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# Evaluation of extracts containing Red Clover in the development of a scalp treatment system targeting hair loss and hair damage

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## INTRODUCTION

Hair loss and hair disorders are common conditions affecting millions around the globe. The causes are complex and not fully understood but links to genetics, lifestyle, intrinsic aging, and environmental impact are all suggested. Prevalence is known to increase with age and response to treatment is notoriously variable. Treatments have a global market value of \$2.5 billion (USD) driven by increasingly long and healthy lifespans, emphasis on aesthetic appearance, and the emotional distress experienced by patients. In-vitro models such as dermal papilla cells (DPC) from hair follicles are powerful tools for screening ingredients for hair growth promoting activities and inhibitory effects while also providing insight into possible mechanistic actions at the cellular level. We screened several ingredient blends containing either panthenol or licorice extract using the DPC model with three different sources of Red Clover Flower Extract. A comparison of the gene expression datasets with statistically significant fold change values  $\geq 1.5$  show unique influences by each extract blend. Using the DPC models to guide ingredient selection, a treatment serum was formulated and tested in 6-month (n=52) in-vivo human clinical studies using subjects with self-described thinning or damaged hair. Qualitative effects to the subject's hair and scalp were evaluated by independent clinical grading vs. baseline. Results showed statistically significant ( $p \leq 0.05$ ) improvements in several categories including volume and scalp coverage. Analysis of broken and intact hair counts from Brush Friction Hair Count Method and Macro Photography of a 2x2 cm area also show statistically significant improvement of 71.7%, 80.8%, and 31.5% respectively after 6 months. Of note, the percentage of positive responders reported by an independent clinical grader and self-perception was  $>80\%$  and  $>90\%$  respectively.

## OBJECTIVES

- Assessment of ingredient blends containing either panthenol or licorice with different sources of Red Clover Extract was tested on dermal papilla cells (DPC) for its effect on induction of hair follicle and hair regeneration.
- Clinical study to evaluate a serum composition containing blend of Red Clover Extract and dipotassium glycyrrhizate (DG) to improve hair volume, thickness, and reduction of broken hair.

## IN VITRO ASSAYS

### Ingredient blend combinations tested on DPCs for its capacity to induce cell proliferation and expression of hair morphogenesis genes

Blend	Composition
Blend 1	Panthenol + Trifolium Pratense (Clover) Flower Extract-1
Blend 2	Panthenol + Hydrolyzed Yeast Protein
Blend 3	Panthenol + Dipotassium Glycyrrhizate (DG)
Blend 4	Dipotassium Glycyrrhizate (DG) + Trifolium Pratense (Clover) Flower Extract
Blend 5	Dipotassium Glycyrrhizate (DG) + Hydrolyzed Yeast Protein
Blend 6	Dipotassium Glycyrrhizate (DG) + Trifolium Pratense (Clover) Flower Extract-2
Blend 7	Dipotassium Glycyrrhizate (DG) + Trifolium Pratense (Clover) Flower Extract-3

**Table 1.** Red Clover Extracts 1, 2, and 3 were procured from 3 different manufacturers. Red Clover Extract-1 was combined with Panthenol only for preparation of Blend 1. Red Clover Extracts 1, 2, and 3 were combined with DG to prepare Blends 4, 6, and 7.

## CLINICAL STUDY

### Evaluation of a serum composition (Key Ingredients: 1% Hydrolyzed yeast protein, 3% Trifolium Pratense (Clover) Flower Extract, 0.68% Panthenol, 0.5% DG) for its capacity to improve hair volume and thickness

A clinical study was conducted on subjects (n=52) with thin or damaged hair over 6-month period. The subjects applied the serum twice daily following hair washing for up to 6 months. The subjects were evaluated for changes from baselines 1, 2, 3, 4, 5, and 6 months in several parameters which included clinical grader assessment, self perception questionnaire, and Macro Photography hair counting. The subjects were also measured for broken and intact hair count using Brush Friction Hair Count Method at baselines 2, 4, and 6 months. The study protocol was reviewed and approved by IRB prior to study initiation.

