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Research article

Hair growth-promotion effects at the cellular level and antioxidant activity of the plant-based extract Phyllotex™

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ABSTRACT

Hair loss and predominantly female hair loss is a common dermatologic condition with serious psychosocial consequences. Effective treatments remain scarce mainly due to the multifactorial elements involved in the onset of this chronic condition. The approved drugs available are based on molecules designed towards a single pharmacological target and do not interact with the various biochemical mechanisms involved in alopecia. Phytochemical compounds and their derivatives represent a plethora of biologically active agents, which act in synergism and simultaneously activate different biochemical pathways. Here we present an herbal formulation composed of herbs, vitamins, and minerals acting on hair regrowth and hair micro vascularization.

This study aimed at evaluating the potential of Phyllotex™ to treat multifactorial androgenetic alopecia (AGA) in males and females, as well as delving into its molecular mechanisms of action. *In vitro* studies showed that the herbal formula stimulates cell proliferation of both dermal papilla and HaCaT cells and increases the phosphorylated form of the extracellular signal-regulated kinase 1 and 2 (ERK1/2), a well-known marker for cell proliferation. Surprisingly, expression of TGF-β1 was significantly suppressed without blocking DHT production. Additionally, the formula was able to rescue cells from the oxidative stress conditions generated by 2,2′-Azobis (2-amidinopropane) dihydrochloride (AAPH), a high oxidative agent. This data supports the potential use of this formulation as a hair growth-promoting agent for the treatment of both male and female AGA due to its multifactorial composition, which grants it the ability to cope with the different mechanisms involved in alopecia.

1. Introduction

Hair is a unique structure of the skin and is one of the defining characteristics of humans. The hair follicle (HF) is subject to constant turnover in the course of perpetual cycles through various stages of proliferation (anagen), involution (catagen), and resting (telogen) (Stenn and Paus, 2001). The cycling and regeneration of each HF depends on specialized human dermal papilla cells (HDPCs) and proliferating matrix cells located at the base of the follicle, which is mediated by several regulation and growth factors (Ozeki and Tabata, 2003). In addition to growth factors, androgen hormones are also involved in the hair cycle. Androgens include testosterone and dihydrotestosterone. These indirectly control hair growth by triggering hair loss through the stimulation of the expression of transforming growth factor-β1 (TGF-β1) in HDPCs, which results in epithelial cell growth inhibition (Rho et al., 2005). Another group of cells essential to hair maintenance is the keratinocytes, which reside mainly in the bulge region of the hair follicle, playing a critical role in hair follicle regeneration (Kamimura et al., 1997).

Hair loss is not a severe disorder but often causes significant mental stress since the most important function of the hair shaft is to act as a physical medium of social intercourse; in fact, scalp, facial, and body hairs are essentially the only body parts an individual can shape to influence social interaction. This point emphasizes the importance of hair and the psychosocial consequences of its pathology that affect both men and women, particularly from the age of 50 (McElwee and Sinclair, 2008; Stenn and Paus, 2001). Female pattern hair loss manifests diffuse hair thinning mostly on the top of the scalp but not completely bald like in male AGA, from which one could infer that the severity in females is relatively less than in males (Ludwig grade III vs. Hamilton-Norwood grade VII) (Dinh and Sinclair, 2007). However, psychosocial problems are more expected to occur in women because a smaller proportion of women are affected, thus emphasizing their deviation from the norm. It was found that 13% out of 564 premenopausal women showed hair loss on the scalp, while in men, the percentage was about 45% (HAMILTON, 1951; Venning and Dawber, 1988).

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Drug therapy and human hair transplantation are currently the two available methods for treating androgenic alopecia (AGA), and it is generally agreed upon that neither can reverse hair loss. Topical Minoxidil and oral Finasteride are the most popular and approved drugs for hair loss treatment. Minoxidil promotes hair growth by increasing the duration of anagen phase by opening the potassium channels in the cell membrane and expanding the scalp's blood vessels (Messenger and Rundegren, 2004). Unfortunately, the efficacy of Minoxidil is variable and temporary (Gupta and Charrette, n.d.). Although the most common adverse reactions of the topical formulation are limited to irritant and allergic contact dermatitis on the scalp, it has been reported that a non-arteritic anterior ischemic optic neuropathy caused by topical 5% Minoxidil treatment was resolved after discontinuation of the drug (Aktas et al., 2016). Finasteride is a competitive inhibitor of 5α-reductase (type II) (Mysore, 2012). However, Finasteride has been associated with some sexual dysfunction such as ejaculation and libido disorders and erection dysfunction (Kiguradze et al., 2017). It also may cause malformation of the external genitalia of male fetuses (Sawaya and Shapiro, 2000). For hair transplantation, the limitation comes from the limited transplantable hair and the cost of the treatment (Rose, 2015). Therefore, new drugs or therapies to prevent hair loss and enhance hair growth are still necessary. Nowadays, several natural substances are known for their influence on the expression of hair growth factors (Boisvert et al., 2017; Oh et al., 2014; Patel et al., 2015). Some plants produce anti-hair loss effects by the inhibition of 5α-reductase (Kumar et al., 2012). Recently, the hair care products available on the market have contained more than 20 herbal substances, many of which have been acclaimed for their hair growth promotion (Jadhav et al., 2009).

