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Research Article

The Effects of L-ergothioneine on Skin Hydration, Elasticity, Brightening, Anti-carbonylation and Anti-glycation: A Single Center, Open Label Pilot Study

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Abstract

The increased population of Z-generation who are strikingly willing to spend on skin caring products with solid scientific support has created a tremendous market of beauty from within products. The high popularity and acceptance of L-ergothioneine in the cosmetics market as well as the general awareness of advantages of orally supplemented functional ingredients over topical applications drove us to hypothesize the skin benefits conferred by orally supplemented L-ergothioneine. In our study, we conducted a single-center, open label pilot study investigating the effects of L-ergothioneine (standardized to 1% in Citrine pleurotus extract) on the improvement of skin conditions. We observed the facial skin conditions were significantly improved regarding skin hydration, trans-epidermal water loss, elasticity, wrinkle areas and anti-carbonylation after 30 healthy subjects were orally administered Citrine pleurotus extract for 28 consecutive days. Besides, no adverse events were reported during the trial. The anti-aging efficacies of L-ergothioneine provide us valuable and critical information that in the future functional ingredients traditionally used in cosmetics can be considered as oral supplements with the advantages of higher bioavailability and broader functionality.

Introduction

Human skin is considered to be one of the most important body organs, protecting the body against external jeopardizing factors and maintaining body homeostasis [1]. However, facial skin loss, most easily noticed as facial morphological modifications, might occur at an average rate of about 7% per decade due to internal genetic causes and external environmental exposures [2]. The loss of extracellular matrix (ECM) can be another significant attributor for facial aging. External factors such as inappropriate environmental exposures can be detrimental and cause acute and chronic skin damages [3]. Ultraviolet radiation B (UVB) from sun exposure is absorbed by DNA in the epidermis, directly damaging DNA structures and causing sunburn clinically characterized by redness [4]. Ultraviolet radiation A (UVA) penetrates deeper into dermis and activates melanin deposition and skin pigmentation [4]. The cross-link of the skin tissues caused by internal glycation from reducing sugars and collagen fibers also critically contributes to facial aging [5]. The inevitability of facial aging and its anxiety effects on female individuals enhance their eager willingness to purchase beauty related products such as facial essence. The market of beauty from within products has been attracting a great many loyal consumers, resulting from solid scientific demonstrations regarding their advantageous and positive effects on the improvement of skin conditions. For instance, a human trial showed orally supplemented collagen peptides derived

from fish induced the skin fibroblast to produce extracellular matrix materials characterized by intensified water retention and elasticity recovery [6]. L-ergothioneine is a well-educated ingredient in cosmetics due to its excellent anti-oxidizing and powerful cytoprotective capacity [7]. A clinical study demonstrated the mitigation effect of L-ergothioneine on telomere shortening under oxidative stress conditions [8]. However, there is little literature information regarding the benefits of orally administered L-ergothioneine on the improvement of skin conditions from any clinical trials. In our study, we aimed to demonstrate the efficacies of orally supplemented L-ergothioneine for 28 consecutive days on the improvement of skin conditions including elasticity, skin hydration, anti-glycation and anti-carbonylation by a single center, open label pilot study involving 30 healthy subjects.

Materials and methods

Intervention design

This study was a single center, open label, pilot study in which 30 healthy subjects (from 30 to 50 years old, mean age of 38.8 years) were involved for the investigation of orally supplementation of L-ergothioneine for 28 consecutive days on the improvement of skin conditions regarding the skin hydration, elasticity, brightening, anti-carbonylation and anti-glycation effects. During the 28-day intervention period, all testing subjects were asked to maintain their usual diets, exercise and life patterns without any significant fluctuations. No supplements or functional food related to skin improvement (e.g., collagen peptides) were allowed to be taken beyond the testing supplement. Also, all the testing subjects were required to report their compliance on a daily basis. Any adverse events were required to be reported to the professional physicians instantly. Efficacy measures were conducted at day 0, day 14 and day 28 during the intervention period.

