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RESEARCH ARTICLE

Ultraviolet B–Induced Skin Damage Is Mediated by Suppression of Protein Phosphatase 2B Activity

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A B S T R A C T

Calcineurin (Protein phosphatase 2B, PP2B) is a calcium-dependent serine/threonine phosphatase that regulates cellular survival and stress responses; however, its role in ultraviolet B (UVB)–induced skin damage remains incompletely understood. In the study, we investigated whether PP2B activity modulates UVB-induced apoptosis in skin. Normal epidermal keratinocytes (NHEKs) were exposed to UVB irradiation, followed by the assessment of intracellular Ca^{2+} levels, PP2B activity, and cell viability. At 8-12h post-irradiation, UVB significantly reduced intracellular Ca^{2+} concentration and PP2B activity in NHEKs, accompanied by a decrease in cell viability in NHEKs. Treatment with baicalein markedly attenuated UVB-induced apoptosis and restored PP2B activity. Notably, pharmacological inhibition of PP2B abrogated the cytoprotective effects of baicalein, as evidenced by reduced cell viability and increased caspase-3 activity. In an in vivo mouse model, baicalein administration significantly reduced epidermal thickness and the number of TUNEL-positive cells in UVB-irradiated dorsal skin. Consistently, caspase-3 expression was markedly decreased in baicalein-treated skin tissues following UVB exposure. Collectively, these findings demonstrate that UVB-induced keratinocyte apoptosis is associated with reduced PP2B activity, and that baicalein attenuates UVB-induced skin damage at least in part by preserving PP2B homeostasis. Our results identify PP2B as a critical regulator of UVB-induced skin injury, suggesting it as a potential therapeutic target for preventing photodamage.

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INTRODUCTION

Calcineurin is a protein phosphatase regulated by calcium/calmodulin (Duan *et al.,* 2024). Calcium is an essential ion in keratinocyte differentiation and proliferation. It regulates the expression of genes and the corresponding proteins in keratinocytes (Tsukui *et al.,* 2022). PP2B serves as a crucial regulatory enzyme in calcium (Ca^{2+}) signal transduction (Luan and Wang, 2021; Rusnak and Mertz, 2000), thereby regulating various cellular processes, including inflammation, apoptosis, and tumorigenesis. Additionally, it plays a central regulatory role in skin disorders (Dotto, 2011; Kusumaningrum *et al.,* 2018; Wu *et al.,* 2018). In canine and feline skin, PP2B regulates critical cellular processes including inflammation, apoptosis, and immune responses, making it a key target in atopic dermatitis, vesicular cutaneous lupus erythematosus (VCLE), and keratoconjunctivitis sicca (KCS) (Marsella, 2005; Wichtowska and Olejnik, 2025)

The skin, our body's largest organ, serves as a crucial barrier, safeguarding us from environmental assaults (Sutter *et al.,* 2009; Han *et al.,* 2014; Kwon *et al.,* 2025). Keratinocytes, the predominant cells in the skin, serve as the primary barrier of defense against environmental stressors, including toxins, microbes, and solar radiation (Gutowska-Owsiak and Ogg, 2012; Piipponen *et al.,* 2020; Ortiz-Lopez *et al.,* 2022; Gentry, 2025). Ultraviolet (UV) radiation is the main environmental factor that induces skin damage, skin aging, and cancer (Ansary *et al.,* 2021; D'Orazio *et al.,* 2013; Narayanan *et al.,* 2010; Rittié and Fisher, 2002). Among the types of UV radiation in sunlight, mid-wave ultraviolet-B (280-320nm) is biologically highly active (Parrish *et al.,* 1982; Verma *et al.,* 2024) and cause photodamages triggered by UVB-induced DNA damage, inflammations and oxidative stress (Chen *et al.,* 2025a). Recent study showed that UVB induces epidermal DNA lesions, ROS generation and inflammatory reactions that lead to solar dermatitis and carcinogenesis (Chen *et al.,*

2025a). Moreover, long term UVB exposure causes reduced MiRNAs/lncRNAs causing sking photodamage (Chen *et al.,* 2025b).

Recent studies have demonstrated that PP2B inhibitors can alleviate UVB-induced inflammation and apoptosis across various cell types, underscoring the enzyme's crucial role in cellular stress responses (Yarosh *et al.,* 2005; Xu *et al.,* 2018). For example, Topical calcineurin inhibitors, including pimecrolimus, offer a promising alternative to corticosteroids for managing cutaneous lupus erythematosus (CLE) without causing skin atrophy. They also reduce UVB-induced DNA damage by acting as UVB filters while preserving DNA repair processes (Alshathri *et al.,* 2025). Conversely, Cao *et al.* (2012) reported that the PP2B inhibitor FK506 induced apoptosis of skin fibroblasts. Some reports indicate that the activity of PP2B is reduced by UV irradiation in skin, and this suppression promotes tumor growth in keratinocytes. Another recent study documented psoriasiform lichenoid dermatosis (PLD) in 28 PP2B inhibitor-treated dogs with universal *S. pseudintermedius* association, highlighting CNI complications (Davis *et al.,* 2026). Moreover, calcineurin inhibitors like cyclosporine and tacrolimus impair UV-induced DNA repair, contributing to an increased risk of skin cancer in transplant patients (Ume *et al.,* 2020). Another investigation found that calcineurin inhibitors inhibit the nuclear localization of NFAT induced by UV and impair DNA repair, raising concerns about their potential cancer risk, even when used locally (Canning *et al.,* 2006). Although these findings highlight PP2B's complex role, no studies have conclusively shown how PP2B activity specifically changes in normal human epidermal keratinocytes (NHEKs) after UVB exposure or how this change correlates with apoptosis in these cells.

While PP2B inhibitors have been studied in UVB contexts, no research has examined how PP2B activity itself changes in normal human epidermal keratinocytes (NHEKs) after UVB exposure or whether this correlates with apoptosis in these primary cells**.** In this study, we demonstrate that UVB irradiation induces a marked reduction in PP2B activity, correlating with decreased intracellular Ca^{2+} concentrations and increased apoptosis in NHEKs. Furthermore, PP2B is identified as a key player in UVB-induced skin injury. If we can learn to manage its activity, we could unlock a powerful new strategy for preventing UVB-damage. These results highlight the therapeutic potential of targeting PP2B in protecting skin cells from UVB-related injury.

