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ORIGINAL STUDY

A Comparative Study Examining the Effects of Nifedipine and Minoxidil on Stimulating Hair Growth Using a Mouse Model

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ABSTRACT

Hair loss is a common human scalp condition caused by chemotherapy, chronic diseases, and aging. Currently, it is estimated that approximately 50% of males and 15–30% of females experience hair loss issues. Minoxidil is considered a preferred treatment for hair loss in men and women. Nifedipine is effective in improving blood circulation and blood supply via its vasodilator action. To compare nifedipine and minoxidil in terms of their effect on stimulating hair growth using a mouse model. Fifteen female albino mice, aged 8 to 10 weeks, were randomly assigned to three groups of five mice each, all with shaved dorsal skin, for a 21-day experiment. Group 1 (untreated intact control) consisted of healthy mice that experienced the same conditions as the other groups without receiving any treatment. Group 2 (Positive control) contained mice that received a standard drug, minoxidil solution at a 2% concentration, administered topically once daily for 21 days. Group 3 included mice that received topical treatment with nifedipine gel (0.3% w/w) once daily for 21 days. Hair samples were collected on days 14 and 21. Nifedipine cream and standard minoxidil solution 2% increased hair growth when compared with an untreated control group, and the hair of the mice group treated with nifedipine cream on days 14 and 21 of the treatment course was significantly longer than all the other mouse groups treated with minoxidil solution 2%. The current study shows that nifedipine cream exhibits hair growth-promoting effects, with the best result being observed with the use of nifedipine topical cream in a strength of 0.3%.

Keywords: Hair loss, Calcium channel blockers, Nifedipine, Minoxidil

1. Introduction

Hair loss is a common human scalp condition caused by chemotherapy, chronic diseases, and aging. Currently, it is estimated that approximately 50% of males and 15–30% of females experience hair loss issues. Losing hair becomes a problem when the rate of loss exceeds that of regrowth, leading consequently to alopecia, or in instances when the regrowth rate is weak owing to abnormality in the hair growth cycle, as can be seen in androgenic alopecia (AGA) and chronic alopecia areata (CAA) [1, 2].

Alopecia (baldness), despite not being a life-threatening condition, is one of the most common dermatological disorders in which people lose some or all hair on the scalp and sometimes on the entire body. It has affected an increasing number of people for more than 2000 years and represents a remarkable problem of cosmetology [3, 4].

Hair loss management is essential part of clinical dermatology owing to the prevalence of hair loss disorders and their great impact on patient's mental health, self-respect and overall quality of life [5]. Is new, minimally invasive, office-based procedure that

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is based on injecting autologous blood centrifugation product containing high concentrations of platelets in a small volume of plasma to the area of hair loss to promote hair growth via stimulating angiogenesis and vascularization as well as the entry and prolongation of anagen phase of the hair cycle since PRP contains various growth factors in alpha granules of the platelet that participate in hair growth process as VEGF, epidermal growth factor (EGF), insulin like growth factor (IGF), interleukin-1 (IL-1) and TGF [6].

Several plant extracts proved to exhibit hair growth promoting effects in animals via several mechanisms [7]. The technique involves taking plugs of normal occipital hairs (donor site hair) that are resistant to androgen action and transplanting them into bald areas (recipient site), it is used in AGA and eyebrows transplantation, however, it has several limitations as it is invasive, painful, expensive and involve redistribution of only the small area of remaining hairs to cover a much larger recipient site hence there is shortage in exact number of hairs available for transplantation that requires good and long-term planning to achieve good results [8].

Minoxidil (MXD) is considered a preferred treatment for hair loss in men and women. MXD is a derivative of pyrimidine that acts as a peripheral vasodilator; it was originally approved in 1970 for reducing blood pressure. MXD opens potassium channels, releases nitric oxide (NO), and increases blood flow to hair follicles, altering the pathway of prostaglandins and subsequently suppressing the alopecia pathway. Chronic oral intake of MXD can lead to adverse skin reactions, resulting in therapy discontinuation and reduced patient compliance [9].

Nifedipine is a calcium channel blocker (CCB) that belongs to the third generation, which acts on voltage-gated L-type calcium channels, having a greater ratio of vascular smooth muscle effect relative to cardiac effect [10].

