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In Vitro Evaluation of *Suregro* for Proliferation Assay on Human Keratinocytes (HaCaT) Cell Line: A Study

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Abstract: ***This study evaluated the efficacy of Suregro in inducing proliferation in Human Keratinocytes (HaCaT) cell line through the MTT assay. The primary objective was to determine cell proliferation in response to varying concentrations of Suregro, ranging from 7.8 µg/mL to 1000 µg/mL. Results showed that the highest percentage proliferation was 141.54% at the 1000 µg/mL concentration. These findings suggest that Suregro promotes cell growth in keratinocytes, making it a promising candidate for therapies that require cellular regeneration, especially in skin - related treatments.***

Keywords: Cell proliferation, HaCaT cells, *Suregro*, keratinocytes, MTT assay, cell culture.

1. Introduction

Keratinocytes, the predominant cell type in the epidermis, play a critical role in maintaining the structural integrity and functionality of the skin. These cells form a protective barrier against environmental factors, including pathogens, chemical irritants, and UV radiation, while also participating in processes such as immune response, wound healing, and skin regeneration.1Proper keratinocyte proliferation is essential not only for the maintenance of skin homeostasis but also for the repair of damaged skin, making it a crucial element in recovery from injuries, wounds, and various dermatological conditions.2

The ability of keratinocytes to proliferate and differentiate is especially important in the context of wound healing, where rapid regeneration of skin cells is necessary to restore the protective barrier.3 In dermatological treatments aimed at skin rejuvenation, anti - aging, and healing, enhancing the proliferation of keratinocytes is often a therapeutic goal.4 As the demand for natural and plant - based products in skin care increases, there has been growing interest in the exploration of botanical formulations for their ability to promote keratinocyte growth and skin repair.5

Suregro, a proprietary formulation composed of natural ingredients, has been developed with the aim of improving skin health. Given the importance of keratinocyte proliferation in skin rejuvenation, this study was designed to investigate the potential of Suregro to promote the growth of keratinocytes, thereby supporting skin recovery and regeneration. The HaCaT cell line, an immortalized human keratinocyte model, was used to assess the effects of Suregro on keratinocyte proliferation. The HaCaT cell line is a well - established in vitro model for studying skin biology, keratinocyte behavior, and responses to various compounds due to its ability to mimic normal human skin cells while retaining the capacity for continuous growth.6

To quantitatively measure keratinocyte proliferation in response to Suregro, the MTT assay was employed.7 The MTT assay is a standard colorimetric method used to assess cell viability and proliferation by measuring the metabolic activity of cells. This assay is based on the reduction of MTT (a yellow tetrazole) to formazan (a purple dye) by mitochondrial enzymes in metabolically active cells, providing a reliable indication of cell growth and proliferation.8

The objective of this study was to evaluate the ability of Suregro to stimulate keratinocyte proliferation at different concentrations, thus determining its potential for use in skin rejuvenation and repair treatments. If proven effective, Suregro could offer a natural, plant - based alternative to existing dermatological products aimed at enhancing skin regeneration and improving overall skin health.

2. Materials and Methods

2.1 Study Design

This study was conducted at Radiant Research Services Pvt. Ltd., Bengaluru, and was designed to evaluate the in vitro effects of *Suregro* on the proliferation of human keratinocytes. The MTT assay was used to measure cellular metabolic activity, which is directly related to cell proliferation.

Test Product
The test product, *Suregro*, was provided by Essbrains Pharma Pvt Ltd., Ernakulam, Kerala. It was supplied in liquid form and stored at room temperature (RT).

- **Product Name:** *Suregro*
- **Batch Number:** SG1230
- **Storage Condition:** Room temperature (RT)

