Antispot's whitening efficasy

H E L L E N I C   R E P U B L I C

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Skin whitening Test Protocol

Evaluation of change of skin Melanin content after application of the product

Product to evaluate: Anti spot / Company name: BELVITA Natural Health and Body Care IKE

Experimental design

Panel: 10 volunteers (Male and Female adults 30 to 55 years old.

Volunteers excluded: if allergies are reported, or under medication treatments, or having skin diseases (according to medical history form completed by subject).

The volunteers were informed on the protocol and potential risks of the study and signed an informed consent statement.

Instructions to volunteers: The volunteers were instructed not to wear any cosmetic product or smoke 12 hours before and during the test

Application site: right or left forearm skin (about 600 cm2) was picked randomly

The opposite forearm skin was served as control (untreated)

Product application: Once daily (evening). 2-3 pumps and rubbing by gentle hand movement

Duration: 60 days

Measurement time points: before application (T0) at 30 and 60 days (T60, T60 respectively).

Measurement site: one reading on each forearm at each time point.

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<u>Biophysical Measurements</u>: Melanin content

<u>Instrumentation</u>: Soft plus, Callegari, Italy

This instrument measures the melanin content of the epidermis, through a double wavelength reflectance photometer ($\lambda 1$=875 nm, $\lambda 2$=660 nm). The skin melanin units are arbitrary (range 0-100 c.u, resolution 1 c.u.).

<u>Data statistical evaluation</u>: the mean value and standard deviation (SD) of the measurements on all volunteers, at each time point is calculated. The melanin content change at each time point is calculated subtracting the melanin content of the initial measurements from the respective at each time point. Finally the effect of the product in test will be evaluated subtracting the melanin content of the respective untreated area from the treated area (normalized values). The statistical significance of the acquired data will be evaluated using t-test, setting as confidence interval 95% (statistical significance: p value<0,05).

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RESULTS

At each time point, one measurement per volunteer were done at each forearm (treated and untreated). The results of the skin Melanin test are summarized on tables 1 and 2.

Table 1: Skin Melanin of treated and untreated areas					
Time	Anti spot (treated)			Untreated		
a/a Volunteer	T_0	T30	T60	T_0	T30	T60
1	12	7	5	11	15	16
2	11	7	8	10	11	13
3	20	19	16	21	24	26
4	24	23	18	23	24	25
5	6	4	3	9	8	9
6	6	4	3	6	8	11
7	11	10	8	19	20	24
8	10	7	5	10	13	18
9	17	15	14	15	17	18
10	12	11	10	19	20	22
Mean value	12,90	10,70	9,00	14,30	16,00	18,20
SD	5,80	6,41	5,40	5,87	6,00	6,00

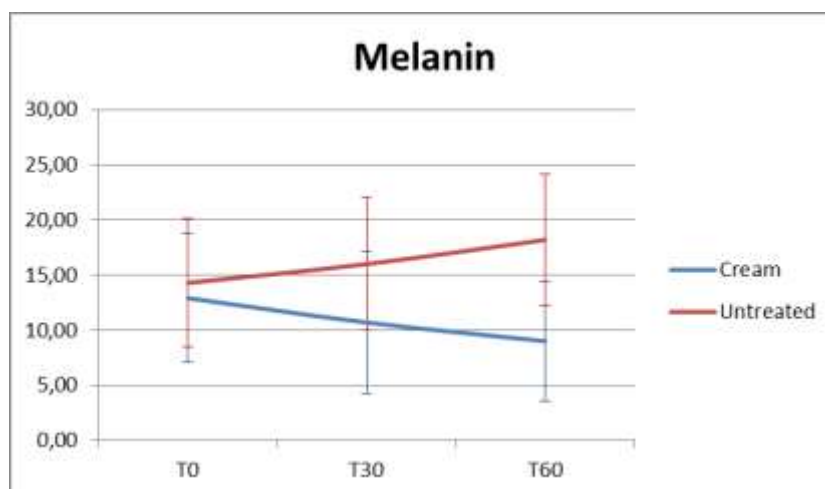The melanin content mean values of treated and untreated groups at each time point is depicted on Fig. 1.

Figure 1: Skin Melanin Mean content for treated (blue line) and untreated (red line) groups of volunteers

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In order to assess the effect of the product without the impact of the initial values of Melanin the *ΔMelanin* value is calculated.