MATERIALS AND METHODS

Cell culture: Normal human epidermal keratinocytes (NHEKs; neonatal; Lonza) were seeded on collagen-coated plates at a density of 3.5×10^3–1×10^4 cells/cm² and used when they reached 70–90% confluence.

Animals and experimental design: BALB/c male mice (21–25g) were used in this animal study, mice were maintained in four groups (n=5 per group): control, UVB alone, UVB+0.5mM baicalein, and UVB+1mM baicalein. UVB irradiation (400mJ/cm²) was applied to a shaved dorsal skin area (1×1.5cm²) once every 3 days. Baicalein was topically administered daily for 10 days following UVB exposure. Skin samples were collected 24h after the final irradiation. Baicalein concentrations (0.5 and 1mM) were selected based on direct extrapolation from our in vitro cytoprotective range (25-100μM) that aligns with previous topical mouse skin studies (Zhang *et al.,* 2014) demonstrating UVB protection with preliminary experiments confirming excellent local tolerability (no irritation or systemic toxicity).

Ethics committee report: The study was approved by the Animal Experiments Local Ethics Committee of Jeonbuk National University (Approval No: (JBNU-2020-0150).

UVB irradiation and treatments in vitro: NHEKs were seeded at 1×10^5 cells/well in 24-well plates. Prior to UVB exposure, culture media were replaced with PBS, and cells were irradiated with UVB (100mJ/cm²) using a CL-1000UV crosslinker calibrated with radiometer regularly following the manufacturer's protocol. Fresh medium containing baicalein was added immediately after irradiation. For PP2B inhibition, cells were pretreated with FK506 (1μM) or cyclosporine A (1μM) for 1h before and after baicalein treatment. The method was influenced by prior studies (Khalil and Shebaby, 2017; Gag *et al.,* 2023).

Measurement of intracellular Ca^{2+}: Intracellular Ca^{2+} levels were assessed using Fluo-4 AM (Hong *et al.,* 2024). UVB-exposed NHEKs were treated with Fluo-4 AM for 40min, washed with HBSS, and analyzed using a flow cytometry-based detection system (excitation/emission: 488/530nm)

PP2B activity assay: Calcineurin (PP2B) activity was measured in a commercial assay kit (Moon *et al.,* 2021). Following UVB exposure, cells were lysed, and phosphatase activity was quantified by measuring the release of free phosphate at 620nm using a malachite green-based method.

Cell viability and cytotoxicity assays: Cell viability was evaluated by crystal violet staining, followed by solubilization in SDS and measurement of absorbance at 550nm (Hong *et al.,* 2023). Cytotoxicity was assessed by measuring lactate dehydrogenase (LDH) release into culture supernatants using a commercial LDH detection kit (Zinnah *et al.,* 2025).

Western blot analysis: Total proteins were quantified by BCA assay, separated by SDS-PAGE, transferred to NC membranes, and bound with antibodies against β-actin, caspase-3, and phospho-Bcl-10. Whole process was carried out following the previously explained protocol (Hong *et al.,* 2024).

Draize scoring and histological analysis: Skin damage was evaluated using a Draize scoring system (range: 0-8) (Yamate *et al.,* 2017). Skin sections were subjected to hematoxylin and eosin after fixation with 4% paraformaldehyde. Epidermal thickness was measured using ImageJ software.

TUNEL assay: Apoptotic cells in skin tissues were detected using a fluorometric TUNEL assay. The

fluorescence intensity was assessed using ImageJ (Hong *et al.,* 2024).

Statistical analysis: Data are presented as Mean±SD from at least three independent biological experiments. For cell-based assays, each biological experiment included triplicate wells per condition. Western blot band intensities were quantified from three independent experiments using densitometry, normalized to β actin, and expressed as fold change relative to control. Statistical significance was assessed by one way ANOVA followed by Tukey's post hoc test, with $P<0.05$ considered significant.

RESULTS

UVB irradiation reduced calcineurin activity in NHEKs: Calcineurin is regulated by intracellular Ca^{2+} concentration (Rusnak and Mertz, 2000). NHEKs were irradiated at 100mJ/cm^2 and then stained with a calcium indicator at the indicated time points. UVB irradiation reduced the intracellular Ca^{2+} concentration in NHEKs at 8 h and 12 h post-treatment (Fig. 1A and B). Likewise, a time-dependent significant reduction of PP2B activity was observed in the UVB-irradiated NHEK pellets at 8 and 12h post-treatment (Fig. 1C). Notably, our measurements were performed at relatively late time points (1–12 h) after UVB exposure. Thus, the observed reduction in intracellular Ca^{2+} likely reflects a delayed phase of Ca^{2+} dysregulation rather than the immediate transient Ca^{2+} increase reported in earlier studies (Masaki *et al.,* 2009; Seo *et al.,* 2020). To further confirm the reduction of PP2B activity, we evaluated the level of p-Bcl-10, which is specifically dephosphorylated at ser-138 by PP2B (Thys *et al.,* 2018). In line with the reduction of PP2B activity, the levels of p-Bcl-10 increased in a time-dependent manner in the UVB-irradiated cells (Fig. 1D and E). These findings demonstrate that UVB treatment reduces the intracellular Ca2+ concentration and subsequently decreases PP2B activity in NHEKs.

UVB irradiation induced apoptosis in NHEKs: Using crystal violet staining, we evaluated the viability of UVB-irradiated normal human epidermal keratinocytes (NHEKs). Both morphological assessment and staining results revealed that UVB-irradiated cells exhibited a significant, time-dependent reduction in cell viability compared to non-irradiated cells (Fig. 2A, B, and C). We also measured lactate dehydrogenase (LDH) release into the media as an indicator of cell membrane damage. A time-dependent elevation in LDH was observed, with levels significantly rising from 4h after UVB irradiation (Fig. 2D). UVB irradiation also induces apoptosis in the epidermis. To study UVB-irradiated apoptosis, we assessed the levels of caspase-3 (cas-3). The total form of cas-3 (35 kDa) decreased, and the fragmented forms of cas-3 appeared (17 and 19 kDa) after 4h of UVB irradiation (Fig. 2E). These results indicate that UVB irradiation reduces cell viability, compromises membrane integrity, and triggers apoptotic pathways in NHEKs.

