The Moisturizing Efficacy of a Proprietary Dermo-Cosmetic Product (CLS02021) Versus Placebo in a 4-week Application Period

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ABSTRACT

Background: Studies suggest that applying probiotic skincare products may have beneficial effects on the skin due to bacteria competitiveness and specific metabolites produced by probiotics. The cream CLS02021 is a postbiotic blend of metabolites, including organic acids, enzymes, and peptides that are a result of the co-fermentation of three proprietary probiotic strains and is specific for its high concentration of the enzyme Sphingomyelinase which plays a significant role in cell signaling pathways and ceramide production.

Objective: The aim of this study is to evaluate the cosmetic effects of probiotic-derived Ingredient CLS02021 and its safety in healthy volunteers.

Methods: Fifty healthy volunteers between 18 and 69 years old were recruited to participate in this multicentric, randomized, intra-individual, double-blind group study. Following a face-split design, volunteers applied both face cream containing CLS02021 on one side of their face and a placebo cream on the other side of their face for a total duration of four weeks, two times a day. Evaluation of the cosmetic effects included instrument measurements of moisturizing, elasticity, wrinkle depth, sebum production, pore size, melanin production, sensitivity, and side effects.

Results: A significant difference of CLS02021 over the placebo group was observed for moisture and elasticity increase (both p<0.001), pore size (p<0.01), and a wrinkle depth decrease (p<0.05). No significant differences were noted in sebum production, melanin level, skin cleanliness, and sensitivity between groups.

Conclusion: CLS02021, as a probiotic-derived ingredient, showed strong beneficial effects on skin moisture and elasticity increase, wrinkle depth, and pore size reduction. Face cream containing CLS02021 is well tolerated and could be an organic choice for cosmetic hydrating and antiaging skincare treatment.

Keywords: probiotics, skin care, cosmetic product, antiaging.

1. BACKGROUND

The human skin is an essential defense barrier against environmental factors such as various toxins and microbial pathogens. To achieve this function, our skin hosts a variety of protective microbiota. In healthy skin, the microbiota is in homeostasis and balance. Due to physical, chemical, and other external and internal factors, our microbiota can be easily disrupted, leading to skin conditions including acne, atopic dermatitis, psoriasis, rosacea, or even premature aging (1-3). Beneficial skin properties of probiotic bacteria can be assigned to bacteria competitiveness with harmful bacteria and bacteria-specific metabolite production. In more simple terms, beneficial bacteria drive the harmful bacteria from the skin and restore its balanced environment while producing various organic acids, polysaccharides, and proteins - mostly enzymes that participate in the normal metabolic processes of the skin such as hyaluronic acid production, ceramide synthesis, melanocyte stimulation, and many others. A further emphasis is attributed to Lactic Acid Bacteria (LAB) compounds such as cell wall fragments and their metabolites (4). Different studies have shown that LAB metabolites such as hyaluronic acid, lipoteichoic acid, sphingomyelinase, and lactic acid have promising cosmetic and therapeutic effects on the skin (5-8). The proprietary cream CLS02021 is a postbiotic blend of metabolites, including organic acids, enzymes, and peptides, that are a result of the co-fermentation of three proprietary probiotic strains. These strains are lactic acid bacteria (LAB) strains isolated from native fermented dairy products from the Balkan region. Genomic tools were used to fully characterize and denominate the strains as Lactobacillus.
Hyaluronic Acid (HA)

HA consists of a basic unit of two sugars, glucuronic acid, and N-acetylglucosamine, polymerized into large macromolecules of 2000–25,000 repeating units (11). HA is essential for maintaining the normal structure of the stratum corneum and conserving epidermal barrier functions. Topical application of HA for 60 days showed significant improvement in skin hydration and elasticity. Application of low-molecular-weight HA resulted in a significant reduction in wrinkle depth, which was attributed to better penetration (12). Furthermore, low-molecular-weight HA has also been reported to increase epithelial defense by inducing one of the most common types of antimicrobial peptides, β-defensin-2, via Toll-like receptors (TLR), participating in the host response against bacterial infections (13). HA is commercially obtained by extraction from rooster combs and by fermentation by LAB (14).

