RESEARCH ARTICLE

In-vivo Study of the Effect of 5 and 10% Nebivolol Cream on Hair Growth in Mice Models

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ABSTRACT

Background: Hair growth promotion via the use of vasodilators represents an important approach in hair loss management as they have proven to be effective in promoting hair growth. Nebivolol is a potent vasodilator which also exhibits anti-apoptotic, anti-inflammatory, and antioxidant effects, yet its role in hair growth induction is not well known.

Objective: The present study aims to investigate hair growth-promoting effect of the topical application of nebivolol cream in two strengths 5 and 10% in mice models.

Methods: Active hair growth (Anagen) induction by the topical use of nebivolol cream has been evaluated in mouse model. Fifty male swiss albino mice (8 weeks aged) dorsal skins were shaved and these mice were divided randomly and equally into untreated control group with which other experimental groups compression was done and 4 treated groups (n=10) with nebivolol 5% cream, nebivolol 10% cream, minoxidil 2% topical solution and vehicle (1:1 vaseline and lanoline). The topical treatment continued for 21 days during which the visual observation of mice was done for qualitative and quantitative evaluation of hair growth, then, following 21 days, the mice were euthanized and skin samples were harvested for the purpose of hair weight measurement, histological and immunohistochemistry evaluations.

Results: The results of current study revealed that nebivolol as 5 and 10% topical cream accelerated hair growth induction and prolonged active hair growth phase via peri-follicular vasodilation and via up-regulating the mRNA expression of vascular endothelial growth factor (VEGF) following their topical application in mice models.

Conclusion: This study has demonstrated for the first time the potential effect of topical nebivolol on hair growth promotion which may help in the treatment of hair loss disorder.

Keywords: Alopecia, Antioxidant, B-blockers, Hair growth promotion, Hair loss, Nebivolol, Vasodilators.

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INTRODUCTION

Hair loss disorder (alopecia) is a medical condition of various etiologies in which case people lose some or all hairs on scalp and sometimes on entire body, this disorder despite of not being a life-threatening condition, represents a remarkable problem of cosmetology and on the top of challenging issues for dermatologists around the world since most of the available therapeutic modalities proved to exhibit limited efficacy and safety beside demanding long term commitment for the occurrence of positive outcome.[1]

The currently researched treatments of hair loss disorder focus mainly on agents that can stop hair loss and induce hair growth by promoting blood circulation by causing peri-follicular vasodilation.[2]

It is known that nitric oxide (NO) causes greater arterial distension and greater blood supply as being an endothelium-dependent acute vasodilator[3] that can also increase the expression of vascular endothelial growth factor (VEGF)[4] which is an autocrine growth factor for dermal papillae cells and key angiogenesis promoting factor that causes an increase in the peri-follicular vascularization and in the vascular permeability leading to an increase in hair follicle size and hair shaft length, (VEGF) mRNA is strongly expressed in the keratinocytes of the outer root sheath of hair follicles during early and mid-anagen (active growth) and regress during catagen (regression) and telogen (resting) phases.[5,6]

Nebivolol is a long-acting, third-generation and highly selective beta-1 (β1) adrenergic receptor blocker[7] that causes direct vasodilation via causing an increase in NO in the L-arginine-NO pathway by stimulating the endothelial nitric oxide synthase (eNOS) mediated via its β3 receptor agonistic effect,[8] furthermore nebivolol also exhibits antioxidant[9] and anti-inflammatory effects.[10]

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The improvement of perifollicular microcirculation is well known to play important role in the induction of hair growth and in causing hypertrichosis.[11] Previous *in vivo* and *in vitro* studies with several other vasodilators had proven their effectiveness in hair growth promotion.[12,13]

Based on previous studies carried out on vasodilators, it is hypothesized that nebivolol may also induce hair growth via its vasodilator effect, so in the current study evaluation of nebivolol effect on hair growth in Swiss albino mice models was done.

