Cellium® GC: evaluation of a new natural active ingredient in 210 mg/ml topical solution, through scalp biopsy

Avaliação por biópsias de couro cabeludo da atividade de novo ingrediente ativo natural, o "Cellium® GC", formulado em solução tópica de 210mg/mL

Original Article

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ABSTRACT

Introduction: Androgenic alopecia is a progressive alteration of the scalp with few treatment options, which motivates the search for new local or systemic medications to control this pathology.

Objective: To evaluate patient tolerance for and identify the action mechanism of the Cellium® GC compound in the treatment of androgenic alopecia.

Methods: Male patients (n = 20) with androgenic alopecia participated in this open prospective study. The compound was used on the scalp twice a day, at home, for 12 consecutive weeks. Biopsies were carried out before and after treatment to evaluate the alterations in the cutaneous immune response, cellular proliferation and anti-apoptosis activity. Questionnaires were administered to evaluate efficacy and patient satisfaction.

Results: Nineteen patients completed the study, with an average satisfaction of 8.3 out of 10. Immunohistochemical analyses of scalp biopsies showed a significant increase in the cutaneous immune response after treatment: 73.9% increase in CD1A+ Langerhans cells (p = 0.003, t paired test), 41.7% increase in the cellular proliferation marker Ki-67+ (p = 0.012), and an 89% increase in BCL-2+ anti apoptotic proteins (p = 001). The product was also found to be tolerable and safe. Conclusions: Cellium® GC improved the skin's immune defense and the proliferation of keratinocytes, and produced high levels of patient satisfaction in the treatment of androgenic alopecia.

Keywords: alopecia; biopsy; keracinocytes; minoxidil.

RESUMO

Introdução: *A alopecia androgênica é alteração progressiva do couro cabeludo com poucas opções terapêuticas. Justifica-se, portanto, a pesquisa de novas drogas de uso local ou sistêmico direcionadas ao controle desta patologia. Objetivo: Avaliar a tolerância e identificar o mecanismo de ação do composto Cellium® GC no tratamento da alopecia androgênica.*

Métodos: *Estudo prospectivo e aberto em 20 portadores de alopecia androgênica. O produto foi utilizado no couro cabeludo duas vezes ao dia em regime domiciliar por 12 semanas consecutivas. Foram realizadas biópsias antes e depois do tratamento para avaliar as alterações da resposta imune cutânea, da proliferação celular e da atividade antiapoptose. A avaliação da efetividade e do grau de satisfação dos pacientes foi realizada por meio de questionários.*

Resultados: *Dezenove voluntários do sexo masculino completaram o estudo, com grau médio de satisfação de 8,3/10. Análises imuno-histoquímicas das biópsias de couro cabeludo revelaram aumento significativo da resposta imune cutânea depois do tratamento: 73,9% de aumento de células de Langerhans CD1A+ (p = 0,003, teste t pareado), 41,66% de aumento de Ki-67+, marcador de proliferação celular (p = 0,012), 89% de aumento de proteínas antiapoptóticas BCL-2+ (p = 0,001). O produto também foi bem tolerado e seguro.*

Conclusões: *Cellium® GC melhora as defesas imunológicas da pele e a proliferação dos queratinócitos, e confere satisfação aos voluntários no tratamento da alopecia androgênica.*

Palavras-chave: *alopecia; biópsia;queratinócitos; minoxidil.*

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This study was carried out at the Dermatology Outpatient Department of the Hospital das Clínicas da Universidade de São Paulo, São Paulo, Brazil.

Conflicts of interests: The authors wish to thank Legacy Healthcare (Epalinges, Switzerland) for providing the topical solution (Cellium® GC) used in this study.

