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Coreopsis tinctoria Extract: A Natural Remedy for UVA-Induced Photodamage via Mitigation of Endoplasmic Reticulum Stress

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1. Introduction

Skin photodamage results from prolonged UV radiation exposure, leading to issues like erythema, inflammation, photoaging, and skin cancers [1]. UV radiation is categorized into UVA (320–400 nm) and UVB (280–320 nm), with UVA penetrating deeper and generating reactive oxygen species (ROS) that cause indirect DNA damage and activate inflammatory pathways. Effective strategies to mitigate UVA-induced damage are essential.

Endoplasmic reticulum (ER) stress arises from protein folding disruptions, leading to misfolded protein accumulation and potential cell apoptosis if excessive [2]. UV exposure induces ER stress through ROS, activating the unfolded protein response (UPR) in dermal fibroblasts and keratinocytes [3].

Coreopsis tinctoria (CT) is known for its antioxidant and anti-inflammatory effects due to bioactive flavonoids [4]. While okanin from CT protects against UVB damage [5], research on CT's effects against UVA damage is limited. This study explores CT’s potential to mitigate UVA-induced photodamage in human keratinocytes HaCaT by alleviating ER stress and apoptosis.

Materials and Methods

2.1 Cell Culture and UVA Radiation

HaCaT cells were cultured in 6-well plates with DMEM, 10% FBS, and 1% penicillin-streptomycin at 37°C in a 5% CO_2 atmosphere. Before UVA exposure, cells were washed and treated with 1 mL PBS. They were irradiated with 5 J/cm² using a UVA crosslinker, followed by treatment with extracts from CT.

2.2 Cell Apoptosis Assay

The apoptosis ratio was analyzed using the Annexin V-FITC Apoptosis Detection Kit (Beyotime, C1062L). Cells were harvested and resuspended in a buffer with Annexin V-FITC and propidium iodide per the manufacturer's instructions. Flow cytometry analysis was conducted using a Beckman CytoFlex flow cytometer, and data were analyzed with CytExpert software.

2.3 RNA Extraction and Quantitative PCR (qPCR) Analysis

Total RNA was extracted from the cells using the TransZol Up Plus RNA Kit (Transgen). The RNA was reverse-transcribed into cDNA with the 5X Evo M-MLV RT Reaction Mix (Agbio, AG11728). The cDNA was then used as a template for quantitative PCR (qPCR) analysis, performed with SYBR Mix (Yeasen, 11202ES).

2.4 Western Blot Analysis

Total proteins were extracted using RIPA buffer (Beyotime, P0025) with MG-132. Extracts were denatured in 5× SDS loading buffer, separated by SDS-PAGE, and transferred to PVDF membranes (Millipore). Membranes were blocked with 5% non-fat milk and incubated with primary antibodies, including anti-IRE1, anti-CHOP, and anti-NRF2. HRP-conjugated secondary antibodies were applied. Bands were visualized with ECL reagent and analyzed using ImageJ for protein expression quantification.

2.5 Bulk RNA Sequencing

HaCaT cells were treated as previously described, and total RNA was extracted using TRIzol™ Reagent (Gibco). mRNA was enriched with VAHTS mRNA capture beads. RNA was fragmented to 250-450 bp and converted into a cDNA library, with quality assessed using Qubit 4.0 and ABI QuantStudio. RNA-seq was conducted on the Illumina Novaseq™ 6000 platform, quantifying gene abundance using FPKM.

2.6 Statistical Analysis

Statistical analysis was performed using GraphPad Prism (version 9.0). Differences between groups were assessed using one-way ANOVA. Data are expressed as means ± standard deviations, with $p < 0.05$ considered statistically significant; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Results

