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MINOXIDIL AND NEOPTIDE TOPICAL APPLICATION REINFORCED BY LOW-LEVEL LASER THERAPY ON AN ANIMAL MODEL OF ALOPECIA

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ABSTRACT. This study investigated on a rat model the hair regrowth effects of Low-Level Laser Therapy as monotherapy or concomitant therapy with topical application of two chemical treatments that are used in human patients with hair loss: Minoxidil 2% - Hairgrow (Dar Al Dawa Pharma/Amman, Jordan) and Neoptide (Ducray/ Boulogne, France).

Results of hair regrowth evaluated by macroscopical images (photographs), trichoscopy (with a dermatoscope) and grown hair weight (from a surface area of 1cm²) revealed that Minoxidil 2% therapy was more efficient than Neoptide. Topical treatments were less efficient than LLLT exposure alone. Both combined therapies: LLLT plus Minoxidil 2% and LLLT with Neoptide induced better hair regrowth than topical applications. Our study proves that not all the products recommended for human use have the same hair regrowth efficiency on an animal model and that a combined therapy (laser plus topical substance) may bring supplementary benefits.

Keywords: *hair regrowth, low-level laser therapy, combined treatment, Minoxidil 2%, Neoptide, trichoscopy*

INTRODUCTION

More than half of the population worldwide suffers from a hair loss disorder [1]. Androgenetic alopecia (AGA) is the most common cause of hair loss, affecting more than half of the males over 40 years old and females over

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65 [1]. AGA is a chronic dermatological disorder defined as thin diffuse hair loss over the vertex and frontal area of the scalp in females and it is accompanied by hairline recession at the temples in males. The disease is caused by androgen excess (testosterone and its derivate dihydrotestosterone) and it is due to the susceptibility of hair follicles to androgen miniaturization. Heredity also plays a part [1].

Minoxidil 2% topical therapy in female patients, as well as Minoxidil 5% and Finasteride treatment in males represent the only FDA approved drugs for AGA. As currently used therapies proved not to be effective in all patients, scientists focused on finding new quality therapies for hair loss and treatment. Laser has thus become an interesting and encouraging field of research as it has already been demonstrated that red or near-infrared light determines tissue repair. Low Level Laser Therapy (LLLT) may provide a promising treatment option for patients who do not respond to classical treatment. LLLT represents a type of laser which produces low power, coherent monochromatic red light, promotes regeneration and stimulates cellular activity [2]. The device was previously tested and recorded positive results on wound healing, nerve regeneration, joint pain relief, stroke recovery and mucositis treatment [3,4,5,6,7,8,9]. LLLT delivers fluences of 1-10J/cm² with a power density of 3-90mW/cm² and has demonstrated beneficial effects in various skin conditions as well [10].

In the late 1960's the usual wavelengths ranged from 500-1100 nm, therefore Endre Mester used a low-power ruby laser (694nm) on a mice animal model. During his study on cargiogenic potential of lasers, he discovered that the therapy applied determined an unexpectedly significant hair regrowth on the test areas [11,12]. LLLT was since considered a potential treatment for hair loss [13]. The photobiomodulation produced by LLLT stimulates epidermal stem cells in the follicle bulge and determines the shifting of the follicles from telogen (resting phase) to the anagen (active) phase of the hair growth cycle [1]. Laser phototherapy is thought to prolong the duration of anagen and prevent premature catagen development (hair falling phase) [14,15]. LLLT alters cell metabolism through photodissociation of inhibitory nitric oxide (NO), leading to increase adenosine triphosphate (ATP) production and modulation of reactive oxygen species (ROS) [16]. It also determines induction of transcription factors that cause protein synthesis, triggering cell proliferation, alteration of cytokine levels, growth factors, inflammatory mediators and significant tissue oxygenation [17]. LLLT has been proved to produce vasodilation and increased blood flow with important therapeutic effect in alopecia [17-19].

Recently, a large number of commercial devices using LLLT have been promoted with increased media attention and significant marketing budgets.

Professional and home-based LLLT devices may have a hand-held design or consist of a therapy helmet for hands-free use. Most of the studies claim that LLLT of the scalp at 655nm significantly improves the hair counts in males with AGA, with an increase in the number of terminal hairs and in shaft diameter, together with a decrease of vellus hairs [20, 21]. Few clinical trials have been conducted on these LLLT devices and results are controversial. The therapeutic effect remains unclear due to the lack of independent long-term placebo-controlled clinical studies and due to analyzing issues [21, 22]. Further research is required to establish the efficacy on hair regrowth in comparison to classic topical solutions [22, 23].