Lactic Acid

Lactic acid is an organic acid with one hydroxyl group attached to the alpha position of the acid. It has been widely used for many years in cosmetic regimens and skincare products such as moisturizers, exfoliants, and emollients (15, 16). The use of lactic acid in skincare in different concentrations leads to the improvements in both the epidermal and dermal components providing useful treatment for rejuvenating photo-damaged skin (17). Lactic acid is regularly produced through microbial fermentation and is a predominant end-product of LAB carbohydrate metabolism.

Sphingomyelinase (SMase)

Sphingomyelinase (SMase) is an enzyme that is responsible for ceramide production. Ceramides are lipids (fats) found naturally in high concentrations in the uppermost layers of skin, making over 50% of the skin’s composition. Ceramides help retain the skin together by forming a protective layer that limits moisture loss and protects against visible damage from pollution and other environmental stressors. A decrease in ceramide in the stratum corneum causes water loss and barrier dysfunction in the epidermis, including a loss of protection against antigens and bacteria (18).

SMase is detected in bacteria, with great variations in SMase activity among different bacterial strains. Bacterial SMase is a secretory protein released from cells into the media. In vitro studies have confirmed that keratinocyte cell lines treated with sonicated S. thermophilus significantly increase ceramide generation. Further examinations proved that increased levels of ceramide production were due to SMase (>0.1 mU/mL) obtained from sonicated cells of S. thermophilus. These results were further confirmed using in vivo testing on 17 healthy Caucasian volunteers with a cream formulation containing sonicated S. thermophilus applied twice daily. After seven days of treatment, S. thermophilus induced a very significant increase in ceramide levels as compared to controls (19). Based on the mechanisms of action of these specific metabolites, it can be hypothesized that the primary effect of daily use of a topical product with CLS02021 is an increase in overall skin moisture levels.

The chemical composition of Metabiotic Complex provides a promising base for other beneficial effects on the skin such as anti-inflammatory effects, stimulation of collagen production, increase in skin elasticity, brightening of skin complexion, reduction of fine lines and wrinkles, and general improvement of skin appearance.

There is a growing body of evidence, and ongoing research focus on the benefits of technologies based on probiotics for cosmetic purposes (19). However, there is a lack of well-designed Randomized Controlled Trials (RCTs) for the study of cosmetic products and their measurable effects on the skin.

The aim of this randomized and controlled study is to explore the cosmetic effects of the ingredient “Metabiotic Complex,” developed by Anbiome Labs. It is hypothesized that Metabiotic Complex-based formulations may have beneficiary effects on a wide array of cosmetic appliances.

2. OBJECTIVE

The aim of this study is to evaluate the cosmetic effects of probiotic-derived Ingredient CLS02021 and its safety in healthy volunteers.
3. SUBJECTS AND METHODS

Study subjects

Fifty healthy volunteers without any prior skin-related or health-related conditions, between 18 and 69 years old, were recruited to participate in this study. The exclusion criteria included pregnancy, nursing, and planning a pregnancy. All volunteers signed a written informed consent in accordance with the study procedure approved by the Bioethical Committee of the Sarajevo Medical School at the Sarajevo School of Science and Technology and were free to discontinue participation at any time without any obligation. Volunteers were required to follow specific instructions for four weeks according to the study design.

Study design

This was a randomized, intra-individual, double-blind controlled study performed between July 2021 and September 2021 in Sarajevo, Bosnia, and Herzegovina. The study was performed in agreement with the principles of the Declaration of Helsinki and recommendations of Good Clinical Practice ICH E6(R2). Furthermore, the study was applied and registered at ClinicalTrials.gov, receiving an Identifier code: NCT05048121.

