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Collagen Formula Contained Hydrolyzed Fish Collagen, Pitaya, Rosa Rugosa, and H. Pluvialis, Gamma Aminobutyric Acid Improved Skin and Sleep Condition

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ABSTRACT

The combination of Chinese herbal medicine and fish collagen has the function of skin anti-aging. However, there are few clinical studies on whether the health care products composed of hydrolyzed fish collagen, pitaya, rosa rugosa, and H. pluvialis, gamma aminobutyric acid have skin anti-aging effects. This study used LISAVEI collagen beverage composed of fish collagen, pitaya, rosa rugosa, H. pluvialis and GABA, to explore whether collagen beverage can improve skin and sleep condition. The study recruited 50 subjects and divided into a placebo group (n = 30) and a LISAVEI collagen beverage group (n = 30) for 8 weeks and then examined skin and sleep condition at 0, 4, 8 weeks. The results showed that collagen, elasticity, brightness, moisture significantly increased by 5.9%, 1.8%, 2.2%, 3.7% respectively, compared to placebo group, and pores, melanin, redness decreased by 8.1%, 4.4 %, 2.5 % respectively, compared to the placebo group. And the subjects felt that the overall skin and sleep condition improved by questionnaire. LISAVEI collagen beverage rich in hydrolyzed fish collagen, pitaya, rosa rugosa, H. pluvialis and GABA can improve skin and sleep.

Keywords: Hydrolyzed Fish Collagen; Pitaya Extract; Rosa Rugosa; Haematococcus Pluvialis; Gamma Aminobutyric Acid; Skin

Introduction

Aging is a global trend, and the most obvious change after aging is the change of skin appearance (Clatici, et al. [1]). Therefore, active ingredients that delay skin aging are particularly attractive. Nowadays, many products on the market focus on natural ingredients, and the demand for natural ingredients is increasing day by day. Traditional Chinese herbal medicines are mainly made from natural plants (Goyal, et al. [2]). Chinese herbal medicines and natural animal products have the effect of treating skin diseases, sun protection, and enhancing skin nutrition (Pu, et al. [3]). Therefore, now it's mainstream in the market that the addition of Chinese herbal extracts into health foods and cosmetics. Skin appearance depends on the collagen skeleton, for instance, wrinkle formation has been associated to decreased collagen synthesis and increased collagenase activity (Sanchez, et al. [4]). Some approaches to prevent or retard the apparition of wrinkles in humans are to use cosmetics or to intake nutritional supplements which help to maintain collagen molecules in the skin at optimum (Sanchez, et al. [4]). Some of these products include, as an active ingredient, collagen or hydrolyzed collagen. Traditional sources of collagen for cosmetics are skins and hides from pigs and cows (Leon Lopez, et al. [5]). However, nowadays, collagen from marine origin is preferred, since it is free from animal species, causing religious concerns (Lim, et al. [6]). Fish collagen is more easily absorbed than porcine collagen, has a low molecular weight, and is preferable to the industry due to low inflammatory reactions. Also, type I collagen is abundant in marine organisms (Lim, et al. [6]). Pitaya belongs to the plant of triangular prism cactus genus, with high nutritional ingredients are rich in polysaccharide composition (Luu, et al. [7]). In the stem of Pitaya, it has many health benefits to human skin by using of extraction and manufacturing, which are widely used in various healthy foods for cosmetic products, not only anthocyanins, flavonoids, polyphenols but also other ingredients, they may provide good the effect on the health for human physiology (Anand Swarup, et al. [8]).

