

ORIGINAL ARTICLE

Efficacy and Safety of *Pueraria lobata* Extract in Gray Hair Prevention: A Randomized, Double-Blind, Placebo-Controlled Study

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Background: Graying of hair—a sign of aging—raises cosmetic concerns. Individuals with gray hair often look older than others their age; therefore, some dye their hair for aesthetic purposes. However, hair colorants can induce many problems including skin irritation, allergic reaction and hair-breakage. **Objective:** This randomized, double-blind clinical trial was performed in order to examine the effects of APHG-1001, a compound including an extract from *Pueraria lobata*, on graying hair. **Methods:** A total of 44 female subjects were randomly treated with either APHG-1001 or placebo twice daily for 24 weeks. Using the phototrichogram analysis, a count of newly developed gray hair was estimated. Investigator assessment and subject self-assessment were also performed in order to evaluate the efficacy of the compound. **Results:** The mean number of newly developed gray hair at 24 weeks was 6.3/cm² in the APHG-1001 group and 11.4/cm² in the placebo group; the difference was statistically significant ($p < 0.05$). However, the investigator assessment and subject self-assessment did not show any significant change in the gross appearance of hair grayness by the end of the study. No severe adverse events in either group were observed. Moreover, the

incidence of adverse events did not differ between the groups. **Conclusion:** This clinical trial revealed that APHG-1001, which contains an extract of *P. lobata*, could prevent the development of new gray hair without any remarkable adverse effects. Thus, it can be considered as a viable treatment option for the prevention of gray hair. (**Ann Dermatol 25(2) 218 ~ 222, 2013**)

-Keywords-

Agging, Antioxidants, Gray hair, Hair, *Pueraria lobata*

INTRODUCTION

Graying hair is a distinct feature of aging^{1,2}. It is known that gray hair often first appears during one's thirties, and half of the people in their 50s have 50% gray hair³⁻⁵. In Korea, the prevalence of gray hair is 95.3% for people in their 50s⁶. Although gray hair does not cause any medical problems, it can make people appear older than their actual age⁷. Currently, with the extended human lifespan, the aged population is increasing. Hence, cosmetic demands for creating younger appearances are also increasing. Cosmetic concerns induced by gray hair are particularly prominent in Korean individuals, whose original hair color is very dark (close to black). Half of the Korean individuals over 50 years of age with gray hair dye their hair in order to cover the grayness⁸. However, hair colorants are composed of various chemicals and carry the risk of eliciting irritative and allergic contact reactions. For example, *p*-phenylenediamine—a major chemical component of hair dye—is well known to induce contact dermatitis in its unpolymerized state⁹.

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Pueraria lobata is a climbing, deciduous perennial vine, native to eastern Asia. Its extract reportedly include isoflavones puerarin, daidzein and genistein¹⁰, which have antioxidant properties^{11,12}. In addition, we found that the extract from *P. lobata* promotes the expression of microphthalmia transcription factor (MITF) *in vitro* and prevents hair graying in MITF^{vit/mi} mice (Park, 2012, unpublished data). Considering that MITF is a master transcriptional regulator of melanocytes, and that oxidative stress may be associated with hair graying^{13,14}, the extract from *P. lobata* could potentially prevent graying of hair in humans.

Currently, there are no medicines proven to prevent gray hair in humans. In this study, we performed a randomized, double-blind, placebo controlled study in order to examine the efficacy and safety of APHG-1001, a topical agent including extracts from *P. lobata*, in subjects with gray hair.

MATERIALS AND METHODS

Subjects

Healthy women (aged 35~65 years) with gray hair were enrolled in this study. The exclusion criteria were as follows: significant systemic or chronic disease, hair loss greater than Ludwig type I, any hair or scalp disease, a history of any treatment for hair loss or hair graying within the previous 6 months, or a history of hair dyeing within the previous 1 month. The study protocols were approved by the Institutional Research Board of Seoul National University Hospital (H-0911-037-301) and written informed consent was obtained from all subjects.

