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Can a cosmetic formula exert a phisiological anti-ageing activity and/or contribute to the healing of skin diseases?

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SUMMARY

Aim: This research was aimed to determine the activity of a phospholipid and N-Acetyl-Glucosamine (NAG) based serum by assessing the immediate re-epithelizing and restoring activity of the study product as well as its anti-ageing activity.*

Methods: 20 female volunteers (45 to 65 years) were enrolled in this open, single center study, under dermatological control. The product effectiveness was assessed clinically and by non-invasive instrumental evaluations through two distinct studies. A short-term study evaluated the re-epithelizing and restoring activity of the study product applied one single time on experimentally damaged skin by repeated tape stripping on the forearm (volar surface) and the activity on skin smoothness of the face by one single application. A long-term study evaluated the anti-ageing activity of the same cosmetic formula, applied at night on the face, for an interrupted period of 1 month.

Results: The cosmetic formulation under study showed on the forearm, already 1 hour after a single application on experimentally induced damage, a very important increase of superficial hydration, a low variation of epicutaneous pH (control and normalization activity) and a clinically relevant restoration of skin microrelief (re-epithelizing/restoring activity). The same product determined on the face, after the first application, a significant reduction of crow's feet wrinkles (lifting effect) and after 1 month-treatment determined an important improvement on skin hydration (moisturizing activity), skin roughness around the eyes (anti-wrinkles activity), as well as on face volume and skin firmness (redensifying and revolumizing effect).

Conclusion: Results obtained by short and long-term studies, confirm the anti-age efficacy and the immediate re-epithelizing and restoring activity of the tested product, together with the good/excellent tolerability. The most part of included subjects confirmed the efficacy of the tested serum.

KEYWORDS

phospholipid, N-Acetyl-Glucosamine, NAG, cosmetic, non invasive evaluation

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** Skinius, Fospid Active Serum*

INTRODUCTION

The skin is the largest organ of the body and the seat of essential functions for life. Barrier functionality is one of the most important ones. Skin barrier activity is twofold: on the one hand it prevents the loss of water and mineral salts, essential for life, from the organism to the external environment and on the other it plays a defense role against the chemical and physical aggressions of the environment. The epidermis and above all its most superficial layer, the stratum corneum, represents the structure most responsible for the correct development of the skin barrier.

That's why it is compared to a wall made of bricks and cement. The most superficial cells, the "dead" cells, constitute the bricks of this wall, while the fatty substances which are found in the space between cell and cell, make up the cement. For a wall to be solid it is important that there is a correct overlap of the bricks and a meticulous interposition of the cement; a possible disintegration of these constituents would be able to destroy the wall. When this wall, anything but inert, is broken up, the skin becomes dry, cracked, flaky, hyper-reactive. On the contrary, when this wall is reinforced with new cement, similar to the existing one, the conditions described above are adequately treated. Factors capable of causing an alteration of the skin barrier can be intrinsic to the skin itself, due to diseases such as eczema, atopic dermatitis, psoriasis but also due to aging or they can be external such as cold, wind, too aggressive detergents, chemical solvents. How is an altered skin barrier cured? With moisturizing and / or emollient products, able to restore the functionality of the wall. Hydration can be defined as a process through which the water content of the skin is restored to its physiological level. Hyaluronic acid, and better still its precursor, N-Acetyl-Glucosamine are the substances that perform most of all this natural function of hydration. Emollient, however, is a cosmetic feature that gives the skin surface softness, smoothness, spreadability. It is usually associated with the lipid component present in the formulation. In light of what has been described so far, in order to obtain physiological emolliency and hydration, it is necessary that the lipids contained in the formulations intended for contact with the skin are similar to those found naturally at this level. The best lipids used for this purpose are derived from plants: ceramides, sphingolipids, vegetal oils.

When hydration and emollience are achieved in this way, the application of a cosmetic product takes on a value that goes beyond the cosmetic act: in fact, a soothing and regularizing action is obtained that helps to treat various inflammatory conditions, with improvement not only of the smoothness but also the color and softness of the skin. On the contrary, if we attack the skin with exfoliants, scrubs and lightening substances, if we use petrochemical derived oils such as paraffin and vaseline or silicones as emollients, the skin will in the long run become rough, irritated, stained.

METHODS

Participants

The study aimed to enroll a total of 20 female subjects aged 45-65 years, who gave written informed consent for the study procedure including the specific requests for keeping the same habits on food, exercise, make-up, cosmetics, detergent and avoiding strong ultraviolet exposure. Main exclusion criteria were pregnancy, lactation, sensitivity to the test product or its ingredients, participation in a similar study during the previous 3 months, dermatological diseases on the tested area, as well as general diseases. Additional exclusion criteria were the use of drugs.

