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A Preliminary Study on the Effects of Vitexin on the Promotion of Hair Growth in Mice

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ABSTRACT

In this study, the hair-growth-promoting effects of topical vitexin were compared with those of topical minoxidil in a mouse model. Six-week-old male C57BL/6 mice were randomly treated with 0.2% vitexin, 5% minoxidil, or 60% ethanol as a control. Topical application of vitexin did not result in a significant difference of hair regrowth among groups. In contrast, histopathological data showed an increase in the number of hair follicles for the vitexin-treated group, with an accumulation in the early anagen phase in the dermis. The minoxidil-treated group showed a predominance of late anagen phase in the deep dermis and hypodermis, with some newly formed capillaries and vessel dilation. The hair-growth-promoting effects of vitexin were not significantly different from those of minoxidil. Importantly, vitexin slowly promoted anagen phase but did not induce systemic side effects. This study helps the development of a new therapeutic option for non-scarring alopecia.

Keywords: Androgenetic alopecia; Hair growth; Minoxidil; Mouse model; Vitexin

1. Introduction

Hair loss is a prevalent dermatological condition affecting both men and women. Androgenetic alopecia (AGA) is one of the most common types of non-scarring alopecia and is characterized by a progressive shortening of anagen phase duration and a premature catagen phase, resulting in the progressive shrinking of hair. These effects are related to the presence of dihydrotestosterone in the hair follicle, causing ectopic activation of androgen receptor signaling. This mainly occurs in the dermal papilla, thereby modifying the

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expression of paracrine factors related to hair growth [1, 2].

In addition to androgen-dependent processes, genetic and pathogenic factors, including microbial flora, stress, and micro-inflammation, are involved in the pathogenesis of AGA. Approved therapeutic treatments for AGA, including oral finasteride and topical minoxidil, are still limited in efficacy and are associated with adverse effects, leading to poor compliance. Recently, the discovery of vitexin, a naturally occurring flavonoid polyphenol produced by species of the *Vitex* genus, was reported. In China, vitex spp. have been used in traditional herbal medicine to treat cough, asthma, rheumatism, and arthritis [3, 4] and have been shown to exert various pharmacological effects, including anti-inflammatory and antioxidant effects [5, 6]. Moreover, a recent *in vitro* study showed that vitexin compound-1, which is isolated from vitex, can augment Wnt/β-catenin signaling in human dermal papilla cells (hDPCs) and significantly promote the proliferation of hDPCs [2]. However, the mechanisms and efficacy of vitexin are unclear, and further studies are required.

Accordingly, in this study, the efficacy of hair-growth promotion by vitexin was examined using a mouse model. The results may aid in the development of novel therapeutic agents for patients with non-scarring alopecia.

2. Materials and Methods

2.1 Animals

Five-week-old C57BL/6 male mice were housed under strict hygienic conditions, controlled environmental conditions (12:12-h light:dark cycle, light at 130–325 lux, temperature at approximately 22 ± 1°C, relative humidity at 30–70%), and were provided standard laboratory food and water ad libitum for 1 week. Subsequently, the mice were randomly assigned to the vitexin-treated group ($n = 10$), minoxidil-treated group ($n = 10$), or ethanol-treated group ($n = 10$). The study protocol was approved (approval no. LACTU-C026/2562) by the Animal Ethical Committee of Thammasat University, Thailand.

2.2 Materials

Vitexin was purchased from Sigma-Aldrich (St. Louis, MO, USA; CAS number: 3681-93-4) and dissolved in 60% ethanol for a final vitexin concentration of 0.2%. The positive and negative controls were 5% minoxidil and 60% ethanol, respectively.

2.3 Study design

Mice were anesthetized with inhaled isoflurane (Attane; Piramal, USA) 1 day before the experiment (day 0), and an area of hair on the dorsal skin was shaved under sterile conditions. From days 1 through 21, mice were topically treated once daily with 0.2% vitexin, 5% minoxidil, or 60% ethanol (0.1 mL/mm^2). Photographic data were recorded weekly for 3 weeks using a digital camera (Sony A7 III; Sony Corporation, Japan). Photographic data were analyzed using Photoshop 6.0 software to calculate the surface area ratio of regrown hair to still denuded. At the experimental endpoint, mice were humanely euthanized with maintenance of inhaled isoflurane (Attane; Piramal) to the deep stage of anesthesia, followed by cervical dislocation. After euthanasia, necropsies were performed, and spleen and thymus samples were collected from each mouse for comparison of absolute organ weights between groups.

