"Safety and Efficacy Evaluation of a Repairing Essence in Maintaining Hair and Scalp Healthy"

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1. Introduction

Recently, the frequency of hair loss and thinning has been increasing, even among young people, due to nutritional imbalance and changes in lifestyle [1]. Alopecia is among the most prevalent dermatologic disorders. It has been estimated that 32 % of adult males and 28 % of females suffer from alopecia in Europe and the United States, whereas its rate in Asian countries is 25 % and 22 %, respectively. Furthermore, the number of people suffering from alopecia areata has been increasing [2,3]. There are many causes of hair loss, such as physical stimuli, trauma, chemotherapy, excessive dihydrotestosterone, increased sebum production, and dysregulation of endogenous growth factors [4]. This directly or indirectly results in a dysfunctional follicular growth cycle in the skin [5,6]. Minoxidil is a drug used for talopecia. It can shorten the resting phase and prolong the hair follicle growth anagen phase. However, it has been associated with withdrawal syndrome, and its discontinuation often leads to more severe alopecia and adverse symptoms such as pruritus dandruff, and contact dermatitis [7,8]. Therefore, it is important to identify a green, safe, and effective anti-alopecia product. The scalp serves as the foundation upon which hair relies for survival, therefore, maintaining a balanced ecological environment is crucial for promoting healthy hair growth. This research study aimed to develop a scalp essence containing complex peptides and multiple plant extracts, exhibiting excellent efficacy, strong targeting, and minimal side effects to improve the effectiveness of hair loss treatment.

2. Materials and Methods

2.1. Materials

Heat-inactivated fetal bovine serum for embryonic stem cells and trypsin-EDTA solution were procured from Gibco, USA; disodium hydrogen phosphate dodecahydrate and sodium dihydrogen phosphate dihydrate were obtained from China National Pharmaceutical Group Chemical Reagent Co., Ltd.; high-glucose medium (DMEM) was acquired from Beijing Solarbio Science & Technology Co., Ltd.; dimethyl sulfoxide (DMSO) was bought from Beijing Solarbio Science & Technology Co., Ltd.; 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Changzhou Bairuiji Biomedical Co., Ltd.; lipopolysaccharide (LPS) was sourced from Sigma-Aldrich (Shanghai) Trading Co., Ltd.; mouse macrophage RAW264.7 was obtained from Shanghai Cell Bank, Chinese Academy of Sciences; mouse tumor necrosis factor (TNF)-α ELISA kit, interleukin (IL)-1β ELISA kit were purchased from Nanjing Jiancheng Bioengineering Institute. The Thermo Scientific Multiskan FC microplate reader and Steri-Cycle CO_2 incubator were acquired from Thermo Fisher Scientific (China) Co., Ltd.; the TopPette pipette was bought from Dragon Lab Instruments (Beijing) Co., Ltd.; the DS-1 dermoscope was obtained from Suzhou Guoke Yingrui Medical Technology Co., Ltd.; the SB-030-Revolve electron microscope was sourced from Beijing

Deep Blue Cloud Biotechnology Co., Ltd.; the VISIA CR facial image analysis system was procured from Canfield, USA; the Dermalab Series Skin Lab Combo skin tester was acquired from Cortex, Denmark. The materials used in this study were at least cosmetic grade, and deionized water was used for all experiments.

2.2. Formulation

The test sample is a scalp essence that contains complex peptides and multiple plant extracts, formulated as follows in table 1. The complex polypeptides are composed of myristyl pentapeptide-4, palmitoyl tripeptide-1, acetyl tetrapeptide-3 and tripeptide-1 with certain proportion. And the complex plant extracts are composed of panax ginseng root extract, anemarrhena asphodeloides root extract, zingiber officinale (ginger) root extract, angelica polymorpha sinensis root extract, biota orientalis leaf extract, polygonum multiflorum root extract with certain proportion.