Test supplement

In our study, natural L-ergothioneine of the Citrine pleurotus extract was produced by pure water extraction, and its functional ingredient of L-ergothioneine was standardized to 1%. The testing supplement was prepared by Shanghai Powell R&D team in a cleanroom of 100K magnitude. The testing supplement was made in a sachet of stick powders in which Citrine pleurotus extract rich in ergothioneine (standardized as 1%) and fillers were included.

Participants

All the subjects received paper informed consent in accordance with regulations, explaining the nature, purpose and the potential risks of participating in the study. All the subjects could withdraw from the study at any time for any reason. They had sufficient time to consider before signing all informed consent signatures prior to the start of the study.

Inclusion criteria also include:

a. had wrinkles around the eyes

b. agreed not to use any cosmetics, drugs and health products that may affect the results.

Exclusion criteria were as follows:

a) those who used antihistamines in the past week or tried immunosuppressants in the past month

b) any anti-inflammatory drug was applied to the test site in the past two months

c) those who suffered from skin diseases (such as psoriasis, eczema, psoriasis, skin cancer, etc.)

d) insulin dependent diabetes patients

e) asthma or other chronic respiratory diseases patients who were under treatment

f) those who received anti-cancer chemotherapy in the past 6 months

g) patients with immune deficiency or autoimmune diseases

h) lactating or pregnant women

i) bilateral mastectomy and bilateral axillary lymph node resection

j) scar, pigment, atrophy, fresh erythema nevus, uneven skin color, folliculitis or other defects on the skin, which affected the judgment of the test result

k) applied vitamin A, α- Hydroxy acid, salicylic acid, hydroquinone in recent 3 months, or prescription drugs (antibiotics, vitamin A α- Hydroxy acids and steroids).

Efficacy outcome measures

For each test, the subjects wiped their skin with dry tissue paper, sat in a room with a temperature of 21 ± 1 °C and a humidity of 50 ± 10% for 30 minutes before professional physicians conducted these measures. The efficacies of the skin hydration, elasticity, brightening, anti-carbonylation, and anti-glycation were evaluated based on data analysis tested in the eye area and the face in combination with the pictures.

Measurement of elasticity

The elasticity was measure by the Cutometer® that was based on the suction method, where negative pressure deformed the skin mechanically. The resistance of the skin to the negative pressure (firmness) and its ability to return into its original position (elasticity) were displayed as curves (penetration depth in mm/time) during the measurement, which were used to calculate the parameters related to elastic and viscoelastic properties of skin surface.

Measurement of hydration at skin surface

The hydration level of the skin surface (stratum corneum) was characterized by Corneometer® CM825 that allowed very quick measurement (1 s) and avoided any occlusion. Substances on the skin such as salts or residues of topical applied products only had minimal impact due to capacitance measurement. Larger value reflected higher extent of hydration at skin surface.

Trans-epidermal Water Loss and Skin Barrier Function

Trans-epidermal water loss was characterized by the Tewameter® TM 300 with its "open chamber" principle. This is an indispensable parameter for the evaluation of the water

barrier function of the skin and a basic measurement in all kinds of applications. Even the slightest damage in the skin water barrier can be determined at an early stage.

Measurement of facial images

Multi-modality facial images of the subject's frontal, left-side, and right-side views were captured at day 0, day 14, and day 28 using the VISIA-CR. The VISIA-CR analysis provided the wrinkle areas and their proportions.

Measurement of melanin

The Mexameter® MX 18 (Courage+Khazaka electronic GmbH), a very easy, quick and economical tool, was applied to measure melanin index from the intensity of absorbed and reflected light at specific wavelength as recommended. Larger value reflects higher melanin in the skin.

Measurement of skin gloss

The overall pigmentation or skin gloss was determined by a system called ITA° (individual topology angle). By using the method of the L*, a*, b* color space, it was possible to calculate the so-called ITA°. L* stood for lightness (100 corresponds with white and 0 with black), a* denoted red-green coloration and b* denoted blue-yellow coloration. The lighter the skin, the higher the ITA°. The exact ITA° was determined by colorimeter CL400.