Investigational product (study agent)

APHG-1001 was provided by AMOREPACIFIC R&D Center and prepared as a colorless tonic spray containing 1.0% *P. lobata* ethanolic extract, 0.5% *Pleuropterus multiflorus* extract and 0.5% ginkgo leaf extract. All plant extracts were kindly provided by Bioland Co. (Cheonan, Korea). A 50% ethanol solution was used as the vehicle. The placebo was also a colorless spray, without any active ingredients.

Study design

This was a randomized, double-blind, placebo-controlled study. A total of 44 subjects were randomly assigned to APHG-1001 or placebo for 24 weeks. They were instructed to use the spray twice daily, with 2 pumps (2 ml) for each dose. In order to remove any confounding effects of other topical agents, we provided standard shampoo to all study subjects and instructed them not to apply any other agents. The subjects returned to the clinic at 12 and 24 weeks after their initial visit.

Efficacy assessment

The primary end point was the development of new gray hair during the course of the 24-weeks treatment, assessed by a phototrichogram analysis. Macro photographs of a 1 cm² circular area, 2 cm from the vertex, were taken at 0, 12 and 24 weeks. At the first visit of each subject, the center of this scalp circle was marked with a small tattoo so that the same area could be photographed at each subsequent visit. At the conclusion of the study, we compared the macro photographs at 12 and 24 weeks with the baseline photograph and identified gray hair that had been dark-colored at the baseline (Fig. 1). New gray

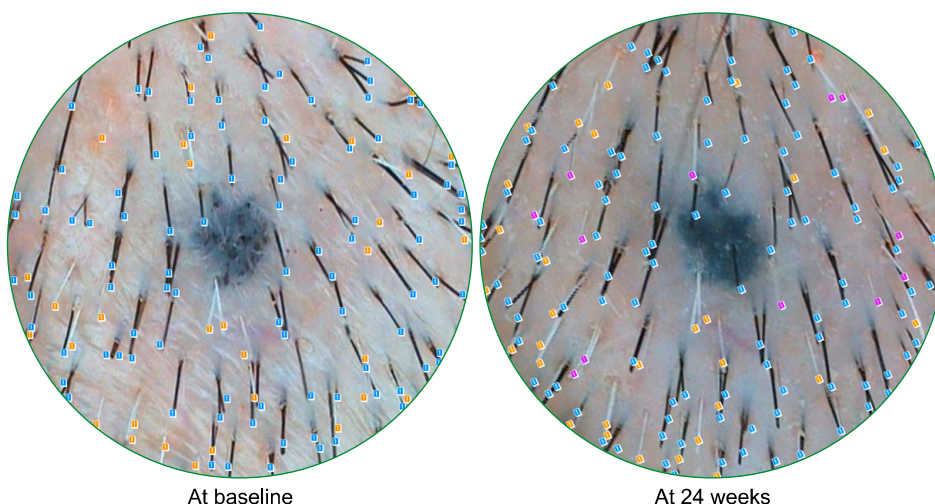


Fig. 1. An example of counting new gray hair developed during the treatment using the phototrichogram analysis. Macro photographs of the 1 cm² circular area were taken at baseline and at 24 weeks. We marked the pigmented hair with blue, existing gray hair with orange, and new gray hair with purple.

hair developed during treatment were counted and statistically assessed between APHG-1001 and placebo groups. A camera system developed by Canfield Scientific Inc. (Fairfield, NJ, USA) was used in this study.

In order to perform investigator assessments, photographs of the temporal area were taken at baseline and at 24 weeks. Hair on the temporal area of the scalp, 6 cm above the external auditory canal, was parted centrally with a comb. The separated strands of hair were fixed on either side of the parting with hair clips in order to photograph the area. Using a series of reference photographs showing increasing degrees of graying from 1 (all hair pigmented) to 10 (all hair white), a panel of 2 dermatologists independently scored the grayness by comparing the subject's photographs at baseline and at 24 weeks with the reference photographs. The changes in score during the treatment were calculated for each subject and compared between groups.