The ingredients of Suregro oil are Eclipta alba, Azadirachta indica, Ixora coccinea, Aloe vera, Phyllanthus embilica extracts infused into Coconut oil. Eclipta alba, or Bhringraj, is widely recognized in Ayurveda for its potent hair growth - promoting properties. Studies suggest that it stimulates hair follicles, prevents hair loss, and may promote the regrowth of new hair. Roy, R. K., Thakur, M., & Dixit, V. K. (2008). demonstrated that the ethanolic extract of Eclipta alba significantly increased hair follicle count and hair growth rate in albino rats, comparable to the standard treatment with minoxidil.[9] Azadirachta indica (Neem) is known for its antifungal and antibacterial properties, making it effective in treating scalp infections, which can inhibit hair growth. By keeping the scalp healthy and free from infections, Neem indirectly supports stronger, thicker hair growth. Pandey, R., & Mishra, A. (2010) found that Neem's antifungal properties help treat fungal infections like dandruff, which indirectly promotes hair growth by maintaining a healthy scalp.[10] Ixora coccinea has been traditionally used to treat skin and scalp conditions, but it also has potential hair benefits due to its antioxidant and anti - inflammatory properties. These effects help to improve scalp health, creating an optimal environment for hair growth. Though specific hair growth studies are scarce, Shirwaikar, A., & Rajendran, K. (2007) highlighted the strong antioxidant potential of Ixora coccinea, which is crucial in reducing oxidative stress on the scalp and promoting a healthy environment for hair growth.[11] Aloe vera is a well - known remedy for soothing scalp irritation and moisturizing hair, contributing to a healthier scalp environment that promotes hair growth. Aloe vera's proteolytic enzymes repair dead skin cells and enhance scalp health, creating a nourishing environment for hair follicles to thrive, thus promoting hair growth.[12] Phyllanthus emblica (Amla) is rich in vitamin C, which helps boost collagen production and promotes hair growth. Its antioxidant properties also prevent damage to hair follicles and reduce oxidative stress on the scalp, leading to healthier hair. Kapoor, V. P., & Verma, A. showed that the high concentration of vitamin C and antioxidants in amla helps boost collagen production, leading to increased hair strength and reduced hair loss, contributing to hair growth.[13] Coconut oil is packed with fatty acids, primarily lauric acid, which deeply penetrates the hair shaft, reducing protein loss and protecting the hair from damage. It also has antifungal properties, which help treat dandruff and scalp infections, further promoting healthy hair growth. Rele, A. S., & Mohile, R. B. concluded that coconut oil effectively penetrates the hair shaft and reduces protein loss, significantly contributing to the prevention of hair damage and enhancing hair growth.[14]

Cell Line and Culture Medium
Human keratinocyte (HaCaT) cells were obtained from AddexBio, USA, and cultured in Dulbecco's Modified Eagle Medium - High Glucose (DMEM - HG), supplemented with 10% inactivated foetal bovine serum (FBS), penicillin (100 IU/mL), streptomycin (100 µg/mL), and amphotericin B (5 µg/mL) in a humidified atmosphere of 5% CO_2 at 37°C. The cells were subcultured as needed and used when they reached 70 - 80% confluence.

MTT Assay
The MTT assay was employed to assess cell viability and proliferation. HaCaT cells were seeded at a density of 100, 000 cells/mL in 96 - well plates. After a 24 - hour incubation, the medium was replaced with different concentrations of *Suregro* (7.8 µg/mL to 1000 µg/mL), and the cells were incubated for an additional 24 hours. Untreated cells were used as controls. Following incubation, 100 µL of MTT solution was added to each well, and the plate was incubated for 3 hours at 37°C. The resulting formazan crystals were dissolved in dimethyl sulfoxide (DMSO), and absorbance was measured at 570 nm using a microplate reader.

Statistical Analysis
The percentage proliferation was calculated by comparing the absorbance values of treated wells to those of control wells. Data were presented as mean ± standard deviation (SD) for triplicate experiments. The results were analyzed using one - way analysis of variance (ANOVA), with significance set at $p<0.05$.

3. Results

The proliferation assay demonstrated that *Suregro* promoted cell proliferation in a concentration - dependent manner. The highest cell proliferation (141.54% ± 3.59) was observed at a concentration of 1000 µg/mL, indicating a strong proliferative effect. The results for all concentrations tested are summarized in Table 1.

Table 1: Proliferation of Human Keratinocytes (HaCaT) after Treatment with *Suregro*

Concentration (µg/mL)	Percentage Proliferation (%)
1000	141.54 ± 3.59
500	139.23 ± 5.13
250	138.46 ± 0.51
125	135.51 ± 1.92
62.5	128.33 ± 1.92
31.25	122.95 ± 4.23
15.625	119.62 ± 0.38
7.8	112.95 ± 2.69

Suregro showed a significant increase in keratinocyte proliferation compared to the untreated control, particularly at higher concentrations, which may be beneficial for skin regeneration and healing.

