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Research Article

Hydrogen's Antiaging and Whitening Effects on Skin

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Abstract: This study was conducted to confirm the suitability of hydrogen as a functional cosmetic ingredient by evaluating the anti-aging and whitening effect of hydrogen, known as an excellent antioxidant. Cosmetic hydrogen powder was prepared to investigate its inhibitory activity on elastase, collagenase, and tyrosinase, and a hydrogen pack was produced using the prepared cosmetic powder to conduct clinical trials. The results showed that hydrogen powder had excellent anti-aging activation and whitening effect. In clinical trials, skin elasticity was increased by 15%, skin sagging decreased by 4.53%, and skin tone improved by 4.86%, compared to the control group. These results suggest that hydrogen has excellent anti-aging and whitening effect. Therefore, it is highly likely that hydrogen can be used as a functional cosmetic ingredient for wrinkle improvement and skin whitening.

Keywords: Hydrogen, antiaging, whitening, elasticity, functional cosmetic

1. Introduction

According to the free radical theory of aging, free radicals cause aging by damaging cells and inducing oxidative stress [1]. The human body is constantly exposed to free radicals owing to metabolism occurring in organelles, such as mitochondria and endoplasmic reticulum [2]. Among these free radicals, hydroxyl (HO·) and peroxynitrite (NO_3·) radicals are the most dangerous and powerful radicals, which attack the main components of the human body, including DNA, protein, and lipids. This causes permanent damage and lead to aging[3]

Many studies have already investigated the antioxidant activity of hydrogen. It is observed that as the smallest molecule, hydrogen easily enters the mitochondria and cell nucleus to remove the free radicals. In particular, hydrogen is confirmed to exhibit antioxidant activity by specifically eliminating HO· having the highest reactivity among reactive oxygen species (ROS) [4]. H_2 is a tasteless and odorless gas that is neutrally charged. Thus, if the gas is administered using a hydrogen therapy machine, it can be used for therapeutic purposes such as in the form of antioxidant agents, anti-inflammatory agents, and cellular signaling molecules [5,16]. In sports science, hydrogen gas is used to remove oxidative stress caused by exercise by consuming hydrogen water, inhaling hydrogen gas, showering using hydrogen water, and intravenously injecting hydrogen. Moreover, H_2 is used in the medical field to treat psoriasis and acute inflammatory diseases via hydrogen-water baths [6]. Although the antioxidant properties of hydrogen are widely used in various fields, only few studies have investigated their effects and use in cosmetic products.

Therefore, in this study, we evaluated the in vitro skin antiaging and whitening effects using hydrogen powder and conducted a clinical test to demonstrate the beneficial effects of hydrogen on the skin. We believe that this study willfacilitate the development of new functional cosmetics in the future.

2. Methods

2.1 Sample preparation

In the experiment, hydrogen powder was provided by Boyaz Energy Co., Ltd. (Korea). The powder was processed using ICID-registered ingredients for its use in cosmetics. The ingredients are listed in Table 1. The physical properties of the as-prepared powder were determined and used in the in vitro experiment.

For clinical tests, the sample was prepared by applying the fabricated powder to a facial mask sheet and drying it. Before using the sample for the clinical experiment, water was sprayed to generate hydrogen.

Table 1. The ingredientsof hydrogen power

ICID name	Unit(%)
ZnO	20
Zea Mays(Corn) Starch	70
Helianthus Annuus seed oil	3
Citric acid	1

Magnesium Sulfate	3
Silica	3

1.2 In vitro experiment

Antiaging experiment Hydrogen powder was diluted to a final concentration of 1, 10, 100, and 1000 μg/mL. Elastase and N-succinyl-(L-Ala)3-p-nitroanilide were dissolved in 50 mM Tris-HCl buffer (pH 8.6) based on the corresponding concentrations. Further, 40 μL of 2.5-U/mL elastase was added to 40-μL of the samples of each concentration, followed by the addition of 80 μL of 0.5-mg/mL N-succinyl-(L-Ala)3-p-nitroanilide. After reacting at 37 °C for 30 min, the absorbance was measured at 445 nm using a microplate reader. The elastase inhibition rate was calculated according to the following equation:

$$Inhibition\ rate\ (\%) = \left(1 - \frac{absorbance\ of\ the\ sample}{absorbance\ of\ the\ control}\right) \times 100$$

