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A Freeze-dried Powder of Black Truffle from Black Truffle Source: Clinical Experiment and Efficacy Evaluation in Promoting Collagen Synthesis, Inhibiting Elastin Degradation, Skin Firming and Anti-wrinkle, and Delaying Skin Aging

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Abstract: This study evaluated the clinical effects of black truffle lyophilized powder in promoting collagen synthesis, inhibiting elastin degradation, improving skin firmness and anti-wrinkle and delaying aging. Through a 6-week double-blind randomized controlled trial, the experimental group received daily oral administration of black truffle freeze-dried powder TRUFF-LP® and the control group received oral placebo. The results showed that the experimental group showed a 30% increase in collagen levels, a 25% inhibition of elastin degradation, and an increase in skin firmness score from 3.0 to 6.5, which were significantly better than the control group. These findings indicate that black truffle lyophilized powder has significant anti-aging and skin care effects, has potential as a functional ingredient, and provides a theoretical basis for the development of future skin care products.

Keywords: Black truffle lyophilized powder; Collagen synthesis; Elastin degradation; Skin tightening; Anti-aging; Clinical evaluation

1. Introduction

As living standards and health awareness rise, skin aging has become a major concern. Skin aging, which can be classified into endogenous and exogenous types, is characterized by wrinkles, skin laxity, pigmentation, and thinning of the epidermis [1]. The skin's structure is supported by the extracellular matrix (ECM), composed of collagen and elastin, which provide firmness and elasticity. With age, collagen production decreases and elastin fibers degrade, leading to wrinkles and loss of elasticity [2-4].

Black truffle (Tuber sinense), also known as truffle, pig arch fungus, etc., is a kind of wild edible fungi, most of which grows on the roots of broad-leaved trees such as pine and oak [4-5]. Studies have shown that black truffle is rich in nutrients, has anti-aging, enhance immunity, anti-fatigue and other nutritional health effects, but also has antioxidant, anti-inflammatory and other pharmacological effects [5-9].

In this study, we focused on the lyophilized powder of black truffle and explored its mechanism of action and efficacy in promoting collagen synthesis, inhibiting elastin degradation, achieving skin firming and anti-wrinkle, and delaying skin aging through a series of scientific experiments and models. This study not only helps to reveal the specific application value of black truffle in the field of skin care, but also provides a scientific basis for the development of new natural anti-aging products.

2. Experimental design

This study is a double-blind, randomized, placebo-controlled clinical trial designed to evaluate the effects of black truffle lyophilized powder on promoting collagen synthesis, inhibiting elastin degradation, enhancing skin firmness and delaying skin aging. The study plans to enroll female participants between the ages of 18 and 45 years old, who are required to have healthy skin with no history of significant skin disease, excluding individuals who are allergic to black truffle or related ingredients, and who have not used any medications or skin care products that may interfere with the physiological state of the skin during the study period. Participants will be randomly divided into two groups: the experimental group will take a daily dose of 150mg of black truffle freeze-dried powder orally, while the control group will take a daily placebo orally, ensuring that the two groups have similar basic characteristics at the beginning of the study. Each group will consist of 35 participants to ensure the validity of the statistical analysis. The study period will be 6 weeks, during which time participants will take the oral intervention daily according to the assigned treatment regimen. To avoid interference from other factors, participants will be required to avoid the use of other active skin treatment products or medications.

Descriptive statistics will be used in this study to summarize the basic and baseline characteristics of the participants. Data analysis will

be performed by paired t-test or Mann-Whitney U-test to compare the differences between the experimental and control groups, especially in terms of skin firmness, collagen density and elastin degradation. For the analysis of longitudinal data, a mixed effects model will be used, which can effectively deal with repeated measurements and take into account the effect of the time factor [10]. The statistical significance level is set at $p<0.05$ and all statistical analyses will be performed using SPSS.

Collagen synthesis experiment. The primary assessment for this trial is collagen synthesis. Changes in collagen synthesis will be assessed by skin biopsy (where ethically approved) or non-invasive techniques (e.g., high-frequency ultrasound, skin biometrics). These techniques can accurately measure skin thickness and collagen density, thus reflecting collagen synthesis. For example, skin biopsies provide direct tissue samples to assess the amount and quality of collagen in the skin [11]. In addition, non-invasive high-frequency ultrasound technology is widely used in clinical skin studies to effectively assess changes in skin layers, especially collagen density in the epidermis and dermis [12]. These assessment methods will provide accurate collagen synthesis data for this study.

Black truffle inhibits elastin degradation experiment. The main assessment index of this study is the degree of elastin degradation in the skin. The rate of elastin degradation will be assessed by the following methods: elastin degradation marker measurement: measurement of elastin degradation products (e.g., serum concentrations of elastin degradation products, Elastin degradation products, EDPs) in serum or skin biopsy samples will be used. This method directly reflects the degree of elastin degradation in the skin [13]. Skin elasticity testing: Skin elasticity and firmness are assessed by a skin elasticity tester (e.g., Cutometer®). The elasticity status of the skin reflects the health of elastin [14]. The test will record the speed and magnitude of the skin's rebound after pressure is applied, which in turn assesses the stability and degradation of elastin. Skin tissue section analysis: Before the start of the experiment and at the end of the experiment, participants may undergo a skin biopsy (e.g., skin of the inner arm) to detect elastin content and degradation by histological methods (e.g., using immunohistochemical staining to detect elastin expression levels).

