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Efficacy of a newly developed hydroxylated grapeseed oil on hair growth stimulation

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1. Introduction

Hair loss, or alopecia, is a prevalent and multifactorial condition that affects both men and women across all age groups. Its etiology is complex and often involves a combination of genetic predisposition, hormonal imbalances, psychological stress, nutritional deficiencies, and environmental exposures [1, 2]. Beyond its physiological consequences, alopecia can deeply affect psychological well-being and social interactions, leading to decreased self-esteem and a diminished quality of life [3].

Androgenetic alopecia, the most common form of hair loss, is characterized by progressive follicular miniaturization and a shortened anagen phase [4]. It is linked to genetic and hormonal factors, particularly dihydrotestosterone (DHT). Pharmacological treatments such as Minoxidil and Finasteride are frequently prescribed, but their efficacy varies, and they require long-term use, which may be associated with side effects or poor compliance [5].

To address the growing demand for effective hair loss treatments, various natural strategies have been explored. Among them, castor oil has been traditionally used for its potential benefits on hair growth. Castor oil is particularly rich in ricinoleic acid, a hydroxylated fatty acid that has been described to possess soothing properties [6]. However, despite its wide use in cosmetics, scientific evidence supporting castor oil's effectiveness in promoting hair growth remains limited.

Therefore, the demand for well-tolerated, and naturally derived solutions that can support hair growth remains a key area of research in cosmetics.

In this study, we developed a hydroxylated grapeseed oil enriched in 13-hydroxy-9-octadecenoic acid, a hydroxylated fatty acid, and demonstrated its effects on promoting hair growth on various biological models.

2. Materials and Methods

2.1. Obtention of hydroxylated grapeseed oil

Hydroxylated grapeseed oil is enriched in 13-hydroxy-9-octadecenoic acid, a hydroxylated free fatty acid. It was obtained from grapeseed oil, through a two-step enzymatic method. First, grapeseed oil was hydrolyzed with a lipase to release free fatty acids. Then, specific hydroxylation of linoleic acid into 13-hydroxy-9-octadecenoic acid was performed with a 13-hydratase.

2.2. Effect on the secretion of Prostaglandin E2

Dermal papilla cells from hair follicles were cultured on a 6-well plate at 37°C, 5% CO2 and treated with either hydroxylated grapeseed oil (HGSO) or castor oil. 48 hours after treatment, the culture supernatants were collected. The amount of secreted prostaglandin E2 was measured using PGE2 ELISA Kit (Enzo Life Sciences) and normalized by cell viability.

2.3. Effect on VEGFA mRNA expression

Hair follicle dermal papilla cells were seeded at the density of 600 000 cells in a 100mm petri dish. After 24 hours, cells were treated with either HGSO or Minoxidil (Sigma Aldrich). After 24h hours of treatment, cells were washed, detached and RNA was extracted using Trizol™ (Invitrogen) and Chloroform (Sigma-Aldrich). RNA was quantified then reverse transcribed in cDNA using OneScript® RT Mix for qPCR w/gDNAOut kit (Ozyme). Quantitative PCR was performed to assess VEGFA mRNA expression using iTaq Univer SYBR Green SMX 500 kit (Bio-Rad) in a thermocycler (Bio-Rad).

2.4. Effect on the growth of hair follicles in culture

Hair follicles were dissected and seeded in 24-well plates in culture medium containing or not HGSO or the market reference ingredient (used in a commercialized product) at 0.0005%. Hair follicles were treated for 10 days with treatment renewals after 3 and 7 days of incubation. Length measurements were performed after 0, 1, 3, 7 and 10 days of culture, using optical microscopy and image analysis (Image J). 12 hair follicles were used per condition.

2.5. Effects on hair growth on skin explants

Skin explants (female donor, 77 years old) containing at least 3 hair follicles each were cultured for 5 days. Topical treatment (2mg/cm²) was performed daily with formulas containing either no active ingredient (placebo), 0.1% castor oil or 0.1% HGSO. Hair length was measured after 2 and 5 days of treatment. 3 explants were used by condition.
After 5 days of culture, mRNA extraction was performed in Trizol reagent (Invitrogen). After quantification and quality control, RNA was reverse transcribed into cDNA and quantitative PCR was performed. mRNA expression of Fibroblast Growth Factor 7 (FGF7), Proliferating Cell Nuclear Antigen (PCNA), Hypoxia-Inducible Factor-1α (HIF-1A) and β-catenin (CTNNB1) was studied.

