IFSCC 2025 full paper (IFSCC2025-1545)

"Photoprotective and Post-Sun Repairing Effects of an Up-cycled Complex Consisting of Camellia japonica Pericarp Extract and 3-O-Ethyl-L-Ascorbic Acid"

Hayeon Kim [2], Cha Young Yoo [1], Yong Woo Kim [1], Suwon Jeon [2], Deokhoon Park [2], Sang-Keun Han [1] and Eunsun Jung [2],*

[1]R&D Center, Kolmar Korea, Seoul, 06800, South Korea; [2]Biospectrum Life Science Institute, A-1805, U-TOWER, 767, Sinsu-ro, Suji-gu, Yongin-si, Gyeonggi-do, 16827, Republic of Korea

1. Introduction

Ultraviolet radiation (UVR) is a major environmental factor affecting the skin, and prolonged exposure leads to photoaging, inflammation, DNA damage, and oxidative stress [1]. UVB, in particular, penetrates the entire epidermal layer and induces the formation of cyclobutane pyrimidine dimers (CPDs) and reactive oxygen species (ROS), which contribute to inflammation and the activation of extracellular matrix-degrading enzymes such as collagenase [2,3]. In addition, UVB enhances angiogenesis and microvascular permeability, resulting in erythema and edema [4].

Sunscreen has been widely used to protect skin from the harmful effect of UVR. However, several studies have reported that reagents used in sunscreen are linked to cancer, contact eczema, and allergies [5]. In addition, nanoparticle inorganic filters was reported to exert neurotoxic effect through oxidative stress and apoptosis-related cytotoxicity [6]. For this reason, active research is being conducted on sunscreen materials derived from natural sources, which are expected to be safer for the skin compared to conventional chemical agents. Many studies revealed that antioxidant agents such as flavonoids, polyphenol and ascorbic acid can exhibit photoprotective capacity by inhibiting oxidative stress and inflammation [7,8].

Vitamin C, a representative antioxidant, is known for its effects on improving skin aging, whitening, and providing photoprotection. Previous studies have reported that it protects the skin by scavenging UV-induced reactive oxygen species (ROS) and suppressing pro-inflammatory factors [9]. 3-O-ethyl-L-ascorbic acid is a stabilized derivative of vitamin C. It has been reported to exhibit its anti-aging and anti-hyperpigmentation properties [10]. However, its potential role in photoprotection has not yet been elucidated.

Camellia japonica (C. japonica) belongs to the *Camellia* genus and grows naturally in Korea, Japan, and China. Active compounds such as polyphenol, vitamin E, saponin, triterpenoids, and fatty acids can be found in *Camellia japonica.* Several studies reported *that Camellia japonica* extract shows antioxidant, wound healing, and anti-inflammatory activities [11,12].

In this study, we investigated the photoprotective effect of multi-complex(CE-EA complex), consisting of 3-O-ethyl-L-ascorbic acid and *Camellia japonica* pericarps extract, on UVB radiation-induced skin damage. Initially, the protective effects of CE-EA complex on UVB-induced keratinocyte damage were assessed by evaluating DNA damage, apoptosis, and the expression of inflammatory mediators. To further investigate its paracrine effects on neighboring cells, keratinocyte-conditioned medium was applied to vascular endothelial cells and melanocytes, and the subsequent changes in vascular permeability and pro-inflammatory cytokine levels in endothelial cells, as well as melanin synthesis in melanocytes, were analyzed.

2. Materials and Methods

Materials and reagents

Human Epidermal Keratinocytes, neonatal (HeKn), EpiLife medium, Human Keratinocyte Growth Supplement (HKGS), Human Epidermal Melanocytes, darkly pigmented donor (HeMn-DP), M254 medium and Human Melanocyte Growth Supplement (HMGS), MCDB131 medium were obtained from Thermo Fisher Scientific (MA, USA). Human dermal microvascular endothelial cells(HMEC-1) and BALB/c 3T3 cells were purchased from ATCC(VA, USA). MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide), dimethyl sulfoxide (DMSO), 3-O-ethyl-L-ascorbic acid, Propidium Iodide (PI), RNase A and hydrocortisone were purchased from Sigma-Aldrich (St. Louis, MO, USA). Recombinant human EGF were obtained from R&D Systems (MN, USA). Fetal bovine serum (FBS), Bovine Calf Serum (BCS), and Penicillin/Streptomycin were purchased from WELGENE (Korea) and glutamine from Gibco (CA, USA). Ethanol was obtained from Millipore (Merck). The CE–EA complex was prepared by mixing 3-O-ethyl-L-ascorbic acid and hot water extract of *Camellia japonica* pericarps in a 1:1 ratio.