3.1 Bulk mRNA Sequencing to Identify the Biological Function of CT upon UVA Exposure

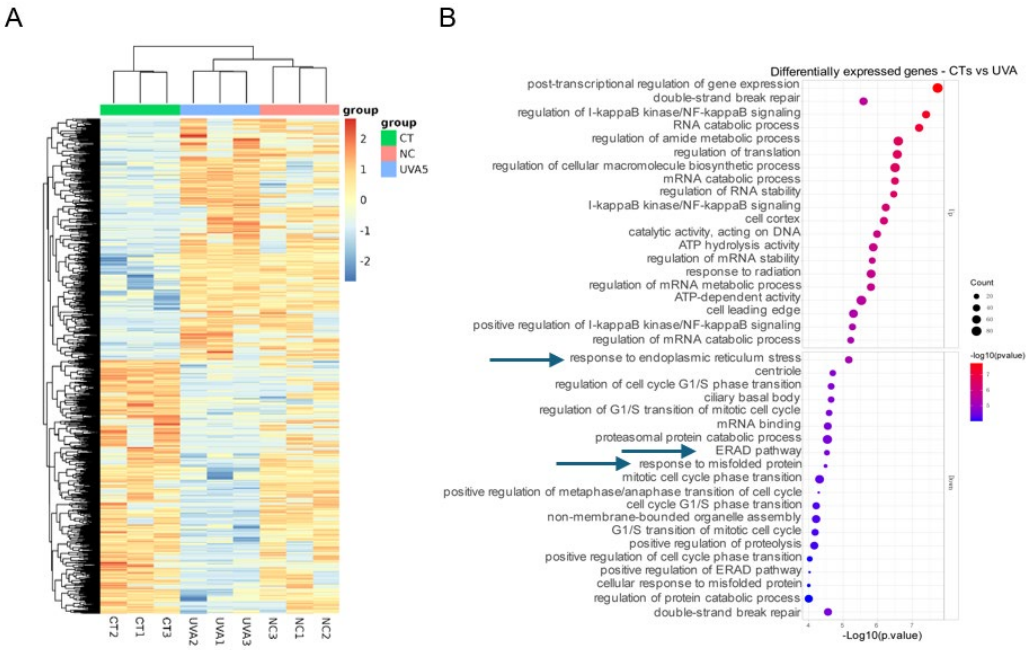
To find out the how CT is able to counteract the UVA photodamage, we performed mRNA sequencing on three groups: 5J UVA-irradiated HaCaT cells, CT-treated UVA-irradiated HaCaT cells, and non-irradiated control HaCaT cells. As illustrated in Figure 1A, heatmap indicated that the UVA-irradiated HaCaT cells, CT-treated UVA-irradiated HaCaT cells, and control HaCaT cells formed distinct clusters, separate from their respective controls (Figure 1A). Additionally, the DEGs underwent Gene Ontology (GO) enrichment analysis, revealing that the categories ‘response to ER stress’, ‘cellular response to misfolded proteins’, and the ‘ERAD

(ER-associated degradation) pathway' were significantly enriched among the DEGs between UVA-irradiated HaCaT cells and CT-treated UVA-irradiated HaCaT cells (Figure 1B).

Figure 1

(A) Heatmap based on UVA-irradiated HaCaT cells, CT-treated UVA-irradiated HaCaT cells, and control HaCaT cells.

(B) GO enrichment analysis of DEGs between UVA-irradiated HaCaT cells and CT-treated UVA-irradiated HaCaT cells.