MATERIAL AND METHOD

Material and Chemicals

Standard minoxidil 2% topical solution supplied by Medpharma, United Arab Empire was purchased from a local pharmacy. Raw powder of nebivolol was purchased from Baoji Guokang Bio-Technology Co.,China. Oleic acid supplied by Sigma-Aldrich/Germany was used as solvents, and vaseline supplied by Sigma-Aldrich, Germany and lanoline supplied by China were mixed and used as vehicle in the topical formulation of nebivolol cream.

Preparation of Topical Cream

The topically applied nebivolol cream in strength 5% and in strength 10% were freshly prepared using 5 grams of nebivolol raw powder dissolved in 1-mL of oleic acid in case of 5% cream preparation and 10 grams of nebivolol raw powder dissolved in 2 mL of oleic acid in case of 10% cream preparation which were then mixed with cream base made up of a mixture of vaseline and lanolin in a ratio (1:1) with continuous stirring by stirrer at room temperature until getting a uniform cream.

Experimental Animals

Fifty healthy Swiss albino mice (weighing 18–20 grams, aged 8 weeks) were purchased and housed in the Animal House of Baghdad University, College of Veterinary Medicine in a spacious cage and allowed to acclimatize to the surrounding environment for seven days with making adjustments of all conditions in term of temperature 25 ± 2°C, humidity 40–60%, 12 hours of dark/light cycle was ensured, feeding with standard pellet and water was provided. This study was approved by the Institutional Review Board (IRB) in College of Medicine. Al-Nahrain University

The Process of Depilation and Classification of Experimental Animal Groups

For investigating hair growth, method reported by Matias & was followed[14] with some slight modifications. The dorsal skin of all mice groups was shaved (4 cm length x 2 cm width) via an electrical animal clipper and the use of hair removal cream a day before conducting the experiment which resulted in the synchronization of hair follicles in telogen phase as evidenced by pink skin. All mice were randomly and equally divided into 5 groups (10 mice per group) based on treatment option: untreated group (intact control), vehicle (vaseline and lanolin) treated group (negative control group; NC group), minoxidil 2% solution treated group (positive control group; PC group), nebivolol 5% cream treated group and nebivolol 10% cream treated group, each agent was applied once daily topically for a period of 21 days with the initiation of treatment on the day following depilation.

Qualitative Evaluation of Hair Growth

Evaluation of the minimum time taken for hair growth initiation and completion on the shaved dorsal skin was done via the visual observation and comparison of time taken to grow new hair and time taken to cover the denuded dorsal skin of all mice groups by taking photographs on days 1,7,14 and 21 of the treatment course.

Quantitative Evaluation of Hair Growth

The condition of hair growth cannot be obviously observed via photograph, therefore for the evaluation of hair growth state and for the conformation of hair growth-promoting effect of nebivolol, we have done qualitative studies that involved the determination of hair length and the determination of hair weight.

Determination of Hair Length

All mice were anesthetized via intraperitoneal injection of ketamine/xylazine and hair samples were pulled haphazardly on days 14 and 21 of treatment course via using sterile forceps from the previously shaved areas of all mice groups, the length of the hair was measured in millimeters via using digital vernier caliper and the results were reported as the mean length ± standard deviation (SD) of 20 hairs.

Determination of Hair Weight

Hair weight measurement was performed after the 21 days of the treatment course, following euthanasia of the mouse via an overdose of diethyl ether, a small portion (1 cubic centimeter) of previously shaved dorsal skin was harvested from the same position in all mice by a sterile surgical blade, the weights of the skin with hair and without hair were measured using sensitive electrical balance, the differences in the weights were recorded and reported in milligrams as the net weights of the newly regrown hairs.

Histological Evaluation of Hair Growth

At the end of the experiment, a dorsal skin sample of about 2 cm area from the same position of each mouse was harvested parallel to the vertebral line after long hairs shaving and preserved in 10% phosphate-buffered formalin for the period of 24 hours, followed by paraffin wax embedding using standard techniques, longitudinal sections of the skin were prepared and stained with hematoxylin and eosin and then observed for different parameters used in the evaluation of therapeutic responses in mice hair follicles. The measurements of hair follicle length and hair follicle bulb diameter at the level of the largest diameter ("Auber's line") of the hair follicle bulb represent the easiest parameters for classifying hair follicle growth stage, these measurements were done for nearly 300 hair follicles via the use of the light microscope and graduated lens (magnification 40X). The number of hair follicles in 2 mm area was recorded and reported as follicular density (number of

follicles/millimeter), the number of hair follicles in the active growth (anagen) phase and those in the resting or quiescent (telogen) phase were also counted microscopically at 10X magnification, and the ratio of anagen/telogen hair follicles were determined.

