

Effects of a Fermented Grape Combined with Specified Fruits and Vegetables or Their Extracts on Anti-aging and Anti-inflammatory Status: A Randomized Controlled Clinical Trial

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Abstract Vegetables and fruits had antioxidant capacity. Fermented grapes had a higher content of anthocyanins and flavonoids, and reduced skin wrinkles. There were not more clinical studies on whether fermented grapes with fruit and vegetable extracts had anti-aging and anti-inflammatory activities. The current study used fermented grapes beverages made from fermented grapes combined with fruit and vegetable extracts to explore anti-aging and anti-inflammatory. 60 subjects were recruited and divided into a placebo and another fermented grape drinks group. The subjects drank 50 ml, 1 bottle a day for 8 week. After drinking for 8 weeks, a skin test and blood inflammatory factor analysis were performed. Compared to the placebo group, skin brightness, moisture, elasticity was significantly increased by 3.6%, 4.9%, 5.4%, respectively. Fermented grape drinks decreased skin spots, and wrinkles by 7.0%, and 19.0%, respectively. TNF- α (Tumor Necrosis Factor) and IL-6 (Interleukin-6) expression were decreased, also, as a function of fermented grape drinks intakes. This study indicated that fermented grapes combined with fruit and vegetable extracts can significantly improve the brightness, moisture, elasticity, wrinkles, pores, and decrease TNF- α and IL-6 expression.

Keywords: fruit, vegetable, grapes, inflammation, skin aging

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1. Introduction

Modern medicine is advanced, and human life is extended, but it still has to face the threat of many diseases. Healthy skin provides a protective interface between the internal and external environment of the body to adapt the environment. However, many different factors can exacerbate the aging process of the skin, including aging, radiation, consumption of an unbalanced diet, and stress-related defects in micronutrients, leading to dullness, wrinkles, and spots in the skin [1]. In recent years, natural compounds, such as vegetables and fruits, have been pointed out as natural antioxidants that are beneficial to human health and have attracted great attention [2]. In

addition, multiple fruit and vegetable intake may be additive or synergistic effects to produce a better response in the prevention of disease [3].

Vegetables and fruits contain a variety of phytochemicals, which are the best and natural source of antioxidants, especially dark-colored fruits and vegetables, which have stronger antioxidant ability [4]. Grapes are one of the most valuable fruits in the world. Grapes had many beneficial effects, such as antioxidants, anti-cancer, anti-inflammatory, anti-skin aging and anti-inflammatory [5]. It is worth noting that many compounds have been identified in grapes, including polyphenol, flavonoids (anthocyanins, flavanols, and flavonols) and trans-resveratrol [6]. In particular, the resveratrol compound which was found in grape peel, had a strong antioxidant activity [7]. The grape extract can effectively prevent the formation of skin

wrinkles caused by ultraviolet rays [8]. Grape extract can reduce reactive oxygen species in cells, and resveratrol from grape exhibits anti-cancer, anti-inflammatory, antibacterial [5]. The grape extract reduced UVB-induced oxidative damage in mice and hence can play a protective role in skin photo-damage [9]. The fermented grapes had higher anthocyanins and flavonoids compared with unfermented grapes, it can reduce the wrinkles and can also alleviate the deterioration of the eyesight of the elderly [10]. However, there were not more studies on whether fermented grapes had anti-aging and anti-inflammatory properties.

In the current study, fermented grape drinks prepared of fermented grapes with fruit and vegetable extracts were used to explore whether it can improve the skin's condition and achieve anti-inflammatory effects. Sixty subjects were recruited and divided into placebo group and fermented grape drinks group. After 8 weeks drinking, skin test and blood biochemical analysis were performed.

2. Materials and Methods

2.1. Clinical Design

The clinical study had been approved by Antai-Tian-Sheng memorial Hospital Institutional Review Board, and the study had been registered on ClinicalTrials.gov Identifier: NCT04805697. 60 adult subjects (20-65 years old) were recruited in this trial between October 2019 and October 2020, and informed consent was obtained from all subjects before the study at Chia Nan University of Pharmacy & Science. The subjects divided into a placebo group (n=30) and an experimental group (n=30). Each subject was informed to intake a bottle of grape drink labeled 50ml, or a placebo drink daily for 8 weeks and was not allowed to take any other supplement during the intervention period, and was confirmed by telephone interview. The exclusion criteria included: i) skin disorders; ii) liver diseases; iii) kidney diseases; iv) allergy to cosmetics, drugs, or foods; v) pregnant and lactating women; vi) people who had any cosmetic procedures (intense pulse light, medical peelings, or laser therapy) before 4 weeks of the study; and, vii) people who took collagen supplements in the past 3 months.

