An Innovative Study on Rose Petal Extract (*Rosa centifolia L.*) for resisting skin stress towards Epidermal Keratinocytes

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Introduction

The dynamic interplay of tissue and organogenesis represents an exquisite molecular and cellular orchestration. The mammalian skin exemplifies this complexity through its multilayered architecture and functional heterogeneity. Recent investigations have established that the skin is a dual-functional organ that acts as a primary sensor of environmental stressors and a key target of systemic stress responses[1]. Environmental stressors, including ultraviolet radiation (UV)/air pollutants and intrinsic physiological factors, such as psychosocial stress, can synergistically induce cutaneous damage through distinct molecular pathways, leading to compromised epidermal barrier function and cosmetic deterioration[2, 3]. As the primary interface between an organism and the external environment, the epidermis functions as a biomechanical barrier, mitigating physical trauma, microbial invasion, and transepidermal water loss via structured collagen networks and lipid-rich stratum corneum[4]. Keratinocytes, which form the outermost layer of the skin, can sense exogenous stresses and generate endocrine peptides.

UVR exposure, as an exogenous stressor, is the leading determinant of skin pigmentation, which is regulated by multiple extrinsic (primarily UV) and intrinsic (primarily hormonal) factors[5]. Keratinocytes and melanocytes in the basal layer of the epidermis primarily regulate skin pigmentation. UVR exposure induces overexpression of the tumor suppressor gene p53 in keratinocytes, which subsequently upregulates transcription of the proopiomelanocortin (POMC) gene. This process yields biologically active peptides, including α-melanocyte-stimulating hormone (α-MSH) and adrenocorticotropic hormone (ACTH). These peptides exert their effects on melanocytes by binding to melanocortin 1 receptor (MC1R), a functional receptor expressed on melanocyte membranes. The activation of the MC1R signaling cascade promotes melanogenesis. Concurrently, endothelin-1 (End-1), a potent mitogenic peptide secreted by keratinocytes, further amplifies melanogenic responses by binding to the endothelin B receptor (EDNRB) on melanocytes. This interaction enhances melanocyte sensitivity to α-MSH and ACTH via the upregulation of MC1R surface expression, thereby synergistically intensifying melanin production. Collectively, these UV-induced molecular pathways highlight the complex interplay between keratinocyte-melanocyte crosstalk and melanocortin signaling in photoadaptive mechanisms[6, 7].

The skin and its appendages are key targets of endogenous stress mediators. As a multifaceted organ, the skin can respond to neurotransmitters, neuropeptides, and other signaling molecules predominantly originating from the hypothalamic-pituitary-adrenal (HPA) axis. Notably, cortisol—a glucocorticoid hormone secreted by the adrenal cortex—interacts

extensively with the skin. This hormone binds to glucocorticoid receptors, which are highly expressed in keratinocytes and fibroblasts, thereby modulating epidermal homeostasis[1, 8]. While cortisol maintains systemic stress responses, its prolonged elevation disrupts cutaneous barrier integrity. Recent mechanistic studies revealed that chronic glucocorticoid exposure delays transepidermal water loss and impairs lipid synthesis in the stratum corneum, thereby prolonging wound re-epithelialization[2, 9].

Rosa centifolia L., a member of the Rosaceae family and Rosa genus, is among the most widely distributed plant species worldwide. Beyond its ornamental value, this species has significant culinary and medicinal applications, particularly the petals[10]. The physiological properties of *Rosa centifolia* can be attributed to the rich presence of bioactive phytochemicals, such as phenolic compounds, flavonoids, and anthocyanins[11]. Extensive scientific research has highlighted the biological functions of these compounds, including their potent antioxidant, antimicrobial, anti-inflammatory, and antiallergic properties[12, 13]. Recent studies have demonstrated that rose petal extract (RPE) exhibits significant depigmentation effects via tyrosinase inhibition, as evidenced by clinical trials showing increased skin brightness[14]. However, despite their wide availability, the link among the RPE, keratinocytes, and skin stress remains underexplored. In this study, we determined the whitening and repair mechanisms of UV- and cortisol-treated keratinocytes, supporting their utility as botanical ingredients in neurocosmetics.

