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Hair Growth Promoting Effect of Trichoxidil™: A New Natural Compound for Hair Loss

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Abstract

Background: Androgenetic alopecia (AGA) is a most common condition of hair loss. Thus, the present study aimed to investigate the effect of Trichoxidil™, a phytocomplex obtained from a blend of essential oils, in the treatment of hair loss caused by AGA. **Methods:** The CCD1072Sk cells were cultured for the 24-hour cell viability assessment and cytotoxicity of Trichoxidil™. The expression of mRNA levels from KGF, IGF-1, and VEGF in fibroblasts was evaluated by RT-qPCR. Thirty-three volunteers, diagnosed with AGA, men and women, aged between 25 and 50 years, were divided into Control group, without treatment (n = 5); Trichosol™ vehicle group, without active (n = 5); Hydroalcoholic vehicle group, without active (n = 4); Trichosol™ vehicle group, with 2.5% minoxidil (n = 5); Hydroalcoholic vehicle group, with 2.5% minoxidil (n = 5); Trichosol™ group with 2.5% Trichoxidil™ (n = 5) and Hydroalcoholic vehicle group with 2.5% Trichoxidil™ (n = 4) to dermoscopic and histologic. **Results:** Fibroblasts exhibited higher proliferation when treated with higher concentrations of Trichoxidil™. Trichoxidil™ significantly increased the expression of KGF, IGF-1, and VEGF mRNA in fibroblasts cells. Analysis of the capillary density showed that Trichoxidil™ associated with Trichosol™ vehicle, was the most effective association. In addition, it was observed an increased more effectively the percentage of anagen phase and reduction of the telogen when compared to other formulations. **Conclusion:** Trichoxidil™ promoted proliferative effects and positively modulated the expression of growth factors IGF-1, VEGF, and KGF, being a promising candidate for the treatment of hair loss caused by AGA.

Keywords

Trichoxidil™, Hair Growth, Anagen, Telogen, KGF, IGF-1

1. Introduction

Androgenetic alopecia (AGA) is a most common condition of hair loss [1] [2]. It is characterized by a progressive loss of diameter, length, and pigmentation of the hair that causes serious psychological impacts [2] [3].

Clinical treatments have variable responses and require long-term care, a factor that reduces patient follow-up [2]. Classic clinical treatment is recommended and may or may not be associated with surgical treatments such as hair transplantation. Topical minoxidil and oral finasteride are among the most used medications [2] [4]. However, new clinical treatments are necessary [2]. The use of natural products treatment as an alternative or adjuvant therapy has been carried out for a long time [5].

Thus, the present study aimed to investigate the effect of Trichoxidil™, a phytocomplex obtained from a blend of essential oils, on hair loss caused by AGA, analyzing the proportion of anagen and telogen follicles, as well as changes in other characteristics dermoscopic conditions observed in hair disorders, such as follicular units and capillary density. In addition, the study also quantified, in scalp fragments from volunteers, terminal follicles, *vellus* follicles and fibrous tract.

2. Material and Methods

2.1. *In Vitro* Studies: Reagents

Dulbecco's modified Eagle's medium (DMEM), keratinocyte serum-free medium, fetal bovine serum (FBS), penicillin-streptomycin, and phosphate-buffered saline (PBS) were obtained from Gibco BRL (Grand Island, CA, USA). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Sigma-Aldrich (St. Louis, Mo., USA). Trichoxidil™, Trichosol™, and minoxidil solution were supplied by Fagron™ (Brazil). The purity and quality of the raw materials used, as well as the formulation of Trichoxidil™ and Trichosol™ were monitored by the Fagron™ Brazil quality control department.

2.2. Culture and MTT Assay

The CCD1072Sk cell line was obtained from Rio de Janeiro Cell Bank (CCD1072Sk - ATCC CRL2088). The cells were cultured in a monolayer using IMDM (Gibco) supplemented with 10% fetal bovine serum (FBS), 100 UI/mL penicillin/streptomycin, and 0.25 μg/mL Fungizone (Gibco) in a humidified atmosphere at 37˚C in 5% CO_2. These cells were trypsinized three times per week using 0.25% trypsin/EDTA (Cultilab, Brazil). For the 24-hour cell viability assessment, the control and treated cells were centrifuged and resuspended in equal parts medium and trypan blue (0.05% solution) and counted using a hemocytometer. To evaluate the cytotoxicity of Trichoxidil™, the essential oil was dissolved in the culture medium in appropriate concentrations. The cell viability of control and Trichoxidil™ (0.05% - 5.0%)-treated fibroblasts cells were measured using a standard MTT assay. Briefly, 5×10^4 viable cells were seeded into

clear 96-well flat-bottom plates (Corning) in IMDM medium supplemented with 10% fetal bovine serum (FBS) and incubated with different concentrations of the extract for 24 h. Then, 10 µL/well of MTT (5 mg/mL) was added and the cells were incubated for 4 h. Following incubation, 100 µL of 10% sodium dodecyl sulfate (SDS) solution in deionized water was added to each well and left overnight. The absorbance was measured at 595 nm using a FlexStation 3 Multi-Mode Benchtop Reader (Molecular Devices, Sunnyvale, CA, USA).

