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Research Article
Effect of Natural Plant Extracts on Hair Loss Prevent in People with Alopecia

[1]Jun-Bo Sim, [1]Sang-O Park, [1]Byung-Sung Park and [2]Ga-Yeong Noh

[1]College of Animal Life Science, Kangwon National University, Chuncheon, Gangwondo, 200-701, South Korea
[2]NJY Life Science, Republic of Korea

Abstract

Background and Objective: In experiments using laboratory animals, Natural Plant Extracts (NPE) containing sweet flag have been found to have anti-atopic and hair growth effect. However, whether NPE could clinically prevent hair loss in people with alopecia is unknown. Therefore, the objective of this study was to determine whether NPE could prevent hair loss in people with alopecia. **Methodology:** The NPE was applied to a total of 8 subjects with alopecia for 10 months. When the time duration of NPE application was increased, the number of hair loss from subjects was decreased, while the percentage of hair loss prevention was significantly ($p<0.05$) increased 82.67% compared to before NPE application. **Results:** At 60 days after applying NPE, 80.24% of subjects had great satisfaction on its effect on hair loss prevention. The density and thickness of hair were significantly ($p<0.05$) increased to 128 and 233% respectively, compared to before NPE application. **Conclusion:** These results implies that NPE containing sweet flag could prevent hair loss in people with alopecia.

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Approximately an average of 50~100 hairs are fallen off daily from the skin of normal people. However, patient with alopecia will have more than 100 hairs fallen off from the skin continuously on a daily basis[1]. Approximately 2% of the world population has hair loss problem[2]. About 10 million people have alopecia in Korea with a market value of almost 360 million dollars. Approximately 80% of male alopecia is known to be caused by mutation in testosterone receptor, with stress as an important factor that causes gray hair and hair loss[3]. Due to stresses from diet, child birth, excessive study and increased drinking, hair loss is increasing in younger age women[4].

The number of follicles that enable scalp hair to grow is estimated to be over 100,000. The density of hair will decrease gradually due to aging[5]. Hair loss is known to be part of the process of aging. Its main cause is genetics. However, various factors including stress (social, psychological and mental), eating habit and living habit can affect hair loss[6]. Hair is made of protein filament created and grown in the follicle inside the dermis. The hair grown from the follicle will pass 4 stages of hair cycle (anagen, catagen, telogen and exogen) to repeat hair growth and loss[7]. In the anagen stage, follicle is grown in the dermal papilla layer of the dermis. In the catagen stage, the thickness of the dermis is reduced and the follicle comes closer to the epidermis. The dermal papilla cell existing inside the follicle has to be lost to begin the new hair growth cycle[8]. Alopecia is the phenomenon of hair not growing normally (hair is lost and becoming thinner). Therefore, the anagen gets shorter while the telogen gets longer at the same time[9].

One method to prevent alopecia is by improving blood flow around the follicle to supply essential nutrients to the root of hair to enable hair growth and by using lymphocytes gathered in great numbers around the follicles that were lost to improve the immune function to activate the follicle[8]. Natural products have been applied in cosmetics and hair care industry. Many studies have been conducted for alopecia using different plant extracts. Natural Plant Extracts (NPE) from morus bark (*Mori cortex radices*), sweet flag (*Acorus calamus* Linn.), licorice (*Glycyrrhiza uralensis*), pine needles (*Pinus densiflora*), sophora root (*Sophora angustifolia*), cnidium (*Ligusticum chuanxiong* Hort) and korean angelica root (*Angelica gigas*) have been reported to be effective in preventing alopecia and gray hair, treating inflammation and improving blood circulation on the scalp[10]. The main ingredient of the above seven natural NPE has been found to be asacurin 100 that has atopy prevention effect through its anti-inflammatory activity[11]. It has been demonstrated to be able to promote hair growth using bio-genetic control in C57BL/6 mouse[10]. In this study, the preventative effect NPE on hair loss in people with alopecia was determined.

