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Efficacy of a Topical Application of *Ageratum Conyzoides* on increasing Hair Growth and in Males and Females: A Randomized Double-Blind Placebo-Controlled Study

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ABSTRACT

Background
Alopecia affects both males and females and can cause significant psychological distress. *Ageratum conyzoides*, traditionally used to treat a multitude of conditions including skin disorders, gastrointestinal problems, headache, and pneumonia, has been also found to have good efficacy in increasing hair growth and decreasing hair loss. Importantly, its good safety profile makes it advantageous over the current drug treatments for hair loss; Finasteride and Minoxidil, both of which are associated with adverse effects.

Objectives
A 12-week double-blind, randomized, clinical trial investigated the efficacy and safety of a topical application of *A. conyzoides* in males and females over 18-years of age.

Methods
A. Conyzoides topical gel of 0.5% strength was administered daily for 12-weeks to 80 otherwise healthy males and females over 18-years of age who self-reported hair loss. Hair growth was assessed by measuring hair density using HairCheck® and calculating the hair loss ratio (HLR). Hair loss was assessed by the mean number of hairs lost during a one-minute combing test and a hair tug or pull test. Other hair measures included the Hamilton-Norwood scale for men and Savin scale for women. Participants' quality-of-life (QoL) was evaluated by self-assessment questionnaires. Biochemical and haematological parameters were also assessed.

Results
Our study found a significant increase in hair density and significant decrease in HLR following topical application of *A. conyzoides*. At 12-weeks, hair density in the *A. conyzoides* treated group was significantly higher and HLR was significantly lower than the placebo group. No significant changes were found in the one-minute combing test or hair pull test or assessment by the Hamilton-Norwood and Savin hair loss scales. QoL measures and biochemical and haematological parameters showed no significant changes throughout the study.

Conclusion
The results from our study demonstrate a net increase in hair growth following topical application of *A. conyzoides*.

Keywords
Ageratum conyzoides; Hair loss; Hair growth; Alopecia; Topical application.

INTRODUCTION

Alopecia is a common condition experienced by both males and females. Although not debilitating, it can cause significant psychological distress and billions of dollars are spent on hair loss treatments per year. The most common type of male and female pattern baldness is androgenetic alopecia (AGA), a genetically determined androgen-induced pattern baldness.[1] It affects up to 80% of men and 40% of women during their lifetime, and although its prevalence increases over time, it can occur at almost an age.

Dihydrotestosterone has been identified as an underlying driver of AGA, and inhibition of 5-alpha-reductase, an enzyme responsible for converting testosterone to dihydrotestosterone, is a recognised treatment approach.[1] Recent studies have found Prostaglandin D2 synthase (PTGDS) and its enzymatic product, PGD2 to be increased in the bald scalp of men with AGA,[2,3] giving rise to new avenues of intervention.

Available treatments for AGA include surgery and pharmacological approaches utilizing topical gels/creams and oral medications. The two most commonly used are Finasteride and Minoxidil. Finasteride is a type 2 5-alpha reductase inhibitor which blocks the conversion of testosterone to dihydrotestosterone and improves scalp hair growth in men by reducing dihydrotestosterone (DHT) levels in the scalp.[4] It does not provide the same benefit in women. Minoxidil, a vasodilator initially used as a treatment for high blood pressure, is thought to enhance hair growth *via* activity at potassium channels and/or prostaglandin levels.[2] Surgical treatments are expensive and invasive, and the drugs in current use combine relatively low efficacy with a non-insignificant risk of adverse effects.