2.2. Grape Yeast Compound Fruit Vegetable Beverage (Shanghai Daerwei Biotechnology Co., Ltd.)

Fermented grape drinks contains: 3% fermented grape from red grapes, 3% aronia, 1% black currant, 1% phyllanthus emblica, 1% blueberry, 2% purple perilla seed, 0.005% sucralose, 0.05% citric acid, 0.4% corn syrup, 0.3% xylitol, water. Placebo drink contains: 0.005% sucralose, 0.05% citric acid, 0.4% corn syrup, 0.3% xylitol, water. Each subject was required to undergo skin condition checks at 0, 4 and 8 weeks, and blood was drawn at 0 and 8 week to analyze inflammatory factors.

2.3. Skin Measurement

Chroma Meter MM500 (Mino Ita, Japan) was used to measure the skin brightness of upper check; *L* value (in

the range of 1-100) defined by International Commission on Illumination indicates the change of skin brightness. Corneometer CM825 (CK, Germany) was employed to analyze the skin moisture of upper check based on the skin conductance response. Cutometer MPA580 (CK, Germany) was used to measure skin elasticity of upper check by suction method; the instrument create a negative pressure to deform skin and the mechanical property of skin is analyzed. VISIA Complexion Analysis (Canfield Scientific, US) was used to obtain the values of skin wrinkles and pores [11].

2.4. Inflammatory Factor Detection

The measurement of IL-6, TNF- α in blood was based on the Enzyme-linked immunosorbent assay (ELISA). The ELISA kits were obtained from Cloud-Clone Corp. (US). All experimental procedures were following the recommended protocols [12].

2.5. Statistical Analysis

The experimental data analysis was first calculated by the normal distribution, and then calculated by the paired t-test check; the non-normal distribution was calculated by the Wilcoxon signed-rank test. $p < 0.05$ level was considered statistical significance.

3. Results and Discussion

3.1. Effects of Fermented Grape Combined with Fruits and Vegetables or Their Extracts on Improved Skin Status Improvements

The Figure 1 showed that after 8 weeks intake period of the tested formula, skin brightness was significantly increased by 3.6% than the placebo group, and the moisture content was significantly increased by 4.9% than the placebo group, and the elasticity was significantly increased by 5.4% than the placebo group. In addition, skin spots were significantly decreased by 7.0% than the placebo group, and wrinkles were significantly decreased by 19.0% than the placebo group. According to the above results, fermented grapes combined with fruits and vegetables extracts can increase brightness, moisture, elasticity, and decrease spots and wrinkles. Recently, it had been reported that fermented juice had anti-oxidant and whitening effects. Since the microbial fermentation process can increase the equivalent components of phenols, anthocyanins, resveratrol, and tannins, it had a positive effect on health improvement [13]. The studies pointed out that light and temperature can enrich the accumulation of flavonols and anthocyanins in grapes [14]. In addition, fermented grape beverages can improve the skin may be due to the effects of tannins and polyphenols [15]. Resveratrol can inhibit the UVB radiation caused damage in skin [7]. Subjects taking resveratrol can increase the skin brightness and reduce wrinkles and spots [16]. The possible mechanism of Resveratrol was to increase antioxidant transcription factor, NF-E2-related factor 2 (Nrf2), and reduced the Matrix metalloproteinase

(MMP) expression, thereby reducing the wrinkles formation. In addition, fermented grapes can increase the aquaporin-3 expression and enhance skin moisture [17]. In addition to grapes, other vegetable and fruit extracts including aroma, black currant, phyllanthus emblica extract, blueberry, purple perilla seed extract were also rich in antioxidant effects [18,19]. These studies results suggested that fermented grape combined with fruits and vegetables had skin care potential.