Study design and objectives

This open, single center study, under dermatological control was conducted at DERMING S.r.l. Clinical Research and Bioengineering Institute, Milan, Italy. The product effectiveness was assessed clinically and by non-invasive instrumental evaluations through two distinct studies (long and short-term study).

Long-term study

The primary objective of the long-term study was to evaluate the anti-age activity of the serum. A fixed quantity of the tested product was applied by the subjects on the face, including the submental area, once a day, at the evening (preferentially always at the same hour), with a mild massage for an interrupted period of 1 month.

The trial foresaw a basal visit before product application (T0), a visit 12 hours after the 1st evening application (T12h) and a final visit after 1 month-treatment (T1M). At T12h it was performed skin roughness measurement (crow's feet profilometry), to define the immediate product lifting activity. After 1 month-treatment the following clinical evaluations were performed: skin firmness, skin roughness (crow's feet profilometry), skin superficial and deep hydration (corneometer Courage and Khazaka, moisture meter Delphin), 3D mid-face volume evaluation (Vectra H1).

The secondary objectives were to evaluate the product efficacy by the volunteers and tolerance both by investigator and volunteers. Moreover, to highlight the serum efficacy on skin imperfection as dark spots and scars, 2D pictures of some selected cases were taken at T0 and T1M.

Short-term study

The primary objective of the short-term study was to evaluate the immediate soothing and re-epithelizing activity of the serum in comparison to a reference product on the market and versus untreated control area. To investigate the activity of the study formulation, skin's stratum corneum on 3 different adjacent areas of the forearms (volar surface) was experimentally damaged by "repeated tape stripping" technique. This technique is used in dermatological research to selectively and exhaustively remove the skin's stratum corneum. In particular the skin of the forearms (volar surface) was stripped with repeated applications of clear tape 15 mm (Scotch® 3M – Italy).For each skin tested area 40 tape stripping were performed by the same specialised technician, then a fixed quantity of 2 mg/cm2 of both products (study and reference products) were applied on the assigned skin area; control area was left untreated. Re-epithelizing and restoring activity was defined by the measurement of: transepidermal water loss (TEWL), skin superficial and deep hydration, epicutaneous pH and surface microrelief profilometry. All evaluations were carried out at baseline (T0 - immediately after stripping execution), 1 (T1h), and 24 (T24h) hours after the products application, except for the surface microrelief profilometry performed on silicon skin replicas only at T0 and T24h by image analysis.

Statistical analysis

The statistical analysis was performed on the data of 20 included subjects. For the long-term study, clinical data were compared versus basal conditions by non-parametric test (Wilcoxon signed rank test), instrumental data by parametric test (Paired t test).

For the short-term study the comparison of the different study times (T1h and T24h) versus T0 for each study area and among the 3 skin tested areas time by time were performed by ANOVA test for repeated-measures, followed in case of statistically significant result by Holm-Sidak Adjusted t tests.

RESULTS

No drop-outs occurred during the trial, therefore the statistical analysis was performed on 20 included subjects. No other important event which may have interfered to the test results occurred during the study period.

Long-term study

Skin firmness (skin resistance to pinching, resistance to traction and recovery after pinching) was performed at level of cheek (malar region) according to the following score: 0 = very important, 1 = important, 2 = moderate, 3 = weak, 4 = very weak. Study product determined after 1 month-treatment a statistically (Wilcoxon signed rank test T1M vs T0) and clinically significant improvement of all the skin tonicity evaluations. In **Tab. 1** the variation percentages (mean value) of the considered items versus T0 were summarized (a reduction of the clinical score corresponds to an increase in the skin firmness).

Clinical evaluation	Variation (%) versus T0 T1M
Resistance to pinching	- 25% (**)
Recovery after pinching	- 28% (***)
Resistance to traction	- 30% (***)

Wilcoxon signed rank test ** p<0.01 and ***p<0.001 vs T0

Tab. 1. *Percentage improvement in skin firmness clinical scores.*

Obtained results highlighted at T1M a statistically significant increase of superficial hydration (Paired t test p<0.01 vs T0) of 14%, index of an important moisturizing activity of the serum.

Although no significant increase of deep skin layers hydration measured at 0.5 mm of depth was observed, it is important to underline a trend improvement of 4% of this parameter.