Hydrogen powder was diluted to a final concentration of 1, 10, 100, and 1000 μg/mL. 4-Phenylazobenzyloxycarbonyl-Pro-Leu-Gly-Pro-D-Arg was dissolved in 0.1 M Tris-HCl buffer (pH 7.5) containing 4 mM $CaCl_2$. Further, 250 μL of 0.3-mg/mL 4-phenylazobenzyloxycarbonyl-Pro-Leu-Gly-Pro-D-Arg was added to 100 μL of the samples of each concentration, followed by the addition of 150 μL of 0.2-mg/mL collagenase. After reacting at room temperature for 20 min, 500 μL of 6% citric acid and 1.5 mL of ethyl acetate were added to the reaction mixture. The absorbance was measured at 320 nm using a microplate reader and calculated using the same equation above

Whitening experiment Hydrogen powder was diluted to a final concentration of 1, 10, 100, and 1000 μg/mL. Then, 200 μL of 100 mM sodium phosphate buffer (pH 6.5) and 20 μL of 2000-U/μL mushroom tyrosinase were added to 20 μL of the samples of each concentration. Further, 40 μL of 1.5 mM levodopa was added to the resulting solution, and the solution reacted at 37 °C for 15 min. After the reaction, the absorbance was measured at 490 nm using a microplate reader. The tyrosinase inhibition rate was calculated according to the following equation:

$$Inhibition\ rate\ (\%) = \left(1 - \frac{absorbance\ of\ the\ sample}{absorbance\ of\ the\ control}\right) \times 100$$

2.3Clinical trial

After stating the purpose and contents of this study, 10 women in the age group of 40–50 years voluntarily filled the consent form and were recruited. The trial was conducted with the approval of the institutional review board. The experimental group applied the facial mask prepared in this study, and the control group used a commercially available hydrogen facial mask for 4 weeks. Measurements were performed before the test, 2 weeks after application, and 4 weeks after application. The used measuring devices were as follows: Visia-CR® (Canfiled Imaging System, USA) for whitening, F-ray (Beyoung, Korea) for skin laxity, and Ballistometer BLS780 (Dia-Stron Ltd., United Kingdom) for skin elasticity.

2.4Data processing

SPSS Ver.26 (IBM, USA) was used to test all calculated data for statistical significance. The Mann–Whitney U method test was used to test the homogeneity between the groups ($p > 0.1$). The Wilcoxon signed-rank test was used to compare the effects before and after using the masks for evaluation criteria, and the Mann–Whitney U method test was used to test the level of change between the groups ($p < 0.05$).

3. Results and Discussion

3.1 Properties of hydrogen power

The manufactured powder is white and odorless. After storing it at −10°C, 0°C, 25°C, and 45°C for 24 hours, the powder did not show any change in coagulation, agglomeration, extraction, color, etc., confirming its stability. The results also showed that hazardous substances such as lead, arsenic, and mercury were not detected in it, confirming its safety.

3.2 In vitro test

Anti-aging test Figure 1 shows the results of measuring the inhibitory effect of hydrogen powder on elastase and collagenase. Elastase inhibition assay was performed at the sample concentrations of 3.50 ± 2.04, 4.89 ± 1.17, 13.27 ± 1.13, and 54.84 ± 4.39 at 1, 10, 100, and 1000 μg/mL.

Collagenase inhibition assay, measured at the same sample concentration, showed 2.37 ± 1.05, 7.00 ± 1.32, 15.65 ± 0.84, and 40.33 ± 1.19. All results showed concentration-dependent trends.Elastase is a non-specific enzyme that breaks down elastin, an insoluble fiber protein. It is capable of causing wrinkles by digesting collagen and elastin and destroying the network structure [7]. If IC50 of oleanolic acid used as a functional raw material for wrinkle improvement and 89.0 μg/mL of hydrogen powder are compared in their inhibitory activities on elastase, hydrogen powder has higher inhibitory activities [8].

Collagenase produced by fibroblasts is called MMP-1 and serves as a prototype of all interstitial collagenases. Since collagenase is an MMP-1 that acts specifically on collagen, as MMP-1 activation increases, collagen is decomposed, creating wrinkles [9]. The inhibitory activity of ascorbic acid on collagenase was 74.5% at 1000 μg/mL, respectively, and hydrogen powder showed 50% efficacy compared to the control group.