Table 1. The scalp essence formulation

Ingredients	Content(w/%)
Deionized water	Up to 100
Complex polypeptides	5
Complex plant extracts	5
Phenoxyethanol	0.45
Ethylhexylglycerin	0.05

2.3. Inflammatory factor inhibition test

The MTT assay was conducted to assess the effects of essence on cell viability and proliferation. And to investigate the potential anti-inflammatory properties of the scalp essence, a LPS-stimulated model was employed: Mouse macrophages were seeded at a density of 5×10^3 cells per well in 96-well culture plates and stimulated with varying concentrations of LPS solution at 37 °C and 5% CO_2 for 24 hours. The optimal conditions for LPS stimulation were determined by assessing cell viability and inflammatory factor levels to establish the inflammatory model;

Determination of the product inhibitory effect on cellular inflammatory factors: Mouse macrophages were seeded at a density of 5×10^3 cells per well in 96-well culture plates and stimulated with a specific concentration of LPS. Different concentrations of product were then added and incubated at 37 °C and 5% CO_2 for 24 hours. The supernatant was collected to measure cytokine content and calculate the inhibition rate.

$$\text{Cell survival rate(\%)}=\frac{OD_{sample}-OD_{blank}}{OD_{cell\ control}-OD_{blank}}\times100\%$$

2.4. Cellular ROS detection assay

According to the manufacturer's instructions, L929 mouse fibroblasts (1×10^4/well) were seeded into a 96-well microplate. After overnight incubation, the media were removed and cells were washed with buffer. Subsequently, cells were divided into four groups. First, the protection group was exposed to 200 µM H_2O_2 for 2 hours, after which the culture medium was discarded. The wells were subsequently washed three times with phosphate-buffered saline (PBS), followed by the addition of training samples to each well for a 24-hour incubation period. Following this, the repair group was initially cultured under standard conditions. After 24 hours, the culture medium was removed, the wells were washed three times with PBS, and the cells were exposed to 200 µM H_2O_2 stimulation. Cell viability was assessed using the MTT assay, with absorbance measurements recorded at 490 nm using a microplate reader.

2.5 Clinic trial

2.5.1 Test requirments

Prior to formal testing, all participants underwent a standardized training session to familiarize themselves with the process. The study included 20 subjects experiencing scalp sensitivity and hair loss, including 5 males and 15 females with an average age of 33.12 ± 12.11 years. Throughout the test, participants were instructed to apply a suitable amount of serum to their scalps daily as required. The testing phase lasted 28 days, followed by a 7-day washout period. The shampoo used by participants during both the washout and test phases was supplied by the company's research and development laboratory and contained only basic surfactants, without any active ingredients. Participants were required to wash their hair at least twice, and up to three times, weekly. During the test period, they were not allowed to use any other scalp nourishing products or engage in activities that could influence the test results, such as swimming, using a sauna, sun exposure, or any other activities that might irritate or cause discomfort to the scalp. The scalp serum was to be applied at a rate of 5 ml per application, twice daily, for 28 days, with a gentle massage until fully absorbed. The Dermalab skin detector was utilized to measure the scalp cuticle's water content, sebum content and trans epidermal water loss (TEWL) before treatment (T_0), at the 14-day midpoint (T_1), and at the 28-day endpoint (T_2), while the dermatoscope was used to observe and photograph the scalp's condition. The ambient temperature was maintained at (25±1) ℃, and the relative humidity was kept at (50±10) %. Informed consent was obtained from all subjects prior to testing.

2.5.2 Evaluation of subject satisfaction and efficacy

Following the test, subjects self-assessed their dandruff, scalp tightness, pruritus, hair loss, and redness. The subject self-assessment data were recorded and analyzed by staff. The satisfaction survey employs a 5-point scale, where 5 points indicate very satisfied, 4 points indicate satisfied, 3 points indicate general satisfied, 2 points indicate dissatisfied, and 1 point indicates very dissatisfied. The effectiveness (%) is calculated as (very satisfied + satisfied) cases divided by the total cases, multiplied by 100%. The scalp condition was documented using a dermoscope to capture data from the subject's scalp in the vertical plane.

2.5.3 Safety assessment

The subjects applied the tested product as indicated on the product label. Skin reactions were monitored by observing the subject directly and through weekly follow-up calls. The grading of skin reactions was conducted in accordance with the Technical Code for Cosmetic Safety (2015 edition), where grade 0 signifies no skin reaction, grade 1 indicates weak erythema, grade 2 represents erythema with infiltration and papules, grade 3 corresponds to erythema, edema, papules, and blisters, and grade 4 signifies erythema, edema, and the presence of blisters. subjects were tracked for any discomfort symptoms experienced during product use, and any adverse reactions were documented.