Image analysis of the area around the eyes ("Crow's Feet") showed at T12h an important and visible serum lifting activity and at T1M a very significant anti-wrinkles product efficacy **(Fig. 1)**.

More precisely the reduction of the following parameters versus baseline was highlighted: Ra (average roughness of the analysed profile) reduction of 15.8% at T12h and 28.3% at T1M (Paired t test p<0.001 T12h and T1M vs T0), index that the area around the eyes is generally less wrinkled **(Fig. 2)**; Rt (wrinkles total high) reduction of 9.2% at T12h and 23.9% at T1M (Paired t test p<0.05 T12h vs T0 and p<0.01 and T1M vs T0), index that wrinkles are less marked; Rv (wrinkles maximum depth) reduction of 14.3% at T12h and 29.4% at T1M (Paired t test p<0.01 T1M vs T0), sign that wrinkles are less deep.

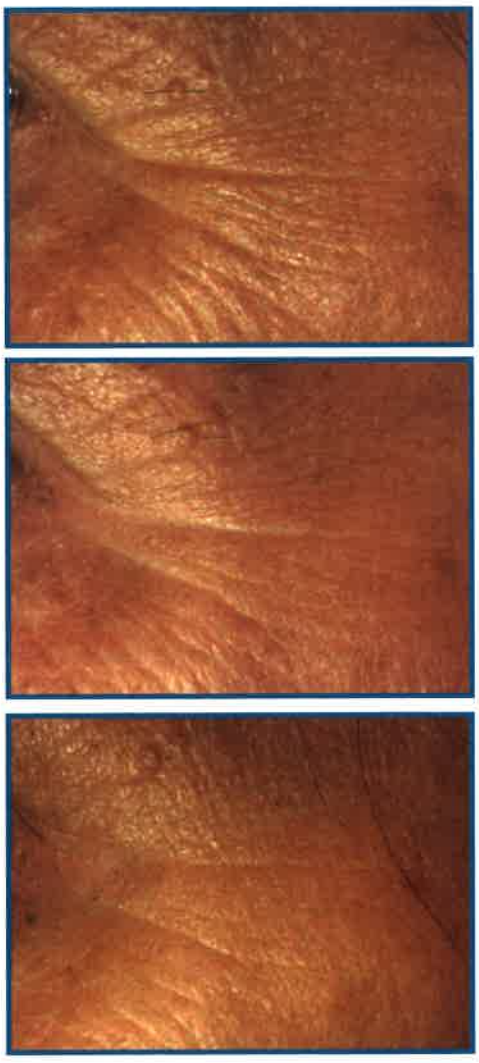


Fig. 1. *Clinical pictures of subject n. 7 showing the effect of the study product on the crow's feet wrinkles.*

T0 (baseline)

T12h (12 hours after the 1st evening application)

T1M (after 1 month-treatment)

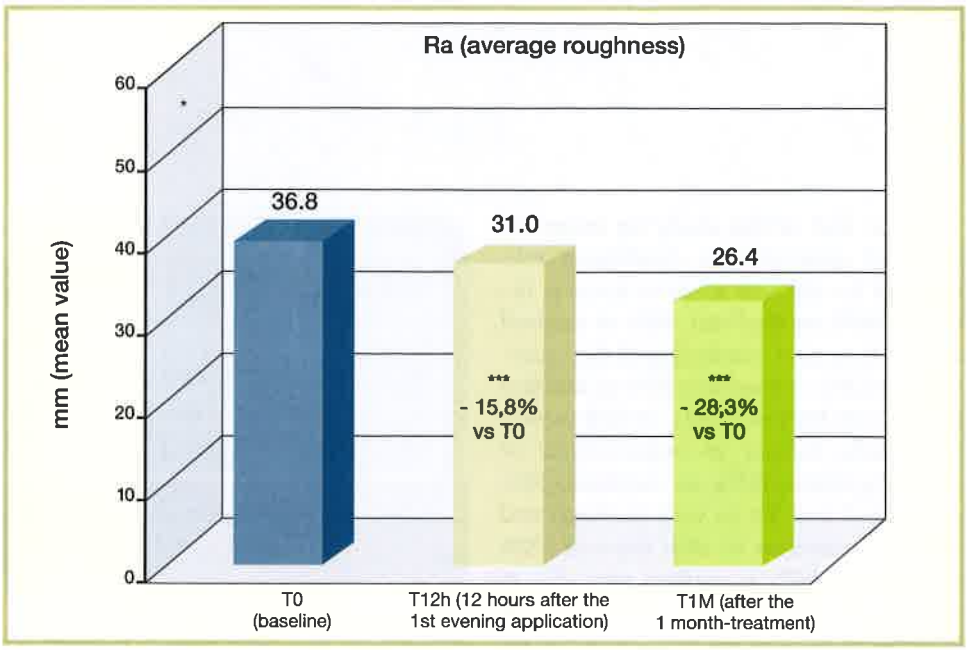


Fig. 2. *Percentage reduction in crow's feet average roughness value.*

T0
(baseline)

