



STUDY RESULTS OF PHARMACOLOGICAL EFFECTS OF HAIR GROWTH SOME PLANTS EXTRACTS AFFECTING HAIR GROWTH

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KEYWORDS

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ABSTRACT

To investigate the effect of various plant extractions on hair growth in Wistar rats, experimental animals were randomized into eight groups (n = 4). The study evaluated the hair-promoting activity of extracts from *Urtica cannabina* L, *Achillea millefolium*, *Glycyrrhiza glabra*, Saw palmetto, and *Tribulus terrestris*. A 12 cm² area (with horizontal length of 3 cm and longitudinal length of 4 cm) of hair was shaved from the dorsal portion of rats whose hair follicles were synchronized in the telogen stage.

Comparing the experimental groups with the hair growth of the control group, the study found that rats treated with *Tribulus terrestris* L. extract exhibited reduced skin inflammation, increased hair growth compared to other groups, and showed enhanced hair regeneration. Additionally, all animals treated with *Urtica cannabina* L. extract, *Glycyrrhiza glabra* extract, and *Tribulus terrestris* L. extract for 28 days exhibited uniform hair growth compared to other groups.

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These findings suggest that specific extracts of *Tribulus terrestris* and *Urtica cannabina* L. are superior in promoting hair growth compared to other variants.

Further research could explore optimal ratios for combining these extracts into formulations for hair growth preparations.

INTRODUCTION

A person loses 50-100 hairs per day. It usually falls off when combing, washing, getting up from sleep, and sticking to clothes every day. The life cycle of the hair is over, and the metabolism is going on, which is about to push out new hair. In this way, even if the hair falls out, it regenerates and grows back. Therefore, your hair should not become thin or bald. But if it really falls out more than normal, it is called hair loss disorder. Hair loss can affect a person's emotional well-being and attraction. There are many reasons for hair loss, including nutritional deficiencies, aging, hormonal imbalances, and stress etc^{1,2}. To date, the number of people suffering from hair loss or baldness has increased dramatically, regardless of age or gender³. The search for treatment results into few drugs of synthetic origin, but side effects associated with them cannot be neglected^{4,5}. Although the hair loss drugs finasteride and minoxidil have been approved by the Food and Drug Administration, their efficacy is limited and transient due to unpredictable efficacy and side effects⁶. Therefore, it is urgent to develop more and better treatment options, and the search for natural products with hair growth promoting potential is continuing. Natural products in the form of herbal formulations are available on the market and are used as hair tonics, hair growth promoter, hair conditioners, hair-cleansing agents, antidandruff agents, as well as for the treatment of alopecia and lice infections⁷. Many of the medicinal and therapeutic effects of medicinal plant are due to their active ingredients. However, the active ingredients of the same type of medicinal plants grown in different countries vary. It is inextricably linked with the geographical system of the region. In this, some herbs that are believed to reduce the rate of hair loss and at the same time stimulate new hair growth, increased blood supply, follicular enlargement, and prolong the anagen phase, as well as aromatherapy by which some plants extract and their phytoconstituents inhibit the hair loss or promote hair growth^{8,9}. In fact, it is important to

note that these plants are recommended in traditional medicine¹⁰. Therefore, in our research, we aimed to compare some plant extracts grown in Mongolia to promote hair growth.

MATERIALS AND METHODS

Most plants were collected from Botanical Garden of Drug Research Institute in Mongolia. The plant was confirmed by the Institute of Biology of the Mongolian Academy of Sciences (Ulaanbaatar, Mongolia). Minoxidil were purchased from Sigma Chemical company (Korea). Saw palmetto (45 and 25% fatty acid) dry extract were purchased from China.

Preparation of extractions: The plants of *Urtica cannabina* and *Tribulus terrestris* were extracted with 30% ethanol by maceration method and liquid extract was dried by spray drying method. *Glycyrrhiza glabra* was dried under the shade, then in an oven at 30°C, and thereafter reduced to powder form. The powdered material (100 g) was extracted in a Soxhlet apparatus with 50% ethanol (60-80°C) for 50 h. The extract was filtered and dried under reduced pressure to give a dry yield of 1.6 %. w/w. *Achillea millefolium* leaves are finely ground and extracted in hot water for 4 hours in a refrigerator. Repeat this process 4 times. Cool the extract and filter. The filtrate was dried in a vacuum evaporator at 40°C.