Many forms of alopecia are common in the general population (Gupta and Mysore, n.d.). Therefore it would not be unexpected for two or more forms to occur together in the same patient, resulting in multifactorial alopecia (Gupta and Mysore, n.d.). Effective treatment of multifactorial alopecia, including women's alopecia, is still an unmet need. Most of the approved drugs available today are single molecules designed to deal with only one of the various alopecia leading mechanisms. In seeking to address this multifactorial issue of AGA, this study has tested an herbal formulation namely, Phyllotex™, which is composed of 4 plant extracts (Euterpe oleracea, Olea europea, Tabebuia impetiginosa and Coffea arabica),micronutrients (Zinc, magnesium, pantothenic acid and vitamin D3) and p-coumaric acid, a well-known antioxidant and anti-inflammatory natural agent (Mozaffari Godarzi et al., 2020; Rice-Evans et al., 1996). The mechanism of action of this product is believed to be built on the synergistic effects of the formula constituents acting simultaneously in different mechanisms. These include stimulating cell proliferation of hair tissue by upregulating cell growth-related proteins and promoting vasodilation in peripheral microcirculation, improving the nutrition of the hair follicles, and restoring damaged cells from oxidative stress, and promoting the growth of the hair bulb. Additionally, it was found that Phyllotex™, contrary to finasteride, can significantly reduce the expression of transforming growth factor-β1 (TGF-β1) in HDPCs without affecting DHT production. The formula was capable of producing in vitro results simultaneously acting on different hair loss-related mechanisms thus covering the multifactorial pattern of most alopecia disorders found in the population, including women AGA.

2. Materials and methods

2.1. Cell culture and treatment

Adherent human cell lines were cultured according to standard mammalian tissue culture protocols and sterile technique. Human follicle dermal papilla were purchased from Promocell (Catalog number: c-12071) and cultured in human follicle dermal papilla cell growth medium, ready to use, low-serum (Promocell, catalog number: c-26501), supplemented with growth medium supplement Mix (Promocell, catalog number: c-39625). The catalog number for male or female human follicle dermal papilla cells is the same (c-12071). The lot number specifies whether the cells are from male or female.

Keratinocyte cells (HaCaT) were cultured in high glucose Dulbecco's Modified Eagle Medium with 10% serum (Biological industries, catalog number: 01-052-1A). Normal human fibroblasts such as HFF and BJ were culture in BIOAMF-2 complete medium (Biological Industries, Israel, Catalog number: 01-194-1A). All media was supplemented with streptomycin (100 mg/ml), penicillin (100 U/ml), and Nystatin (12.5 U/ml). Cells were incubated in 5% CO_2 at 37 °C. All tissue cultures were maintained in 25cm^{2}Nunc™ cell culture treated EasYFlask™ (Thermofisher scientific), and all the media and supplements were obtained from Biological Industries. Treatments were performed by plating cells in a Nunc™ 96 micro well delta surface plate (Thermofisher scientific) in a starting confluence of 0.5×10^4 cells/well. After 24h of incubation, the cells were treated with the formula Phyllotex™, as displayed in the results section.

2.2. MTT assay

The viability of the cells following treatment was determined using a commercially available MTT assay kit (ABCAM, ab146345) and performed according to the manufacturer's instructions. Briefly, cells were seeded in 96-well plates at a density of 0.5×10^4cells/well (n = 4). After overnight plating, cells were exposed to varying concentrations of Phyllotex™ (9–0.07 mg/ml). Then, plates were incubated in a humidified atmosphere containing 5% CO_2 in air at 37 °C for 24 h. According to the MTT standard protocol, after 24 and 48h treatment, the media was removed. All cells were incubated with serum-free media containing 0.5 mg/ml MTT for 4 h at the incubator. The MTT purple crystals formed by the viable cells were diluted using isopropanol containing 0.04 mol/L HCL. The quantification was determined by measuring the optical density at 570nm in an enzyme-linked immunosorbent assay (Spark, Tecan) reader. Data were presented as proportional viability (%) by comparing the treated group with the untreated cells, which is assumed to be 100%.

2.3. Protein extraction and Western blot analysis

The whole-cell lysate was prepared by washing cell pellets with 1X Phosphate buffer saline (Biological Industries), resuspending the pellet in ice-cold T lysis buffer [50mM Tris-Cl (pH 7.5), 150mM NaCl, 1mM EDTA, 1% Triton X and 1X halt™ protease and phosphatase inhibitor cocktail] and incubating for 30 min in ice. The lysate was then centrifuged at 13,800g for 10 min at 4 °C to clear the cellular debris. Total protein was quantified using the Bradford protein assay kit (Biorad, Hercules, and CA). An equal amount of protein was resolved on precast Bolt™ 4–12% Bis-Tris Plus polyacrylamide gel (Invitrogen), electro-transferred to precast nitrocellulose stacks using iBlot®2 system (Invitrogen), and Western blot analysis was performed using the antibodies described above. Immuno-detection was performed by blocking the membranes for 1 h in TNT buffer [10mM Tris-Cl (pH 7.5), 150mM NaCl, 0.05% Tween-20] containing 5% powdered non-fat milk followed by the addition of the primary antibody (as indicated) in TNT for 2 h at room temperature. Specifically bound primary antibodies were detected with peroxidase-coupled secondary antibodies and developed by enhanced chemiluminescence (Biological Industries) according to manufacturer's instructions and quantitated using ImageQaunt LAS 4000 mini, General Electric). All experiments were performed at least three times using independent biological replicates.