Immunohistochemistry

The immunostaining process was performed on 5-µm paraffin-embedded tissue sections obtained from previously denuded dorsal skin. The paraffin-embedded tissue sections were deparaffinized in xylene, rehydrated in an ethanol series, and equilibrated in Townes-Brocks syndrome (TBS) for 5 minutes at room temperature. Following the blocking with 10% E-IR-R217A- normal goat serum at room temperature for 10 minutes, the sections were incubated with anti-VEGF A antibody [E-AB-22215] at 20~37°C for 30 minutes in accordance to the manufacturer protocol, following the wash of the slides with phosphate-buffered saline (PBS) three times for 5 minutes of each, the secondary antibody E-IR-R217B (polyperoxidase-anti-Mouse/Rabbit IgG) was added and incubated at room temperature or at 37°C for 20 minutes then the sections were treated at room temperature with 3,3-diaminobenzidine (DAB) working solution for 5 minutes and were counterstained by hematoxylin and dehydrated and covered slip with dibutylphthalate polystyrene xylene (DPX).

Then the positively charged slides were examined under the light microscope at 40X to identify the immune-reactivity for VEGF and scoring system was used, these scores that had been recorded were the average intensity of the expression as follow:

Table 1: hair growth initiation and completion time in each study group

Study group	*Hair growth*	
	Day of initiation	*Day of completion*
Nebivolol 10% cream	5th	20th
Nebivolol 5% cream	6th	22th
Minoxidil 2% solution	5th	20th
Vehicle only	9th	27th
Untreated control	10th	27th

Absence of immune-reactivity had the score 0, Weak immune-reactivity had the score 1, Moderate immune-reactivity had the score 2, strong immune-reactivity had the score 3.[15]

Statistical analyses

The collected data was analyzed using Statistical Package for Social Sciences (SPSS) version 26 and presented as mean standard deviation and ranges. Categorical data is presented by frequencies and percentages. Independent t-test and Analysis of Variance (ANOVA) (two-tailed) was used to compare the continuous variables accordingly. A level of p value < 0.05 was considered to be significantly different.

RESULTS

Qualitative Study of *In vivo* Hair Growth.

The minimum time taken for hair growth initiation and completion was shortened upon treatment with nebivolol 5% cream and nebivolol 10% cream when compared to untreated control group and their effects were comparable to the