2.3. Reverse Transcription-Quantitative PCR (RT-qPCR)

The effect of Trichoxidil™ on the expression of mRNA of KGF; IGF-1; and VEGF in fibroblasts was evaluated by RT-qPCR. Cells were treated with Trichoxidil™ (2.5% and 5.0%) for 24 hours. Total RNA extracted from cells samples was converted to cDNA using a SuperScript® III RT kit (Invitrogen, Carlsbad, CA), according to the manufacturer's protocol. The concentration of RNA was detected using a NanoDrop 2000 (Thermo Fisher Scientific, Inc.). GAPDH was used as the internal control. The thermocycling conditions were as follows: 95˚C for 10 min followed by 36 cycles of 94˚C for 15 sec and 57˚C for 40 sec. The 2-ΔΔCq method was used to quantify the relative gene expression levels of the target genes. Relative standard curves were generated by serial dilutions and all samples were run in triplicates. Following, the sequence of primers used in the qRT-PCR analysis: primer forward (5'-AGAGACCCTTTGCGGGGCTGA-3') and reverse primer (5'-CTTCTGAGTCTTGGGCATGT-3') for IGF-1; primer forward (5'-ATCAGGACAGTGGCAGTTGGA-3') and reverse primer (5'-AAC ATTTCCCCTCCGTTGTGT-3') for KGF, primer forward (5'-CTTTAGAGATC AGCCCAACC-3') and reverse primer (5'-CTACCCAGAGGGAAGAAATAAC-3') for VEGF; and primer forward (5'-GGAAGGTGAAGGTCGGAGTC-3') and reverse primer (5'-CTCAGCCTTGACGGTGCCATG-3') for GAPDH.

2.4. *In Vivo* Studies: Volunteers and Study Groups

This study was carried out after approval by the local Ethics Committee and written consent was obtained from each subject (3.150.582 - approbation number). After obtaining informed written consent, all the patients were subjected to a detailed medical history followed by thorough general physical, dermatological and systemic examinations. Both the image acquisition by the Trichoscan equipment and the cylindrical fragments, 33 volunteers, diagnosed with androgenetic alopecia, men and women, aged between 25 and 50 years, were evaluated. Exclusion criteria were: 1) volunteers who were not diagnosed with AGA; and 2) volunteers who were not in the age range (25 and 50 years). The subjects were divided into four groups and assigned the drugs in a randomized manner. Control group, without treatment (n = 5); Trichosol™ vehicle group, without active (n = 5); Hydroalcoholic vehicle group, without active (n = 4); Trichosol™ vehicle group, with 2.5% minoxidil (n = 5); Hydroalcoholic vehicle group, with 2.5% minoxidil (n = 5); Trichosol™ group with 2.5% Trichoxidil™ (n = 5) and

Hydroalcoholic vehicle group with 2.5% Trichoxidil™ (n = 4). They were instructed to apply the solution to the balding area with a calibrated dropper twice daily at 12-hour intervals. The volunteers were evaluated in 2 phases throughout the study. Time zero (T = 0), the first evaluation and time ninety (T = 90), the second evaluation, after 90 days of treatment with the formulations developed for the study. In the evaluation, T = 0, data and images were obtained using the Trichoscan, followed by the collection of the cylindrical fragment for extraction of the total mRNA and histological analysis. The same procedure was repeated after 90 days of treatment (T = 90).

2.5. Dermoscopic Analysis (Trichoscan)

The FotoFinder Trichoscale softwear was used to evaluate the parameters of the hair growth phases, as the anagen and telogen phases. All patients were assessed and subjected to photographic records with a 10x magnification dermatoscope and a digital camera with 20x and 40x magnification on the small area of the shaved headscalp. The dermoscopy findings, for example, the numerical data report, as well as the photos generated by the equipment, were stored for statistical analysis and compared between the groups evaluated.

2.6. Scalp Fragments-Histologic Evaluation and RT-qPCR

Cylindrical scalp fragments were obtained on the vertex region. After trimming 1.0 cm^2 of hair, 4-mm punch biopsies were taken at T = 0 and T = 90. The punches were obtained parallel to the direction of hair growth. For follicular peribulbar evaluation and molecular biology analysis, scalp fragments were fixed for four h to 48 h, using 10% buffered formalin, and embedded in pure paraffin. To histologic analysis, the scalp fragments were sectioned and stained with hematoxylin and eosin. The following parameters were evaluated: total follicles, vellus follicles, terminal follicle and fibrous tract. The presence of peribulbar changes (*i.e.* inflammation) were assessed by a dermatologist. Part of the scalp fragments were used to extract total RNA followed by the quantification of mRNA of KGF; IGF-1; and VEGF, as described above in the in vitro tests. All tissues were stored at −80˚C until the RNA extraction procedure.

2.7. Statistical Analysis

The obtained results were expressed as the mean ± standard error of mean (SEM) from at least three independent experiments, unless stated otherwise. Paired data was evaluated by Student's t-test. One-way analysis of variance (ANOVA) was used for multiple comparisons. A p value of <0.05 was considered significant.

