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*Full Length Research Paper*

# Anticarcinogenic effect of ferulic acid on ultraviolet-B irradiated human keratinocyte HaCaT cells

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**The key role of ultraviolet (UV) radiation in skin cancer development has been confirmed by abundant evidences. The human epidermis is the main defense against UV radiation. Ferulic acid is a potent ubiquitous plant antioxidant. The purpose of the present study was to investigate whether Ferulic acid could inhibit ultraviolet-B (UVB) induced carcinogenesis and its possible underlying mechanisms. The human keratinocyte HaCaT cells were treated by UVB irradiation and Ferulic acid. And the cellular viability, secretion of IL-6 and TNF-α, apoptosis and cell cycle, formation of cyclobutane pyrimidine dimers (CPDs), mRNA expression of p53, p21 and c-fos, protein expression of p53, proliferating cell nuclear antigen (PCNA), and replication protein A (RPA) were investigated. Ferulic acid treatment inhibited the UVB-induced cytotoxicity, apoptosis and CPDs formation. Ferulic acid also attenuated the mRNA levels of apoptosis-regulatory gene (p53- p21 and c-fos) the protein levels of p53, PCNA and RPA and the secretion of cytokines (IL-6 and TNF-α). These results indicate that Ferulic acid may have the potential anti-carcinogenic properties on the UVB induced epidermic tumor development by blocking the relevant cytokine secretion and expression of p53, p21, c-fos, PCNA and RPA genes.**

**Key words:** Ferulic acid, ultraviolet-B irradiation, keratinocyte, skin cancer.

## INTRODUCTION

Overexposure to solar ultraviolet (UV) radiation, especially ultraviolet-B (UVB) (290~320 nm) component, induces a multitude(multiple or many) of acute and delayed responses in human skin, including the generation of free radicals and relevant reactive oxygen species (ROS), secretion of inflammatory cytokines, immunosuppression of topical and general immune systems, DNA damage and mutation, at last contributes to photo carcinogenesis development (Krutmann, 2000; Gruijl et al., 2001; Kawachi et al., 2008). And the formation of photo products, predominantly cyclobutane pyrimidine dimers (CPDs), is critical to the initiation of UV-induced skin carcinoma (Hidema et al., 2007).

For the past few decades, there has been great interest in chemoprevention of photo damage by using naturally botanical agents to reduce the risk. It requests the agents which have potent anticarcinogenic bioactivity that intervene one or more steps among the initiation, development and progression of cancer, including mutation, proliferation, differentiation, apoptosis and metastasis (Bickers and Atha, 2000). Ferulic acid is a ubiquitous plant antioxidant and has been confirmed owning many biological activities, including improvement of microcirculation, elimination of oxygen-free radicals and anti-inflammation (Zhou et al., 2000; Liu et al., 2002; Liu et al., 2002; Li et al., 2002; Barone et al., 2008). There are some reports that shows protective effect of Ferulic acid on UVB radiation, but its mechanism has not been investigated adequately, yet IL-6 and TNF-α are mainly produced and secreted by keratinocytes, which play an important part in cellular responses to UVB (Schwarz et al.,1995; Grandjean-Laquerriere et al.,2003; Wozniacka et al., 2008). In addition, DNA photo lesions would contribute to cell cycle arrest, DNA repair and apoptosis. A lot of studies have demonstrated that p53/p21 is responsible for these adaptive protective responses. Moreover, p53 also takes part in the initiation and regula-

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tion also takes part in the initiation and regulation of DNA repairing procedure directly (Melnikova and Ananthaswamy, 2005; Seo and Jung, 2004; Fotedar et al., 2004; Beattie et al., 2005; Dhanalakshmi et al., 2005). In mammalian cells, the photo lesions are mainly removed by nucleotide excision repair (NER) pathway, while p53, PCNA and RPA proteins are involved in this process (Lao et al., 2000; Stelter and Ulrich, 2003; Mortusewicz and Leonhardt, 2007). At last, the expression of c-fos gene induced by UVB irradiation plays a key role in proliferation and tumor development (Kaina, 2003; Christmann, et al., 2006).