All volunteers were assigned a Subject Identification code for easier monitoring and to maintain subject anonymity. Each volunteer was given a set of two identical tubes, one tube containing CLS02021 and another one containing a placebo, and they were instructed to apply a product cream in an amount of a hazelnut size while avoiding the eye region. The study products were applied twice a day, for four weeks, to the left and right sides of the face using the indication on the tubes shown as “Left” and “Right,” a method widely known as split-face testing. Figure 1 briefly provides an overview of the study design.

Study points and parameters

During the four-week study, a total of three measurements were performed at the following points: baseline point (W0), point Week 2 (W2), and endpoint Week 4 (W4). Initial measurement prior to the study beginning served as a baseline point. After the measurement of endpoint W4, the study was concluded. At every study point, with the aim to assess the safety profile, volunteers were prompted to report any side effects that might have stemmed from the product use. All of the measurements were carried out by an independent cosmetologist, completely blinded to the procedure.

The measurement process was performed in two designated pharmacies. The API Skin 100 instrument (ARAM HUVIS, South Korea) was used for measurement and evaluation of the following skin parameters: moisturizing and elasticity using corneometry (Bioelectrical Impedance Analysis); wrinkle depth by photometry (shadow method and picture processing); sebum production by photometry; pore size by photometry (polarized light and picture processing); melanin production by photometry (light absorption); sensitivity by photometry (light reflection); cleanliness by photometry (polarized light and picture processing).

Statistical analysis

Data analysis is conducted using SPSS Statistics for Windows, Version 23.0 (IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp). Descriptive statistics were performed for the characterization of demographic characteristics of the volunteers and included frequency (n), mean, a standard deviation of mean, minimum, median, and maximum values. Frequency and percentages were provided for categorical variables. Mann-Whitney U Wilcoxon test was used to compare the efficiency of test and control products between measurements. All results were evaluated at a confidence level of 95%, and the significance level was set at p<0.05. The per-protocol (PP) population included all randomized volunteers who fulfilled inclusion criteria.

| Parameter | Active | Placebo | p     |
|-----------|--------|---------|-------|
| Moisture  |        |         |       |
| ΔMin-Max  | -30-76 | -48-80  |       |
| ΔMean±STD | 11,94±9,09 | -4,45±21,47 | 0,000** |
| ΔMedian   | 10     | 0       |       |
| Pore      |        |         |       |
| ΔMedian   | 3      | 3       |       |
| ΔMin-Max  | -35-50 | -36-61  |       |
| ΔMean±STD | 6,11±18,17 | 7,94±21,11 | 0,850  |
| W0-W2     |        |         |       |
| ΔMedian   | -97-86 | -97-89  |       |
| ΔMin-Max  | -3,94±14,65 | 3,74±16,29 | 0,008** |
| ΔMean±STD | 11,87±15,64 | 1,91±19,45 | 0,001** |
| Sebum T zone |        |         |       |
| ΔMedian   | 0      | 0       |       |
| ΔMin-Max  | -57-97 | -49-97  |       |
| ΔMean±STD | 4,79±36,56 | 6,21±36,34 | 0,814  |
| W0-W4     |        |         |       |
| ΔMedian   | 0      | 2       |       |
| ΔMin-Max  | -97-86 | -97-89  |       |
| ΔMean±STD | 0,79±25,23 | 6,79±28,9 | 0,421  |
| Sebum U zone |        |         |       |
| ΔMedian   | 0      | 0       |       |
| ΔMin-Max  | -57-97 | -49-97  |       |
| ΔMean±STD | 4,79±36,56 | 6,21±36,34 | 0,814  |
| W0-W4     |        |         |       |
| ΔMedian   | -97-86 | -97-89  |       |
| ΔMin-Max  | -3,94±14,65 | 3,74±16,29 | 0,008** |
| ΔMean±STD | 11,87±15,64 | 1,91±19,45 | 0,001** |