The objective of our study was to determine whether treatment with a low-level laser device, the US FDA-cleared HairMax Lasercomb, is efficient in inducing hair growth in vivo, on an animal model in single usage (standard parameters, 3 minutes therapeutic exposure) and in combination with topical therapy: Minoxidil 2% (Hairgrow, Dar Al Dawa Pharma/Amman, Jordan) and Neoptide (Laboratoires Dermatologiques Ducray/ Boulogne, France).

RESULTS AND DISCUSSION

A) Macroscopic and Microscopic Evaluation

Macroscopic examination of the skin surface on day 30 of treatment enabled us to assess the *in vivo* hair regrowth effect induced by topical daily application of Minoxidil 2% in Group I. We comparatively evaluated the test area and the control area of each rat from this group and the majority of rats showed positive hair regrowth under this therapy. Regarding the macroscopic score, 4 rats had Type 4 hair regrowth on the test area (marked increased hair regrowth, full, thick fur), 2 rats had Type 3 (moderately increased hair growth with no visible skin area), one rat showed Type 2 (low hair density, with the visualization of the skin) and another one Type 1 (uneven hair growth on the test area, skin easily seen). Trichoscopic examination of the same study group revealed an equal distribution (3:3 ratio) between Type 4 and Type 3 hair regrowth pattern.

Group II (treated with Neoptide 0.3 ml topical daily application) had a moderate hair growth effect, as the test area presented Type 3 hair regrowth in 4 rats, Type 2 hair regrowth in 3 rats and only 1 rat recorded a maximum hair regrowth result (Type 4 hair regrowth pattern). Tricoscopy evaluation however showed that Type 4 pattern was misinterpreted and, in fact, it was a case of moderately increased hair growth.

LLLT laser therapy in Group III (applied for 3 minutes, three times per week) enhanced hair regrowth. Both microscopic and macroscopic assessment revealed that half of the rats in the study group had Type 4, marked increased hair regrowth on the test area. Type 3 pattern was diagnosed in 2 rats and an equal number had Type 2 pattern.

When evaluated by macroscopic aspect, the majority of animals in Group IV (LLLT plus Minoxidil 2%) revealed marked increased hair regrowth (Type 4 in 5 rats), suggesting a powerful therapeutic effect. Moderately increased hair regrowth (Type 3) was encountered in 2 rats on the test area, and only one had low hair density (Type 2). Tricoscopy evaluation enabled us to correctly assess the hair regrowth result and to notice that one Type 3 pattern was misinterpreted as marked increased hair growth. 75% of the animals in this group recorded an efficient hair growth effect induced by this combined therapy.

Group V, treated with LLLT plus Neoptide, recorded similar results to LLLT laser Group III: 4 rats with Type 4 hair regrowth, 2 rats with Type 3 and 2 with Type 2. The results were arrived at by macroscopic and trichoscopic assessments.

The comparative evaluation of the experimental groups show that the lowest hair regrowth effect was noticed in Group II (Neoptide) ($p>0.05$). As far as topical treatment is concerned, we found out that topical Minoxidil 2% application (in Group I) was significantly better in inducing hair regrowth, even thought the p-value was over 0.05 (Table 1, Table 2).

Table 1. The macroscopic score from the treated area and the control area, expressed in the 0-5 scale described in literature. Results are expressed with Exact Wilcoxon-Signed-Rank Test * = $p<0.05$.

Treatment	**Macroscopic score Median Percentile (25^{th}-75^{th})**	***Mean ± SD***	***P value***
Group 1	3.00 (2.25-3.75)	*2.88±0.991*	
Minoxidil 2%			*0.102*
Control	2.50 (1.25-3.00)	*2.38±1.061*	
Group 2	3.00 (2.00-3.00)	*2.75±0.707*	
Neoptide			*0.257*
Control	2.00 (1.25-3.75)	*2.38±1.188*	
Group 3	3.00 (2.25-3.75)	*3.00±0.756*	
HairMax			*0.034**
Control	2.00 (1.25-3.00)	*2.25±1.035*	
Group 4	4.00 (3.00-4.00)	*3.50±0.756*	
HairMax + Minoxidil 2%			*0.038**
Control	2.50 (2.00-3.00)	*2.50±0.926*	
Group 5	3.50 (2.25-4.00)	*3.25±0.886*	
HairMax + Neoptide			*0.023**
Control	2.00 (1.25-3.00)	*2.25±1.035*	

By comparing the outcomes of LLLT performed with HairMax Laser Comb, we noticed that in Group III (treated only with the laser device) it had a significant hair growth effect ($p<0.05$). Still, LLLT monotherapy was less efficient on hair regrowth than the combined treatment regimen with daily topical application of the Minoxidil or Neoptide compounds.

