	0.0%
	AT	6	14.0%	3	12.5%	3	15.8%
	Total	43	100.0%	24	100.0%	19	100.0%
PARTICIPANT WITH MELASMA?	Yea	43	100.0%	24	100.0%	19	100.0%
	Not	0	0.0%	0	0.0%	0	0.0%
	AT	0	0.0%	0	0.0%	0	0.0%
	Total	43	100.0%	24	100.0%	19	100.0%
SMOKING PARTICIPANT CHRONIC? (>10)	Not	43	100.0%	24	100.0%	19	100.0%
	Yea	0	0.0%	0	0.0%	0	0.0%
	Total	43	100.0%	24	100.0%	19	100.0%
NORMAL TERMINATION OF ES-ALL	Yea	0	0.0%	0	0.0%	0	0.0%
	Not	8	100.0%	1	100.0%	7	100.0%
	Total	8	100.0%	1	100.0%	7	100.0%

Table 2

Parameter	kinetics	Treatment (PI + Sunscreen)					Control (sunscreen only)					comparison of *group p*-value (significance)
		Average	median	SD	Average % Change	*P*-value (Significance)	Average	median	SD	Average % Change	*P*-value (Significance)	
Intensity of coloring melasma	D0	2.0	2.0	0.7			1.6	1.0	0.8			
	D56	1.3	1.0	0.6			1.6	1.0	1.0			
	D84	1.1	1.0	0.7			1.2	1.0	0.6			
	D56 - D0	- 0.7	- 1.0	0.5	- 35.6	**<0.001 (S)**	- 0.1	0.0	0.8	- 1.3	0.763 (NS)	**0.004 (S)**
	D84 - D0	- 0.9	- 1.0	0.7	- 44.7	**<0.001 (S)**	- 0.5	0.0	0.5	- 29.0	**0.003 (S)**	0.051 (LS)
Affected area fur melasma	D0	2.1	2.0	0.9			1.6	2.0	0.6			
	D56	1.8	2.0	0.8			1.8	2.0	0.7			
	D84	1.5	1.0	0.9			1.6	2.0	0.6			
	D56 - D0	- 0.3	0.0	0.4	- 14.1	**0.014 (S)**	0.2	0.0	0.5	12.6	0.180 (NS)	**0.007 (S)**
	D84 - D0	- 0.6	- 1.0	0.6	- 27.5	**<0.001 (S)**	0.0	0.0	0.0	0.0	AT	**<0.001 (S)**
Skin tone uniform	D0	1.7	2.0	0.8			2.3	2.0	0.7			
	D56	2.1	2.0	0.7			2.1	2.0	1.0			
	D84	2.4	3.0	0.8			2.4	3.0	0.7			
	D56 - D0	0.4	0.0	0.6	24.7	**0.007 (S)**	- 0.2	0.0	0.6	- 8.8	0.257 (NS)	**0.006 (S)**
	D84 - D0	0.7	1.0	0.8	41.5	**<0.001 (S)**	0.1	0.0	0.3	4.5	0.157 (NS)	**0.001 (S)**
hydration	D0	1.8	2.0	0.5			1.7	2.0	0.5			
	D56	2.3	2.0	0.6			1.9	2.0	0.6			
	D84	2.9	3.0	0.3			1.9	2.0	0.4			
	D56 - D0	0.5	1.0	0.5	28.6	**0.001 (S)**	0.3	0.0	0.6	15.5	0.059 (LS)	0.084 (LS)
	D84 - D0	1.1	1.0	0.5	60.5	**<0.001 (S)**	0.3	0.0	0.5	15.6	**0.025 (S)**	**<0.001 (S)**
Softness	D0	1.9	2.0	0.6			1.6	1.0	0.8			
	D56	2.5	2.0	0.5			2.4	2.0	1.0			
	D84	3.2	3.0	0.4			2.3	2.0	0.6			
	D56 - D0	0.6	1.0	0.7	32.2	**0.002 (S)**	0.8	1.0	1.1	51.3	**0.006 (S)**	0.809 (NS)
	D84 - D0	1.3	1.0	0.6	68.9	**<0.001 (S)**	0.8	1.0	0.5	44.3	**<0.001 (S)**	**0.006 (S)**
luminosity	D0	1.6	2.0	0.6			1.5	2.0	0.5			
	D56	2.3	2.0	0.5			1.9	2.0	0.8			
	D84	2.9	3.0	0.3			2.0	2.0	0.6			
	D56 - D0	0.7	1.0	0.5	39.1	**<0.001 (S)**	0.4	0.0	0.7	23.8	**0.038 (S)**	0.063 (LS)
	D84 - D0	1.3	1.0	0.5	76.9	**<0.001 (S)**	0.5	0.0	0.5	31.0	**0.003 (S)**	**<0.001 (S)**
Oiliness	D0	1.6	2.0	0.5			1.5	2.0	0.5			
	D56	1.7	2.0	0.5			1.8	2.0	0.9			
	D84	1.3	1.0	0.5			1.2	1.0	0.4			
	D56 - D0	0.1	0.0	0.4	4.3	0.317 (NS)	0.3	0.0	0.7	16.5	0.102 (NS)	0.470 (NS)
	D84 - D0	- 0.3	0.0	0.5	- 15.8	**0.034 (S)**	- 0.3	0.0	0.5	- 20.7	**0.014 (S)**	0.719 (NS)