Table 1. Distribution of study subjects
A total of fifty healthy volunteers were enrolled in this study. However, during the first two weeks, three participants withdrew from the study; therefore, forty-seven participants completed the study (Figure 1). Baseline demographic data of volunteers are presented in Table 1. All of the volunteers reported the use of facial creams in the past and no prior skin-related or health-related conditions (Table 2). During the study, three side effects were reported; two volunteers reported pimple breakdown, which occurred in the first week of product application, and one volunteer reported redness on the forehead during the first 3-4 days of use. Further analysis shows that reported redness occurred on the side of the face where the active product was applied for one subject and in the other subject where the placebo was applied, which may be caused by the base cream formulation and not the CLS02021. Overall, the safety and tolerability results of CLS02021 were comparable with those of the placebo and local tolerability was good for both treatments. A comparison of treatment groups, based on a difference in measured scores (Delta Δ) at baseline point W0, point W2, and after endpoint W4, is presented in Table 3. Sebum T zone and Sebum U zone parameters were measured not at point W2. Our results indicate a significant difference between CLS02021 and the placebo groups in the following parameters: Moisture (p<0.0001), Elasticity (p<0.0001), Pore Size (p<0.01), and Wrinkle Depth (p<0.05). However, the change was not statistically significant from the placebo group in the following parameters: Sebum U and T zone, Melanin, Cleanliness, and Sensitivity. Statistically significant increases in moisture (p<0.0001) are observed in the CLS02021 group at W0 to W4 (11.49±19.09) compared to the Placebo group (-4.45±21.47). A similar effect has been observed in the CLS02021 group with parameter Elasticity (p<0.0001), where the mean improvement at W0 was 11.04±17.62, while the Placebo group recorded a mean decrease of -3.51±18.32 at W4 (Table 3). Statistically significant reduction of pore size and decrease in wrinkles depth (p<0.05) was observed from W4 to W0, while no significant difference was observed between W2 and W0. Obtained results suggest improvement of the following skin parameters: moisture, elasticity, and wrinkle depth over a period of four weeks. Furthermore, a comparison of mean values at points W0, W2, and W4 demonstrates a positive effect on moisture, elasticity, pore size, and wrinkle depth of the tested product versus the placebo (Graph 1). Although there was a positive trend, no statistically significant difference between the CLS02021 group and the placebo group was observed in the following measured parameters: sebum, melanin, cleanliness, and sensitivity. However, volunteers using test tubes containing CLS02021 showed a rapid and constant decrease in melanin levels as well as sensitivity improvement. Regarding sebum levels in the U zone, the CLS02021 group shows a lower and slower decrease in sebum levels and lower levels of sebum in the T zone.

5. DISCUSSION

Recent studies have shown that the intestinal microbiome plays an essential role in modulating systemic inflammation and skin diseases by modulating the host im-

| Table 2. Comparison of treatment groups based on difference of scores measures on baseline, W2 and W4. Mann-Whitney U Test; *p<0.05; **p<0.01 |
| ΔMin-Max | ΔMean±STD | ΔMin-Max | ΔMedian | ΔMin-Max | ΔMedian | ΔMin-Max | ΔMedian | ΔMin-Max | ΔMedian |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| **Wrinkle**     | **ΔMin-Max**    | **ΔMean±STD**   | **ΔMin-Max**    | **ΔMedian**     | **ΔMin-Max**    | **ΔMedian**     | **ΔMin-Max**    | **ΔMedian**     | **ΔMin-Max**    |
| W0-W2 ΔMean±STD | -5.94±26.95     | -2.83±25.66     | 0.922           | 0               | 4                | 0                | 0               | 0               |
| ΔMedian         | -7.62           | -8.64           |                | -4              |                  |                  |                  |
| ΔMin-Max        | -9.43±31.12     | -5.96±24.15     | 0.994           | -2              |                  |                  |                  |
| ΔMin-Max        | -8.71           | -68.64          |                |                  |                  |                  |