The study subjects also performed a self-assessment. Each subject was requested to evaluate her own graying of hair using a visual analogue scale (VAS) from 0 (all hair pigmented) to 10 (all hair white) at baseline and at 24 weeks of treatment.

Safety assessment

Safety of the treatment was assessed by the results from a physical examination and by the subjects' self-reporting of adverse events.

Statistical analysis

Subjects who completed all study schedules for 24 weeks were included in the study. The Paired t-test and chi-square test were used, respectively, for the change of efficacy variables during the study and the analysis of the incidence of adverse effects between groups. Analyses were performed using Statistical Package for the Social Sciences version 17.0 (SPSS Inc, Chicago, IL, USA). *p*-values of <0.05 were considered significant.

RESULTS

A total of 44 subjects were recruited at screening. We randomly assigned 22 subjects to APHG-1001 and the other 22 subjects to placebo treatment. One subject was dropped out because of withdrawal of the consent; the remaining 43 subjects (21 in the APHG-1001 group and 22 in the placebo group) were included for statistical analysis.

Demographic characteristics

Subjects' demographics are summarized in Table 1. The

mean age of the study participants was 48.9 years in the APHG-1001 group and 49.1 years in the placebo group. At baseline, the numerical counts of total hair and gray hair were measured at a 1 cm² circular area of the scalp, 2 cm from the vertex. There was no statistical difference between the two groups.

Newly developed gray hairs

By comparing the macro photographs at 12 and 24 weeks with those taken at baseline, we identified newly developed gray hair in the 1 cm² circular scalp area (Fig. 1). Although there was no difference in the number of new gray hair at 12 weeks, there were significantly fewer gray hair in the APHG-1001 group (6.3/cm²) than in the placebo group (11.4/cm²) at 24 weeks of treatment (*p*<0.05) (Fig. 2).

Investigator assessment and subject self-assessment

The gross change in hair grayness during the 24-week-study was assessed by both study investigators (Fig. 3) as well as the subjects themselves. Two different investigators assessed the temporal scalp area and found that the grayness score changed slightly during the study period in

Table 1. Demographic characteristics at baseline

Variable	APHG-1001 (N=21)	Placebo (N=22)	<i>p</i> -value
Age (yr)	48.9±6.4	49.1±6.1	0.68
Total hair count in 1 cm ² *	170.2±31.2	174.3±34.8	0.69
Gray hair count in 1 cm ² *	10.6±15.6	14.7±14.1	0.38

Values are presented as mean±standard deviation. *These data were obtained from macrophotographs of a 1 cm² circular area, 2 cm from the vertex.

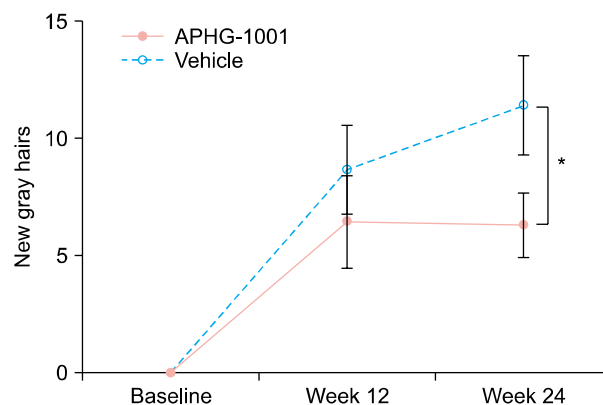


Fig. 2. New gray hair developed after 12 and 24 weeks of treatment with APHG-1001 or with the vehicle. At week 24, there were fewer new gray hair in the APHG-1001 group than in the placebo group. **p*<0.05, mean±standard error.



Fig. 3. Photographs of the temporal area in a subject treated with APHG-1001. Investigators did not find any visible change in gross hair grayness during the 24-week treatment.