LLLT plus Minoxidil 2% (in group IV) and LLLT with Neoptide (in Group V) seem to be equally successful therapies in inducing hair regrowth, facts revealed both by macroscopic and trichoscopic assessment ($p<0.05$). Out of the five study groups, the best hair regrowth effect was noticed by macroscopic and trichoscopic assessment in Group IV (Laser + Minoxidil 2%) ($p<0.05$) (Table 1, Table 2).

Table 2. Trichoscopy score from the treated area and the control area, expressed in the 0-5 scale defined by using a dermatoscope. Results are expressed with Exact Wilcoxon-Signed-Rank Test. * = $p<0.05$.

Treatment	Trichoscopic score Median Percentile (25^{th}-75^{th})	*Mean ± SD*	*P value*
Group 1	3.00 (2.25-4.00)	*3.00±1.069*	
Minoxidil 2%			*0.102*
Control	2.5 (1.25-3.00)	*2.38±1.061*	
Group 2	3.00 (2.25-3.00)	*2.75±0.463*	
Neoptide			*0.157*
Control	2 (1.25-3.00)	*2.25±1.035*	
Group 3	3.00 (2.25-3.75)	*3.00±0.756*	
HairMax			*0.034**
Control	2 (1.25-3.00)	*2.25±1.035*	
Group 4	4.00 (3.25-4.00)	*3.63±0.744*	
HairMax + Minoxidil 2%			*0.024**
Control	2.5 (2.00-3.00)	*2.50±0.926*	
Group 5	3.50 (2.25-4.00)	*3.25±0.886*	
HairMax +Neoptide			*0.023**
Control	2 (1.25-3.00)	*2.25±1.035*	

B) HAIR WEIGHT DETERMINATION

Regarding the hair growth differences recorded between the test area and control area of the same animal, hair weight evaluation showed that the sum of differences in the Minoxidil study group was of 40.6 mg/cm². The weight of newly grown hair was the lowest in the Neoptide treatment protocol (Group II), the difference in hair weight being of 38,3 mg/cm². Both Minoxidil and Neoptide topical compounds induced a statistically significant hair regrowth on the test areas ($p<0.05$) (Table 3).

Compared to the control area from the same rat, the hair weight increased significantly in the LLLT treated groups (Group III, IV and V). Group III had a hair weight difference of 45.4mg/cm² between the test and control area of the rats. The highest hair weight was recorded for LLLT plus Minoxidil treated animals (Group IV), with a sum of hair weight differences of 57.1mg/cm². Group V (LLLT and Neoptide) has a lower, but still significant hair growth result, with a total of hair increase, (evaluated by hair weight difference) of 51.8 mg/cm². Statistic analysis enabled us to say that LLLT therapy (in monotherapeutic regimen or in combination with Minoxidil or Neoptide) has induced a better regrowth effect than topical therapy alone ($p<0.05$) (Table 3).

Table 3. The hair weight removed from one cm^2 in the treated area and the control area, expressed in mg/cm^2. Results are expressed with Exact Wilcoxon-Signed-Rank Test. * = $p<0.05$.

Treatment	Hair weight (mg/cm^2) Median Percentile (25^{th}-75^{th})	*Mean ± SD*	*P value*
Group1	42.40 (38.37-48.15)	*42.53±6.03*	
Minoxidil 2%			*0.012**
Control	38.25 (31.25-42.47)	*37.46±6.09*	
Group 2	44.10 (42.02-47.15)	*44.51±2.83*	
Neoptide			*0.012**
Control	39.25 (36.00-42.95)	*39.72±3.99*	
Group 3	44.25 (42.30-51.62)	*45.48±5.68*	
HairMax			*0.012**
Control	38.85 (35.5-43.47)	*39.81±5.47*	
Group4	48.50 (44.87-52.3)	*48.70±4.77*	
HairMax + Minoxidil 2%			*0.012**
Control	40.35 (36.87-45.82)	*41.56±5.29*	
Group5	45.15 (41.82-49.27)	*44.80±5.01*	
HairMax +Neoptide			*0.012**
Control	39.00(34.37-42.22)	*38.32±5.74*	

In the attempt to discover an effective treatment for hair loss, new therapies for alopecia have been studied on rats, hamsters and even stump-tailed macaque [24, 25].

