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Beneficial Effects of Fermented Cricket Powder as a Hair Growth Promoting Agent in a Mice Model

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Insects have been proposed as new protein sources for human nutrition, and protein availability is affected by insect characteristics. Fermentation can be used to obtain a variety of insect-based ingredients and products with unique properties, but its effect on protein availability is unknown. Fermented cricket (*Gryllus bimaculatus*) powder consists mainly of protein, and its oral administration has been reported to improve hair growth in androgenetic alopecia. The purpose of this study was to evaluate the hair-promoting activity of fermented cricket powder in an animal model using male C57BL/6 mice (25-30 g). The abdominal hair of the mouse (2x2.5 cm) was gently removed, and the groups fed as follows: Intact controls (no cricket powder); cricket powder only; and fermented cricket powder only. Food was applied daily for 11 weeks. Observational and physical examinations were performed and the results of the different groups compared. The application of fermented cricket powder significantly (p<0.01) promoted hair growth compared to the intact controls. The C57BL/6 results confirmed increased growth after seven weeks when the proportion of anagen follicles had increased by about 125% and 120% in the control and cricket powder groups, respectively. In conclusion, fermented cricket powder can be seen as a promising alternative alopecia treatment because it promotes hair growth, and, given the powder's composition, trace elements such as amino acids may have contributed to these effects.

Key words : Alopecia, cricket powder, fermented cricket powder, *G. bimaculatus*, hair growth

Introduction

Eating insects and foods containing insects is a common practice in many parts of Asia, Africa and the United States [1, 13]. The Food and Agriculture Organization of the United Nations (FAO) reports that more than 1,900 species of insects are eaten worldwide, including meal larvae, crickets, ants, grasshoppers, and flies. Insects are a source of high-value, digestible protein with low environmental impact, which could be a cost-effective and more sustainable alternative to animal-based protein in the human diet [10]. Crickets are included in the list of most likely food insects by the European Food Safety Authority (EFSA) [17]. They include significant amounts of protein (up to 70 g/100 g can reach dry matter), fats (especially polyunsaturated fatty acids), and fiber (due to the presence of chitin), as well as vitamins (mainly group B) and minerals (ex. iron, selenium, zinc) [17]. However, mainly due to its appearance, consumer acceptance of insect-based foods is low and their use as potential ingredients for the development of foods with improved nutritional properties is diminishing. The introduction of insects into the diet is limited.

Insects are proposed as a new protein food because of their low environmental impact, such as greenhouse gas production, land requirements, water consumption, and nutrient composition. In addition to the high protein content (50-82%) and balanced AA profile, the quality of insect protein also depends on digestibility [1]. Edible insect species such as yellow mealworms (*Tenebrio molitor*) and crickets are currently being industrially bred and investigated in Western countries to feed humans, livestock and zoo animals.

Recently, experimental evidence supporting the effectiveness of crickets has been accumulated in various biological fields. Many studies have demonstrated that crickets exhibit anti-inflammatory [5], antiviral [11], antibacterial [15], anticancer [14], and antiallergic effects [3]. A physicochemical

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study of crickets was conducted on its bioactive constituents. In addition to the above biological effects, crickets are traditionally used orally to treat patients with alopecia. Although experimental evidence for its hair regrowth effect has not yet been established, crickets are widely used as hair promoters in Asian countries, especially in South Korea, due to their anecdotal efficacy.