In the present study, we thus investigated whether Ferulic acid has the potential protective efficacy on UVB induced skin carcinogenesis and its underlying mechanisms through measuring the relevant cytokine secretion, production and elimination of CPDs, expression of p53/p21, c-fos, PCNA and RPA in cell culture system.

## MATERIALS AND METHODS

### Cell culture and ferulic acid treatment

Human immortalized keratinocyte HaCaT cells were provide by Dr Gu (Department of Dermatology Changhai Hospital, Shanghai, China) which have been used widely in dermatological research (Fusenig and Boukamp, 1998). Cells were cultured in RMPI-1640 medium with 10% fetal bovine serum and 100 units/ml penicillin/streptomycin at 37°C in the incubator containing 5% $CO_2$. When the cells grew to 70 - 80% confluence, they were serum-starved for 24 h before UVB and Ferulic acid treatment. Cells were irradiated in phosphate-buffered saline (PBS) using the UVB lighter (SIGMA company, China). The 1 kW xenon arc lamps was adjusted to $8 \times 10^{-5}$ W $cm^2$ and metered by the radiometer fitted with a UVB probe at (285 ± 5) nm. Cells were irradiated with 30, 60 or 90 $mJ/cm^2$ during the experiment. Control cells were placed under the lamps but shielded from the UV light by an opaque sheet of aluminium foil. After UVB irradiation, cells were given serum-free medium with 200 µg/ml concentration of Ferulic acid (the National Institute for the Control of Pharmaceutical and Biological Products, Beijing, China, purity > 98%).

### Cell viability assay

The cellular viability was measured by MTT [3-(4,5-dimethyl-thiazol-2-yl)- 2,5-diphenyl-diphenyl-tetrazoliumbromide] assay. 20 µl of 5 mg/ml MTT solution was added per 100 µl medium. After incubating for 4 h, 100 µl of dimethylsuloxide (DMSO) was added in each well and the absorbance at 490 nm were recorded on the microplate reader.

### Immunohistochemical staining analyses

CPDs amount were detected by immunohistochemical staining analyses at different phases (0, 0.5, 2, 4, 12 and 24 h) after 30 $mJ/cm^2$ UVB irradiation. We gave the intervention of Ferulic acid immediately post irradiation, and the influence was showed at 2 h phase. The manipulation was conducted strictly according to the protocols of kits (Boster Biological Engineering Company, Wuhan, China). All specimens were incubated with monoclonal mouse anti-human CPDs (1:1000 dilutions) for 1 h at 37°C, then with biotin-conjugated biotin-conjugated goat anti-mouse IgG at 37°C for 20 min. The positive cells with brown nuclei were calculated in 200 cells in 5 - 10 successive high power fields.

### ELISA analysis

Cell-free supernatants were collected and then stored at -20°C for enzyme-linked immunosorbent assay (ELISA). Detection of IL-6 and TNF-α were carried out using Human Cytokine Sandwich ELISA Kit (JingMei Bioengineer Company, Shenzhen, China). The cytokine levels were measured following the manufacturer's protocol.

### DNA flow cytometric analysis

DNA flow cytometric analysis was used to determine the cell cycle. Cells were harvested by trypsin–ethylene diamine tetra-acetic acid (EDTA) and fixed in 1% paraform aldehyde. Half an hour before flow cytometric analysis, the cells were resuspended in a 1 mL Propidium Iodide (PI) DNA staining solution. Flow cytometric analysis was performed with flow cytometer (EPICS, USA). The level of apoptotic cells was determined.