Materials and Methods

2.1 RPE

RPE was provided by Jinan Zerun Biotechnology Co., Ltd. (Shan Dong, China). The dried flower petals of *Rosa centifolia L.* were crushed and extracted using flash extraction with 30% 1, 3-butanediol. The extract was then filtered using coarse and fine filters to remove residues and other large insoluble matter. Finally, the extract was sterilized and mixed with preservatives to obtain the final RPE.

2.2 Human epidermis equivalents cultivation

The three-dimensional (3D) Reconstructed Human Epidermis (RHE) and Reconstructed Human Pigmented Epidermis (RHPE) models (EpiKutis® & MelaKutis®, Guangdong Boxi Biotechnology Co., Guangzhou, China) were inoculated into 6-well plates. The 3D skin model was randomly divided into the control, model, and REP (0.05–0.1%) groups with three replicates per group. All tissue samples were maintained under standard cell culture conditions (37 °C, 5% CO_2) throughout the experiment.

2.3 UV treatment

UV irradiation was performed using ultraviolet B (UVB) lamps (Philips, Netherlands). RHE equivalents in a medium containing 10% fetal bovine serum (FBS) were stimulated by UVB irradiation at 600 mJ/cm^2 using a UVB lamp. After treatment, RPE was added (or not) under liquid, and the tissues in 6-well plates were incubated (37 °C, 5% CO_2) for 24 h. RHPE equivalents in a medium containing 10% FBS were irritated by UVB irradiation at 50 mJ/cm^2 combined with 5 nM End-1 and α-MSH for 9 d. During processing, RPE was continuously added (or not) under liquid conditions from day 3 to 8. Each condition was evaluated using

three different explants and was compared with the control (untreated equivalents). Equivalent amounts of culture supernatant were collected for evaluation.

2.4 Cortisol stimulation

RHE equivalents were stressed by adding 1 nM of cortisol to the culture medium. RPE, diluted in tissue medium, was simultaneously added (or not), and skin samples were cultured in a 5% CO_2 incubator at 37 °C for 24 h. Each condition was evaluated on three different explants and compared with the control (untreated equivalents). Subsequently, equivalents were collected for paraffin sectioning.

2.5 Enzyme-linked immunosorbent assay (ELISA)

Following treatment, culture supernatants from the RHE and RHPE models were collected. ELISA was used to measure the release of α-MSH, ACTH, and ET-1. Human α-MSH (CUSABIO, China), ACTH (Abcam, England), and ET-1 ELISA kits (BOSTER, China) were used according to the manufacturer's instructions. Experiments were conducted at least in triplicate.

2.6 Sample preparation and silver staining

After treatment, fixed specimens in chilled 4% paraformaldehyde (Fisher Scientific, American) for 24 h were embedded in paraffin and sectioned. All samples were stained simultaneously under the same conditions, according to a modified silver staining protocol (Solarbio, China). After staining, slices were photographed under a microscope (Olympus, Tokyo, Japan).

2.7 Evaluation of skin condition

Apparent colorimetric analysis was conducted using a camera configured with a focal length of 5.8 mm, dual-aperture settings (f/8 and f/22), shutter speed of 1/80 s, and ISO 1600. Subsequently, L* values were measured by vertically orienting a spectral aperture toward the model surfaces, with triplicate readings for each specimen to ensure data consistency for individual tissues.

2.8 Histological examination

The paraffin-embedded sections were used for immunofluorescence (IF) and immunohistochemical (IHC) staining. IF staining results were observed using a fluorescence microscope (Olympus, Tokyo, Japan). Differentiation markers were determined using filaggrin (FLG, 1:500, Proteintech, China) and loricrin (LOR, 1:500, Abcam, England). The secondary antibody used was Alexa Fluor 488 IgG (1:2000, Abcam, England). Sections were mounted, covered, and visualized under a microscope.