3. Results

3.1. Effects of Trichoxidil™ on Cellular Viability

Figure 1 shows the different concentrations of Trichoxidil™, which were tested

for cell proliferation on fibroblast cell line. The highest concentrations tested induce cell proliferation (*p < 0.01).

3.2. Trichoxidil™ Regulates KGF, IGF-1, and VEGF mRNA Levels in Fibroblasts Cells

To investigate whether Trichoxidil™ regulates KGF, IGF-1, and VEGF expression at transcriptional level, RT-PCR was performed. As shown in **Figure 2**, Trichoxidil™ significantly increased the expression of KGF, IGF-1, and VEGF mRNA levels in fibroblasts cells. Trichoxidil™ was superior to the normalized control (arbitrary unit 1.0). It was also shown to be superior to minoxidil, considering the levels of KGF and IGF-1 mRNA (a p < 0.05) and (#p < 0.05), respectively. In addition, Figure 2 shows the dose-response effect of both Trichoxidil™ (C) and minoxidil (D) on the mRNA levels of the growth factors evaluated.

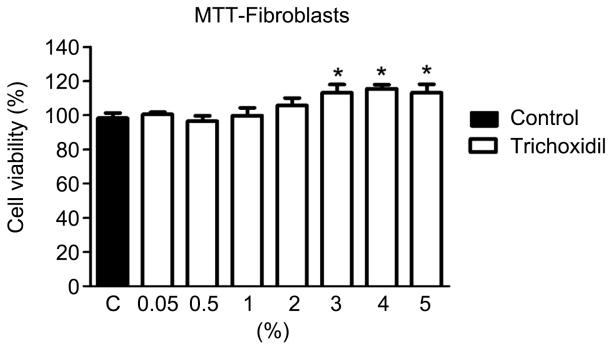

Figure 1. Effects of Trichoxidil™ on fibroblast cell line viability. Cells were treated with different concentrations of Trichoxidil™ for 24 h. Date shown are representative of three independent experiments. The values are expressed as mean ± SEM and *p < 0.01 indicates statistical difference (unpaired t-test).

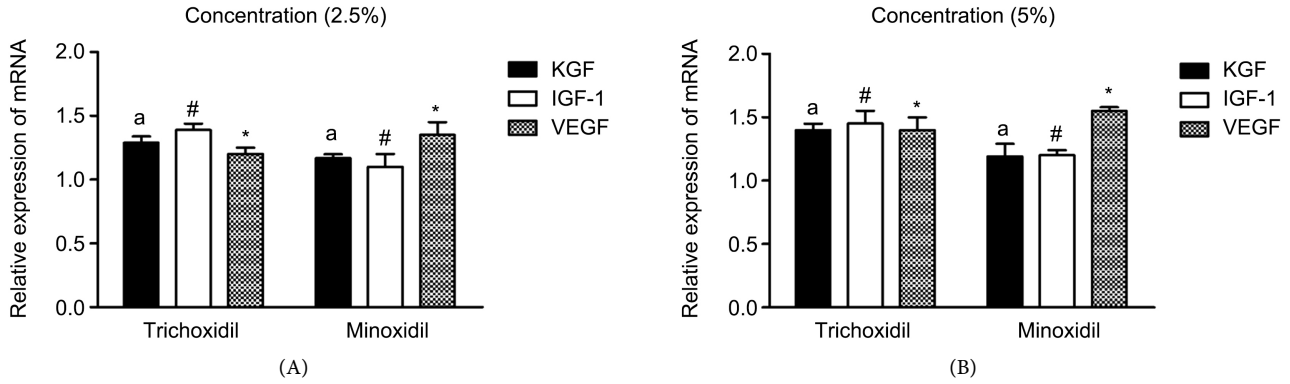

Figure 2. Effects of Trichoxidil™ on the expression of mRNA of KGF, IGF-1, and VEGF from fibroblasts cells. (A) Both Trichoxidil™ and minoxidil increased the levels of mRNA of KGF, IGF-1, and VEGF, when compared to normalized control (data not showed) however, in relation to KGF (a p < 0.05) and IGF-1 (#p < 0.05), Trichoxidil™ showed a significant increase when compared to minoxidil-treated cells. (B) Similarly, the concentration of 5.0% of Trichoxidil™ was higher than minoxidil to IGF-1 (#p < 0.05) and KGF (a p < 0.05) mRNA levels. GAPDH was used as an internal control. The mRNA levels were determined using real-time RT-PCR. Values are expressed as means ± SEM. (#) p < 0.05 Trichoxidil™-treated cells vs. minoxidil-treated cells to IGF-1 mRNA levels. (a) p < 0.05 Trichoxidil™-treated cells vs. minoxidil-treated cells to KGF mRNA levels. (*) p < 0.05 Trichoxidil™-treated cells vs. minoxidil-treated cells to VEGF mRNA levels.