LLLT has been tested on animal models and significant hair regrowth has been reported, but the optimum wavelength and dosimetric parameters still need to be determined [26]. According to literature LLLT stimulates hair regrowth in mice under chemotherapy-induced alopecia. LLLT applied on a rat animal model determined a significant hair regrowth in the HairMax treated groups, 5 days earlier than in the control and sham-control animals, as reported by a Satino study [27].

HairMax Laser Comb Professional 12 was screened for safety and received the US-FDA approval for medical use since 2007 [28]. In clinical use it is recommended for the scalp, 8 minutes, three times per week and its good compliance is due to the fact that it does not leave residue on the scalp, as topically applied substances do [10].

The incidence of adverse effects of LLLT is rather low: dry skin (5.1%), pruritus (2.5%), skin tenderness (1.3%), irritation (1.3%) and warm sensation at the site of application (1.3%) [1,10]. In our study no side effects were recorded in the LLLT treated groups (III, IV and V).

Minoxidil 2%, that we used in two of our study groups (I and IV) was not specially devised for hair loss problems and its hair growing effect has accidentally been discovered as a side effect.

The mechanism underlying the hair growth effect is therefore not completely clear yet. The drug action is directed to the mesenchymal cells from the follicular dermal papilla of the hair follicle (DP) which controls the growth and differentiation of hair matrix cells [26]. DP associated stem cells are the site of expression of genes related to hair growth, being under the negative effect of androgen mediated events, and under the positive stimulation of Wnt proteins and wound growth factors [29].

Possible indirect drug action is represented by the vasodilation produced by Minoxidil that generates an increased blood flow to the DP [26]. Minoxidil contains an N-oxide group able to release NO, an important cellular signaling molecule, also functioning as a vasodilator [30]. Minoxidil is also a potassium channel blocker, which also leads to vasodilation of the scalp blood vessels. This direct mechanism of action involving the ATP sensitive K+ channels was highly investigated [31]. New hair growth induced by Minoxidil can also be due to local irritation related to it or some components of the vehicle [26]. Researchers also suspect that Minoxidil may act in inducing hair regrowth through the immune system, as it stimulates prostaglandin synthethase-1 and the subsequent production of PGE(2) [32,33].

Literature did not mention any dysplastic or atypical changes in follicular germinal epithelium during or after application of topical minoxidil, and did not reveal the development of new follicles (follicular neogenesis) [26]. Studies performed on animal models with Minoxidil topical therapy described the shortening of telogen (resting phase), a premature entry in anagen phase (active) of the resting follicles, an action meant to prolong the anagen and leading to hair regrowth [34,35]. Larger hair follicles were reported in the morphometric evaluation of human scalp biopsies after treatment with topical minoxidil in a vehicle made up of propylene glycol, water and ethanol [26]. The alcohol and propylene glycol present in topical preparations can dry the scalp, causing dandruff and contact dermatitis. Nanosome formulation reduces the rate of contact dermatitis from the vehicle [36,37].

A systemic review of the side-effects of Minoxidil as hair loss treatment for AGA patients, done by Cochrane, underlined significant differences between 2% and 5% formulation, the latter determining an increased rate of general side-effects, such as: pruritus, skin irritation, dermatitis with a slightly elevated rate of hair growing in places other than the scalp, burning or irritation of the eye, redness, unwanted hair growth elsewhere on the body and even temporary hair loss or exacerbation of hair loss [38,39,40]. Serious side effects such as: severe allergic reactions, chest pain, dizziness, fainting, tachycardia, unexplained weight gain, or swelling of the hands and feet, were rarely reported [41]. Our study findings are in agreement with literature reports as no side effects were recorded in the Minoxidil 2% treated animal groups (I and IV).

The three types of evaluation methods applied in our study support the idea that Minoxidil 2% has a good hair growth inducing effect in the topical application regimen on animal model. Our results correlate with other literature data on Minoxidil efficacy. An Uno and Kurata study, performed on fuzzy rats in 1993, reported that topical application of Minoxidil, Diazoxide and Copper peptide produced a conversion from short vellus to long terminal hairs, an enlargement of the follicular size and a prolongation of anagen phase [31,42]. In human studies, hair loss reduction and growth of new hair is noticed after 4 months of 1 ml application/day Minoxidil topical treatment and after 6 months there was an increased in hair count with 13.2% [43,44,45]. Unfortunately, even though Minoxidil is the most commonly used topical hair growth compound, the monotherapy has a positive response rate in just 20-56% of the cases and AGA continues to progress [31,45].