Cell culture

Human Epidermal Keratinocytes, neonatal (HeKn) were cultured in EpiLife medium supplemented with Human Keratinocyte Growth Supplement (HKGS) at 37℃ in 5% CO_2. Human dermal microvascular endothelial cells (HMEC-1) were cultured in MCDB131 medium supplemented with 10 ng/ml EGF, 10 mM Glutamine, 1 μg/ml Hydrocortisone, 10% fetal bovine serum, and 1% penicillin-streptomycin at 37℃ in 5% CO2. The BALB/c 3T3 cells were cultured in DMEM containing 10% Bovine Calf Serum (BCS) and 1% penicillin/streptomycin at 37℃ in 5% CO2. Human Epidermal Melanocytes, neonatal, darkly pigmented donor (HeMn-DP) were cultured in M254 medium supplemented with human melanocyte growth solution (HMGS) at 37℃ in 5% CO2.

3. Results

Synergistic protective effects of EA and CE co-treatment on UVB-induced damage

To assess the protective effects of 3-O-ethyl-L-ascorbic acid (EA) and *Camellia japonica* pericarps extract (CE) co-treatment against UVB-induced cytotoxicity, HeKn cells were pretreated with EA alone or combined with CE before UVB exposure. Cell viability, measured by the MTT assay, decreased significantly to 43% in the UVB-treated group. Treatment with 100 ppm EA alone improved viability to 60.5%. Co-treatment with 50 and 100 ppm CE in combination with EA increased viability to 76% and 82.5%, respectively (Figure 1A), indicating that EA and CE together provide better protection against UVB-induced damage than EA alone.

The protective effect of EA alone or in combination with CE against UVB-induced DNA damage was evaluated by measuring the formation of cyclobutane pyrimidine dimers (CPDs). Figure