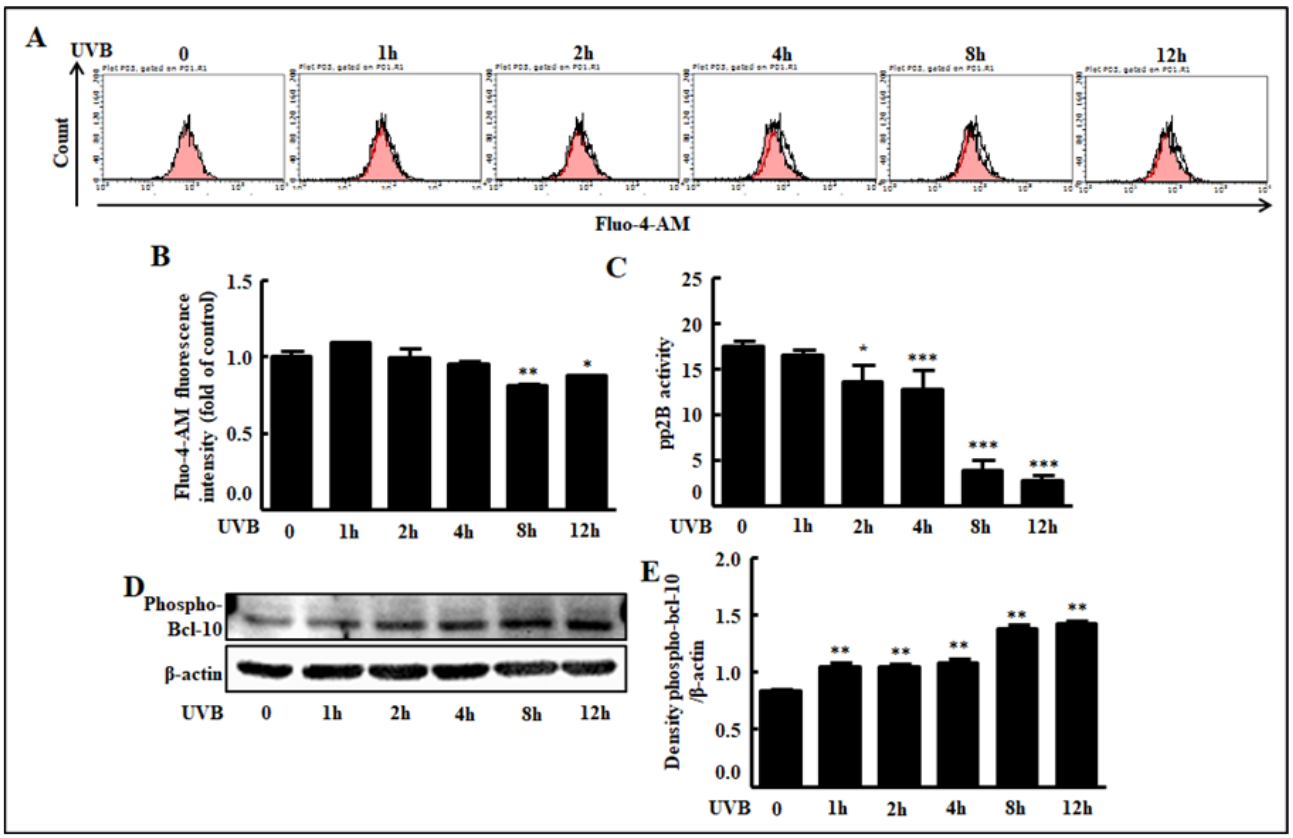

Fig. 1: UVB irradiation reduced PP2B activity in NHEKs. NHEKs were irradiated with 100mJ/cm^2 UVB. Unexposed NHEKs were the control (0 h). (A, B) At indicated time points (1, 2, 4, 8, and 12 h), intracellular Ca^{2+} concentration was measured by staining with Fluo-4-AM (5 μM) for 30 min. After staining, the changes in the intracellular Ca^{2+} concentration were evaluated using flow cytometry. (C) PP2B activity was measured with the PP2B (calcineurin) activity assay kit at the same time points after irradiation. (D, E) Levels of p-bcl-10 were assessed by western blotting; the band densities were measured. The bar graph indicates the mean ± SD (n=3). *P<0.05 and **P<0.01 indicate significant differences between the control and each treatment group.

Inhibiting PP2B reduction prevented the UVB-induced apoptosis in NHEKs: We assessed the effect of baicalein on PP2B/Calcineurin activity in UVB-irradiated NHEKs. NHEKs were treated with baicalein (100μM) for 8h after 100mJ/cm^2 UVB irradiation. Baicalein treatment inhibited the reduction in intracellular Ca^{2+} concentration (Fig. 3A and B) and effectively suppressed the UVB-induced decline of PP2B activity (Fig. 3C). Additionally, western blot analysis of whole cell lysates from UVB-irradiated NHEKs, subsequently treated with baicalein, revealed that baicalein inhibited the UVB-induced increase in p-Bcl-10 levels (Fig. 3D and E).

To evaluate the role of baicalein on UVB-induced apoptosis, NHEKs were treated with varying concentrations of baicalein (0, 25, 50, and 100μM) after 100mJ/cm^2 UVB irradiation. Cell viability was assessed 12 hours after UVB irradiation. In Fig. 4, baicalein treatment effectively improved cell viability, as indicated by morphological observations (Fig. 4A), crystal violet staining (Fig. 4B and C), and a reduction in LDH levels (Fig. 4D). Furthermore, western blot analysis indicated that baicalein reduced the levels of cleaved caspase-3 while increasing the total caspase-3 (Fig. 4E) in UVB-irradiated NHEKs. The findings suggest that baicalein protects against UVB-mediated damage in NHEKs.