IHC detection of Cyclobutane pyrimidine dimer (CPD) in equivalent paraffin sections was performed as previously described[15]. Bound anti-CPD antibody (CosMoBioCo. Ltd., Japan) was detected by incubation with biotinylated goat anti-mouse IgG1, followed by peroxidase-labeled streptavidin. After washing, the sections were incubated with diaminobenzidine and counterstained with hematoxylin and eosin. Staining results were observed using an inverted microscope (Olympus, Tokyo, Japan).

2.9 Statistical analysis

Image Pro Plus (IPP) software was used for analyzing silver staining and immunofluorescence images, and GraphPad Prism was used for plotting. Statistical analysis was conducted using R-3.6.3 software. The data were presented as mean ± standard deviation (SD). Two-tailed t-tests were performed, and a significance level of $p < 0.05$ was considered a statistically significant difference.

Results

3.1 RPE inhibited melanocyte-stimulating factor secretion on 3D RHE and RHPE equivalents

To study the effect of RPE on UVB-induced melanocyte-stimulating factor secretion in the epidermis, we analyzed the expression of ACTH, α-MSH and End-1 in 3D RHE equivalents using ELISA. The secretion of ACTH, α-MSH and End-1 increased significantly after UVB treatment compared with those in the untreated controls ($p < 0.05$). However, 0.05–0.1% RHE suppressed the release of ACTH, α-MSH, and End-1 in 3D RHE equivalents ($p < 0.05$). The results are shown in Fig. 1. The same results were validated using 3D RHPE equivalents (Fig. 2).

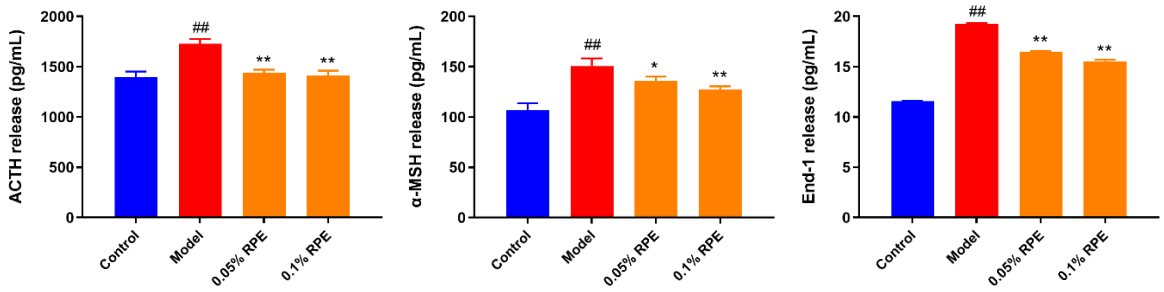


Fig 1. Effects of RPE on ACTH, α-MSH, and End-1 release of 3D RHE equivalents. The results are presented as mean ± SD of three independent experiments (##, $p < 0.01$, the model group compared with the control group; *, $p < 0.05$, **, $p < 0.01$, the RPE group compared with the model group).