Immunoblot analysis was performed using antibodies against ERK1 phospho T202 + ERK2 phospho T186 (1:1000 dilution; ab201015, Abcam (, GAPDH (1:6000 dilution; ab128915, Abcam), β-actin (1:1000 dilution; 101173, Abcam), TGF-β (1:500 dilution; ab179695, Abcam). Species-specific HRP-labeled secondary antibodies were then added. The blots were visualized using enhanced chemiluminescence (biological industries) and quantitated using ImageQaunt LAS 4000 mini (General Electric).

2.4. ELISA for DHT detection

Dihydrotestosterone (DHT) detection was performed using the Aviva Systems Biology dihydrotestosterone ELISA kit (OKEH02531). The technique is based on a competitive binding enzyme immunoassay. A 96 well plate was pre-coated with an antibiotinylated dihydrotestosterone antibody. 50 µl of male HDPCs control and treated samples were added to the wells along with 50 µl of 1X dihydrotestosterone-Biotin complex. After incubation at 37 °C for 60 min, the wells were washed three times with 1X Wash buffer, and 100 µl of Avidin-HRP conjugate solution was added to each well. The plate was incubated at 37 °C for 45 min and washed with 1X wash buffer. 90 µl of TMB subtract was added, and each well was followed by incubation in the dark at 37 °C for 15–30 min. Finally, 50 µl of a stop solution was added, and the quantification was determined by measuring the optical density at 450nm in an enzyme-linked immunosorbent assay (Spark, Tecan) reader.

2.5. Oxidative stress assay

HDPCs were plated in a Nunc™ 96 delta surface microwell plate (Thermofisher scientific) in a starting confluence of 0.5×10^4 cells/well. After 24h of incubation, the cells were co-treated with different concentrations of Phyllotex™ and 10mM of AAPH or only with AAPH (control cells) as elucidated in the experiments displayed in the results section. The concentration of AAPH selected to perform this assay was determined by the minimal concentration to reduce cell viability to 30% (EC30). After another 24h incubation period at 37 °C, 5% cell viability was determined using a commercially available MTT assay kit (ABCAM, ab146345) and performed according to the manufacturer's instructions.

2.6. Phyllotex™ sample preparation

Capsules of the commercial product Phyllotex™ (500 mg) were extracted in ethanol and water (50:50). The solution was well mixed for 10 min and centrifuged at 15000G for 10 min. The supernatant was evaporated until dried. P-coumaric acid (Sigma-Aldrich, Israel, c9008) was add at 2, 3 or 5% of the final dried weight and the final mixture was then resuspended in cell media to the desired concentrations.

2.7. Statistical analysis

We used Student's t-test for statistical analysis in Microsoft Excel. Data were considered significantly different from control when $p < 0.05$.

3. Results

3.1. Growth-promoting effect of the formula Phyllotex™ in HDP and HaCaT cells in vitro

After 24 h of incubation with Phyllotex™ formula at concentrations 2–0.12 mg/ml, the proliferation of female HDPCs increased up to 48% when treated with 2 mg/ml (Figure 1). The growth effect in male HDPCs was moderate, with proliferation increasing up to 36.5% when treated with 2 mg/ml (Figure 2). Formula variants containing 3% of p-Coumaric acid did not show significant changes in the proliferation effect of both male and female HDPCs (Figures 1 and 2). Phyllotex™ supplemented with 5% p-Coumaric acid showed a high toxicity in female HDPCs even in small concentrations (Figure 1). Therefore, 2% of p-coumaric acid was selected to perform the forward experiments. Proliferation of HaCaT cells was significantly increased up to 118% over the control when treated with 0.12 mg/ml of the formula (Figure 3). Likewise, treated HFF and BJ normal human fibroblasts showed a robust increase in cell proliferation (125% and 97.8% respectively) when treated with the higher concentration of the formula (2 mg/ml). The proliferation effect decreased proportionality to the tested concentrations, however, showing significant effects even in the lower concentration (22.5% and 5.3% respectively) (Figures 4 and 5). HFF and BJ cell lines were also treated with Minoxidil at 0.5 µg/ml, which failed to stimulate cell proliferation (light gray bars in Figures 4 and 5).

Moreover, after 24h of treatment with 0.56 mg/ml, male HDPCs showed significant activation of extracellular signal-regulated kinase (ERK) protein as indicated by the increase of its phosphorylated form (Figure 6 and S1).