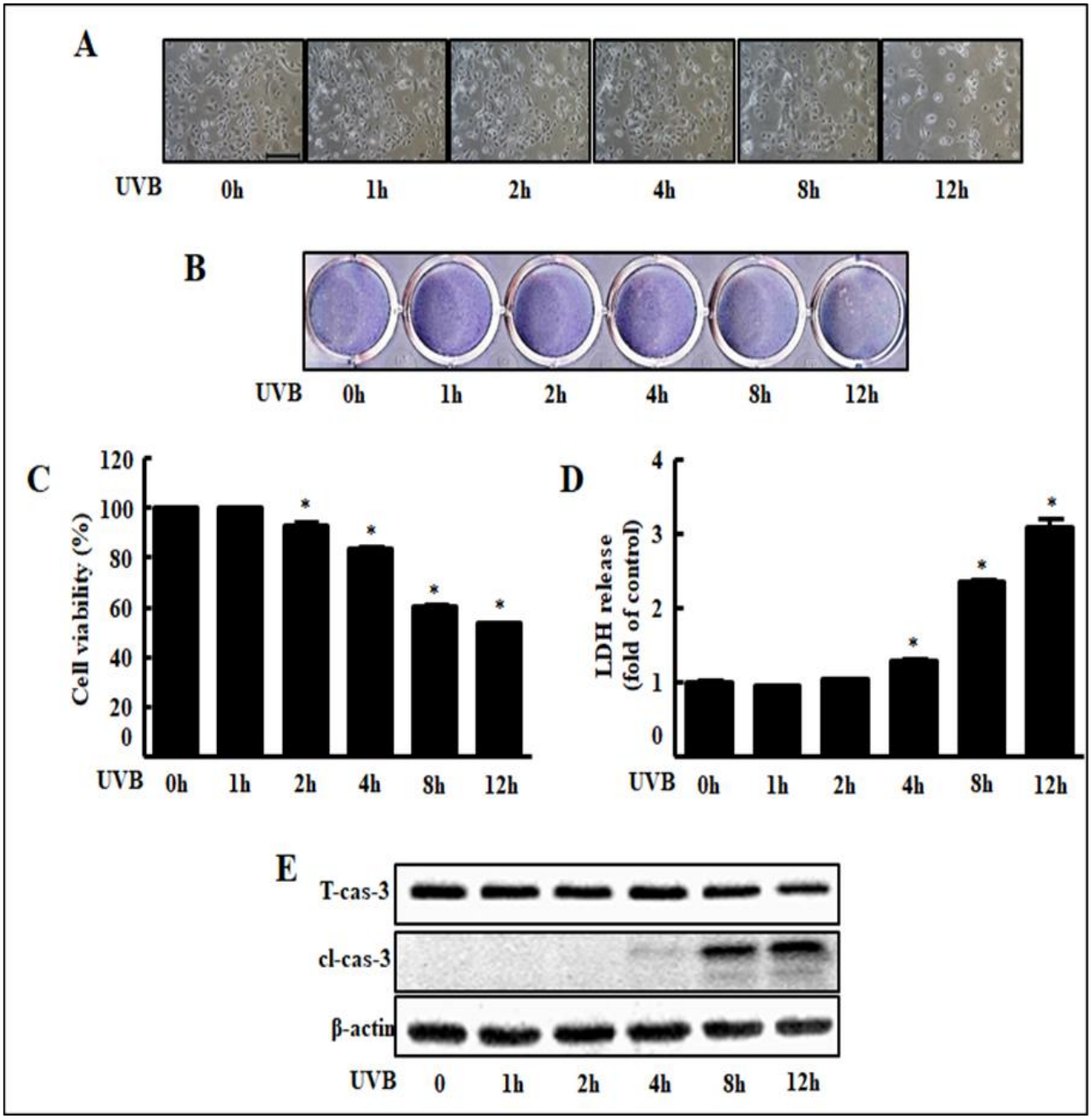

Fig. 2: UVB irradiation induced apoptosis in NHEKs. NHEKs were irradiated with 100mJ/cm^2 UVB. Control: Unexposed NHEKs (0 h). (A) At the indicated time points (1, 2, 4, 8, and 12 h), cell morphology was photographed using a light microscope (×100), with a scale bar of 50 μm. (B) Morphological image after staining with crystal violet. (C) Viability of the control cells was set at 100%, and the relative viability of the treatment was measured (D) Lactate dehydrogenase (LDH) assay was used to measure the LDH released into the medium. LDH release of control was set at 1 (E). Western blot indicates the caspase-3 activity after UVB irradiation. β-actin was used as a loading control. The bar graph indicates the Mean±SD (n = 3). *P<0.05, indicating a significant difference between the control and each treatment group.