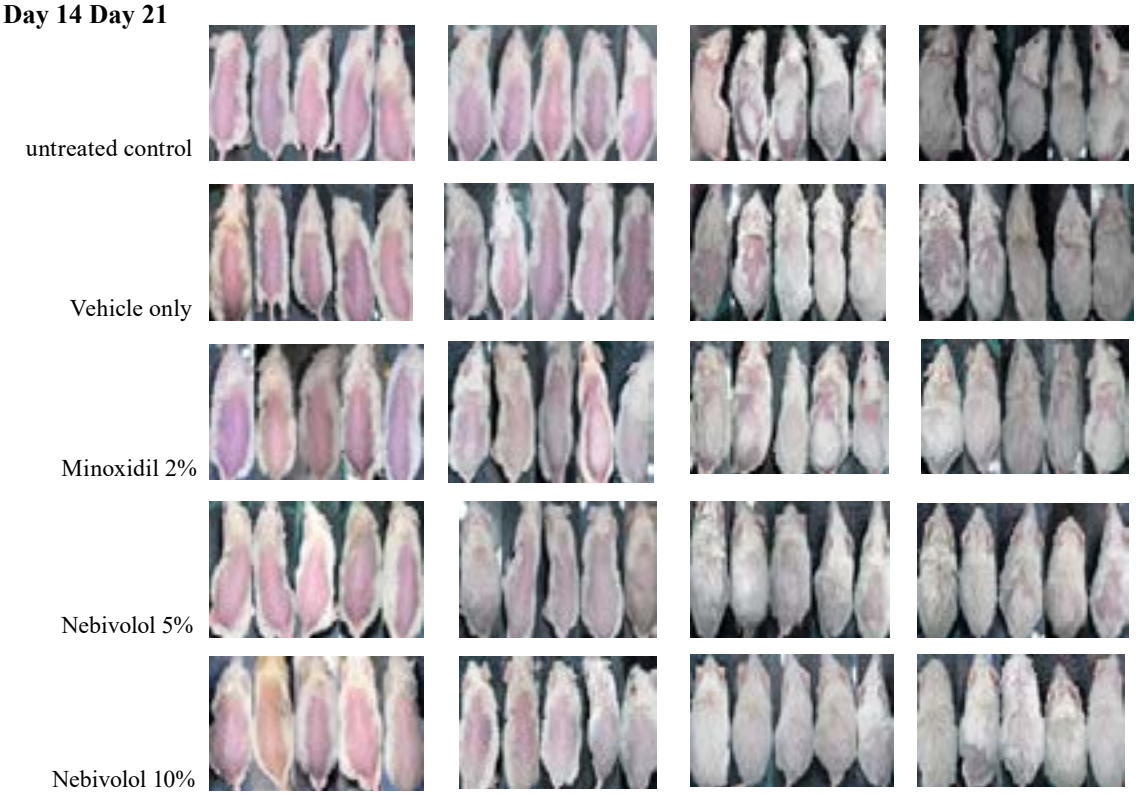

Figure 1: Gross observation of dorsal skins in albino mice during first, seventh, fourteenth and twenty first day following the shaving and the topical application of agents of study for 21 days except in intact control, the dorsal skin hair was shaved and no treatment applied topically on the back skin.

standard minoxidil 2% solution. The minimum time taken for hair growth initiation was on day 5 in mice groups treated with nebivolol 10% cream and with standard minoxidil 2% solution and on day 6 in mice group treated with nebivolol 5% cream, while in cases of untreated control and negative control groups the visible hair growth was noticed on days 9 and 10, respectively.

The minimum time taken for hair growth completion was on day 20 in mice groups treated with nebivolol 10% cream and with standard minoxidil 2% solution, while it was noticed to occur on day 22 in mice group treated with nebivolol 5% cream in untreated control and negative control groups the complete hair growth was observed to occur on day 27 since the initiation of treatment course (Table 1 and Figure 1).

Quantitative Evaluation of Hair Growth:

Determination of Hair Length

The length of 20 plucked newly grown hairs from treated mice groups (except vehicle group) on days 14 and 21 (Figure 2) since the initiation of therapy was significantly longer ($p < 0.05$) than the untreated control group (4.28 mm ± 0.13 on day 14 and 5.76 mm ± 0.13 on day 21). The mean hair length of mice groups treated with nebivolol 10% cream and nebivolol 5% cream on day 14 (5.65 mm ± 0.24 and 5.27 mm ± 0.3 respectively) and on day 21 (6.71 mm ± 0.32 and 6.88 mm ± 0.22) of treatment course was comparable to the mean hair length of mice group treated with the standard minoxidil 2% solution (5.83 mm ± 0.31 on day 14 and 6.72 mm ± 0.46 on day 21) revealing comparable efficacy.

Determination of Hair Weight

The hair weight of all treated mice groups (except vehicle group) following 21 days of the treatment course (Figure 3) was significantly heavier than the hair weight of untreated control group (43.6 mg ± 1.3). Mice groups treated with nebivolol 10% cream and nebivolol 5% cream exhibited nearly same mean hair weights (50.3 mg ± 1.9 and 50.3 mg ± 27, respectively) which were slightly less than the mean hair weight of mice group treated with the standard minoxidil 2% solution (51.6 mg ± 3.5).

