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Research Article

Utilization of HaCaT-derived psoriatic skin models in assessing the therapeutic potential of pharmacological agents

Intira Pathtubtim[1,4], Thanchanok Muangman[1,*], Sasithon Temisak[2], Celine Viennet[3], Jarupa Viyoch[4,*]

[1] Expert Centre of Innovative Health Food, Thailand Institute of Scientific and Technological Research (TISTR), Techno Polis, Klong Luang, Pathum Thani 12120, Thailand
[2] National Institute of Metrology (NIMT) PathumThani 12120
[3] UMR 1098 RIGHT INSERM EFS FC, DImaCell Imaging Resource Center, University of Marie&Louis Pasteur, Besancon 25000, France
[4] Department of Pharmaceutical Technology, Faculty of Pharmaceutical Sciences and Center of Excellence for Innovation in Chemistry (PERCH-CIC), Naresuan University, Phitsanulok 65000, Thailand

ABSTRACT

Psoriasis is a prototypic example of an immune-mediated skin disorder, characterized by abnormal keratinocyte differentiation, barrier dysfunction, and excessive production of pro-inflammatory cytokines. Here, a psoriasis-like human reconstructed epidermis (RHE-Pso) model was established by cytokine treatment of HaCaT keratinocytes with a cytokine mixture (IL-1α, TNFα, IFN-γ). This model manifested a psoriasis-like phenotype, characterized by reduced TER values, altered epidermal histomorphology, as well as a reduction in Filaggrin (FLG) expression at the protein as well as mRNA levels, accompanied by a marked enhancement in the release of cytokines like IL-1α, IL-8, TNFα, and IFN-γ. Later, this RHE-Pso model has been employed to assess various pharmacologic as well as natural agents. Clobetasol propionate emerged as one of the most effective agents in reclaiming TER values, increasing *FLG* mRNA expression (3.40 ± 0.37-fold of control), as well as curtailing the release of cytokines. Retinoic acid increased *FLG* mRNA (2.85 ± 0.35-fold of control) as well as moderately inhibited cytokine secretion, whereas calcipotriol partially relieved barrier restoration. A comparative analysis of natural agents revealed that quercetin increased *FLG* mRNA as well as inhibited TNFα & IFN-γ levels, whereas resveratrol relieved TER values as well as specifically inhibited cytokine release. Conversely, curcumin exhibited minimal activity. Taken together, this data propose that the HaCaT-derived RHE-Pso model can act as a promsing pre-clinical *in vitro* model in defining anti-psoriasis drug leads as well as natural bioactive agents.

Keywords:
HaCaT; Psoriasis-like 3D skin model (RHE-Pso); Pharmacological agents.

1. INTRODUCTION

The pathogenesis of psoriasis involves the interaction of multiple factors, including environmental stimuli, genetic predisposition, and immune dysfunction. Clinically, psoriasis appears as erythematous, scaling plaques as a result of the persistent inflammation, malformed keratinocyte hyperproliferation, and dysfunctional cell differentiation. Keratinocytes, the predominant cell population of the epidermis, play a leading role as both passive targets and active players in the inflammatory process by producing cytokines and chemokines, such as interleukin -1α (IL-1α), IL-6, IL-8 tumor necrosis factor α (TNF-α), and interferon-γ (IFN-γ) [1,2]. These cytokines perpetuate chronic inflammation, disrupt epidermal barrier function, and suppress the expression of key structural proteins involved in terminal keratinocyte differentiation and intercellular cohesion,

***Corresponding author:**
* Jarupa Viyoch Email: jarupav@nu.ac.th

including filaggrin (FLG)[3]. Although a variety of therapies, such as corticosteroids, vitamin D analogues, and retinoids, are available, long-term therapy is constrained by side effects and variability of efficacy. Concurrently, naturally derived compounds with anti-inflammatory and barrier-reconstituting properties, notably polyphenols and flavonoids, have demonstrated therapeutic potential as adjunctive treatment strategies. Accordingly, well-defined preclinical models are essential for systematically evaluating and comparing the efficacy of established pharmacological agents and candidate natural compounds. Since animal models and *ex vivo* human skin present ethical concerns and exhibit limited translatability[4,5], *in vitro* cell and tissue models, such as three-dimensional reconstructed human epidermis (RHE), have been developed as screening tools to assess the therapeutic potential of promising agents for psoriasis treatment. Recently, we reported the reconstruction of a psoriasis-like epidermal tissue model (RHE-Pso) using HaCaT keratinocytes stimulated with a cytokine cocktail, which effectively recapitulated the characteristic features of psoriasis, including hyperinflammation, barrier breakdown, and downregulated FLG expression [6].Notably, this model responded to clobetasol propionate, a corticosteroid that exhibits marked anti-inflammatory action[7], thereby demonstrating its translational potential for screening psoriasis treatment efficacy. The present study therefore extends previous work by evaluating the therapeutic potential of selected pharmacological agents, both synthetic and naturally derived, for psoriasis treatment using the established RHE-Pso model. The agents investigated include clobetasol propionate, a corticosteroid with potent anti-inflammatory activity; calcipotriol, a vitamin D analog that regulates keratinocyte proliferation and differentiation while exerting anti-inflammatory and immunomodulatory effects[8]; retinoic acid, a retinoid that modulates keratinocyte proliferation and differentiation; and the flavonoids and polyphenols curcumin, genistein, resveratrol, and quercetin, which mediate cytokine modulation and confer barrier-supportive effects[9, 10, 11]. The findings not only demonstrate the utility of the RHE-Pso model as a reliable screening platform for psoriasis therapeutics but also highlight the therapeutic promise of the tested natural compounds for psoriasis management.

2. MATERIALS AND METHODS

2.1. Materials

An immortalized human keratinocyte cell line (HaCaT) was obtained from CLS Cell Lines Service GmbH (Germany; lot no. 3004934619). Cells were maintained using Dulbecco's Modified Eagle Medium (DMEM) and DMEM/Nutrient Mixture F-12 (DMEM/F12), supplemented with fetal bovine serum (FBS) and penicillin–streptomycin, all purchased from GIBCO® (New York, USA). A keratinocyte growth supplement kit was provided by ATCC (Virginia, USA). For three-dimensional culture, Transwell® inserts (24-well format) were supplied by Corning (Arizona, USA). Calcipotriol (99% purity) was obtained from Biosynthesis (Ballerup, Denmark), while clobetasol propionate (98% purity), retinoic acid (98% purity), curcumin (94% purity), genistein (98% purity), resveratrol (99% purity), quercetin (95% purity), and paraformaldehyde solution (4%) were sourced from Sigma-Aldrich (Missouri, USA). FSC-22 clear frozen section compound was purchased from Leica (Illinois, USA). For immunostaining, a mouse monoclonal anti-filaggrin (FLG) primary antibody was obtained from Invitrogen (California, USA), and goat anti-mouse IgG Alexa Fluor 568 secondary antibody was purchased from Abcam (Cambridge, UK). In addition, ELISA kits for IL-1α, IL-8, IFN-γ, and TNF-α were also acquired from Abcam (Cambridge, UK).