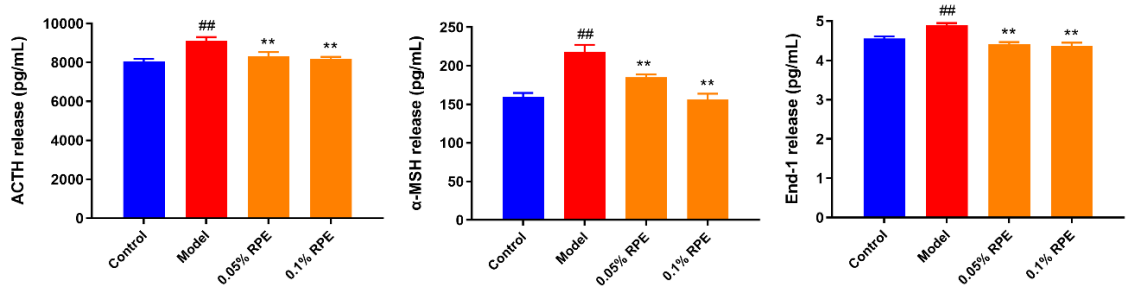


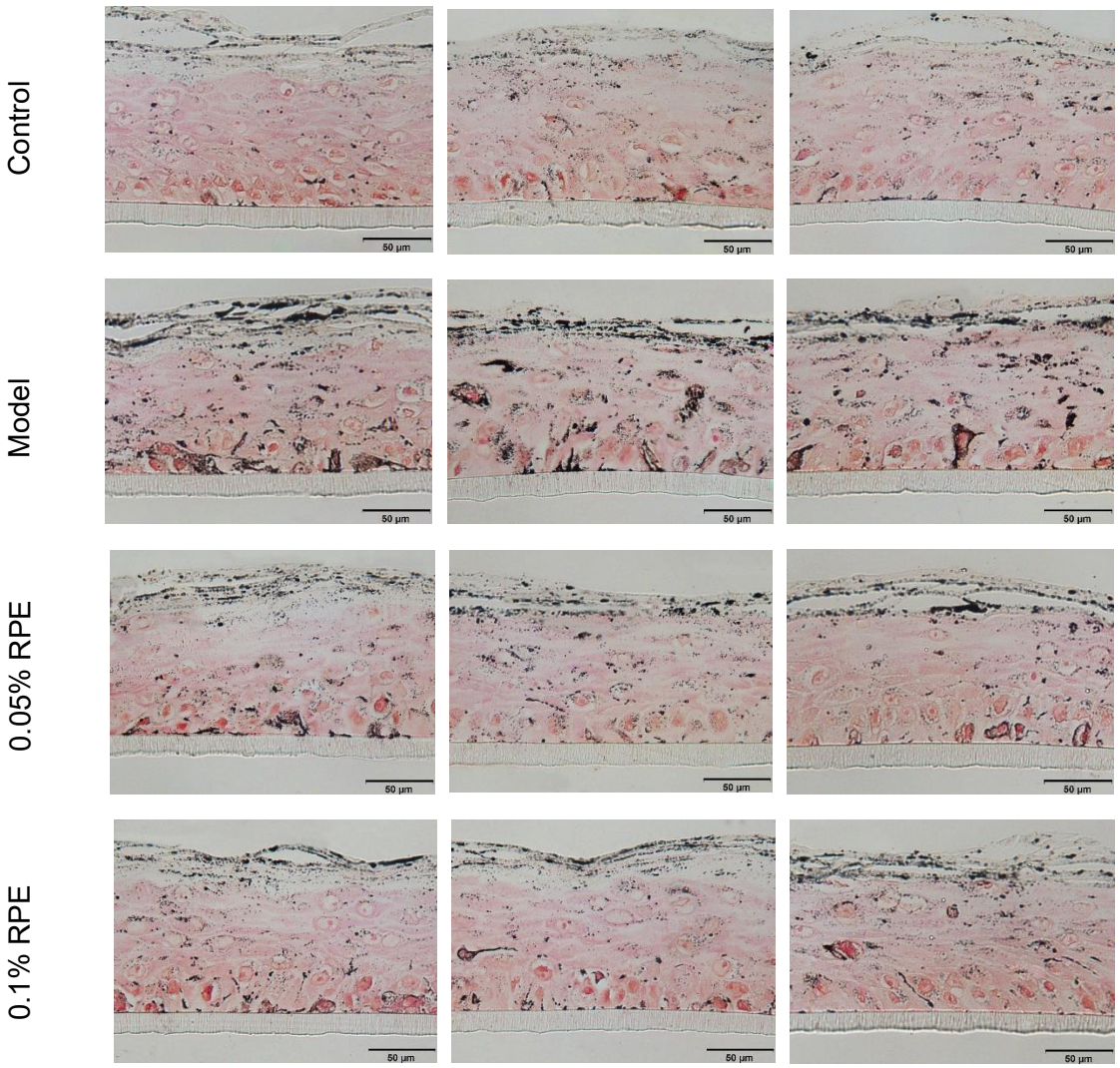
Fig 2. Effect of RPE on ACTH, α-MSH, and End-1 release of 3D RHPE equivalents. The results are presented as mean ± SD of three independent experiments (##, $p < 0.01$, the model group compared with the control group; **, $p < 0.01$, the RPE group compared with the model group).

