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Ageratum conyzoides L. extract inhibits 5α-reductase gene expression and prostaglandin D_2 release in human hair dermal papilla cells and improves symptoms of hair loss in otherwise healthy males and females in an open label pilot study.

Paul Clayton1*, Ruchitha Venkatesh2, Nathasha Bogoda3 and Silma Subah3

[1]*Institute of Food, Brain and Behaviour, Beaver House, 23-28 Hythe Bridge Street, Oxford OX1 2EP, UK*

[2]*University of Hong Kong, Department of Medicine, Hong Kong, China*

[3]*Gencor Pacific Limited, Discovery Bay, Lantau Island, New Territories, Hong Kong*

Abstract

Background: Hair loss is a debilitating condition often encountered by older adults. Common hair loss treatments such as Minoxidil and Finasteride are associated with potentially severe adverse effects. *Ageratum conyzoides L.*, an annual herb shown to inhibit pathways associated with hair-loss, is a potential safe and effective alternative treatment for hair loss

Objective: A pilot, open-label, randomized, parallel and *in vitro* study assessed the efficacy and safety of an Ageratum conyzoides formulation on hair loss.

Methods: 28 otherwise healthy males and females over 18 years of age exhibiting pattern baldness received either a 0.5% or 1% strength *A. conyzoides* gel formulation to be applied topically twice per day for 8 weeks. Hair growth as measured by temporal recession distance and participants' quality of life was assessed by the Hair Distress Questionnaire. The effect of an *A. conyzoides* extract on gene expression of 5α-reductase and release of Prostaglandin D2 (PGD2) in Human Hair Dermal Papilla Cells (HHDPC) was also assessed to determine mechanisms of action.

Results: Temporal recession in men reduced from 9.1 cm to 8.6 cm in the 1% group and from 8.6 to 7.5 cm in the 0.5% group. The normal distance in men without recession is between 6-6.5cm. Among males, hair distress questionnaire scores fell by 6 and 3.6 points from baseline for the 0.5% and 1% product respectively. In the 1% treatment group, 64% of men and 100% of women reported an improvement in their hair loss symptoms. Over 65% of participants indicated a significant improvement in their hair related quality of life ($p < 0.05$). In the *in vitro* study, the *A. conyzoides* extract significantly inhibited PGD2 release and 5α-reductase type 1 expression in HHDPC.

Conclusion: These results indicate that *A. conyzoides* may be an effective treatment option for facilitating hair growth and inhibiting hair loss.

Keywords: Ageratum conyzoides • hair • hair loss • alopecia • androgenetic alopecia • 5α-reductase • prostaglandin D2

Abbrivations: AGA, Androgenetic Alopecia; PGDS, Prostaglandin D Synthase; PGD2, Prostaglandin D2; HHDPC, Human Hair Dermal Papilla Cells; DHT, Dihydrotestosterone.

Introduction

Billions of dollars are spent on hair loss treatments per year [1]. While the primary function of hair is protection from sunlight, cold and physical damage, it also has key roles in social and sexual communication, often signalling health, self-expression, desirability and youthfulness [2,3]. This explains why hair loss can be associated with negative psychological well-being and many seek treatment to halt or reverse this phenomenon [4-6]. There are also negative health consequences to early onset AGA, such as increased exposure of the scalp to sun damage, as well as associations with obesity, early-onset coronary heart disease and metabolic syndrome [4].

The main causes of hair loss are Androgenetic Alopecia (AGA) or male pattern hair loss in men, and female pattern hair loss in women. These conditions affect at least half of white men and women by the age of 50 years [2,7]. AGA can affect all men of all ages after puberty, while in women the incidence of female pattern hair loss increases after menopause.

AGA is characterised by follicular miniaturization and reduced hair density, and is accompanied by a shortened growth phase of the follicle (anagen), resulting in a greater percentage of microscopic hairs at the resting phase (telogen) remaining on the scalp [8,9]. Dihydrotestosterone (DHT) has been implicated as a major driver of androgenetic alopecia. Levels of DHT are elevated in the balding scalp, as are DHT receptors on hair follicles and 5-alpha reductase, the enzyme that converts testosterone to DHT [10-13]. Another likely driver system for AGA involves Prostaglandin D_2 Synthase (PTGDS), its enzymatic product PGD_2, and PGD_2 receptor expression [6,14].

The two most commonly used hair loss treatments are Minoxidil and Finasteride. Finasteride, a selective type 2 5-alpha reductase inhibitor, blocks the conversion of testosterone to DHT and has been shown to improve hair growth in men by reducing DHT levels in the scalp [15,16]. It is not as effective in women [17]. Minoxidil is an antihypertensive vasodilator. Its targets in preventing hair loss are unclear, but may involve ATP synthase-

***Corresponding Author:** Dr. Paul Clayton, Beaver House, 23-28 Hythe Bridge Street, Oxford OX1 2EP, UK, Tel: +44 800 644 0322; E-mail: paulrclayton@gmail.com*

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induced stem cell differentiation in the hair follicles [18]. Both drugs are associated with adverse effects, which may rarely be severe.