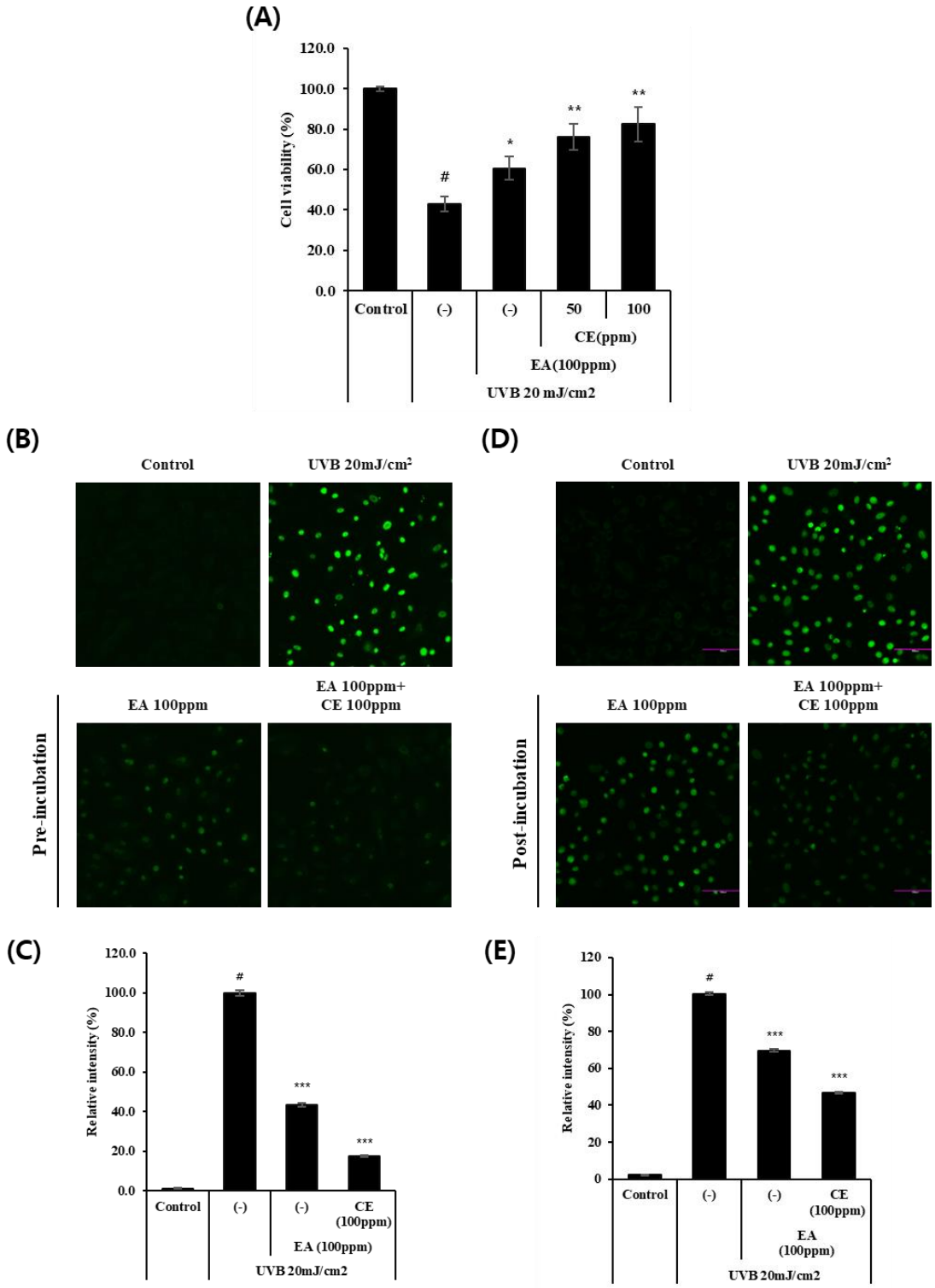

Figure 1. Synergistic effects of EA and CE co-treatment on UVB-induced damage. (A) Cell viability was assessed by MTT assay after UVB irradiation. Cells were pretreated with EA alone or combined with CE before UVB exposure. (B, D) Immunofluorescence staining for cyclobutane pyrimidine dimers (CPD) to evaluate DNA damage following UVB exposure. (B) CPD levels in pre-incubation. (D) CPD levels in post-incubation. (C, E) Quantification of CPD fluorescence intensity in (B) and (D), respectively. Data are presented as mean ± SD (#$p < 0.05$ vs. control group; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs. UVB-treated group).

1B shows CPD levels, a marker of DNA damage. The UVB-treated group exhibited a significant increase in CPD formation. Pre-incubation with 100 ppm of EA alone or combined with 100 ppm of CE reduced CPD formation by 56.8% and 82.5%, respectively, with the co-treatment showing a stronger effect. Figure 1C shows a graphical representation of CPD formation after pre-incubation. In Figure 1D, CPD levels following post-incubation are shown. Treatment with 100 ppm EA alone reduced CPD formation by 30.8%, whereas co-treatment with 100 ppm CE resulted in a 52.8% reduction, indicating a synergistic protective effect against UVB-induced DNA damage. Figure 1E shows a graphical representation of CPD formation after post-incubation.

These findings demonstrate that EA and CE co-treatment provides enhanced protection against UVB-induced cytotoxicity and DNA damage, offering both preventive and reparative benefits. The synergistic effects of EA and CE contribute to more effective protection and recovery from UVB-induced damage in keratinocytes. In this study, the CE-EA complex was prepared by combining EA and CE in a 1:1 ratio, with a final concentration of 100 or 200 ppm for the complex.

CE–EA complex protects against UVB-induced DNA damage

To evaluate the protective and restorative effects of the CE–EA complex on UVB-induced DNA damage, CPD formation was assessed under both pre- and post-incubation conditions. As shown in Figure 2A and 2B, CE–EA complex treatment at 100 and 200 ppm markedly reduced CPD levels compared to the UVB-exposed group. The graphical representation of CPD intensity revealed a significant reduction in the pre-incubation condition, with decreases of 62.5% and 67.2% at 100 and 200 ppm, respectively (Figure 2C). Similarly, post-incubation with the CE–EA complex led to a substantial decrease in CPD formation, with reductions of 71.1% and 82.9%, respectively (Figure 2D). These results indicate that the CE–EA complex effectively prevents and repairs UVB-induced DNA damage when applied both before and after UVB exposure.

To further assess DNA strand breaks, a comet assay was performed. As shown in Figure 2E, UVB exposure resulted in pronounced comet tail formation, indicating extensive DNA damage. Treatment with the CE–EA complex at 100 and 200 ppm significantly reduced comet tail length, suggesting efficient DNA protection. The quantification of comet tail moment, shown in Figure 2F, revealed a reduction of 66.4% and 68.9% at 100 and 200 ppm, respectively, further confirming the DNA-protective effect of the CE–EA complex.

Collectively, these results indicate that the CE–EA complex effectively mitigates UVB-induced DNA damage by suppressing CPD formation and minimizing DNA strand breaks, as reflected by reduced comet tail lengths, in both pre- and post-treatment conditions.

CE-EA complex reduces UVB-induced erythema and inflammation

Erythema, characterized by skin redness resulting from vasodilation and increased blood flow, is closely linked to elevated prostaglandin E_2 (PGE_2) production under UVB exposure [13]. To examine the inhibitory effect of CE–EA complex on erythema, PGE_2 levels were measured in UVB-irradiated HeKn cells. As shown in Figure 3A, UVB exposure markedly increased PGE_2 release, whereas treatment with CE–EA complex significantly reduced PGE_2 levels in a dose-dependent manner, suggesting that CE–EA complex effectively suppresses UVB-induced erythema.