### Reverse transcription-polymerase chain reaction

RNA was extracted using trizol (Invitrogen, USA) according to the supplier's instruction. Then cDNA was synthesized by using a Superscript pre-amplification system (Promega, USA) according to manufacturer's instructions. Polymerase chain reaction (PCR) was performed to amplify the cDNA fragments of p53, p21, c-fos and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) genes. The specific primers used were as follows: p53 sense primer: 5'-GGACAGCCACGTCTGTGACTTG-3' and anti-sense primer: 5'-CCAGTGGTTTCTTCTTTGGCTG-3'; p21 sense primer: 5'-CCGAAGTCAGTTCCTTGTGGAG-3' and anti-sense primer: 5'-TTAGGGCTTCCTCTTGGAGAAG-3'; c-fos sense primer: 5'-TCAACGCGCAGGACTTCTGCAC-3' and anti-sense primer: 5'-TGGTCTGTCTCCGCTTGGAGTG-3'; GADPH sense primer: 5'-AACCATGAGAAGTATGACAACAGC-3' and anti-sense primer: 5'-CATGTGGGGCCATGAGGTCCACCAC-3'. The specific primer yielded expected PCR products of 643, 567, 386 and 580 bp for p53, p21, c-fos and GADPH genes, respectively. PCR reaction was conducted in an automatic heat-block DNA thermal cycle instrument (PTC-200, MJ RESEARCH, USA) for 35 cycles: denaturing at 95°C for 60 s; annealing at 58 - 64°C for 45 - 60 s; extension at 72°C for 45 s to 120 s depending upon the characteristics of each set of primers. The amplified PCR products were electrophoresed on a 2% agarose gel in TAE (40 mM Tris acetate, 1 mM EDTA) and visualized by ethidium bromide staining. The mRNA expression level was shown as the band intensity ratio between purposed gene and house keeping gene after each amplified product was normalized to the corresponding GAPDH product.

### Western blotting analysis

The total protein was extracted by trizol protocol at 4 h after UVB irradiation. The 20 µg of each protein sample was analyzed with SDS-PAGE and electro blotted to PVDF membranes. The membranes were incubated overnight at 4°C with the specific monoclonal antibodies (Boster Biological Engineering Company, Wuhan, China) to p53, PCNA, RPA respectively (1:500 dilution). Then the membranes were washed and incubated with goat anti-mouse IgG (1:1000; Beijing ZhongShan Biotechnology, Beijing) for 20 min at 37°C. After staining with DAB, band intensity was detected by GS700 densitometer and analyzed by molecular

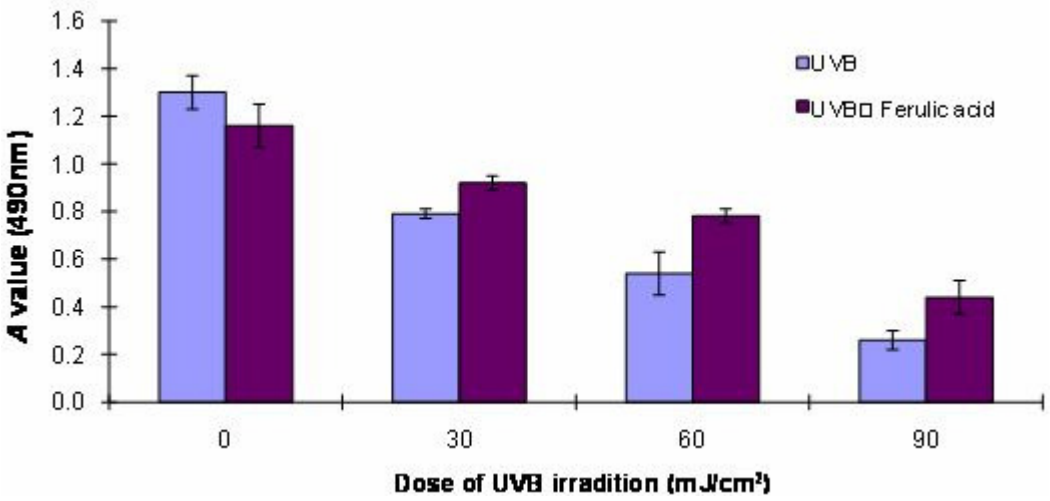


**Figure 1.** Photoprotective effect of Ferulic acid on the UVB-induced cell photodamage in cultured HaCaT cells. Cells were irradiated by 0 - 90 mJ/cm$^2$ and cultured with 200 µg/ml of ferulic acid for 24 h. MTT assay for cellular activity was performed as described. The values expressed as mean ± SD, and the difference was statistically significant.

analyst software (Bio-Rad Laboratories, Hercules, CA, USA).