Thus, the improvement in the local microcirculation and enhancement of HF blood supply represents one of the important mechanisms to treat hair loss, and based on this, several studies carried out using the vasodilators calcium channel blockers revealed induction of hair growth through in vitro and in vivo experiments via improving peri-follicular microcirculation [11].

In such way and based on previous studies, it is hypothesized that nifedipine may promote hair growth via their vasodilator property. Drug also exhibit antioxidant, anti-apoptotic and anti-inflammatory effects which proved to play roles in hair growth promotion.

2. Materials and methods

2.1. Experimental procedures

This experimental control study was conducted at the Pharmacy College of Tikrit University in approval number SREC20. Fifteen (female) albino mice to avoid hormonal disorders (androgenic alopecia), aged 8 to 10 weeks, were randomly divided into three groups and housed in spacious polypropylene cages with wood shavings for bedding, which were replaced weekly. Proper ventilation and experimental conditions, including suitable humidity, an ambient temperature of 25 ± 2°C, and 12-hour light/dark cycles, were maintained as consistently as possible throughout the entire experimental period, with normal feeding using standard pellets and water provided. Any stressful conditions that could impact the results were avoided, and no experiments were conducted during the first week to allow the animals to acclimatize to their shelter and adjust to the environment by local and international scientific ethical standards [12].

2.2. Experimental design

Each group consisted of five mice, whose dorsal skins were shaved and subjected to the experiment for 21 days. The mice's dorsal hair, which covered an area of approximately 4 centimeters in length and 2 centimeters in width, was shaved using an electric shaver a day before the experiment, followed by smearing the shaved area with a hair removal cream to eliminate all hairs. Topical formulations of drugs were applied once daily, possibly at the same time, on the shaved area of the dorsal skin of two groups of mice for 21 days, while the intact control group received no treatment. The nifedipine formulation was protected from photodegradation due to its photosensitivity to UV light [13].

Fifteen female albino mice, aged 8 to 10 weeks, were randomly assigned to three groups of five mice each, all with shaved dorsal skin, for a 21-day experiment.

The groups were defined as follows:

- Group 1 (untreated intact control), consisting of healthy mice that experienced the same conditions as the other groups without receiving any treatment.
- Group 2 (Positive control) contained mice that received a standard drug, minoxidil solution at a 2% concentration, administered topically once daily for 21 days.

- Group 3 included mice that received topical treatment with nifedipine cream (0.3% w/w, India MART) once daily for 21 days.

The measurement of hair length was performed on days 14 and 21 of the treatment course. 20 hairs were pulled indiscriminately from the previously hairless region of all mice dorsal skin using sterile forceps after being anesthetized with ketamine/xylazine (at a dose 50 milligrams per kilogram of ketamine and 10 milligrams per kilogram of xylazine) via intraperitoneal (IP) route [14].

Then the average lengths of the randomly chosen 20 hairs were measured in millimeters via the digital vernier caliper. Hair weight measurement was done following 21 days of treatment course. After euthanasia of the animals via an overdose of diethyl ether, a small portion (1 cubic centimeter) of previously shaved and treated dorsal skin region was dissected by a sterile surgical blade from the same position as much as possible in all mice, and the weight of the skin with hair was measured using a sensitive electronic balance and recorded in grams. The skin was smeared with hair removal cream to ensure the removal of all hair. The weight of skin without hair was then measured, and the difference in the weight was recorded and reported in milligrams as the net weight of the new regrown hair [15].

3. Results

3.1. Average weight

In comparison between the untreated control and the treated groups, the mean weight of mice was higher among all treated groups when compared to the untreated control group due to hormonal modulation or improved peripheral circulation.

Table 1. Average weight of mice.

Groups	Day of initiation	Day of completion
Group control	21 g	23 g
Minoxidil 2%	20 g	26 g
Nifedipine 0.3%	22 g	28 g

3.2. Quantitative evaluation of hair growth

In comparison between the untreated control and all treated groups, the mean hair weight following 21 days of treatment initiation was higher in all treated groups than in the untreated control group.

The data reported in Table 3 suggests a statistically significant increase in hair growth across all groups throughout time, with the Nifedipine group displaying the highest mean hair growth on both day 14 (6.32 ± 0.13 mm) and day 21 (9.40 ± 0.12 mm).

Table 2. Average hair weight growth of three groups of mice.