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INTRODUCTION

Androgenetic alopecia (AGA) is biologically natural process that, under normal circumstances, has no negative impact on the clinical state of humans; however, it has a negative impact on quality of life. It affects more than 50% of men aged 50 as well as a significant proportion of women.[1] The development of AGA is dependent on the interaction of genetic and hormonal factors, with a multifactorial etiology having been advanced.[2]

Two drugs are indicated for the treatment of the condition, based on scientific evidence: minoxidil and finasteride. Nevertheless, the search for new substances with a similar purpose is ongoing.[3]

A new, biologically active substance (Cellium® GC; Legacy Healthcare, Epalinges, Switzerland), the combination of active principles extracted from plants, has been developed into a solution for topical use for the treatment of excessive hair loss. In the concentration of 210 mg/ml, Cellium® GC has been shown to be effective in preventing hair loss and promoting hair growth. A preliminary clinical study showed that Cellium® GC in a concentration of 210 mg/ml significantly increases the number of hairs in the anagen phase and significantly reduces the number of hairs in the telogen phase, leading to normalisation of the anagen/telogen ratio 6 weeks post-treatment.[4] In addition, a different study involving male patients who presented with AGA showed that topical application of Cellium® GC to the scalp is well tolerated and accompanied by a high degree of satisfaction with regard to its efficacy.[5]

Bearing this in mind, it is important to understand the action mechanisms of this new active agent. When tested in vitro using endothelial cells, Cellium® GC showed the ability to stimulate angiogenic response6, while an in vivo study showed a significant increase in the concentration of perifollicular collagen.[5] Nevertheless, it is proving difficult to link the results of the two studies to the efficacy of the active substance with regard to either hair growth or hair loss. Therefore, further testing, aimed at identifying any additional action mechanisms responsible for the efficacy y of Cellium® GC at a concentration of 210 mg/ml in patients affected by AGA, is warranted.

Action mechanisms which were investigated further include the topical effect of the substance on cutaneous immunological defenses, keratinocyte proliferation and anti-apoptotic activity.

Study design

Participants of this open, monocentric, prospective 12-week study were their own witnesses.

Study population

Twenty volunteers presenting with clinically diagnosed AGA were recruited by the Dermatology Ambulatory of the Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil from 14 January to 20 June 2008. The study was carried out in accordance with the Declaration of Helsinki, while laboratory analyses complied with the principles set out by the Best Laboratory Practice guidelines as defined by the Instituto Nacional de Metrologia, Normalização e Qualidade Industrial (Inmetro; National Institute of Metrology, Standardization and Industrial Quality, Brazil).

Participants had to meet the following inclusion criteria: men, aged 20–40 years, Fitzpatrick skin phototypes II and III, presenting with AGA, classified as type I–VII according to the modified Norwood–Hamilton scale, absence of treatment with minoxidil or finasteride for at least 5 months prior to recruitment, and absence of modifications in habits and hair styling during the study period. The exclusion criteria were: women, treatment with minoxidil or finasteride in the 5-month period preceding recruitment, iatrogenic or traumatic alopecia, concomitant use of other scalp treatments, seborrheic dermatitis, psoriasis, or any scalp dermatitis.

METHODS

The study was approved by the ethics committee of the patient care service and all volunteers signed an informed consent form. Participants had to attend two clinical examinations for data collection and scalp biopsies [the first time before the start of treatment (W0 phase) and the second 12 weeks after treatment (W12 phase) with Cellium® GC 210 mg/ml (Legacy Healthcare, Epalinges, Switzerland)]. Additionally, questionnaires completed by the investigator, based on answers given by both the volunteers and their families, were collected (in the W12 phase).

Side effects and cosmetic tolerance were evaluated by the investigator using a scale of 1–3 for intensity and 1–8 for the dermatological examination.

Biopsies from the vertex scalp were obtained out using a 4 mm punch. The samples were fixed in phosphate-buffered formalin (pH 7.2) at room temperature for 24 h, and were later embedded in paraffin wax. The paraffin slides were hematoxylin and eosin stained for histological examination.