3.2. Anti-oxidative effect of Phyllotex™ in HDPCs co-treated with AAPH

Male HDPCs were co-incubated with different concentrations of Phyllotex™ and 10 mM of 2,2′-Azobis (2-amidinopropane) dihydrochloride (AAPH) for 24h to evaluate whether the formula would be able to neutralize the oxidative stress generated by AAPH by protecting the cells from oxidative damage and death. Cell viability was reduced to 30% when cells were treated only with 10mM AAPH (data not shown). However, co-treatment with 1 and 2 mg/ml of Phyllotex™ was enough to overcome the toxic conditions generated by AAPH and rescue from oxidative stress through cell growth (Figure 7). Interestingly, the treatment with 2 mg/ml of Phyllotex™ was so protective that apart from eliminating the toxic effect generated by AAPH, it increased cell proliferation by 32% compared to control cells not treated with AAPH (Figure 7).

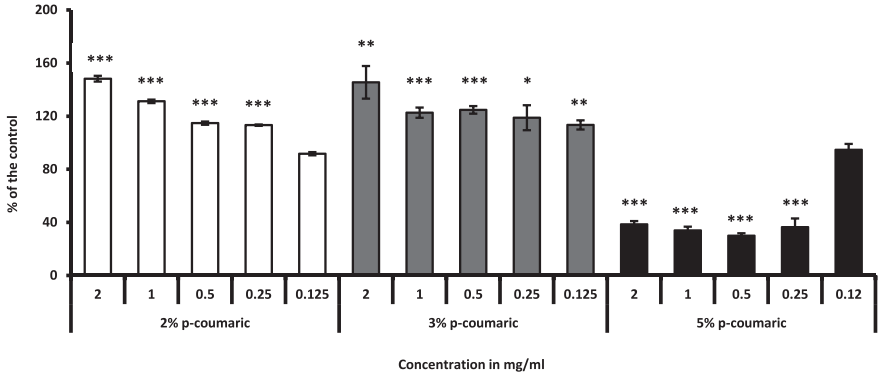

Figure 1. MTT viability assay. Female HFDP cells were treated with Phyllotex™ including 2, 3 and 5 % of p-coumaric acid as indicated. The incubation time after plating was 24 h. MTT assay shows a significant increase in female HFDP cell viability after treatment. Data are presented as the mean ± SE (n = 4). *P < 0.05; **P < 0.01; ***P < 0.001 by Student t-test.

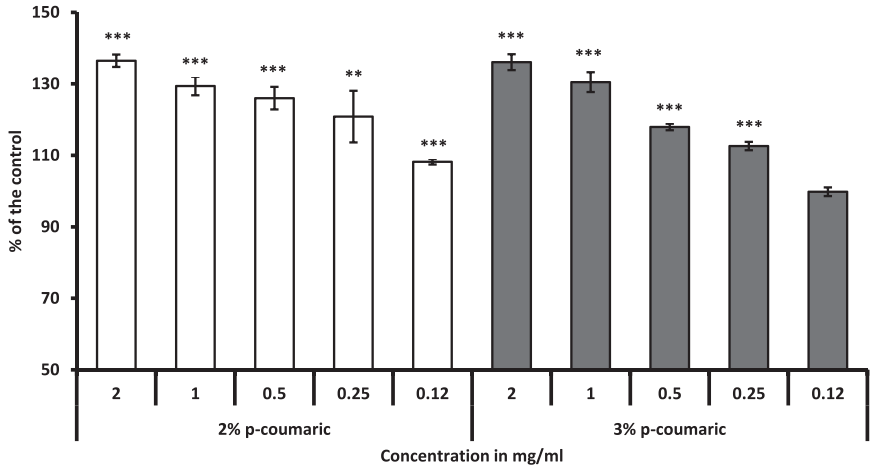

Figure 2. MTT viability assay. Male HFDP cells were treated with Phyllotex™. The incubation time after plating was 24 h. MTT assay shows a significant increase in HFDP male cell viability after treatment. Data are presented as the mean ± SE (n = 4). *P < 0.05; **P < 0.01; ***P < 0.001 by Student t-test.

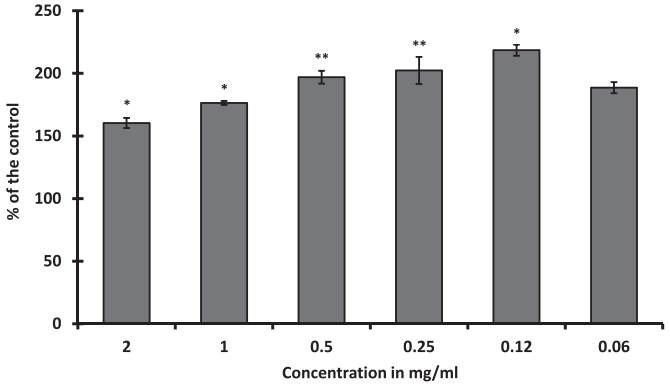

Figure 3. MTT viability assay. Keratinocytes cells were treated with Phyllotex™ including 2% p-coumaric acid. The incubation time after plating was 24 h. MTT assay shows a high increase in Keratinocytes viability after treatment. Data are presented as the mean ± SE (n = 4). *P < 0.05; **P < 0.01; ***P < 0.001 by Student t-test.