2.2. Reconstruction of psoriasis-like human epidermis (RHE-Pso) from HaCaT cells

The generation of psoriasis-like features from HaCaT cells was performed as previously reported[6] with minor modifications. Briefly, HaCaT cells at passages 10-15 were seeded onto Transwell® culture inserts (24-well) at a density of 2×10^5 cells per well. These cells were cultured in DMEM/F12 medium supplemented with a keratinocyte growth kit. The cell cultures were maintained at 37°C in an atmosphere containing 5% CO_2. On day 3 of culture, the medium was replaced with fresh medium containing 2 mM $CaCl_2$ to create a high-calcium environment. The cells were then cultured for an additional 4 days. On day 7, the culture conditions were changed to an air-liquid interface setting. On day 8 of cultivation, one day after transitioning to the air-liquid interface, a cocktail of psoriasis-associated cytokines (30 ng/mL TNF-α, 30 ng/mL IL-1α, and 50 ng/mL IFN-γ) was added to the high-calcium medium. The samples were continuously incubated in cytokine-enriched high-calcium medium for a total of 72 hr, with the medium replaced daily using freshly prepared cytokine-enriched high-calcium medium. The complete process of psoriasis-like reconstructed human epidermis construction is illustrated in Figure 1. For the control group, after transitioning to the air-liquid interface, tissues were cultured in high-calcium medium without cytokines until day 13 of cultivation.

2.3. Assessment of responses of the developed RHE-Pso to pharmacological agents

On day 11 of cultivation, RHE-Pso tissues were treated with pharmacological agents delivered in

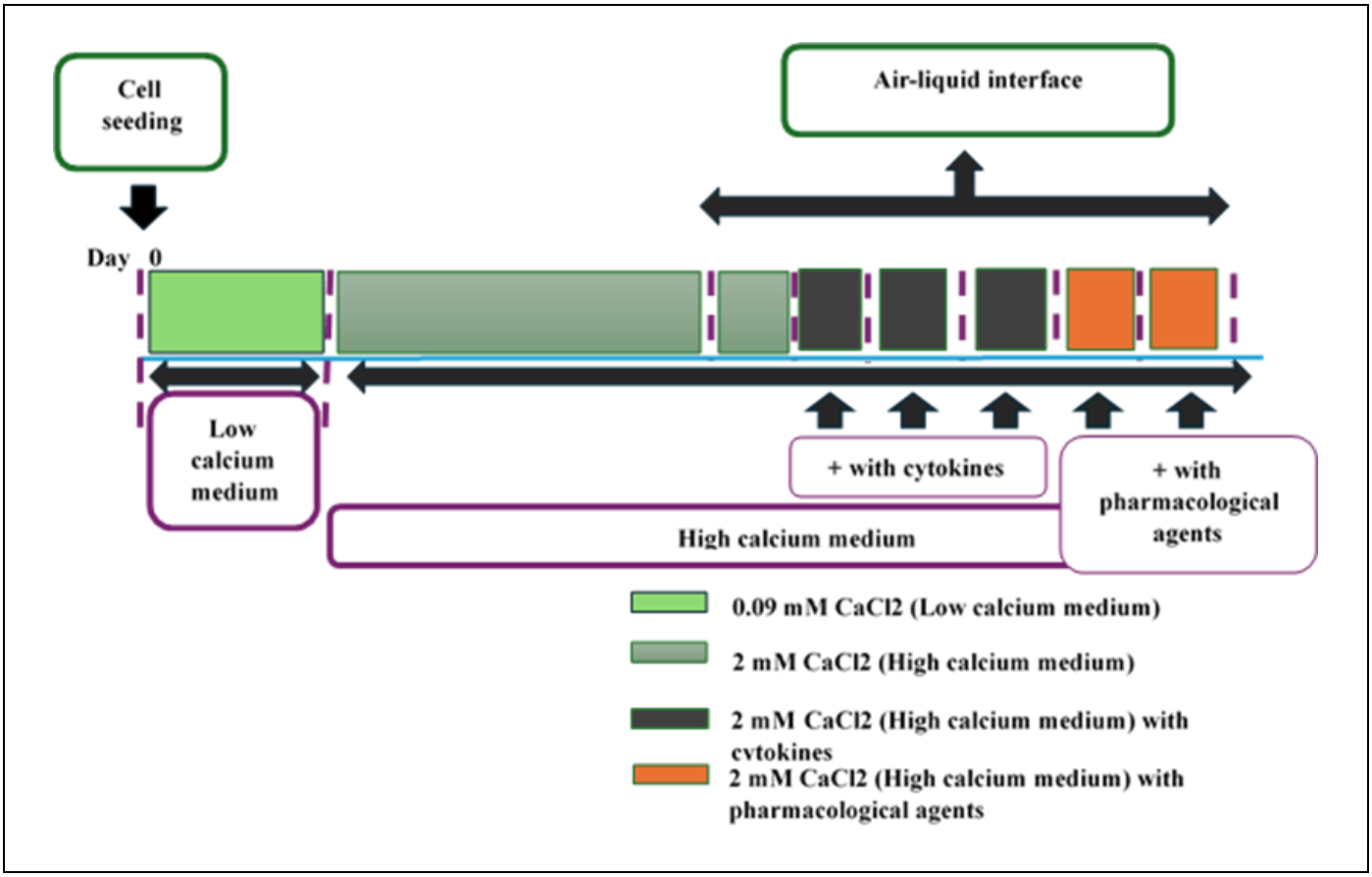

Figure 1. Diagram illustrating the construction of psoriasis-like epidermal tissue (RHE-Pso) along with treating by pharmacological agents including calcipotriol, clobetasol propionate, retinoic acid, curcumin, genistein, resveratrol, and quercetin.

culture medium at two concentration levels: 10 µg/mL for the reference compounds (calcipotriol, clobetasol propionate, and retinoic acid) and 30 µg/mL for the test phytochemicals (curcumin, genistein, resveratrol, and quercetin). All treatment concentrations were established based on prior screening cytotoxic studies (data not shown). All compounds were dissolved in dimethyl sulfoxide (DMSO) to a final concentration of 1% (v/v) in the culture medium, except for calcipotriol, curcumin, resveratrol, and quercetin, which were prepared in ethanol at a final concentration of 1% (v/v). The tissues were then cultured for an additional 48 hr with daily replacement of freshly prepared medium containing the test pharmacological agents, as shown in Figure 1. For the cytokine induction group, the tissues were continuously exposed to cytokines for further 48 hr. The study was conducted in triplicate, and the culture medium and tissue samples were collected on the final day for further investigation as follows.

2.3.1. Transcutaneous electrical resistance (TER)

Transepithelial electrical resistance (TER) of RHE-Pso and RHE-Pso treated with the test pharmacological agents was measured to determine skin barrier function strength. Electrical resistance measurements were performed with a Millicell-ERS system (Millipore, Massachusetts, USA), utilizing a paired chopstick-type electrode configuration. Resistance values were recorded in ohms (Ω), with background resistance from cell-free Transwell® inserts subtracted from each measurement. TER was monitored daily from day 8 to day 12 of cultivation, encompassing both the RHE-Pso induction phase and the subsequent pharmacological treatment period. Results were expressed as a normalized ratio relative to baseline, calculated as follows[12]:

$$\text{TER Ratio} = \frac{\text{TER (time point)}}{\text{TER (baseline)}}$$

To assess the capacity of each test agent to restore barrier function, a percentage recovery value was calculated as follows

$$\% \text{ Recovery} = \frac{\text{TER at 24 h post-treatment - TER at 72 h cytokine incubation}}{\text{TER at 24 h post-treatment}} \times 100$$

2.3.2. Morphology

The test samples were immersed in a 4% paraformaldehyde solution for 15 min at room temperature. After that, they were rinsed three times in 1X phosphate-buffered saline (PBS), with each rinse lasting 5 minutes, and then embedded in FSC-22 clear frozen section compound to create five µm-thick vertical slides. The sectioned samples underwent staining with hematoxylin and eosin (H&E) to enhance the observation of tissue morphology using an inverted microscope.