Sections of 3μ were prepared from the paraffin-fixed samples and the longitudinal biopsy slides were processed using the avidin-biotin-peroxidase (ABC) immunohistochemistry technique.7 Following deparaffinization, the slides were hydrated and incubated in 0.3% H_2O_2 in methanol for 20 minutes to reduce endogenous peroxidase activity. The slides were then incubated at 4 °C overnight with primary antibodies (Dako Denmark A/S, Glostrup, Denmark) diluted in tris-buffered saline solution (TBS) containing 0.5% bovine serum albumin. The primary antibodies used in the study were: cluster of differentiation 1a (CD1A) in Langerhans cells; antigen identified by monoclonal antibody Ki-67 (a cellular proliferation marker), heat shock protein 47 (HSP47), B-cell lymphoma 2 (BCL2) (an apoptosis regulator protein). The slides were then washed twice in TBS and then incubated with goat-anti-mouse/goat-anti-rabbit biotinylated antibodies [Dako Duet streptABComplex/HRP kit (code number: K 0492), Dako Denmark A/S, Glostrup, Denmark]. After incubation for 1 h at 37 °C with the second antibody, the slides were incubated using

the VECTASTAIN ABC kit (Vector Laboratories, Burlingame, CA, USA) at room temperature for 30 minutes, then developed with diaminobenzidine (Sigma-Aldrich, Barcelona, Spain) and embedded in Entellan® (catalogue number: 107961; Merck KGaA, Darmstadt, Germany). The slides were then counterstained with Mayer's hematoxylin for 2 minutes. All histological slides were processed simultaneously for each marker. The negative controls were slides without primary antibody. The positive controls were slides of other tissues showing positive reactions to each specific antibody.

Microscopic analysis was carried out using a CCD Sony camera linked to a Zeiss Axioplan optical microscope. Images were processed using the Kontron 300 image analyzer (Zeiss, Feldbach, Switzerland). Ten different fields were randomly selected and the dermal area was determined through the analysis of images (200x magnification). The immunohistochemical reaction threshold level was set for each slide after the contrast had been enhanced so that the cells could be easily identified. The area occupied by the cells was determined by means of digital densitometric recognition, by adjusting the measurement threshold level up to the gray density.

TREATMENT

The investigator supplied each volunteer with a specific treatment in its commercial version (without randomization or coded label). The treatment set included two 110 ml clear glass vials supplied with a pumping system and containing a brownish solution. Specifically, the solution contained 210 mg/ml of Cellium® GC, a combination of active principles extracted from four plants (Allium cepa, Citrus medica limonum, Paullinia cupana and Theobroma cacao). The treatment began after the S0 phase, with volunteers applying approximately 1 ml of the topical solution on either dry or wet scalp twice a day (total daily dose: 2 ml), at 12-h intervals over a period of 12 weeks. Previous clinical trials4 showed that the effective target-dose is 1 ml, applied twice a day. Patient adhesion to treatment was assessed by analysing product consumption for each patient.

STATISTICAL ANALYSIS

Statistical analyses were carried out using the paired t-test, unidirectional variance analysis, the Kruskal–Wallis, Tukey's, and Dunnett's tests, using the SigmaStat software (Jandel Corporation, California, USA) for the immunohistochemical analyses. The data were deemed significant when $p < 0.05$.

RESULTS

Twenty participants of an average age of 32.5 years (range: 23–40 years) were recruited to take part in the study. Five participants refused to undergo scalp biopsy in the W12 phase, while one participant did not answer the questionnaire about efficacy. All of the participants applied the topical solution twice daily according to the protocol. Average daily consumption of Cellium® GC (Legacy Healthcare, Epalinges, Switzerland) was 1.45 g (range: 0.87–1.95 g).