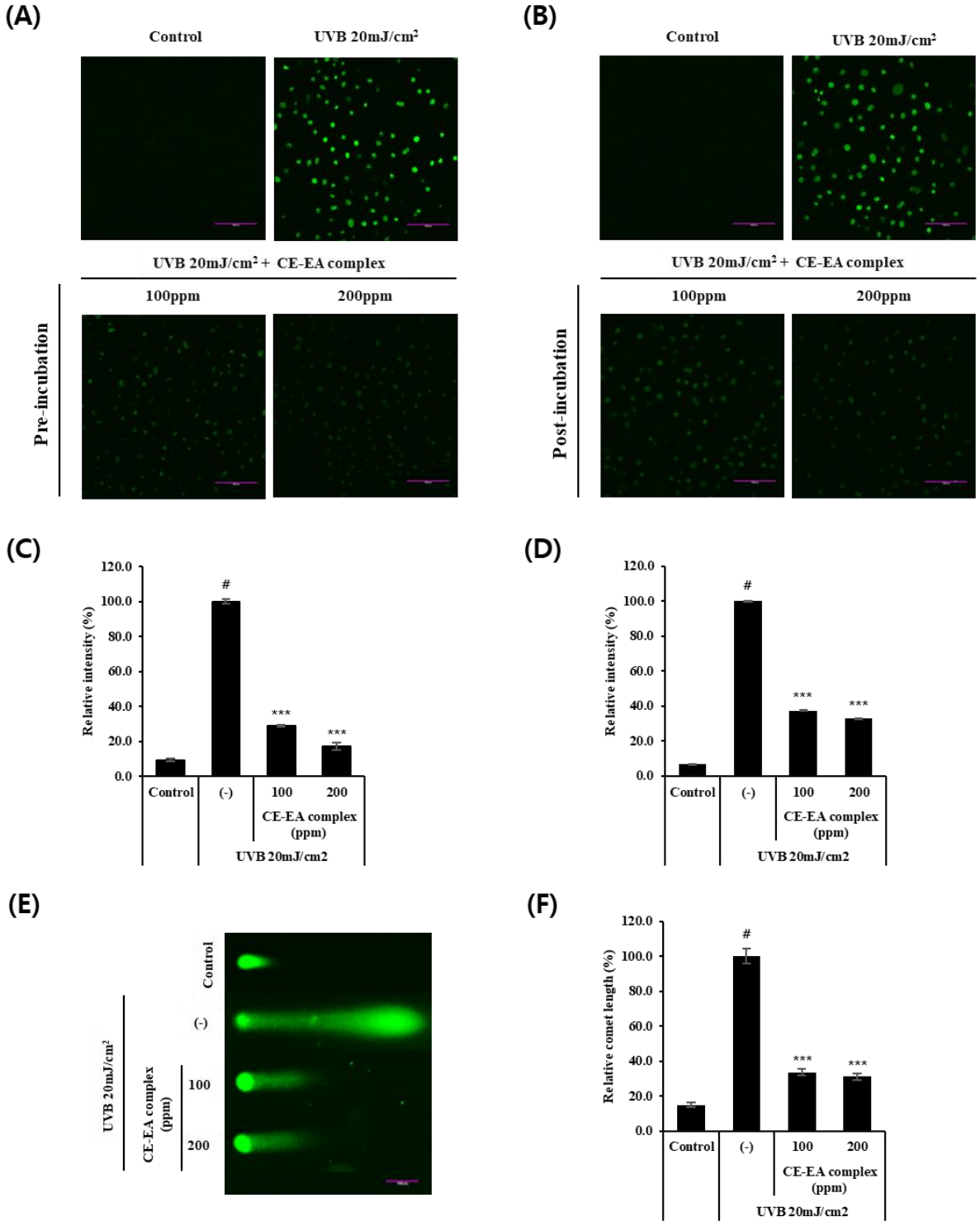

Figure 2. CE–EA complex protects against UVB-induced DNA damage. (A, B) Immunofluorescence staining for CPD to evaluate DNA damage following UVB exposure. Cells were treated with CE–EA complex (100 or 200 ppm) either before (pre-incubation) or after (post-incubation) UVB irradiation. (C, D) Quantification of CPD intensity. (E) Comet assay images showing DNA strand breaks induced by UVB and their attenuation by CE–EA complex. (F) Quantification of comet tail length. Data are presented as mean ± SD (#$p < 0.05$ vs. control group; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs. UVB-treated group).

Given that PGE_2 is a key mediator of UVB-induced inflammation, we further assessed the anti-inflammatory potential of the CE–EA complex by analyzing mRNA expression of pro-inflammatory cytokines in UVB-exposed keratinocytes. UVB exposure induced a significant increase in the expression of TNF-α, IL-1β, IL-6, and IL-10, indicating an inflammatory response (Figure 3B). Among the pro-inflammation cytokines, TNF-α exhibited the most substantial decrease following CE–EA complex treatment.

These findings suggest that the CE–EA complex effectively alleviates UVB-induced erythema and inflammation by suppressing PGE_2 production and modulating cytokine expression.