Rosa rugosa, a member of Rosaceae, which is widely planted all over the world and distributed in many places in China. R. rugosa is often used in the field of food and medicine (Xie, et al. [9]). Its petals and buds are often used to make flower tea, jam, fruit wine or other food. Some studies reported that R. rugosa application in cosmetics has the effects of antioxidant, anti-aging, whitening, moisturizing. R. rugosa contains a lot of flavonoids, polyphenols, polysaccharides and other components, suggesting R. rugosa as a potential anti-aging and anti-aging plant (Andrzej Cendrowski, et al. [10]). Haematococcus pluvialis is a freshwater species of Chlorophyta from the family Haematococcaceae (Suseela, et al. [11]). Astaxanthin is one kind of carotenoid, and it is the strongest antioxidant activity among the natural materials (Suseela, et al. [11]). H. pluvialis has the highest astaxanthin content, which is important in aquaculture, and cosmetics (Pertiwi, et al. [12]). H. pluvialis has high antioxidant activity, can remove free radicals, stimulate immune response, and has anti-cancer effects, which has high medical value (Pertiwi, et al. [12]). In addition, γ-aminobutyric acid (GABA) was discovered in the extract of mammalian brain and identified to be an inhibitory neurotransmitter (Smart, et al. [13]). GABA works not only as a mediator in the neuronal system but is also involved in the skin (Galanopoulou [14]). The study had showed that GABA stimulated the synthesis of hvaluronic acid (HA) and enhanced the survival rate of the dermal fibroblasts when fibroblasts were exposed to H2O2, an oxidative stress agent (Ito, et al. [15]). Clinical study showed that GABA can increase skin elasticity and improve sleep (Hokazono, et al. [16]). However, there was

still not much clinical studies on hydrolyzed fish collagen, pitaya, rosa rugosa, H. pluvialis and GABA for skin. In this study, we used LISAVEI collagen beverage, formulated primarily with hydrolyzed fish collagen, pitaya, rosa rugosa, H. pluvialis and GABA, to explore whether LISAVEI collagen beverage can improve skin condition. The study recruited 50 subjects and divided into a placebo group (n = 30) and a LISAVEI collagen beverage group (n = 30) for 8 weeks and then examined skin and sleep condition at 0, 4, 8 weeks.

Methods

Clinical Trial Design

The clinical study had been approved by Human Trial Committee of Antai Hospital (TSMH-IRB 21-102-B), and the study had been registered on ClinicalTrials.gov Identifier: NCT05182814. Sixty adult subjects were recruited in this trial between August 2021 and May 2022. Informed consent was obtained from all subjects before the study at Chia Nan University of Pharmacy and Science. The subjects were divided into a placebo group (n=30) and a collagen beverage group (n=30). Each subject was informed about intaking a bottle of collagen beverage labeled 50ml or a placebo drink daily for 8 weeks and was not allowed to take any other supplement during the intervention period. Inclusion criteria included: healthy men and women aged > 20. The exclusion criteria included:

- 1. Skin disease, liver cirrhosis, or chronic renal failure
- 2. Allergy to cosmetics, drugs, or foods
- 3. Pregnant and breastfeeding
- 4. Taking chronic drugs

5. People who had any cosmetic procedures (intense pulse light, medical peelings, or laser therapy) before 4 weeks of the study.

Test Sample

LISAVEI collagen beverage contains 10% hydrolyzed fish collagen, 4% pitaya, 0.4% gamma aminobutyric acid, 0.02% rosa rugosa, and 0.0002% H. pluvialis, sucralose, citric acid, water. Placebo beverage contains sucralose, citric acid, and water.