Ageratum conyzoides Linn., (*A. conyzoides*) commonly known as Billy Goat Weed, is a widely available annual herb which belongs to the family Asteraceae, tribe Eupatoriae. It has long-standing medicinal use in the tropical and subtropical regions[5] especially in Africa, Asia and South America where it has been traditionally used for skin disorders, gastrointestinal complaints, headache, rheumatism, pneumonia and wound healing.[6] The plant possesses antimicrobial, antioxidant, anti-inflammatory and analgesic pharmacological activities, and has allelopathic properties useful in horticulture.[6]

The plant has known toxic effects due to low but measurable levels of pyrrolizidine alkaloids.[7] An alkaloid-free *A. conyzoides* extract showed no adverse effects *in vitro* and *in vivo* acute and semi-acute models, nor in a 90-day repeated-dose oral toxicity study.[8] The same alkaloid-free extract was devoid of fetotoxic and teratological toxicity in a prenatal developmental toxicological study in rats.[9]

Constituents within *A. conyzoides* inhibit 5-alpha-reductase gene expression and were therefore considered likely to reduce dihydrotestosterone production.[10] In recently published open-label and *in vitro* studies, daily topical *A. conyzoides* application for a period of 8-weeks was confirmed to inhibit 5α-reductase, reduce levels of PGD2 and act as an effective and safe treatment option for hair loss in men and in women.[11]

The results of these studies were encouraging and warranted further research. This investigation was designed to examine the effectiveness of orally-dosed or topically applied *A. conyzoides* over a longer time period, and in a larger group of subjects.

METHODS

The study was a double-blind, randomised, clinical trial with a 12-week treatment duration. It was conducted by RDC Clinical between September 2020 and March 2022 in Queensland, Australia in compliance with the International Conference on Harmonization (ICH) Guideline for Good Clinical Practice (GCP).

Eighty (80) healthy males and females over 18-years of age, self-reporting hair loss were enrolled in this study. 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. Scalp conditions or any other genetic disease or issue that could contribute to baldness, current use of hair growth formulations, history of radiotherapy to scalp for cancer treatment, current or past history of cicatricial alopecia, participation in another hair growth trial 3-months before the start of this study, alternation in hair style, extreme hair types (eg dreadlocks, afro), colouring, bleaching, straightening or curling. Females with clinical diagnosis of menstrual and/or endocrine disorders, Polycystic ovary syndrome (PCOS), hyperandrogenism, pregnant, up to 12-months post-partum or lactating, or not on a suitable form of birth control (i.e., oral contraceptive pill) were excluded. Males who have used or continue to use antihypertensives, steroids, spironolactone, ketoconazole, cytotoxic compounds, anticonvulsant drugs, oestrogens or progesterone within the last 6-months were also excluded.

Participants were randomized into two groups: *A. conyzoides* topical gel 0.5% strength (supplied by Gencor Pacific Ltd) and topical gel placebo. They were required to apply 2 teaspoons of gel onto the scalp twice a day (morning and evening) for the duration of the study. Participants were required to attend the study site for hair density assessment tests, hair tug and pull test and blood tests for measurement of blood serum markers (DHT, insulin-like growth factor 1 (IGF-1), high-density lipoprotein (HDL), Erdr1, PTGDS, PGDT, GPR44, full blood count (FBC), E/LFT, thyroid stimulating hormone (TSH), ferritin and Vitamin D). Quality-of-life (QoL), diet, and improvement in hair were recorded through self-reported questionnaires.

Change in hair growth over a 12-week period was determined by measurement of hair density using the HairCheck® instrument. Other assessments included the Norwood/Hamilton scale (for males) and Ludwig Savin scale (for females) and photographs of affected scalp regions.

Decrease in hair loss over a 12-week period was measured by the mean number of hairs lost during a one-minute combing

test. In this test>150 hairs was graded as 'poor', 100-150 hairs was graded 'fair' and 50-100 hairs was graded 'good'. Hair tug or pull test and photographs of affected regions of the scalp were also used to measure the decrease in hair loss. Change in hair color over a 12-week period was determined by comparing photographs of the scalp at baseline and at 12-weeks. Participants monitored hair quality evaluation and QoL impact using self-assessment questionnaires.

Statistical Analysis

Analysis of variance (ANOVA) and *t*-tests or Mann-Whitney test were conducted to compare change from baseline to 12-weeks data where statistical significance was defined as $p<0.05$.