Paracrine protection by CE-EA complex against UVB-induced endothelial dysfunction

Since PGE_2 is a well-established vasodilatory and pro-inflammatory mediator, its upregulation strongly correlates with vascular permeability at the cellular level [13]. Endothelial layer disruption has been associated with an increase in microvascular permeability. To evaluate the paracrine impact of UVB-irradiated HeKn cells on endothelial function, HMEC-1 cells were cultured on transwell inserts and treated with conditioned medium (CM) from UV-irradiated HeKn cells, with or without CE-EA complex treatment. As shown in Figure 4A, exposure to CM from UVB-irradiated HeKn cells enhanced endothelial permeability compared to the untreated CM control. However, when HMEC-1 cells were exposed to HeKn CM treated with the CE-EA complex, the permeability increase was markedly alleviated, suggesting that the CE-EA complex mitigates UVB-induced vascular barrier disruption via paracrine mechanisms.

In addition, we examined the effects of CM derived from UV-irradiated HeKn cells treated with the CE-EA complex on endothelial gene expression in HMEC-1 cells. We first analyzed the mRNA expression of vascular endothelial growth factor (VEGF), a potent inducer of angiogenesis and vascular permeability. As shown in Figure 4B, VEGF expression was significantly increased by UVB irradiation but markedly suppressed by treatment with the CE-EA complex, suggesting attenuation of erythema-associated vascular activation. Similarly, the expression levels of VE-cadherin (Figure 4C) and Claudin-5 (Figure 4D), crucial components of vascular barrier integrity, were decreased upon UVB exposure but recovered following treatment with CE-EA complex-containing HeKn CM. Notably, Claudin-5 expression was restored to levels comparable to those of the untreated control group, indicating a protective effect on endothelial function.

We further investigated the impact of CE–EA complex-treated CM on inflammatory responses associated with erythema in HMEC-1 cells. The expression of VCAM-1 (CD106), a key adhesion molecule involved in immune cell recruitment, was significantly increased by UVB irradiation but was not affected by CE-EA complex treatment (Figure 4E). In contrast, ICAM-1 (CD54) expression, which was also elevated upon UVB exposure, was notably reduced by CE-EA complex-containing CM (Figure 4F), suggesting its involvement in mitigating inflammatory cell adhesion and vascular inflammation.

Furthermore, we analyzed the expression of pro-inflammatory cytokines, including IL-6 (Figure 4G), IL-8 (Figure 4H), TNF-α (Figure 4I), and IL-1β (Figure 4J). These cytokines were upregulated in HMEC-1 cells treated with conditioned medium from UVB-irradiated HeKn cells, whereas treatment with the CE-EA complex led to a substantial reduction in IL-6 and IL-1β levels.

These results collectively indicate that the CE-EA complex mitigates UVB-induced vascular barrier dysfunction and inflammatory activation in endothelial cells by modulating paracrine signals from keratinocytes, thereby suppressing key inflammatory mediators involved in erythema development.