Measurement of anti-carbonylation and anti-glycation

The cuticle samples from the testing subjects were collected with cuticle sampling tape. Then DSquared films with cuticle sample were immersed into 40 μ Mol/L fluorescein. After fluorescence staining and PBS solution washing, the film was observed in a fluorescence microscope and pictures from three random fields were taken (magnification: 100; FTZ excitation wavelength: 492 nm, incident wavelength: 516 nm). Image Pro Plus 7.0 software was used to calculate its average fluorescence intensity, which represented the protein carbonylation level. The higher the fluorescence intensity, the higher the degree of skin carbonylation is. Commercially available ELISA kit was used to detect CML (a non-fluorescent and non-crosslinking AGEs produced by lysine with GO (glyoxal) as a glycation intermediate to reflect the skin glycation. The higher the CML content, the more serious the skin glycation is.

Statistical analysis

Statistical analysis software was used for statistical analysis of data. The measurement data was expressed as mean ± standard deviation, and the normal distribution test was carried out. If the data was normally distributed, the t-test method was used to analyze the changes of the skin data before and after the use of the test supplement, and the conclusion of whether the test supplement was effective was made according to the above statistical analysis. The two-sided test and test level α= 0.05。

Results

The Effects orally administered L-ergothioneine on the improvement of skin conditions are shown in Table 1. After 14 days of oral administration of the test supplement, the moisture content of the stratum corneum significantly increased by 8.57% (p=0.046). Trans-epidermal water loss decreased by 7.82% while skin elasticity R2 increased by 1.23% compared to that of the baseline, but there were no significant differences (p=0.461 and p=0.827 respectively). After 28 days of oral administration, the moisture content of the stratum corneum significantly increased by 9.71% (p=0.026) while the trans-epidermal water loss significantly decreased by 11.94% (p=0.038), compared to those of the baseline. Skin elasticity R2 significantly increased by 6.35% (p=0.042). The skin gloss ITAo at day 14 and day 28 increased by 1.81% and 3.70% respectively compared with those of the baseline (p=0.66 and 0.532 respectively). Skin melanin at day 14 and day 28 decreased by 5.44% and 4.76% respectively (p=0.492 and 0.367 respectively). The content of CML in skin decreased by 0.34% and 1.50% respectively after 14 and 28 days, but there were no significant differences (p=0.984 and p=0.932 respectively). There are significant improvements of skin wrinkles after 28 days supplementation. After 14 days, the area of skin wrinkles significantly decreased by 16.27% (p=0.044) while the proportion of skin wrinkle area significantly decreased by 20.53% (p=0.033) (Figures 1 & 2). After 28 days, the area of skin wrinkles significantly decreased by 22.65% (p=0.008), and the proportion of skin wrinkle area significantly decreased by 27.96% (p=0.027) (Figures 1 & 2). The VISIA-CR showed the significantly reduced fine lines around eye corners (Figure 3). After 14 days of oral administration of the test product, skin carbonylation decreased by 20.39% (p=0.009) (Figure 4). After 28 days of oral administration, skin carbonylation decreased by 29.23% compared with that of the baseline (p=0.003) (Figure 4).

Table 1: Effects of orally administered L-ergothioneine on the improvement of skin conditions at different time.

Skin Conditions	Day 0	Day 14	Day 28
Skin Hydration	53.62±8.91	58.22±7.16*	58.82±9.05*
Trans-Epidermal Water Loss	15.07±4.44	13.89±3.46 n.s.	13.27±3.29*
Skin Elasticity R2	0.57±0.07	0.57±0.07 n.s.	0.60±0.07*
Wrinkle Area	8.79±5.83	7.36±5.40*	6.80±4.90**
Wrinkle Area Proportion	9.45±6.27	7.51±4.89*	6.81±4.54*
The Skin Gloss It A°	39.60±6.43	40.32±8.88 n.s.	41.07±10.98 n.s.
Skin Melanin	167.17±34.27	158.07±45.86 n.s.	159.20±39.89 n.s.