T1M
(after 1 month-treatment)

Fig. 3. *Clinical pictures of subject n. 17 showing the effect of the study product on dark spot.*

Face volume image analysis was carried on the 3D pictures taken at T0 and T1M by Vectra H1. In particular Vectra analysis module (VAM) merges and compares the images taken at two different times and automatically calculates the volume difference using a colour distance map. Obtained results highlighted at T1M an average increase of volume versus T0 of 0.439 cc, index of a revolumizing anti-aging performance of the tested treatment.

Photographic documentation of some selected cases whose presented at the inclusion hyperchromic spots and/or skin scars, also of old onset, highlighted an important aesthetic performance of the study product on these skin imperfections. In fact the dark spots and scars appeared, at the end of the trial, less marked and visible **(Fig. 3 and 4)**.

T0
(baseline)

T1M
(after 1 month-treatment)

Fig. 4. *Clinical pictures of subject n. 6 showing the effect of the study product on scar.*

At the end of the study an improvement of general skin conditions was referred by 100% of subjects for skin firmness (40% as medium, 45% as marked and 15% as very marked) and skin smoothness (30% as medium, 55% as marked and 15% as very marked), as well as 95% of subjects noticed an improvement of skin brightness (35% as medium, 55% as marked and 5% as very marked) and 90% of volunteers of skin dryness (20% as medium, 55% as marked and 15% as very marked). Moreover 65% and 85% of subjects noticed the efficacy of the serum on deep (35% as medium and 30% as marked) and superficial wrinkles (50% as medium and 35% as marked), while 100% and 75% of the cases underlined a lifting effect (50% as medium, 45% as marked and 5% as very marked) and reshaping of face silhouette (50% as medium and 25% as marked). Reduction of dark spots and scars was confirmed by 59% and 62.5% of subjects respectively. At the end of the trial the investigator's and volunteers' judgement about the tolerability of the tested cream is good/excellent in 100% of treated cases.

Short-term study

Stripping corneum determined on all skin study areas an increase of TEWL (transepidermal water loss) mean value; the alteration of skin barrier resulted similar for all tested areas; in fact no clinical or statistical difference was found at T0 among the 3 compared areas. In **Tab. 2** the variation percentages (mean value) of the considered items versus T0 were summarized.

Study product application determined, starting from T1h, a significant and important reduction of TEWL mean value (Holm-Sidak Adjusted t tests $p<0.05$ T1h and T24h vs T0) corresponded respectively to 32.5% after 1 hour and 34.2% after 24 hours; these reductions were clinically different from the one obtained on the untreated control area (-24.2% at T1h and -23.2% at T24h), sign of a faster restoration of the skin barrier physiological conditions; moreover at T24h the study product efficacy was more marked to the one highlighted on the skin area treated with the reference product.

TEWL	Variation (%) vs T0	
	T1h	T24h
Study product	- 32.5% (*)	- 34.2% (*)
Reference product	- 36.5% (*)(°)	- 27.1% (*)
Untreated control area	- 24.2% (*)	- 23.2% (*)

Holm-Sidak Adjusted t tests *p<0.05 vs T0 and °p<0.05 vs untreated control area

Tab. 2. *Percentage variation of TEWL value.*

A single application of the study and reference formulations determined at T1h a very significant increase (Holm-Sidak Adjusted t tests $p<0.05$ T1h vs T0) of superficial skin hydration mean value vs T0, index of an immediate and important moisturizing activity, that decreases within 24 hours.

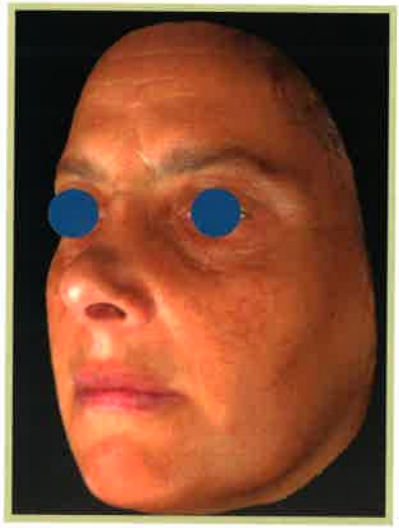
In particular at T1h the hydrating activity of the study product resulted clinically more marked and statistically different from the one highlighted for the reference product, as well as still significantly present at T24h (long lasting efficacy); on the contrary for the untreated control area a clinically significant reduction of basal values was showed **(Tab. 3)**.