Questionnaire assessment (n = 19) indicated good efficacy with regard to hair growth, as described by both volunteers and their families. Volunteers' satisfaction with the product reached an average of 8.3/10. Evaluation by the volunteers showed good effectiveness and cosmetic acceptance given that 90% of participants observed new hair growth, 63–73% observed faster hair growth, 84% observed more hair, 68% experienced a pleasant sensation following application, and 65% classified the product as good or very good.

With regard to product safety, dermatological examinations of the scalp in the W0 and W12 phases did not reveal adverse reactions to the product.

Scalp biopsies showed the epidermis as being of normal thickness and also revealed inflammatory infiltrate of mononuclear cells around vessels and annexes (n = 15), while elastosis was observed in the dermis. The histomorphometric analyses of the slides, carried out once the slides had been processed with the biomarkers outlined previously (namely antibodies for the detection of CD1A protein in Langerhans cells, monoclonal antibody Ki-67, HSP47 and BCL2), will be detailed next. The data shown include an increase of statistically significant percentages as well as microphotographs of the slides.

Histomorphometric analyses

The histomorphometric analysis of CD1A+ Langerhans cells was carried out by determining the fraction of CD1A+ Langerhans cells in the epidermis. There was a statistically significant increase of 73.9% in the fraction of CD1A+ Langerhans cells in the epidermis after treatment with 210 mg/ml Cellium® GC topical solution ($p = 0.003$, paired t-test) (Table 1 and Figure 1). The result was confirmed by the Kruskal–Wallis test ($p = 0.003$).

The analysis of Ki-67+ cells was carried out by determining the fraction of Ki-67+ cells in the epidermis. There was a statistically significant increase of 41.7% in the fraction of Ki-67+ cells in the epidermis after treatment with 210 mg/ml Cellium® GC topical solution ($p = 0.012$, paired t-test) (Table 2 and Figure 2). That result was confirmed by the ANOVA ($p = 0.003$) and Tukey's ($p < 0.05$) tests.

The analysis of HSP47+ cells was carried out by determining the fraction of HSP47+ cells in the dermis. No statistical differences were observed in the fraction of HSP47+ cells in the dermis before and after treatment with 210 mg/ml Cellium®

Table 1: Fraction of epidermal CD1A+ Langerhans cells before and after treatment in 15 participants

Parameter	Average (%)	SDDP
Fraction of CD1A+ Langerhans cells before treatment	7,3	2,23
Fraction of CD1A+ Langerhans cells after treatment	12,7	5,55