Ageratum conyzoides L., also known as "goat weed" or "billy goat weed", is an annual herb belonging to the family Asteraceae [19]. It is traditionally used in the treatment of skin disorders, ulcers, burn wounds, diarrhea, infectious diseases, headaches and gynaecological diseases [19-21]. Studies have also reported its anti-inflammatory, antioxidant and analgesic properties [22,23]. Recently, an ethyl alcohol extract of *A. conyzoides* was shown to reduce 5α-reductase type 1 and type 2 activity in human prostate epithelial cells [24].

Pyrrolizidine alkaloid-free *A. conyzoides* extracts meet international safety standards. Null results have been demonstrated in the bacterial reverse mutation test, and *in vitro* and in vivo mammalian chromosomal aberration tests [25]. 90-day oral toxicity and teratogenicity studies have shown a similarly clean profile [23,26]. In clinical use, Detering et al's study found *A. conyzoides* to have a good safety profile, with no notable adverse effects associated with 250mg/d supplementation for 12 weeks. There were no effects on hematological and biochemical parameters, lipids or blood glucose levels and no observed suppression of testosterone, DHT levels or sexual functioning [24].

We conducted *in vitro* investigations in human hair dermal papilla cells to measure changes in 5α-reductase expression after 48 hours and inhibition of PGD_2 after 6 hours of treatment with *A. conyzoides*. An open-label clinical study was also conducted to assess the efficacy and safety of two dose levels of an alkaloid-free *A. conyzoides* topical gel in increasing hair growth and decreasing hair loss in males and females.

Activity Modulation of 5α-reductase gene expression study

Study design and aim

The study was designed to quantify the change in expression level of 5α-reductase type 1 enzyme in Human Hair Dermal Papilla Cells (HHDPC) after 48 hours of treatment with *A. conyzoides*. Nucleic acid levels were subsequently quantified using real time qPCR.

Test materials

Ageratum conyzoides paste (Batch: NC/HGP/13001) was obtained from Gencor Pacific Limited, New Territories, Hong Kong. The sample was solubilized directly in the DMEM culture medium at 0.1 mg/ml and 0.025 mg/ml concentration to make the final test products.

Cell culture and treatment

Human Hair Dermal Papilla Cells (HHDPC) with fibroblast-like morphology (Source: Inno prot – P10881) were directly solubilized in the culture medium at 0.1 and 0.025 mg/ml in DMEM culture medium, 10% fetal bovine serum, glutamine, gentamicin and penicillin-streptomycin.

Treatment and exposure

The cells were seeded in 6 well plates at 14000 cells/well for 24 hours. The cultures were subsequently treated with 10 ng/ml testosterone for 24 hours, followed by addition of fresh medium and supplementation with 0.1 mg/ml and 0.025 mg/ml concentration of *A. conyzoides*. Untreated cells were used as negative control and cells treated with 10 mg/ml of *Serenoa repens* were used as positive control. All samples were tested in duplicate. After 48 hours of exposure, total RNA was purified from cells by trizol protocol.

RNA extraction and retro-transcription

Total RNA was extracted using a guanidine thiocyanate-based reagent according to manufacturer's instruction (TRIZOL-INVITROGEN). After precipitation and centrifugation (30' 12000 rpm, 4°C), RNA was resuspended in 20 ml sterile water and its concentration was determined spectrophotometrically. 300 ng of total RNA were retro-transcribed into cDNA using random primers at 37°C for 2 hours in thermo cycler following manufacturer's instruction (Applied Biosystems, Foster City, CA).

Analysis of gene expression profile by real time RT-PCR

Changes in gene expression profiles were analyzed by real time PCR technology using Sybr-green based chemistry. Primer pair sequences for tyrosinase analysis were designed across intron-exon spanning regions and screened against a non-redundant database (GENBANK) to verify the unicity of amplified regions across the genome. The increase in this signal is directly proportional to the amount of PCR products in the reaction. Therefore, by recording the amount of fluorescence emission at each cycle it was possible to monitor the PCR reaction during exponential phase where the first significant increase in the amount of PCR product correlates to the initial amount of target templates (Figure 1).

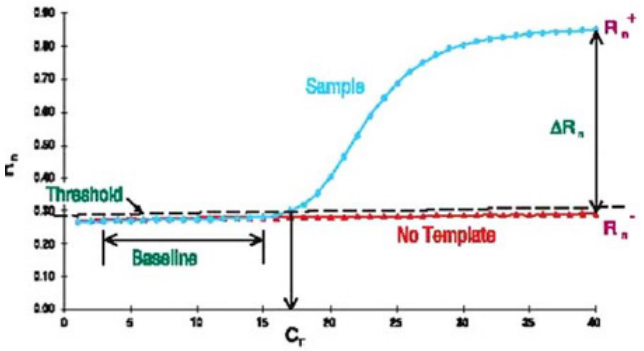

Figure 1. Analysis of 5α-reductase type 1 gene expression in human hair dermal papilla cells profile by RT-qPCR. Representative amplification plot: plot of fluorescent signal versus cycle number. Initial plot: The initial cycle of the PCR shows little change in fluorescent signal. This defines the baseline for the amplification plot. An increase in fluorescence above the baseline indicates the detection of accumulated PCR product. A fixed fluorescence threshold can be set above the baseline. The Cycle Threshold (Ct) is defined as the number of cycles required for the fluorescent signal to cross the threshold (i.e. exceeds 10 times the background level). Ct levels are inversely proportional to the amount of target nucleic acid in the sample. So, the higher the initial amount of the sample, the sooner accumulated product is detected in the PCR process as a significant increase in fluorescence, and the lower the Ct value. Ct values are very reproducible in replicates because the threshold is picked to be in the exponential phase of the PCR, where there is a linear relation between log of the change in fluorescence and cycle number and the reaction components are non-limiting.