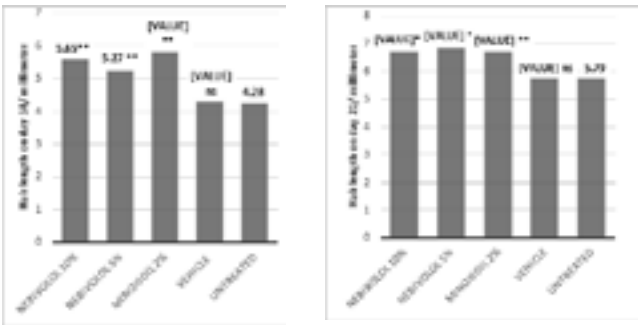

Figure 2: Bar charts show the differences in hair lengths measured in millimeter via digital vernier caliper on days 14 and 21 of treatment course in the five experimental groups. Data introduced as mean ± SD. **: highly significant difference $p < 0.01$ ns: no significant difference compared with untreated control group (n=10).

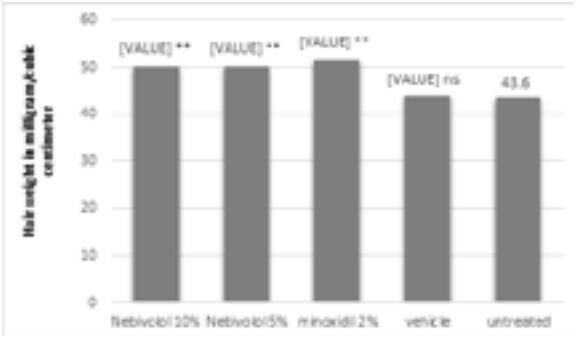

Figure 3: Bar charts illustrate the differences in hair weight following 21 days since the initiation of topical treatment course in the five experimental groups. Results are graphed as mean ± SD. **: highly significant difference $p < 0.01$ ns: no significant difference compared with untreated control group (n=10).

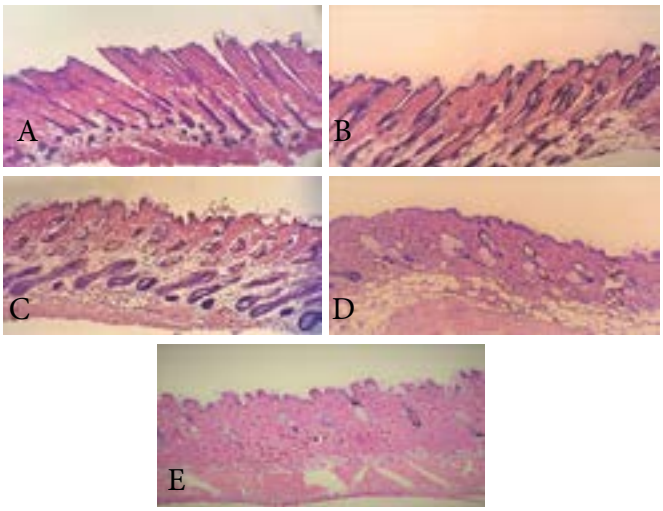

Figure 4: Effects of drugs of study on the hair follicles in mice models were analyzed using hematoxylin-eosin (H&E) staining of the longitudinal dorsal skin tissue section at 10X following 21 days of treatment course where A) Nebivolol 10% treated group, B) Nebivolol 5% treated group, C) Minoxidil 2% treated group, D) Vehicle treated group, E) Untreated control group.

Histological Evaluation of Hair Growth

Histological analysis in addition to the quantitative techniques, provides more precise results in the evaluation of hair growth status[16] It revealed that 3 weeks of topical application of nebivolol 10 % cream, nebivolol 5% cream and of minoxidil 2% solution have promoted hair follicle elongation from dermis to sub-cutis layer of the skin via growth pressure derived by the proliferation of the epithelial cells (Table 2) and that the treatment with nebivolol 10 % cream significantly increased hair follicle bulb diameter (109.0 µm ± 13.5) with efficacy being superior to the standard minoxidil 2% solution (103.5 µm ± 15.3). In case of mice groups treated with nebivolol 5% cream also revealed an increase in hair follicle bulb diameter, but it was insignificant (103 µm ± 18.9) when compared to the untreated control group (88.6 µm ± 12.4) (Table 3). The increase in hair follicle length and bulb diameter suggests anagen phase prolongation.