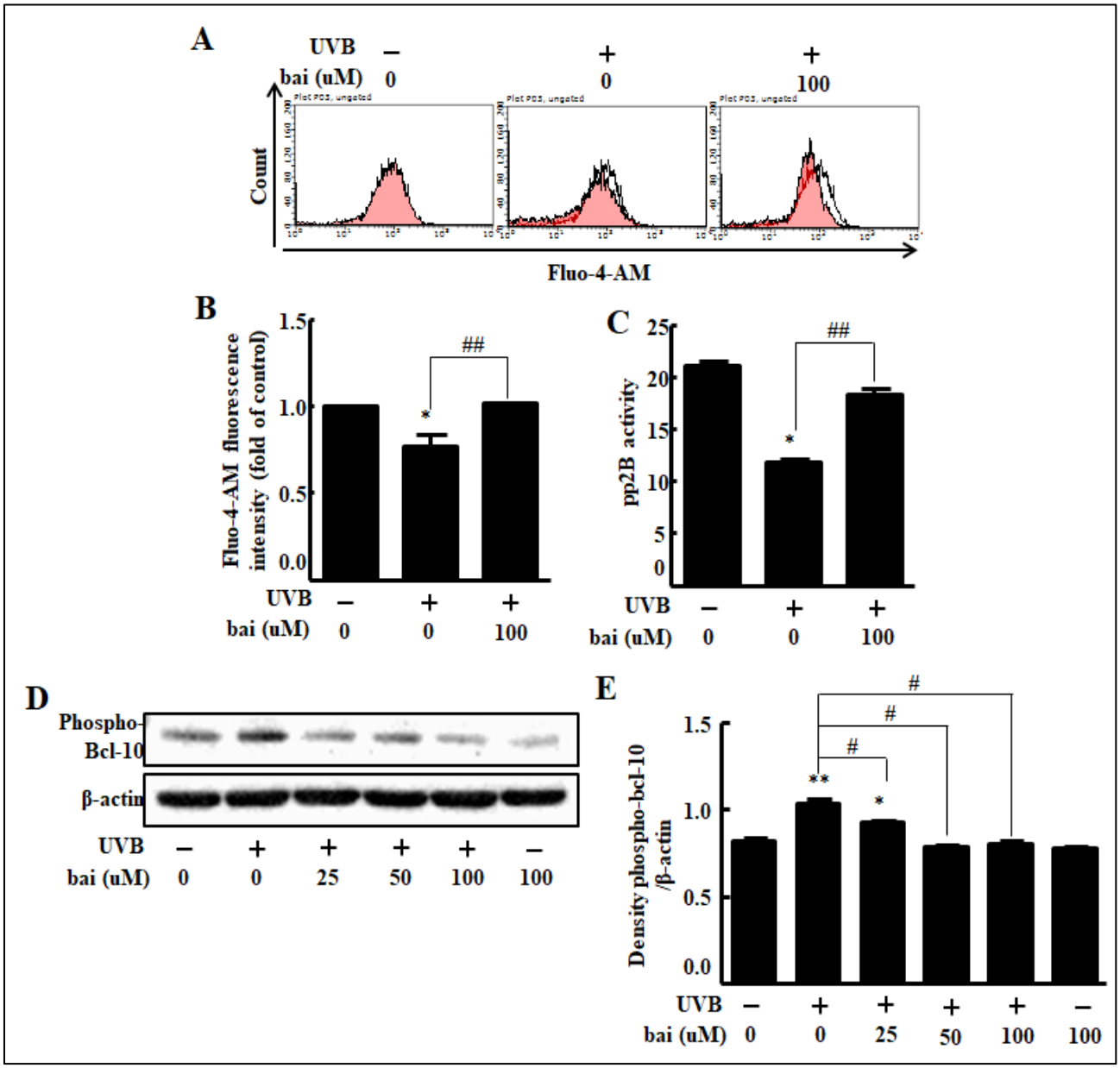

Fig. 3: Baicalein treatment inhibited the reduction of PP2B activity in NHEKs. NHEKs were exposed to 100mJ/cm^2 UVB, and then treated with baicalein (100 μM). Control: unexposed NHEKs without baicalein. (A, B) For intracellular Ca^{2+} concentration, NHEKs were stained with Fluo-4-AM (5 μM) for 30 min at 8 h after UVB irradiation and evaluated using flow cytometry. (C) PP2B activity was measured with the PP2B (calcineurin) activity assay kit at 8 h after irradiation. (D, E) NHEKs were incubated with baicalein (0, 25, 50, and 100 μM) 8 h after UVB exposure. Levels of p-bcl-10 were assessed by Western blot. The bar graph indicates the Mean±SD (n = 3). *P<0.05, **P<0.01 indicates a significant difference between the control and each treatment group, #P<0.05, ##P<0.01 indicates a significant difference between the UVB-irradiated group and the baicalein treatment group.

PP2B inhibitors suppressed the effects of baicalein in UVB-irradiated NHEKs: We investigated the role of PP2B/Calcineurin in UVB-induced apoptosis following baicalein treatment using the PP2B-specific inhibitors FK506 and Cys A. The cell viability significantly decreased in the viability of baicalein-treated NHEKs pretreated with FK506 or Cys A compared to those treated with baicalein alone (Fig.s 5A-C). Additionally, these pretreated cells exhibited a marked increase in LDH release, indicating enhanced cellular damage (Fig. 5D). Moreover, the administration of FK506 or Cys A counteracted the baicalein-induced reduction in caspase-3 cleavage, restoring its activity to levels observed in untreated cells (Fig. 5E). These findings underscore the crucial role of PP2B in mediating the protective effects of baicalein against UVB-induced apoptosis.

Baicalein attenuated the UVB-induced skin damage: To complement our *in vitro* studies, we assessed the *in vivo* protective effect of baicalein on UVB-induced skin damage in mice. Shaved dorsal skin was exposed to 400mJ/cm^2 with or without baicalein treatment. UVB irradiation induced skin erythema and edema in the control mice that were not treated with baicalein. However, the skin symptoms were inhibited by baicalein treatment, as confirmed by the Draize score (Fig. 6A and B). The dorsal skin samples were stained with H&E for histological analysis. The epidermis of UVB-irradiated mouse skin was thicker than that of non-irradiated skin (Fig. 6C and D). However, this damage was attenuated by baicalein treatment (Fig. 6C and D). To assess apoptosis, UVB-irradiated skin samples were analyzed using the TUNEL stain and western blotting for cas-3. The number of

TUNEL-positive cells, an indicator of apoptosis, increased with UVB irradiation; in contrast, baicalein administration reduced the TUNEL-positive cells (Fig. 6E and F). Fragmented cas-3 levels were significantly elevated in the UVB-irradiated skin samples but lowered in the baicalein-treated samples (Fig. 6G). In conclusion, these findings confirm that baicalein protects against UVB-induced skin damage and apoptosis in both *in vitro* and *in vivo* models. This highlights baicalein's potential as a therapeutic agent for preventing UVB-induced skin injuries.