#### Statistical analysis

The experimental data were expressed as mean ± standard deviation and analyzed by SPSS 11.0 software. Chi-square criterion, Paired T-test and U analysis were used in the study. $P < 0.05$ was considered statistically significant.

## RESULTS

### Ferulic acid inhibited ultraviolet-B induced photo-damage

Herein, the cytotoxicity of Ferulic acid was analyzed in cell culture system. A series of concentrations of Ferulic acid (0, 50, 100 and 200 µg/ml) were added into the medium and incubated for 24 h. The similar cell proliferation curves were observed for all tested concentrations, which implied there was no marked cytotoxicity for Ferulic acid solution to HaCaT cells ($P > 0.05$) (data is not shown).

As shown in Figure 1, UVB-induced photo damage occurred after irradiation of different dosage. HaCaT cells were exposed to 0~90 mJ/cm$^2$ of UVB, and then the cellular activity was detected 24 h after UVB treatment. Compared with the sham group, the cell survival rate was decreased in a dose-dependent manner. Incubation with Ferulic acid could improve cellular activity partly. The protective efficiency of Ferulic acid was apparent even under 90 mJ/cm$^2$ UVB irradiation ($P < 0.05$).

### Ferulic acid prevents CPDs formation

Formation of CPDs was observed only in irradiated cells with brown nuclei (Figures 2a - d) (200X). After 30 mJ/cm$^2$ UVB irradiation, the positive cells were counted at 0, 0.5, 2, 4, 12 and 24 h phase respectively. The production of CPDs arrived at peak 0.5 h after irradiation. And the removed speed for CPDs was more rapid during the first 4 h, while almost all CPDs were cleared till the 24 h. In addition, the amount of CPDs in the irradiated group and Ferulic acid treated group 2 h after UVB irradiation were 12 and 4 positive cells ($U_{Ferulic\ acid} = 2.000 > 1.96$, $P < 0.05$).

### Ferulic acid affected the secretion of cytokines

The concentration of IL-6 and TNF-α secreted from HaCaT cells increased in UVB dose variation, being (98.6 ± 0.87) – (403.53 ± 1.01) pg/ml in IL-6 and (11.27 ± 0.68) – (31.27 ± 0.64) pg/ml in TNF-α, respectively. After irradiated HaCaT cells were co-incubated by Ferulic acid, IL-6 secretion was reduced on every dosage of UVB irradiation (Figure 3a). Compared with IL-6, Ferulic acid also had the inhibition effect on TNF-α secretion at various doses of UVB irradiation (Figure 3b) ($P < 0.001$).

### Ferulic acid inhibited ultraviolet-B induced apoptosis

We also observed the effect of Ferulic acid on UVB induced apoptosis. Apoptosis level was determined 24 h after UVB treatment. UVB caused conspicuous apoptosis in cultured HaCaT cell line, and the apoptosis rate was dose-related (from 0.21% at 0 mJ/cm$^2$ to 71.18% at 90 mJ/cm$^2$). Ferulic acid decreased apoptotic rate in every group, showing protective effect on UVB-induced apoptosis ($P < 0.05$) (Table 1).