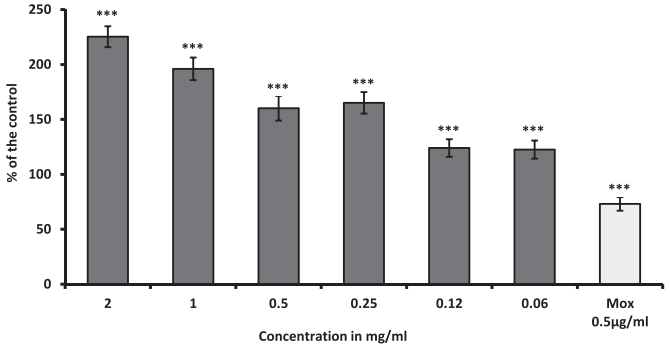

Figure 4. MTT viability assay. Human skin cells HFF were treated with Phyllotex™ including 2% p-coumaric acid. The incubation time after plating was 24 h. MTT assay shows a high increase in HFF cells viability after treatment. Data are presented as the mean ± SE (n = 4). *P < 0.05; **P < 0.01; ***P < 0.001 by Student t-test.

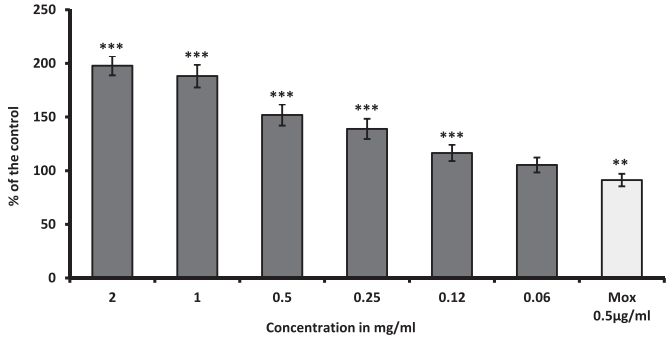

Figure 5. MTT viability assay. Human skin cells BJ were treated with Phyllotex™ including 2% p-coumaric acid. The incubation time after plating was 24 h. MTT assay shows a high increase in BJ cells viability after treatment. Data are presented as the mean ± SE (n = 4). *P < 0.05; **P < 0.01; ***P < 0.001 by Student t-test.

3.3. Non-androgenic related effect of Phyllotex™ in downregulation of TGF-β

The expression of TGF-β type I was significantly decreased in both male and female HDPCs after treatment with 2 mg/ml of Phyllotex™ for 24h (Figures 8,S2 and 9, S3). TGF-β1 downregulation was more remarkable in male HDPCs showing a 5.2 fold decrease in protein expression when treated with the formula (Figure 8). Female HDPCs showed a moderate but still significant decrease in TGF-β1 expression (1.45 fold) after 24h of treatment (Figure 9). The same down-regulation patterns of TGF-β1 expression were observed in HaCaT cells upon treatment for 24h; a 1.5 fold decrease was observed (data not shown).

In order to understand whether this downregulation effect of TGF-β1 expression is androgenic related, we further tested the influence of the formula Phyllotex™ in dihydrotestosterone production (DHT). Previous treatment of male HDPCs with 5μM testosterone was mandatory for stimulation of DHT production. After 8h of incubation with testosterone, 2 mg/ml of Phyllotex™ was added to the cells. Testosterone did not alter