2.6. Statistical analysis

The SPSS 26.0 software was utilized for statistical analysis. Measurement data with a normal distribution were expressed as mean ± standard deviation, and a paired T-test was employed to compare the data before and after treatment. Count data were represented as case percentages (%). A bilateral test was conducted at a significance level of $\alpha=0.05$.

3. Results

3.1 MTT assay results

An MTT assay was utilized to determine the cytotoxic effects of the essence on mouse macrophages cells and mouse fibroblasts. Cells were exposed to different concentrations (0.05%–5%) of the essence for 24h. The results revealed that the cell viability of mouse macrophages cells remained almost 80%, showing an increasing trend when treated with concentrations below 1% compared to the control group. A sharp reduction in cell viability was observed with 5% stimulation (Figure 1a). While, as shown in Figure 1b, essence promoted cell viability in mouse fibroblasts in a dose-dependent manner. Cell viability

remained above 100% even at 1% concentration. This demonstrates that essence has limited cytotoxic effects in mouse fibroblasts. The results indicate that the sample at concentrations of 0.5% and below can be used for subsequent cell efficacy test experiments.
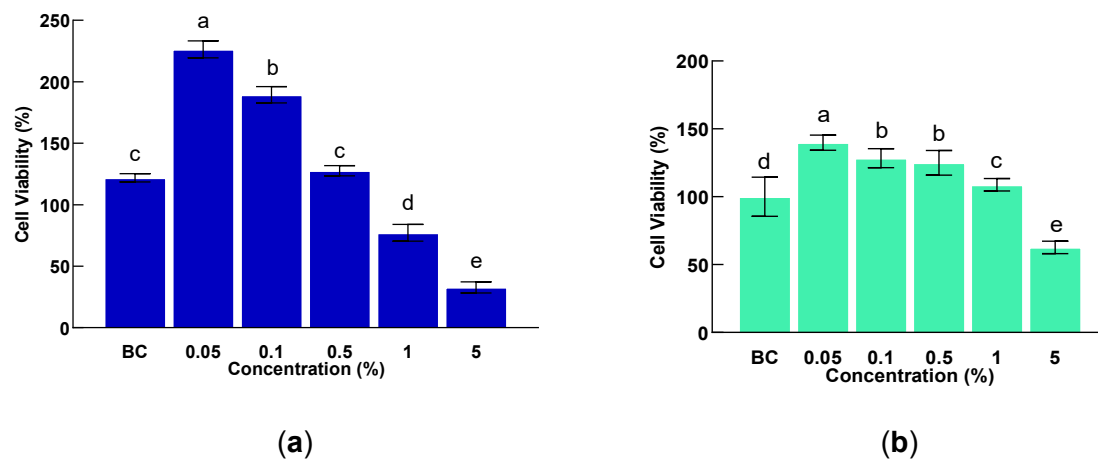

(a)   (b)

Figure 1. MTT assay results. (a) Viability of mouse macrophages cells (b) Viability of mouse fibroblasts. Different letters indicate significant differences between groups ($P < 0.05$)

3.2 H_2O_2 Induced cell death in cultured L929 mouse fibroblasts

H_2O_2 induces hair loss through its cytotoxic effects and the ability to induce oxidative stress [9]. In this study, H_2O_2 was used to induce oxidative stress in L929 mouse fibroblasts.The negative control consisted of mouse fibroblasts maintained in standard culture medium, demonstrating 100% cell viability (set as baseline), which confirmed the excellent baseline viability of this cell batch. As shown in Figure 2, In the model group exposed to 200 µM H_2O_2, cell viability decreased to 48.72%. Statistical analysis revealed an extremely significant difference compared to the negative control ($P < 0.01$), validating the success of the oxidative stress induction protocol. The experimental anti-dehydration formulation was tested at five concentrations: 5%, 1%, 0.5%, 0.1%, and 0.05%. Notably, the 5% and 1% concentration groups exhibited markedly reduced cell viability (attributable to concentration-dependent cytotoxicity), whereas the 0.5%, 0.1%, and 0.05% groups demonstrated significant cytoprotective effects. Compared to the model group, these three lower concentrations showed highly statistically significant improvements in cell survival ($P < 0.01$), indicating potent protection against H_2O_2-induced oxidative damage.
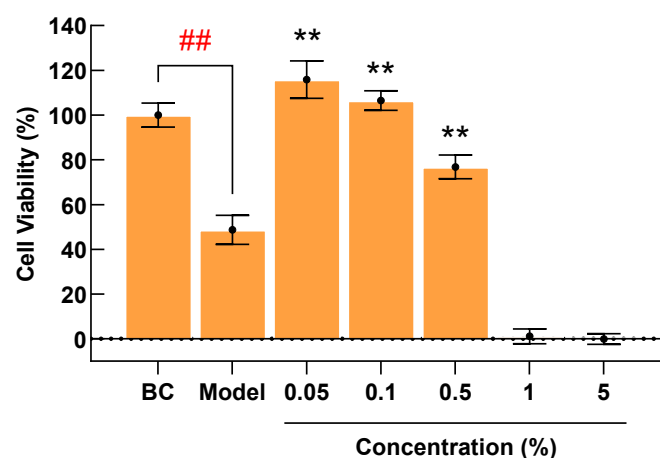