Skin Carbonylation	11.29±4.67	8.99±5.43**	7.99±3.56**
Skin Glycation	11.36±4.52	11.33±5.03 n.s.	11.19±5.73 n.s.

n.s. denotes not significant

*Denotes significant differences (0.01<p<0.05)

**Denotes significant differences with p<0.01

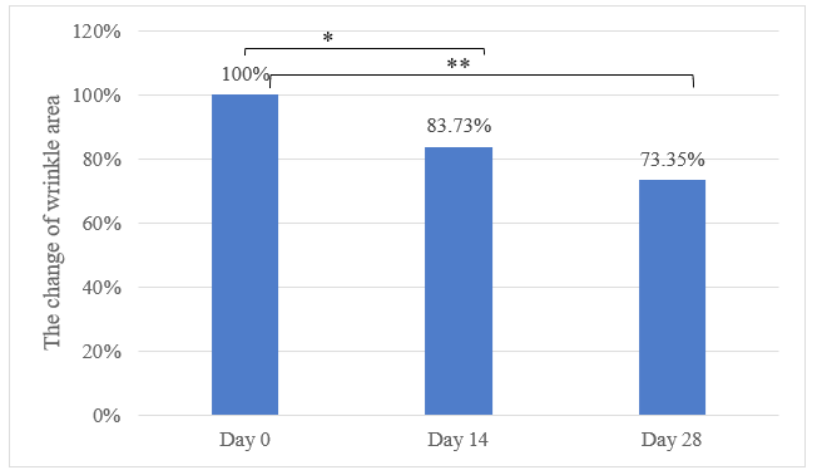

Figure 1: Effect of L-ergothioneine on the wrinkled areas at different time.

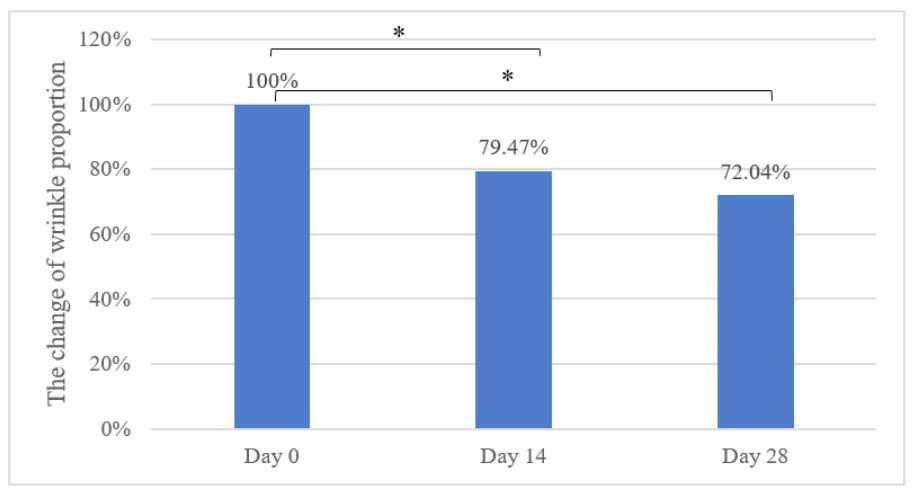

Figure 2: Effect of L-ergothioneine on the wrinkle area proportions at different time.