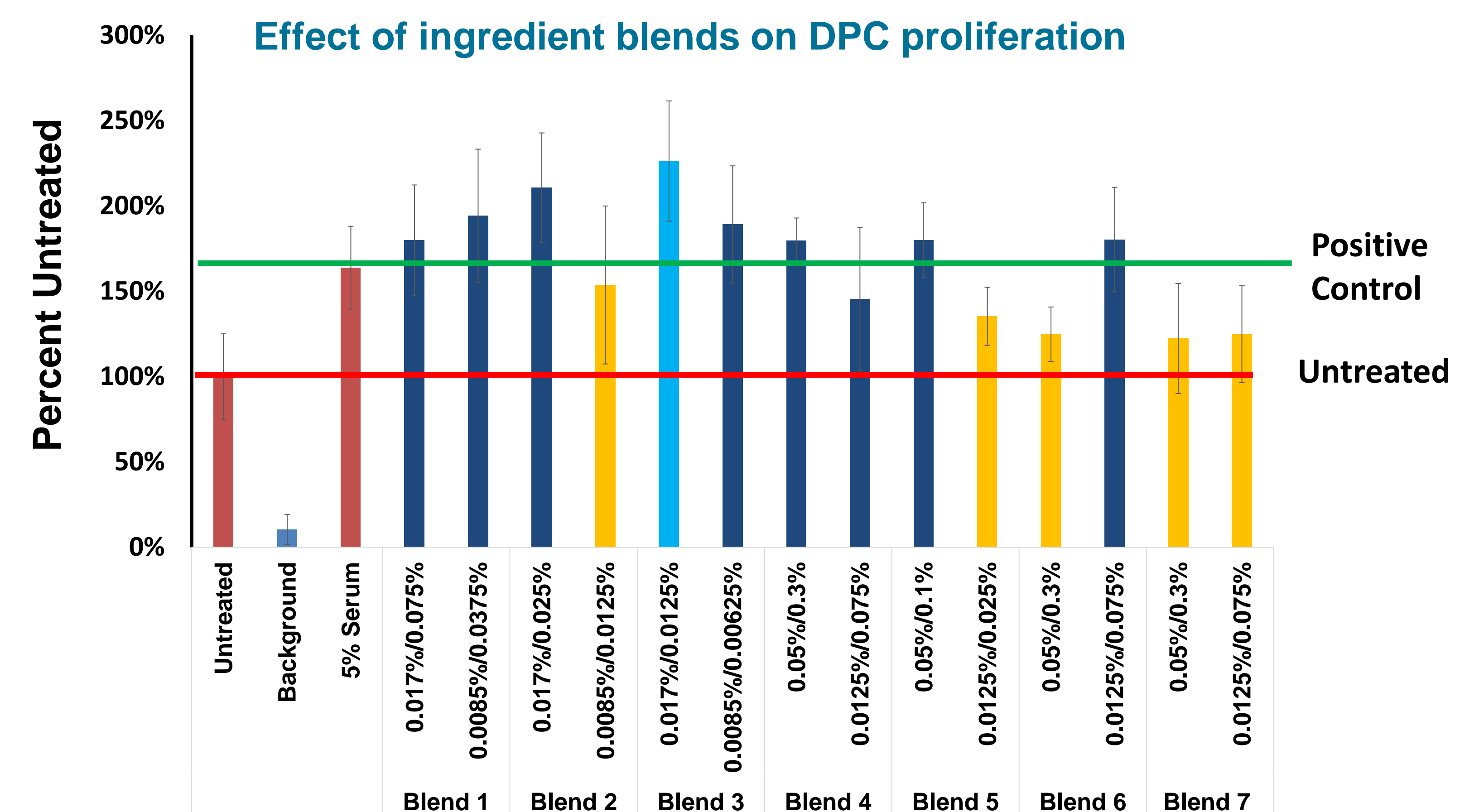
## CONCLUSIONS

- Blends containing Red Clover Flower Extract in combination with DG increased expression of genes in DPC involved in hair follicle morphogenesis and regeneration. Three of the blends (4, 5, and 7) increased COL1 A1 gene expression.
- Clinical study with twice daily application of serum containing a blend of red clover with DG (n=52) resulted in improvement in hair volume and thickness in 75-85% of subjects after 6 months. Broken hair count by Brush Friction Count Method showed reduction in broken hair in 71% of subjects after 6 months of treatment.