Figure 2. H_2O_2 induces cell apoptosis. Viability of L929 mouse fibroblasts after exposure to 200 µM H_2O_2 with different concentrations of essence for 24 h. ** $P < 0.01$: Compare with model, ## $P < 0.001$: Compare with blank control.

3.3 Essence shows a signaficant anti-inflammatory effect in mouse macrophages cells

It is widely accepted that ROS promotes inflammatory responses. Besides, pro-inflammatory cytokines, such as IL-1β and TNF-α, are enhanced in the scalps of individuals with AGA, exacerbating follicular miniaturization. As shown in Figure 3,the essence significantly reduced these cytokines content with different concentrations. These findings suggest that essence exerts notable anti-inflammatory effects.
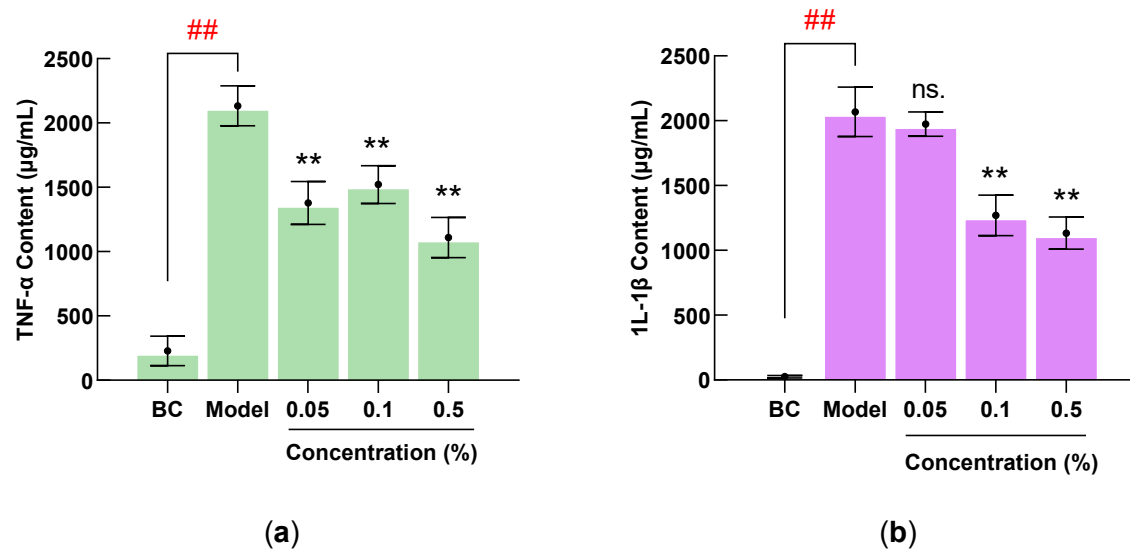

(a)   (b)

Figure 3. Mouse macrophages cells were exposed to 5 µg/m LPS with different concentrations of the essence. (a) TNF-α content (b) IL-1β content. Data are presented as mean ± SD. ** $P < 0.01$: compare with model. ## $P < 0.001$: compare with blank control.