MATERIALS AND METHODS

Preparation of NPE: Morus bark, sweet flag, licorice, pine needle, sophora root, cnidium, (*Portulaca oleracea* L.), guava (*Psidium guajava*), gotu cola (*Centella asiatica*) and Korean angelica root were mixed in a ration provided by NJY Biotech Co., LTD. A heating mantle was used for hot water extraction (1:10 w/v). The extract was filtered with a filter study (Whatman No. 2). A rotary vacuum evaporator (EYELA, Rikakiki Co., Tokyo, Japan) was used for vacuum concentration. After vacuum concentration, 7.5% of hot water extract was obtained. It was then mixed with an ointment base provided by NJY Biotech Co., LTD to produce the NPE.

Application of NPE: This study was conducted after obtaining approval from the Institutional Review Board of Kangwon National University, Republic of Korea (IRB, KW-141027-1). A total of 12 subjects with alopecia were enrolled in this study, including 8 men and 4 women in the age of 20-60 years old. They had daily hair loss of more than 100. The NPE was applied twice daily for 10 months to examine its effect on hair loss prevention and hair growth promotion.

Prevention effect on hair loss: At 30, 40 and 60 days after applying NPE, hair lost from individual subject was gathered inside a plastic box twice daily to count the number of hairs. The number of hair loss was expressed as a percentage to that on the initiation date of NPE application. The images related to hair loss and hair growth of the subject before and after NPE application were obtained. Satisfaction over the NPE application by the subject was measured using a 10 point method (1-4 points: Unsatisfactory, 5-7 points: Satisfactory, 7-10 points: Very satisfactory).

Measurement of hair density, length and thickness: At 30, 40 and 60 days after applying NPE, a hair analysis system (PSI 2003, Hair and Sclap Microscope System, SIF-1, Korea) was used to measure the density, length and thickness of the hair with a microscope at $300\times$ magnification. To obtain hair density, the number of hair per 0.6 mm^2 was measured. The length and thickness of hair were measured by the default programs inputted in the device.

Statistical analysis: Data were statistically processed by using SAS program[12]. The average and standard error of each treatment plot were calculated and subjected to analysis of variance. Duncan's multiple range test was used to determine the significance level. Statistical significance was considered when p value was less than 0.05.

RESULTS AND DISCUSSION

At 30, 40 and 60 days after NPE application, the number of hair, hair loss prevention rate and satisfaction level over hair loss prevention were determined. Results are shown in Fig. 1-4. Compared to those at the NPE initiation day, the number of hair loss from the subject was decreased significantly (p<0.05) as the number of days of NPE application was increased. Hair loss prevention rate was increased significantly (p<0.05). After applying NPE for 30, 40 and 60 days, hair loss prevention rates were increased to 33.33, 82.67 and 82.67% respectively, reaching a plateau point after 40 days of application. Regarding the satisfaction over hair loss prevention effect of NPE, 80.24% subjects reported it to be very satisfactory.

Controlling hair loss can be done by controlling follicle cell activity in the anagen using another substance. To prevent alopecia, improving the blood flow around the follicle to supply essential nutrients to the hair root to enable hair growth and using lymphocytes gathered in great numbers around the follicles to improve the immune function and activate the follicle have been used[13,14]. The NPE is a natural plant extract that containing sweet flag[11]. It can stimulate hair growth. It contains asacurin 10 that has great apolecia prevention effect. In Korea, there is a tradition to wash hair in water infused with sweet flag to enable lustrous hair and prevent hair loss. Sweet flag is also known as bacha. The main ingredient of sweet flag extract of essential oil is asarone that has anti-bacterial effect[15]. It has been used in soap exclusively for scalp[16]. The main ingredients of morus bark (*Mori cortex radicis*) are α-amyrin, kaempferol, morin, quericetin and umbellifer one that are known to have hair growth promotion effect on alopecia[17]. For licorice (*Glycyrrhiza uralensis*), its main ingredients are flavonoids, including liquiritigenin, liquiritin, licuraside and glycyrrhizin[18]. The main ingredients of pine needle (*Pinus densiflora*) extract are cumaric acid, terpen, polyphenolic tannin and proanthocyanidin that are known to have alopecia prevention effect[19]. Sophora root (*Sophora angustifolia*) contains various alkaloids and flavonoids, including d-matrine and d-oxymatrine that have effect on hair growth[20]. *Cnidium* (*Ligusticum chuanxiong* Hort) contains volatile essential oil and alkaloids, such as
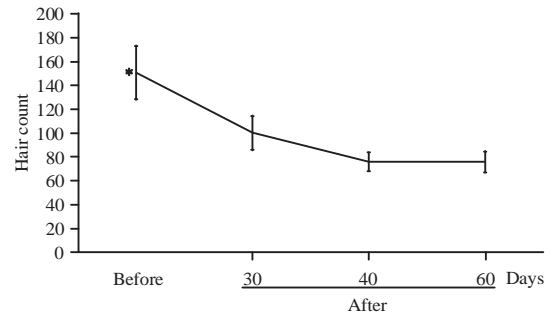