3.3. Evaluation of Hair Growth of Volunteers by Dermoscopy (TrichoScan)

The results obtained at times T = 0 and T = 90 days are shown in Table 1. Anagen and telogen follicles, follicular units and capillary density were evaluated. Both Trichoxidil™ and minoxidil increased all parameters evaluated. Analysis of the capillary density showed that Trichoxidil™ associated with Trichosol™ vehicle, was the most effective association (Table 1). Figure 3(A) shows that the formulations containing Trichoxidil™ and minoxidil, which used Trichosol™ as vehicle, significantly increased the percentage of follicular units (*$p < 0.05$). Figure 3(B) shows that Trichoxidil™ formulated with Trichosol™ increased more effectively the percentage of anagen phase and reduction of the telogen when compared to other formulations.

3.4. Histological Evaluation of Transverse Scalp Sections

Total follicles, vellus follicles, terminal follicles and fibrous tract were evaluated, shown in Table 2. Figure 4(A) shows the percentage of total follicles in volunteers who used Trichoxidil™ or minoxidil formulations. Total follicles percentage was higher in volunteers treated with Trichoxidil™ associated with Trichosol™,

Table 1. Parameters obtained by dermoscopy using TrichoScan. Anagen and telogen follicles were analyzed, as well as follicular units and follicle density. (*) $p < 0.05$, significant in relation to T = 0. Student's t test.

Group	Dermoscopy by TrichoScan (mean ± SEM)				
	Time (days)	Anagen follicles	Telogen follicles	Follicular units	Hair density
Control	T = 00	55.6 ± 3.7	54.4 ± 2.4	59.3 ± 5.1	89.3 ± 2.2
	T = 90	61.3 ± 2.4	38.7 ± 8.6	65.0 ± 5.3	106.5 ± 8.7
Alcohol	T = 00	67.0 ± 2.5	32.9 ± 5.5	116.6 ± 1.5	201.6 ± 10.2
	T = 90	70.2 ± 3.5	29.8 ± 3.8	**103.6 ± 2.2***	193.8 ± 6.1
Trichosol	T = 00	75.6 ± 1.2	24.4 ± 1.2	111.0 ± 16.0	167.9 ± 9.2
	T = 90	**82.9 ± 2.5***	**17.1 ± 2.0***	119.1 ± 16.4	**222.9 ± 12.8***
Minoxidil + alcohol	T = 00	56.8 ± 3.8	43.2 ± 6.8	106.6 ± 5.2	198.5 ± 6.0
	T = 90	**69.8 ± 2.1***	**30.2 ± 3.1***	**117.0 ± 2.3***	201.8 ± 4,5
Minoxidil + trichosol	T = 00	56.2 ± 1.3	43.8 ± 2.3	79.6 ± 3.5	106.2 ± 5.1
	T = 90	**71.1 ± 4.7***	**29.9 ± 2.1***	**92.3 ± 4.8***	**234.0 ± 19.8***
Trichoxidil + alcohol	T = 00	59.7 ± 1.0	40.2 ± 1.2	87.5 ± 3.8	189.5 ± 7.8
	T = 90	**72.1 ± 2.6***	**28,9 ± 2.6***	**97.0 ± 2.7***	**215.4 ± 13.1***
Trichoxidil + trichosol	T = 00	57.2 ± 6.0	42.8 ± 7.0	106.3 ± 7.1	245.6 ± 7.9
	T = 90	**73.2 ± 5.9***	**26.7 ± 6.1***	**121.3 ± 1.3***	**269.3 ± 6.5***

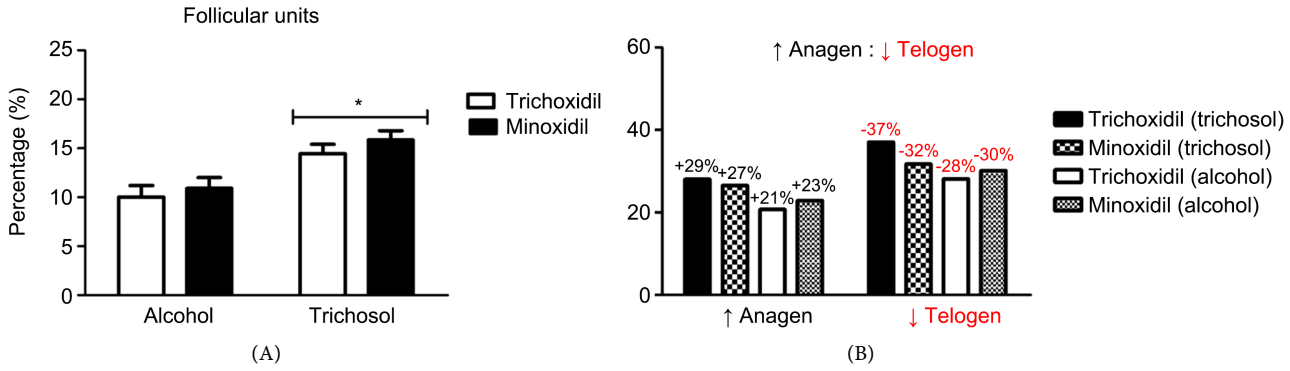

Figure 3. Trichoxidil™ and minoxidil formulations in Trichosol™ or alcohol vehicle (T = 0 and T = 90). (A) Porcentage of follicular units after 90 days of treatment. Note the significant increase in formulations that used Trichosol™ as a vehicle instead of alcohol (*p <0.05). There was no statistical difference between Trichoxidil™ and minoxidil in Trichosol™ vehicle (B) Percentage of anagen and telogen phases. Trichoxidil™ formulated with Trichosol™ increased more effectively the percentage of anagen phase and reduced the telogen phase when compared to other formulations.