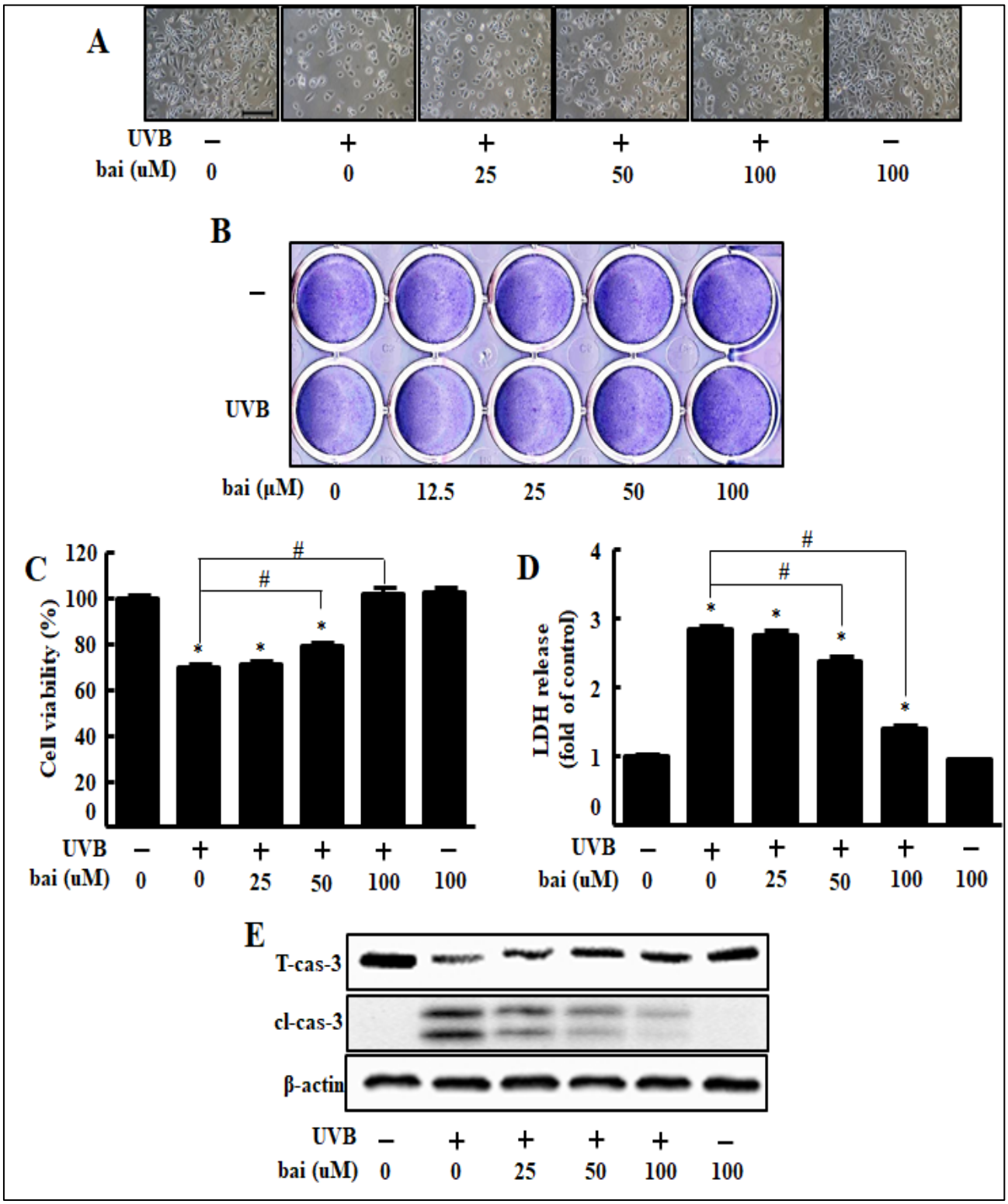

Fig. 4: Baicalein attenuated UVB-induced apoptosis in NHEKs. NHEKs were exposed to 100mJ/cm^2 UVB and were treated with baicalein (0, 25, 50, and 100μM). Unexposed NHEKs without baicalein and only baicalein (100μM) administered NHEKs without UVB were the controls. (A) Cell morphology under light microscope (×100), scale bar 50μm. (B) Morphology after staining with crystal violet. (C) Viability of the control cells was set at 100%, and the relative viability was measured. (D) Lactate dehydrogenase (LDH) assay was used to measure the LDH release. The LDH release of control was set at 1. (E) Western blot indicates cas-3 activity after UVB irradiation with or without baicalein treatment. β-actin was used as a loading control. Bar graph indicates Mean±SD (n=3). *P<0.05 indicates a significant difference between the control and each treatment group; # P<0.05, between the only UVB-irradiated group and each baicalein treatment group.