3.4 Efficacy evaluation of water content, TEWL value and sebum content

Following treatment, the stratum corneum (SC) of the scalp exhibited significant improvements in hydration parameters. Specifically, we observed a marked increase in water content ($P < 0.001$), accompanied by a substantial reduction in transepidermal water loss (TEWL) values ($P < 0.001$) and decreased sebum secretion levels ($P < 0.001$) when compared to baseline measurements. These statistically significant differences across all measured parameters are comprehensively detailed in Table 2.

Table 2. Clinical outcomes at baseline, 14 days, and 28 days post-treatment.($\bar{x} \pm s$, n=20)

Group	Water content （C.U.）	TEWL value （g/(h·m²)）	Sebum content （µg/cm²）
Baseline	42.89±1.89	19.03±0.96	112.28±5.62
14 days post-treatment	48.77±2.43	15.34±0.78	97.12±4.86
28 days post-treatment	53.85±2.69	15.15±0.74	86.75±4.29
t value[a]	5.360	8.510	7.340
P value[a]	<0.001	<0.001	<0.001
t value[b]	4.910	7.730	9.030
P value[b]	<0.001	<0.001	<0.001

*[a] $P < 0.001$: 14 days post-treatment compare with baseline; [b] $P < 0.001$: 28 days post-treatment compare with baseline

3.5 Case clinical profile

3.5.1 Essence's impact on optimizing the scalp microenvironment

Typical subject 1(Figure 4a): A 48-year-old male presented with severe scalp dysfunction, characterized by compromised epidermal barrier integrity. Dermoscopic evaluation at baseline revealed prominent desquamation and perifollicular telangiectasia, accompanied by subjective reports of persistent scalp tightness, dysesthesia, and diffuse alopecia. At 14 days post-treatment, quantitative analysis demonstrated an obvious reduction in the desquamation index and erythema severity compared to baseline. By 28 days post-treatment, complete restoration of stratum corneum integrity was confirmed via transepidermal water loss (TEWL) measurements. subject-reported outcomes showed resolution of sensory symptoms, with trichoscopy confirming follicular repatterning and capillary normalization.

Typical subject 2(Figure 4b): A 34-year-old female presented with seborrheic scalp dysfunction. Baseline dermoscopy identified diffuse erythema and follicular hyperkeratosis ("yellow dot" sign), consistent with compromised barrier function. 14 days after treatment, scalp sebum production was improved, hair follicle opening was reduced, sebum secretion was reduced, scalp redness was alleviated, and barrier function was improved. After 28 days of use, the yellow spots of the hair follicle basically disappeared, the mouth of the hair follicle closed, the scalp redness basically disappeared, and the scalp barrier function returned to health.
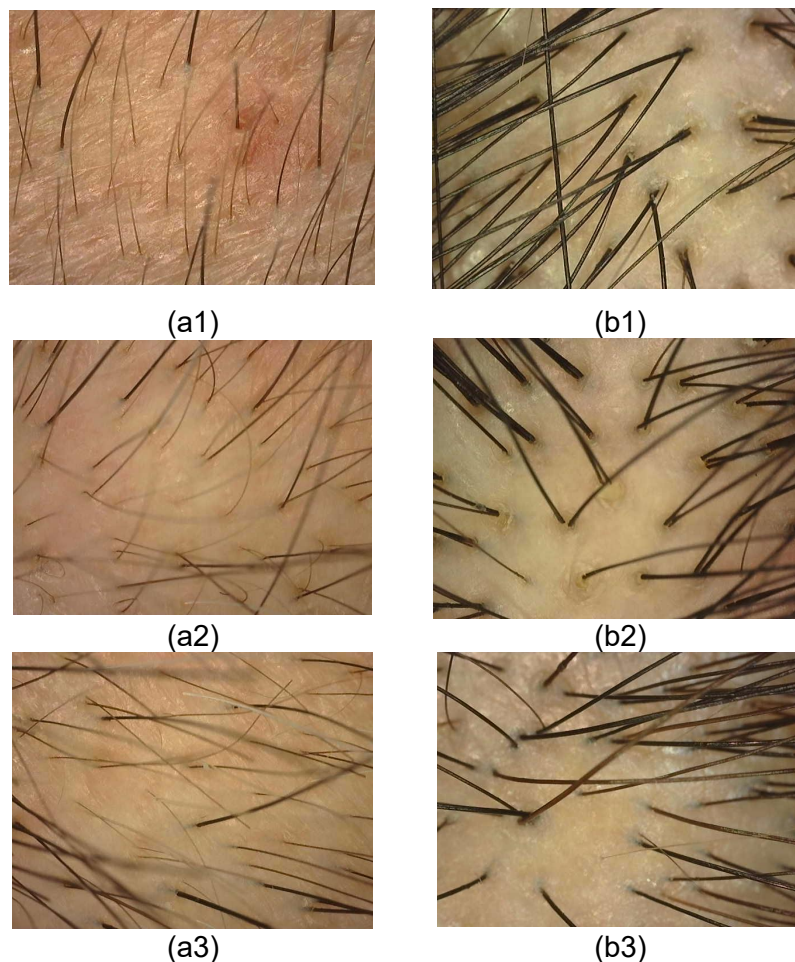