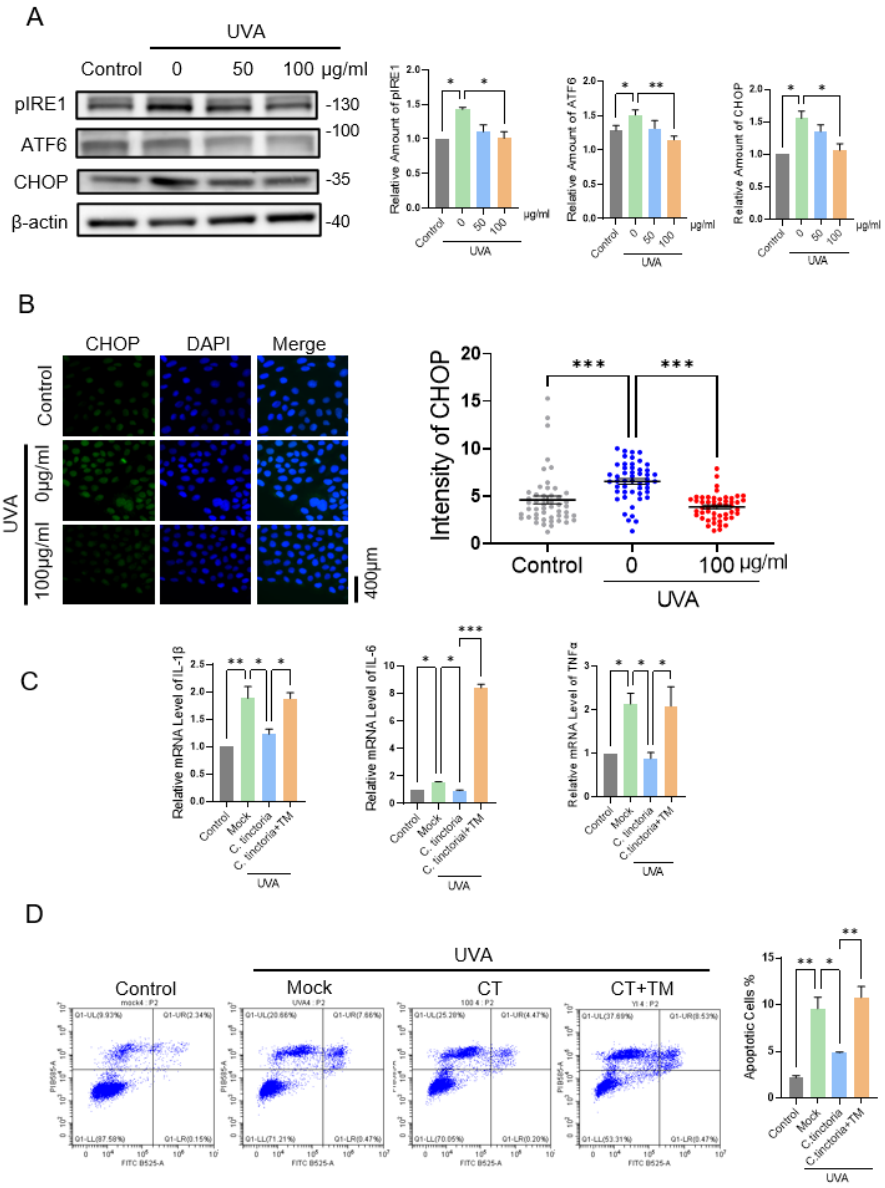


3.2 CT Decreases UVA-Induced ER Stress and ER Stress-induced Inflammation and Apoptosis

Previous studies indicate that UV-induced apoptosis in skin cells is triggered by excessive ER stress, activating the unfolded protein response [3]. To explore how CT modulates ER stress from UVA radiation, we treated HaCaT cells with UVA and various concentrations of CT. We measured markers of ER stress, including phosphorylated IRE1 (pIRE1), ATF6, and CHOP. Treatment with 100 μg/ml of CT significantly reduced UVA-induced ER stress by downregulat-

ing pIRE1, ATF6, and CHOP levels (Fig. 2A). Given CHOP's role in ER stress-related apoptosis, we conducted an immunofluorescence assay, showing reduced CHOP expression intensity after CT treatment (Fig. 2B).

We then introduced tunicamycin (TM), an antibiotic that induces ER stress, post-CT treatment. Since the unfolded protein response is linked to inflammation, we assessed inflammatory cytokine expression in HaCaT cells following UVA exposure and CT treatment. TM negated the CT-induced reduction in IL-1β, IL-6, and TNF-α mRNA expression (Fig. 2C), indicating that



photodamage-related inflammation is connected to ER stress induction. Furthermore, TM diminished CT's protective effects against UVA-induced apoptosis (Fig. D). These findings confirm that CT alleviates UVA-induced ER stress in HaCaT cells.