Table 2. Parameters obtained from fragments obtained from volunteers. Histological examination evaluated total follicles, follicular vellus, terminal follicles and fibrous tract. (*) p <0.05, significant in relation to T = 0. Student's t test.

Group	Punch-biopsy (mean ± SEM)				
	Time (days)	Total follicles	Vellus follicles	Terminal follicles	Fibrous tract
Control	T = 00	29.6 ± 7.5	5.3 ± 0.8	8.1 ± 2.8	5.4 ± 1.7
	T = 90	27.6 ± 7.1	6.6 ± 1.7	5.3 ± 4.1	3.4 ± 1.1
Alcohol	T = 00	26 ± 8.0	8.0 ± 1.3	4.8 ± 3.2	6.0 ± 1.5
	T = 90	25 ± 0.5	6.9 ± 2.6	9.6 ± 5.7	5.0 ± 2.6
Trichosol	T = 00	28.6 ± 1.4	9,8 ± 4.0	3.6 ± 3.2	4.6 ± 0.8
	T = 90	**33.0 ± 1.3***	8.7 ± 4.8	7.6 ± 1.7	5.0 ± 3.1
Minoxidil + alcohol	T = 00	29.3 ± 3.1	9.8 ± 0.8	4.3 ± 0.9	5.6 ± 3.5
	T = 90	**37.1 ± 4.5***	**7.4 ± 1.3***	**5.6 ± 2.7***	5.0 ± 2.0
Minoxidil + trichosol	T = 00	25.0 ± 4.1	7.4 ± 1.1	14 ± 2.6	3.3 ± 1.1
	T = 90	**35.3 ± 5.7***	**5.0 ± 1.3***	**18.2 ± 0.6***	2.1 ± 0.4
Trichoxidil + alcohol	T = 00	25.0 ± 2.6	8.9 ± 2.7	9.9 ± 2.7	3.67 ± 0.8
	T = 90	**33.3 ± 7.1***	**6.2 ± 1.8***	**12.7 ± 1.1***	**1.21 ± 1.7***
Trichoxidil + trichosol	T = 00	16.6 ± 3.5	6.1 ± 2.1	5.9 ± 1.3	8.9 ± 2.0
	T = 90	**23.9 ± 2.7***	**3.9 ± 1.1***	**7,9 ± 0.6***	**5.7 ± 1.8***

when compared to other formulations (*p < 0.05). Trichoxidil™ also significantly reduced (*p < 0.05) the percentage of vellus follicles when compared to minoxidil, regardless of the vehicles used in the formulations (Figure 4(B)). The percentage increase in terminal follicles was significant (*p < 0.05) in the volunteers who used the formulation of Trichoxidil™ in Trichosol™ (Figure 4(C)).

Figures 5(A)-(G) shows histological sections of anagen terminal follicles (capillary channel diameter greater than the inner root sheath), without signs of apoptosis in the outer root sheath. Finally, the results described in Table 2 showed that the volunteers who received formulations with Trichoxidil™, both in Trichosol™ vehicle and hydroalcoholic vehicle, showed a significant reduction (*p < 0.05) in the number of fibrous tracts.