Whitening test Figure 2 shows the results of tyrosinase inhibition assay using hydrogen powder. Sample concentrations of 1, 10, 100, and 1000 μg/mℓ showed an inhibition rate of 3.62 ± 0.12, 3.82 ± 0.73, 13.78 ± 4.98, and 61.46 ± 5.79, showing concentration-dependent trends. In comparison to the results from Kang [10], the inhibitory activity of hydrogen powder on tyrosinase is similar to the inhibitory activity of fermented algae and arbutin. In particular, the inhibition rate of 61.46 ± 5.79% at 1000 μg/mL by hydrogen powder showed higher activity than did arbutin.

A series of oxidation processes produces melanin pigment. Hydrogen is already known as an excellent antioxidant, and it has an excellent whitening effect that can inhibit the production of melanin pigment [11].

Tyrosinase, a copper (Cu) oxidase, is an enzyme that initiates the first step of melanogenesis. It oxidizes the tyrosine substrate through DOPA (3,4-dihydroxyphenilalanine) into dopaquinone and DOPAchrome, thereby activating the synthesis of eumelanin (black brown) and pheomelanin (red yellow) [12].

Inhibition assay of tyrosinase, a critical component of melanin production, using hydrogen powder in this study showed the effects similar to that of arbutin, the positive control group, suggesting that hydrogen powder has a high potential for use as a whitening agent.

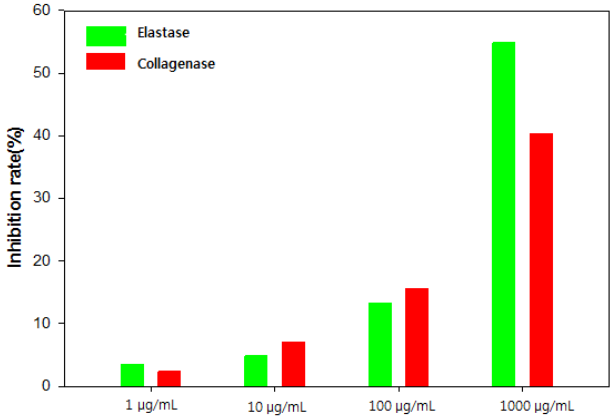

Figure2. Antiaging effects of hydrogen power

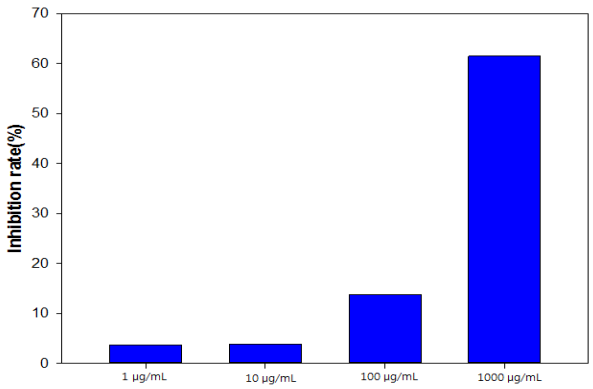

Figure2. Whitening effects of hydrogen power

3.3 Clinical trial

Prior to the experiment, a primary skin irritation test was performed on the region of the clinical subjects' skin that did not have pigmentation or damage. After 24 h, the IQ Ultimate chamber was removed and the extent of skin reaction was visually assessed. Based on the Frosch & Kligman's CTFA guidelines, skin reactivity was assessed and the skin irritation index was calculated. When the degree of skin irritation by the hydrogen mask pack was checked using the skin irritation index table generated via the Draize method, there was no anomalies found [13].

To verify the homogeneity in skin conditions prior to the use of the product between the control and experimental groups, homogeneity indexes were calculated using the results of the subjects' pre-experiment skin condition analysis. The two groups were not significantly different in terms of pre-trial skin conditions.

To improve the pigmentation of the specimen and to evaluate skin tone (brightness), the face of the subjects was photographed using Visia-CR® (Canfiled Imaging System, USA). Pigmented regions were selected in the photo of the face taken in the cross-polarized mode.

An image analysis software (Image-Pro Plus, USA) was used to determine the average V values in the pigmented regions in the photo of the face. Measurements were performed before the test and 2 and 4 weeks after the test. Skin color analysis revealed that skin pigmentation gradually faded after 2 and 4 weeks of use compared with that before use. Changes in skin color (brightness, V value) in the pigmented region were analyzed using Image-Pro Plus. The pigmented region was improved by 4.86% after 4 weeks and by 0.403% in the control group, and the difference was statistically significant.