Quantification of inhibition of PGD_2 production in Human Hair Dermal Papilla Cells (HHDPC)

Study design and aim

This study aimed to assess the inhibition of production of PGD_2 in Human Hair Dermal Papilla Cells after 6 hours of treatment with *A. conyzoides* paste. The final PGD_2 inhibition analysis was performed by competitive EIA.

Test material

A. conyzoides paste (Batch: NC/HGP/13001) was obtained from Gencor Pacific Limited, New Territories, Hong Kong.

Cell culture methods and media

HHDPC were obtained from the European Collection of Cell Cultures (ECACC), and were preserved and cultured according to the protocols provided by the manufacturer and confirmed by scientific publications. HHDPCs were seeded in 24-well plates at a 20000 cells/well concentration, and cultured at 37°C, 95% humidity, 5% CO2, using high Glucose Dulbecco's Modified Eagle's Medium (DMEM) containing 4.5 g/L D-glucose

4mMol L-Glutamine, and supplemented with 10% fetal bovine serum, 1.2 g/L sodium bicarbonate and 0.1 mg/ml Penicillin, Streptomycin and Kanamycin. Cell cultures were allowed to adhere and grow for 24 hours before the tests.

Preliminary baseline PGD_2 quantification

For assessment of baseline PGD_2 levels, untreated cells were plated as previously described and grown for 24 hours. Half the cell cultures were treated for 6 hours with 0.05 mg/ml 2, 2'-Azobis (2-methylpropionamidine) dihydrochloride, a known COX activator, in order to confirm the cells' response when stimulated to produce PGD_2. Cell culture supernatants were removed, and the cells were detached and re-suspended in 0.1 M potassium phosphate. The cell solution was exposed to sonication for 25 min in order to obtain a cell lysate containing PGD_2. The lysate was tested as a 1:10 dilution in EIA buffer, showing an acceptable baseline production for the final test. The EIA test was performed according to the manufacturer's indications, and the readings were taken and analyzed as reported below.

Samples preparation

The sample (*A. conyzoides* extract) was dissolved in complete HHDPC medium at a 5 mg/ml concentration. 50 mg of the sample were carefully weighted in a 15 ml sterile vial. 10 ml of complete HHDPC medium was used. Two sample dilutions (1:10 and 1:50 respectively) were prepared from this starting solution in order to prepare the final sample concentrations used in the tests.

Final PGD_2 inhibition assay

The final PGD_2 inhibition assay was performed on cell lysates, obtained as previously described from the following cell cultures:

- Sample 1: Cell cultures treated for 6 hours with 0.5 mg/ml *A. conyzoides* Paste dissolved in growth medium
- Sample 2: Cell cultures treated for 6 hours with 0.1 mg/ml *A. conyzoides* Paste dissolved in growth medium
- Negative control: Untreated cell cultures (baseline PGD_2 secretion).
- All sample cultures were run in quadruple repeats.

Six hours before the test, the sample cell cultures were treated with 0.5 mg/ml and 0.1 mg/ml *A. conyzoides* Paste directly dissolved in the culture medium. Untreated cultures were used as a negative control. Two cultures from each sample were then used for PGD_2 quantification. Following exposure, the supernatant was removed, each culture was harvested and lysates were prepared. The samples obtained were diluted in EIA buffer (1:10 ratio). The three samples obtained (0.5 mg/ml, 0.1 mg/ml and untreated control) were then run in the PGD_2 competitive EIA assay together with standards containing known concentrations of PGD_2 for comparison. After an18 hour incubation, the samples and standards were exposed to Ellman's reagent and their absorbance at a 405 nm wavelength was read using a spectrophotometer (Tecan Sunrise). All the data were then corrected for blank readings, non-specific binding and baseline diluted-medium absorbance. The absorbance obtained from the standard was plotted vs. known concentration in order to identify a concentration-to-absorbance function, and the readings from the samples were tested through regression on a logistic-fit in order to determine the concentration of PGD_2 contained in each supernatant.

Open Label Study

Study design

This was a pilot, open-label, randomized, parallel study lasting 8 weeks to determine the effectiveness of 2 dose levels of a topically applied *A. conyzoides* gel on increasing hair growth and decreasing hair loss in males and females, over 18 years of age. 28 healthy males and females were enrolled in this study. This study was conducted by RDC Clinical, a contract research organisation, between October 2017 and March 2018 in Queensland, Australia.