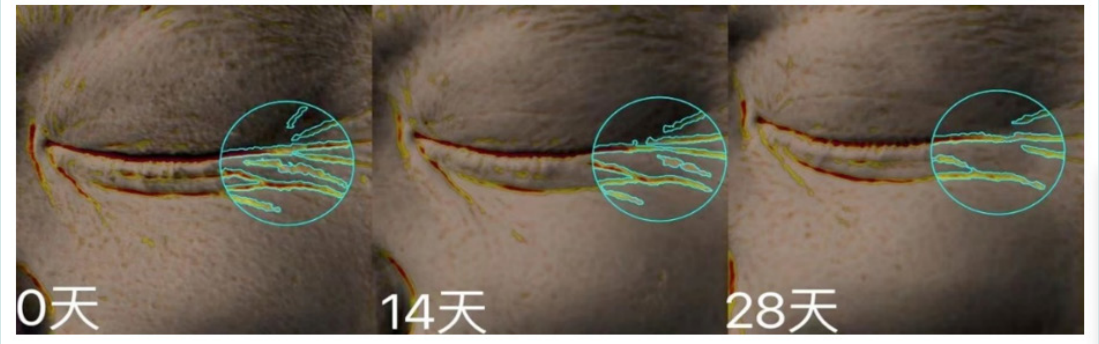

Figure 3: Effect of L-ergothioneine on the wrinkles by VISIA-CR at different time.

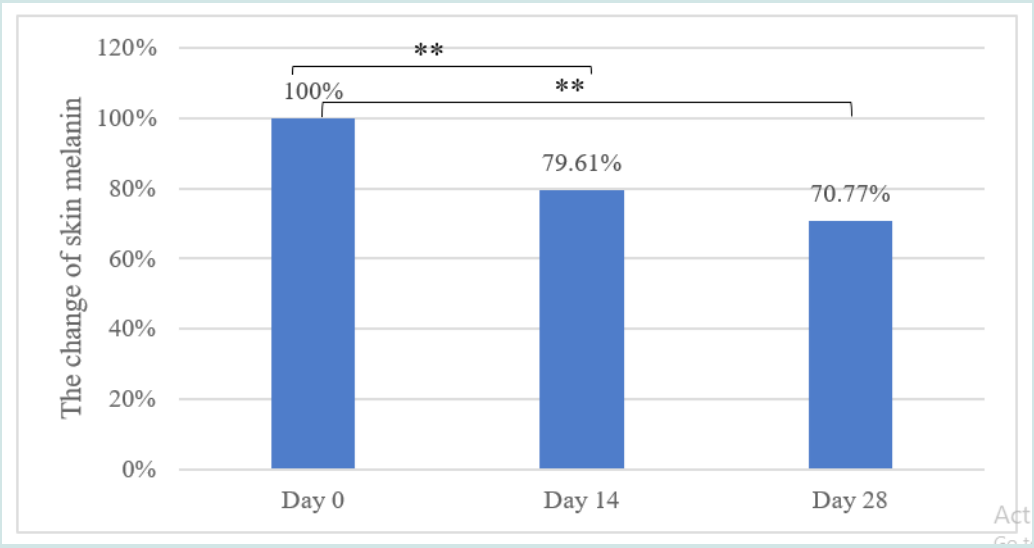

Figure 4: Effect of L-ergothioneine on the anti-carbonylation at different time.