RESULTS

Eighty (80) participants enrolled in the study. Fifteen (15) dropped out or were lost to follow-up. Participants who completed at least week 4 were included as intention to treat, one participant was excluded as an outlier as their baseline measures were over 3 times standard deviation (SD). Data for 67 participants are presented below.

Baseline Demographics

There were no statistical differences between groups at baseline or week 4, 8 or 12 for any of the demographic or anthropometric measures in Table 1.

Table 1. *Participant Details*

	***A. conyzoides* (n=36)**	**Placebo (n=31)**
Age (years)	41.19 (10.26)	41.12 (10.98)
Gender	9 females, 27 males	10 females, 21 males
Systolic BP	115.88 (16.02)	116.11 (19.35)
Diastolic BP	76.44 (7.44)	74.28 (8.29)
Heart rate	68.38 (9.42)	68.17 (9.34)
Weight (kg)	83.11 (16.87)	84.94 (19.49)
Height (cm)	175.48 (10.00)	175.48 (8.58)
BMI kg/m^2	27.01	27.60

Hair Density (as Measured by Hair Check Device)

The hair mass index is a measure of hair density in an area of the scalp. The hair mass measured in the thinning area is calculated against an area of control hair (not thinning) to generate the hair loss ratio.

The hair density significantly increased from baseline in the *A. conyzoides* group from week 8, and the change was significantly different between groups at week 12.

The Hair loss ratio was also significantly different from baseline in the Ageratum group at week 8 and 12 and there was a significant difference between groups at week 12 (Table 2).

Table 2. *Hair Density and Hair Loss Ratio*

	***A. conyzoides* (n=36)**	***A. conyzoides***	**Placebo (n=31)**	***A. conyzoides***
Ageratum				
Control reading	69.33 (22.91)			
Hair mass index (density)	31.75 (18.41)	33.91 (18.69)	34.36$^{\#}$ (19.42)	34.39$^{*\#}$ (18.21)
Hair loss ratio (HLR)	53.80 (21.65)	50.96 (21.27)	50.16$^{\#}$ (21.33)	49.99$^{*\#}$ (20.60)
Placebo				
Control reading	78.12 (22.71)			
Hair mass index (density)	36.77 (24.89)	36.74 (24.33)	36.71 (24.72)	36.45 (25.16)
Hair loss ratio (HLR)	54.21 (25.65)	53.98 (25.81)	53.91 (25.57)	55.06 (24.70)

$^{\#}$Significant ($p<0.05$) change from baseline, *significantly different change between groups

Table 3. *Hair Fall (comb test) and Hairline Measures*

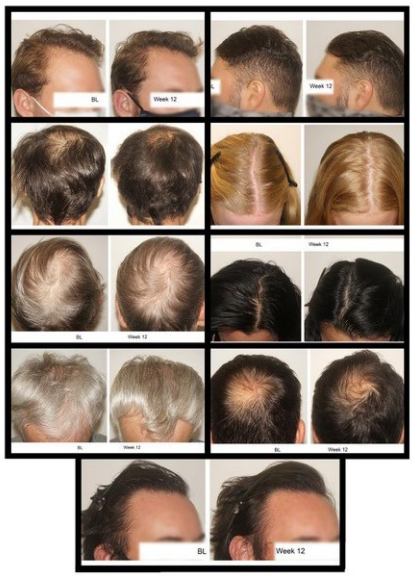
	Baseline	**Week 4**	**Week 8**	**Week 12**
A. conyzoides				
Hair comb test	16.83 (15.45)	20.25 (22.50)	22.80 (24.78)	22.72 (2.14)
Hairline measure	8.04 (2.15)	8.00 (2.16)	7.96 (2.11)	7.91 (20.60)
Placebo				
Hair comb test	17.47 (13.90)	23.93 (2.11)	18.1 (14.02)	22.30 (2.47)
Hairline measure	8.18 (2.65)	8.30 (2.49)	8.27 (2.49)	8.27 (24.70)

The hair fall (comb) test measures the number of hairs lost.The hairline measure is from the eyebrow to first hair growth at hairline (cm)

Other Hair Measures

No significant differences were seen in either group from baseline or between groups for hair fall (comb test), the hair pull test or the Hamilton-Norwood and Savin hair scales (Table 3) (Figure 1).