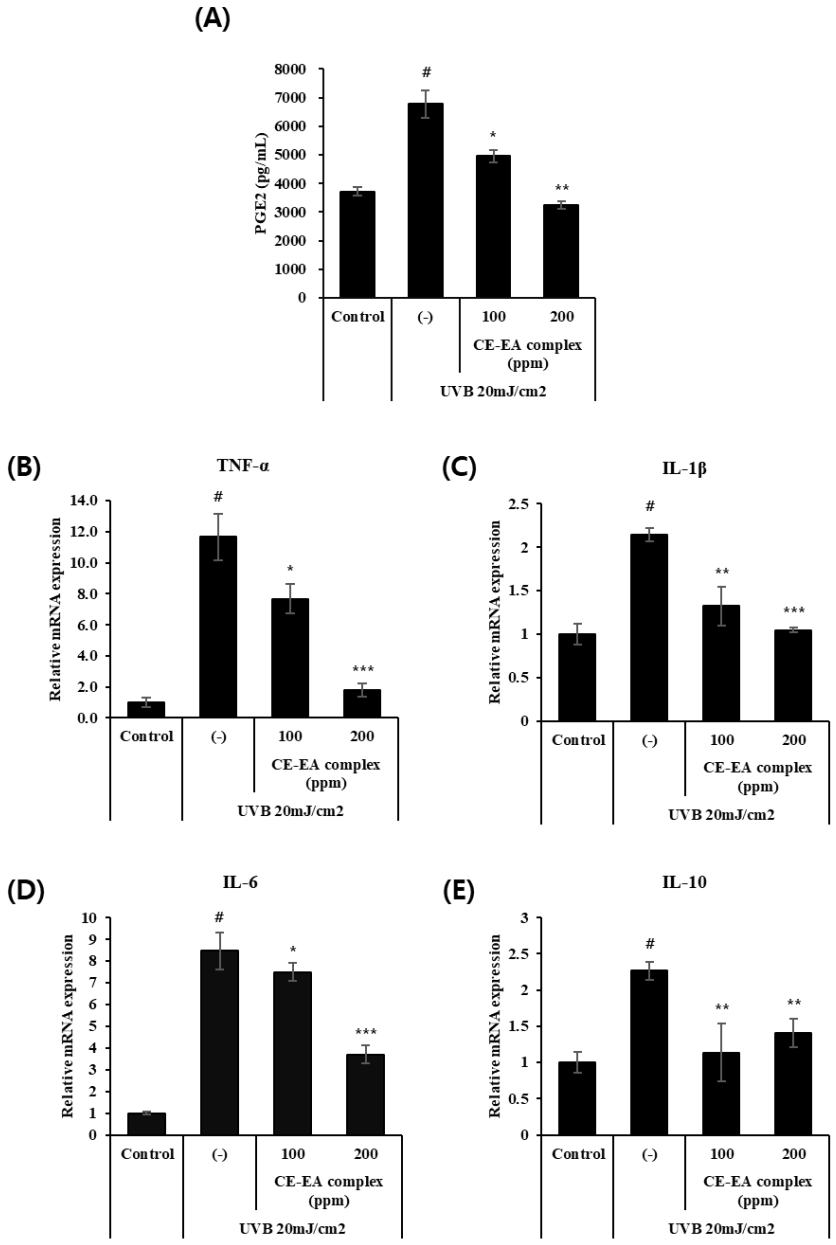

Figure 3. CE-EA complex reduces UVB-induced erythema and inflammation. (A) Enzyme-Linked Immunosorbent Assay (ELISA) of PGE_2 in UVB-treated HeKn cells with or without CE-EA complex treatment. (B) mRNA expression levels of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6 and IL-10) in UVB-irradiated HeKn cells treated with CE-EA complex. Data are presented as mean ± SD (#p < 0.05 vs. control group; *p < 0.05, **p < 0.01, ***p < 0.001 vs. UVB-treated group).

Inhibitory effect of CE-EA complex on UVB-induced inflammatory hyperpigmentation (PIH)

Post-inflammatory hyperpigmentation (PIH) refers to darkening of the skin that occurs as a result of inflammation or injury [14]. This condition is often persistent because melanin pigments, once produced, can migrate to deeper layers of the skin, such as the dermis, where they are more difficult to remove. To model PIH, conditioned medium (CM) from UVB-irradiated HeKn cells was applied to melanocytes after UVB exposure, which promotes pigmentation.