Clinical Skin Efficacy Assessment

Skin brightness was measured using a skin color difference analyzer (Chroma Meter MM500, Minolta, Japan). The standard colorimetric method formulated by the CIE (Commission Internationale de L'Eclariage) system is used to obtain the quantification of the color L* value. L* value - the value range is: 0-100, which is a gray scale, and the higher the value, the brighter it is. Detection position: the upper cheek. Skin redness was detected using a skin color difference analyzer (Chroma Meter MM500, Minolta, Japan). The standard colorimetric method developed by the CIE (Commission Internationale de L'Eclariage) system is used to obtain the quantitative a* value of the color. The higher the a* value, the more red and inflamed the skin. Detection position: the upper cheek. Skin melanin was detected using the Schute skin tester (Soft Plus, Callegari 1930, Italy). Skin red and melanin levels were analyzed using dual wavelength measurement 505 nm green light and 875 nm infrared light. Detection position: the upper cheek. Skin moisture was measured using a skin moisture meter Corneometer CM825, CK, Germany. Based on the amperometric method, the skin's moisturizing ability is tested. Detection position: the upper cheek. Skin elasticity was measured using Cutometer MPA580, CK, Germany. Using the principle of negative pressure suction, the performance of skin elasticity is detected, and the restoring force is measured through the different depths of light penetrating the skin and the resistance caused by the skin being inhaled by negative pressure. Detection position: the upper cheek. Skin transepidermal water loss was measured using a transepidermal water loss meter (Tewameter TM300, CK, Germany). The structural integrity of the stratum corneum of the skin is inferred from the evapotranspiration of the skin. Detection position: the upper cheek. Skin wrinkles were detected using VISIA Micro-Analysis Skin Image Analyzer (VISIATM Complexion Analysis, U.S.A). Closed face photo studio (unified light source), and 36-million-pixel photo images, analyze and compare image data. Using standard white light to detect changes in skin shade, quantify the distribution and number of skin wrinkles. Dark green represents deeper wrinkles, light green is the opposite. Detection position: full face. Skin texture was detected using VISIA Micro-Analysis Skin Image Analyzer (VISIATM Complexion Analysis, U.S.A). Closed face photo studio (unified light source), and 36-million-pixel camera images, analysis and comparison of image data. Measure skin smoothness using standard white light to detect changes in skin shading. The raised parts of the skin surface are shown in yellow, the concave parts are shown in blue, and the less yellow and blue, the smoother the skin surface. Detection position: full face.Skin pores were detected using VISIA Micro-Analysis Skin Image Analyzer (VISIATM Complexion Analysis, U.S.A). Closed face studio (unified light source). Use standard white light and 36 million pixels to take pictures, analyze and compare image data. Use standard white light to detect the shadows produced by the sunken skin pores to evaluate the number of pores and the location of their subdivisions. Detection position: full face. Skin collagen

was detected using a subcutaneous collagen scanner (DermaLab® Series SkinLab Combo, Cortex Denmark. High-frequency ultrasound imaging instrument was used to scan subcutaneous collagen to quantify collagen density. Detection location: on the upper cheek.

Clinical Sleep Assessment

Measures of sleep disturbances included self-reported insomnia symptoms and sleep duration. Information about perceived symptoms of insomnia was obtained by self-report using the Insomnia Self-assessment Inventory (ISAI) questionnaire, which was designed by the World Health Organization worldwide project on sleep and health. Each question was scored with a 4-point (0–4) Likert-type scale. For the sleep status of the subjects before and after taking the product, the ISAI was used to investigate. The total score of 5-9 points was judged as mild sleep disorder, and the total score of more than 10 points was sleep disorder.

Statistical Analysis

Before and after comparison within the group was performed by Student's t test. *, p 0.05 indicates a statistically significant difference. Values are percent change compared to the control group.

Results

LISAVEI Collagen Beverage Increased Skin Collagen, Elasticity, Brightness, Moisture

After the subjects took collagen beverage for 8 weeks, the skin collagen density significantly increased by 10.4% compared to baseline (week 0); the collagen density significantly increased by 5.9% compared to placebo group (Figure 1A). Skin elasticity significantly increased by 3.7% compared to baseline (week 0); skin elasticity significantly increased by 1.8% compared to placebo group (Figure 1B). Skin brightness significantly increased by 2.6% compared to baseline (week 0); skin brightness significantly increased by 2.6% compared to baseline (week 0); skin brightness significantly increased by 2.2% compared to placebo group (Figure 1C). Skin moisture significantly increased by 4.5% compared to baseline (week 0); skin moisture significantly increased by 3.7% compared to the placebo group (Figure 1D). These results indicated that collagen beverages included hydrolyzed fish collagen, pitaya, rosa rugosa, H. pluvialis and GABA can increase collagen density, elasticity, brightness, moisture in the skin.