The progression of hair follicle in hair growth cycle and the assessment of anagen phase induction were done by the determination of follicular density and by specifying the phase of hair follicle growth (Table 4). The results revealed were significant increase in follicular density in mice groups treated with nebivolol 10% cream, nebivolol 5% cream and minoxidil 2% solution when compared to the untreated control group, the denser hair follicles were observed in mice groups treated with nebivolol 10% and minoxidil 2% solution (6.4 ± 1.1 and 6.4 ± 0.96 respectively) while in case of nebivolol 5% cream, the performance of which on the follicular density (5.9 ± 1.1) was less than the minoxidil 2% solution.

In case of hair follicle growth phase (Figure 4), the majority of hair follicles in mice treated with nebivolol 10% cream, nebivolol 5% cream and with standard minoxidil 2% solution were in the active growth (anagen) phase with the best result being observed in mice treated with nebivolol 10% cream (4.7 ± 0.94) and with nebivolol 5% cream (4.5 ± 0.97) when compared to the untreated control and positive control groups. In cases of untreated control and negative control groups the majority of hair follicles were in the resting (telogen) phase with just one or two follicles being in the active growth phase, anagen/telogen ratio was significantly higher in all treated groups (except vehicle group) than the untreated control, with the highest anagen/telogen ratio occurring in mice groups treated with nebivolol 10% cream (3.48 ± 1.8) and with nebivolol 5% cream (3.6 ± 1.4) when compared to the other experimental mice groups.

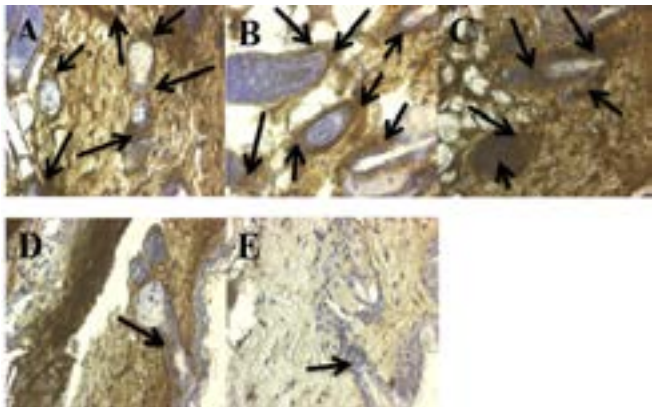

Figure 5: Representative sections of VEGF-A immune-reacted dorsal skin tissue of the previously shaved Swiss albino mice following 21 days of the topical treatment examined under the light microscope at 40X where A) group treated with nebivolol 10% cream, B) group treated with nebivolol 5% cream, C) group treated with minoxidil 2% solution, D) group treated with the vehicle, E) untreated control group. (Black arrows) point to the expression of VEGF in the hair follicles.

Table 2: Comparison between untreated control and all other groups in the mean of hair follicle length measured under the light microscope via the graduated optical lens at magnification 40X following 21 days of topical treatment course

Study group	*Hair follicle length (μm) Mean ± SD*	*p - value*
Nebivolol 10%	831.5 ± 303.5	0.005
Untreated control	483.2 ± 176.4	
Nebivolol 5%	806.4 ± 241.8	0.003
Untreated control	483.2 ± 176.4	
Minoxidil 2%	766.0 ± 284.3	0.014
Untreated control	483.2 ± 176.4	
Vehicle	466.3 ± 141.5	0.816
Untreated control	483.2 ± 176.4	

Immunohistochemistry

The immunohistochemistry staining for the expression of VEGF in the hair follicles following 21 days since the initiation of topical treatment was observed and immunohistochemical analysis revealed that the expression of VEGF increased significantly in all treated mice groups (except vehicle group) when compared to the untreated control group in which few VEGF expressing cells were seen in the hair follicles examined at 40X magnification (Figure 5). In mice groups treated topically with nebivolol 10% cream, nebivolol 5% cream and with minoxidil 2% solution many VEGF expressing cells were observed in the hair follicles, revealing an increase in the VEGF