Fig. 1: Changes in hair count before and after application of NPE in peoples with alopecia. Bars represent standard deviation of mean values (n = 8). Significant difference between before and after NPE application (p<0.05)
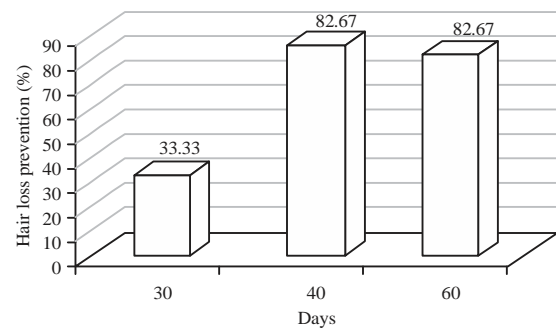

Fig. 2: Percentage of prevention of hair loss after application of NPE in peoples with alopecia
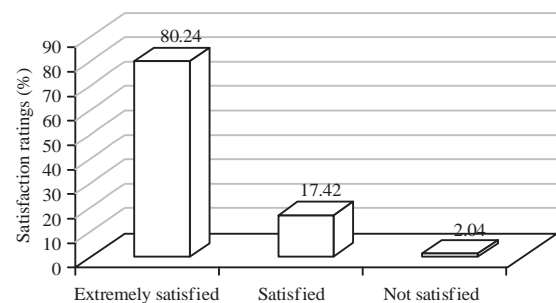

Fig. 3: Satisfaction percentage of NPE application in peoples with alopecia for 30 days (n = 8)

dl-tetrahydropalmatine, protopine, ferulic acid, ligustilide and butylidenpthalide[21]. The main ingredients of Korean angelica root (*Angelica gigas*) are coumarin derivatives (such as decursin, decursinol and nodakenin) and essential oils (α-pinene, limonene, β-eudesmol and elemol) that can keep scalp healthy[22]. As the hair enters anlagen stage, the skin is changed into gray or black[23]. When C57BL/6 mice are applied with NPE, hair starts to grow and the skin is changed from pink to gray flag[11]. It is found that the subject's hair

Before treatment   After treatment

3 months

Age 23
male

3 months

Age 27
male

3 months

Age 33
male

3 months

Age 47
female

10 months

Age 60
male

Fig. 4: Clinical picture before and after NPE application in peoples with alopecia

maintained black color as it grew and their hair maintained strongly without falling out easily.