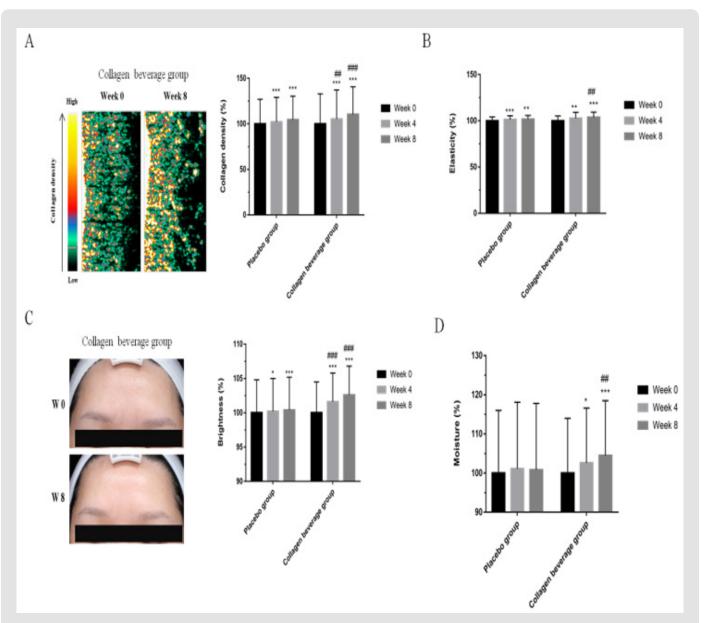


Figure 1: LISAVEI collagen beverage increased skin collagen, elasticity, brightness, moisture. 60 subjects were recruited and divided into 2 groups, one was the placebo group (n=30) and the other was the collagen beverage group (n=30). Take one bottle of beverage daily for 8 weeks, and then examined

- A. Collagen
- B. Elasticity
- C. Brightness
- D. Moisture

Error bars represent \pm standard deviation. Significantly different from baseline (week 0): *, p < 0.05; **, p < 0.05; **, p < 0.001; Significantly different from placebo: #, p < 0.05; ##, p < 0.01; ###, p < 0.001).

LISAVEI Collagen Beverage Decreased Skin Pores, Melanin, Redness

After drinking collagen beverage for 8 weeks, the skin pores decreased by 7.0% compared to baseline (week 0); the skin pores decreased by 8.1% compared to the placebo group (Figure 2A). Skin melanin significantly decreased by 4.7% compared to baseline

(week 0); skin melanin significantly decreased by 4.4 % compared to the placebo group (Figure 2B). Skin redness significantly decreased by 4% compared to baseline (week 0); skin redness significantly decreased by 2.5 % compared to the placebo group (Figure 2C). These results indicated that collagen beverage included hydrolyzed fish collagen, pitaya, rosa rugosa, H. pluvialis and GABA can decrease skin pores, melanin, redness.