3.2 RPE suppressed pigmentation on 3D RHPE equivalents

To examine the effect of RPE on UVB-induced melanin synthesis in the epidermis, we analyzed the pigmentation in UVB/α-MSH + End-1 stimulated 3D RHPE equivalents using

silver staining. The number of melanin particles in 3D RHPE models increased significantly after UVB/α-MSH + End-1 treatment compared with those in the untreated controls ($p < 0.05$). 0.05–0.1% RPE significantly inhibited melanin particles irritated by UVB/α-MSH + End-1 in 3D RHPE models ($p < 0.05$; Fig. 3).

A



B

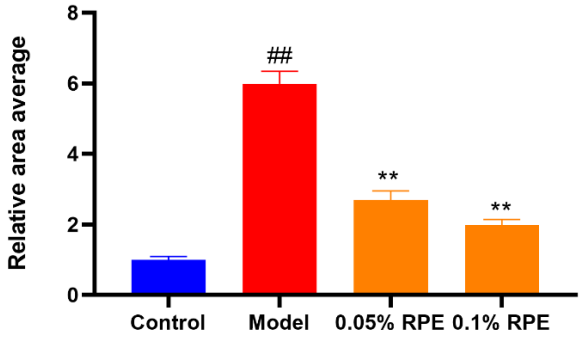


Fig 3. Effect of RPE on melanin distribution of UVB/α-MSH + End-1-treated 3D RHPE equivalents. (A) The silver-stained images of melanin in the 3D RHPE models. (B) Relative area of melanin particles based on the silver staining images was analyzed using IPP software. (##, $p < 0.01$, the model group compared with the control group; **, $p < 0.01$, the RPE group compared with the model group).

3.3 RPE elicited a skin whitening effect on 3D RHPE models

In this study, the skin-whitening effect of RPE was further confirmed by UVB/α-MSH + End-1-stimulated 3D RHPE models by apparent colorimetric and apparent luminance analysis. As observed in Figure 4, RPE reversed the darkening chromaticity induced by UVB/α-MSH+End-1-stimulated 3D RHPE equivalents. Subsequent analyses revealed that the application of 0.05% and 0.1% RPE demonstrated a statistically significant enhancement in skin brightness by 9.17% and 11.19%, respectively, compared with the model group ($p < 0.05$).