Discussion

The market of facial skin care has been expanding rapidly due to the increased population of Z-generation who are not sensitive to prices of anti-aging products that have firm scientific evidence. Beauty from within products are being recognized by their advantages over topical cosmetics from the perspectives of absorption, bioavailability, and functionality. For instance, orally supplementation of trans-resveratrol conferred a lot more benefits compared to topical applied trans-resveratrol that was significantly compromised by its unfavorable transformation into cis-resveratrol [9,10]. A non-negligible reason for facial skin aging lies in the sun exposure of the facial skin. Ultraviolet A (UVA), which comprises 90-95% of the ambient sunlight and has a wavelength ranging from 315 – 400 nm, can penetrate from epidermis to dermis and generate huge amount of reactive oxygen species (ROS) [11]. Reactive oxygen species activate signaling pathways for the production of metalloproteinases that are responsible for the degradation of extracellular matrix including collagen, elastin fibers, proteoglycans and consequently death of the skin fibroblast cells [12]. On the other hand, they indirectly damaged DNA and cellular structures, which activates the melanin production by melanocytes and its deposition in keratinocytes to prevent skin cells from further injury [13]. The UVA related oxidative stress is also one of the risk factors for skin cancer due to a cascade of inflammatory cytokines through NF-kB pathways activations and the immunosuppressive effects. Thus, scavenging ROS is considered a priority and strikingly efficient for the protection of skin photoaging. In our study, we investigated the effects of orally administered L-ergothioneine on the improvement of skin conditions in a single center, open label pilot study. It was found the skin conditions including skin hydration, trans-epidermal water loss, wrinkle reduction and carbonylation were significantly improved. The beneficial effects should be mostly attributed to the powerful anti-oxidizing capability of L-ergothioneine. There was a decreasing trend of oxidative damage biomarkers when L-ergothioneine was orally administered to healthy human subjects, which was consistent with animal studies [14]. Also, it was important to note that L-ergothioneine plays a major role as an antioxidant perhaps only under conditions of oxidative stress, as a certain level of oxidative species could generate health benefits [15]. Compared to other antioxidants such as Vitamins, the retention of L-ergothioneine is high from the perspectives of bioavailability, half-life and urinary excretion [16]. Telomeres, providing dynamic protection and stability of the ends of chromosomes, finally become critically short that cells stop dividing. This telomere senescence can be repaired by an enzyme called telomerase that can keep the length of telomeres by adding DNA sequences to its ends [17]. Internally, facial skin aging lies in the loss of skin cells caused by the slow-down of cell division because of telomere loss [18]. Ergothioneine could decrease the rate of telomere shortening and preserving telomere length under oxidative stress conditions by transiently increasing relative telomerase activity after 24 h ($p<0.05$ for all concentrations) [8]. Therefore, we proposed the beneficial effects of L-ergothioneine also came from its activation capability of telomerase that potentially stimulated the skin cell division.

Carbonylated proteins (CPs) are synthesized by reactions between amino groups and reactive aldehyde compounds (RAC) initiated by reactive oxygen species (ROS) from even slight UV radiation and increase the intracellular ROS levels and the synthesis of intracellular CPs, which sets up a viscous circle that finally contributes to alterations of the dermal matrix [19]. CPs are detected in a higher frequency at sun-exposed sites of the skin especially in elderly subjects [20]. Our finding of reduction of CPs by orally administered L-ergothioneine was consistent with other research findings in which antioxidants as α-tocopherol and β-carotene suppressed the synthesis of RAC during lipid peroxidation which resulted in the reduction of UVA-induced CPs in the stratum corneum [19]. The correlation between the production of advanced

glycation end products (AGE-products) and skin aging should also be highly appreciated for facial anti-aging. However, in our study we did not observe significant inhibition effects of L-ergothioneine on anti-glycation. As the Maillard reaction commonly known as glycation involves a cascade of complex reactions, the information of whether L-ergothioneine could inhibit any of the involved steps remains further investigation. In our study, no adverse event such as vomiting, diarrhea or any chronic uncomfortable feelings were reported. Its safety was also evaluated and corroborated by the European Food Safety Authority [21]. We still have some limitations. First of all, the experimentation design is not a randomized, double-blinded, placebo-controlled trial, which is stricter and more scientific for the demonstration of the L-ergothioneine's effects for the improvement of skin conditions. Also, we did not have stricantioxidants such diet patterns that might contain high level of antioxidants as L-ergothioneine enriched mushrooms. The extent of sun exposure and season were also crucial factors we should consider in the futuristic studies.

Conclusion

Based on our clinical research and investigation, we concluded that orally supplementation of L-ergothioneine for 28 consecutive days could improve skin conditions regarding skin hydration, trans-epidermal water loss, skin elasticity, wrinkle area and anti-carbonylation without any reported adverse effects. Due to the advantages of oral supplements over topical applications given their high bioavailability and their synergistic effects combing with cosmetics, more traditionally used cosmetic ingredients should be further evaluated for their efficacies and safety for the benefits of facial skin anti-aging.

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