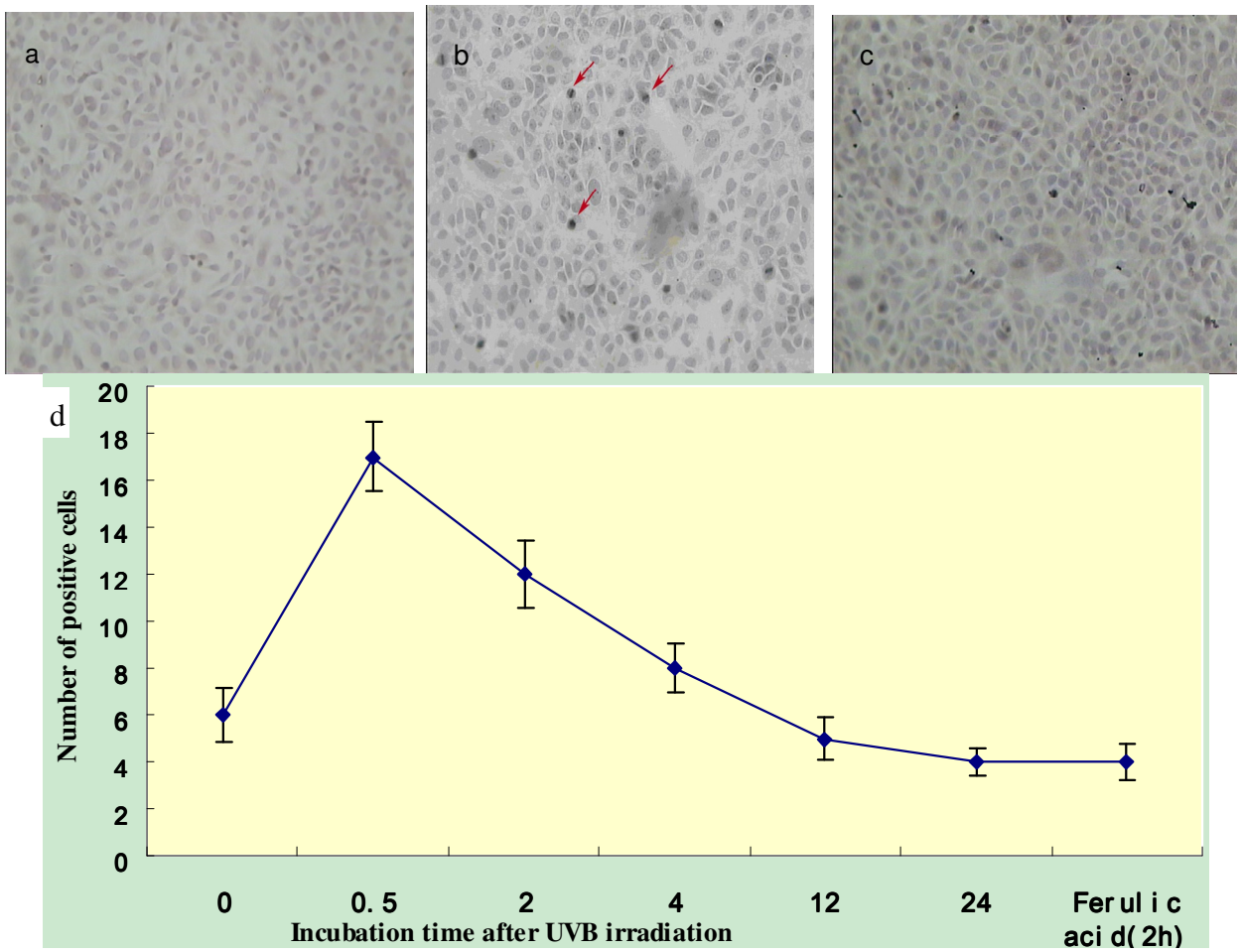


**Figure 2.** (a) sham irradiated group (b) 2h group (c) 2c Ferulic acid+UVB group (d) CPDs in the irradiated group and Ferulic acid treated group. Effect of UVB irradiation on production of CPDs. There was no positive cell found in sham irradiated group. Production of CPDs could be induced by 30 mJ/cm$^2$ UVB irradiation, shown as the representative positive cells with brown nuclei. With intervention of Ferulic acid, only 4 positive cells were found (200X).

### Ferulic acid down-regulated ultraviolet-B induced p53/p21 and c-fos mRNA expression

To evaluate whether Ferulic acid had inhibitory effects on p53, p21 and c-Fos gene expression, the mRNA levels were determined by RT-PCR. After UVB irradiation, the mRNA expression of p53, p21 and c-fos genes reached the highest level at 8, 12and 4 h, respectively. Compared to the control, the highest expression levels of these genes increased 225, 80 and 312% respectively. Ferulic acid intervention partly decreased higher mRNA levels induced by UVB irradiation. All the difference have the statistically significance ($P < 0.05$) (Figure 4).