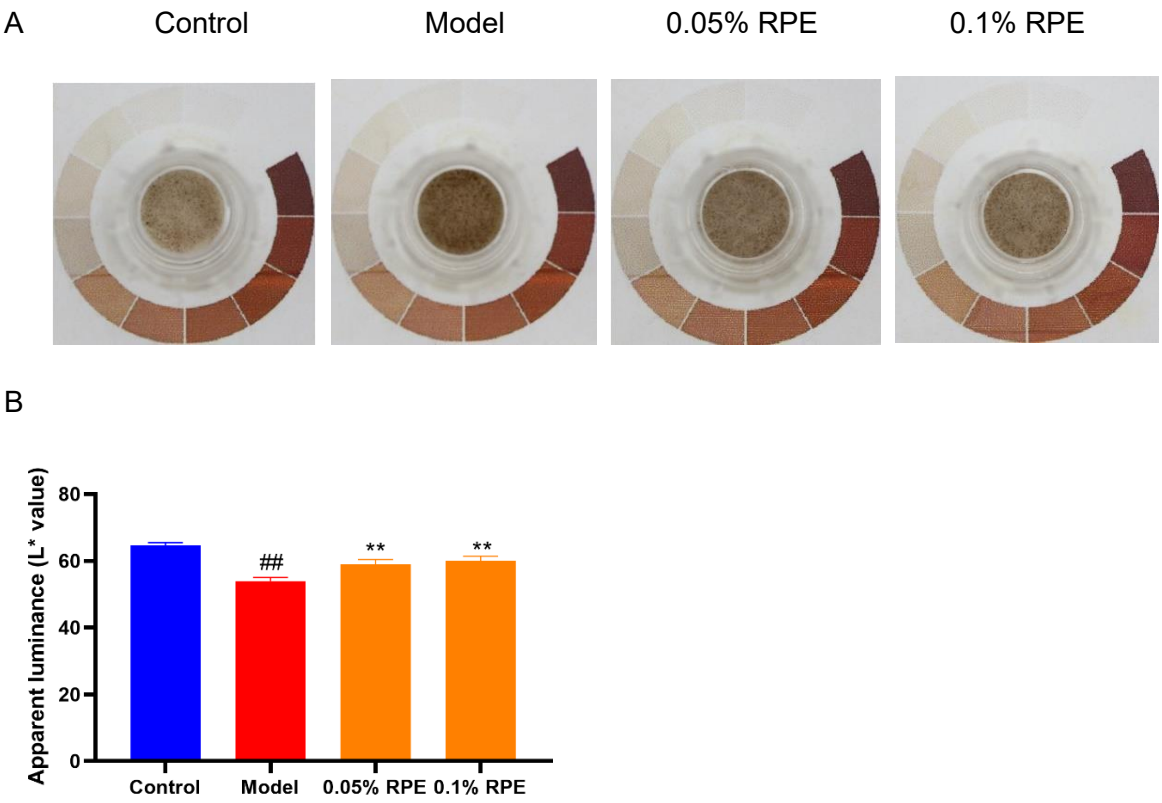


Fig 4 Whitening effect of RPE on UVB/α-MSH+End-1-treated 3D RHPE equivalents. (A) Apparent colorimetric images, (B) apparent luminance analysis in the 3D RHPE models. (##, $p < 0.01$, the model group compared with the control group; **, $p < 0.01$, the RPE group compared with the model group).

3.4 RPE prevented damage in cortisol-induced 3D RHPE models

An ex vivo cortisol-induced stress model, based on 3D RHPE equivalents, was designed to study the protective effects of RPE against skin injury caused by chronic stress. Three indicators of skin chronic stress damage were evaluated using IF and IHC staining. The results revealed that cortisol treatment triggered a stress reaction in the skin equivalents, as shown by the significantly decreased expression of FLG and LOR ($p < 0.05$) and a

significant increase in the expression of DNA-damaged CPD ($p < 0.05$). Co-treatment with cortisol and RPE promoted the downregulation of FLG and LOR expression ($p < 0.05$) and inhibited CPD upregulation ($p < 0.05$) (Fig. 4).