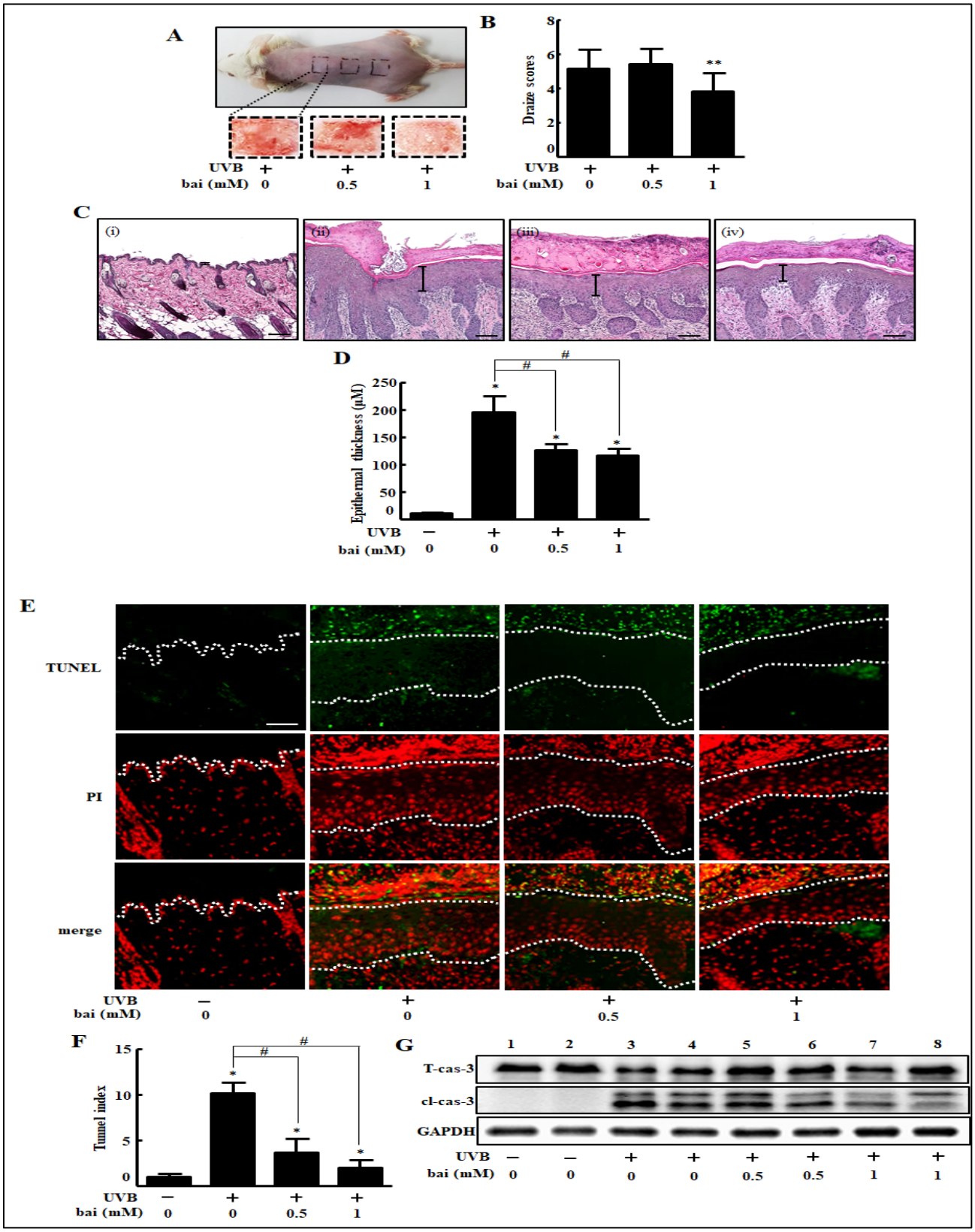

Fig. 6: Baicalein protected against UVB-induced skin damage. Seven-week-old male BALB/c mice were subjected to four groups (Group 1: normal control; Group 2: UVB-irradiated without baicalein treatment; Group 3: With 0.5 mM baicalein and UVB; Group 4: With 1 mM baicalein and UVB; n=5). Baicalein was administered daily to the dorsal skin of mice, and skin samples were harvested 24 hours after the last UVB irradiation. (A) Morphological images of the dorsal skin of mice before sample collection. (B) Skin damage was investigated with the Draize scoring system by five researchers. The bar graph indicates the Mean±SD (n=5). (C) Harvested skin samples were stained with H&E, scale bar 100 μm, and (D) Thicknesses of epidermis measured with Image J. Bar graph indicates the Mean±SD (n=3). *P<0.001 indicates a significant difference between the control and each treatment group; # P<0.001, between the only UVB-irradiated group and each baicalein treatment group. (E, F) Skin samples assessed with TUNEL stain. The nucleus was stained with PI, scale bar 100 μm. The TUNEL-positive cells were counted, and the bar graph indicated the fold change compared to the control (non-irradiated skin), with the Mean±SD (n=3). (G) Western blot data indicate cas-3 activity in skins after UVB irradiation with or without baicalein treatment. β-actin was used as a loading control. *P<0.05, **P<0.01 indicates a significant difference between the control and each treatment group; # P<0.05, between the just UVB-irradiated group and each baicalein treatment group.

DISCUSSION

In this study, we found that UVB irradiation reduced PP2B activity in NHEKs, and this process was correlated with UVB-induced apoptosis. UVB irradiation, similar to ionizing radiation therapy, induces acute skin inflammation mediated by multiple inflammatory pathways including NF-κB and COX-2 activation (Lee *et al.,* 2024). Previous studies report rapid Ca^{2+} increases immediately after UVB (Katsuyama *et al.,* 2021). Our 1-12h measurements capture a delayed Ca^{2+} decline that coincides with reduced PP2B activity and induced apoptosis, suggesting a biphasic Ca^{2+} dynamics linking late-phase dysregulation to cell death rather than initial UVB sensing. Inhibiting this reduction in PP2B activity using baicalein prevented UVB-induced apoptosis, and suppressing the blocking effect of baicalein with PP2B inhibitors reversed the apoptosis in UVB-irradiated NHEKs. The signaling pathway was further analyzed in an *in vivo* study, where baicalein administration was shown to prevent skin damage. Thus, we concluded that the activity of PP2B, which is regulated by Ca^{2+}/calmodulin, serves as a key determinant of UVB-mediated skin injury.

Calcium ions play a crucial role in signal transduction in keratinocytes (Lee *et al.,* 2018; Park *et al.,* 2024). Previous studies reported that the intracellular Ca^{2+} concentration increased immediately after UVB exposure (Huang *et al.,* 2017; Park *et al.,* 2019). Recent studies have investigated the effects of low extracellular Ca^{2+} concentration on UVB-induced apoptosis in keratinocytes (Kumar *et al.,* 1999; Sasaki *et al.,* 2005; Seo *et al.,* 2020) and depletion of ER Ca^{2+} stores (Farrukh *et al.,* 2014). PP2B inhibition can be considered as an alternate in skin damage therapy (Marsella, 2005; Banovic *et al.,* 2017). However, conflicting results have been reported for the effects of UV exposure on PP2B activity in the skin (Smit *et al.,* 2010; Musson *et al.,* 2011). Many PP2B inhibitors show adverse reactions to the skin, especially of companion animals (Davis *et al.,* 2026). Our results indicated that UVB irradiation reduced the activity of PP2B from 2 to 12h after UVB irradiation in NHEKs, which was also in line with the changes in intracellular Ca^{2+} concentration. As a protein phosphatase, PP2B dephosphorylates Bcl-10 on S138 (Rusnak and Mertz, 2000; Seumen *et al.,* 2021). Bcl-10 is a member of the CAR-BCL 10-MALT1 signalosome complex and is involved in NF-κB activation, which is a transcription factor of several pro-survival genes (Bhattacharyya *et al.,* 2011; Pires *et al.,* 2018; Seumen *et al.,* 2021). We found that there was an increase in p-Bcl-10 from 2 to 12h after UVB irradiation, indicating a decrease in PP2B activity. Furthermore, the viability of UVB-irradiated NHEKs decreased with increasing treatment duration. The cleavage of the apoptotic indicator cas-3 also occurred in a time-dependent manner. The results suggest that the activity of PP2B decreases under UVB exposure and that it facilitates UVB-induced apoptosis. The malachite green assay's sensitivity to free phosphate is complemented by our stringent Ca^{2+}/calmodulin-dependent conditions, complete inhibition by FK506/cyclosporine A, and concordant p-Bcl-10 changes, collectively confirming PP2B as the predominant phosphatase measured under our experimental conditions. Calcineurin classically regulates NFAT through dephosphorylation and nuclear translocation (Brun *et al.,* 2013; Lee *et al.,* 2018). Although NFAT localization or transcriptional activity was not directly examined in this study, future work incorporating NFAT immunolocalization or reporter assays will help further define whether PP2B-mediated protection under UVB stress is primarily transmitted through NFAT, Bcl-10/NF-κB, or additional downstream targets.