Table 2. Summary of adverse events

Adverse event	APHG-1001	Placebo	<i>p</i> -value
Scalp irritation	1 (4.8)	3 (13.6)	0.32
Coarse hair	2 (9.5)		0.14

Values are presented as number (%).

both groups; the mean change was +0.33 in the APHG-1001 group and +0.52 in the placebo group. The subjects were asked to self-assess their hair grayness using the VAS system at each visit; however, neither group showed any significant change; the mean change during the study was +0.52 in the APHG-1001 group and +0.17 in the placebo group.

Safety assessment

Although 3 of the 21 subjects in the APHG-1001 group and 3 of the 22 subjects in the placebo group complained of a certain discomfort with treatment, there were no systemic or serious adverse events (Table 2). The total number of reported complaints did not differ between the groups ($p=0.95$). Scalp irritation followed by coarse hair were the most common complaints.

DISCUSSION

Graying of hair occurs when melanin is absent in the hair shaft. Melanins are synthesized in melanosomes, which are cytoplasmic organelles found in melanocytes, and the melanosomes are subsequently transferred to keratinocytes. In pigmented hair, the hair bulb melanocytes supply melanosomes to the keratinocytes located in the hair shaft. However, active hair bulb melanocytes are markedly reduced in gray hair¹⁵. Although the molecular mechanisms

underlying the decreased number of melanocytes in the hair bulb are not yet fully elucidated, a 'free radical theory of graying' has been proposed¹⁴. Melanogenesis, a complex melanin-synthesizing pathway, generates high oxidative stress via the hydroxylation of tyrosine and the oxidation of DOPA and thus, melanocytes in the hair bulb may be particularly vulnerable due to their extraordinary melanogenic activity during the anagen phase of hair growth. This theory is supported by the accumulation of hydrogen peroxide in the hair shaft of human white scalp hair¹³. Theoretically, hair graying could be prevented if reactive oxygen species are adequately removed by effective antioxidants.

The topical agent used in this study, APHG-1001, contains an extract of *P. lobata* as its major constituent. *P. lobata*, a kudzu-vine, is known to have antioxidant activity *in vivo*. In a diabetic rat model, oral administration of a quantified 50% EtOH root extract of *P. lobata* for 3 weeks reduced the plasma level of malondialdehyde—a marker of oxidative damage to lipids¹⁶. The antioxidant activity of *P. lobata* is due to isoflavonoids, such as puerarin and daidzein. Puerarin, in particular, is reported to be correlated with the antioxidant activity of *P. lobata*¹². In addition, our unpublished data indicate that the extract from *P. lobata* promotes the expression of MITF and melanogenesis *in vitro*, and its topical application prevents hair graying in MITF^{vit/mi} mice (Park, 2012, unpublished data).

In the present study, topical application of APHG-1001 for a period of 24 weeks prevented subjects from developing new gray hair growth. The number of new gray hair developed during the study was significantly smaller in the APHG-1001 group compared to the placebo group. However, the investigators' assessment and subjects' self-assessment did not reveal any marked change in hair

grayness during the 24-week treatment. There are several reasons that may account for this discrepancy. First, treatment response may differ according to different regions of the scalp. In this study, the macro photographs for the identification of new gray hair growth were taken near the vertex; however, the investigators' assessment was performed at the temporal area. This discrepancy was inevitable because the vertex area, clipped for macro photographs, was not suitable for the investigators' assessment. Second, the number of new gray hair was too small to produce a visible change in gross hair grayness. Because APHG-1001 cannot restore the original color of gray hair, the difference between the study groups indicates only the prevention of new gray hair. However, hair graying is a very slow aging process, usually taking more than 20 years for all scalp hair to become white from the first appearance of gray hair⁶; thus, only a few gray hair are expected to be developed during the course of 24 weeks under normal conditions. In order to identify the effect of APHG-1001 on the overall appearance of grayness, a study with a longer duration may be needed. In conclusion, a 24-week-treatment of the scalp with topical APHG-1001, a major constituent of which is *P. lobata* extract, prevented the development of gray hair. Moreover, no systemic or serious adverse events were observed. Therefore, topical APHG-1001 could be considered as a treatment option for the prevention of gray hair.

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