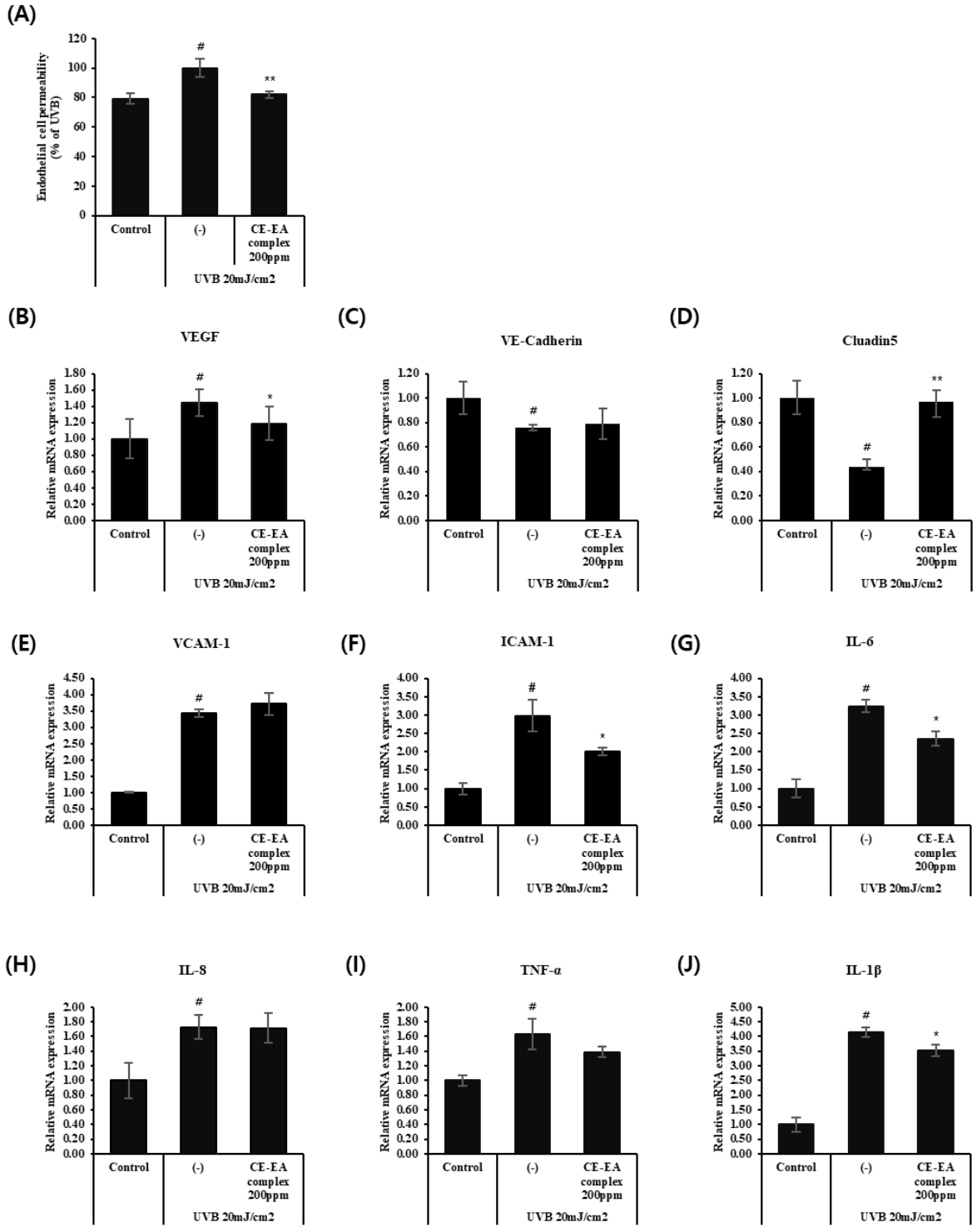

Figure 4. Paracrine protection by CE-EA complex against UVB-induced endothelial dysfunction. (A) Endothelial permeability was assessed using a transwell system after treatment with conditioned medium from UVB-irradiated HeKn cells with or without CE-EA complex. (B–F) mRNA expression levels of VEGF, VE-cadherin, Claudin-5, VCAM-1 and ICAM-1 were measured to evaluate vascular activation and barrier integrity. (G–J) mRNA expression of IL-6, IL-8, TNF-α, and IL-1β were examined to assess inflammatory responses. Data are presented as mean ± SD (#$p < 0.05$ vs. control group; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs. UVB-treated group).

To assess whether the CE-EA complex has a direct whitening effect on melanocytes, HeMn-DP were treated with 200 ppm CE-EA complex alone. As shown in Figure 5A, treatment with CE-EA complex did not affect melanin content, suggesting that the CE-EA complex does not have intrinsic whitening effects on melanocytes. However, to investigate its potential effect on post-inflammatory hyperpigmentation (PIH), melanocytes were treated with conditioned medium (CM) from UVB-irradiated HeKn cells. As shown in Figure 5B, UVB exposure significantly increased melanin content in melanocytes, confirming the development of PIH. Treatment with 200 ppm of CE-EA complex in this model effectively reduced the increased melanin levels to baseline, indicating that the CE-EA complex has a significant inhibitory effect on PIH. This suggests that the CE-EA complex could be a potential therapeutic agent for PIH, particularly in cases where pigmentation has deeply affected the skin and is more difficult to fade.

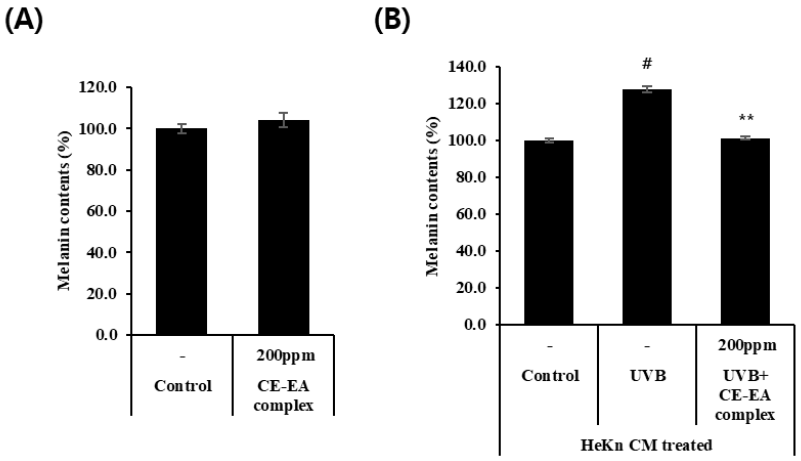

Figure 5. Inhibitory effect of CE-EA complex on UVB-induced inflammatory hyperpigmentation (PIH). (A) HeMn-DP treated with CE-EA complex (B) HeMn-DP treated with conditioned medium from UVB-irradiated HeKn cells with or without CE-EA complex. Data are presented as mean ± SD (#$p < 0.05$ vs. control group; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs. UVB-treated group).

4. Discussion

Recent studies have highlighted the limitations of conventional sunscreens, which provide only prophylactic photoprotection and lack efficacy in repairing DNA damage once it has occurred [15]. The use of a compound mixture represents a promising strategy for achieving both photoprotection and repair of UV-induced skin damage. A previous study demonstrated that a combination of rutin and ascorbic acid protected keratinocytes and fibroblasts from UVA and UVB-induced damage through antioxidant and anti-apoptotic mechanisms [16]. In the present study, co-treatment with the CE–EA complex showed superior cytoprotective effects compared to individual compounds. Notably, the complex significantly inhibited cyclobutane pyrimidine dimer (CPD) formation when applied both before and after UVB exposure. These results indicate that the CE–EA complex not only prevents UVB-induced damage but also promotes DNA repair, offering dual functionality that may overcome the limitations of conventional chemical sunscreens.