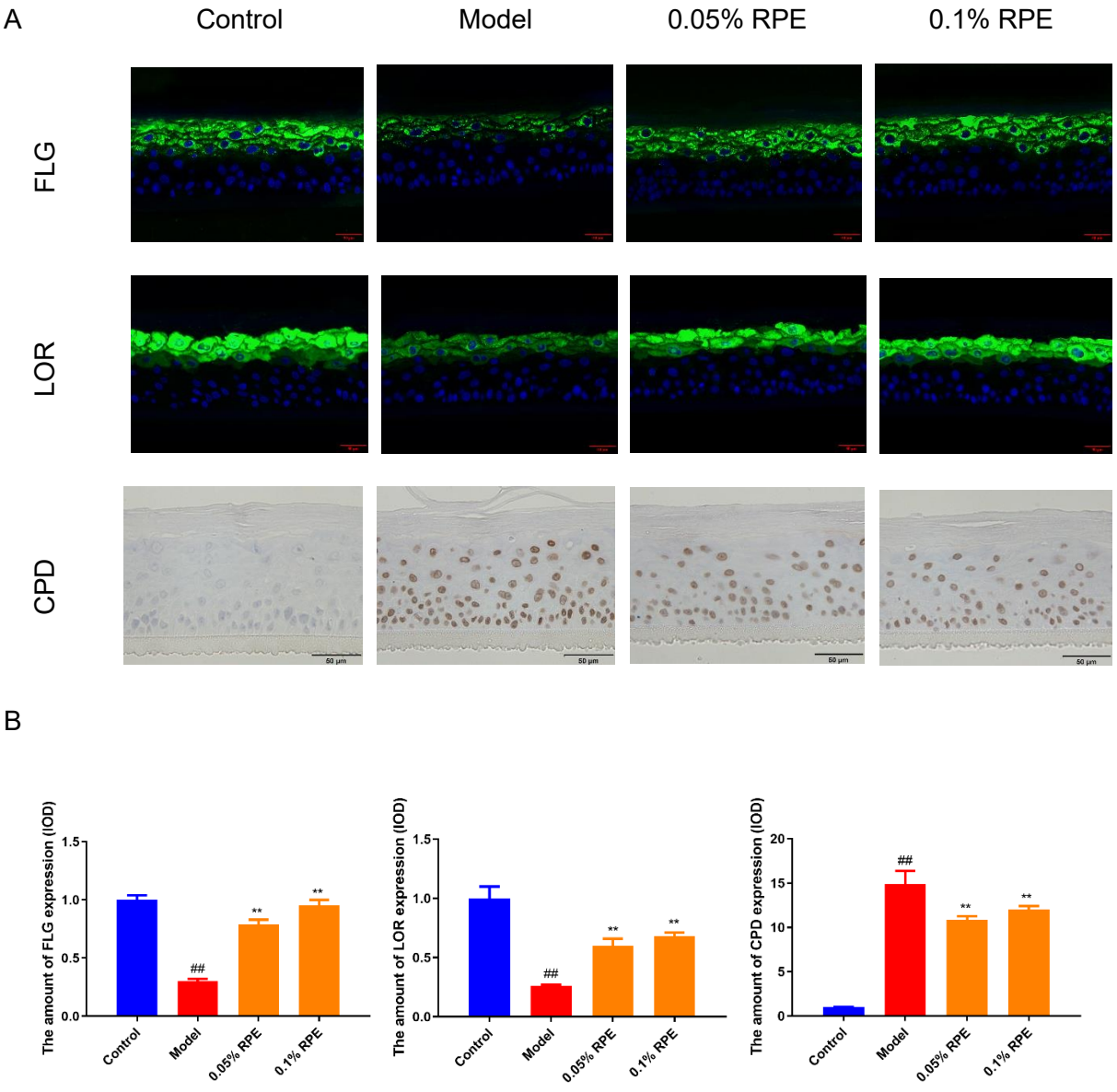


Fig. 5: The repairing effect of RPE on cortisol-treated 3D RHPE equivalents. (A) IF staining for FLG, LOR, and IHC staining for CPD in untreated and complex-treated 3D RHPE equivalents. Cell nuclei were labeled with DAPI (blue fluorescence), while FLG and LOR were labeled with 488 (green fluorescence) in IF staining. CPD^+ cells were shown in dark brown in IHC staining. (B) Quantitative analysis of FLG, LOR, and CPD expression levels in untreated and complex-treated 3D RHPE equivalents (##, $p < 0.01$, the model group compared with the control group; **, $p < 0.01$, the RPE group compared with the model group).

Discussion

The skin, which constitutes the body's natural integumentary system, protects against exogenous and endogenous factors that can trigger harmful biological responses. As the largest and most exposed organ, the integumentary system integrates barrier integrity,

immune surveillance, and neuroendocrine-immune crosstalk to maintain redox homeostasis between external influences and internal tissues[1]. Keratinocytes, which form the outermost layer of the skin, sense exogenous stresses and generate endocrine peptides. Given the positive effects of the rose extract on human health and its widespread application in cosmetics, we studied its effects on exogenous and endogenous factors that induce stress in keratinocytes.