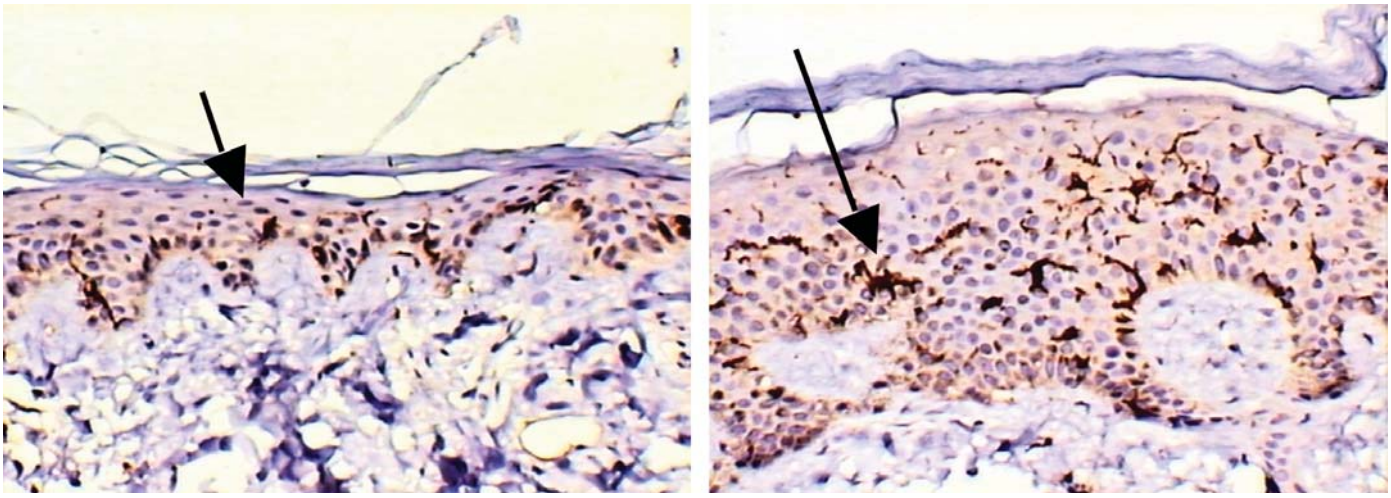

Figure 1: Fraction of epidermal CD1A+ Langerhans cells (arrows) in patient No. 9 before and after treatment

Table 2: Fraction of epidermal Ki-67+ cells before and after the treatment in 15 participants

Parameter	Average (%)	SD
Fraction of Ki-67+ cells before treatment	12	4,17
Fração de células Ki-67+ pós-tratamento	17,8	5,69

GC topical solution (p = 0.938, paired t-test) (Table 3 and Figure 3). The result was confirmed by the ANOVA test (p = 0.942).

The analysis of BCL2+ cells was carried out by determining the fraction of BCL2+ cells in the epidermis. There was a statistically significant increase of 89% in the fraction of BCL2+ cells in the epidermis before and after treatment with 210 mg/ml Cellium® GC topical solution (p = 0.001, paired t-test) (Table 4 and Figure 4). The result was confirmed by the Kruskal–Wallis test (p = 0.006) and by the Dunnett's test (p < 0.05).

DISCUSSION

Application of the 210 mg/ml Cellium® GC (Legacy Healthcare, Epalinges, Switzerland) topical solution twice daily for a duration of 12 weeks was not followed by an increase in the response of thermal shock proteins or in the collagen-specific molecular chaperone HSP47. The latter is expressed in inflammatory cells. Only a few inflammatory cells were observed in the dermis during the study, which supports the findings of Keagle et al.[8] who also observed this. This agrees with the response of the inflammatory cells observed during the course of the study, with HSP47+ cells showing diffused location in the dermis, mainly around the vessels. The study's results also show that a 12-week application of Cellium® GC 210