Figure 1. *Biochemical And Haematological Analysis*



Quality-of-Life

No differences were seen between groups for the quality-of-life questionnaires.

Biochemical and Haematological Analysis

No significant differences were observed between groups either at baseline or at week 12. All measured blood serum markers remained within the normal physiological levels (Table 4).

DISCUSSION

The results of this double-blind, randomized, placebo-controlled clinical trial confirmed the results of an earlier pilot study.[11] It demonstrated that a topical *A. conyzoides* formulation increased hair growth in males and females over a period of 12-weeks. There was a significant increase in hair density in affected areas in participants who applied a 0.5% strength *A. conyzoides* topical gel twice daily when compared to both baseline hair density levels and participants who applied a placebo gel twice daily for 12-weeks. Significant changes in hair density from baseline levels in the *A. conyzoides* group were seen as early as 8-weeks, while the placebo group showed no difference from baseline at any time-point. Hair growth was assessed by the use of HairCheck®, a sensitive and validated tool which measures hair mass by cross-section trichometry.[12]

Hair density scores were used to determine the hair loss

Table 4. *Serum Measures*

	A. conyzoides		Placebo	
	Baseline	**12-weeks**	**Baseline**	**12-weeks**
25 OH Vit D (ng/mL)	29.94 (8.81)	27.89 (8.19)	30.85 (16.02)	30.75 (13.78)
IGF-1 (ng/mL)	166.92 (52.25)	172.23 (49.38)	169.01 (39.72)	170.16 (38.69)
TSH (mIU/L)	1.80 (1.03)	1.94 (1.20)	1.81 (0.99)	1.92 (1.44)
Albumin (g/L)	45.63 (2.25)	45.19 (2.20)	46.35 (2.31)	45.30 (2.27)
Alk Phos (U/L)	76.38 (24.41)	79.94 (35.88)	76.19 (21.53)	78.81 (31.09)
ALT (U/L)	25.11 (12.36)	27.03 (14.80)	28.79 (15.15)	29.35 (10.73)
AST (U/L)	29.10 (9.94)	37.22 (35.17)	35.14 (16.15)	34.80 (13.49)
Cholesterol (mmol/L)	5.69 (1.09)	5.37 (1.04)	5.85 (1.38)	5.62 (1.35)
Ferritin (ng/mL)	130.80 (107.92)	113.88 (104.61)	155.43 (179.59)	105.65 (71.66)
HDL (mmol/L)	1.40 (0.31)	1.41 (0.33)	1.46 (0.42)	1.43 (0.40)
LDL (mmol/L)	3.87 (0.92)	3.80 (0.91)	4.18 (1.25)	4.04 (1.34)
Triglycerides (mmol/L)	1.48 (1.05)	1.47 (0.88)	1.36 (0.88)	1.53 (1.00)
Total Protein (g/L)	76.40 (4.11)	75.32 (4.80)	75.84 (4.02)	74.67 (4.74)
GGT (U/L)	28.44 (20.33)	31.13 (32.71)	40.10 (51.52)	36.45 (50.23)
Bilirubin (µmol/L)	14.08 (7.17)	12.99 (5.18)	13.46 (5.25)	13.63 (5.36)
Creatinine (µmol/L)	75.63 (21.97)	74.61 (24.34)	77.44 (21.17)	74.45 (23.14)
Glucose (mmol/L)	5.59 (0.55)	5.44 (0.65)	5.50 (1.31)	6.04 (1.66)
PGEM (pg/mL)	14.54 (9.56)	13.91 (8.79)	14.40 (9.81)	13.80 (9.05)
Testosterone (ng/mL) Male	4.92 (1.67)	4.98 (1.79)	4.72 (2.20)	4.90 (1.64)
Testosterone (ng/mL) Female	0.14 (0.07)	0.14 (0.07)	0.17 (0.09)	0.17 (0.09)
Dihydrotestosterone (pg/mL) Males	329.5 (136.7)	319.7 (111.0)	343.0 (86.74)	331.3 (73.48)
Dihydrotestosterone (pg/mL) Females	42.00 (37.69)	39.01 (36.68)	40.89 (25.26)	49.56 (29.29)