Study Population

Participants were recruited from databases and through social media advertising. Participants were eligible to enroll in this study if they were over 18 years old and exhibiting male or female pattern baldness according to the Norwood/Hamilton Scale or Ludwig scale, respectively. Exclusion criteria included history of clinically significant medical conditions including, but not limited to, cardiovascular, neurological, psychiatric, renal, immunological, endocrine (including uncontrolled diabetes or thyroid disease) or haematological abnormalities that are uncontrolled. Other reasons for exclusion were current use of hair growth formulations, history of radiotherapy to scalp for cancer treatment, current or past history of cicatricial alopecia, use of hair dye and alterations in hair style. Also excluded were women with clinical diagnosis of menstrual and/or endocrine disorders, PCOS, hyperandrogenism and men who have used or continue to use antihypertensives, steroids, spironolactone, ketoconazole, cytotoxic compounds, anticonvulsant drugs, estrogens or progesterone within the last six months. Additionally, women using hormone therapy including oral contraceptives were excluded.

Trial Product

The investigational product was a gel formulation of *A. conyzoides*, of either 0.5% or 1% strength. *A. conyzoides* gel was provided by Gencor Pacific Limited, New Territories, Hong Kong. Participants were randomly allocated to the 1% group or the 0.5% in a 2:1 ratio. Randomisation was conducted using https://www.randomizer.org. Participants were required to apply the gel topically twice daily (morning and night) for the duration of the study.

Procedure

Participants were required to apply a teaspoon quantity of the gel topically twice a day (morning and night) for the duration of the study. Every two weeks, participants were required to attend the study site for a hair measurement assessment. Participants recorded Quality of life scores and self-assessed improvement in hair through self-reported questionnaires.

Outcome measures

Increase in hair growth over an 8-week period. Hair growth was determined by the temporal recession distance (measured as the distance in cm from top of eyebrow to start of hairline), and the hair card test for evidence of new growth (yes or no).

Decrease in hair loss over an 8-week period. Hair loss was determined by the mean number of hairs lost during a one-minute combing test (females only).

Self-reported measures of improvement. The Hair Loss Quality of Life (Distress questionnaire) consists of 10 questions evaluating how the loss of hair affects quality of life. Answers are scored on a Likert scale from 0 = not a problem, to 4 = very distressing. The Self-Assessment Questionnaire is a specifically designed questionnaire to rate the improvement in hair characteristics as a result of treatment. Answers are reported from very satisfied/effective to very unsatisfied/ineffective.

Statistical Analysis

Paired-samples t-tests were conducted to compare baseline to week 8 data where significance was defined as $p < 0.05$.

Results

Modulation of 5α-reductase type 1 gene expression in

HHPD cells

The gene expression profile of 5α-reductase type 1 was evaluated after 48 hours of HHPD cell exposure with *A. conyzoides* paste at two different concentrations of the product. Treatment with *A. conyzoides* for 48 hours significantly reduced the expression of 5α-reductase type 1 compared to the negative control (arbitrarily set to 1). There was a 3-fold reduction (good reduction) in gene expression at 0.1 mg/ml concentration of *A. conyzoides* compared to the untreated control (from 1.0 to 0.33 ± 0.08). The positive control, *Serenoa repens*, had a reduction in gene expression of 5α-reductase type 1 (1 to 0.11 ± 0.05) relative to the negative control (Figure 2). There was no reduction in gene expression at 0.025 mg/ml of *A. conyzoides* in human hair dermal papilla cells.

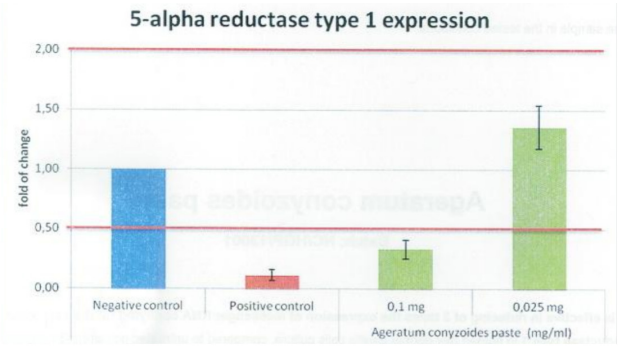

Figure 2. Effect of 0.025 mg/ml and 0.1 mg/ml A. conyzoides on 5α-reductase type 1 gene expression in human Hair Dermal Papilla Cells with Serenoa repens as positive control.

Effect on Prostaglandin D_2 Production in HHDPC

After 6 hours treatment with *A. conyzoides*, there was a marked significant inhibition of PGD_2 release by HHDP cells relative to the negative control (1478.99 ± 30.27 pg/ml). In cells exposed to 0.5 mg/ml *A. conyzoides* paste PGD_2 release was inhibited by 80.58 ± 2.19% (287.18 ± 2.15 pg/ml), and in cells exposed to 0.1 mg/ml *A. conyzoides* paste, PGD_2 release was inhibited by 78.27 ± 2.63% (423.31 ± 8.61 pg/ml). No positive control was used in this experiment (Figure 3).