Figure 2

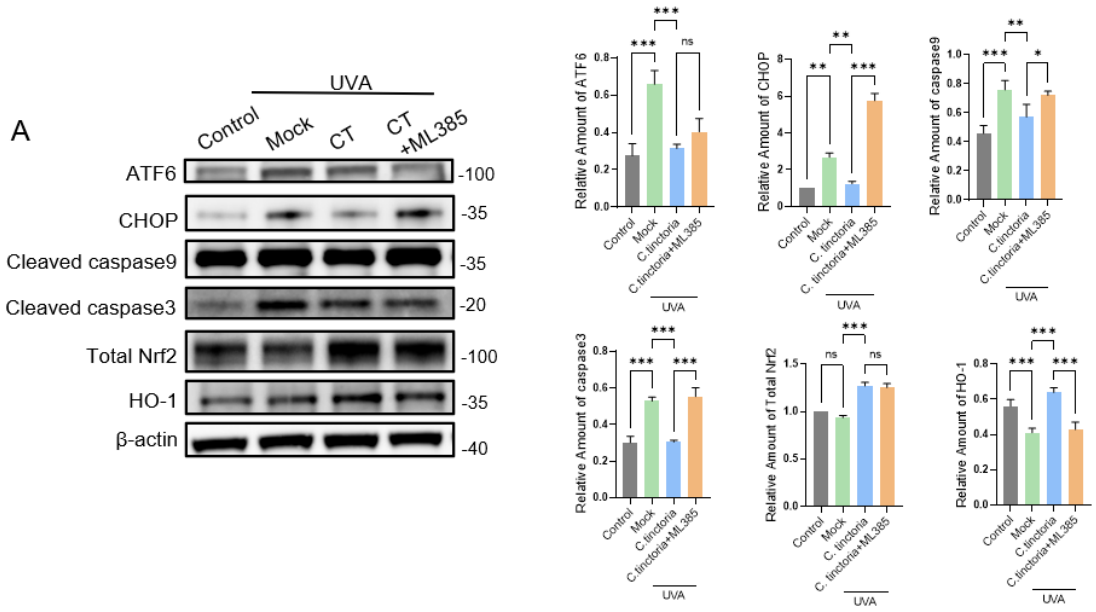
(A) Western blot analysis.
(B) Immunofluorescence assay.
(C) RT-qPCR assays were performed to determine the mRNA levels
(D) Flow cytometry analysis was performed to determine cell apoptosis.
N=4. * P ≤ 0.05; **P ≤ 0.01, ***P ≤ 0.001.

3.3 CT Alleviates UVA-Induced ER Stress via the Antioxidant Nrf2/HO-1 Pathway

Given that UVA exposure induces prolonged ER stress and pro-apoptotic signaling, we assessed CT's potential to mitigate ER stress-related apoptosis. The Nrf2/HO-1 pathway is crucial for regulating ER stress [6]. We introduced ML385, a specific Nrf2 inhibitor, to evaluate the effects of UVA irradiation and CT treatment on Nrf2/HO-1 pathway proteins in HaCaT cells. As shown in Figure 3A, ML385 negated the CT-induced upregulation of nuclear NRF2 and HO-1. Treatment with 100 μg/ml of CT significantly reduced apoptotic markers, including cleaved caspase-3 and -9, but ML385 reversed these effects, increasing ER stress and apoptotic markers. These results indicate a significant link between the Nrf2/HO-1 pathway and ER stress during CT action.

Figure 3

(A) Western blot assays.
N=4. * P ⩽ 0.05; **P ⩽ 0.01, ***P ⩽ 0.001.



3.4 Mitigation of UVA-Induced ER Stress by the CT Constituent Marein

To identify the key compound responsible for CT's anti-photodamage effects, we conducted ultra-performance liquid chromatography-mass spectrometry (UPLC-MS) analysis, revealing marein as the predominant flavonoid in CT (Fig. 4A,B). We then investigated marein's ability to counteract UV-induced ER stress using an ER tracker. The results showed significant up-regulation of proteins linked to the antioxidant Nrf2/HO-1 pathway after marein treatment, while ER stress markers were notably downregulated (Fig. 4C). In conclusion, our findings indicate that marein, as the primary flavonoid in CT, is crucial for modulating the Nrf2/HO-1 pathway and alleviating UVA-induced photodamage in HaCaT cells by effectively reducing ER stress.