(a1) (b1)

(a2) (b2)

(a3) (b3)

Figure 4. Clinical and dermatoscopic progression of scalp lesions during treatment: (a) Typical subject a.(a1: Pronounced erythema on the scalp at the pre-treatment stage; a2: Partial resolution of erythema observed at 14 days post-treatment; a3: Complete remission of erythema achieved by 28 days post-treatment; (b) Typical subject b.(b1: Pre-treatment clinical examination revealed pronounced scalp erythema with active inflammation and excessive sebum production; b2: At 14 days post-treatment, reduced sebum secretion and partial follicular ostia contraction were observed; b3: By 28 days post-treatment, near-complete closure of follicular ostia and resolution of erythema were achieved

3.5.2 Essence's impact on optimizing hair loss condition

The subject was a 39-year-old female presenting with sparse hair in the frontal scalp area and self-reported significant hair loss (Figure 5). During the one-week follow-up, the participant reported reduced hair shedding. Comparative VISIA imaging analysis revealed notable improvements: pre-treatment image showed clearly visible scalp areas, whereas post-treatment evaluation at 28 days demonstrated a great increase in hair coverage density with a corresponding obvious reduction in exposed scalp surface area, indicating clinically observable hair volume restoration.
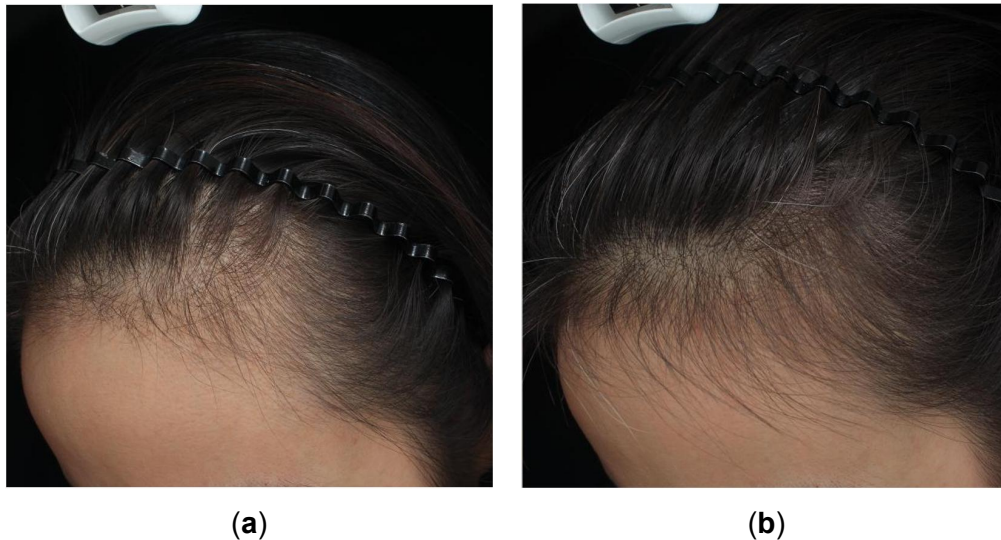

(**a**)   (**b**)

Figure 5. VISIA imaging analysis (a) pre-treatment (b) 28 days post-treatment.