Therefore, biological methods using fermentation are naturally in the limelight. Fermented eggs, lactic acid bacteria, mold, yeast, ripe C57BL/6 organisms give new physiological functional activity from the efficacy of existing foods, increase various useful ingredients [16], increase absorption in the intestine of C57BL/6. The fermented strain used in this study contained *Bacillus subtilis* and was fermented. The natural product fermentation process using *B. subtilis* is easy to maximize the increase in active ingredients and bioactivity of the powder. It is also a method that can be used and is widely known in many studies [6]. *B. subtilis* degrade carbohydrates and proteins, citric acid, and acid-tolerant saccharifying enzymes [7]. It also produces organic acids well, which makes it easier to make entries and lowers the pH. There is a merit that it becomes safe by changing to acid. *B. subtilis*, one of the typical bacteria, has an antithrombotic effect. As C57BL/6 organism with excellent white matter decomposition power, it promotes the growth of bacteria during fermentation. At the same time, it dissolves due to the action of enzymes produced by the strain. The content of exudate increases and the protein is converted to low molecular weight protein. It exhibits various physiological activities such as catabolism [12].

Currently, the FDA has approved only two treatments for androgenetic alopecia [9]: oral finasteride and topical minoxidil. However, the effects of this drug are variable and temporary, and several side effects such as pruritus, dermatitis, and irritation have been reported [4]. As a result, there is still an unmet need for the development of new and ideal hair growth promoters with minimal adverse effects.

Among the different species of animals, mouse models are the most widely reported in hair growth promotion studies due to their extensive database and availability of specific variants such as nudity, hairless, and rhinoceros. Growth is much more consistent and less susceptible to iatrogenic. The disadvantages associated with the mouse model are the high hair follicle density and the rodent hair cycle progressing in a wave pattern that sweeps posteriorly and dorsally, unlike the mosaic pattern found in humans. Trunk pigmentation in C57BL/6 mice is completely dependent on follicular melanocytes. This type of trunk epidermis lacks melanocytes, which are closely linked to the growth phase of hair growth.

The cricket powder used in this study has been reported to be effective in improving liver function as well as being protein-rich and antioxidant in rugs [5], providing a treatment for diabetes. In addition, it has several pharmacological effects. In conclusion, the application of fermented powder has shown promoted hair growth, so it may be considered a promising alternative for the treatment of androgenetic alopecia. Also, given its composition, trace components such as amino acids and phytoestrogens may contribute to this effect.

Materials and Methods

Microbial preparation

The microorganisms (*B. subtilis*) used in this experiment were obtained from the Korean Agricultural Culture Collection (KACC, No.19623). A liquid medium suitable for the growth of the *B. subtilis* is prepared and the *B. subtilis* are cultured as follows. LB media (distilled water 200 ml, 2.5% LB, during 24 hr, at 30-40℃, 50% humidity).

Fermentation of *G. bimaculatus* Powder

For smooth fermentation of *B. subtilis*, the pairwise cricket powder prepared above is immersed and drained. After high-pressure sterilization, fermentation was performed. Independently inoculate *B. subtilis* in the liquid medium (200 ml liquid medium and 1 kg *G. bimaculatus* powder) under specific fermentation conditions 30-40℃, humidity 50% for 2 weeks.

Animal experiment

6-week-old male C57BL/6 mice were supplied by Orient Bio Inc. (Seongnam, Korea). Mice were housed in cages and acclimatized for 1 week by maintaining them in a controlled environment of 22±1℃, 50±10% relative humidity on a light-dark cycles of 12 /12 hr. During acclimation, mice were fed a two-spotted cricket and two-spotted cricket fermentation powder and water. Animal experiments were carried out in accordance with the Guide for the Care and Use of Laboratory Animals, provided by Experimental Animal Center of Daegu-Gyeongbuk Medical Innovation Foundation (DGMIF), Daegu, Republic of Korea (DGMIF-21071903-00).

C57BL/6 mice were randomly divided into 3 groups, 5 per group. To synchronize the phases of telogen stage [2], the hair on the dorsal area (2x3 cm) was shaved with an animal clipper without sham group. The mice were fed a two-spotted cricket and two-spotted cricket fermentation powder for 11 weeks. After starting the feed application, the amount of hair growth of the mice was measured every week, and photographs were taken using APMPRO dermoscope.