A study on the mechanism of NPE on hair growth acceleration and alopecia prevention action supports biogenetic revelation control[10]. In other words, NPE can up-regulate the mRNA levels of Vascular Endothelial Growth Factor (VEGF) and Keratinocyte Growth Factor (KGF) to stimulate hair growth, while down regulating
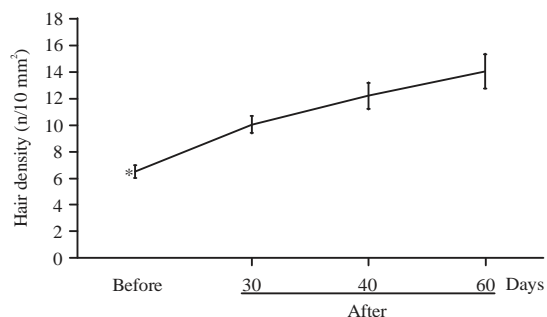
Fig. 5: Changes in hair density before and after application of NPE in peoples with alopecia. Bars represent standard deviation of mean values (n = 8). Sinificant difference between before and after NPE application (p<0.05)
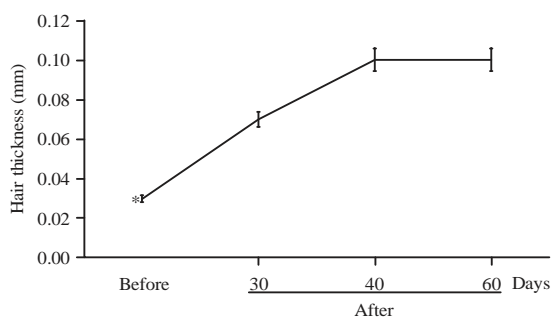

Fig. 6: Changes in hair thickness before and after application of NPE in peoples with alopecia. Bars represent standard deviation of mean values (n = 8). Sinificant difference between before and after NPE application (p<0.05)

Transforming Growth Factor-β1 (TGF-β1) mRNA levels, thus suppressing alopecia[10].

After 20 days of applying NPE, black and strong hair began to grow in this study. After 30, 40 and 60 days of NPE application, images of hair growth and changes in hair density, hair length and hair thickness were obtained. Results are shown in Fig. 5 and 6. After 30, 40 and 60 days of NEP application, the density and thickness of hair were increased significantly. Compared to those before applying NPE, the density of hair after 30, 40 and 60 days of NPE application were 62.92, 97.88 and 128.1% respectively and the thickness of hair were increased 133, 233 and 233% respectively. Meanwhile, when the hair grown after 30 days of NPE application was pulled out with a tweezer, it was found that it could not be pulled out easily. Considering these results, it was verified that NPE application maintained the strength of hair root. In a previous study, after apply Abelmo product produced with NPE on BALB/c mice, hair began to grow after 9 days of application. Compared to those

of the negative control group, the density of the hair after 9, 12 and 15 days of NPE application was significantly increased to 599.8, 166.7 and 136.6% respectively. The length of hair was increased 122.2, 171.4 and 116.9%, respectively, while the thickness of hair was increased 181.8, 200.2 and 180.7% respectively[10], in agreement with the results of this study.

Alopecia occurs through insufficient follicle length during the hair growth period. The thickness of the growing hair is determined by the length of the follicle. Therefore, the length of the follicle growing from the initial period to the middle period of hair growth is very important in normal hair cycle[24]. In anagen stage, the skin is developed on the dermis and subcutaneous tissue, while the follicle is grown on the papillary layer of the dermis[25]. In catagen stage, the thickness of the dermis is reduced in the skin to have the follicle come close to the epidermis while the hair papilla cell inside the follicle regenerates the follicle to accelerate the development of the follicle[26,27]. The follicle grown inside the papillary layer of the dermis passes through the catagen to be close to the epidermis, while the hair pailla cell existing inside the follicle begins the new hair growth cycle. Hair growth control of hair papilla cell is known to be related to the size of hair papilla. As for the follicle cycle, it is known that the hair papilla cell atrophied on catagen can become bloated through the proliferation of the epithelial cell. Therefore, a big hair bulb is formed to cause hair growth[8,13]. The NPE might have played a role in the catagen stage or the anagen stage or the follicle cycle. The exact mechanisms of how NPE could stimulate hair growth merits further study.

CONCLUSION

In summary, According to NPE application days passed, the number of hair loss from the subject was decreased, but hair loss prevention rate was increased up to 82. 67%. After 30, 40 and 60 days of NEP application, the density and thickness of hair were increased and when the hair grown after 30 days of NPE application was pulled out with a tweezer, it was found that it could not be pulled out easily. Considering these results, it was verified that NPE application maintained the strength of hair root.

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