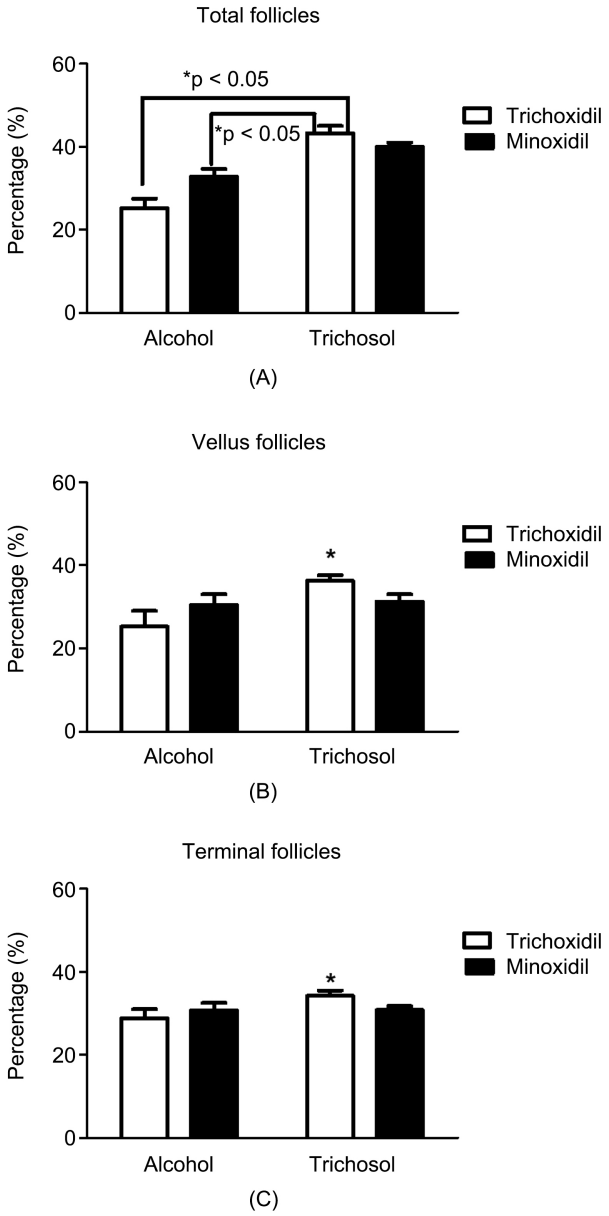

Figure 4. Comparison between the different formulations with Trichoxidil™ and minoxidil. (A) Percentage of total follicles. The formulation Trichoxidil™ in vehicle Trichosol™ was superior to the other formulations (*p < 0.05). (B) Percentage of follicles vellus. Minoxidil in Trichosol™ was able to significantly reduce the percentage of vellus follicles in the volunteers (*p < 0.05). (C) Percentage of terminal follicles. Trichoxidil™ in Trichosol™ increased significantly the percentage of terminal follicles in the volunteers (*p < 0.05).

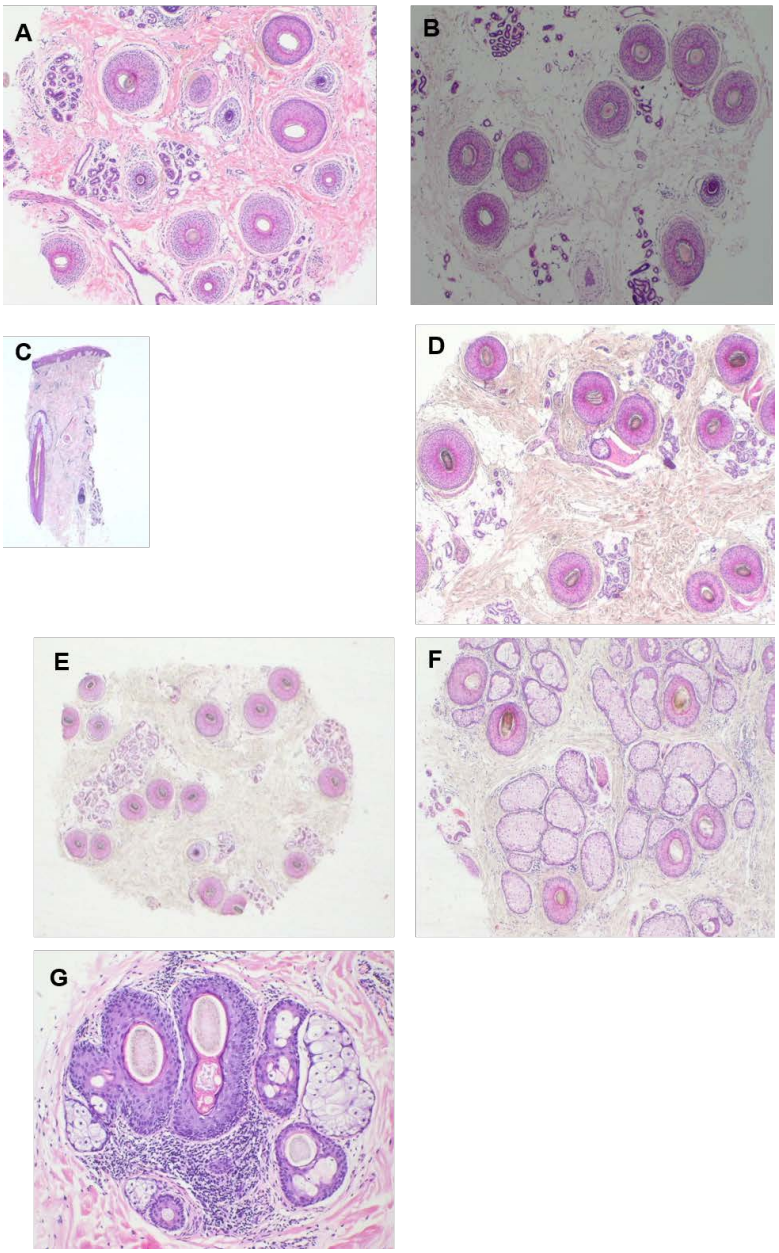

Figure 5. Histological aspect in transverse section of follicles: (A) Control; (B) Trichosol™ vehicle; (C) Alcohol vehicle; (D) Minoxidil in Trichosol™ vehicle; (E) Minoxidil in alcohol vehicle; (F) Trichoxidil™ in Trichosol™ vehicle; and (G) Trichoxidil™ in alcohol vehicle. Terminal anagen with inner and outer root sheath showing growth of new hair (HE, 100×).