Neoptide, on the other hand, is a promising topical treatment for hair regrowth as some human studies revealed. With a proper blend of vitamin B3, amino acids and botanicals, it significantly reduces the hair loss in female patients, simultaneously boosting the hair mass. The association of Neoruscine, Nicotinamide and GP4G facilitates optimal nutritional exchange in the hair bulb and provides an energy-activating role.

In agreement with this, our own study data revealed that just the topical therapy does not offer a proper therapeutic response. We also noticed that LLLT is a powerful hair growth promoter by itself. Our results showed *that* hair density was increased when topical treatment and LLLT therapy were associated. The combination of the two induced a statistically significant hair regrowth which suggests that LLLT reinforces the chemical compound. Another possible hypothesis is that through the vasodilation created by LLLT the substance applied topically on the scalp has a better absorption rate which eases the active compounds delivery to the hair bulb. It will be our purpose, in a next research, to determine through high affinity chromatography, the absorption rate of the topical treatment.

The originality of our study research relies on the fact that LLLT was applied in conjunction with the gold standard treatment of AGA (Minoxidil 2%) and also with a new compound (Neoptide) which is thought to be an effective hair growth inducer in clinical practice. Another innovative aspect is Neoptide application on an animal model of alopecia, which was not done before.

CONCLUSIONS

An overall improvement of the test area with higher hair density was reported in all our study groups, though the results did not reach statistical significance for groups I and II when evaluation by macroscopical images (photographs) or trichoscopy (with a dermatoscope) was performed.

Results of the hair weight assessment (from a surface area of 1cm²) revealed that treatment with Minoxidil 2% induced significant hair regrowth. The lowest hair growth effect was noticed after treatment with Neoptide. Thus our study proves that not all the products recommended for human use are having the same hair regrowth efficiency on an animal model.

We also found a statistically significant difference in the increase of hair density between LLLT treated areas and control areas. Our results suggest that low-level laser treatment is more efficient than single use of topical therapy while the combined therapy (laser plus topical application) brings even supplementary benefits. The applied therapy was well tolerated as no adverse effects were reported on the animal model used.

Research on prevention and reversal hair loss continues to be a challenging subject. Further studies are required not only to compare efficiency of different therapies but also to identify the optimal length of laser treatment, the duration of the response and more importantly the long term safety of the newly implemented LLLT.

EXPERIMENTAL SECTION

Study design

40 adult Wistar-Bratislava rats were preselected for their telogen (resting) phase of the hair cycle according to age (around 120 days) from the Animal Facility of UMF “Iuliu Hatieganu” Cluj-Napoca. The Wistar albino rats of either sex, weighing about 200 g, were acclimatized to the experimental room in the Physiology Department of UMF Cluj, at a temperature of 23 Celsius degrees, in controlled humidity conditions with a 12:12 h light and dark cycle for 14 days prior to the experiment. We housed maximum 2 animals

per cage, offering them free access to standard pellets as basal diet and water ad libitum. Following the experiment they were euthanized according to the current regulations.

The study has obtained ethical committee clearance from the Institutional Animal Ethics Committee (IAEC) of Iuliu Hatieganu University of Medicine and Pharmacy Cluj-Napoca.

In order to create the alopecia animal model we induced general anesthesia and used electric hair clippers for the dorsal animal fur, followed by shaving which allowed us to create two rectangular areas, each of 2 cm width and 4 cm length. The denuded areas were symmetrically situated on both sides of the mid dorsal line, the right area provided for testing and the left as control.

We randomly assigned the animals to 5 experimental groups, each consisting of 8 rats. The treatment was performed for 1 month (Table 4).

Table 4. Experimental groups and treatments

GROUP	PRODUCT	MANUFACTURER/ CITY, COUNTRY	DETAILS	EXPOSURE DOSE	TREATMENT FREQUENCY
I	Minoxidil 2%, HairGrow	Dar Al Dawa Pharma / Amman, Jordan	topical application	0.3 ml	daily
II	Neoptide, Traitement antichute	Laboratoires Dermatologiques Ducray/Boulogne, France	topical application	0.3 ml	daily
III	HairMax Laser Comb 12	Lexington International LLC/ USA	laser exposure 655nm, <5mW	3 minutes	three times/ week
IV	Minoxidil 2% + HairMaxLaser	Dar Al Dawa Pharma, Lexington International LLC	topical laser exposure	0.3 ml 3 minutes	daily three times/ week
V	Neoptide + HairMaxLaser	Laboratoires Dermatologiques, Lexington International LLC	topical laser exposure	0.3 ml 3 minutes	daily three times/ week