2.3.3. Protein expression of filaggrin

The sectioned samples, as previously mentioned, underwent immunofluorescence staining for filaggrin (FLG). Nonspecific antigens were blocked by incubating the samples in 10% bovine serum albumin (BSA) in PBS within a humidified chamber at room temperature for 60 min. The samples were then incubated overnight at 4°C with primary antibodies, including mouse anti-filaggrin. After three rinses in PBS, each lasting 5 min, the samples were incubated with secondary antibodies, including goat anti- mouse IgG (Alexa Fluor 568), for 2 hr at room temperature in the dark. Immunofluorescence images were captured using fluorescence microscopy.

2.3.4. mRNA expression of filaggrin and inflammatory mediators

Total RNA was extracted using the Monarch® Total RNA Miniprep Kit (New England Biolabs, USA) following the manufacturer's instructions. After DNase treatment to eliminate genomic DNA contamination, complementary DNA (cDNA) was synthesized. Quantitative real-time PCR (qPCR) was performed using Luna Universal qPCR Master Mix (New England Biolabs), which included SYBR Green dye for fluorescence detection and facilitated melting curve analysis to verify amplification specificity. Primers used in this study (detailed in Table 1) were obtained from Macrogen (Seoul, South Korea). The expression levels of target genes were normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expression. Relative gene expression levels were calculated using the 2^-ΔΔCT^ method. Data are presented as fold change relative to the control group.

2.3.5. Release levels of inflammatory mediators

On the 13th day after the last treatment, the cultured medium was collected to determine the levels of inflammatory cytokines, including IL-1α, IL-8, IFN-γ, and TNF-α. This determination was performed using ELISA kits, following the recommended procedures provided by the manufacturer.

2.4. Statistical analysis

Data are presented as mean ± standard deviation (SD) of three independent experiments (n = 3). Statistical differences among groups were determined by one-way analysis of variance (ANOVA) with post hoc multiple comparisons. Values were considered statistically significant at $p < 0.05$ relative to the cytokine-induced control group.

3. RESULTS

3.1. TER

As shown in Figure 2, the control group, which received no cytokine stimulation, demonstrated a relatively stable ratio with minimal increase over time. In contrast, all cytokine-exposed groups exhibited a progressive reduction in TER ratio from 24 to 72 hours, ranging from 0.867 ± 0.022 to 0.939 ± 0.047.

Table 1. All primers used in quantitative real time-PCR.

Gene	Primer sequence (5′-3′)
IL1-α	Forward CCACAGACCTTCCAGGAGAATG
	Reverse GTGCAGTTCAGTGATCGTACAGG
IL-8	Forward GAGAGTGATTGAGAGTGGACCAC
	Reverse CACAACCCTCTGCACCCAGTTT
TNF-α	Forward CTCTTCTGCCTGCTGCACTTTG
	Reverse ATGGGCTACAGGCTTGTCACTC
IFN-γ	Forward GAGTGTGGAGACCATCAAGGAAG
	Reverse TGCTTTGCGTTGGACATTCAAGTC
Filaggrin (FLG)	Forward GCTGAAGGAACTTCTGGAAAAGG
	Reverse GTTGTGGTCTATATCCAAGTGATC
GAPDH	Forward AAGGTGAAGGTCGGAGTCAA
	Reverse AATGAAGGGGTCATTGATGG

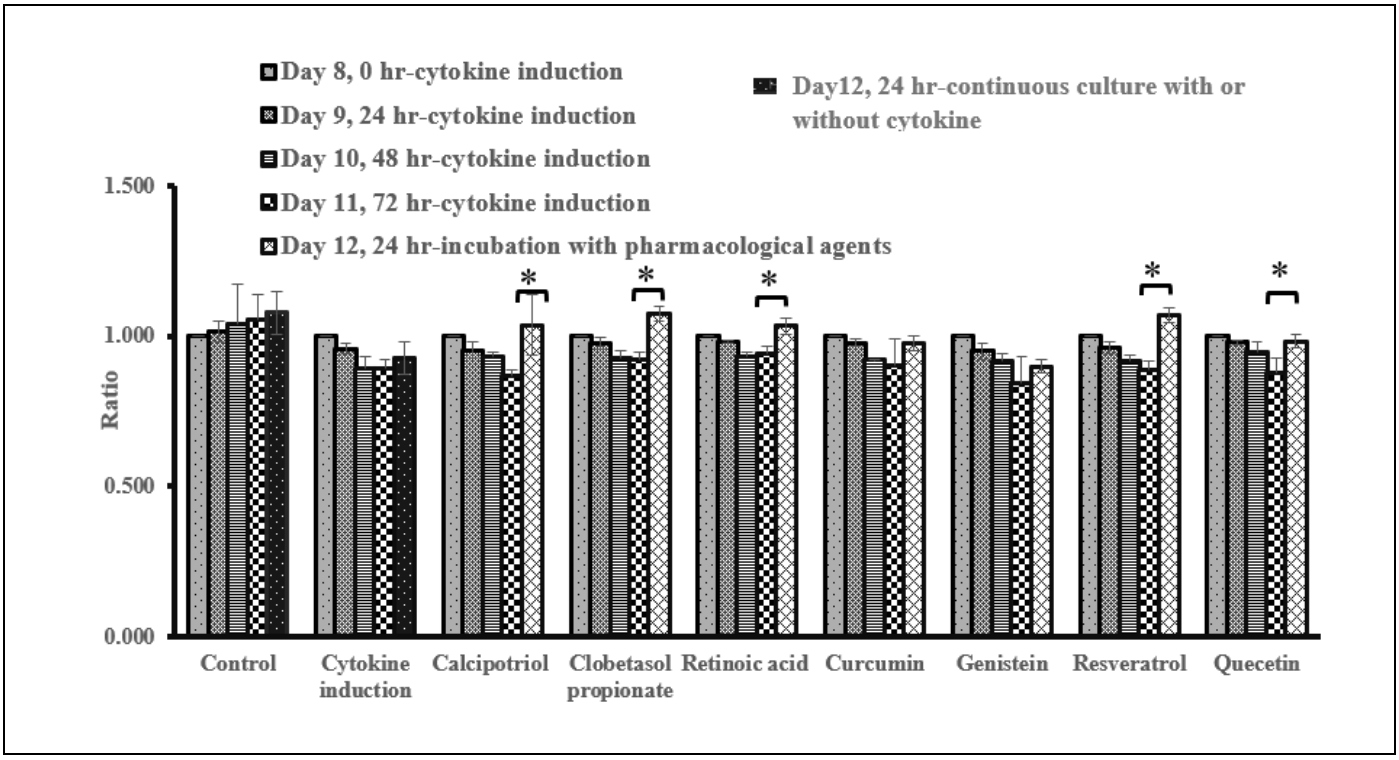

Figure 2. Transepithelial electrical resistance (TER) ratio of reconstructed human epidermis under normal conditions and following with cytokines inducing (RHE-Pso) for 24, 48, and 72 hr. The cytokine mixture consisted of 30 ng/mL TNF-α, 30 ng/mL IL-1α, and 50 ng/mL IFN-γ. Following 72 hr of induction, the RHE-Pso tissues were subsequently treated with various pharmacological agents: calcipotriol, clobetasol propionate, retinoic acid, curcumin, genistein, resveratrol, and quercetin, for 48 hr. Data are presented as mean ± SD (n = 3). *****$p < 0.05$ indicates a significant difference.