Experimental animals: Male Wistar rats (16-week-old, 180-200g) used from Drug Research Institute in the Vivarium (Ulaanbaatar, Mongolia). Rats were housed in cages under a condition of 12-h light/ dark cycle and maintained on standard rat food and water. The cage temperature and humidity were 23±2°C 35-60% respectively¹¹.

Hair length determination: Regrown hairs were plucked from representative areas in shaved dorsal center parts of rats on 21 and 28 days. We calculated the average hair length from 15 hairs per rats^{12,13}.

Experimental studies protocol: Thirty-two animals in 8 randomized groups ($n = 4$) were used some plants to studying the hair promoting activity of *Urtica cannabina* L, *Achillea millefolium*, *Glycyrrhiza glabra*, Saw palmetto and *Tribulus terrestris*. A 12 cm² area (horizontal length, 3 cm; longitudinal length, 4 cm) of hair was shaved from the dorsal portion of a Wistar rat with an animal clipper at 16 weeks of age, at which rats' hair follicles were synchronized in the telogen stage. While animals in the control group received distilled water (200 µl) with an equal volume of mixture containing glycerol and, other groups *Urtica cannabina* L extract, *Achillea millefolium* extract, *Glycyrrhiza glabra* extract, *Glycyrrhiza glabra* ethanol extract, *Serenoa repens* (Saw palmetto) extract, *Tribulus terrestris* extract and Minoxidil (200 µl), respectively, received an equal volume of the same mixture described. Extractions were applied topically to the dorsal skin for 28 days using a syringe plunger with the same strokes. Animals were kept in isolation for a certain amount of time and then housed back to separate cages. At 28th day, rats were sacrificed to obtain skin specimens for histological preparation. Visible hair growth was recorded at 0, 7, 14, 21 and 28 days^{14,15}.

Histological preparation: The dorsal skin of sacrificed rat was fixed with 10% neutral buffered formalin at 4°C for 24 h and washed with PBS (pH 7.4). Fixed samples were dehydrated through an ascending series of graded ethanol, cleared in xylene, and embedded in paraffin blocks. Subsequently, samples were cut either longitudinally or transversely into 5-µm-thick sections and mounted on gelatin-coated glass slides^{14,16}.

Quantitative histomorphometry: Skin biopsies were fixed with 10% neutral formalin for routine histology, paraffin-embedded, and processed for hematoxylin-eosin staining. Individual hair follicles were confined to specific hair cycle stages (telogen or anagen I–VI), based on the classification of Chase. The percentage of

hair follicles in each defined cycle stage at 28 days was calculated 14,6,17.

Hematoxylin-Eosin staining: To observe the histological change after topical application of plant extracts, sections were stained with hematoxylin and eosin. Briefly, sections were deparaffinized with xylene, hydrated in a descending series of graded ethanol, and stained with hematoxylin for 2 min, followed by washes for 2 min and eosin staining for 5s.

RESULTS

The rats of the experimental study groups were evenly shaved to a size of 12 cm² and the test site was prepared. All the eight groups of animals were observed for hair growth initiation and completion time. Extracts treated animals showed significant reduction in hair growth initiation and completion time as compared to control and minoxidil treated animals.

Comparing the experimental groups with the hair growth rats of the control group, the study group used *Tribulus terrestris* L. extract, which has been shown to reduce skin inflammation, grow more hair than other groups, and increase hair regeneration. The skin of untreated control rats was reddened and bruised (Figure 1). Also, it can be observed that all the animals in the 28 days treated *Urtica cannabina* L. extract, *Glycyrrhiza glabra* extract and *Tribulus terrestris* L. are growing evenly compared to other groups.

Wistar rats were shaved and topically applied with control ($n=4$, Ctrl), Fig 1: B-21 days and Fig 1: C-28 days. Hair growth-promoting effects of some plant's extracts. The length of regrown hair after shaved. Values are mean $\pm$ standard deviation ($n=15/\text{rat}$ * $p<0.001$, vs. control group): *Urtica cannabina* L extract ($n=4$, UC extract), *Achillea millefolium* extract ($n=4$, AM extract), *Glycyrrhiza glabra* extract ($n=4$, GG extract), *Glycyrrhiza glabra* ethanol extract ($n=4$, GG ethanol extract), Saw palmetto extract ($n=4$, SP extract), *Tribulus terrestris* extract ($n=4$, TT extract), and Minoxidil ($n=4$).