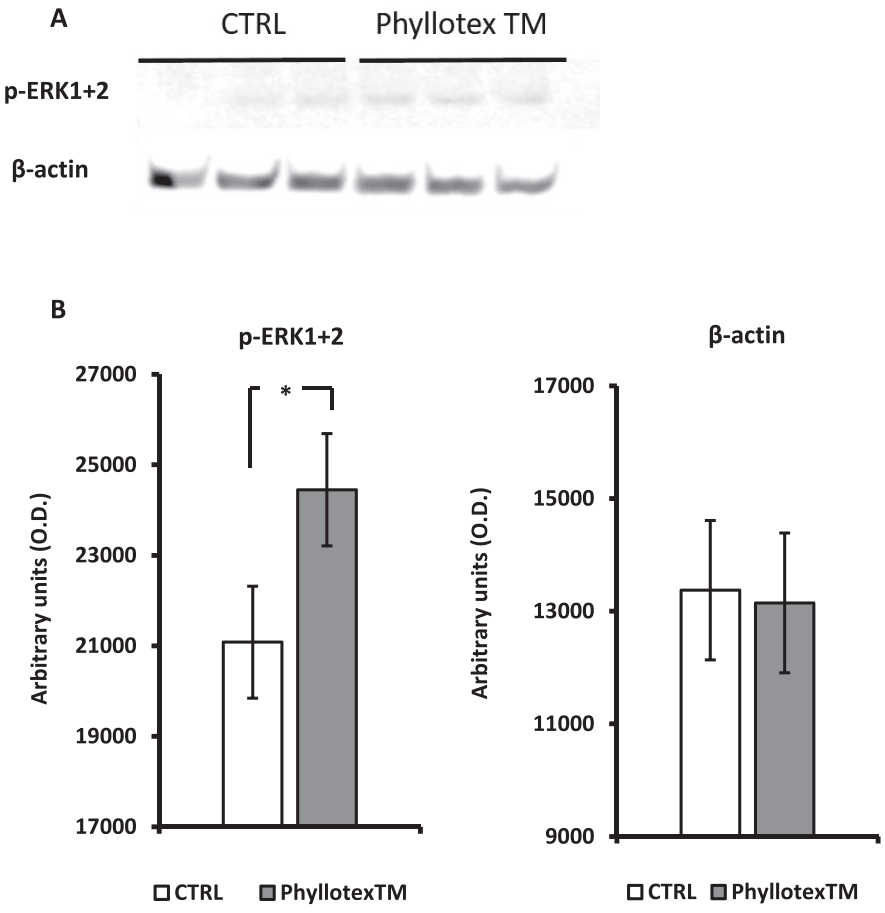

Figure 6. Western blot assay. Phospho Erk1+2 expression in male DPCs after treatment with 0.56 mg/ml Phyllotex™ including 2% p-coumaric acid. A) Expression of phospho Erk1+2 was significant increased after treatment with Phyllotex™ while β-actin protein content was unchanged. Band intensities were quantified using Image J software and plotted in B. The data are mean ± SE (n = 3). Difference between the control and treatment is significant (Student's t-test, *p < 0.05).

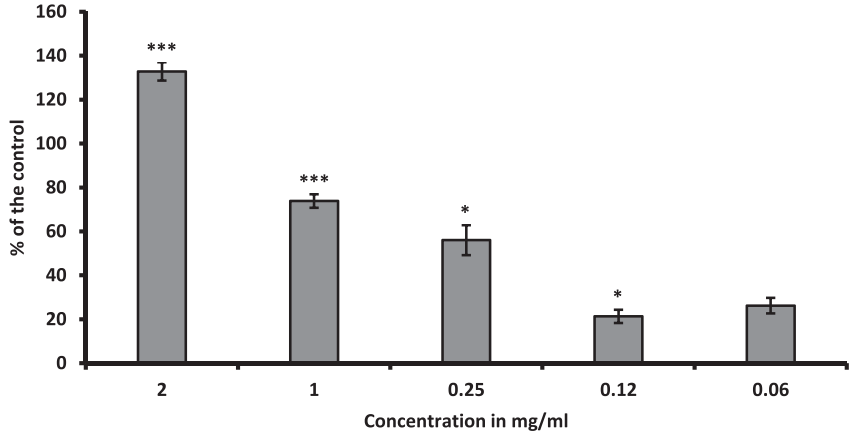

Figure 7. Anti-oxidative effect. Cell viability of male DPCs were evaluated after co-incubation with different concentrations of Phyllotex™ and 10 mM of AAPH for 24h. MTT assay shows a high capacity of Phyllotex™ to protect male DPCs against the toxicity effect of AAPH oxidative agent. Data are presented as the mean ± SE (n = 4). *P < 0.05; **P < 0.01; ***P < 0.001 by Student t-test.

cellular morphology or viability per se (data not shown). After 24h, cell lysates were analyzed by ELISA for DHT detection. As shown in Figure 10, no significant changes in the normal DHT production were observed in cells treated with Phyllotex™ compared to cells treated only with testosterone.

4. Discussion

Human follicle dermal papilla HDPCs and keratinocytes (HaCaT) were used as models in this study due to their relevance to hair growth. HDPCs are major components of hair and responsible for the production