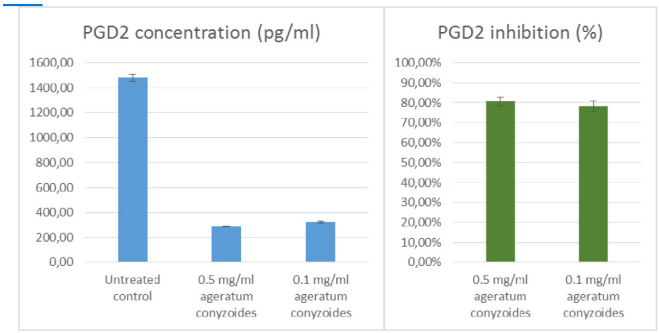

Figure 3. PGD2 concentration (pg/ml) and inhibition percentage (%) in the A. conyzoides (0.5 mg/ml and 0.1 mg/ml) analyzed cell culture.

Open-Label Study

Demographics

Out of the 28 participants enrolled, 23 participants completed the trial; 16 in group A (1% concentration, male:female 14:2), and 7 in group B (0.5% concentration, male:female 6:1). Of 23 males enrolled in the study, three participants in group A dropped out due to headaches. Two of the six male participants in group B were lost to follow-up in Week 8. Therefore, the temporal recession distance and quality of life score at week 8 for males accounted for 18 participants (1%:0.5% 14:4). Of the 5 females, one dropped out for personal reasons (group A) and one was lost to follow-up (group B).

There was no significant difference in the average baseline measurements in terms of Quality of life score and temporal recession distances between the two treatment groups (Table 1).

Average Baseline Measurements	0.5% Concentration	1% Concentration
Quality of life score	M: 13	M: 11.1
	F: 19	F: 23
Temporal recession	M: 8.62	M: 9.13
	F: 7	F: 6.5

Table 1. Average baseline measurements for males and females for 0.5% and 1% of A. conyzoides gel formulation.

Quality of life and temporal recession scores per treatment group

There was a significant reduction ($p<0.05$) in hair distress questionnaire scores after the completion of 8-week trial compared to the baseline for both the groups and gender. Among the males, the distress score fell from 13 (Baseline) to 7 (Week 8) for 0.5% of *A. conyzoides* gel formulation and from 11.1 (Baseline) to 7.5 (Week 8) for the 1% gel formulation ($p < 0.05$) (Figure 4).

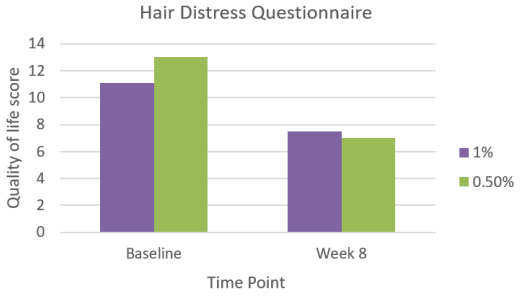

Figure 4. Score on Hair quality of Life Questionnaire for males from baseline to week 8.

Temporal recession in men reduced from 9.1cm to 8.6 cm in the 1% group, and 8.6 to 7.5 cm in the 0.5% group (Figure 5). Although the result was not statistically significant ($p = 0.084$), it trended towards significance. Due to a small sample size, the scores of female participants were not calculated.

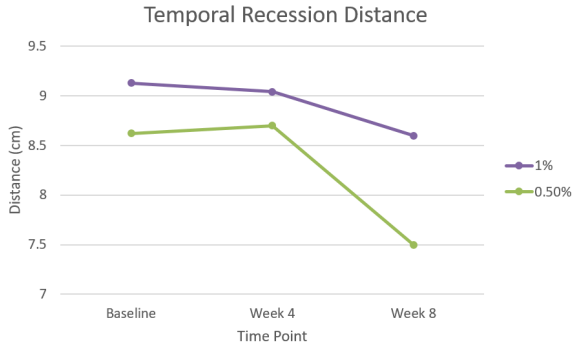

Figure 5. Temporal recession distance of males for 0.5 and 1% of A. conyzoides gel formulation

Self-assessed improvement in hair

64% of men and 100% of women self-reported an improvement in their hair loss symptoms in the 1% treatment group (Tables 2 and 3). Over 65% of both men and women indicated an improvement in their hair related quality of life, which is statistically significant ($p < 0.05$). More than 90% of the men in the 1% group had new hair growth observed in the hair card test (Table 2). Due to low sample size, the data for female participants were pooled together (Table 3).

MALES	0.5% concentration	1% concentration	Total
Quality of life improved at week 8	75%	71%	72.2%
Self-Assessed improvement in hair	50%	64%	61.1%
Reduced Recession Distance (better)	25%	64%	55.6%
New Growth Observed	75%	93%	88.9%

Table 2. Percentage of participants indicating overall improvement in hair loss (males).

FEMALES	Total
Quality of life improved at week 8	66.7%
Self-Assessed improvement in hair	100%
Reduced hair fall	66.7%
New Growth observed	66.7%

Table 3. Percentage of participants indicating overall improvement in hair loss (females).