Following cytokine stimulation, treatment with calcipotriol, clobetasol propionate, retinoic acid, resveratrol, and quercetin significantly restored the TER ratio ($p < 0.05$ compared to the 72-hour cytokine induction group), with recovery observed 24 hours after treatment. Calcipotriol, clobetasol propionate, retinoic acid, and resveratrol demonstrated substantial increases in TER values, with 16%, 15%, 14%, and 18% recovery, respectively, while quercetin showed a 9% increase. For curcumin and genistein, neither the TER ratio nor the percentage recovery reached statistical significance compared to cytokine-induced RHE-Pso tissues at 72 hr.

3.2. Tissue morphology and expression of filaggrin at the protein and mRNA levels

Histological observation using H&E staining revealed pathological characteristics typical of psoriasis in RHE-Pso tissues (cytokine induction group), including epidermal thickening, increased cellular layers, and disrupted epidermal organization compared to the well-stratified control epidermis, as shown in Figure 3A. Immunofluorescence staining demonstrated a marked reduction in FLG protein expression in RHE-Pso tissues compared to the control (non-cytokine induction group). After treating RHE-Pso tissues with pharmacological agents for 48 hr, FLG protein expression clearly increased, particularly in tissues treated with calcipotriol, clobetasol propionate, retinoic acid, and resveratrol.

Regarding *FLG* mRNA expression levels, no significant decrease was observed in cytokine-induced RHE-Pso tissues compared to the control. However, after treatment with pharmacological agents for 48 hours, *FLG* mRNA expression significantly increased compared to untreated RHE-Pso tissues for all agents except genistein and calcipotriol. The most marked increases in expression levels were observed in clobetasol propionate-treated (3.40 ± 0.37-fold of control) and retinoic acid-treated (2.85 ± 0.35-fold of control) RHE-Pso tissues, as shown in Figure 3B.

3.3. mRNA expression and secreted protein levels of cytokines

The mRNA and secreted protein levels of IL-1α, IL-8, IFN-γ, and TNF-α are shown in Figures 4 and 5, respectively. Induction of tissues with the cytokine cocktail without any agent treatment (cytokine induction group) markedly increased the expression of *IL-1α*, *IL-8*, *IFN-γ*, and *TNF-α* mRNA compared to the control (non-cytokine induction group). Subsequent treatment of RHE-Pso tissues with pharmacological agents including calcipotriol, clobetasol propionate, retinoic acid, curcumin, genistein, resveratrol, and quercetin

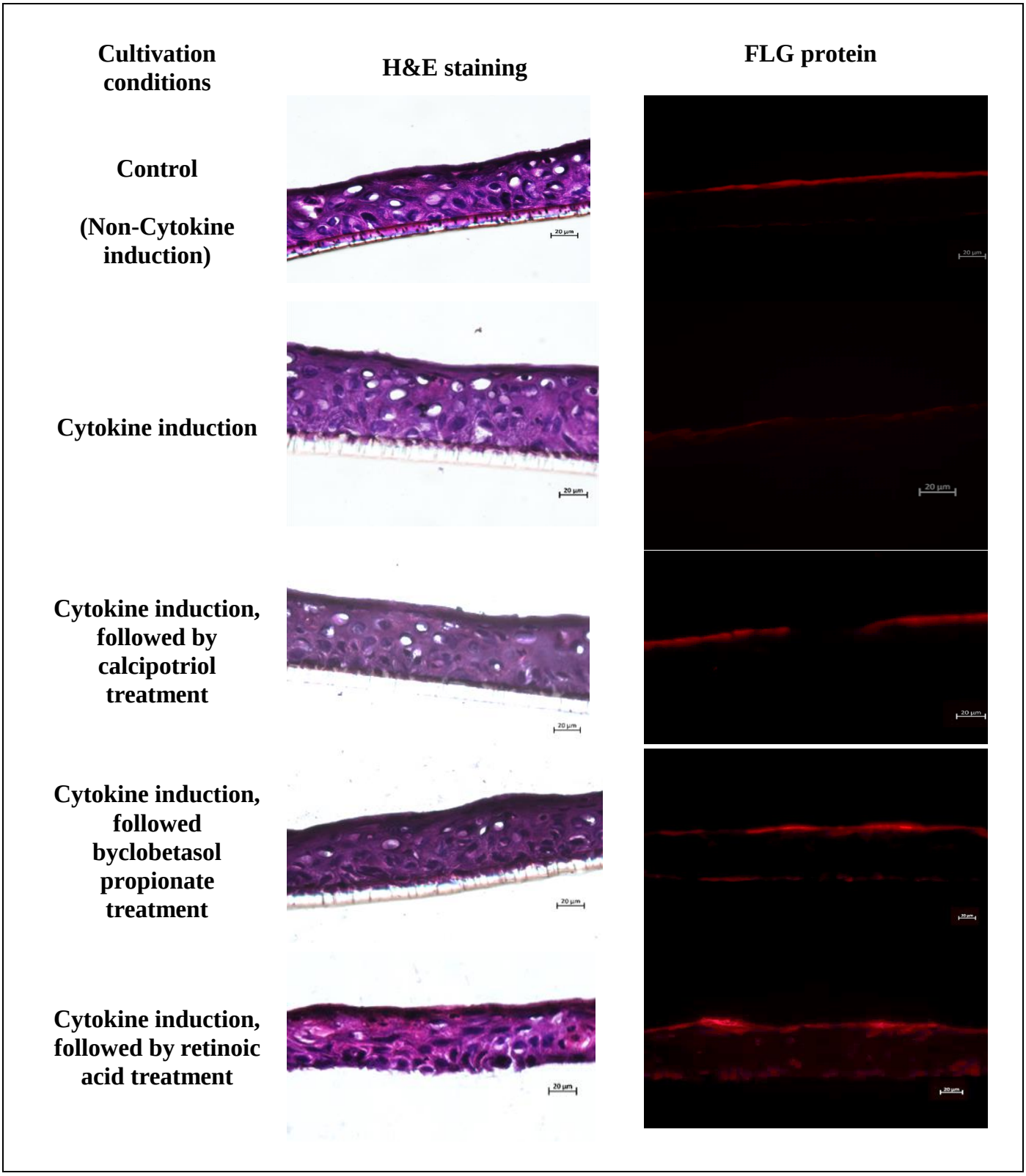

Figure 3. (A) Representative images of hematoxylin and eosin (H&E) and immunofluorescent staining of filaggrin (FLG) protein, and (B) FLG mRNA expression in reconstructed human epidermis under normal conditions and following cytokine-induced psoriatic stimulation (RHE-Pso). Psoriatic conditions were induced using a cytokine mixture comprising TNF-α (30 ng/mL), IL-1α (30 ng/mL), and IFN-γ (50 ng/mL) for 72 hr. Following 72 hr of induction, RHE-Pso tissues were subsequently treated with the following pharmacological agents: calcipotriol, clobetasol propionate, retinoic acid, curcumin, genistein, resveratrol, and quercetin, for 48 hr. Data are presented as mean ± SD (n = 3) and expressed as fold change relative to the untreated control. Statistical analysis was performed using one-way ANOVA with post hoc multiple comparisons. *$p < 0.05$ and **$p < 0.01$ indicate statistically significant differences relative to the cytokine-induced group."