3.2. Effect of Fermented Grape Combined with Fruits and Vegetables on Inflammation

Second, in order to explore whether fermented grape drinks can decrease inflammation, the subject's blood were collected for biochemical analysis. TNF- α and IL-6 are common for inflammatory factors. The Figure 2 showed that after drinking fermented grape drinks for 8 weeks, TNF- α in the blood was significantly decreased by 19.6% than the placebo group, and IL-6 was significantly decreased by 19.0% than the placebo group. Thus, fermented grape combined with fruits and vegetables drink can decrease inflammatory cytokines and effectively

fight inflammation. It had been reported that flavonoids of grape seed extract can regulate the inflammatory factor, TNF and IL-6, by affecting macrophages or immune cells [20]. Grape polyphenols can reduce chronic inflammation by reducing (Reactive Oxygen Species (ROS) levels [21]. Some studies had shown that polyphenols inhibited the expression and secretion of pro-inflammatory molecules in skin cell lines [21]. Grapes contained flavans, anthocyanins, flavonols and resveratrol, shown to prevent LDL oxidation, oxidative stress, dyslipidemia and inflammation [22]. Long-term consumption of high-fat diet in mice, and then using grape extracts can prevent weight gain and reduce inflammatory factors [23]. NF- κ B, a transcription factor, plays a role in the regulation of the expression of inflammatory mediators such as IL-6, inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX2) [24]. The grape extracts attenuated lipopolysaccharide (LPS)-stimulated phosphorylation of NF- κ B [25]. In addition, grape extracts can decrease LPS-stimulated activation of mitogen-activated protein kinase (MAPK), subsequently decreasing the iNOS, COX-2 and other related inflammatory cytokines [25]. These results suggested that fermented grapes combined with fruits and vegetables could inhibit inflammatory responses.