Discussion

The dermal papilla is a highly active group of cells derived from the dermis mesenchyme, located at the base of the hair follicle. It is capable of inducing follicle development from the epidermis and controlling the subsequent cyclical production of hair fiber [27-29]. Hair growth cycles are intricately controlled by chemical signalling and hormonal regulation [5,27,30,31], which coordinate the growth of each hair follicle to an individual's age, development stage and environment [5]. Each hair follicle passes through many cycles consisting of four stages; anagen, the active growth phase where hair is produced, catagen, a transition phase where hair starts getting cut off from its blood supply and stops growing, telogen, the hair follicle's resting phase where it is completely cut off from its blood supply and exogen, where the old strand sheds and a new strand begins to form [5,30-32].

The main hormones regulating hair follicles are the androgens [30]. Increased androgen expression is one of the key factors in the development of AGA [4,5]. It stimulates tiny vellus hair follicles on the axilla, pubis, chest and face to transform into larger, thicker, pigmented hairs while simultaneously inhibiting the growth of larger terminal hairs on the scalp to produce smaller, vellus-like hairs [5]. This is why AGA is more prevalent in men than women [4,5] and often portrays a phenotype of greater chest, axillary and facial hair in conjunction with reduced hair on the scalp [5]. Dihydrotestosterone (DHT), is the main androgen causing AGA [4]. 5α-reductase, an enzyme expressed in skin melanocytes, fibroblasts and keratinocytes [33] is responsible for converting testosterone to the more potent DHT [4,5,33]. Inhibition of 5α-reductase can improve hair growth and slow down hair loss [4]. Apart from hormonal regulation, lipid signaling has also generated interest for its role in controlling hair growth cycles [6,14]. Interestingly, the prostaglandins, which are highly expressed in male genitalia and can induce masculine phenotypes, are found to be testosterone-responsive such that prostaglandin and androgen signalling may influence one another to control hair growth [14].

PGD_2, is a prostaglandin primarily produced by mast cells, that performs several functions including inflammatory modulation and vasodilation [34]. It has important effects in recruiting eosinophils, basophils and Th2 cells [34]. PGD_2 is produced through enzymatic alteration of its Precursor, Prostaglandin H2 (PGH2) by PGDS [34]. There are two isoforms of PGDS; lipocalin-type PGDS localized in the central nervous system, male reproductive system and heart and hematopoietic-type PGDS distributed in the peripheral tissues and localized in antigen-presenting cells, mast cells and megakaryocytes [34,35]. In both human [14] and animal [14,36] models, elevated levels of localized PGD_2 were linked to hair growth-inhibition through the G-protein-coupled receptor 44 (GPCR44) [6,14,37] and high levels of topically applied PGD_2 were demonstrated to inhibit hair growth [6,14]. Both PGD_2 and PGDS were also found to reach much higher levels in balding scalp tissue compared to non-balding tissue [6,14]. Moreover, the PGD_2 pathway is associated with induction of the hair follicle into the resting catagen phase of the hair cycle [14].

As PGD_2 is a ligand for PPARγ, a transcription factor responsible for adipose development, which also supports sebaceous gland function, increased PGD_2 levels could be a reason for sebaceous hyperplasia often occurring with AGA [6]. The role of prostaglandins in inflammatory signalling coincides with increased inflammatory mediators observed in alopecia, including the autoimmune disease Alopecia Areata (AA) [14,37] as well as AGA [6]. In AA, infiltrating lymphocytes and mast cells have been found around miniaturized follicles [14], accompanied by an increase in CD4+ and CD8+ T cells and an impaired function of T regulatory cells [37]. Moreover, mice overexpressing the COX2 enzyme have been shown to develop alopecia [6].

Prostaglandins are thought to be dysregulated in AGA [14], with PGD_2 signalling increased and PGE2 and PGF2α, both of which are shown to facilitate hair growth, decreased [6,14]. Re-balancing these prostaglandins, i.e. decreasing PGD_2 expression and elevating PGE2 and PGF2α, might subsequently be expected to support hair re-growth. Inhibiting PGD_2 signalling in the scalp and simultaneously reducing local androgen expression via 5α-reductase inhibition therefore constitutes a potential therapy for hair loss.

Our in-vitro studies displayed *A. conyzoides* inhibition of Prostaglandin D_2 (PGD_2) and 5α-reductase, both of which are linked to suppression of hair growth and the pathogenesis of Androgenetic Alopecia (AGA) [4,6,14,37], in Human Hair Dermal Papilla Cells (HHDPC) . *A. conyzoides* paste was shown to inhibit PGD_2 release in HHDPC at a concentration of 0.5 mg/ml (80.58 ± 2.19% inhibition) and 0.1 mg/ml (78.27 ± 2.63% inhibition). Moreover, after 48 hours of exposure, *A. conyzoides* paste was found to markedly reduce the expression of 5α-reductase in HHDPC compared to an untreated control. 0.1 mg/ml *A. conyzoides* paste showed a 3-fold reduction of 5α-reductase type 1 mRNA in HHDPC compared to the negative control while 0.025mg *A. conyzoides* didn't show a reduction of mRNA expression.