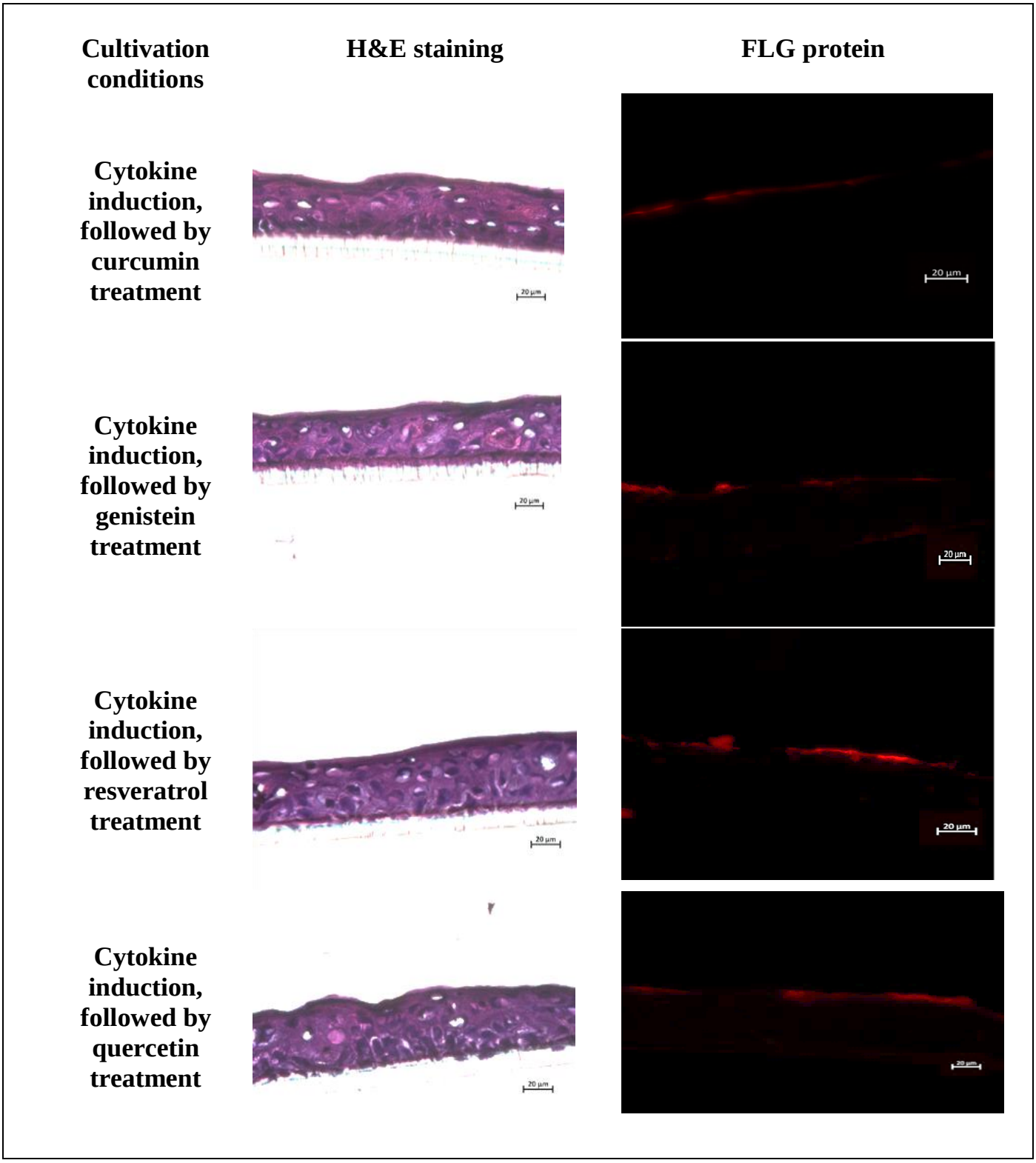

Figure (Continued) 3. (A) Representative images of hematoxylin and eosin (H&E) and immunofluorescent staining of filaggrin (FLG) protein, and (B) FLG mRNA expression in reconstructed human epidermis under normal conditions and following cytokine-induced psoriatic stimulation (RHE-Pso). Psoriatic conditions were induced using a cytokine mixture comprising TNF-α (30 ng/mL), IL-1α (30 ng/mL), and IFN-γ (50 ng/mL) for 72 hr. Following 72 hr of induction, RHE-Pso tissues were subsequently treated with the following pharmacological agents: calcipotriol, clobetasol propionate, retinoic acid, curcumin, genistein, resveratrol, and quercetin, for 48 hr. Data are presented as mean ± SD (n = 3) and expressed as fold change relative to the untreated control. Statistical analysis was performed using one-way ANOVA with post hoc multiple comparisons. *$p < 0.05$ and **$p < 0.01$ indicate statistically significant differences relative to the cytokine-induced group." (Continued).