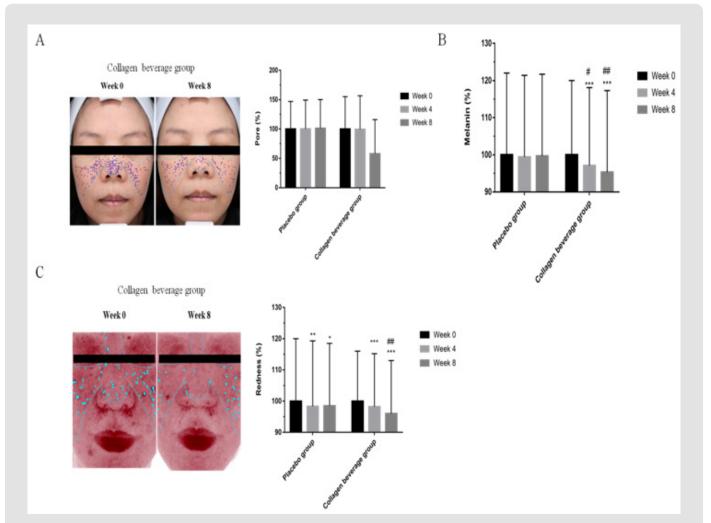


Figure 2: LISAVEI collagen beverage increased skin collagen, elasticity, brightness, moisture. 60 subjects were recruited and divided into 2 groups, one was the placebo group (n=30) and the other was the collagen beverage group (n=30). Take one bottle of beverage daily for 8 weeks, and then examined

- A. Pores
- B. Melanin
- C. Redness

Error bars represent \pm standard deviation. Significantly different from baseline (week 0): *, p < 0.05; **, p < 0.05; **, p < 0.001; Significantly different from placebo: #, p < 0.05; ##, p < 0.01; ###, p < 0.001).

LISAVEI Collagen Beverage Improved Skin and Sleep Condition by Self-Assessment Questionnaire

A self-assessment questionnaire was conducted on the skin condition of the subjects before and after taking the product. The results showed that after drinking collagen beverage for 8 weeks, the subjects felt that the overall skin condition improved (Figure 3A). In addition, the ISAI was used to investigate the sleep part. The results showed that after drinking collagen beverage for 8 weeks, the average ISAI of the subjects was significantly decreased from 9.4 points to 6.7 points (Figure 3B), and subjects felt sleep quality improved (Figure 3C).

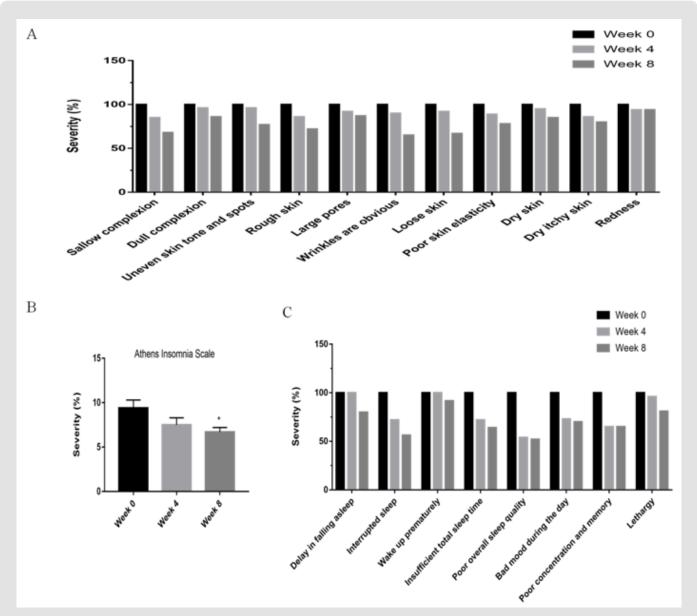


Figure 3: LISAVEI collagen beverage improved skin and sleep.

The subjects consumed the collagen beverage for 8 weeks. Then the questionnaire was filled to survey the

- A. Skin severity condition
- B. Insomnia self-assessment inventory
- C. Sleep severity condition