Table 3: Comparison between untreated control and all other groups in the mean of hair follicle bulb diameter measured under the light microscope via the graduated optical lens at magnification 40X following 21 days of topical treatment course

Study group	*Hair follicle bulb diameter (μm)Mean ± SD*	*p -value*
Nebivolol 10%	109.0 ± 13.5	0.002
Untreated control	88.6 ± 12.4	
Nebivolol 5%	103.0 ± 18.9	0.062
Untreated control	88.6 ± 12.4	
Minoxidil 2%	103.5 ± 15.3	0.025
Untreated control	88.6 ± 12.4	
Vehicle	88.0 ± 11.4	0.911
Untreated control	88.6 ± 12.4	

Table 4: Histological evaluation of follicular density (number of hair follicle/millimeter), hair follicle in anagen (active growth) phase, hair follicle in telogen (resting) phase and anagen/telogen ratio. p - values were compared to the untreated control group. Results are expressed as mean ± standard deviation of the mean

Treatment	*Follicular density*	*Hair follicle in anagen phase*	*Hair follicle in telogen phase*	*Anagen/ telogen ratio*
Nebivolol 10%	6.4 ± 1.1	4.7 ± 0.94	1.7 ± 0.82	3.48 ± 1.8
Nebivolol 5%	5.9 ± 1.1	4.5 ± 0.97	1.4 ± 0.51	3.6 ± 1.4
Minoxidil 2%	6.4 ± 0.96	4.3 ± 0.82	2.1 ± 0.31	2.06 ± 0.41
Vehicle	5.4 ± 0.51	0.6 ± 0.84	4.8 ± 0.78	0.15 ± 0.21
Untreated control	5.0 ± 0.47	1.2 ± 0.91	3.8 ± 0.91	0.4 ± 0.44

expression when compared to the untreated control group. The highest expression of this growth factor was noticed in the hair follicles of mice group treated with minoxidil 2% solution, which is known to cause up-regulation in VEGF expression that represents as one of the mechanisms via which minoxidil promote and improve the growth of hair.

DISCUSSION

For most people, hair is of great importance in terms of self-identity and self-esteem. It represents a sign of beauty and a sign of well-being since long time, so the abnormal loss of hair represents a devastating issue that leads to psychological disturbances.[17] The treatment of this condition is challenging for many physicians due to its variable etiologies, lack of safety and effectiveness of many of the available treatment approaches that mandate continuous search for drugs and treatment options with more promising outcomes.[1]

Hair growth promotion occurs via the induction of anagen (active growth) phase that leans on angiogenesis induction to meet the enhanced nutritional and oxygen demands of the rapidly dividing cells of hair follicles that occur during anagen phase of hair growth cycle, vasodilator as minoxidil causes hypertrichosis and improves hair growth via improving the cutaneous blood flow to the scalp.[14] Based on this, several previous studies have revealed that other vasodilators such as phosphodiesterase inhibitor cilostazol and sildenafil induced hair growth through *in vitro* and *in vivo* experiments[12,13] in such way we hypothesized that nebivolol via its vasodilator action would promote hair growth.

The results of the present study revealed that the topical use of nebivolol cream in strengths 5% and 10% exhibited hair growth promoting and accelerating effects when examined in the Swiss albino mice whose hair follicles were in resting (telogen) phase. The visible growth of hair is normally observed to occur on day 9 in the untreated mice groups,[18] but it occurred sooner in the first week of the treatment course in all mice groups subjected to the topical treatment except the vehicle-treated mice group, this accelerated transition of telogen to anagen phase can be attributed to blood circulation and nourishment improvements of the hair follicles via the vasodilator action of nebivolol19 as had been proven by several previous studies.[11-13,18,20]

Uneven and patchy hair growth seen in some mice following the 21 days of treatment course, this pattern of hair growth had been observed to occur in several other studies carried out on mice models[21] and had been seen in mice treated with minoxidil.[22] This patchy and uneven hair growth can be attributed to the induction of hair growth in the third cycle, which in mice occurs in an asynchronous pattern, unlike the first and second cycles of hair growth that occur in a synchronous pattern.[23]