3.6 Satisfaction and effectiveness of scalp essence for subjects with sensitive scalp

Following 28-day application of the therapeutic essence, the clinical efficacy rates were as follows: 80.0% (16/20) for scalp erythema, 80.0% (16/20) for dandruff, 85.0% (17/20) for pruritus, 90.0% (18/20) for hair loss, and 80.0% (16/20) for scalp tightness. All outcome measures are systematically presented in Table 3. The treatment demonstrated significant clinical efficacy across multiple scalp parameters.

Table 3. *Satisfaction and effectiveness of scalp essence for subjects with sensitive scalp for 28d [cases (%), n=20]*

Classification	Satisfaction					Effectiveness
	Very satisfied	Satisfied	General satisfied	Dissatisfied	Very dissatisfied	
Scalp redness	11（55.0）	5（25.0）	2（10.0）	2（10.0）	0（0）	16（80.0）
Dandruff	8（40.0）	8（40.0）	2（10.0）	1（5.0）	1（5.0）	16（80.0）
Pruritus	9（45.0）	8（40.0）	3（15.0）	1（5.0）	0（0）	17（85.0）
Hair loss	9（45.0）	9（45.0）	1（5.0）	1（5.0）	0（0）	18（90.0）
Scalp tightness	10（50.0）	6（30.0）	2（10.0）	2（10.0）	0（0）	16（80.0）

*Effectiveness(%)=(very satisfied + satisfied) cases/total cases×100%

4. Discussion

Hair is an accessory of the body and has always been a symbol of social, cultural and political climate throughout history. On the other hand, apart from aging and death, hair loss is also associated with loss of attractiveness, personality, stigma and disease [10]. The hair growth cycle includes the anagen phase (anagen phase), the catagen phase (catagen phase), and the telogen phase (catagen phase). Hair loss is a problem of reduced hair growth caused by an imbalance in the hair growth cycle. The underlying process is complex and not yet fully understood. The most important mechanisms are androgen signaling, lack of cell

communication, and insufficient activity of stem cells within hair follicles [11]. Alopecia causes a significant decrease in self-esteem and a negative impact on quality of life [12]. Although topical and oral medications are widely used to treat hair loss, limited research has explored the importance of scalp health in preventing hair loss.

This study emphasizes the anti-inflammatory effect of the essence, indicating that it can significantly reduce the pro-inflammatory cytokines IL-1β and IL-6, further enhancing its therapeutic value. More and more people believe that inflammation is a key factor in hair follicle damage in hair loss, which exacerbates hair loss by disrupting the circulation of hair follicles and promoting fibrosis [13]. By reducing inflammatory responses, the essence protects hair follicles from damage and creates an environment conducive to hair regrowth. This anti-inflammatory effect is consistent with the emerging treatment strategies for hair loss inflammation to improve the therapeutic effect.

Oxidative stress is caused by excessive concentrations of reactive oxygen species (ROS) or free radicals, which can cause oxidative damage to HFs, disrupt the hair cycle, and lead to pathological hair loss. These findings indicate that our product is a new and effective approach to treating oxidative stress-induced cellular dysfunction in scalp cells [9].

The clinical study also confirmed significant improvements in scalp redness, itching, tingling, tightness and dandruff. After 28 days of scalp essence treatment, reductions in TEWL and sebum production suggested enhanced scalp barrier function, which is typically compromised in hair loss due to sebaceous gland hypertrophy and excessive sebum secretion. By restoring scalp hydration and sebum balance, scalp essence may create a more favorable environment for hair growth, mitigating the negative effects of excess sebum, which can lead to scalp inflammation and follicular occlusion. The essence includes diverse types of bioactive peptides,which can address a variety of scalp issues. By utilizing their functions to improve the scalp environment, promote hair growth, polypeptides can interact with the hair and scalp through multiple pathways, enhancing the overall quality and health of the scalp. They possess a range of potential benefits and broad development prospects in scalp anti-aging applications.

However, due to the limited population diversity and small sample size in clinical studies, it is recommended to conduct further research to verify its effectiveness and expand its application in different populations.

5. Conclusion

In conclusion, the scalp essence demonstrates potential as a multifaceted treatment for hair loss by modulating key signaling pathways, exhibiting anti-inflammatory effects, and enhancing scalp and hair health. Further studies are needed to validate its long-term efficacy and safety in larger, diverse populations and to elucidate its molecular mechanisms. These findings support the essence as a potential therapeutic option for scalp healthy and hair loss.

6. References

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