Group	Day 21
Control Group	22 grams
Minoxidil 2%	23 grams
Nifedipine 0.3%	25.5 grams

Table 3. Average length of hair growth of three groups of mice.

Day	Group	Mean ± SE	p-value (ANOVA)
Day 14	Control	4.50 ± 0.07	$P < 0.001$
Day 14	Minoxidil	5.26 ± 0.09	
Day 14	Nifedipine	6.32 ± 0.13	
Day 21	Control	5.00 ± 0.14	$P < 0.001$
Day 21	Minoxidil	7.34 ± 0.11	
Day 21	Nifedipine	9.40 ± 0.14	

The Minoxidil group followed with intermediate results, while the Control group demonstrated the lowest growth (4.50 ± 0.07 mm and 5.00 ± 0.14 mm for day 14 and day 21, respectively).

One-way ANOVA produced p-values less than 0.001 for both time points, confirming significant differences in hair growth among the three treatment groups. The increase in mean hair length from day 14 to day 21 within each group further implies a time-dependent effect of the applied drugs, notably noticeable in the Nifedipine and Minoxidil groups.

Table 4. Comparison of the length of hair growth of three groups of mice.

Day	Comparison	p-value
Day 14	Control vs Minoxidil	$P < 0.001$
Day 14	Control vs Nifedipine	$P < 0.001$
Day 14	Minoxidil vs Nifedipine	$P < 0.001$
Day 21	Control vs Minoxidil	$P < 0.001$
Day 21	Control vs Nifedipine	$P < 0.001$
Day 21	Minoxidil vs Nifedipine	$P < 0.001$

The ANOVA results are further validated by the pairwise t-test results shown in Table 4. All treatment group comparisons (Control vs. Minoxidil, Control vs. Nifedipine, and Minoxidil vs. Nifedipine) showed extremely significant differences at both time points ($P < 0.001$). When compared to minoxidil and the control group, these pairwise differences demonstrate how much more effective nifedipine is at promoting hair growth.

Notably, the consistency of significant p-values across all comparisons and both days improves the trustworthiness of the observed patterns and supports the conclusion that both Minoxidil and Nifedipine have different and improved effects on hair growth, with Nifedipine being the most effective.

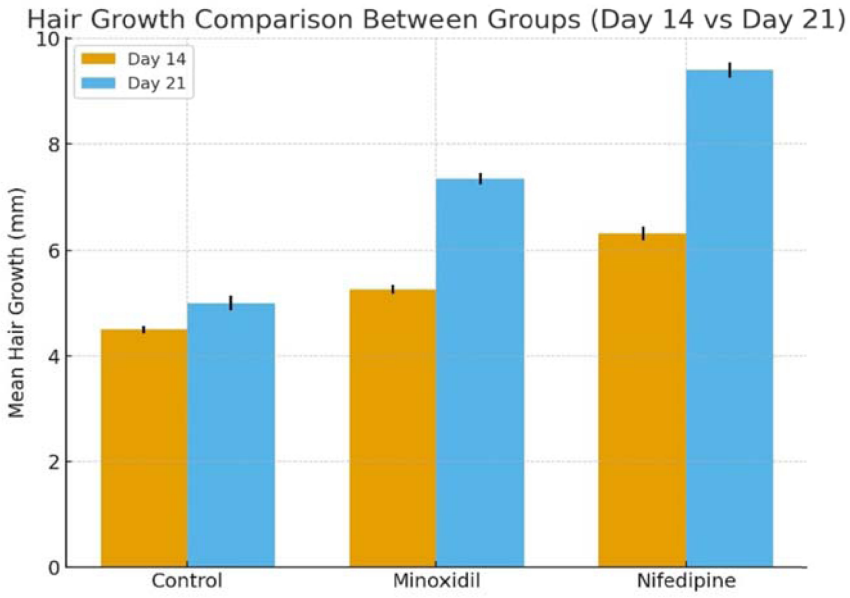


Fig. 1. Hair growth comparison between control, minoxidil, and nifedipine groups.

3.3. Qualitative evaluation of hair growth via the photograph

The minimum time for hair growth promotion and completion on the previously shaved dorsal skin was visually observed via the photograph, and the results are shown in Fig. 1.