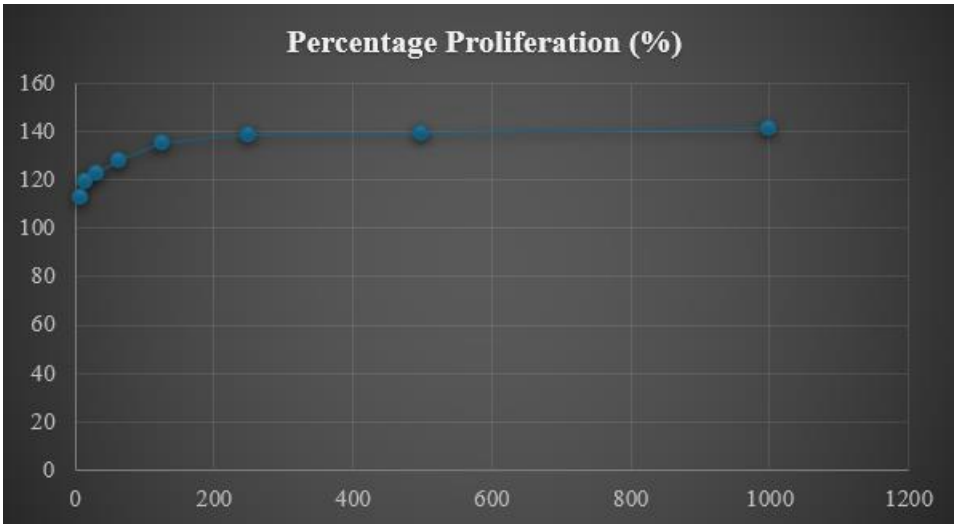

Figure 1

4. Discussion

The study demonstrates a significant increase in keratinocyte (HaCaT) proliferation when treated with Suregro, a plant - based formulation composed of Eclipta alba, Azadirachta indica, Ixora coccinea, Aloe vera, Phyllanthus emblica, and coconut oil. The results show a clear concentration - dependent effect, with proliferation rates increasing as the concentration of Suregro rises. At the highest concentration of 1000 µg/mL, keratinocyte proliferation reached 141.54%, suggesting that the formulation is highly effective in promoting cell growth. This finding has several important implications for skin regeneration and wound healing.

1) Concentration - Dependent Proliferation

The data in Table 1 reveals a steady increase in proliferation with increasing concentrations of Suregro. The lowest concentration of 7.8 µg/mL yielded a 112.95% proliferation rate, which is already higher than the untreated control. At the highest concentration tested (1000 µg/mL), the proliferation rate was 141.54%. This demonstrates that the effect of Suregro is dose - dependent, which is critical information for therapeutic applications. Higher doses may be more effective in accelerating skin regeneration, making it useful in clinical scenarios where rapid cell turnover is required, such as wound healing and post - surgical recovery.

The increase in proliferation at even the lowest concentration (112.95% at 7.8 µg/mL) suggests that Suregro has potent bioactive effects even at minimal doses. This is promising for the development of topical formulations that may require lower concentrations to avoid irritation while still delivering therapeutic benefits.

2) Potential Mechanisms of Action

The plant ingredients in Suregro are individually recognized for their therapeutic properties, particularly in promoting skin health and regeneration. Each component may contribute synergistically to the observed increase in keratinocyte proliferation.

Eclipta alba (Bhringraj) is known for its regenerative properties. Studies have shown that Eclipta alba stimulates hair follicle cells and promotes the growth of new cells in both hair and skin tissues. The inclusion of this herb in Suregro likely plays a role in enhancing cellular turnover in keratinocytes, leading to the observed increase in proliferation.

Azadirachta indica (Neem) is widely used for its antibacterial and antifungal properties. The skin's natural regenerative processes can be hampered by microbial infections and inflammatory responses. Neem's antimicrobial activity helps maintain a healthy environment for keratinocyte proliferation by preventing infections that could otherwise disrupt healing. Neem's ability to reduce inflammation further supports cell growth and regeneration, ensuring that keratinocytes can proliferate without impediment.

Ixora coccinea provides antioxidant protection, which is critical in reducing oxidative stress, a major factor in cellular aging and skin damage. By mitigating the harmful effects of free radicals, Ixora coccinea may help keratinocytes proliferate more efficiently, as oxidative stress often leads to premature cell death or reduced cell viability.