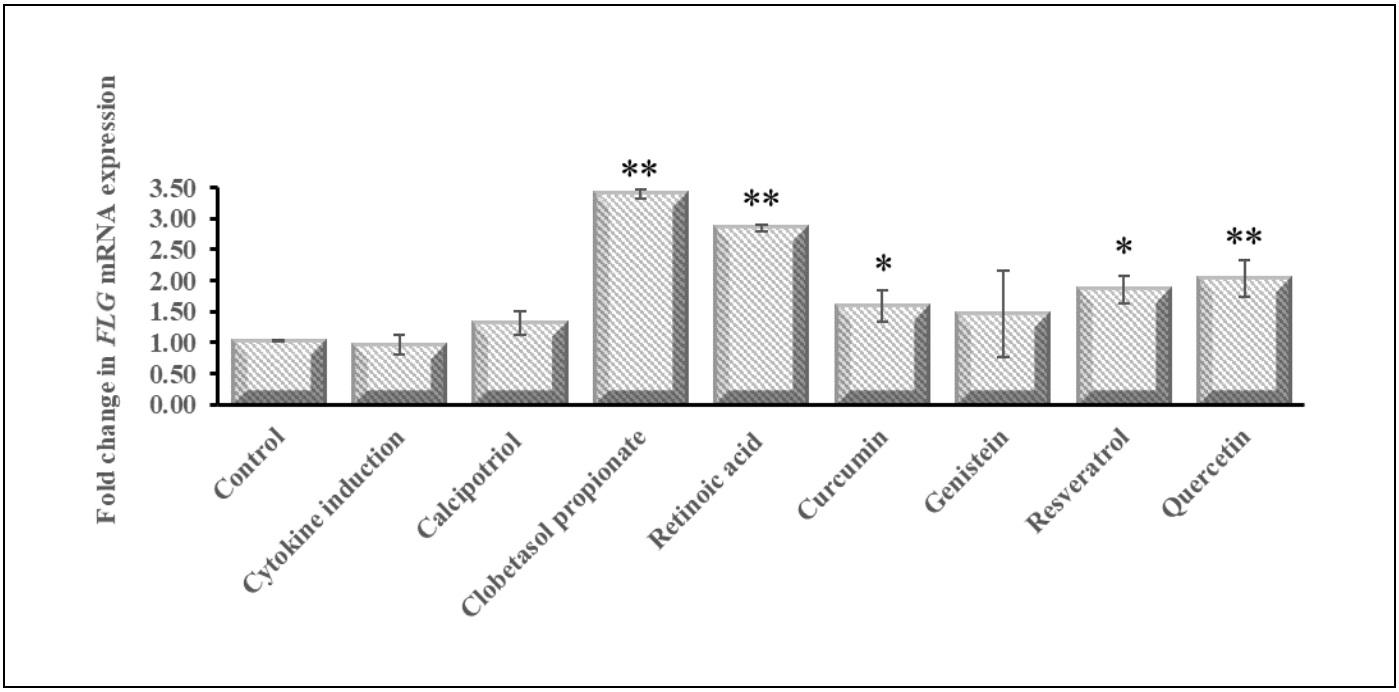

Figure (Continued). 3. (A) Representative images of hematoxylin and eosin (H&E) and immunofluorescent staining of filaggrin (FLG) protein, and (B) FLG mRNA expression in reconstructed human epidermis under normal conditions and following cytokine-induced psoriatic stimulation (RHE-Pso). Psoriatic conditions were induced using a cytokine mixture comprising TNF-α (30 ng/mL), IL-1α (30 ng/mL), and IFN-γ (50 ng/mL) for 72 hr. Following 72 hr of induction, RHE-Pso tissues were subsequently treated with the following pharmacological agents: calcipotriol, clobetasol propionate, retinoic acid, curcumin, genistein, resveratrol, and quercetin, for 48 hr. Data are presented as mean ± SD (n = 3) and expressed as fold change relative to the untreated control. Statistical analysis was performed using one-way ANOVA with post hoc multiple comparisons. *$p < 0.05$ and **$p < 0.01$ indicate statistically significant differences relative to the cytokine-induced group."

for 48 hr significantly suppressed this overexpression. The exceptions were *IL-1α* mRNA in RHE-Pso tissues treated with retinoic acid and *TNF-α* mRNA in RHE-Pso tissues treated with curcumin, which showed no significant reduction.

For cytokine release levels, the control group was set as 1-fold at baseline. In untreated RHE-Pso tissues, the secreted levels of IL-1α, IL-8, IFN-γ, and TNF-α were elevated to approximately 2-, 5-, 4-, and 2.5-fold of control, respectively. Treatment with pharmacological agents suppressed this elevated secretion across all cytokines tested, although the magnitude of suppression varied among the different cytokines.

Most pharmacological agents showed modest effects on IL-1α, IL-8, and TNF-α protein release, with the overall magnitude of reduction being less pronounced compared to IFN-γ. For IL-8, pronounced suppression was observed in RHE-Pso tissues treated with retinoic acid and resveratrol. For TNF-α, marked reduction was seen in clobetasol propionate-, retinoic acid-, curcumin-, and quercetin-treated RHE-Pso tissues. All pharmacological agents clearly suppressed the elevated release of IFN-γ, with relatively similar suppression efficacy across treatments.

Regarding the well-established antipsoriatic drugs, calcipotriol suppressed the release of IFN-γ, clobetasol propionate substantially suppressed the release of IFN-γ and TNF-α, while retinoic acid markedly suppressed IL-8 and IFN-γ. Among the natural compounds tested curcumin, genistein, resveratrol, and quercetin all markedly suppressed IFN-γ release. Notably, resveratrol demonstrated the most pronounced suppressive effect on IL-8 among the natural compounds evaluated.

4. DISCUSSION

HaCaT keratinocytes have been extensively employed as a well established *in vitro* model for reconstructing human epidermis and investigating keratinocyte biology. These cells are capable of forming stratified epidermal-like structures that closely resemble native epidermis and expressing key differentiation markers, including filaggrin.[13]. However, despite these advantages, the HaCaT-based model exhibits limitations in epidermal lipid organization. The stratum corneum barrier relies on highly ordered lamellar structures composed of ceramides, cholesterol, and free fatty acids. In HaCaT-derived models, lipid lamellae formation is often incomplete, with reduced ceramide content and disorganized lipid architecture, leading to compromised barrier function[14].