3.5. Expression of Growth Factors at mRNA Levels in Scalp Fragments

We also investigated the effects of Trichoxidil™ on the mRNA levels of growth factors KGF, IGF-1, and VEGF in scalp fragments. The expression of growth factors at mRNAs levels increased after treatment with Trichoxidil™ and minoxidil formulations. Regarding IGF-1, the results shown in **Figure 6(A)**, indicate that both Trichoxidil™ and minoxidil increased the mRNA levels. Although

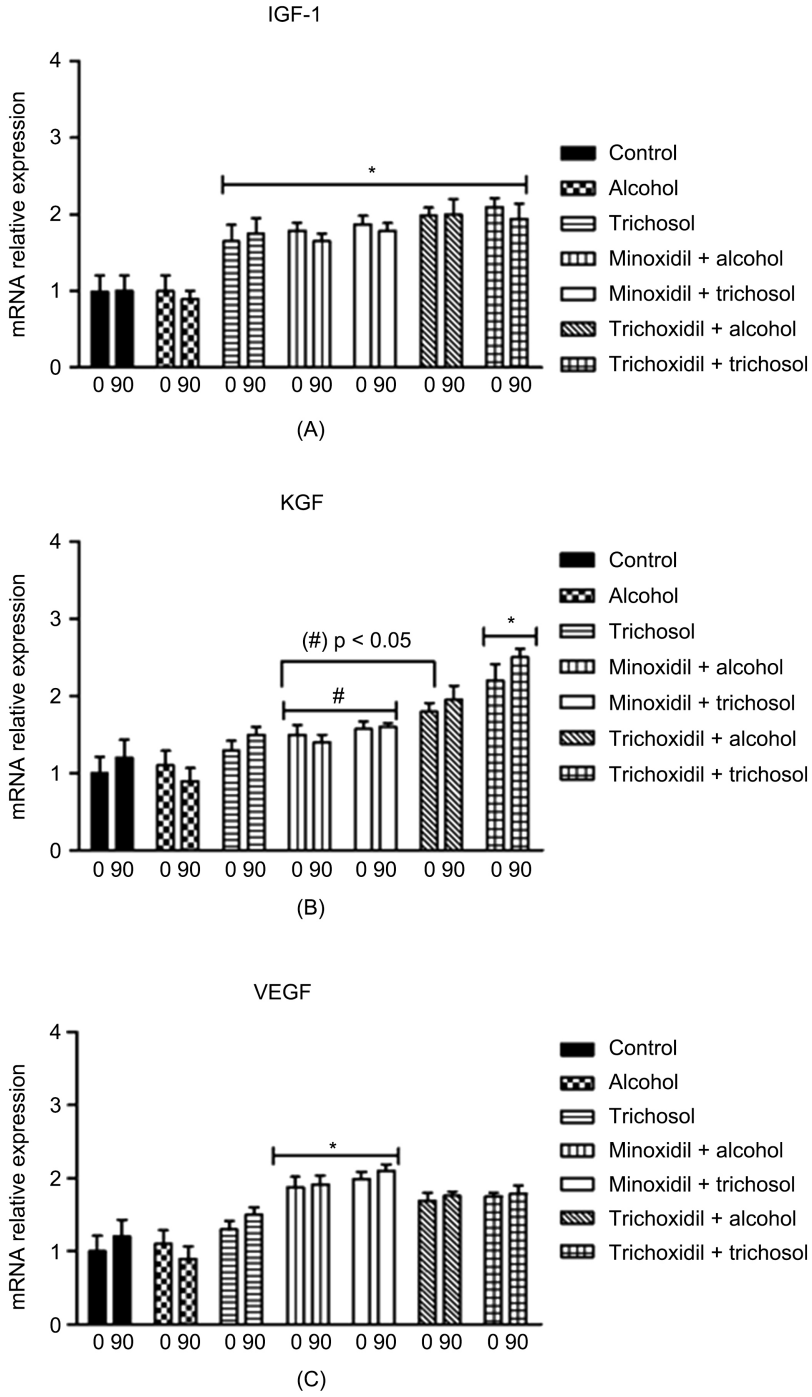

Figure 6. Effects of Trichoxidil™ formulations on the expression of mRNA levels of IGF-1, KGF, and VEGF in scalp fragments. Results obtained before and after using the formulations (T = 0) and (T = 90). (A) mRNA levels of IGF-1 increased significantly (*$p < 0.05$) compared to groups of volunteers who used vehicles only (Trichosol™ or alcohol). Formulations containing Trichoxidil™ or minoxidil showed no significant difference in mRNA levels expression. (B) Trichoxidil™ formulations significantly increased KGF mRNA levels (*$p < 0.05$), when compared to other formulations and control group. Trichoxidil™ formulation, even in an alcoholic vehicle, was superior to formulations with Minoxidil (#$p < 0.05$). (C) VEGF mRNA levels. Formulations containing minoxidil significantly increased the expression of VEGF mRNA levels when compared to formulations containing Trichoxidil (*$p < 0.05$). Real-time RT-PCR was performed to measure the mRNA levels of IGF-1, KGF, and VEGF in scalp fragments. Glyceraldehyde-3-phosphate dehydrogenase (GADPH) was used as an internal control. Data are presented as the means ± SEM of three independent experiments.

formulations with Trichoxidil™ showed an increase, there was no statistical significance. **Figure 6(B)** indicates that Trichoxidil™ independent of the vehicle, significantly increased the expression of KGF when compared to other formulations, including minoxidil formulations (*$p < 0.05$). Finally, **Figure 6(C)** shows that formulations containing minoxidil significantly increased the expression of VEGF mRNA levels compared to the other groups (*$p < 0.05$).