Exposure to stressors, such as UVR, viral or bacterial infections, cold and heat, and endogenous stress mediators, in biological tissues can disrupt homeostasis. Among these, excessive exposure to UVR is a major trigger of hyperpigmentation disorders, such as chloasma and freckles[16]. UVR exposure causes DNA damage in keratinocytes. This damage triggers the activation of p53, which initiates the transcription of POMC[17]. Keratinocytes are equipped with proconvertases I and II, which play crucial roles in processing POMC into its bioactive forms. Hence, the synthesis and secretion of α-MSH and ACTH are increased upon UV exposure. End-1, a powerful mitogen produced by keratinocytes, significantly affects human melanocytes. It enhances the responsiveness of melanocytes to α-MSH and ACTH, further influencing the skin's pigmentation process[18, 19]. Our results showed that 0.05–0.1% RPE significantly decreased the levels of ACTH, α-MSH, and ET-1 in UVB-irradiated 3D epidermal skin models compared with the model group. Moreover, 0.05–0.1% RPE inhibited the secretion of ACTH, α-MSH, and ET-1 in the UVB/α-MSH + End-1-stimulated RHPE models and significantly reduced melanin accumulation, further whitening the apparent chromaticity and enhancing the apparent brightness. Previous studies have shown that RPE dose-dependently suppressed tyrosinase expression and the outcome of melanin synthesis in B16F10 cells in vitro[14]. Other studies have shown that ellagic acid suppressed α-MSH generation and tyrosinase activity in UVA-irradiated keratinocyte HaCaT cells and melanogenesis in α-MSH-stimulated B16F10 cells[20]. Therefore, we speculate that the inhibitory effect of RPE on the secretion of synergistic melanocyte-stimulating factor (ACTH, α-MSH, End-1) and pigmentation in the epidermis is due to its ellagic acid component. However, we cannot rule out the synergistic effects of other polyphenols and flavonoids in the extract. Our results revealed an innovative whitening target for RPE as a botanical cosmetic ingredient.

In addition to releasing endocrine peptides under UV exposure, the skin acts as an effector of endogenous factors, causing a series of adverse effects. Among the endogenous factors, some were correlated with mental health conditions. Cortisol, a glucocorticoid hormone secreted in response to emotional stress and a pivotal component of the HPA axis, is among the intrinsic biological factors implicated in the disruption of skin homeostasis. Its prolonged secretion can exacerbate inflammatory responses, impair cellular DNA damage, and compromise skin barrier function[8, 21]. We developed an in vitro model to observe the epidermal barrier and cellular DNA damage after cortisol stimulation with 3D RHP equivalents. Subsequently, we evaluated the effect of RPE on cortisol levels. We demonstrated that after 24 h of treatment in the presence of cortisol, 0.05–0.1% RPE significantly increased the protein expression of FLG and LOR, and reduced the expression of DNA-damaged CPD. Previous studies have shown that rose extract protects the skin from damage caused by the stress factor epinephrine, produced by the HPA axis, as

demonstrated by several stress indicators[22]. Our research confirmed the repair effect of RPE on skin damage caused by endogenous pressure factors.

Conclusion

Our study revealed an innovative whitening mechanism of RPE for inhibiting POMC biologically active peptides (α-MSH and ACTH) and synergetic End-1 release in keratinocytes, which can secrete endocrine peptides upon exogenous UV stress. Furthermore, our study confirmed the repairing effect of RPE on keratinocyte damage caused by endogenous pressure mediators. Overall, our findings suggest that RPE exhibits an innovative and effective mechanism of action on epidermal keratinocytes under external and internal stress conditions, supporting its potential application as a botanical ingredient for neurocosmetics in skin whitening and repair.

Acknowledgments

None.

Conflict of Interest Statement

None.

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