## REFERENCES

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## RESULTS



**Figure 1.** Comparison of blends on dermal papilla cells

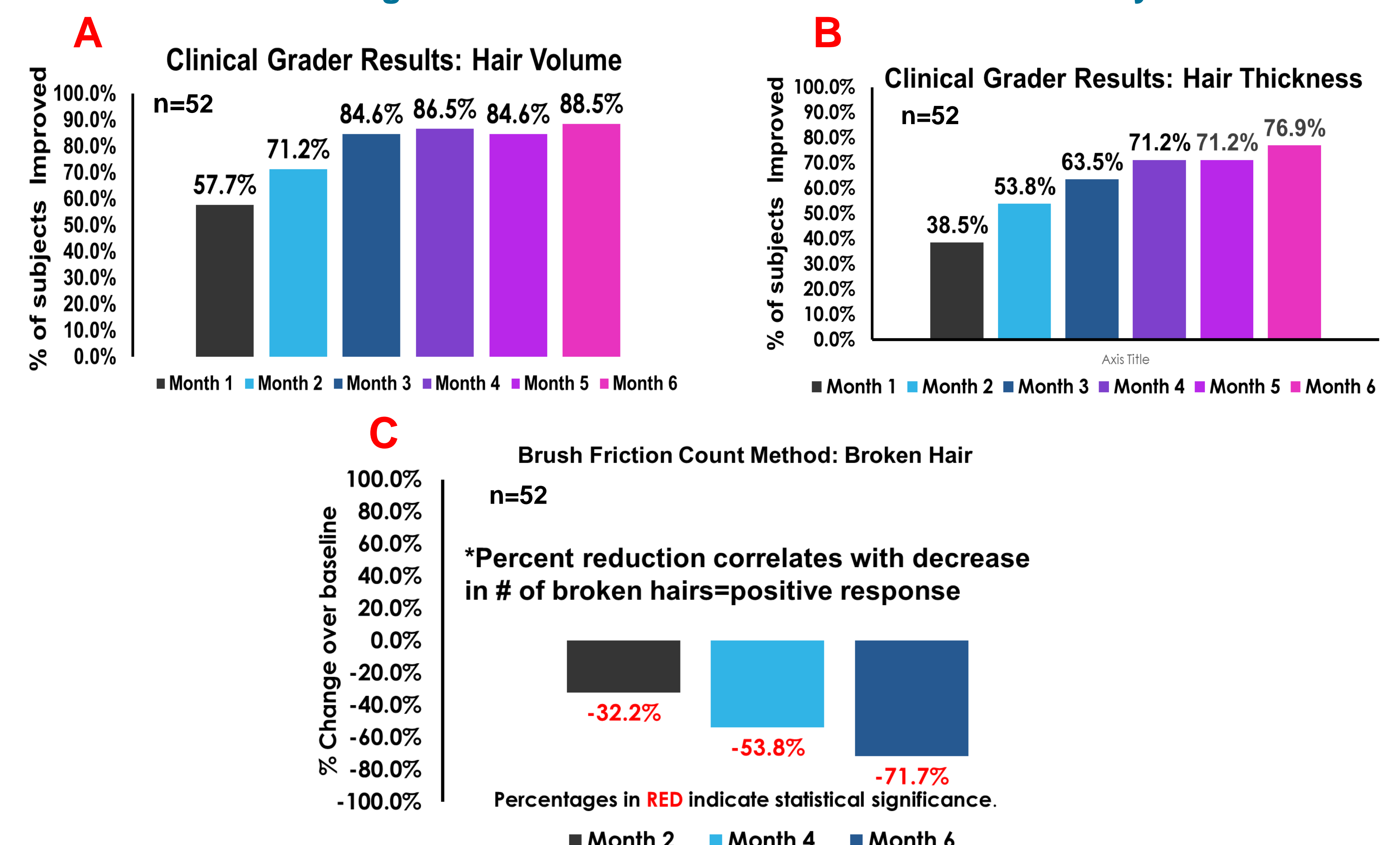
- Increased DP cell proliferation by all blends when compared with 5% fetal bovine serum (FBS).
- Blend 3 showed the highest increase in cell proliferation compared to all other blends.
- Blend 3 was selected for development of serum for performing clinical studies.

### Effect of blends on expression of genes involved in hair follicle morphogenesis (Statistical significance: fold change values $\geq 1.5$ )

Gene	Blend 1	Blend 2	Blend 3	Blend 4	Blend 5	Blend 6	Blend 7
NOG		1.67	1.90	2.34	1.95		
SOX2		1.94	1.32	1.94	1.70		
TGFB2			1.27	1.77	1.64		
COLA41						2.25	
COL1A1			1.16	1.81	1.5		1.84
CD34					1.63		1.87
FGFRL1					2.10		1.80
IL6							1.80
IL8			-2.28	-2.28	-2.63		-2.92
LGR5							1.80
NOTCH1					1.51		1.80
WNT5A			2.41	2.07	2.40		
WNT3A					-1.20	2.25	
WNT11		-1.69					1.80
WIF1		1.73	1.50		1.50		1.80
CCNE2		1.73					1.80

**Table 2.** Combination of Red Clover Extracts derivatives with DG were significantly more effective in stimulating expression of genes involved in hair follicle lineage survival, induction, migration, and regeneration (WNT5A, WIF1, SOX2, TGF beta) and differentiation of inner and outer root sheath cells (NOTCH1). Blend 5 and Blend 7 also expressed CD34, a marker for hair follicle stem cells and for regeneration. IL-8 (pro-inflammatory cytokine) was downregulated in blends containing DG. Blend 1 did not effect any significant change in any of the genes evaluated.

### Clinical Study: Improvement in hair regeneration upon application of serum containing blend of red clover and DG in a 6-month study



**Figure 2.** Treatment with serum containing blend of Red Clover Extract with DG over the course of 6 months twice daily treatment improved hair volume (A), hair thickness (B), and reduction in broken hair (C).