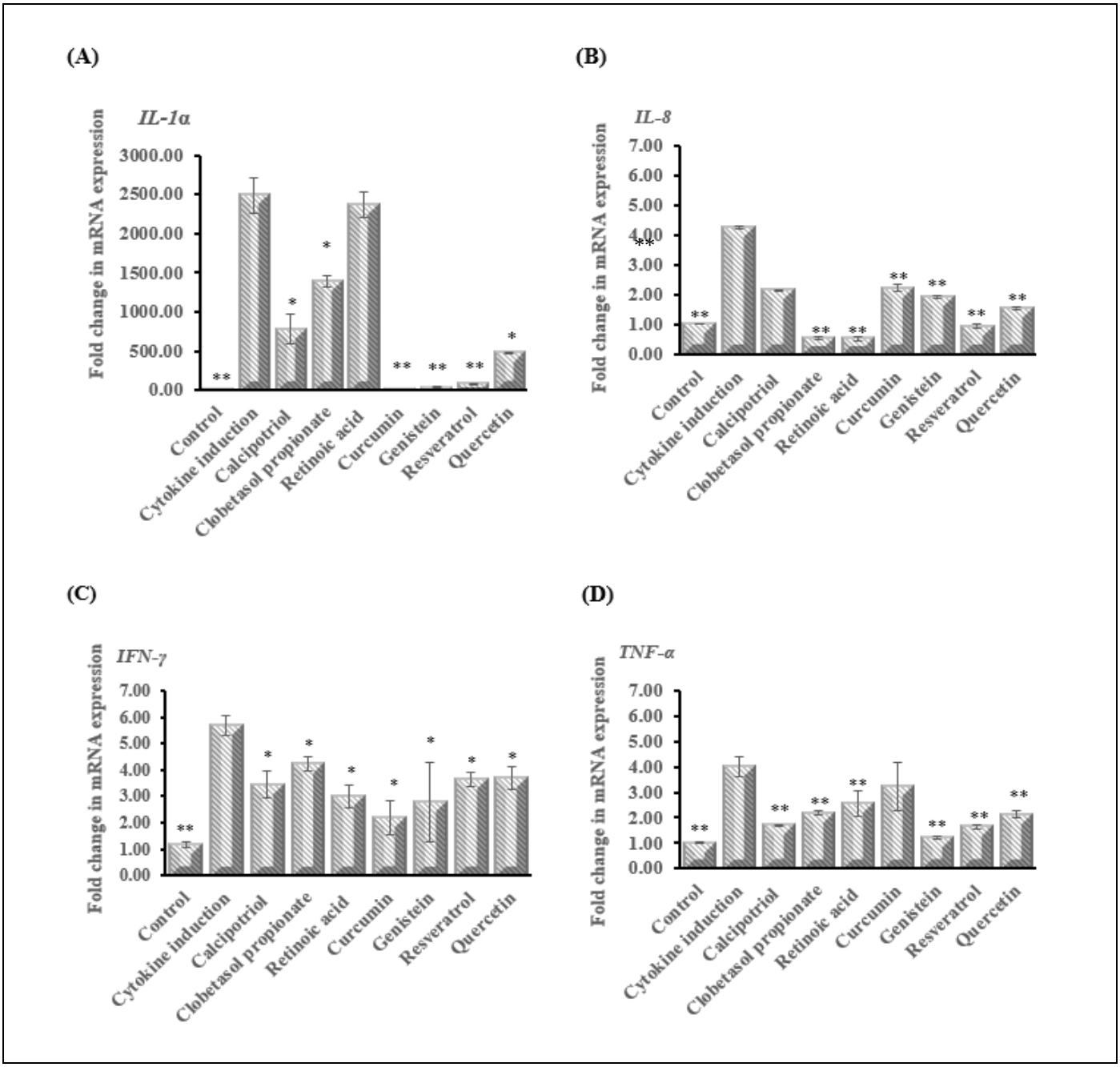

Figure 4. Fold changes in mRNA expression levels of (A) *IL-1α*, (B) *IL-8*, (C) *IFN-γ*, and (D) *TNF-α* in reconstructed human epidermis under normal conditions and following cytokine-induced psoriatic stimulation (RHE-Pso). Psoriatic conditions were induced using a cytokine mixture comprising TNF-α (30 ng/mL), IL-1α (30 ng/mL), and IFN-γ (50 ng/mL) for 72 hr. Following 72 hr of induction, RHE-Pso tissues were subsequently treated with the following pharmacological agents: calcipotriol, clobetasol propionate, retinoic acid, curcumin, genistein, resveratrol, and quercetin, for 48 hr. Data are presented as mean ± SD (n = 3) and expressed as fold change relative to the untreated control. Statistical analysis was performed using one-way ANOVA with post hoc multiple comparisons. *$p < 0.05$ and **$p < 0.01$ indicate statistically significant differences relative to the cytokine-induced group.

Nevertheless, HaCaT cells exhibit robust responses to pro-inflammatory stimuli, mediated through the activation of key signaling pathways such as NF-κB and MAPK, leading to the increased production of pro-inflammatory cytokines, including IL-6 and IL-8[15]. In addition, they play a critical role in skin repair processes due to their capacity for proliferation and migration. Collectively, these properties render HaCaT cells a suitable platform for the development of three-dimensional skin models that recapitulate psoriasis-like conditions.

Consistent with our previous study establishing a cytokine-induced psoriasis-like three-dimensional skin model using HaCaT keratinocytes, which effectively reproduced key pathological features of psoriasis. The model exhibited pronounced inflammatory responses, including elevated secretion of pro-inflammatory mediators and dysregulation of proteins associated with

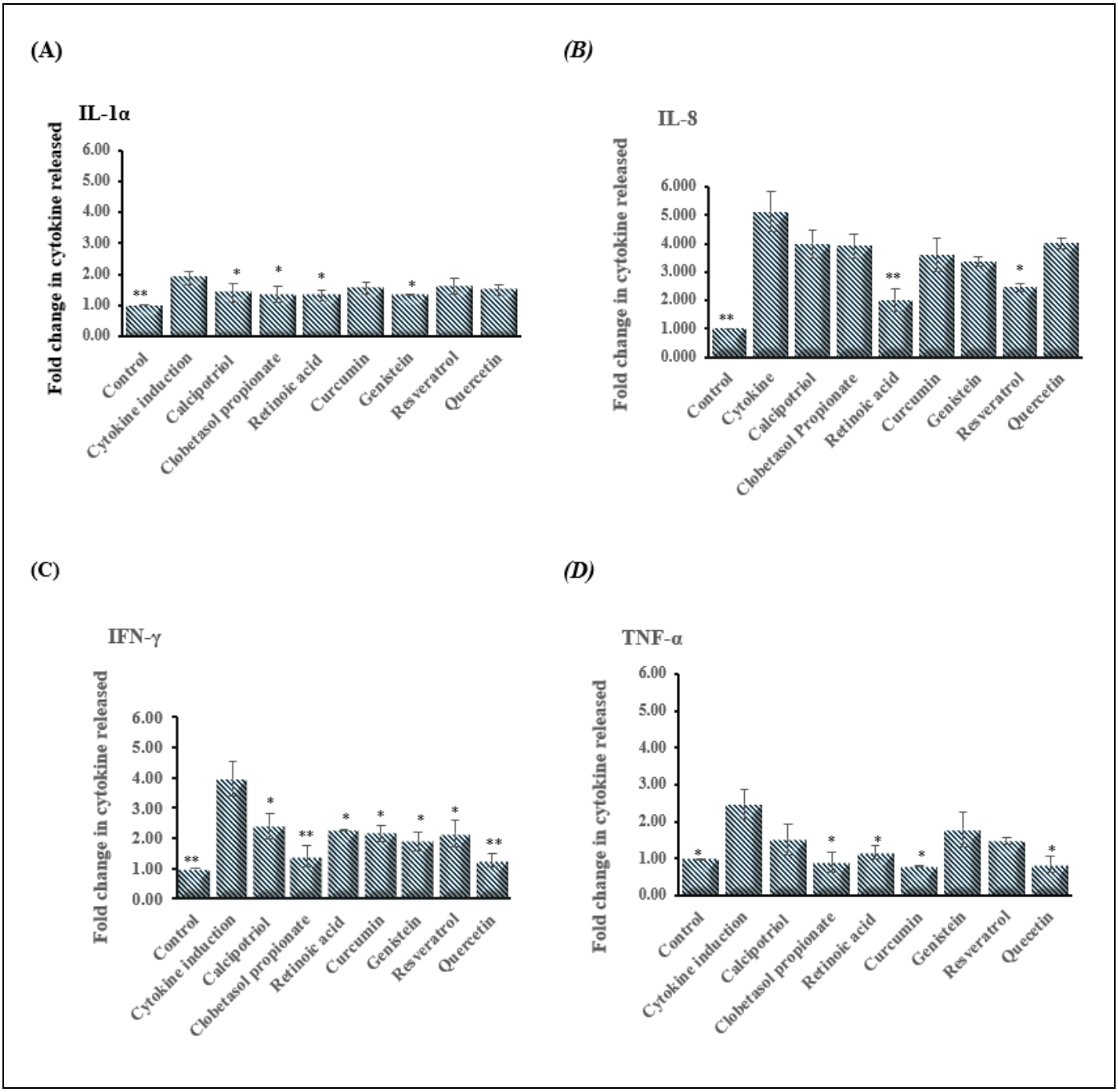

Figure 5. Fold changes in secreted protein levels of (A) IL-1α, (B) IL-8, (C) IFN-γ, and (D) TNF-α released from reconstructed human epidermis under normal conditions and following cytokine-induced psoriatic stimulation (RHE-Pso). Psoriatic conditions were induced using a cytokine mixture comprising TNF-α (30 ng/mL), IL-1α (30 ng/mL), and IFN-γ (50 ng/mL) for 72 hr. Following 72 hr of induction, RHE-Pso tissues were subsequently treated with the following pharmacological agents: calcipotriol, clobetasol propionate, retinoic acid, curcumin, genistein, resveratrol, and quercetin, for 48 hr. Data are presented as mean ± SD (n = 3) and expressed as fold change relative to the untreated control. Statistical analysis was performed using one-way ANOVA with post hoc multiple comparisons. *$p < 0.05$ and **$p < 0.01$ indicate statistically significant differences relative to the cytokine-induced group.

epidermal barrier function, thereby closely mimicking *in vivo* disease conditions. Therefore, the present study aims to evaluate the applicability of the RHE-Pso model as a reliable platform for assessing the therapeutic efficacy of pharmacological agents and standard compounds, with particular emphasis on both anti-inflammatory effects and the restoration of epidermal barrier function at the molecular level.