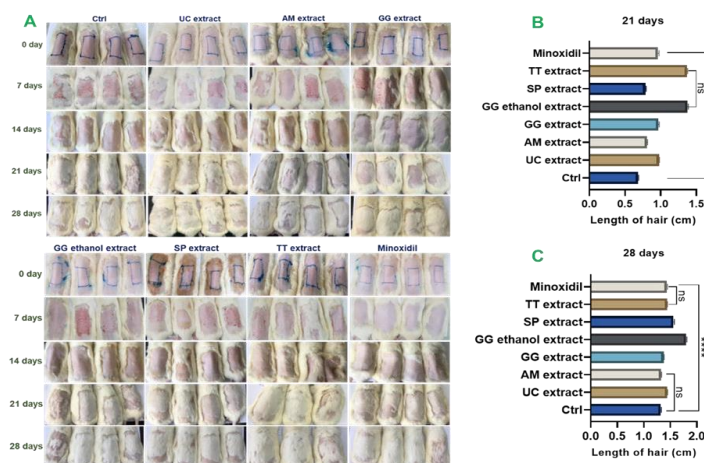


Figure 1. A. Hair growth-promoting effects of some plant's extracts.

According to Figure 1, the length of hair grown in the area prepared on the back of experimental rats at the 21 days when preparations affecting hair growth were applied was 42% greater in the *Urtica cannabina* L extract, 40.5% greater in the *Achillea millefolium* extract, the *Glycyrrhiza glabra* extract has 13% more growth, the *Glycyrrhiza glabra* ethanol extract has 44.9% more growth, the Saw palmetto extract has 42% more growth, the *Tribulus terrestris* extract has 98.5% or almost twice as long growth, and the Minoxidil has 37.6% more hair growth than the control group, respectively. But when

applied all preparation at 28 days experimental rats' hair length was 9.8% more growth in *Urtica cannabina* L extract, 4.5% more growth in *Achillea millefolium* extract, 18.1% more growth in *Glycyrrhiza glabra* extract, and 2.3% more growth in *Glycyrrhiza glabra* ethanol extract 9.1% more growth in Saw palmetto extract, 8.3% more growth in *Tribulus terrestris* extract more hair growth than the control group, respectively. When applied *Urtica cannabina* L. extract to the shaved skin of test rats for 28 days however hair isthmus was not formed to reach the outer surface of the skin yet, and a hair shaft regenerated with hair follicles composed of epithelial.

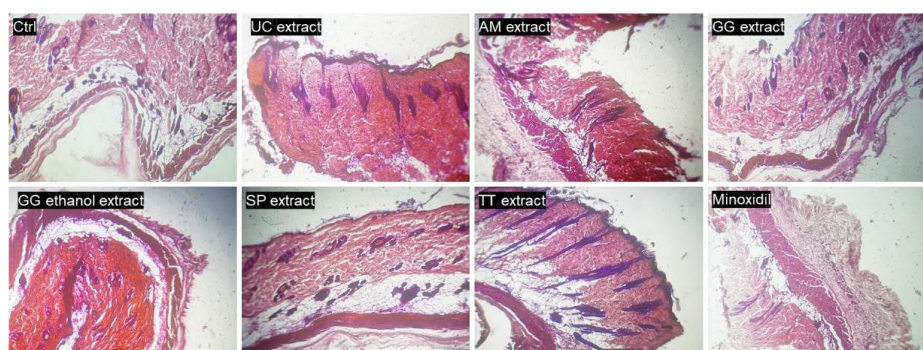


Figure 2. Hair growth-promoting effects of some plant's extracts.

Histological image of Wistar rats' skin were applied with *Urtica cannabina* L extract (n=4, UC extract), *Achillea millefolium* extract (n=4, AM extract), *Glycyrrhiza glabra* extract (n=4, GG extract), *Glycyrrhiza glabra* ethanol extract (n=4, GG ethanol extract), Saw palmetto extract (n=4, SP extract), *Tribulus terrestris* extract (n=4, TT extract), and Minoxidil (n=4).