### Ferulic acid suppresses p53, PCNA and RPA proteins accumulation

As shown in Figure 5 and, we observed the effect of Ferulic acid on NER path related proteins, including p53, PCNA and RPA, by Western blotting. Compared with the high expression for three proteins in irradiated group, lower level of three kinds of protein was observed in (Figure 6) Ferulic acid treated group at 4 h after UVB irradiation. In quantitative analysis, compared with simple UVB exposed control, expression level of p53, PCNA and RPA descended for 24.76, 26.87 and 69.85% respectively with Ferulic acid intervention ($P < 0.05$).

## DISCUSSION

The skin is the biggest organ of human body and has very important barrier function against the harmful effects of UV radiation. UVB (wavelength range between 290 and 320 nm), as the main component of solar irradiation, could induces different various adverse effects in skin, such as sunburn, photo aging and especially skin tumor (Ravanat et al., 2001; Sander et al., 2004). Especially non-melanoma skin cancer has become the most

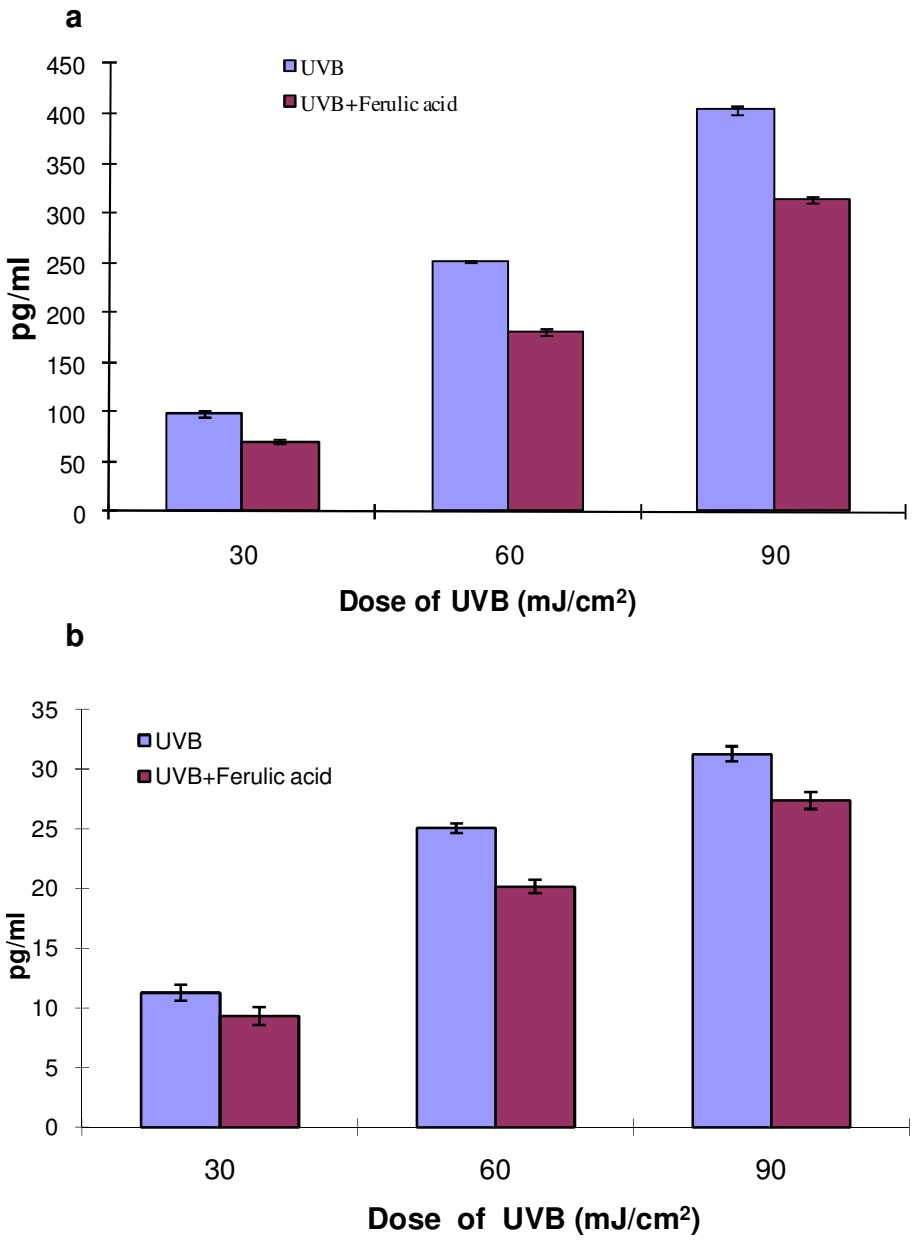