2.4 Histopathological analysis

Samples were fixed in 10% buffered formalin for 24 h, embedded in paraffin, cut into 3 μm-thick slices, and stained with haematoxylin and eosin (H&E). Hair follicles in an examined area of 6 mm^2 were counted and then classified by hair growth cycle stage (anagen, catagen, or telogen). Angiogenesis and the infiltration of inflammatory cells were measured. All histopathological analyses were performed

by two veterinary pathologists under 4× and 10× magnification using optical microscopy.

2.5 Statistical analysis

Repeated ANOVA with post-hoc Bonferroni were used to compare data obtained from each treatment group at day 0 (baseline), 7, 14, and 21. All measured values are written as mean. All statistical analyses were performed using Stata version 13 (StataCorp LP, USA). Statistical significance in all cases was considered as having a p-value <0.05.

3. Results and Discussion

3.1 Health evaluations and body weights of mice

All mice remained healthy until the experimental endpoint. No significant differences in body weight were observed between the groups of mice on days 0, 7, 14, and 21 (P = 0.697, 0.156, 0.202, and 0.147, respectively).

3.2 Hair regrowth observation

On day 21 after treatment, the vitexin-treated group showed no significant difference in hair regrowth area (74.49% ± 10.47%) compared with the minoxidil-treated and ethanol-treated groups (76.78% ± 9.82% and 63.14% ± 17.04%, respectively; P = 0.096) (Fig. 1).

3.3 Spleen and thymus weights

On day 21, after euthanasia, spleen and thymus weights were measured to assess the basic safety of each treatment. The data showed that spleen weight was significantly higher in the minoxidil-treated group than in the vitexin-treated and ethanol-treated groups (0.08 ± 0.02, 0.06 ± 0.02, and 0.06 ± 0.01, respectively; p = 0.017). No significant differences in thymus weights were observed between groups (p= 0.524).

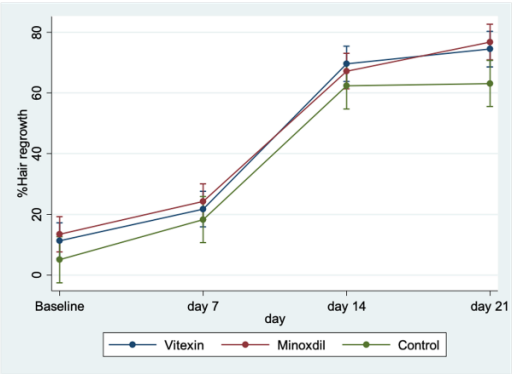

Fig. 1. Comparison of hair regrowth area (%) on days 0, 7, 14, and 21 among the vitexin-treated, minoxidil-treated, and ethanol-treated groups.

3.4 Histopathology

In contrast to the clinical findings, the hair follicle count in the vitexin-treated group was slightly higher than the ethanol-treated group, with most follicles in the early anagen phase in the dermis (60%), followed by late telogen phase in the superficial dermis (40%). In the minoxidil-treated group, the hair follicle count was significantly increased, with most follicles in the late anagen phase in the deep dermis and hypodermis (60%), followed by early anagen phase in the dermis (30%) and telogen phase in the superficial dermis (10%). Ethanol treatment resulted in the fewest hair follicles, with most follicles being in the late telogen phase in the superficial dermis (60%) and few in early anagen phase in the dermis (40%). H&E staining of dorsal skin tissue after 21 days of treatment is shown in Fig. 2 and Table 1.

Table 1. Summary of histopathological results.

Histopathological findings	Vitexin (n = 10)	Minoxidil (n = 10)	Control (n = 10)
Hair Stage			
- Anagen	60%	90%	40%
- Telogen	40%	10%	60%
Number of hair follicles	+	++	=
Blood vessels			
- Newly formed vessels	=	+	=
- Vessel dilatation	+	++	-
Inflammatory cells	+/-	+	+/-
Infundibulum length [ratio]	1.5	2	1

Note: +, slightly increased [increase <50% of baseline]; ++, significantly increased [increase ≥50% of baseline]; -, not found; =, baseline/equal to baseline; +/-, not clearly found.