Discussion

This clinical study showed that LISAVEI collagen beverage rich in hydrolyzed fish collagen, pitaya, rosa rugosa, H. pluvialis and GABA can improve skin and sleep overall. According to the results, after 8 weeks of taking collagen beverage, it can increase collagen density, elasticity, brightness, moisture, and decrease pores, melanin, redness in the skin. In addition, collagen beverages can also improve sleep. Hydrolyzed collagen is a natural protein that is the main substance in animals and is abundant in skin, cartilage and bone (Leon Lopez, et al. [5]). Among them, type I and type III collagens are the most abundant collagens in the skin (X Liu, et al. [17]). Hydrolyzed collagen is a natural protein with perfect biocompatibility and is widely used as medical and health care products (Wang [18]). Studies have shown that hydrolyzed fish collagen can increase skin collagen and elastin, and is rich in glycosaminoglycans and various proteins, which can inhibit the formation of oxidative stress in skin fibroblasts and increase mitochondrial activity (Edgar, et al. [19]). Pitaya is rich in bioactive substances such as betacyanins, phenolic compounds, polysaccharides, and terpenes, which can be used to prevent skin diseases and other inflammatory metabolic diseases (Huang, et al. [20]). Studies had showed that pitaya is rich in vitamin C, which can promote the production of new collagen in fibroblasts, reduce wrinkles, fight free radicals that cause cell damage, and reduce melanin deposition (Vijayakumar, et al. [21]). The vitamin A in pitaya also promoted the skin cells growth, increasing skin elasticity, brightness and moisture (Guimaraes, et al. [22]). Rosa rugosa can effectively scavenge free radicals. The high antioxidant activity of rose extract increased skin moisture and decreased dark spots, redness (A Cendrowski, et al. [23]). Rosa rugosa is rich in vitamin C, carotenoids, polyphenols and various flavonoids with potent antioxidant activity (Czyzowska, et al. [24]). The seeds of rosa rugosa have been shown to contain high amounts of polyunsaturated fatty acids, known to be essential for the skin (Kulaitiene et al. [25]).

Rosa rugosa is rich in carotenoid pigments that inhibit the synthesis of MMP-1, a protein responsible for the breakdown of collagen and helps protect collagen and elastin (Winther, et al. [26]). After taking Rosa rugosa for 8 weeks, subjects can improve wrinkles, skin moisture and elasticity, mainly through improving the cell longevity and obstructing skin aging (Winther, et al. [26]). H. pluvialis promoted cell proliferation, collagen production and antioxidant properties (Chou et al. [27]). H. pluvialis, a ubiquitous green algae, had a high content of astaxanthin, which promoted regeneration of damaged skin (Chou, et al. [28]). Astaxanthin increased skin moisture, hydration, elasticity, as well as promoted skin smoothness and reduced wrinkles (Muzumdar, et al. [29]). Astaxanthin had been shown to increase blood flow, increase cell turnover, enhance elasticity and increase water retention (PH Liu, et al. [30]). After taking H. pluvialis for 8 weeks, it can

reduce skin wrinkles, spots, increase skin elasticity and texture (Tominaga, et al. [31]). It may suggest that astaxanthin derived from H. pluvialis can improve skin condition in all layers such as corneocyte layer, epidermis, basal layer and dermis by combining oral supplementation and topical treatment. GABA is the main inhibitory neurotransmitter in the central nervous system, and oral administration of GABA improves mental conditions such as stress, depression, and insomnia (Hepsomali, et al. [32]). Oral administration of GABA significantly increased the skin elasticity in human study (Hokazono, et al. [16]). GABA stimulated the synthesis of hyaluronic acid and enhanced the survival rate against an oxidative stress in dermal fibroblasts (Uehara, et al. [33]). GABA upregulated the expression of type I collagen and downregulated the MMP-1 expression on human dermal fibroblasts (Uehara, et al. [33]). GABA produced in fermented foods may increase sleep time and decrease sleep onset time. Another recent study showed that the combination of GABA and 5-HTP can work together to improve sleep quality and increase sleep duration (Hong, et al. [34]). Consistent with our results, LISAVEI collagen beverage can increase collagen density, elasticity, brightness, moisture, and decrease pores, melanin, redness in the skin, and improve sleep. This study was demonstrated the LISAVEI collagen beverage rich in hydrolyzed fish collagen, pitaya, rosa rugosa, H. pluvialis and GABA can improve skin and sleep. LISAVEI collagen beverage can be used as one of the health care products for skincare in the future.

Acknowledgment

None.

Conflict Of Interest

The authors declare no conflict of interest.

Data Availability Statement

Not applicable.

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