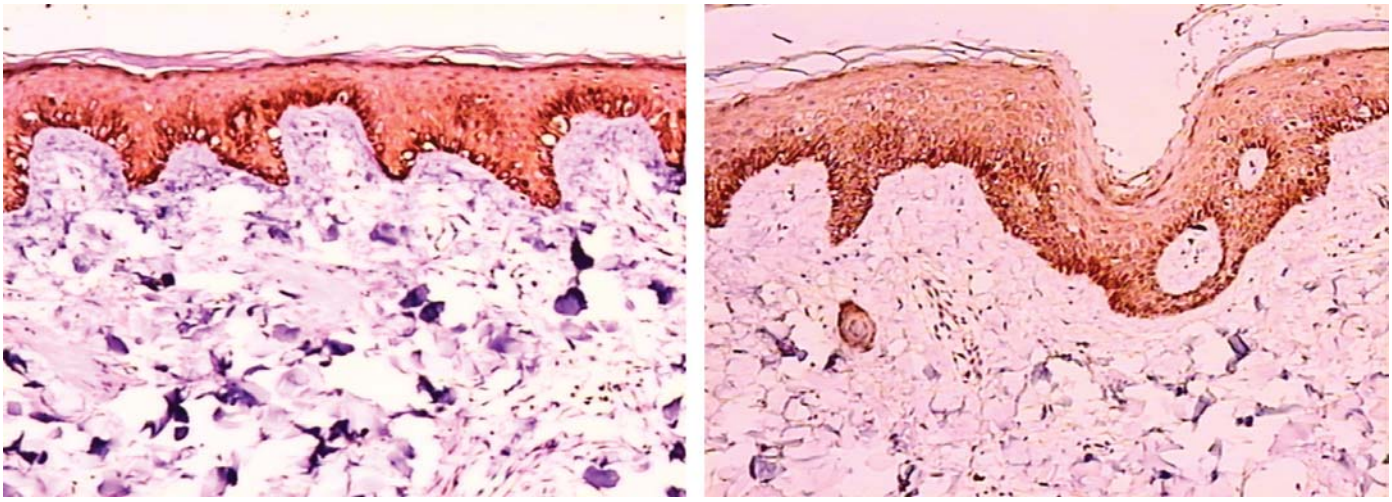

Figure 2: Fraction of epidermal Ki-67+ cells in patient No. 19 (brown) before and after treatment

Table 3: Fraction of dermal HSP47+ cells before and after treatment in 15 participants

Parameter	Average (%)	SD
Fraction of HSP47+ cells before treatment	6.6	1.77
Fraction of HSP47+ cells after treatment	6.7	2.08

Table 4: Fraction of epidermal BCL2+ cells before and after treatment in 15 participants

Parameter	Average (%)	SD
Fraction of BCL2+ cells before treatment	1.722	0.786
Fraction of BCL2+ cells after treatment	3.235	1.624

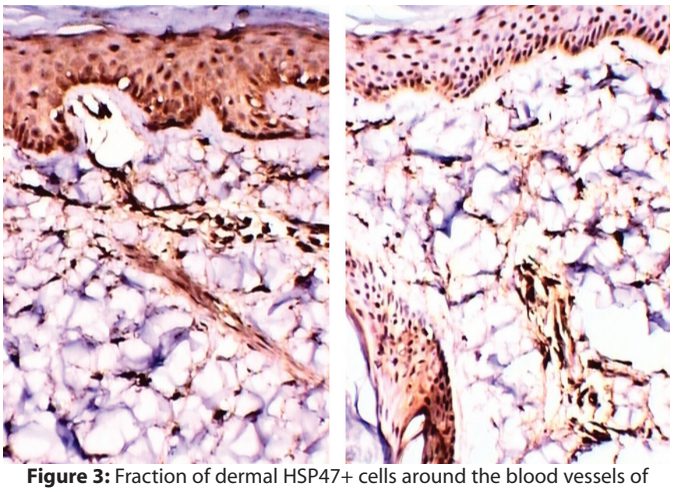

Figure 3: Fraction of dermal HSP47+ cells around the blood vessels of patient No. 16 before and after treatment

mg/ml was followed by an increase in Langerhans cells which play a central role in the skin's immune defense system. Furthermore, an increase in the fraction of Ki-67+ cells (the Ki-67 proliferative index) was observed. Ki-67+ cells in the normal human epidermis are localised mainly in the suprabasal cells layers. In the present study, Ki-67+ cells were either dispersed through all the suprabasal cell layers or agglomerated in specific areas, as also observed by Tilli et al.[9]

BCL2 is a cytoplasmic protein which plays a key role in apoptosis regulation. BCL2 promotes cell survival by inhibiting the mediators needed for the activation of proteases (caspases).10 BCL2 is an essential survival mechanism in normal melanocytes, in correlation with its derivation from the neural crest.11 In several cell types (including melanocytes), the regulation of cell survival and cell death may involve a dynamic interaction between proteins such as BCL2-associated X (BAX) protein, which accelerate programmed cell death, and apoptosis repressors such as BCL2).[12] Overexpression of BCL2 results in cell cycle arrest in the G1 phase of the cell cycle or in a delay in the G1/S transition.[13] In the present study, a significant increase in the expression of BCL2 in the epidermis was observed, probably involving the activity of melanocytes. This finding can be extended to the cells of hair follicles, so that an increase in cellular proliferation, coupled with anti-apoptotic activity, can promote hair growth. In normal skin, however, the proliferation marker Ki-67+ significantly associates with the pro-apoptotic marker protein 53 (p53). Nevertheless, in skins exposed to high UV radiation, Ki-67+ associates with the anti-apoptotic marker BCL2. The imbalance in proliferative/apoptotic signaling can lead to a dysfunctional epidermis which is