4. Discussion

Even though it is a prevalent condition, therapeutic options for AGA are limited and include finasteride and minoxidil, in addition to antiandrogens for female patients [6]. In this study, we introduced Trichoxidil™, a phytocomplex developed from essential oils, that presents as an option for the treatment of hair growth. The in vitro results showed an absence of cytotoxicity by Trichoxidil™ and activation of fibroblast proliferation (CCD1072Sk cell line), besides to promoting upregulation in the expression of mRNAs levels of growth factors related to hair growth, such as insulin-like growth factor 1 (IGF-1) [7], keratinocyte growth factor (KGF) [8] [9] and vascular endothelial growth factor (VEGF) [10]. In the present work, hair growth was evaluated by dermoscopy, histological analysis and mRNA levels quantification. Dermoscopy, a non-invasive diagnostic method, which allows the visualization of microscopic details of skin lesions, besides revealing some structures of the skin below the surface that are not normally visible [11]. Thus, by dermoscopy, the results obtained showed that Trichoxidil™ increased the percentage of anagen and reduced the telogen phase, besides increase significatively follicular units when compared to minoxidil formulations. In addition, Trichoxidil™ showed significative increase of hair density when compared to minoxidil formulations. In the same sense, the histological evaluation revealed that Trichoxidil™ significantly increased total follicles and terminal follicles, whereas the formulation containing minoxidil in Trichosol™ vehicle significantly reduced vellus follicles. Studies have shown that some essential oils contribute to hair growth [12] [13] [14] [15], modulating the expression of growth factors, such as those evaluated in this work, IGF-1 [16], KGF [17], and VEGF [18]. Growth factors that stimulate the anagen phase, comprise fibroblast growth factor 7 (FGF-7 or KGF), hepatocyte growth factor (HGF), IGF-1, prostaglandin E2 (PGE2), and VEGF [19]. Thus, the IGF-1 plays a role in maintaining the anagen phase and its absence can lead to the premature transition from the catagen phase, and in patients with AGA, IGF-1 is dysregulated in regions such as the dermal papilla [20] [21]. Another growth factor present on the development of hair follicles are the members of the fibroblast growth factor (FGF) family, among which is FGF-7 or KGF, an important endogenous mediator of normal hair follicle growth, development, and differentiation [22] [23]. In relation to VEGF, it is important in hair development, promoting growth, and differentiation, of the hair follicle and hair shaft [24]. In addition, VEGF appears to be related with angiogenesis associated to the hair

growth cycle, since VEGF mRNA expression was increased in follicular keratinocytes during the initial anagen-medium growth phase, followed by negative regulation during the involution of catagen [25]. Since, the hair follicle development and morphogenesis requires the intricately controlled regulation of proliferation, differentiation, and apoptosis, we suggest that the results obtained with Trichoxidil™, may be related to the increased expression of the growth factors evaluated in this study. Although the expression of IGF-1 mRNA levels was not greater compared to formulations with minoxidil, the increase in relation to volunteers who participated in the control group or who received only vehicles is notorious. However, KGF showed a significant increase when we compared with formulations containing Minoxidil. KGF is a member of the FGF family, which regulates the proliferation and differentiation of various cell types, including follicular development and hair growth [26] [27]. The activation of the KGF pathway and its time-dependent exposure can influence the morphogenic follicle, including the early morphogenic follicle [28]. In KGF knockout mice, restriction to cells originating from the hair shaft was identified, in addition to changes in hair, but not in epidermal morphology, suggesting a relationship between KGF and hair growth [29] [30]. In addition, KGF appears to exert a cytoprotective effect by reducing DNA damage and decreasing p53 levels after treatment of hair follicles with menadione [31]. Finally, formulations containing Trichoxidil™ and minoxidil, significantly increased the relative expression of mRNA levels of VEGF when compared to control cells or formulations containing only vehicles. When we compared formulations containing minoxidil, Trichoxidil™. However, formulations containing minoxidil, showed a significant increase in relation to other formulations, including those with Trichoxidil™, something, at least in part, expected, since one of the minoxidil mechanism hypotheses is to increase VEGF expression [32] [33] [34]. Although formulations containing Trichoxidil™ and in vitro studies indicated minoxidil's superiority in VEGF mRNA expression levels, it is clear that Trichoxidil™ significantly increased VEGF compared to other experimental conditions, suggesting that positive VEGF modulation is one of the components of multi action target of Trichoxidil™.

5. Conclusion

Trichoxidil™ showed a proliferative effect *in vitro* and *in vivo*, possibly by positive modulation of growth factors, such as IGF-1, VEGF and, especially, KGF, revealing to be a promising candidate for treatment of hair loss caused by AGA.

Limitations of This Study

As limitations of the study, we can mention the lack of a control group without androgenetic alopecia, to obtain normal hair growth parameters.

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Statement of Data Availability

The authors declare that all raw data presented in this manuscript will be available upon request.

Conflicts of Interest

All authors disclose any influence of companies or manufacturers in the present study. In addition, all authors declare that the results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

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