Skin elasticity was measured using a ballistometer (BLS780; Dia-Stron Ltd., United Kingdom). This device uses the principle of vibration of the electromagnet embedded in the probe. When the device is placed on the skin surface, the form (reduction) of the vibration is detected by the control device via rapid vibration, and the value is expressed in arbitrary units. A representative measurement parameter of area (area of the elastic trajectory) was analyzed and evaluated [14]; the higher the value, the better the elasticity. In this test, the left and right cheeks were measured three times: before and after 2 and 4 weeks of use, and the average values were determined. Compared with the pre-treatment group, the skin elasticity of the cheek region in the experimental group increased by 9.40% after 2 weeks and by 15.00% after 4 weeks. On the other hand, the control group showed an increase of 2.37% after 2 weeks and of 5.36% after 4 weeks (Figure 3).

Skin sagging was measured using F-ray (Beyoung, Korea). This device is a three-dimensional surface measurement system (Moire topography system) using moire interference, which occurs due to the wave characteristic of light. When light is irradiated toward the subject with a grid pattern window placed in front of the subject, the periodic grid pattern and grid pattern deformed by the subject intersect with each other to create a moire-like contour line [15]. Through this contour line, the structural changes in the subject's surface can be revealed. In this test, contour images of the left and right cheek regions were taken before and after 2 and 4 weeks of use, and the angles of the contours were analyzed using an image analysis program. Compared with the pre-treatment group, the sagging of the cheek skin in the experimental group (contour angle) decreased by 2.40% after 2 weeks and by 4.53% after 4 weeks. On the other hand, in the control group, it increased by 0.12% after 2 weeks and decreased by 2.14% after 4 weeks. The rate of decrease was significantly higher in the experimental group than in the control group (Figures 4).

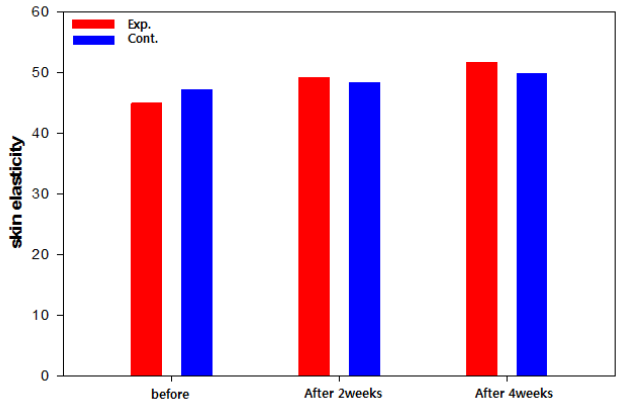

Figure 3. Skin elasticity of experimental group and control group

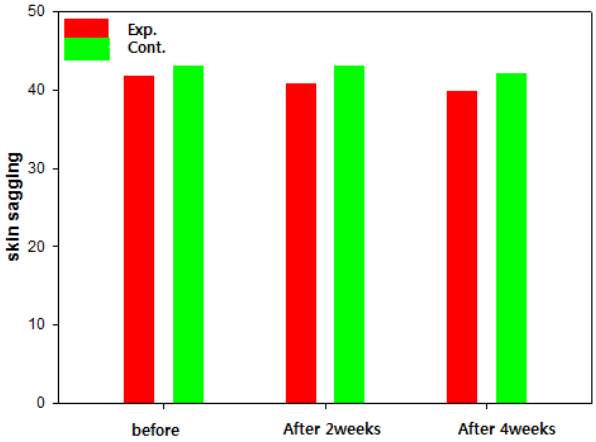

Figure 4. Skin sagging of experimental group and control group

4. Conclusions

This study was conducted to test the skin brightening and anti-aging effect of hydrogen powder and a hydrogen mask pack for the purpose of developing functional cosmetic materials using hydrogen.

The results showed that hydrogen has high antioxidant effects and exhibits excellent inhibitory activities on tyrosinase, elastase, and collagenase.

Therefore, hydrogen seems to be an excellent functional cosmetic material for skin brightening and anti-aging. Given its diverse effects, various formulation products, in addition to the mask pack applied in this study, are highly likely to be used in the future.

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