2.6. Protective effect against heat-induced hair fiber porosity

Hair shafts (3 per condition) from Caucasian donors were exposed to 95°C heat stress for 1 hour (except for control). Then, they were treated or not with either water (stress condition), a formula containing no active ingredient (placebo) or a formula with 0.05% HGSO. Hair shafts were left to dry in open air before being dipped into a fluorescein solution, washed, and

cryopreserved in OCT. Cryo-sections were performed and image acquisition and analysis were achieved with epifluorescence microscopy (Excitation: 465-490 nm; Emission: 520-530 nm) and ImageJ software.

2.7. Effects on hair growth on volunteers

Clinical evaluation of a serum containing hydroxylated grapeseed oil (and a botanical extract with protective effects on hair follicle) was performed on 36 to 42 volunteers aged from 18 to 45 years old. Study included women presenting androgenetic alopecia level I-2 to II-1 according to the Ludwig Savin chart and men presenting androgenetic alopecia maximum level III according to the Norwood-Hamilton scale. The serum was applied everyday for 3 months without rinsing. Clinical evaluation of hair density, thickness and volume was performed by an expert. Hair fall evaluation was assessed by counting the number of hair falling from the root under standardized brushing. Tolerance was also assessed by an expert, supervised by a dermatologist after a month.

3. Results

3.1. Effect of hydroxylated grapeseed oil (HGSO) on the expression of PGE2 and VEGFA

In order to demonstrate the effect of the newly developed HGSO on hair growth, its effect on the expression of factors beneficial to hair growth was investigated.

Firstly, we assessed the impact of HGSO treatment on PGE2 secretion by hair follicle dermal papilla cells (Figure 1a). HGSO significantly stimulated PGE2 secretion at both tested concentrations (+1383% at 0.001% and +1989% at 0.002%). At the same concentrations, castor oil did not demonstrate any stimulating effect on PGE2 secretion.

We also analyzed VEGFA mRNA expression in dermal papilla cells (Figure 1b). Treatment with HGSO at 0.002% resulted in a 20% increase in VEGFA expression, while Minoxidil at 100 µM led to a 30% increase. Despite the numerical difference, statistical analysis revealed no significant difference between the two treatments, indicating comparable efficacy. Interestingly, HGSO at 0.002% contains 27 µM of the active compound, 13-hydroxy-9-octadecenoic acid, approximately three times less than Minoxidil's tested concentration.

