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The Role of a Natural Mollusk Egg-Derived Ingredient in Facial Appearance

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ABSTRACT

New cosmeceutical ingredients that improve skin appearance are of interest to the dermatologist. *Cryptomphalus aspersa* is a snail raised on farms in Spain for its mucinous secretions and eggs. These natural products have been demonstrated in vitro to trigger mesenchymal stem cell differentiation, promote dermal fibroblast and keratinocyte migration, prevent keratinocyte aging, prevent oxidative damage, stimulate the extracellular matrix, and regulate MMPs. This 12-week study enrolled 40 male and female subjects age 40-70 years of Fitzpatrick skin types I-IV with moderate to severe facial aging and Rao-Goldman scores of 4-5 who applied an eye and face anti-aging cream twice daily containing a mollusk egg extract. Dermatologist investigator, subject, and elasticity assessments were performed at baseline, week 8, and week 12. At week 12, the investigator rated a 53% reduction in skin roughness ($P<0.001$), 26% improvement in skin brightness ($P<0.001$), and 12% reduction in skin dyspigmentation ($P=0.033$). The noninvasive elastometer measurements demonstrated an increase in skin elasticity at week 8 of 11% with a continuing elasticity increase at week 12 of 39% ($P<0.001$). The formulation studied included moisturizing, emollient, film-forming, and retinoid ingredients in addition to the mollusk egg extract to produce the clinical improvement.

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INTRODUCTION

Cosmeceuticals aim to improve the appearance of aging skin. It is unique to find a cosmeceutical formulation that delivers a novel active ingredient while providing simultaneous moisturization. The moisturizer serves as the delivery system for the cosmetic ingredient producing smooth, soft skin while creating the optimal environment for barrier repair. Consumers expect cosmeceuticals to provide benefits beyond simple moisturization. Many different categories of ingredients have entered the marketplace to modify the skin, such as hydroxy acids to induce exfoliation or botanical extracts to function as antioxidants. Previous work examined the role of an animal-derived Growth Factor, the secretion of the *Cryptomphalus aspersa,* discovered by Rafael Abad Iglesias MD, a radiation oncologist treating radiation dermatitis.[1,2] The secretion was studied for its histologic effects on photoaging and noted that the mollusk generated a biologically active glycosaminoglycan secretion, as a defense mechanism when exposed to ultraviolet (UV) light and x-rays that assisted in the regeneration of damaged structures of the animal's skin in less than 48 hours.[3] In addition to the secretion's ability to stimulate fibroblast proliferation and rearrange the actin cytoskeleton, Brieva, et al, identified the presence of antioxidants with superoxide dismutase (SOD) and glutathione S transferase (GST) activity within the secretion. Stimulation of extracellular matrix assembly and regulation of matrix metalloproteinase (MMP-1 and MMP-2) activities were also observed.[4] Fabi and Cohen previously demonstrated the activity of this cosmeceutical ingredient on photoaged skin.[5]

A second cosmeceutical ingredient, recently discovered, is derived from the eggs of the *Cryptomphalus aspersa* snail. The eggs are collected on snail farms in Spain and processed to yield an agent with the potential to help delay and reduce the visible signs of aging. Espada, et al, examined the role of *Cryptomphalus aspersa* mollusk egg extract in the promotion of migration and the prevention of cutaneous aging in keratinocytes and dermal fibroblast in vitro.[6] The study measured the effects of the mollusk egg extract on cellular proliferation, migration, distribution of cytoskeletal proteins, production of extracellular components, and the ability to prevent cutaneous ageing due to intrinsic or extrinsic factors (exposure to UVB) by determination of ageing markers. From the obtained results, it was concluded that the mollusk egg extract had the ability to ameliorate a series of functions related to cellular migration, tissue repair, and attenuated age-related morphological changes of human skin cells. Juarranz, et al, examined the in vitro effects of the mollusk egg extract on skin homeostasis, migration, cell survival and MMP regulation.[7] Results showed that the mollusk egg extract promoted epithelial tissue regeneration, being more effective on fibroblasts than on keratinocytes, significantly increased collagen synthesis and fibronectin production while downregulating MMPs in both types of cutaneous cells.