ELISA assay

To analysis of serum cytokines, the blood of C57BL/6 mice were taken at 0, 1, 4, 6, 10 weeks and also harvested new hairs of C57BL/6 mice to measure the level of melanin. The COX-2, TNF-a, IL-6 and melanin levels were measured using ELISA kit according to the manufacturers instructions. Briefly, microplates pre-coated with monoclonal antibodies were washed twice with wash buffer. After that, serum and Biotin-Conjugate were added into the microplate wells and shaken for 2 hr. Then, the microplate was washed 5 times with wash buffer, and Streptavidin-HRP was added to the wells and shaken for 1 hr. Next, TMB reagent added to the microplate wells for 30 min and the absorbance was measured at 450 nm with a microplate reader.

Experimental design

Mice were weighed to the nearest 0.1 g from 8 weeks old ate to 18 weeks by weekly. Mice were allocated to a specific diet every 3-4 days during the experimental period (8-18 weeks of age). The feed intake of each cage was estimated as the difference between the offered feed and the remnant at 3-4 days intervals without considering spillage. Water was offered ad libitum throughout the experiment.

Measurement of hair-growth effect

The two-spotted cricket and two-spotted cricket fermentation powder formulation were applied repetitively to C57BL/6 mice once in three days, and hair growth was monitored weekly using a APMPRO dermoscope. The level of hair density was evaluated with image analyzing software by software of APMPRO dermoscope. In addition, the surface and a cross-section of C57BL/6 mouse skin treated with MXD formulation were also monitored using a digital camera and digital microscope (Bio Medical Science Inc., Tokyo, Japan), respectively.

Statistical analysis

All data were expressed as mean ± SD. and analyzed using one-way analysis of variance. Differences between the groups were analyzed by Dunnetts test, and significance levels were set at $^{*}p<0.05$.

Results

Hair growth-promoting effects of the Fermented *G. bimaculatus* powder

In order to search for hair growth-promoting agents from alternative medicine, we performed *in vivo* experiment with *G. bimaculatus* powder using C57BL/6 mice. The Fermented *G. bimaculatus* powder was superior to the normal *G. bimaculatus* powder tested in terms of hair growth promotion. After oral administration for 10 weeks of C57BL/6 mice , the fermented *G. bimaculatus* powder induced hair growth promotion than did the vehicle control and normal *G. bimaculatus* powder (Fig. 1A, Fig. 1B, Fig. 1C). As shown in Fig. 1, early hair growth promotion was dramatically induced by the fermented *G. bimaculatus* powder in the C57BL/6 mice. This result supports the notion that the fermented *G. bimaculatus* powder induces early onset of anagen and stimulates hair growth.

Effects of the fermented *G. bimaculatus* powder on hair growth promotion

We also tested the effect of the fermented *G. bimaculatus* powder on melanin protein synthesis using ELISA assay. As shown in Fig. 2, the fermented *G. bimaculatus* powder significantly increased the uptake of radio-labeled melanin in the mouse hair grown in mice, resulting in a 125% uptake, relative to the control. Since it has been found that melanin incorporation correlates with hair follicle growth *in vivo*, this result implies that the fermented *G. bimaculatus* powder stimulates hair growth.

To determine whether the fermented *G. bimaculatus* powder had a promoting effect on the hair density, we counted the number of hair. We investigated the effect of the fermented *G. bimaculatus* powder on the growth of hair density. First, we counted the number of hair of vehicle and *G. bimaculatus* powder and fermented *G. bimaculatus* powder using the APMPRO dermoscope. The fermented *G. bimaculatus* powder showed increased hair concentration (Fig. 3). Based on these results, we evaluated increasing ratio. As shown in Fig. 3, the fermented *G. bimaculatus* powder increased the