4. RESULTS

A total of fifty healthy volunteers were enrolled in this study. However, during the first two weeks, three participants withdrew from the study; therefore, forty-seven participants completed the study (Figure 1). Baseline demographic data of volunteers are presented in Table 1. All of the volunteers reported the use of facial creams in the past and no prior skin-related or health-related conditions (Table 2). During the study, three side effects were reported; two volunteers reported pimple breakdown, which occurred in the first week of product application, and one volunteer reported redness on the forehead during the first 3-4 days of use. Further analysis shows that reported redness occurred on the side of the face where the active product was applied for one subject and in the other subject where the placebo was applied, which may be caused by the base cream formulation and not the CLS02021. Overall, the safety and tolerability results of CLS02021 were comparable with those of the placebo and local tolerability was good for both treatments. A comparison of treatment groups, based on a difference in measured scores (Delta Δ) at baseline point W0, point W2, and after endpoint W4, is presented in Table 3. Sebum T zone and Sebum U zone parameters were measured not at point W2. Our results indicate a significant difference between CLS02021 and the placebo groups in the following parameters: Moisture (p<0.0001), Elasticity (p<0.0001), Pore Size (p<0.01), and Wrinkle Depth (p<0.05). However, the change was not statistically significant from the placebo group in the following parameters: Sebum U and T zone, Melanin, Cleanliness, and Sensitivity. Statistically significant increases in moisture (p<0.0001) are observed in the CLS02021 group at W0 to W4 (11.49±19.09) compared to the Placebo group (-4.45±21.47). A similar effect has been observed in the CLS02021 group with parameter Elasticity (p<0.0001), where the mean improvement at W0 was 11.04±17.62, while the Placebo group recorded a mean decrease of -3.51±18.32 at W4 (Table 3). Statistically significant reduction of pore size and decrease in wrinkles depth (p<0.05) was observed from W4 to W0, while no significant difference was observed between W2 and W0. Obtained results suggest improvement of the following skin parameters: moisture, elasticity, and wrinkle depth over a period of four weeks. Furthermore, a comparison of mean values at points W0, W2, and W4 demonstrates a positive effect on moisture, elasticity, pore size, and wrinkle depth of the tested product versus the placebo (Graph 1). Although there was a positive trend, no statistically significant difference between the CLS02021 group and the placebo group was observed in the following measured parameters: sebum, melanin, cleanliness, and sensitivity. However, volunteers using test tubes containing CLS02021 showed a rapid and constant decrease in melanin levels as well as sensitivity improvement. Regarding sebum levels in the U zone, the CLS02021 group shows a lower and slower decrease in sebum levels and lower levels of sebum in the T zone.