Tab. 3. *Percentage variation of superficial skin hydration value.*

SUPERFICIAL SKIN HYDRATION	Variation (%) vs T0	
	T1h	T24h
Study product	+ 34.8% (*)(°)(°)	+12.5% (*)(°)
Reference product	+ 22.5% (*)(°)	+16.5% (*)(°)
Untreated control area	- 15.9% (*)	+3.8%

Holm-Sidak Adjusted t tests *p<0.05 vs T0, °p<0.05 vs untreated control area, °p<0.05 vs reference product

Fig. 5. *3D clinical pictures of subject n.15 showing the effect of the study product on signs of aging.*



T0 (baseline)

T1M (after 1 month-treatment)

Regarding the deep skin hydration measured at 0.5 mm of depth, obtained results highlighted at T1h on the skin area treated with the study product a statistically significant reduction of the analysed parameter (-11.0%, Holm-Sidak Adjusted t tests $p<0.05$ T1h vs T0) index of a control activity of skin irritation/damage induced by tape stripping on the deep skin layers; at T24h the effect was less marked.

Although no statistically significant variation vs T0 was found at any study time, for any tested areas, it is important to note as the stripping corneum determined at T1h a clinically significant increase of pH mean value; this alteration resulted clinically less marked for the tested area treated with study product (+2.6% vs T0) compared to the area treated with reference product and untreated control area (+4.2% and +4.7% respectively); moreover at T24h on this area the pH is almost normalized, while it remained higher on the other two areas (reference product area +2.2%; untreated control area +3.8%).

Skin replicas are obtained using silicone rubber. A picture of each replica was taken at T0 and T24h thanks to Primos compact portable device (GFMesstechnik); the image analysis of surface roughness was performed using the "surface roughness evaluation" function. In particular, the profilometric parameter analyzed in this study was Sa (average roughness of the analyzed profile), that represents an overall measure of the surface texture.

The study product, although not achieving the statistical significance vs T0, determined 24 hours after application an important reduction of Sa profilometric parameter (-5.9% Holm-Sidak Adjusted t tests $p<0.05$ T24h vs untreated control area), index of a clinically significant re-epithelizing activity of the tested formulation not detectable on the untreated control area (+5.9%) and comparable to the one highlighted for the reference product (-7.6% Holm-Sidak Adjusted t tests $p<0.05$ T24h vs T0 and untreated control area).

CONCLUSION

In conclusion, the study formulation determined already 1 hour after the single application on experimentally induced erythema by repeated tape stripping on the forearm: a very important increase of skin superficial hydration, significantly more marked that the one highlighted for the reference product (moisturizing efficacy); a significant reduction of deep hydration and a lower variation of epicutaneous pH, index of a faster control and normalization activity of the skin irritation induced by tape stripping; a clinically relevant restoration of skin microrelief and physiological skin barrier conditions damaged by stripping (re-epithelizing/restoring activity), not detectable on the untreated control area.

At the level of the face, clinical and instrumental results as well as subjects' self-assessment evaluation clearly showed the study product anti-age performance **(Fig. 5)**.

In particular the formulation determined: 12 hours after the first application (T12h) a significant reduction of crow's feet (lifting effect); after 1 month-treatment (T1M) an important increase of superficial skin hydration (moisturizing activity) and a very significant reduction of skin roughness around the eyes (anti-wrinkles activity), as well as an increase of face volume and a visible improvement of skin firmness (redensifying and revolumizing effect). Moreover additional test results suggest how this product is indicated to reduce the skin imperfection as skin spots and scars (anti-spots and restoring activity), improving the skin complexion. The most part of included subjects confirmed the anti-age efficacy of the tested serum.

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MODO D'USO

SIERO: dopo la detersione, applicare mattina e/o sera su viso e collo.

MASCHERA: dopo la detersione, applicare su viso e collo e tenere in posa per 5/10 minuti. Senza risciacquo.

USO LOCALIZZATO: applicare mattina e sera sulle zone da trattare in caso di smagliature, lassità cutanee e inestetismi delle cicatrici.

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