Our open label study demonstrates *A. conyzoides*' efficacy in inhibiting hair loss and facilitating hair growth. 64% of men and 100% of women self-reported an improvement in their hair loss symptoms associated with application of the *A. conyzoides* gel formulation at 1% and 0.5% strength, twice daily for 8 weeks. Over 65% of both men and women indicated an improvement in their hair-related quality of life, which was statistically significant. Temporal recession in men reduced from 9.1cm to 8.6cm in the 1% group and 8.6 to 7.5cm in the 0.5% group, with the normal distance in men without recession being between 6-6.5cm. Although this was not a significantly different result from baseline (p=0.084), it did indicate a trend towards significance. Moreover, while some women indicated a reduced hair fall, the sample size was too small to determine if there was a significant effect. From these results, it is likely that the 1% dose is more effective as the participants had more severe hair loss at baseline.

The product was well tolerated by the majority of participants. Few adverse events occurred in the study; 3 participants reported spots with 1% *A. conyzoides* application and there were 3 dropouts due to headaches. A limitation to the study is there were not enough participants, in particular women. More participants are required to determine conclusive results in both genders as an effect was only seen at 8 weeks. Therefore, a longer study is recommended. In addition, the use of a more quantitative measurement of hair loss and growth, such as a trichometer may give more comprehensive data. Whereas current treatments for hair loss have well-known problems and limitations [4,24,37], pre-clinical and clinical work shows that pyrrolizidine alkaloid-free *A. conyzoides* extracts has an

excellent safety profile [24-26]. Our mechanistic studies and clinical trial results are in line with pre-existing data, and indicate that *A. conyzoides* is an effective treatment option for hair loss with minimal adverse effects.

Conclusion

Taken together, the results of the *in vitro* studies and the open label study indicate that topical *A. conyzoides* application is a viable and safe treatment option for hair loss. The *in vitro* inhibition of PGD_2 and 5α-reductase and the clinically observed improvement of hair loss symptoms and hair-related quality of life are highly congruent. The results of these studies are encouraging and warrant further research.

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Author Contributions

R. Venkatesh, N. Bogoda and S. Subah conducted background research. P. Clayton, R. Venkatesh, N. Bogoda and S. Subah prepared the Manuscript. P. Clayton edited the manuscript.