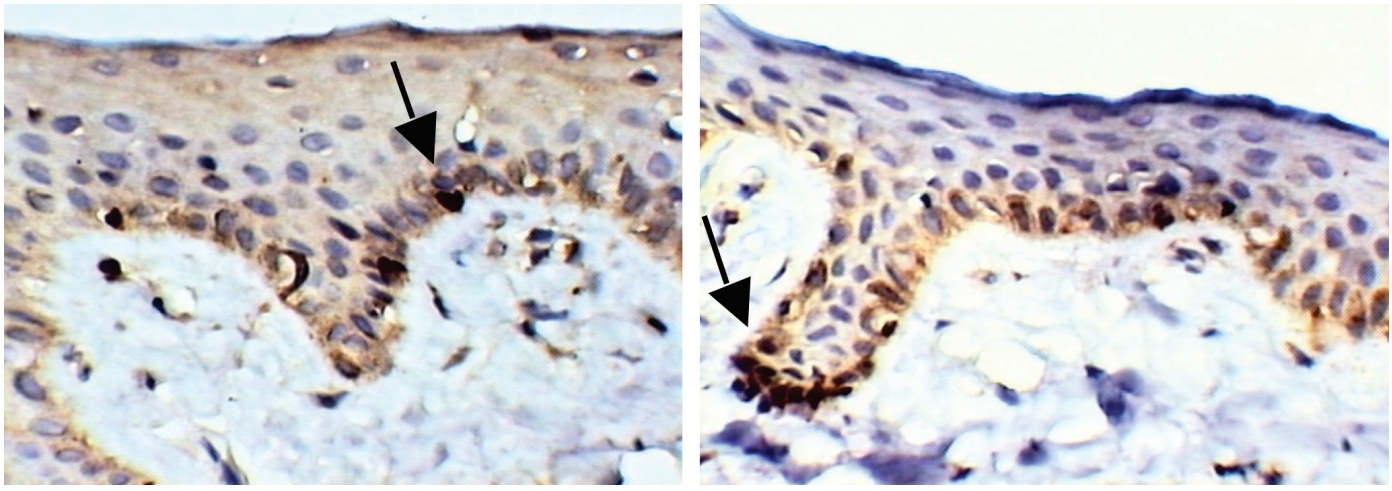

Figure 4: Fraction of epidermal BCL2+ cells (arrow) in patient No. 5 before and after treatment

permissive to aberrant proliferation. In response to genotoxic agents, wild-type p53 accumulates and induces apoptosis. It is then suggested that expression of the pro-apoptotic marker p53 should be investigated when treatment with Cellium® GC 210 mg/ml is applied.

CONCLUSIONS

In light of the results obtained in the present study, the application of the Cellium® GC 210 mg/ml topical solution on the scalp twice daily for 12 consecutive weeks, as carried out by 19 male volunteers presenting with AGA (classified as type I–VII according to the modified Norwood–Hamilton scale), was shown to provide participants with satisfaction regarding hair growth (average satisfaction = 8.3/10). In addition to subjective evaluation, the immunohistopathological analyses of scalp skin biopsies revealed an increase in the skin's immunological defenses, and in the proliferation and anti-apoptotic action of keratinocytes in the dermis and epidermis. Thorough analysis of both clinical and subjective evaluation should ensure that the combination of active ingredients extracted from plants and made available in solution form, are well tolerated, safe and effective. Therefore, a topical solution containing Cellium® GC 210 mg/ml is seen as a viable option in the treatment of AGA.

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