Conditions for hair growth have been established, or hair follicles for hair regeneration have started to form, but it is observed that the regeneration of the bark, medulla, and scales of the scalp is slow. The hair follicles are not fully raised to the surface of the skin. *Tribulus terrestris* extract applied to the hair structure and skin layers of experimental rats showed division of matrix cells and the morphogenesis of hair infundibulum that migrated from the hair bulb to the skin surface. The hair follicle cells are regenerated, and the epithelial cells are seen to be transformed into the follicle. A hair follicle is formed, and the hair shaft rises to the surface of the skin. The stem cells of the epithelial sac have divided and formed a matrix of the correct shape.

DISCUSSION

Hair loss disorders, although not life-threatening, are a debilitating condition that leaves patients vulnerable. Minoxidil has been reported to be effective in promoting hair growth by transitioning hair follicles into the anagen phase in androgenetic alopecia, but the drug has side effects such as itching, dryness, patches, and dermatitis irritation. On the other hand, because of the drugs unwanted side effects and less effective treatment of hair loss and thinning, the use of conventional drug therapy is limited. There is a growing focus on herbal remedies that have hair-promoting properties with little or no side effects or toxicity. Due to many of the medicinal and therapeutic effects of medicinal plants are due to their active ingredients. In this some herbs that are believed to reduce the rate of hair loss and at the same time stimulate new hair growth increased blood supply, follicular enlargement and prolongation of anagen phase, as well as aromatherapy by which some plants extract and their phytoconstituents inhibit the hair loss or promote hair growth.^{9,18,19}. Although in our study, when determining the effects of the plant extracts that we use on hair growth, the hair growth activity of each drug was found proportional to the concentration range tested and compared with standard (2% minoxidil ethanolic solution) by an enlargement of follicular size and prolongation of the anagen phase. It holds the promise of potent herbal alternative for minoxidil.

Certainly, the plants we researched are mentioned in the results of many foreign and domestic studies. For instance, *Glycyrrhiza glabra* L Deb Roy Saumendu and Sukirti Upadhyay have been used to treat *G. glabra* with hydro-alcoholic extract and petroleum ether extract of *G. glabra* roots. The results showed longer hair than those control which minoxidil^{20,21}. Among the plants used in the formulation, the group Urticaceae is the most widely studied. Stinging nettle (*Urtica dioica* L.) belongs to the family Urticaceae. *Urticadioica* L chemical components that are mainly soluble in ethanol are flavonoid, polyphenol, lectins, sterol, lignans, beta carotene, ketone, palmitic and steric acid, oleic acid, etc. Previous research has shown polyphenols and some flavonoids have been reported to hair growth promoting effect by the proliferation of hDPCs with ROS scavenging and increasing of growth factors IGF-1 and VEGF, also confirmed hair re-growth promotion in mice. *Urticadioica* L ethanol extracts enhanced hair shaft elongation, reduced catagen transition and significantly increased the Ki-67+ matrix keratinocytes compared with the vehicle control and water and hexane extracts²². Yang Liu the positivity rate of M-SH expression in the hair follicle melanocytes was 75% in mice treated with the extract, significantly higher than the rate of only 18.75% in the control group. The positivity rate of M-SH expression in the hair follicle melanocytes was 75% in mice treated with the extract, significantly higher than the rate of only 18.75% in the control group. The aqueous extract of *Tribulus terrestris* can significantly increase M-SH expression in the hair follicle melanocytes by activating tyrosinase activity and promoting melanocyte proliferation, melamine synthesis, and epidermal migration of dormant melanocytes²³. The results of the researchers confirm our research that extracts of *Urtica cannabina* L, *Glycyrrhiza glabra*, and *Tribulus terrestris* plant extracts outperformed the experimental animal control group.

CONCLUSIONS

In conclusion, our report is the first to show that *Tribulus terrestris* extract is used to reduce inflammation,

stimulate hair growth, and accelerate the development of hair growth by anagen phase in telogenic Wistar rats. As well as that *Urtica cannabina* L. extract ranked next in terms of hair growth by stimulating epithelial division and promoting new hair growth by creating a new matrix. Taken together, these results suggest that select extracts of *Tribulus terrestris* and *Urtica cannabina* L, which are superior to other variants in terms of hair growth, and mix them in a certain ratio to form preparations.

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