**Figure 3.** Effects of Ferulic acid on IL-6(3a) and TNF-α (3b) secretion induced by UVB irradiation in cultured HaCaT cells. The conditioned medium was collected after the irradiated cells were cultured with 200 μg/ml of Ferulic acid for 24 h. The values detected by ELISA were expressed as mean ± SD.

**Table 1.** Effects of Ferulic acid (200 μg/ml) on apoptosis induced by UVB irradiation in cultured HaCaT cells (%).

| UVB (mJ/cm$^2$) | UVB group | Ferulic acid group | $X^2$ value | P value |
|---|---|---|---|---|
| 0 | 0.21 | 0.15 | 0.688 | > 0.05 |
| 30 | 4.95 | 3.26 | 29.584 | < 0.01 |
| 60 | 70.92 | 1.09 | 7996.437 | < 0.01 |
| 90 | 71.18 | 3.51 | 6825.076 | < 0.01 |

common malignancy in humans, and its incidence is equivalent to the malignancies in all other organs combined. Besides the current methods of sun avoidance, such as counseling patients to avoid sun exposure and

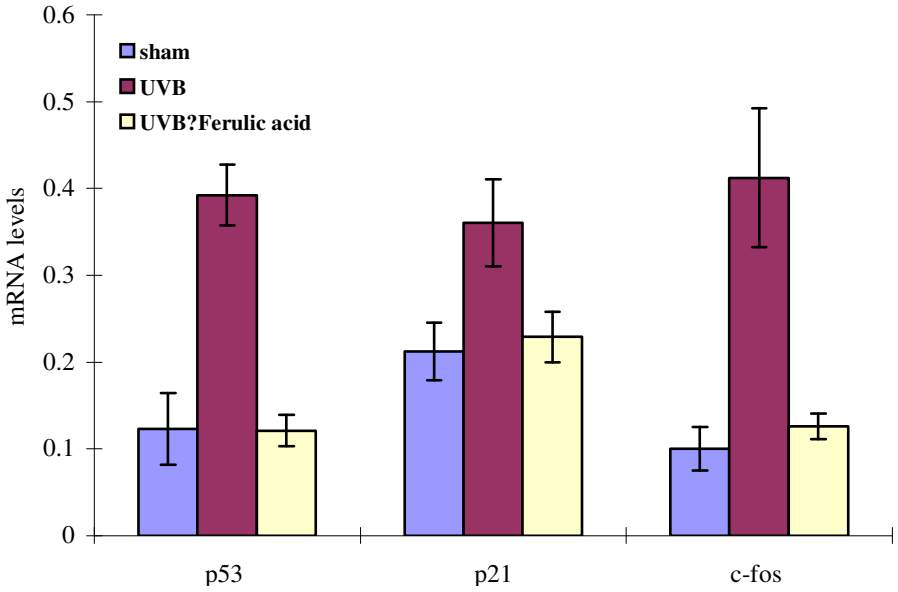


**Figure 4.** Effects of ferulic acid on the UVB-induced mRNA levels of p53 - p21 and c-fos genes in HaCaT cells. The mRNA expression level of p53 - p21 and c-fos genes was shown as the band intensity ratio between purposed gene and house keeping gene after each amplified product was normalized to the corresponding GAPDH product. Each value represents mean ± SD.