Hair length is directly influenced by the duration of the active hair growth (anagen) phase,[24] during which the rapid proliferation of follicular keratinocytes give rise to hair shaft elongation, the significant increase in all treated mice groups hair length (except vehicle group) can be attributed to the premature switch of the hair follicles from resting (telogen) to active growth (anagen) phase of hair growth cycle[25] and to the anagen phase duration prolongation by delaying the progression of catagen phase[18] which can be assured via the increase in hair weight that occurred in all treated mice groups (except vehicle group) as hair weight measures simultaneously the variation in hair length[26] suggesting an increase in hair growth rate and thereby in hair length.[27]

New hair formation and hair growth process is influenced directly by the regeneration and continuous cyclic growth of hair follicles, during which these follicles show distinct morphological features in each phase. Anagen phase is characterized by the increase in hair follicles numbers and continuous increase in their lengths throughout this phase, these follicle's bulb become enlarged and enclose the dermal papillae.[28]

The topical use of nebivolol cream in two strengths significantly increased hair follicle length while in case of hair follicle bulb diameter the increase was significant in mice treated with 10% nebivolol cream but not with 5% nebivolol cream, caused insignificant increase in the bulb diameter when compared to untreated mice group, these effects of nebivolol can be attributed to early hair growth induction (early anagen acceleration) and late active hair growth phase extension (anagen phase prolongation).[29]

The results of the current study revealed significant increase in the follicular density in all treated mice groups (except vehicle group) when compared to untreated control group, this increase can be the outcome of peri-follicular vascularization improvement as has been reported by Yano et al.[30]

The increase in the number of hair follicles in anagen phase relative to hair follicles in telogen phase that occurred in mice groups treated with 5% and 10% nebivolol cream and in group treated with standard minoxidil 2% solution represents a substantial indicator of hair growth promotion by these drugs, this effect had been proven to occur with other vasodilators via cutaneous blood supply improvement.[13,31]

To elucidate the molecular mechanism that underlies the ability of nebivolol to induce hair growth and based on the fact that minoxidil can induce hair growth via up-regulating VEGF expression in the hair follicles, and that VEGF expression rises via NO4 we examined protein expression of VEGF-A in the tissue section of previously denuded dorsal skin via the immunohistochemistry method.

Systemic neutralization of VEGF profoundly retarded hair growth and caused reduction in peri-follicular vascularization and in size of hair follicles, these findings establish that the normal hair follicles cycling and growth in mice require VEGF promoted angiogenesis and that the impairment in the vascularization of hair follicles had been suggested to play a role in hair loss pathogenesis.[30]

The effects of VEGF are mediated partially via the endothelial synthesis of a potent vasodilator nitric oxide

(NO) by the action of nitric oxide synthase (NOS), including endothelial nitric oxide synthase (eNOS).[32,33]

In the current study and following 21 days of the topical treatment course, immunohistochemistry analysis demonstrated that VEGF expression in the hair follicle mainly in the outer root sheath, increased significantly in all treated mice groups (except vehicle group) when compared to the untreated control group.

These results suggest that the topical treatment with nebivolol 5% cream and nebivolol 10% cream can promote hair growth by improving peri-follicular hemodynamic status via vasodilation and via increasing VEGF expression resulting possibly in hair follicles cell proliferation.

These findings agree with several studies, including one carried out by Madaan *et al.*[34] who reported that the increased VEGF expression represents strong supporting mechanism for hair growth induction, as has been proven to occur in another study carried out by Meephansan et al.22 and study carried out by Saansoomchai *et al.*[35]

CONCLUSION

In conclusion, from the observational, histological and molecular findings it can be demonstrated that the present study provides evidence that the topical dosage form of nebivolol in strengths 5% and 10% may represent a promising and novel remedy to treat hair loss disorder without potential adverse effects. Further *in vitro* research and clinical trials are required to elucidate nebivolol's precise mechanisms in promoting hair growth.

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