Aloe vera is a well - known anti - inflammatory and moisturizing agent. It contains proteolytic enzymes that repair dead skin cells and create a favorable environment for keratinocyte proliferation. Additionally, Aloe vera helps to maintain hydration, which is crucial for optimal cell function and division.

Phyllanthus emblica (Amla) is rich in vitamin C, a powerful antioxidant that also supports collagen production, an essential factor for skin integrity and repair. Enhanced collagen synthesis can promote wound healing and skin elasticity, indirectly supporting keratinocyte proliferation by providing a structurally sound extracellular matrix.

Coconut oil deeply penetrates the skin and has been shown to reduce protein loss, which helps maintain the structural integrity of skin cells. Its antimicrobial properties may also support a healthy skin environment, free of microbial disruption, facilitating better keratinocyte growth.

Relevance to Dermatological Applications
Keratinocyte proliferation is central to several key skin processes, including wound healing, skin regeneration, and anti - aging treatments. The ability of Suregro to significantly enhance this proliferation suggests its potential as a therapeutic agent in dermatology.[15]

Wound Healing
In wound healing, rapid keratinocyte proliferation is essential for re - epithelialization, where the skin repairs itself by regenerating new layers. The observed increase in keratinocyte growth at concentrations of 500 µg/mL (139.23%) and 1000 µg/mL (141.54%) implies that Suregro could be particularly effective in formulations intended to accelerate wound closure. Natural products that enhance this process without causing irritation or adverse reactions are in high demand, particularly for patients with sensitive or compromised skin.[16]

Skin Regeneration and Rejuvenation
For anti - aging and skin rejuvenation applications, products that promote keratinocyte proliferation help maintain skin thickness and firmness. As we age, the rate of skin cell turnover decreases, leading to thinning, wrinkling, and a loss of elasticity. By stimulating keratinocyte growth, Suregro could help counteract these aging effects, making it a valuable ingredient in anti - aging creams and serums.[17]

Applications in Cosmeceuticals
The results of this study suggest that Suregro could also be integrated into cosmeceutical formulations, targeting not just skin healing but overall enhancement of skin appearance. Products aimed at improving skin texture, reducing pigmentation, or enhancing glow could benefit from the regenerative properties of Suregro, especially given its natural composition, which appeals to consumers seeking plant - based or non - toxic skincare solutions.

5. Need for Further Research

While the study demonstrates significant in vitro effects, the next step would be conducting in vivo studies and clinical trials to establish its efficacy and safety in human subjects. In vitro models, though valuable, do not always fully capture the complex environment of human skin, where factors like immune responses, microbiota, and environmental exposure come into play. Clinical studies would provide data on how Suregro behaves in real - world conditions, including its long - term effects on skin health, potential side effects, and optimal concentrations for different skin conditions.

Additionally, further research should explore the molecular mechanisms through which Suregro enhances keratinocyte proliferation. Understanding the specific pathways and gene expressions involved could help refine its applications and possibly lead to more targeted treatments in dermatology.

6. Conclusion

This study provides compelling evidence that Suregro significantly promotes the proliferation of human keratinocytes in a concentration - dependent manner, with the highest effect observed at 1000 µg/mL. These findings are highly promising for the development of Suregro as a therapeutic agent in dermatological applications, particularly those aimed at skin regeneration, wound healing, and anti - aging.

The natural, plant - based composition of Suregro further enhances its appeal, given the growing demand for non - toxic and sustainable skincare solutions. Each component, from Eclipta alba to Coconut oil, brings specific regenerative, antimicrobial, and antioxidant properties that contribute to the overall efficacy of the formulation. Together, these ingredients create a synergistic effect that supports keratinocyte proliferation and skin health.

However, to fully realize the potential of Suregro, further in vivo research and clinical trials are necessary to validate these in vitro findings and ensure the formulation's safety and efficacy in real - world dermatological settings. Understanding the molecular mechanisms of action and optimizing formulations for different skin conditions will also be crucial for its future development.

In conclusion, Suregro presents a promising, natural alternative for enhancing skin regeneration and health, with potential applications ranging from wound care to anti - aging products. Its ability to significantly enhance keratinocyte proliferation suggests that it could be a valuable tool in dermatological therapies that require accelerated skin repair and rejuvenation.

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