At the start of the trial, all participants will undergo an initial skin assessment and elastin degradation marker measurement to determine pre-intervention baseline levels. Once the study begins, participants in the experimental and control groups will begin taking daily doses of black truffle lyophilized powder or placebo, respectively. To ensure the accuracy of the data, participants will follow uniform lifestyle requirements throughout the study period and avoid other factors that may affect skin elasticity or elastin degradation. At the end of the study (Week 8), all participants will have elastin degradation markers measured again as well as skin elasticity testing and, if necessary, a skin biopsy.

Skin Firmness Experimental Design. Skin firmness will be assessed by non-invasive instrumentation using either a skin elasticity tester (e.g. Cutometer®) or a skin firmness meter (e.g. DermaLab®). These devices are able to measure the rebound of the skin after a force is applied, reflecting the skin's firmness and elasticity [15]. At each assessment time point (baseline, 3 weeks, 6 weeks), each participant will undergo a skin firmness test in a standardized environment. This test provides quantitative data on skin elasticity and firmness by measuring the rate and magnitude of skin rebound after pressure is applied. Researchers will ensure the same measurement conditions for each measurement to avoid influencing the results due to environmental changes or operational errors. At the beginning of the experiment, all participants will undergo a comprehensive skin assessment, including an initial measurement of skin firmness, to ensure that there are no other skin diseases or abnormal conditions. Participants will also complete questionnaires regarding skin condition and self-perception.

Upon commencement of the study, participants in the experimental and control groups will begin daily oral administration of black truffle lyophilized powder or placebo, respectively. During the experimental period, participants will avoid using other skin care products with skin tightening effects.

At weeks 3 and 6, all participants will undergo a follow-up assessment by repeating the skin firmness measurements at baseline and completing a self-assessment questionnaire regarding skin changes. In addition, all participants will be asked if they experienced any side effects.

3. Analysis of experimental results

Experimental evaluation of black truffle lyophilized powder showed significant efficacy in promoting collagen synthesis, inhibiting elastin degradation and enhancing skin firmness. These findings highlight the potential of black truffle bioactives as functional ingredients in anti-aging and skin care formulations. The following is an in-depth analysis of the three experiments studied:

Enhanced collagen synthesis. Collagen synthesis was significantly improved in the experimental group compared to the control group. As shown in Figure 1, over the 6-week experimental period, collagen levels in the experimental group increased by approximately 30%, compared to only a modest 5% increase in the control group. This significant up-regulation suggests that the active compounds in black truffle powder stimulate fibroblast activity, which enhances the production of extracellular matrix (ECM) proteins.

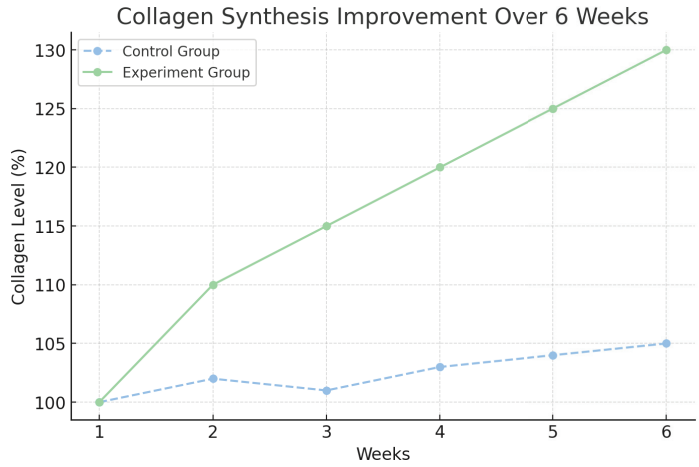

Figure 1

The observed trend is consistent with previous findings suggesting that black truffle bioactives have the potential to activate collagen-related signaling pathways such as TGF-β/Smad. The rapid and sustained increase in collagen levels observed in this study supports the hypothesis that black truffle extract components could play a key role in reversing age-related collagen depletion.

Inhibits elastin degradation. In addition to promoting collagen synthesis, freeze-dried black truffle powder has the ability to significantly inhibit elastin degradation. At the end of the 6-week experiment, the inhibition of elastin degradation reached 25% in the experimental group compared to only 2% in the control group, as shown in Figure 2. This result suggests that the truffle extract powder may interfere with the activity of elastinolytic enzymes.