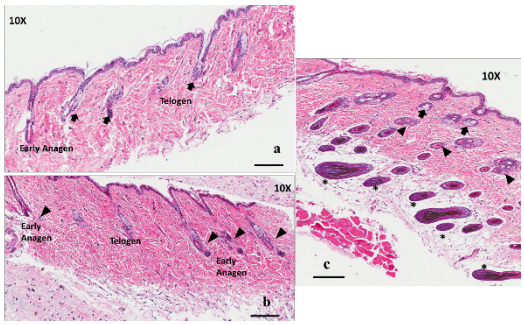

Fig. 2. H&E staining of the dorsal skin tissue after 21 days of topical vitexin, minoxidil, or ethanol application, focusing on growth-cycle stage.
a) Control: mostly found late telogen phase in superficial dermis (arrow) and few early anagen phase in dermis.
b) Vitexin: mostly found early anagen phase in dermis (arrowhead) and some telogen phase in superficial dermis.
c) Minoxidil: mostly found late anagen (asterisk) in deep dermis and hypodermis followed by early anagen phase (arrowhead) in dermis and telogen (arrow) in superficial dermis.

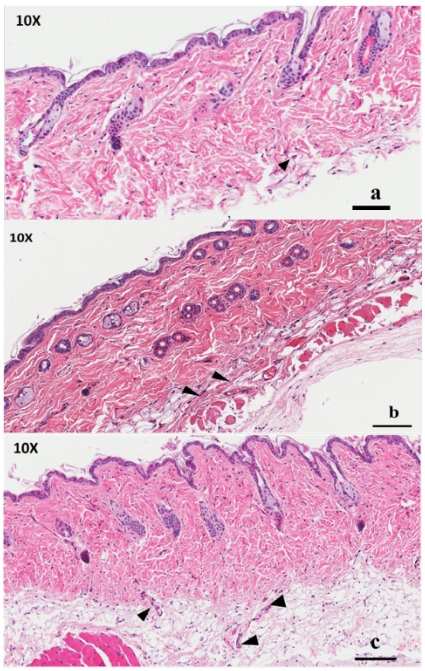

Fig. 3. H&E staining (10× magnification) of dorsal skin tissue after 21 days of topical vitexin, minoxidil, or ethanol application, focusing on newly formed capillaries.
a) Control: normal architecture of blood vessels and sparse newly formed capillaries (arrow).

b) Vitexin: vasodilation (arrowhead) and fewer newly formed capillaries.
c) Minoxidil: some newly formed capillaries and vessels dilation (arrowhead).

H&E staining of skin sections from mice in the vitexin-treated group revealed dilation of vessels and reduced formation of new capillaries when compared with the minoxidil-treated group. In the minoxidil-treated group, some newly formed capillaries and vessel dilation were observed. In contrast, in the ethanol-treated group, few newly formed capillaries were observed, and vessel dilation was not detected. These results are shown in Fig. 3.

Further results of histopathological analyses, focusing on inflammatory cells, showed that inflammatory cell infiltration in the dermis was reduced in the vitexin-treated and ethanol-treated groups. In contrast, in the minoxidil-treated group, inflammatory cell infiltration was reduced in the dermis and was moderately present in the perivascular area.

Finally, the infundibulum length was increased (1.5:1) in the vitexin-treated group compared with those in the ethanol-treated group. The longest infundibulum length was observed in the minoxidil-treated group. The results are shown in Fig. 4.

3.5 Discussion

In contrast to findings of previous studies, topical application of 5% minoxidil solution on shaved dorsal skin of C57BL/6 mice did not induce significant hair regrowth within 21 days of application. However, histopathological results in minoxidil-treated mice showed that most hair follicles had already reached the late anagen phase in the deep dermis and hypodermis. These findings suggested that the minoxidil solution was actively working. In this regard, it is possible that the skin of the mice in this study may have been thicker than that of the mice used in previous studies. As such, the growing hairs would have required a longer period of time to pass through the skin and emerge to cover the area of skin that had been shaved. Supporting this possibility, some studies have shown that significant hair regrowth can occur within 28 days [7]. Thus, in order to observe significant hair regrowth, a longer time period of topical solution application may be required.