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|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| **Wrinkle**     | **ΔMin-Max**    | **ΔMean±STD**   | **ΔMin-Max**    | **ΔMedian**     | **ΔMin-Max**    | **ΔMedian**     | **ΔMin-Max**    | **ΔMedian**     | **ΔMin-Max**    |
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It is suggested that oral probiotics can treat certain skin conditions, such as acne, atopic dermatitis, photoaging, psoriasis, and wound healing (22). Findings from this study suggest that the dermo-cosmetic product CLS02021, with our proprietary cosmetic ingredient “Metabiotic Complex,” has statistically significant beneficial effects on the skin regarding skin moisturizing, elasticity, and wrinkle improvement. These findings are in accordance with the recently reported beneficial effects of probiotics and their metabolites (23). Ceramides are key components in skin barrier integrity maintenance because of their ability to lock in moisture. Furthermore, ceramides contribute to skin elasticity giving it a vibrant, healthy look (24). Studies have shown that topical preparations of precursors can lead to an increase in ceramide levels of Sphingomyelinase, which is naturally produced during the probiotic life cycle and is responsible for the conversion of native skin sphingomyelin into ceramides (25). Metabiotic Complex contains a significant amount of naturally produced Sphingomyelinase, which can be attributed to facilitating the production of ceramides, leading to a measurable increase in skin moisture levels and improvement of skin elasticity. Due to the growing evidence, it is becoming more popular to use topical preparations of probiotics for the treatment of atopic dermatitis, psoriasis, and wound healing but also for photo and skin aging. Recent studies have classified probiotic skincare as one of the latest skincare trends for fighting photoaging and skin aging (26). Probiotics have been shown to have a preventative effect on skin photoaging induced by short-term UV irradiation. Satoh et al. reported that the use of probiotic Bifidobacterium breve B-3 showed its effect on suppressing changes in transepidermal water loss, skin hydration, epidermal thickening, and attenuated the damage to the tight junction structure and basement membrane induced by chronic UVB irradiation (27). In our study, a cream consisting of “Metabiotic Complex,” a probiotic derived ingredient, showed beneficial effects on the main potential risks contributing to the skin aging process. Fast improvement and increase of facial skin moisture, which persists, are one of the effects on overall long-term effects on skin condition. Sharma et al. explain that probiotics can restore acidic skin pH, alleviate oxidative stress, attenuate photoaging, and improve skin barrier function (28). In vitro study of the effects of plant extracts fermented with Lactobacillus buchneri (PELB) on ultraviolet light (UVB)-induced photoaging process reported that PELB could be useful in the cosmetic industry due to its protective effects against UVB-induced photoaging. PELB works by decreasing elastase activity and increasing type I collagen expression, reducing collagenase activity, and promoting the expression of moisture factor and antioxidant enzymes (29).
Our study product showed a significant and persisting increase in skin elasticity which was expressed in the first two weeks of product application. Notay et al. investigated the use of topical Nitrosomonas europa as a cosmetic ingredient for the reduction of facial wrinkles and found a significant improvement in wrinkle depth severity, hyperpigmentation of the forehead, and glabela in the group receiving high topical concentrations of the probiotic formula (30). Hyaluronic acid is a humectant; it binds large amounts of water, more precisely 100 times its weight (31). Its ability to tie water helps rehydrate the skin, thereby filling in fine lines and reducing the appearance of wrinkles (32). Along with lactic acid, hyaluronic acid is a large constituent of a Metabiotic Complex, leading to a reduction of wrinkle depth during four weeks of product use. Topical application of lactic acid has been shown to lead to an increase in skin hydration and elasticity, a decrease in transepidermal water loss, and a decrease in pore size (33). A statistically significant reduction in pore size (p<0.01), was shown in healthy volunteers in the area where the tested product, CLS02021, was applied.

LTA is a natural constituent of lactic acid bacteria cell walls. Through its inhibition of expression of collagen degrading enzyme matrix metalloproteinase-1 (MMP-1), it can be attributed to being an effective agent in skin protection from UV-induced skin damage and phot-aging (34). Four weeks of topical use of a cosmetic product containing Metabiotic Complex infused with LTA has not shown a statistically significant difference in melanin production decrease. However, a melanin production increase was also not observed, which gives cause to hypothesize that prolonged product use could lead to positive results. Considering that the study was performed during the warmest summer months with high UV exposure, melanin production levels could have been significantly influenced. Therefore, further research should be carried out during a different season. In order to achieve more significant scientific data, further evaluations should be performed in more controlled conditions.

6. CONCLUSION

The use of oral and topical probiotics for skincare and the treatment of skin problems has grown in popularity over the last decade. Ongoing research studies attempt to evaluate the efficacy, mechanism of action, safety, and indications of new products when they are introduced to the market. Developed by Anbiome Labs, cream CLS02021 contains “Metabiotic Complex,” a probiotic-derived ingredient, which is a blend of several organic acids, enzymes, and peptides that are a result of a co-fermentation of three proprietary probiotic strains. Our findings have demonstrated strong beneficial effects of face cream containing “Metabiotic Complex” on skin moisture and elasticity increase, wrinkle depth, and pore size reduction during a four-week-long study. Furthermore, face cream containing “Metabiotic Complex” is well tolerated and could be the organic choice for cosmetic moisturizing and anti-aging skincare treatment. However, there is a need to conduct further research with an extended study duration on a larger sample of study subjects.

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The Moisturizing Efficacy of a Proprietary Dermo-Cosmetic Product (CLS02021) Versus Placebo in a 4-week Application Period

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