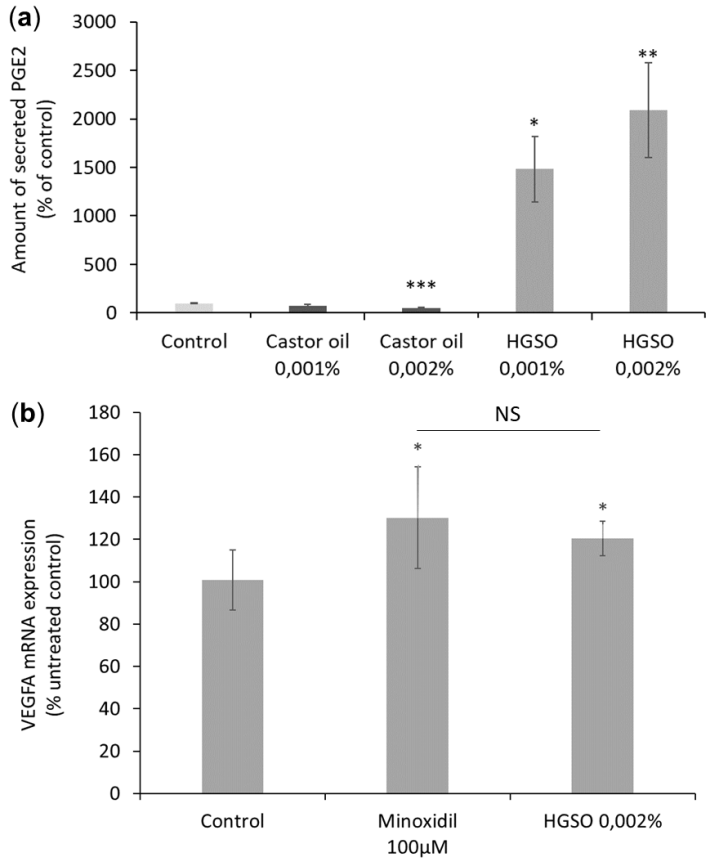

Figure 1. Effect of hydroxylated grapeseed oil on factors involved in hair growth in hair follicle dermal papilla cells. **(a)** Effect on PGE2 secretion, compared to castor oil. Statistical Student's t test: *** p<0.001 **(b)** Effect on VEGFA mRNA expression, compared to 100µM Minoxidil. Statistical Kruskal Wallis test: *p<0.05.

3.2. Effect of HGSO on the growth of hair follicles

Isolated hair follicles were used to assess the effect of HGSO on hair follicle growth, compared to a market reference ingredient.

After 10 days of culture, untreated hair follicles showed a 136% increase in length, while those treated with HGSO grew by 160% and those treated with the reference ingredient by 156% (Figure 2a). Both treatments significantly improved hair growth, with a similar efficacy of 16% compared to the growth of untreated follicles (Figure 2b). However, as shown on Figure 2a, HGSO significantly enhanced hair growth as early as day 7 (with a 14% increase in lengthening compared to untreated follicles) whereas the reference ingredient did not. This suggests that HGSO may have a faster effect on hair growth.

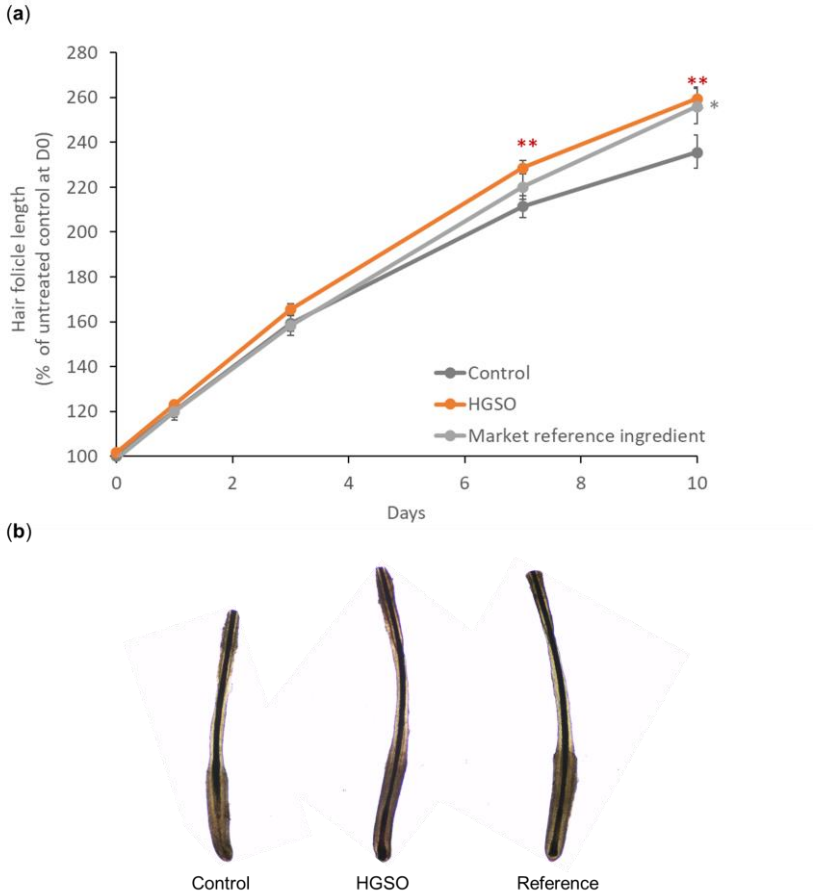

Figure 2. Effect of hydroxylated grapeseed oil on hair follicles growth over 10 days of culture **(a)** Evolution of the length over time of hair follicles treated or not with HGSO or a market reference ingredient. Statistical Student's t test: *p<0.05, **p<0.01. **(b)** Images of hair follicles treated or not with HGSO or the market reference ingredient at day 10.