Furthermore, Espada et al discovered that the mollusk egg extract promoted the migration and regenerative behavior of human keratinocytes and mesenchymal stem cells.[8] The mollusk egg extract induced morphological and phenotypical

changes in mesenchymal stem cells that were consistent with differentiation to a skin cell lineage. This was evidenced by the expression of cytokeratin 14 (CK-14), a specific marker of keratinocytes from the basal membrane, and alpha-smooth muscle actin (a-SMA), a myofibroblast marker.

It was postulated that these in vitro cutaneous effects would be beneficial for aging skin, thus the effect of the mollusk egg extract on skin appearance was studied. The utility of this ingredient, in a well-formulated moisturizer vehicle for the face and eyes, was examined in a monadic study in female and male subjects with Rao-Goldman scores of 4-5 for facial aging who used the products for 12 weeks.

METHODS

40 subjects age 40-70 years of Fitzpatrick skin types I-IV who successfully completed informed consent (Concordia Clinical Research IRB, New Jersey) were enrolled in the 12 weeks study. Subjects underwent an evaluation for inclusion and exclusion criteria. Subjects were excluded who had used retinoids within 3 months of study entry, systemic steroids within 6 months of study entry, underwent facial neurotoxin or filler injections or dermabrasions or laser procedures or chemical peels within 6 months of study entry, used hydroxy acids or active moisturizers within 7 days of study entry, or possessed any facial skin condition that might interfere with the study results. Subjects were required to possess Rao-Goldman scores of 4-5 for facial aging and were forbidden from undergoing facial waxing, bleaching, or using a depilatory cream or self-tanning cream.

Subjects were asked to leave all other skin care products, cosmetics, nutritional supplements, and medications unchanged for the duration of the study, only adding the study eye moisturizer (Tensage Stem Cell Eye Cream with Cellpro Technology, Biopelle, Michigan) and facial moisturizer (Tensage Stem Cell Cream with Cellpro Technology, Biopelle, Michigan) to their routine twice daily. No other moisturizing products were allowed on the face, except for sunscreen as needed. Subjects reported to the research center at baseline for enrollment and at weeks 8 and 12 for assessments. All subjects were asked to wash their face at the research center with a disposable face cloth (Olay, Sensitive Skin Face Cloths, Procter & Gamble, Ohio). At each visit subjects underwent photography with a Nikon D90 camera in 3-point head mount (Canfield, New Jersey) with a fixed F-stop of the front, right, and left face. Subjects were draped in a black collar with their hair pulled back in a black headband. The dermatologist investigator and the subjects completed efficacy and tolerability assessments at baseline, week 8, and week 12. In addition, skin elasticity measurements (Elastometer, Cortex Technologies, Denmark) were performed at baseline, week 8, and week 12.

The investigator evaluated several parameters. Facial photodamage was evaluated separately for the forehead, eye, perioral, and cheek areas on a 5-point ordinal scale (0=none, 1=minimal, 2=mild, 3=moderate, 4=severe). Facial skin condition was evaluated in terms of roughness, brightness, elasticity, pores, and pigmentation on the same ordinal scale. The investigator assessed tolerability by evaluating erythema, desquamation, and edema while querying the subjects for product induced stinging, burning, and itching. Finally, the investigator longitudinally assessed the wrinkles at each visit in terms of (1) No Wrinkles, (2) Shallow, but visible wrinkles, (3) Moderately deep wrinkles, (4) Deep wrinkles with well-defined edges, or (5) Very deep wrinkles with redundant skin folds. This was assessed separately for the forehead, eye, perioral, and cheek areas.

The investigator provided a global assessment at week 8 of the facial appearance on the following scale: (1) = Exceptional Improvement, (2) = Markedly Improved, (3) = Improved, Further Treatment Recommended, (4) = No Change, or (5) = Worsened Compared to Baseline.

Subject assessments were collected for facial skin appearance in terms of skin thickness, fine lines, wrinkles, skin color, broken blood vessels, laxity, tactile roughness, redness, and overall appearance. Subjects also assessed product tolerability in terms of stinging, burning, itching, and irritation. Both assessments were recorded on a 5-point ordinal scale (0=none, 1=minimal, 2=mild, 3=moderate, 4=severe). Compliance was determined by examining product use and reviewing application diaries.