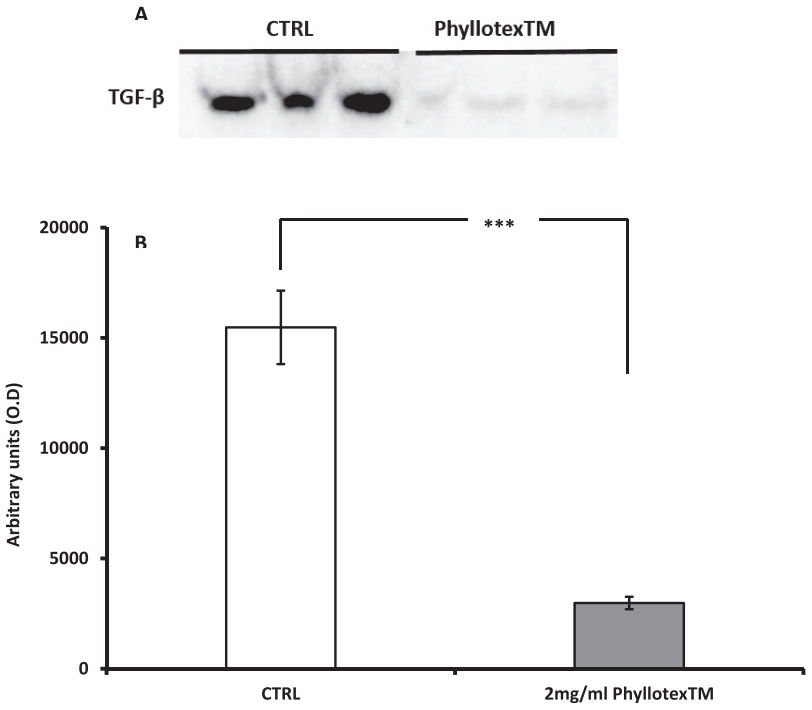

Figure 8. Western blot assay. TGF-β expression in male DPCs after treatment with 2 mg/ml of Phyllotex™ including 2% p-coumaric acid. A) Expression of TGF-β was significant decreased after treatment with Phyllotex™.Band intensities were quantified using Image J software and plotted in B. The data are mean ± SE (n = 3). Difference between the control and treatment is significant (Student's t-test, ***p < 0.001).

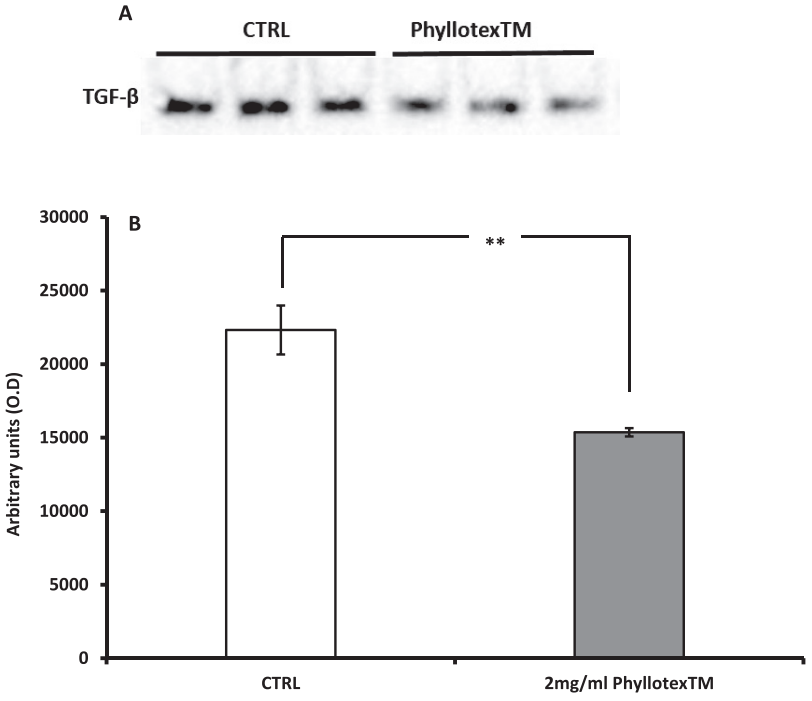

Figure 9. Western blot assay. TGF-β expression in female DPCs after treatment with 2 mg/ml of Phyllotex™ including 2% p-coumaric acid. A) Expression of TGF-β was significant decreased after treatment with Phyllotex™.Band intensities were quantified using Image J software and plotted in B. The data are mean ± SE (n = 3). Difference between the control and treatment is significant (Student's t-test, *p < 0.05).

of essentials growth factors. Among the functions of these growth factors one may include modulation of the follicular epithelium proliferation and acting as a cytokine network controlling follicle development (Rushan et al., 2007). Several studies have shown that dermal papilla size is closely associated with the hair growth cycle, and dermal papilla cell number increases in the anagen phase (Datta et al., 2009; Rho et al.,

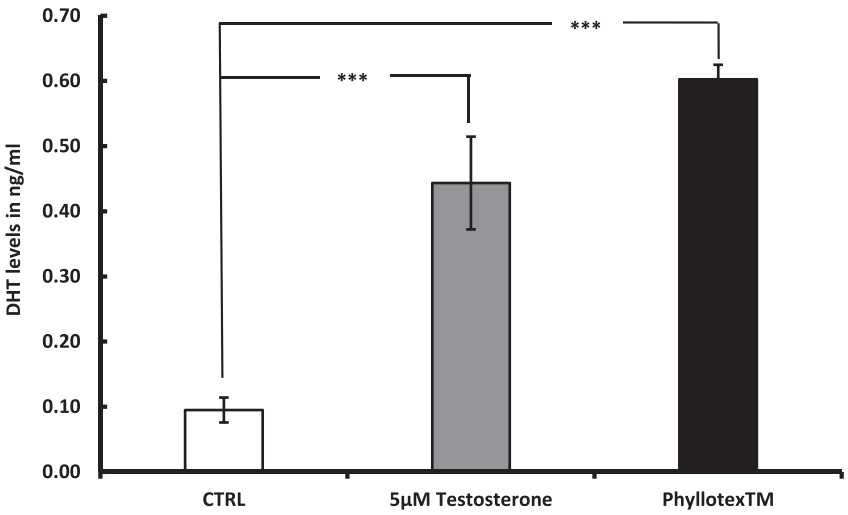

Figure 10. DHT detection assay. Male DPCs were treated with 5μM testosterone previously to treatment with 2 mg/ml of Phyllotex™.After 24 h of incubation, no significant decrease in normal DHT levels were observed compared to cells treated only with testosterone. The data are mean ± SE (n = 4). Difference between the control and treatment is significant (Student's t-test, ***p < 0.001).