The Lasercomb device

LLLT was applied by exposing the test area of each rat in Group III to HairMax Laser, at standard parameters (<5mW, continuous emission) for 3 minutes, with a frequency of three times/week. Group IV received daily

application of 0.3 ml Minoxidil 2% plus LLLT exposure three times per week, while Group V had 0.3 ml Neoptide daily topical application associated with 3 minutes LLLT, three times per week (Monday, Wednesday, Friday).

The HairMax Laser Comb Professional 12 is a hand-held Class 3R lower level laser therapy device. It contains a single laser module that emulates 12 beams: 6 of them at a wavelength of 635nm (±5%) and 6 beams at 655nm (±5%) [10].The device uses a technique of parting the subject's hair by special combs that are attached to it. By aligning the teeth with the laser beams, the hair can be parted, excluding possible obstruction and improving the delivery of distributed laser light energy to the skin surface.

The topical therapy

Topical therapy consisting of 0.3 ml solution was applied on the denuded test area of the rats daily in all test groups, except Group III, restricted to LLLT exposure. Group I received Minoxidil 2% daily application, while Group II was topically treated with Neoptide. Group IV had Minoxidil 2% topical application associated with LLLT for 3 minutes, three times per week (Monday, Wednesday, Friday). Group V also received combined therapy with Neoptide daily application together with laser therapy exposure, in the same specified regimen.

The systematic (IUPAC) name of Minoxidil is 6-Piperidin-1-ylpyrimidine-2,4-diamine 3-oxide. The chemical formula is a Chemical data formula $C_9H_{15}N_5O$, with a molecular mass of 209.251 g/mol. The Pharmacokinetic data include: its biotransformation (metabolism) primarily hepatic (90%), a half-life of 4.2 h and renal excretion [24].

Neoptide (Laboratoires Dermatologiques Ducray/Boulogne, France) is a hair growth promoter containing a peptide complex: Tetrapeptide, Neoruscine, Niconamid and guanosine (5') tetraphospho (5') guanosine abbreviated as GP4G.

Efficacy evaluation

On day 0, during the hair removal procedure with electric clippers, we defined a surface of 1 cm² and gathered the cut hairs in an aluminum foil. The hairs were then weighed with an analytical balance in the Chemistry Experimental Laboratory of the Physiology Department. Once the alopecia animal model was prepared, we photographed the macroscopic aspect and stored the images for each experimental group in electronic folders. We also used a hand held Dermatoscope (Dermlite DL3) to visualize the denuded area of the rats, followed by the capturing of the trichoscopy images and storage in electronic folders.

At the completion of 1 month treatment, the assessment of hair regrowth was evaluated in all study groups. We used both qualitative and quantitative methods, to ensure a correct evaluation process. For qualitative assessments of macroscopic and trichoscopic stored images, an observer, blind to the experiment, was used.

Macroscopic aspect and Trichoscopy are two qualitative assessments with results expressed on a scale of clinical aspect, subjectively perceived. The scale may be either defined in percentage according to the examples provided by literature, or devised by the researcher. We applied an evaluation scoring system based on the comparison of the treated area with the control of the same animal, as it has been successfully used before. The 4 stages of our scoring system were defined as: Type 1 (uneven hair growth on the tested area, skin can be easily seen), Type 2 (low hair density, with the visualization of the skin), Type 3 (moderately increased hair growth with no visible skin area), Type 4 (marked increased hair regrowth, full, thick fur). Both methods are approximations of hair growth that do not provide the possibility of precise measurement of the new grown hairs, as counting the number of hairs per unit and the determination of their diameter are not possible. Macroscopic aspect and Trichoscopy cannot quantify minor increases in hair density, which are considered a reliable sign of hair regrowth.

On the other hand, hair weight evaluation is able to determine slight increases in hair density. Hair weight is a quantitative and objective evaluation technique, based on precise measurement of hair from a predetermined area.

Statistic analysis

Descriptive statistics of the variables were expressed through mean and standard deviations. As variables are either ordinal or scale, but not normally distributed, the median value and the first and third quartile are also presented. Comparison between the treated area and the control one was based on the Wilcoxon Rank Test, a non-parametric test applied for two related samples. Significant effect of the treatment applied was considered when a p-value < 0.05 was obtained.

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