ΔMelanin is calculated at each time point as the difference of skin Melanin content from those at T0.

ΔMelanin = Melanin at each time point – Melanin at T0

The calculated values at each time point per volunteer as well as the mean *ΔMelanin* for all volunteers for treated and untreated groups are summarized on table 2 and Fig.2.

Table 2: ΔMelanin of treated and untreated areas				
Time	**Anti spot (treated)**		**Untreated**	
a/a Volunteer	*ΔHT30-T0*	*ΔHT60-T0*	*ΔHT30-T0*	*ΔHT60-T0*
1	-5	-7	4	5
2	-4	-3	1	3
3	-1	-4	3	5
4	-1	-6	1	2
5	-2	-3	-1	0
6	-2	-3	2	5
7	-1	-3	1	5
8	-3	-5	3	8
9	-2	-3	2	3
10	-1	-2	1	3
Mean value	-2,2	-3,9	1,7	3,9
SD	1,4	1,6	1,4	2,2

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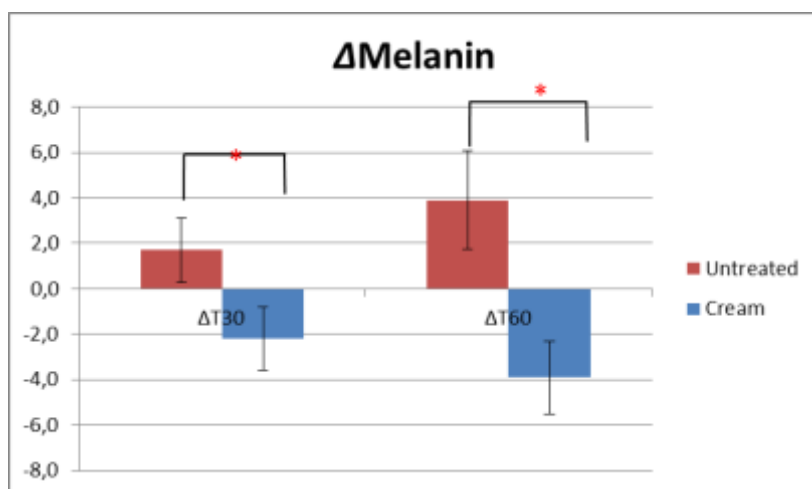**Department of Pharmacy**
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Figure 2: ΔMelanin Mean content for treated (blue line) and untreated (red line) groups of volunteers

ΔMelanin for the group of volunteers treated with the tested product was significantly differed from that of Untreated at 30 days after application (p<0,001). This difference is enhanced (lower melanin content, p<0,000) at 60 days of application.

Finally in order to assess the effect of the product on skin melanin content, the Normalized Melanin values are calculated.

The Normalized Melanin is calculated at each time point by subtracting ΔMelanin of untreated group from ΔMelanin of the treated group as following:

ΔMelanin of the treated group – ΔMelanin of untreated group.

The calculated values at each time point per volunteer as well as the mean Normalized Melanin for all volunteers are summarized on table 3.

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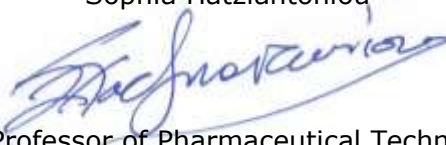| Volunteer	Table 3: Normalized Skin Melanin	
	NormT30	NormT60
1	-9	-12
2	-5	-6
3	-4	-9
4	-2	-8
5	-1	-3
6	-4	-8
7	-2	-8
8	-6	-13
9	-4	-6
10	-2	-5
Mean	-3,9	-7,8
SD	2,4	3,0

The application of the tested product on skin of healthy volunteers results in lowering of skin Melanin by 3,9±2,4 at 30 days of application. At 60 days the Melanin content is diminished by 7,8±3,0 Melanin units. Taking into account the initial melanin units, the percentage of change is calculated 30,23% at 30 days and 72,9% at 60 days of application.

Conclusion

Based on the results of the present study it may be concluded that the application of product **Anti spot** on healthy skin may provide a diminishing of skin Melanin by 7,8±3,0 units (72,9% of initial values) after 60 days of daily application. According to this conclusion the claim "whitening" may be considered as sufficiently supported.

The supervisor of the study

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