3.3. Effect of HGSO on hair growth ex vivo

Hair growth stimulation was also evaluated *ex vivo*, using an adapted phototrichogram model on skin explants. The formulation containing HGSO at 0.1% significantly enhanced hair growth between day 2 and day 5 (Figure 3). However, on this model, the formulation containing 0.1% castor oil did not show any efficacy.

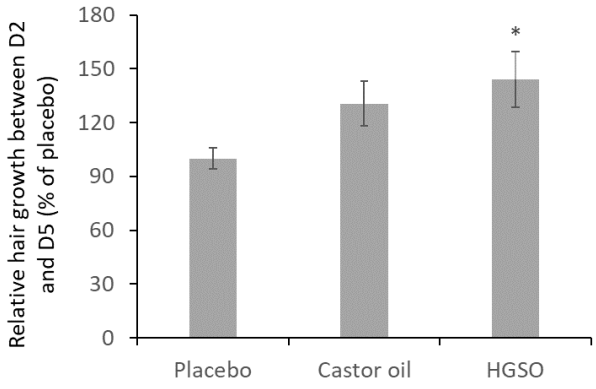

Figure 3. Effect of hydroxylated grapeseed oil on hair growth on a skin explant model treated with formulations containing 0.1% castor oil, 0.1% HGSO or no compound, between day 2 and day 5. Statistical Student's t test: *p<0.05.

On this model, HGSO also significantly stimulated mRNA expression of Fibroblast Growth Factor 7 (FGF7), Proliferating Cell Nuclear Antigen (PCNA), and Hypoxia-Inducible Factor-1α (HIF-1A) (Table 1). At a lower concentration, β-catenin mRNA expression was also significantly increased (results not shown).

Table 1. Effect of HGSO at 0.1%, after 5 days of treatment on scalp explants, on the modulation of various genes involved in the modulation of hair growth. Data are represented as relative expression. Statistical Student's t test: *p<0.05, **p<0.01.

Gene	Placebo	HGSO
FGF7	1.04	3.13**
PCNA	1.05	2.72*
HIF1A	1.01	1.47*

These results confirm the stimulating effect of HGSO on hair growth. Furthermore, the gene analysis suggests that HGSO acts through the promotion of cell proliferation, cell growth and hypoxia signaling.

3.4. Effect of HGSO on hair porosity

HGSO's repairing effect was investigated on hair fibers by evaluating their structural integrity through fluorophore diffusion (Figure 4). Heat stress significantly increased fluorophore diffusion into the fiber, therefore increasing hair porosity. Treatment with a formula containing 0.05% HGSO reduced hair porosity by 92%, with an effect significantly superior to placebo (66%). This result demonstrates that HGSO possesses a repairing effect on hair fiber damaged by thermal stress.