Plant-derived flavonoids protect against skin damage by reducing skin lesions of canine dermatitis (Massimini *et al.,* 2021; Qin *et al.,* 2024) and UVB induced skin damages (Mayangsari *et al.,* 2024). Baicalein, a bioactive flavonoid extracted from *Scutellaria baicalensis* has demonstrated protective effects against UVB irradiation, helping to mitigate skin damage and photoaging. (Oh *et al.,* 2016; Zhang *et al.,* 2014). It also shows therapeutic potential in treating sepsis by reducing TNF-alpha, superoxide anions, and iNOS production, likely through the inhibition of the NF-κB pathway. Additionally, baicalein exhibits antioxidative, anti-inflammatory, and anti-apoptotic properties, which mitigate ischemia-induced neurotoxicity and brain and retinal damage (Pan *et al.,* 2021). It has also been reported to increase intracellular Ca^{2+} concentration (Huang *et al.,* 2016; Lin *et al.,* 2021) and PP2B activity (Wang *et al.,* 2015). We observed that baicalein treatment inhibited the UV-induced reduction in PP2B activity and intracellular Ca^{2+} concentration. In addition, baicalein treatment inhibited the decrease in p-Bcl-10 levels, prevented UVB-induced apoptosis, and allowed extracellular Ca2+ influx. This finding is in agreement with the report that a high extracellular Ca^{2+} concentration blocks UVB-induced apoptosis. While our data demonstrate PP2B preservation as one critical component of baicalein's cytoprotection, these established antioxidant and anti-inflammatory actions likely contribute synergistically. Future studies dissecting these parallel pathways will clarify their relative contributions. These results suggest that inhibiting the reduction of PP2B activity protects against UVB-induced apoptosis. To confirm whether the protective effect of baicalein was mediated through the inhibition of PP2B activity reduction, we used PP2B inhibitors to suppress PP2B activation. Our results showed that treatment with FK506 or Cys A decreased cell viability, which was increased in response to baicalein. Overall, our results suggest that inhibiting the reduction in PP2B activity using baicalein protects against UVB-induced apoptosis. Furthermore, calcium signaling has been studied in the regulation of cell survival pathways under UVB stress. Previous studies have shown that calcium channel blockers can affect UVB-induced apoptosis (Jia *et al.,* 2018). Although we did not investigate the interaction of calcium channel blockers or calcium chelators with baicalein in this study, future studies could examine how calcium signaling blockers affect baicalein's protective role against UVB.

Finally, we investigated the protective effects of baicalein against UVB-induced skin damage in animals by assessing epidermal thickness, TUNEL-stained cells, and the level of caspase-3 fragmentation. UVB directly damages the epidermis of the skin, and the repair process through apoptosis is crucial for defense against UVB damage. Topical administration of baicalein on the dorsal skin decreased epidermis thickness and inhibited apoptosis.

Taken together, our results highlight the protective role of PP2B activity in countering UVB-induced apoptosis, with Ca^{2+} homeostasis playing a key role in this defense. Regulating PP2B activity presents a promising strategy for protecting against UV-mediated skin damage. Additionally, baicalein shows promise as a therapeutic agent by preserving PP2B activity under UVB exposure, warranting further research for its potential clinical applications and development as a skincare product. While our pharmacological approach using well-established calcineurin inhibitors (FK506, cyclosporine A) provides robust functional evidence for PP2B's critical regulatory role, complementary genetic approaches (e.g., siRNA knockdown, CRISPR/Cas9-mediated deletion, constitutively active PP2B) represent logical next steps to further delineate this pathway. These approaches will be essential for translating our mechanistic findings toward therapeutic applications, building directly upon the strong pharmacological foundation established here. In our in vivo model, topical baicalein significantly attenuated UVB-induced skin damage. Consistent with our in vitro findings showing preservation of PP2B activity, baicalein treatment markedly reduced epidermal thickness, TUNEL-positive cells, and caspase-3 activation in UVB-exposed skin. These results support a protective role of PP2B signaling in UVB-induced skin injury. However, direct measurement of PP2B activity in skin tissue was not performed in this study. Future studies including direct enzymatic assays and dose–response analyses will further clarify the mechanistic contribution of PP2B and optimize the therapeutic potential of baicalein in UVB-induced skin damage.

Conclusions: Our study demonstrates that baicalein effectively preserves PP2B activity in UVB-irradiated NHEKs, thereby protecting against UVB-induced skin damage. These findings highlight PP2B as a critical regulator of UVB-induced apoptotic signaling in epidermal keratinocytes. Baicalein emerges as a promising therapeutic candidate for mitigating UVB-mediated skin injury by modulating PP2B activity. Nevertheless, further studies are required to fully define baicalein's pharmacological profile in UVB models, including its antioxidant properties. Future investigations should also examine the effects of calcium channel modulation and genetic inhibition of PP2B to clarify its mechanistic role.

Conflicts of interest: The authors declare no conflict of interest.

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