4. Discussion

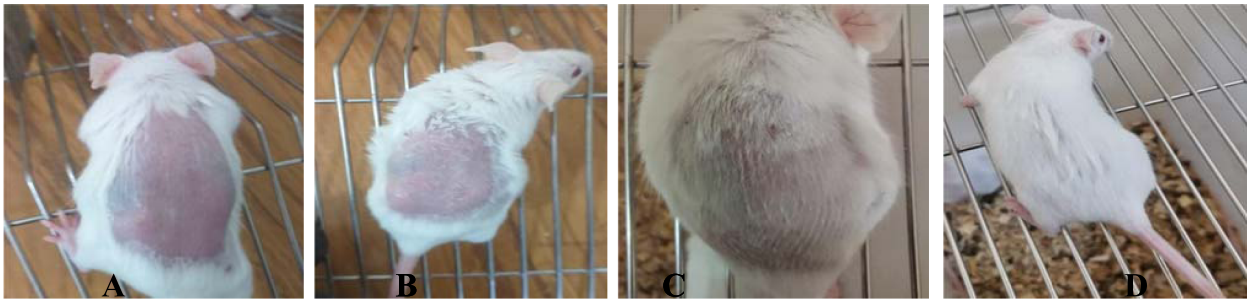
Hair loss disorder is considered one of the issues that should be treated permanently, as people's interest in hair and beauty has largely increased in recent years [16]. Currently, researched hair loss treatment focuses mainly on agents that can prevent hair loss and promote hair growth by enhancing the HF oxygen and nutritional supplement, and by getting rid of the harmful elements via promoting peri-follicular vasodilation, thereby decreasing stress on HFs and by inducing angiogenesis [17].

Measuring the length of newly grown hair represents an important means for the confirmation of hair growth induction by the drugs used in the current study [18]. The results revealed that nifedipine 0.3% and standard 2% minoxidil solution significantly increased hair length when compared to untreated control group and that the hair of mice groups treated with nifedipine 0.3% on days 14 and 21 of treatment course was significantly longer than all mice groups, even longer than the hair of mice group treated with the standard 2% minoxidil solution as revealed to occur to hair of mice treated with minoxidil by other study [19].

The results of the current study agree with the previous study carried out by Juhász and Mesinkovska on the naturally occurring phosphodiesterase inhibitor caffeine, which resulted in hair elongation via enhancing local skin microcirculation. It also finds agreement with a study carried out on the vasodilator cilostazol, which, via improving cutaneous blood flow, resulted in hair growth promotion and elongation of HS [20].

Nifedipine is known to cause vasodilation via different mechanisms dependent on the spontaneous synthesis and bioavailability of nitric oxide (NO), which is an endothelium-dependent acute vasodilator that was identified by Furchgott and Zawadzki and known to cause greater arterial distension and reduction in peripheral vascular resistance and hence greater blood supply [21].

Furthermore, nifedipine exerts its vasodilator effect (mainly on arterioles) by binding to voltage-dependent L-type calcium channels, thereby inhibiting calcium influx into vascular smooth muscle cells [22], where calcium combines with intracellular calmodulin. The calcium-calmodulin complex subsequently converts myosin light-chain kinase (MLCK) enzyme to its active form (MLCK*), which is responsible for the phosphorylation of myosin light-chain, which interacts with actin, ultimately leading to vasoconstriction [23].



Control group: A (one day). B (after 7 days). C (after 14 days). D (after 21 days).

Minoxidil 2% group: A (one day). B (after 7 days). C (after 14 days). D (after 21 days).

Nifedipine cream 0.3% group: A (one day). B (after 7 days). C (after 14 days). D (after 21 days).

Fig. 2. Gross observation of albino mice on days one, seven, fourteen, and twenty-one from the beginning of the experiment.

The vasodilation-mediated induction of hair growth by nifedipine agrees with several studies carried out by Choi and Mingsan, who confirmed that the improvement of cutaneous blood flow via relaxing the vasculature smooth muscle had induced hair growth.

The weight of the hair in mice groups treated with nifedipine 0.3% and with standard minoxidil 2% solution was significantly higher than both untreated controls. The result of this study concerning hair weight comes into agreement with other studies conducted by [24], who reported that the increase in hair weight is the outcome of accelerated telogen to anagen transition, which is responsible for the hair growth induction.

5. Conclusion

The current study shows that nifedipine topical cream in a strength of 0.3% exhibits significant hair growth-promoting effects.

Conflicts of interest

No conflicts of interest.

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