A Mann Whitney two-tailed two-sided longitudinal analysis representing change from baseline was used to analyze the nonparametric ordinal data. A Student t test was used to analyze the numerical elasticity data. Statistical significance was defined as p less than or equal to 0.05. The safety population included all subjects exposed to the study products who provided any post-treatment safety information. Adverse event analyses were conducted as an overall study analysis.

RESULTS

40 of 40 (36 females, 4 males) subjects successfully completed the study. No tolerability issues were observed by either the dermatologist investigator or the subjects. The largest improvement in global facial wrinkling was seen after 12 weeks of product use with an 11% reduction in eye photodamage (P=0.009) accompanied by a 6% reduction in forehead and cheek wrinkles.

The investigator also assessed subject facial skin condition. At week 8, there was a 26% reduction in skin roughness (P<0.001) and a 12% improvement in skin brightness (P=0.006). Improvement continued into week 12 with a 53% reduction in skin roughness (P<0.001), a 26% improvement in skin brightness (P<0.001), and a 12% reduction in skin dyspigmentation (P=0.033). The investigator evaluated facial wrinkles on the

forehead, eye, perioral, cheek, and forehead. While wrinkles in all areas were reduced, the most statistically significant improvement (P=0.004) occurred at week 12 with a 13% reduction in eye wrinkles. Other facial areas also showed wrinkle reduction at week 12: forehead wrinkles by 10%, perioral wrinkles by 3%, and cheek wrinkles by 4%.

The noninvasive elastometer measurements demonstrated an increase in skin elasticity at week 8 of 11% with a continuing elasticity increase at week 12 of 39% (P<0.001).

DISCUSSION

The mollusk egg extract containing moisturizer produced improvement in skin appearance as assessed by investigator assessments, subjects assessments, and noninvasive skin elasticity measurements. Skin elasticity measurements are obtained using double stick tape to create a seal between the skin and an electronic suction cup over the malar eminence on the target cheek. The same technician places the cup and performs the measurement for consistency. The elasticity machine provides negative pressure, stretching and pulling the skin into the cup until a light beam at the top of the suction cup is broken. At this point, the vacuum pump turns off and the skin relaxes. The elasticity machine evaluates both the stretching and relaxation phases of skin performance to create a stress/strain curve. The study moisturizer demonstrated continuous incremental elasticity increase to 39% at week 12.

In addition to the mollusk egg extract, the moisturizer formulation contained moisturizing, emollient, and cosmeceutical ingredients. Some of the visual skin appearance improvement may have been due to this ingredient combination. The facial formulation contained shea butter, dimethicone, and meadow foam seed oil as occlusive agents to retard facial transepidermal water loss and propylene glycol and butylene glycol as humectant moisturizers to attract and hold water in the skin. It contained a number of emollients to make the skin feel smooth and soft: hydrogenated palm glycerides, grape seed extract, cetearyl nonanoate, isostearyl isostearate, and glyceryl stearate. Other skin modifying agents included ceramides, retinol, and diaminopropionoyl tripeptide-33. Thus, the formulation relied on many different mechanisms of action to produce improvement with occlusives, humectants, emollients, retinoids, peptides, and ceramides all contained within the cream.

The eye cream formulation was modified for safety around the eye area and increased skin conditioning. This formulation contained a higher concentration of occlusive moisturizers and film forming agents to minimize fine lines under the eye. These included the silicone derivatives cyclopentasiloxane, dimethicone, stearoxy dimethicone, and shea butter along with beeswax. The film forming agents to reduce wrinkling were also dimethicone based: dimethicone/vinyl dimethicone crosspolymer and diemthicone/polyglycerin-3 crosspolymer. Glycerin was added as a humectant to hold water and zinc oxide along with titanium dioxide were added to opacify the dark undereye tissue providing a temporary cosmetic benefit. Retinol, caffeine and peptides were also included.