Accordingly, the progressive decline in TER observed in cytokine-exposed tissues (from 0.867 ± 0.022 to 0.939 ± 0.047 over 24–72 h) reflects a substantial impairment of epidermal barrier function, a hallmark of psoriatic skin. This functional disruption is closely associated with reduced filaggrin protein expression, which plays a critical role in keratinocyte differentiation and maintenance of barrier integrity

through the formation of natural moisturizing factors[16,17]. Consistent with clinical observations, cytokine-induced downregulation of filaggrin protein further supports the pathophysiological relevance of the RHE-Pso model. Notably, the absence of significant changes in filaggrin mRNA despite reduced protein levels suggests that regulatory mechanisms may predominantly occur at post-transcriptional or post-translational stages [18]. In parallel, cytokine stimulation resulted in a coordinated increase in pro-inflammatory mediators, with IL-8 and IFN-γ levels elevated by approximately 5-fold and 4-fold, respectively, compared to the control. This inflammatory profile is characteristic of Th1/Th17-driven responses and reflects keratinocyte hyperactivation, a central feature of psoriasis pathogenesis. The responsiveness of the model to pharmacological treatments was demonstrated by TER recovery ranging from approximately 14% to 16% following treatment with calcipotriol, clobetasol propionate, and retinoic acid. These effects were accompanied by suppression of IFN-γ and TNF-α, consistent with known mechanisms involving vitamin D receptor-mediated differentiation and glucocorticoid-induced inhibition of NF-κB signaling[19]. In addition, clobetasol propionate and retinoic acid markedly increased filaggrin protein expression (3.40 ± 0.37-fold and 2.85 ± 0.35-fold of control, respectively), highlighting the importance of restoring keratinocyte differentiation in barrier repair.

Among the natural compounds, resveratrol exhibited a comparatively higher TER recovery (18%), along with pronounced suppression of IL-8. This combined effect suggests a coordinated role in both barrier restoration and inflammatory modulation. Mechanistically, these effects may be attributed to the pleiotropic activity of resveratrol, including inhibition of NF-κB signaling, activation of SIRT1, and modulation of oxidative stress via the Nrf2/HO-1 pathway[20,21]. These pathways collectively contribute to the stabilization of tight junction proteins, such as ZO-1 and occludin, thereby enhancing epithelial integrity.

Quercetin demonstrated moderate TER recovery (9%) and consistently suppressed TNF-α at both mRNA and protein levels, indicating a coherent anti-inflammatory effect. Its activity is likely mediated through modulation of NF-κB and JAK/STAT signaling pathways, along with the promotion of keratinocyte differentiation via MAPK/AP-1 signaling[22,23]. These combined mechanisms link inflammatory suppression with restoration of epidermal homeostasis.

In contrast, curcumin and genistein showed minimal TER recovery and limited effects on certain inflammatory markers, despite their reported anti-inflammatory properties [28]. This observation suggests that suppression of inflammation alone may be insufficient to restore barrier function, emphasizing the importance of dual-target mechanisms involving both inflammatory control and differentiation processes.

The discrepancy between mRNA and protein expression levels observed for certain cytokines provides further mechanistic insight. For example, while IL-1α mRNA expression was suppressed across most treatments, corresponding protein levels showed only modest changes. Conversely, TNF-α protein levels were significantly reduced even in cases where mRNA levels remained unchanged, indicating the involvement of post-transcriptional regulation, such as mRNA stability, translational control, or protein secretion dynamics. These findings highlight the importance of integrating multi-level analyses for accurate interpretation of compound activity. Importantly, restoration of filaggrin protein expression was consistently associated with improvements in TER, supporting its role as a functional biomarker of barrier integrity in this model. The observed increase in filaggrin mRNA following treatment, particularly with clobetasol propionate and retinoic acid ($p < 0.05$), may reflect a compensatory transcriptional response rather than direct transcriptional regulation. Collectively, these findings suggest that effective antipsoriatic agents should simultaneously modulate inflammatory pathways and normalize keratinocyte differentiation.

The concordance between the observed responses in this model and the known clinical efficacy of standard antipsoriatic agents supports its predictive validity. Calcipotriol and clobetasol propionate demonstrated robust activity across multiple endpoints, including TER, filaggrin expression, and cytokine suppression, consistent with their established clinical performance[24]. The model also captured the distinct profile of retinoic acid, reflecting its unique mechanism of action and therapeutic characteristics. Despite these strengths, certain limitations should be acknowledged. The absence of immune cell components, such as T cells, dendritic cells, and neutrophils, limits the ability of the model to fully recapitulate the complex immunological interactions involved in psoriasis. Furthermore, the relatively short treatment duration (48 h) may not adequately reflect long-term therapeutic effects or toxicity. Future studies incorporating co-culture systems or extended treatment protocols (7–14 days) are warranted to enhance the physiological relevance of the model.

5. CONCLUSIONS

This study successfully established a cytokine-induced RHE-Pso model that recapitulates key pathological hallmarks of psoriasis, including barrier dysfunction, filaggrin downregulation, and inflammatory cytokine elevation. The model demonstrated robust predictive validity through its ability to correctly

identify the efficacy of established antipsoriatic drugs (calcipotriol, clobetasol propionate, and retinoic acid) while effectively differentiating superior natural compounds, particularly resveratrol, from less effective candidates such as curcumin and genistein.

The multi-parameter assessment approach integrating barrier function (TER), differentiation markers (filaggrin protein and mRNA), and inflammatory mediators (IL-1α, IL-8, IFN-γ, and TNF-α at both protein and mRNA levels) provides comprehensive efficacy evaluation that captures the multifactorial nature of psoriasis. This integrated assessment proved superior to single-endpoint assays by revealing mechanistic insights through mRNA-protein discordance patterns and establishing filaggrin protein restoration as a reliable biomarker correlating with barrier recovery.

Based on our findings, we propose specific screening criteria for identifying promising antipsoriatic candidates: ≥10% TER recovery, significant suppression of at least three out of four key cytokine proteins, and restoration of filaggrin protein expression. Resveratrol exhibited relatively higher TER recovery (18%) compared to other natural compounds and pronounced IL-8 suppression, suggesting its potential for further development as a topical antipsoriatic agent. The cytokine-induced RHE-Pso platform represents a valuable, physiologically relevant, and efficient tool for early-stage screening and mechanistic investigation of psoriasis treating drug candidates. This 3D tissue model bridges the gap between simple cell-based assays and resource-intensive animal studies, offering a practical and ethical approach to accelerate the discovery of novel topical therapeutics for psoriasis management.

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Author contribution

Intira Pathtubtim designed the study, analyzed data, and prepared the manuscript. Sasithon Temisak, Celine Viennet, Thanchanok Muangman, and Jarupa Viyoch suggested the experimental design. All authors contributed to the study and approved the final version.

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Conflict of interest

None to declare

Ethics approval

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