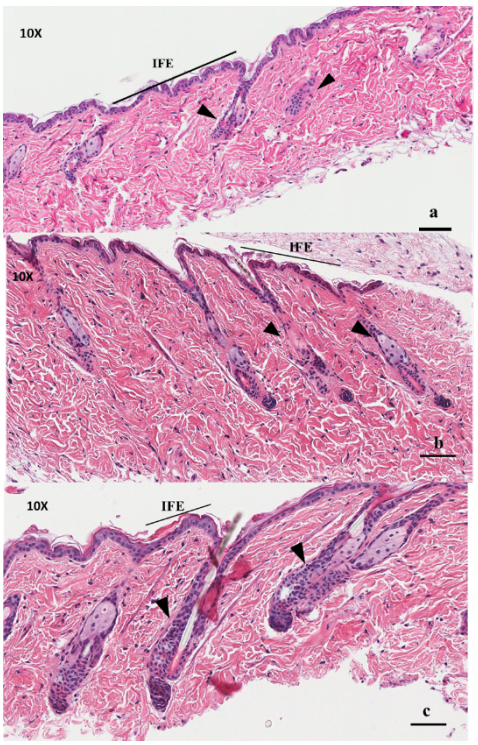

Fig. 4 H&E staining results (10× magnification) for dorsal skin tissues after 21 days of topical ethanol (a), vitexin (b), and minoxidil (c) application, focusing on the interfollicular epidermal thickness (IFE) and infundibulum length (arrowhead).

Although visual analysis of hair regrowth did not reveal statistically significant differences compared with the positive and negative control groups, vitexin treatment did increase the number of hair follicles and result in a predominance of early anagen phase follicles in the deep dermis. Thus, the histopathological results showed that the hair growth-promoting effects of topical vitexin solution were comparable to those of topical minoxidil

solution. The length of the hair infundibulum on day 21 was higher in the minoxidil- and vitexin-treated groups, and was greater in hairs in the anagen phase than it was for other phases of growth. No increase in hair regrowth was observed in the ethanol-treated group.

Further histopathological analyses also showed that minoxidil exhibited properties characteristic of an antihypertensive vasodilator drug and caused an increase in vasodilation when compared with vitexin and ethanol. Minoxidil can also upregulate the expression of vascular endothelial growth factor [8-13]. Consistent with these findings, minoxidil resulted in the greatest number of newly formed vessels when compared with vitexin and ethanol, and although some vessel dilation was observed in the vitexin-treated group, the number of newly formed vessels did not differ between the vitexin- and ethanol-treated groups. Accordingly, vitexin may exert vasodilatory effects via the activities of various cytokines, such as nitric oxide [14-15].

Interestingly, few inflammatory cells were found in the dermis in all groups. However, in the minoxidil-treated group, inflammatory cells were moderately abundant in the perivascular area. In previous studies, vascular injury with perivascular inflammatory cell infiltration has been shown to occur in swine and dogs treated with minoxidil [16, 17]. Thus, topical application of minoxidil may cause some vascular injury to the treated area.

According to histopathological results, vitexin treatment stimulated hair growth by causing the hair follicles to progress from the telogen phase to the anagen phase. However, the hair growth-promoting effects of vitexin were lower than those of minoxidil in this study. This result may be related to the concentrations of the two chemicals used in this study. Indeed, vitexin was applied at a concentration of only 0.2%, whereas minoxidil was applied at a concentration of 5%. Thus, the use of a higher concentration of vitexin solution may enhance the hair growth-promoting effects.

Interestingly, minoxidil treatment also caused significant changes in spleen weight, whereas no differences were observed in the vitexin- and ethanol-treated groups. For topical medications with local effects, systemic side effects are unacceptable. Concerning the topical minoxidil solution, a previous study showed that minoxidil did not localize in specific cells of hair follicles but rather accumulated in other organs, including the spleen, liver, and kidneys [18]. Moreover, in another study that looked at the hair growth-promoting effects of topical lavender oil application compared with minoxidil in C57BL/6 mice [7], it was shown that minoxidil treatment resulted in significant changes in spleen weights. As such, it may be that topical application of minoxidil has systemic side effects on the spleen.

4. Conclusion

In conclusion, the hair regrowth observations, histopathological analysis, and organ weight results from this study demonstrated that vitexin does not exert hair growth-promoting effects that were significantly different from those of minoxidil; however, vitexin treatment did result in a slower transition to the anagen phase, while also having no systemic side effects involving spleen enlargement. These findings could support carrying out further studies allowing open access to application in human clinical trials. The limitations of this study were low sample sizes, missing tests of dose response effects, and the experiment animal not being a model of AGA. Further studies should focus on hair growth-related proteins and growth factor expression according to vitexin treatment which will provide further insight on the underlying hair growth-promoting mechanism.

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