The photographic images obtained by the dermatologist investigator during the study provide a pictorial diary documenting the incremental improvement. Figure 1 demonstrates the reduction in the nasolabial fold over time from baseline to week 8 and on to study conclusion at week 12. Figure 2 examines the upper eyelid fold and crow's feet reduction in the periorbital area. The reduction in forehead lines from baseline to week 12 is demonstrated in Figure 3. An overall look at the changes in photoaging in a male subject is captured in Figure 4 with the imaging showing improvement in facial roughness and dyspigmentation.

Mollusk egg extract represents a novel natural ingredient safe for human use and suitable for topical application. It has been

FIGURE 1. The nasolabial fold area is examined in this photographic diary (baseline, week 8, week 12) with softening noted of the nasolabial folds and a reduction in overall perioral skin roughness.

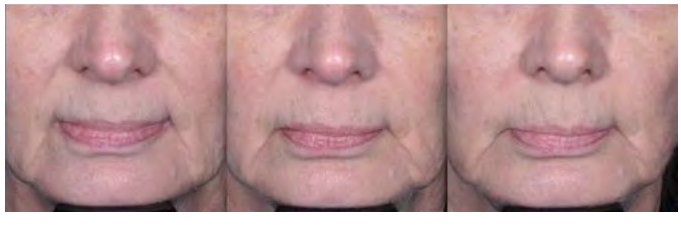


FIGURE 2. The periorbital area is examined (baseline, week 8, week 12) with less redundancy of the upper eyelid fold skin and softening of the crow's feet extending inferiorly on the upper cheek.

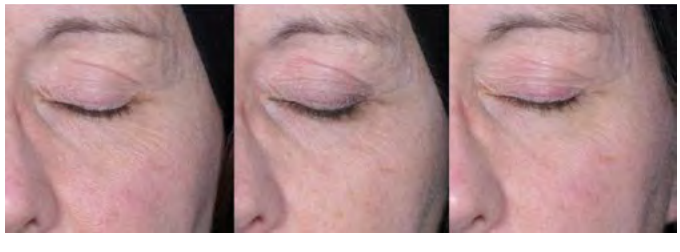


FIGURE 3. The forehead wrinkles are examined with softening noted at week 12.

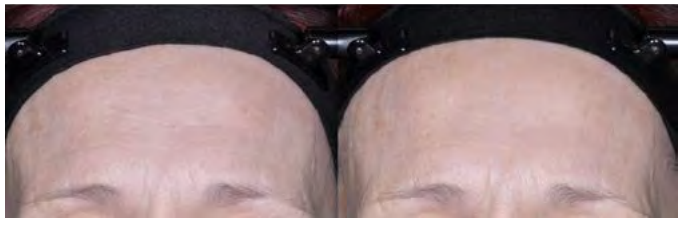
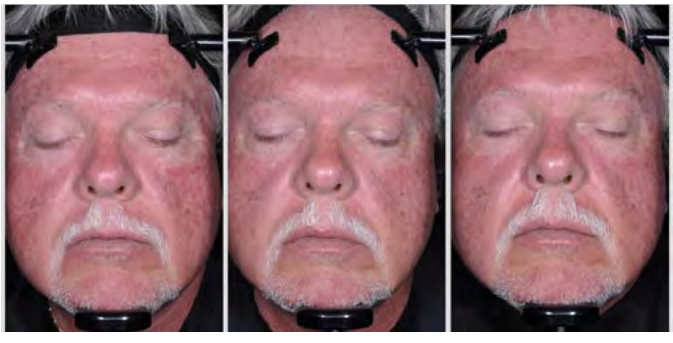


FIGURE 4. Overall appearance improvement in a male subject with severe photoaging is demonstrated (baseline, week 8, week 12).



demonstrated in this in vivo research to induce improvement in skin roughness, brightness, dyspigmentation, and wrinkling. This skin benefit probably represents a combination of the moisturizer, emollient, film-forming, retinoid, and mollusk egg extract effects of the formulation. More research is needed to better understand how the mollusk egg extract triggers the differentiation of mesenchymal stem cells to a skin-like cell lineage, as demonstrated by the expression of the CK-14 marker for keratinocytes and the a-SMA marker for fibroblasts.

DISCLOSURES

The study product and the funds to perform the research were provided by Biopelle located in Ferndale, Michigan.

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