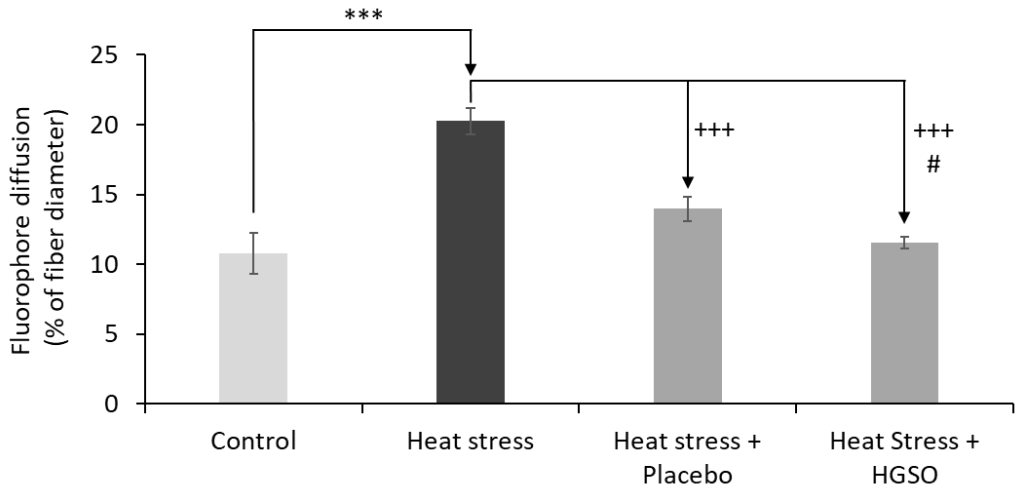

Figure 4. Effect of hydroxylated grapeseed oil on the fluorophore diffusion into hair fiber exposed to thermal stress. Dunnett's post-hoc test for multi-comparisons versus Stress or Placebo: ***p<0.001 vs control. +++ p < 0.001.vs heat stress. # p<0.01 vs Placebo.

3.5. Effect of HGSO on hair growth in vivo

Finally, the effect of a serum containing hydroxylated grapeseed oil was assessed on volunteers suffering from androgenetic alopecia of low severity. Clinical evaluations revealed that the serum significantly increased hair density, volume and thickness after 90 days of

treatment (by respectively 37%, 39% and 87%). Furthermore, a significant effect on hair fall was noticed with a reduction of 53% after 3 months of treatment.

The serum was well-tolerated with no subjects reporting any adverse events.

4. Discussion

This study demonstrates the promising potential of hydroxylated grapeseed oil (HGSO) as a bioactive compound for promoting hair growth and preserving hair fiber structural integrity. Thanks to a multi-model approach, including *in vitro*, *ex vivo*, and *in vivo* systems, we observed significant beneficial effects of HGSO on key biological markers and clinical parameters associated with hair growth.

At the molecular level, HGSO significantly stimulated PGE2 secretion and VEGFA expression in dermal papilla cells. Both factors are well-described mediators of hair follicle activity. PGE2 has been shown to prolong the anagen phase and counteract catagen-inducing prostaglandins such as PGD2 [7], while VEGFA promotes perifollicular angiogenesis, enhancing nutrient and oxygen delivery to the follicle, which is essential for the maintenance of anagen phase [8]. Notably, castor oil, despite its wide use in hair care, did not demonstrate similar responses, highlighting HGSO's higher activity in this context. Interestingly, VEGFA upregulation by HGSO was comparable to that induced by Minoxidil, a standard treatment for androgenetic alopecia described to upregulate VEGFA [9]. This effect was achieved with a threefold lower concentration of active compound (27 µM vs. 100 µM), suggesting that HGSO may exert its effects with higher potency or through complementary mechanisms.

In the hair follicle elongation assay, HGSO significantly enhanced follicle growth over 10 days. Its efficacy was comparable to a market reference ingredient, but with a faster effect. This early stimulation may be clinically interesting, as rapid onset is often associated with higher patient compliance. Ex vivo studies further confirmed HGSO's efficacy to increase hair growth while castor oil again showed no significant effect. Gene expression analysis revealed upregulation of FGF7, PCNA, and HIF-1α, involved in cell proliferation, follicular development, and hypoxia signaling, respectively. These results suggest that HGSO promotes hair growth not only by enhancing angiogenesis and prostaglandin signaling but also by stimulating cell proliferation and metabolic adaptation within the follicle.

Beyond hair growth stimulation, HGSO also demonstrated a repairing effect on hair fiber structure in a thermal stress model. This suggests that HGSO may help repair or reinforce the cuticle layer.

Finally, clinical evaluation in volunteers with mild androgenetic alopecia confirmed the relevance of our findings, supporting the interest of using HGSO in hair loss treatment.

5. Conclusion

Taken together, these results demonstrate that HGSO is a promising multifunctional ingredient for hair care applications. Its ability to stimulate key growth factors, follicular activity, and repair hair fibers suggests a broad spectrum of action that could benefit individuals experiencing hair thinning, hair loss or damage.

In conclusion, our study demonstrates the beneficial effect of hydroxylated grapeseed oil to stimulate hair growth, which constitutes a natural, effective and well-tolerated alternative for hair loss treatment.

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