References

1. Nabahin, Amal and AbouEloun Alaa. "Hair Loss Diagnosis and Treatment Knowledge Based System." *Int J Eng Inf Syst* 1(2017): 160-169.
2. Stough, Dow, Stenn Kurt, Haber Robert and Parsley William M, et al. "Psychosocial effect, pathophysiology, and management of androgenetic alopecia in men." *Mayo Clin Proc* 80(2005): 1316-1322.
3. Cash Thomas F. "The psychosocial consequences of androgenetic alopecia: a review of the research literature." *Br J Dermatol* 141(1999):3-398.
4. Clarke Philip. "Male Baldness." Aust Fam Physician 45(2016):186-188.
5. Randall Valerie Anne. "Androgen and hair growth." *Dermatol Ther* 21(2008):314-328.
6. Nieves, Ashley and Garza Luis A. "Does prostaglandin D2 hold the cure to male pattern baldness? " *Exp Dermatol* 23(2014):224-227.
7. Ablon, Glynis and Kogan Sophia. "A six-month, randomized, double-blind, placebo-controlled study evaluating the safety and efficacy of a nutraceutical supplement for promoting hair growth in women with self-perceived thinning hair." *Journal of Drugs in Dermatology* 17(2018):558-565.
8. Ellis, Justine A, Sinclair Rodney and Harrap Stephen B. "Androgenetic alopecia: pathogenesis and potential for therapy." *Expert Rev Mol Med* 4(2002):1-11.
9. Paus R and Cotsarelis G. "The biology of hair follicles." *N Engl J Med* 341(1999):491-497.
10. Sawaya ME and Price VH. "Different levels of 5a-reductase type I and II, aromatase, androgen receptor in hair follicles of women and men with androgenetic alopecia." *J Invest Dermatol* 109(1997):296-300.
11. Schweikert HU and Wilson JD. "Regulation of human hair growth by steroid hormones. I. Testosterone metabolism in isolated hairs." *J Clin Endocrinol Metab* 38(1974): 811-819.
12. Dallob AL, Sadick NS, Unger W and Lipert S, et al. "The effect of finasteride, a 5 alpha-reductase inhibitor on scalp skin testosterone and dihydrotestosterone concentrations in patients with male pattern baldness." *J Clin Endocrinol Metab* 79(1994): 703-706.
13. Hibberts NA, Howell AE and Randall VA. "Balding hair follicle dermal papilla cells contain higher levels of androgen receptors than those from non-balding scalp." *J Endocrinol* 156(1998): 59-65.
14. Garza, Luis A, Liu Yaping, Yang Zaixin and Alagesan Brinda, et al. "Prostaglandin D_2 inhibits hair growth and is elevated in the bald scalp of men with androgenetic alopecia." *Sci Transl Med* 126(2012): 125ra34-126ra34.
15. Kaufman, Keith D, Olsen Elise A, Whiting David and Savin Ronald, et al. "Finasteride in the treatment of men with androgenetic alopecia." *J Am Acad Dermatol* 39(1998):578-589.
16. Leyden J, Dunlap F, Miller B and Winters P, et al. "Finasteride in the treatment of men with frontal male pattern hair loss." *J Am Acad Dermatol* 40(1999): 930-937.
17. Price VH, Roberts JL, Hordinsky M and Olsen EA, et al. "Lack of efficacy of finasteride in postmenstrual women with androgenetic alopecia." *J Am Acad Dermatol* 43(2000):768-776.
18. Goren A, Naccarato T, Situm M and Kovacevic M. "Mechanism of action of minoxidil in the treatment of androgenetic alopecia is likely mediated by mitochondrial adenosine triphosphate synthase-induced stem cell differentiation." *J Biol Regul Homeost Agents* 31(2017):1049-1053.
19. Okunade Adewole L. "*Ageratum conyzoides L*. (Asteraceae)." *Fitoterapia* 73(2002): 1-16.
20. Adebayo, HA, Zeng Guang-Zhi, Fan Jun-Ting and JI Chang-Jiu, et al. "Biochemical, hematological and histopathological studies of extract of *Ageratum conyzoides L*. in Sprague Dawley rats." *Journal of Medicinal Plants Research* 4(2010): 2264-2272.
21. Kaur R and Dogra N. "A review on traditional uses, chemical constituents and pharmacology of *Ageratum conyzoides L*. (Asteraceae)." *Int J Pharm Biol Arch* 5(2014): 33-45.
22. Kamboj, Anjoo and Saluja Ajay Kumar "*Ageratum conyzoides L*.: A review on its phytochemical and pharmacological profile." *Int J Green Pharm* 2(2008).
23. Lakmichi, Hind, Bakhtaoui Fatima Zahra, Gadhi Chemseddoha A and Aicha Ezoubeiri, et al. "Anti-inflammatory and chronic toxicity study of the leaves of *Ageratum conyzoides L*. in rats." *Phytomedicine* 12(2005):138-142.
24. Detering, Matthew, Steels Elizabeth, Koyyalamudi Sundar Rao and Elena Allifranchini, et al. "*Ageratum conyzoides L*. inhibits 5-alpha-reductase gene expression in human prostate cells and reduces symptoms of benign prostatic hypertrophy in otherwise healthy men in a double-blind randomized placebo controlled clinical study." *Biofactors* 43(2017):789-800.
25. Palmer, Philip A, Bryson Jessica A, Clewell Amy E and Endres John R, et al. "A comprehensive toxicological safety assessment of an extract of Ageratum conyzoides." *Regul. Toxicol. Pharmacol* 103(2019):140-149.
26. Subah, Silma, Bogoda Nathasha, Glávits Róbert and Ruchitha Venkatesh, et al. "Prenatal developmental toxicity study of an alkaloid-free Ageratum conyzoides extract powder in rats by oral administration. Regul." *Toxicol Pharmacol* 117(2020): 104748.
27. Messenger AG "The control of hair growth: an overview." *J Invest Dermatol* 101(1993): 4S-9S.
28. Driskell, Ryan R, Clavel Carlos, Rend Michael and Watt Fiona M. "Hair follicle dermal papilla cells at a glance." *J Cell Sci* 124(2011):1179-1182.
29. Yang, Chao-Chun and Cotsarelis George. "Review of hair follicle dermal cell." *J Dermatol Sci* 57(2011):2.
30. Bernard Bruno A. "Advances in understanding hair growth." *F1000Res* 5(2016).
31. Hoover, Ezra, Alhajj Mandy and Flores Jose L. "Physiology, Hair." *StatPearls Publishing* (2020).
32. Hirt, Penelope A and Paus Ralf. "Healthy Hair (Anatomy, Biology, Morphogenesis, Cycling, and Function) in: Alopecia." *Elsevier* (2019):1-22.
33. Chen W, Zouboulis CC, Fritsch M and Blume-Peytavi U, et al. "Evidence of heterogeneity and quantitative differences of type 1 5a-reductase expression in cultured human skin cells-evidence of its presence in melanocytes." *J Invest Dermatol* 110(1998):84-89.
34. Arima, Masafumi and Fukuda Takeshi. "Prostaglandin D_2 and TH2 inflammation in the pathogenesis of bronchial asthma. Korean." *J Intern Med* 26(2011):8-18.
35. Silpa SR. "Prostaglandin and its type." *Pharmatutor* 2(2014):31-37.

36. Nelson, Amanda M, Loy Dorothy E, Lawson John A and Katseff Adiya S, et al. "Prostaglandin D_2 inhibits wound-induced hair follicle neogenesis through receptor GPR44." *J Invest Dermatol* 133(2013):881-889.

37. Falto-Aizpurua, Leyre, Choudhary Sonal and Tosti Antonella. "Emerging treatments in alopecia." *Expert Opin Emerg Drugs* 19(2014):545-556.

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