UV-induced skin damage affects not only the skin barrier but also neighboring cells, such as dermal vascular endothelial cells, and contributes to the acute burning sensation following UV exposure [17]. In this study, the CE–EA complex significantly reduced the expression of inflammatory mediators—including TNF-α, PGE_2, IL-10, IL-1β and IL-6—in keratinocytes. To assess paracrine effects, conditioned media from UV-irradiated keratinocytes were applied to endothelial cells. Media from UV-irradiated keratinocytes increased vascular permeability

and elevated inflammation-related markers such as VEGF, IL-6, and IL-1β. In contrast, media from CE–EA-treated keratinocytes suppressed these effects. These results suggest that the CE–EA complex mitigates vascular inflammation by downregulating keratinocyte-derived pro-inflammatory mediators. VEGF, a key mediator of UVB-induced burning, promotes angiogenesis and increases UV sensitivity [4]. Vascular permeability can also rise due to reduced expression of endothelial junction proteins, including tight junction protein claudin-5, adherens junction protein VE-cadherin [18]. In this study, conditioned media from UV-irradiated keratinocytes reduced claudin-5 and VE-cadherin expression in endothelial cells, while CE–EA treatment restored claudin-5 levels. These findings indicate that the CE–EA complex protects both the skin barrier and adjacent vascular cells, demonstrating multifaceted protective effects against UV-induced damage.

Post-inflammatory hyperpigmentation (PIH) is a common outcome of UV-induced inflammation, where inflammatory mediators such as PGE2 stimulate melanogenesis [19]. In PIH condition, melanosome can migrate into the dermis, which can make pigment removal more difficult [14]. In this study, melanocytes treated with conditioned media from UV-irradiated keratinocytes showed increased melanin production, whereas treatment with media from CE–EA-treated keratinocytes significantly reduced melanin levels. Direct treatment of melanocytes with the CE–EA complex at the same concentration did not significantly reduce melanin production compared to control group. These results suggest that the CE–EA complex more effectively suppresses melanin production in an inflammatory microenvironment, likely via modulation of keratinocyte-derived signals induced by UVB. Further studies are needed to clarify the underlying mechanisms of CE–EA complex on PIH.

5. Conclusion

The CE–EA complex, composed of *Camellia japonica* pericarp extract and 3-O-ethyl-L-ascorbic acid, provides multifaceted protection against UVB-induced skin damage. It not only prevents DNA damage and inflammation in keratinocytes but also mitigates secondary effects such as vascular permeability and melanogenesis through modulation of paracrine signaling. By combining protective and reparative functions, the CE–EA complex demonstrates strong potential as a functional ingredient in after-sun care products aimed at alleviating erythema, and reducing the risk of post-inflammatory hyperpigmentation.

6. References

[1] Fisher GJ et al., Arch Dermatol. 2002;138(11):1462-70.
[2] A.P. Schuch et al., Free Radic Biol Med. 107 (2017) 110-124.
[3] Muhammad M. Bashir et al., J Invest Dermatol. 2009 April ; 129(4): 994–1001
[4] Hirakawa S et al., Blood. 2005;105(6):2392-2399
[5] R. Schreurs et al., Arch Toxicol. 76 (2002) 257–261.
[6] J.A. Ruszkiewicz, et al., Toxicol Rep. 4 (2017) 245-259.
[7] S.W. Shin et al., PLoS One. 8 (2013) e61971.
[8] Chieh-Chen Huang et al., Molecules. 2007 Aug 14;12(8):1845-58.
[9] Saki Kawashima et al., Sci Rep. 2018 Nov 1;8(1):16199.
[10] Zerbinati N et al., Life (Basel). 2021 Apr 29;11(5):406.
[11] Lee, H.H. et al., Biotechnol. 52, 270-277 (2011).
[12] Teixeira et al., Molecules 26, 2178 (2021).
[13] Trompezinski S et al., Inflamm Res. 2001 Aug;50(8):422–427.
[14] Park, J.Y. et al., Clinical and Experimental Dermatology, 2016 , 41(8), 857-860.
[15] Hanna Luze et al., Photodermatol., 2020 Aug 27;36(6):424–432
[16] Agnieszka Gęgotek et al., Arch Dermatol Res. 2019 Apr;311(3):203-219.
[17] Panich, U. et al., Stem Cells International, 2016, 7370642.
[18] Markus Puhlmann et al., J Transl Med. 2005 Sep 30; 3:37
[19] Tomita Y et al., J Invest Dermatol. 1991 Dec;97(6):1005-1011.