Graph 1



D0 D56 D84

coloring intensity for Melasma

0: lighter

4: Darker

D0 D56 D84

Area affected

0: Small

4: Very extensive]

D0 D56 D84

uniformity of tone

skin

0: Not uniform,

4: Very uniform,

D0 D56 D84

hydration

0: Dry skin

4: Very hydrated skin]

D0 D56 D84

Softness

0: Rough skin

4: Very soft skin]

D0 D56 D84

brightness

0: dull skin

4: Shiny skin,radian

D0 D56 D84

Oiliness

[0: little skin oily

4: very oily]

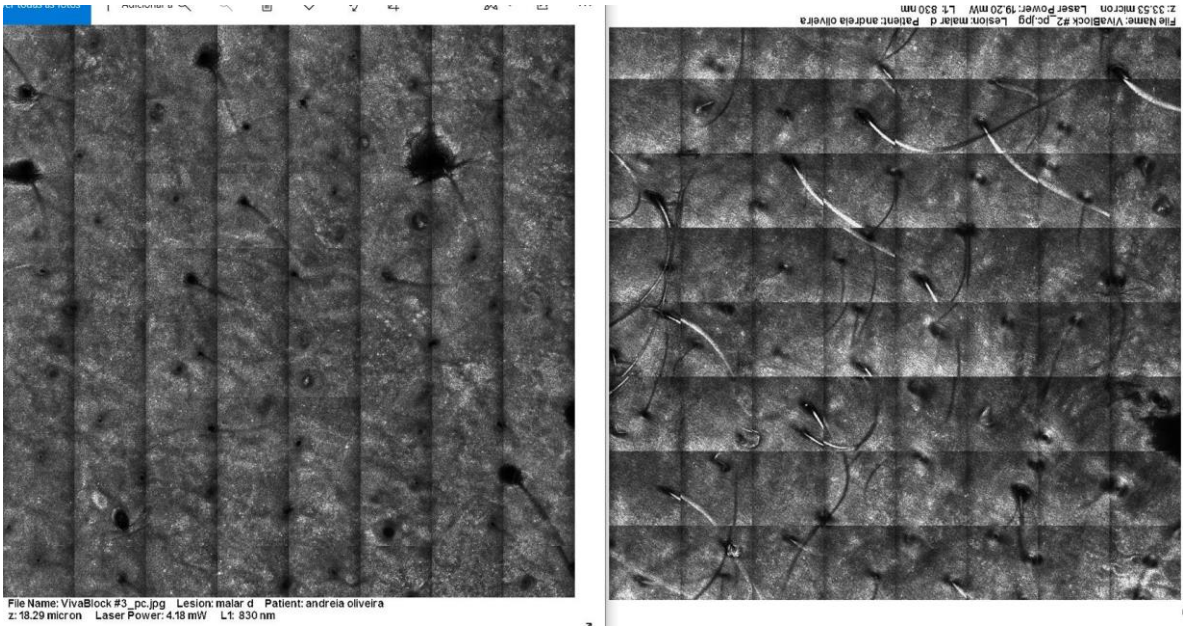
There was a statistically significant difference when comparing the treated and control groups in:

- Intensity of melasma staining (p-value = 0.004) after 56 days of treatment;
- Area affected by melasma after 56 and 84 days of treatment (p=0.007 and <0.001 respectively);
- Uniform skin tone after 56 and 84 days of treatment (p=0.006 and 0.001 respectively);
- Hydration (p value <0.001) after 84 days of treatment;
- Softness (p-value = 0.006) after 84 days of treatment;
- Luminosity (p-value <0.001) after 84 days of treatment

Table 2: Descriptive statistics, evolution over time and group comparison for 'MELASQOL questionnaire'

kinetics	Treatment (PI + Sunscreen)					Control (sunscreen only)					group comparison
	Average	median	SD	Average % Change	*P*-value (Significance)	Average	median	SD	Average % Change	*P*-value (Significance)	*P*-value (Significance)
D0	49.1	52.0	13.9			47.2	46.0	15.7			
D28	38.0	43.0	15.4			39.6	36.0	18.8			
D56	35.1	42.0	15.8			41.8	44.5	16.6			
D84	31.0	24.5	17.2			40.9	45.0	17.7			
D28 - D0	- 11.1	- 11.5	9.6	- 22.6	**<0.001 (S)**	- 7.5	- 8.0	8.0	- 16.0	**0. 001 (S)**	0.197 (NS)
D56 - D0	- 13.1	- 13.0	12.5	- 28.6	**<0.001 (S)**	- 4.3	- 5.5	12.9	- 11.4	0.173 (NS)	**0.033 (S)**
D84 - D0	- 18.2	- 16.5	13.5	- 37.0	**<0.001 (S)**	- 6.3	- 5.0	10.8	- 13.3	**0.021 (S)**	**0.003 (S)**

Picture 1



Discussion

During the study, it was possible to observe during the comparison between two groups, a significant improvement of melasma hyperpigmentation intensity after 56 days (p 0.004) using investigational product plus sunscreen and a significant improvement of the affected area by melasma (p <0.001), uniformity of the skin tone (p 0.001), skin hydration (p <0.001), smoothness (p 0.006) and luminosity (p <0.001) after 84 days using investigational product plus sunscreen when compared to the group sunscreen only.

During the study, 7 participants representing 13.72% of the inclusion panel reported 7 local events outside the product application area. All events were excluded from the use of study products.

Regarding the local intolerances that occurred at the assessment site (face), 7 participants representing 13.72% of the inclusion panel reported 8 events associated with investigational product use. All were of mild to moderate intensity, only 1 had a probable relationship with the use of the investigational product and no event was considered relevant.

Regarding local intolerances associated with the use of sunscreen, 5 participants representing 9.80% of the inclusion panel reported 7 events in total. All were of mild intensity and only 1 had a probable relationship with the use of the investigational product. This was the only one considered relevant since it referred to a participant who already had a previous history of

local intolerance to sunscreen in the past and who was discontinued from the study for meeting one of the non-inclusion criteria.

Conclusion.

The cosmetic product with nano encapsulated cysteamine improves the melasma hyperpigmentation intensity and affected area by melasma when compared to the control group, with a good tolerability profile.

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Conflict of Interest Statement. NONE.

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