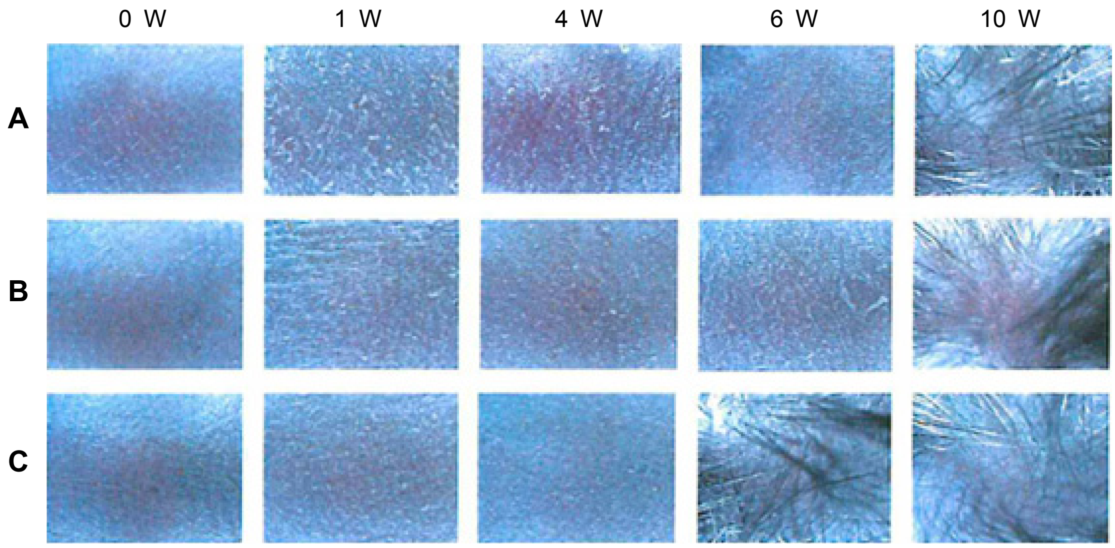

Fig. 1. Hair growth-promoting effects of the fermented *G. bimaculatus* powder in C57BL/6 mice. The ventral skins of the mice were shaved and the *G. bimaculatus* powder and fermented *G. bimaculatus* powder was administered. (A) Control, (B) *G. bimaculatus* powder, (C) fermented *G. bimaculatus* powder. W represent week (0 W means 0 week).

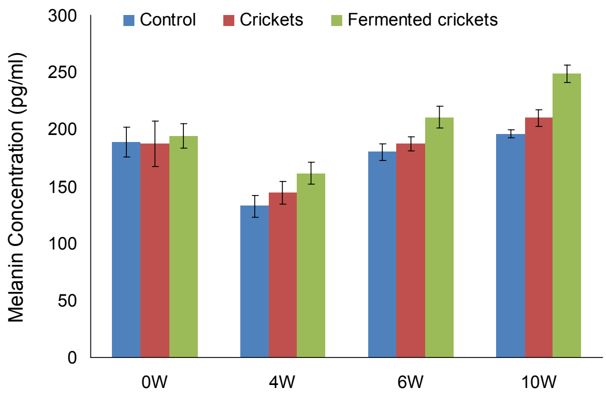

Fig. 2. Effects of *G. bimaculatus* powder and fermented *G. bimaculatus* powder on melanin incorporation in hair. C57BL/6 mice were administered with *G. bimaculatus* powder and fermented *G. bimaculatus* powder and incubated for 10 weeks. The results are shown as the mean values with SD. of 5 numbers of individuals.

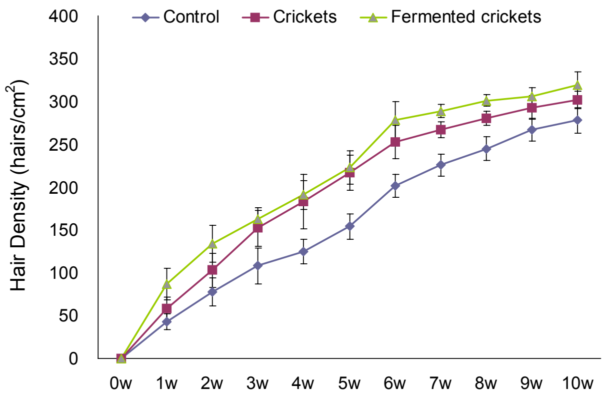

Fig. 3. Effects of the fermented *G. bimaculatus* powder on hair growth promotion. Hair density measured by APMPRO dermoscope. The results are shown as the mean values with SD.

hair density to 106.5% and 127.6%, respectively, as compared to the normal *G. bimaculatus* powder and control.