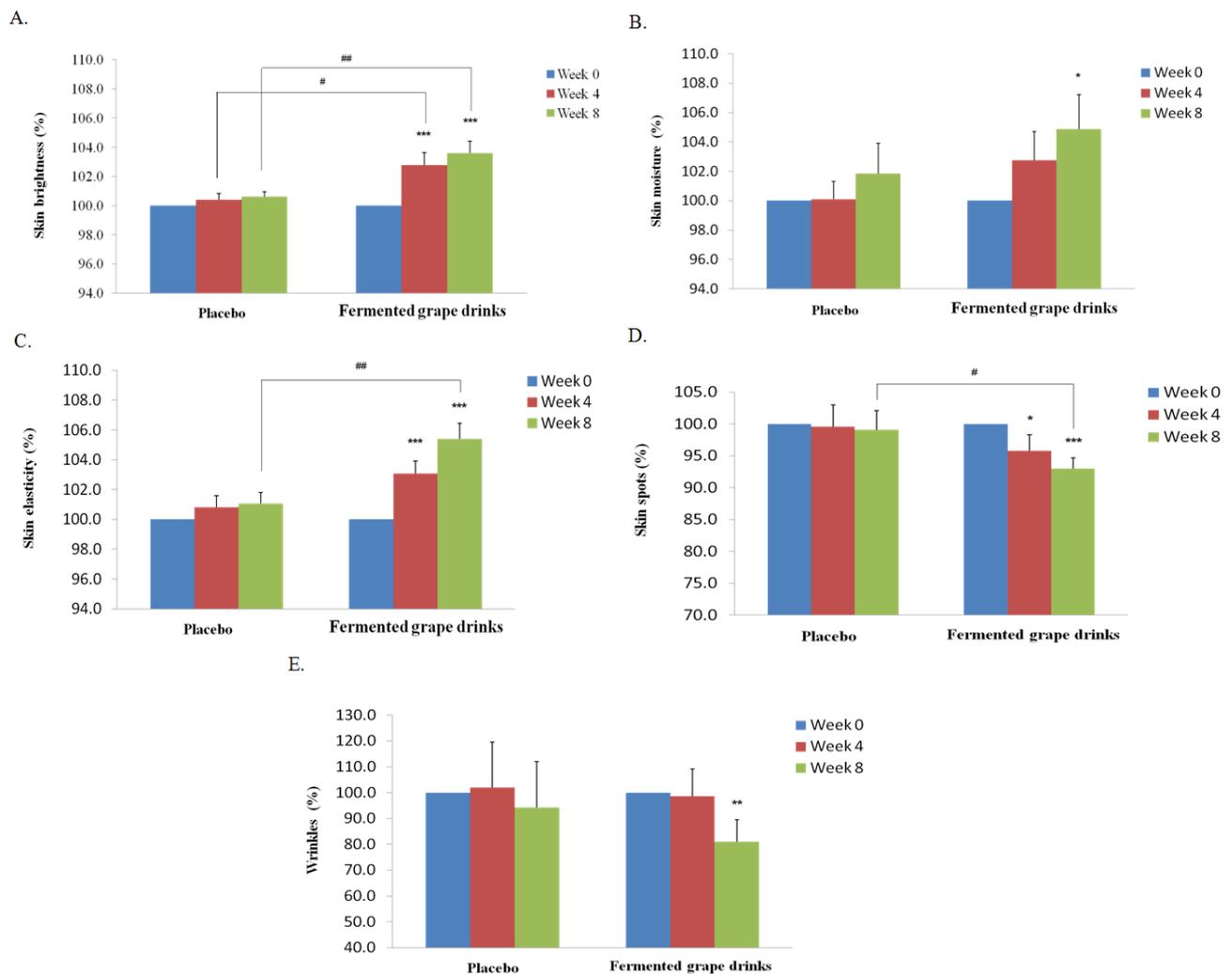


Figure 1. Fermented grape can improve skin condition. The subjects were recruited and divided into placebo group and fermented grape drinks group. After drinking for 8 weeks, the subjects were examined to skin (A) brightness, (B) moisture, (C) elasticity, (D) spots, (E) wrinkles. (n = 60; mean value \pm S.E.M.) (*, compared with before using. #, compared with placebo) (**, p < 0.01, ***, p < 0.001) (#, p < 0.05, #, p < 0.01)

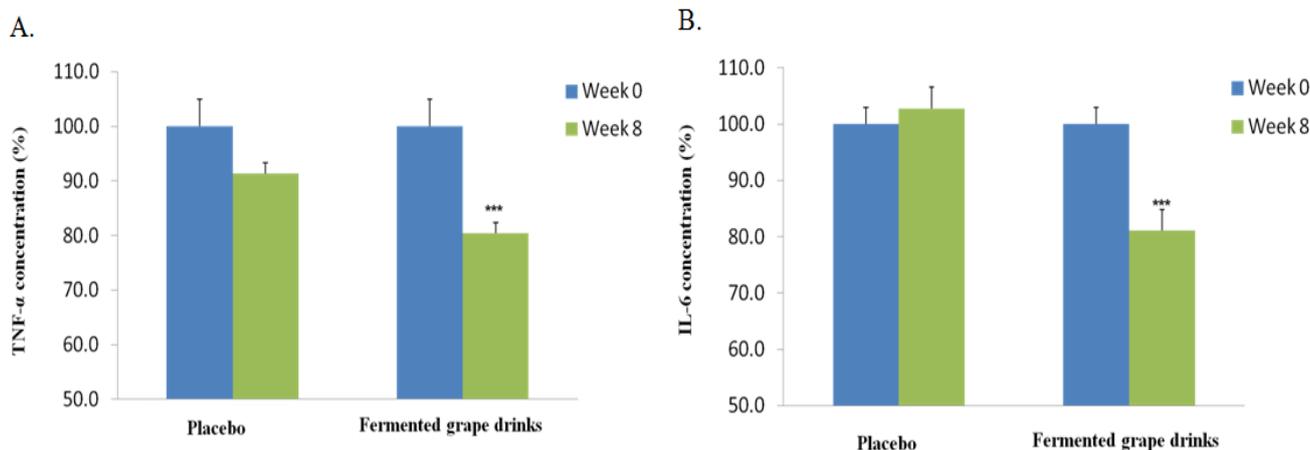


Figure 2. Fermented grape can reduce inflammation. The subjects were recruited and divided into placebo group and fermented grape drinks group. After drinking for 8 weeks, the subject's blood was collected. (A) TNF- α , (B) IL-6 (n = 60; mean value \pm S.E.M.) (*, compared with before using) (***, p < 0.01)

4. Conclusion

This clinical study demonstrated the effects of fermented grapes with a variety of fruit and vegetable or their extracts can significantly on improving the brightness, moisture, elasticity, wrinkles and pores of the skin. Compared with other similar studies, fermented grape drink can comprehensively improve skin condition for most subjects after 8 weeks of ingestion, reduce inflammation, and show competitive efficiency. It could be concluded that fermented grapes drink can delay the skin aging process and decrease skin inflammation.

Statement of Competing Interests

The authors have no competing interests.

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