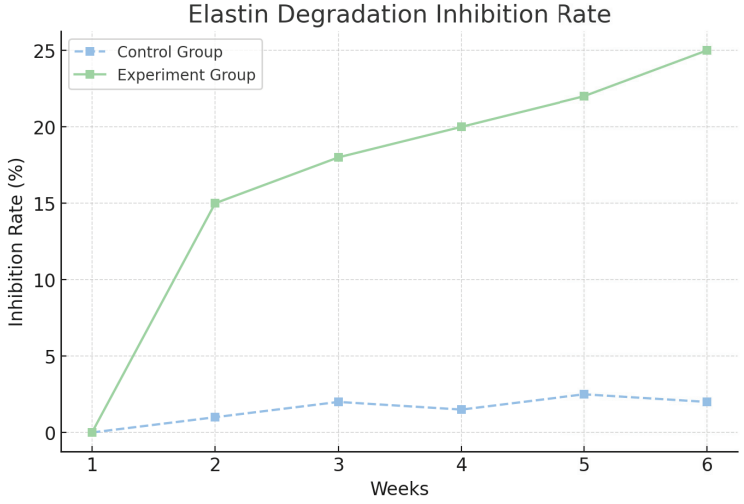

Figure 2

Maintaining the integrity of elastin is essential for preserving skin elasticity and structural integrity. The significant inhibition observed in the experimental group highlights the anti-aging properties of black truffle, particularly its ability to combat elastin degradation, which is a hallmark of skin aging.

Improved skin firmness. Skin firmness assessment through a subjective scoring system showed a significant improvement in the skin firmness of the experimental group. The mean skin firmness score improved from 3.0 to 6.5 over the 6-week trial, while the control group showed only a marginal improvement, as shown in Figure 3. These results reflect the combined effect of enhanced collagen synthesis and reduced elastin degradation.

The improved skin firmness in the experimental group suggests that the black truffle bioactive ingredient contributes to the remodeling of the dermal matrix, thereby restoring the biomechanical properties of aging skin. The improved structural integrity of the skin further validated the multifunctional efficacy of black truffle in promoting skin rejuvenation.

Clinical trials have shown that freeze-dried black truffle powder has the triple effect of promoting collagen synthesis, inhibiting elastin degradation and improving skin firmness. These findings suggest that black truffle bioactive components have great potential for use in ad-

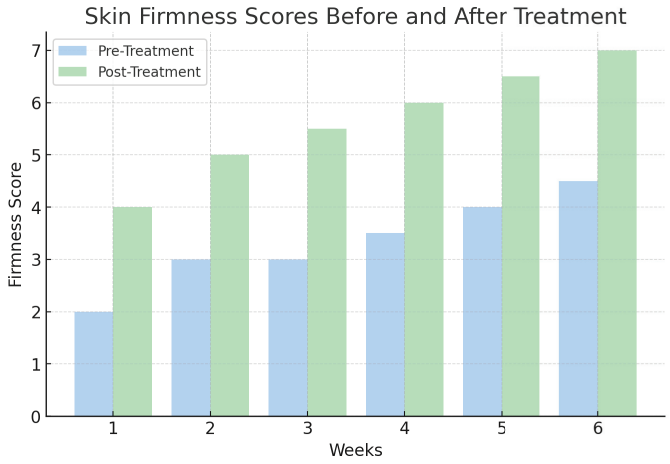

Figure 3

vanced anti-aging skin care products. Further molecular studies could elucidate the exact mechanisms underlying these observed effects, paving the way for the development of targeted formulation strategies.

4. Conclusion

The aim of this study was to evaluate the clinical effects of black truffle lyophilized powder in promoting collagen synthesis, inhibiting elastin degradation, improving skin firmness, and anti-wrinkle and delaying skin aging. The experimental results showed that black truffle lyophilized powder had significant efficacy in these areas, reflecting its potential as an anti-aging skin care ingredient.

First, in terms of promoting collagen synthesis, the experimental group showed a significant effect, with an increase in collagen levels of about 30%, which was significantly higher than the 5% in the control group. This result suggests that black truffle lyophilized powder may promote the synthesis of extracellular matrix proteins by activating fibroblasts, which in turn improves the structure and elasticity of the skin.

Secondly, black truffle lyophilized powder also showed significant effects in inhibiting elastin degradation. The inhibition rate of elastin degradation reached 25% in the experimental group and only 2% in the control group. This indicates that the bioactive components of black truffle can effectively interfere with elastase activity, thus protecting skin elasticity and slowing down the aging process.

In terms of skin firmness, the subjective scores of the experimental group also improved significantly, with the skin firmness score increasing from 3.0 to 6.5, further demonstrating the multiple benefits of black truffle lyophilized powder in promoting skin remodeling and restoring the structural integrity of the skin.

Taken together, black truffle lyophilized powder has significant anti-aging, anti-wrinkle and delayed aging effects by promoting collagen synthesis, inhibiting elastin degradation and enhancing skin firmness. This study provides strong evidence to support the application of bioactive ingredients of black truffle origin in highly effective skincare products, and future studies will help to further reveal their molecular mechanisms and lay the foundation for precise skincare formulation design.

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