Data presented as mean (standard deviation)

ratio (HLR), which compares the hair in the affected area to an area of control (not thinning) hair. The HLR is a measure of hair growth in an affected area. *A. conyzoides* treatment significantly decreased the HLR between baseline and week 12, and the HLR was significantly lower in the *A. conyzoides* group compared to the placebo group. Representative photographs show observable increases in hair mass in areas of thinning, at week 12 compared to baseline.

There were no significant differences seen in either group from baseline or between active and placebo groups for hair fall, as assessed by a one-minute combing test, a hair pull test and assessment by the Hamilton-Norwood and Savin hair loss scales. No differences were seen between groups for participant's QoL. Assessed biochemical and haematological remained within normal parameters in both groups, with no significant changes between groups either at baseline or the end of the study.

Ageratum conyzoides is believed to improve symptoms of hair loss through inhibition of 5α-reductase and reduction of prostglandin PGD2, both of which are implicated in androgenetic alopecia (AGA).[1-3,13] While 5α-reductase converts testosterone to the potent AGA-causing androgen Dihydrotestosterone (DHT),[1,14,15] PGD2, has been linked to hair growth inhibition through G-protein-coupled receptor 44 (GPCR 44) signalling[2,3,13] and up-regulation of pro-inflammatory mediators.[16] DHT and PGD2 are elevated in the balding scalp and correlate with reduced hair growth.[2,3,17]

Previously, we demonstrated that an *A. conyzoides* extract dose-dependently inhibited 5α-reductase gene expression and PGD2, release, in human hair dermal papilla cells.[11] These are in line with other *in vitro* studies, which have shown *A. conyzoides* inhibition of PGD2 precursor, prostaglandin D synthase (PGDS) and 5α-reductase mRNA expression.[10,18] The absence of change in serum testosterone and DHT in this study reflects basic pharmacokinetics; the topical application of small amounts of *A. conyzoides* extract would not be expected to exert systemic effects and their absence here can be regarded as a secondary safety indication.

Our open-label pilot study on 28 adult males and females exhibiting pattern baldness documented an improvement in temporal recession in men, self-reported hair loss symptoms and QoL in men and women after applying 0.5% or 1% strength *A. conyzoides* topical gel formulation twice daily over 8-weeks.

The pilot study was limited by its open-label design, lack of placebo control, small sample size and low number of female participants. Our current clinical trial has a more robust design with a greater number of participants. Female participants were still considerably fewer than males, which reflects the increased prevalence of hair loss among males.

Compared to Finasteride and Minoxidil, *A. conyzoides* has a good safety and tolerability profile.[1,10,13,19] The lack of adverse effects *in vitro* and in pre-clinical toxicology testing is consistent with our previous pilot study[11] and the current clinical study, in which the product was well tolerated by the participants. We conclude that *A. conyzoides* topical gel formulation is a safe and effective treatment for enhancing hair growth in adults.

CONCLUSION

The results of the present study demonstrate the safety and efficacy of a short-term topical *A. conyzoides* application in both men and women. While no significant changes were reported in overall hair loss, there was a clear improvement in hair growth in affected areas, indicating net hair growth due to application of *A. conyzoides*.

INSTITUTIONAL REVIEW BOARD PERMSSION

Yes.

CONSENT

The authors have received written informed consent from the patient.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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