2005). Keratinocytes are part of the hair follicle and hair shaft and produce the major protein of hair, keratin (Klíma et al., 2005). Based on its supposed proliferative effect, Phyllotex™ was investigated for its hair growth-promoting effect by analyzing cell viability and at the different protein expression levels on both male and female HDPCs and HaCaT cells. The formulation showed a remarkable effectiveness in stimulating cell growth in all types of HDPCs and HaCaT cells analyzed. Western blot result analysis revealed two proteins that could be directly related to the observed proliferation effect, one of them the extracellular signal-regulated kinase 1 and 2 (ERK1/2), which showed a significant increase in its phosphorylated active form upon treatment with Phyllotex™. Studies on hair growth have shown that several signaling proteins, such as Wnt/β-catenin and extracellular signal regulated kinases (ERK), were upregulated in dermal papilla cells after Minoxidil treatment and led to the proliferation of dermal papilla cells (Dastan et al., 2016; Kwack et al., 2011). The ERK pathway has been shown to affect cellular functions, including cell proliferation and apoptosis (Gunda et al., 2017; Molavi et al., 2017). Several hair growth activators, such as the vascular endothelial growth factor, placental growth factor, and adenosine, have displayed ERK-mediated hair growth effects (Hwang et al., 2012; Li et al., 2012). Another protein that has a function that is determinant in cell growth is the transforming growth factor 1 (TGF-β1), a member of the cytokine superfamily in mammals that regulates apoptosis in many cell types.

TGF-β1has have recently been dubbed a hair follicle assassin, probably by virtue of playing a central role in the inflammation associated with hair follicle miniaturization, fibrosis, epithelial cell growth inhibition, and eventual loss (Inui et al., 2002; Li et al., 2006). We have shown in this study that treatment with Phyllotex™ leads to significant suppression of TGF-β1 expression in both male and female HDPCs, although the effect in female cells was less accentuate. Additionally, TGF-β1 downregulation was also observed in HaCaT cells. Another FDA-approved drug for AGA treatment with a similar effect regarding TGF-β1 expression is Finasteride, type II 5α-reductase inhibitor that has been shown to cure androgenetic alopecia by reducing DHT levels in the scalp (Dallob et al., 1994; Kaufman et al., 1998). However, it has been reported that the use of finasteride can cause weaknesses, including transient action and infertility problems, due to its interference in DHT production (Rossi et al., 2016; Tu and Zini, 2011). In humans, DHT is essential in inducing the production of epidermal growth factor (EGF), keratinocyte growth factor (KGF), and insulin-like growth factors (IGFs), all of which stimulate cellular proliferation (Dicker et al., 2005). According to the results presented in this study, Phyllotex™ suppresses TGF-β1 expression in male and female HDPCs in a manner that is similar to finasteride without interfering with physiologic DHT production.

Another issue related to alopecia is oxidative stress. Studies suggest that as we age, our endogenous antioxidant capacities become impaired and cannot keep up with neutralizing both naturally-occurring free radicals and those induced from environmental factors (such as cigarette smoke and UV radiation) (Rahman et al., 2012). Certainly, age-associated dietary deficiencies may contribute to decreased natural antioxidant production since many vital nutrients (e.g., copper, manganese, selenium, and zinc) are needed to produce these enzymes. In fact, one of the symptoms of these nutrient deficiencies is often thinning hair (Guo and Katta, 2017). When antioxidant molecules do not inhibit free radicals within the body, the oxidative damage accumulates and is magnified, thus contributing to and causing effects of aging and further impairment of the endogenous antioxidant defense system. Evidence from scientific experiments indicates that this happens in hair follicles, promoting hair loss (whether temporary or permanent) (Trüeb, 2009). In this study, we show that male HDPCs treated with Phyllotex™ were able to recover from the oxidative stress conditions generated by AAPH and overcome the deleterious effects by even stimulating cell proliferation. This effect was probably generated due to the micronutrient compounds present in the formula, such as Zinc and vitamin D3, responsible for calcium homeostasis, essential for normal cell metabolism and enzyme activity.

In conclusion, according to the results presented in this study, Phyllotex™ possesses biological activity on HDPCs via stimulation and activation of the transcription factor ERK1/2, protection from oxidative stress, and suppression of TGF-β1 expression in both keratinocytes and HDPCs without blocking DHT production. Phyllotex™ also increased cell proliferation with a higher effect than that of Minoxidil in the tested concentration. The findings of this study at the cellular level shed light on the biochemical mechanism of action of the Phyllotex™ herbal formulation. The biological effects of the formula constituents act simultaneously by different mechanisms, including stimulation of cell proliferation of HDPCs, antioxidant protective action and upregulation of cell growth-related proteins.

Declarations

Author contribution statement

Raphael Serruya: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Yehoshua Maor: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

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