Effect of the fermented *G. bimaculatus* powder on on inflammatory effect

In this experiment, we used serum ELISA analysis to confirm for inflammatory factors that were affected by the *G. bimaculatus* powder and fermented *G. bimaculatus* powder. We measured the amount of COX-2 (inflammatory effect by testing product). There were significant changes in the inflammatory factor expression (Fig. 4). To further verify the induction of inflammatory molecules of IL-6 and TNF-a, we also performed serum ELISA analysis (supplementary table 1). *G. bimaculatus* powder and fermented *G. bimaculatus* powder changed respectively as compared to control group (Fig. 4).

Discussion

Many people suffer from hair loss and thinning, despite the development of some medical treatments. Therefore, it is important to develop new treatments that prevent hair loss and improve hair growth. In that respect, alternative

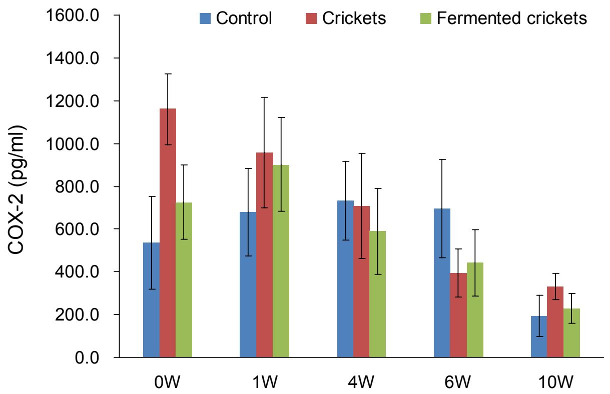

Fig. 4. Effects of *G. bimaculatus* powder and fermented *G. bimaculatus* powder on the induction of inflammatory molecules in serum. The serum ELISA is performed with C57BL/6 mice serum. The results are shown as the mean values with SD. of 5 numbers of individuals.

medicine is attracting attention. Alternative medicine has not yet been incorporated into the mainstream of medicine, but its incomplete knowledge of the mechanisms associated with limited scientific evidence has made it an increasingly attractive approach worldwide. Fermented *G. bimaculatus* is a traditional herbal medicine used in Korea and China to treat various illnesses such as after-stomatitis, local pain, toothache and gingival inflammation [12, 18]. It is known to have several pharmacological effects, including analgesic and anti-inflammatory effects, and protective effects against brain cell damage [8].

Recently, several groups have investigated the role of apoptosis in alopecia [2, 19, 20]. Finasteride, a type IL-6 inhibitor, also affects the expression of caspase and melanin in hair follicle cells and transmits induction signals for the anagen, the active growth stage of the hair cycle. Further studies on the anti-cell death effect of fermented *G. bimaculatus* powder are needed to elucidate the exact biological mechanism of its effect on hair growth. Unfortunately, however, fermented *G. bimaculatus* powder did not show an inhibitory effect on COX-2 expression in this study. Fermented *G. bimaculatus* flour looks like a good candidate for promoting hair growth.

In this reports show that fermented *G. bimaculatus* powder has excellent hair growth-promoting effects in rodent models. In addition, fermented *G. bimaculatus* powder regulates growth factor expression *in vivo* experiments. This result implies that the fermented *G. bimaculatus* powder stimulates hair growth.

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The Conflict of Interest Statement

The authors declare that they have no conflicts of interest with the contents of this article.

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초록 : 생쥐 모델에서 쌍별 귀뚜라미 발효 분말의 발모 촉진제로서의 유익한 효과

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