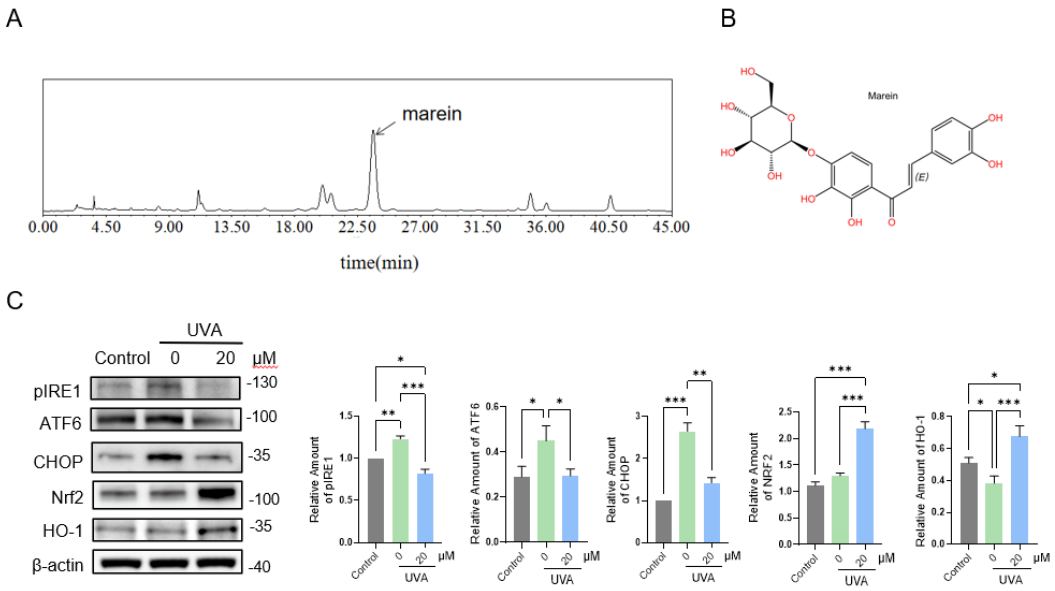


Figure 4

(A) Schematic representation of the experimental design to characterization of CT constituents by HPLC. The top six abundant flavonoids compounds in CT extract were profiled.

(B) Chemical structure of marein

(C) Western blot assays.

N=4. * P ≤ 0.05; **P ≤ 0.01, ***P ≤ 0.001.

4. Discussion

UVA significantly damages both epidermal and dermal structures, contributing to photodamage and photoaging. Unlike UVB, which directly affects DNA, UVA generates reactive oxygen species (ROS) through natural photosensitizers, leading to genotoxic effects [7]. UVA also mediates programmed cell death in keratinocytes, making antioxidant supplementation crucial to mitigate UVA-induced skin damage [1]. Recent studies have explored botanical extracts for their protective effects against UVA photodamage [8]. Additionally, CT extract has demonstrated potential in alleviating UVB-induced photodamage in hairless mice [5]. However, the effects of CT extract on UVA irradiation and its underlying mechanisms remain unexplored. This study aims to elucidate the protective effects of CT extract against UVA-induced photodamage in HaCaT cells.

Using bulk RNA sequencing, we identified a unique anti-photodamage signature associated with CT, focusing on the ER stress response pathways: IRE1α, PERK, and ATF6 [9]. UV exposure induces ER stress via ROS, activating the UPR and causing cellular damage, including

apoptosis [10, 11]. Komori et al. showed that UVA activates these pathways in human dermal fibroblasts through pATF6α(N), IRE1-mediated splicing of XBP1 mRNA, and PERK-mediated phosphorylation of eIF2 [12]. Various plant extracts have exhibited anti-apoptotic effects related to ER stress, such as *Euphorbia supina*, which reduces manganese-induced neurotoxicity [13]. In our study, CT and marein significantly influenced the expression of phosphorylated IRE1α, eIF2α, and ATF6, impacting CHOP expression and modulating BCL-2 family proteins and caspases to alleviate UVA-induced photodamage.

Nrf2 is a key transcription factor that regulates cellular homeostasis by controlling various downstream molecules, including HO-1, which has a promoter rich in antioxidant response elements (AREs) [14]. The Nrf2/HO-1 axis interacts with the PERK pathway to modulate ER stress in various contexts [15]. Our study confirmed that both CT and marein enhance the Nrf2/HO-1 signaling pathway, providing protective effects against ER stress and UVA-induced damage.

5. Conclusion

In summary, our research shows that CT, a flavonoid-rich natural extract, significantly reduces UVA-induced apoptosis and inflammation in human keratinocytes. CT effectively mitigates ER-associated apoptosis from UVA photodamage, with marein identified as a key component that regulates the Nrf2/HO-1 axis. These findings establish CT as a promising candidate for cosmetic and pharmaceutical applications targeting UV photodamage.

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