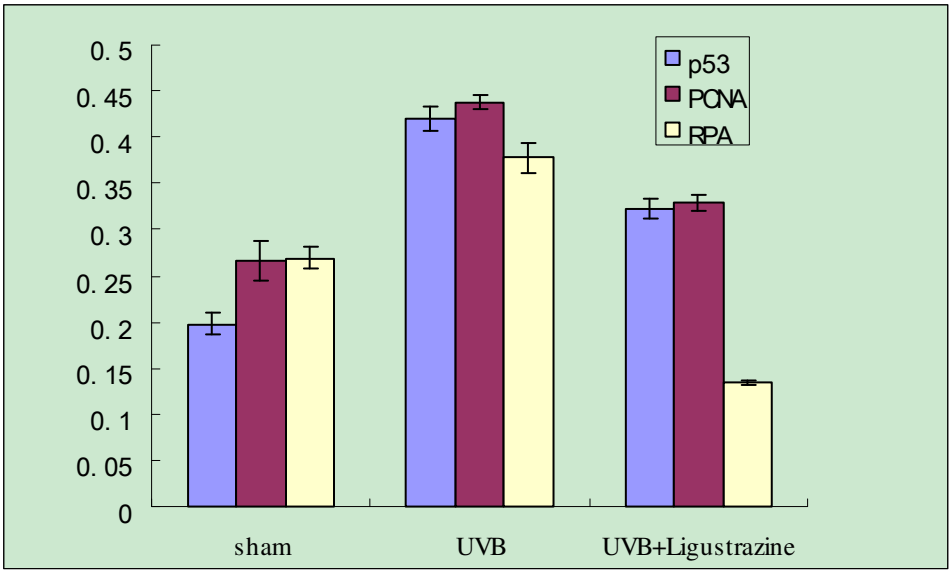


**Figure 5.** Effects of Ferulic acid on the expression of p53, PCNA and RPA. The expression levels of three proteins in Ferulic acid treated group were lower than that in only irradiated group. With correction with GAPDH, the expression amount of p53, PCNA and RPA decreased by 23.1, 24.9 and 64.3% respectively ($P < 0.05$).

sunscreens, additional methods should be taken to prevent the initial UV-introduced damage. DNA photo products, including CPDs and (6 – 4) PPs, form at sites of adjacent pyrimidine bases by the direct absorption of UVB (Svedruzic et al., 2005). CPDs form more abundantly and repair less efficiently than the (6 - 4) PPs, so it is considered as the predominant DNA photo lesions and responsible for the initiation of human skin cancer (Vink et al., 1994; Bendesky et al., 2006). In the present study, the cellular proliferation activity decreased in a UVB

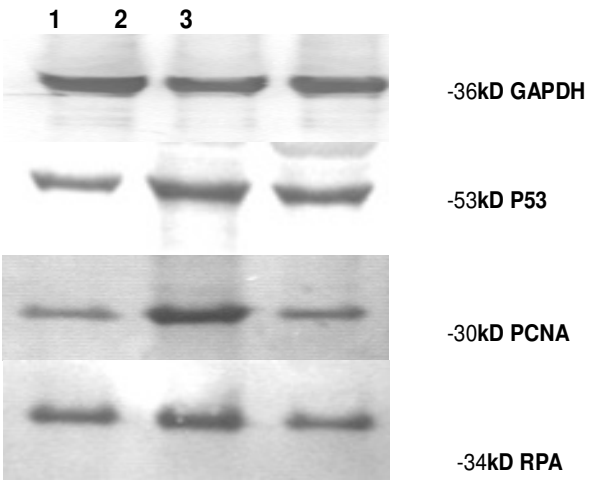


**Figure 6.** (1) Sham irradiated group, (2) only UVB group, (3) Ferulic acid treated group (UVB + Ferulic acid).

dose-dependent manner. And CPDs formation was found following 30 mJ/cm$^2$ UVB irradiation. Moreover, we observed the repairing process for 24 h, and the amount of CPDs reached the peak at about 0.5 h phase. Removing speed of CPDs was more rapidly during the initial 4 h and most of CPDs had been cleared at 24 h post-irradiation. Treatment with Ferulic acid apparently inhibit the cytotoxicity of UVB and resulted in a lower amount of CPDs, which shows that Ferulic acid could directly protects the cells against UVB-induced damage ($P < 0.05$).

Then we investigated whether Ferulic acid had the protective effect on UVB induced apoptosis. The results showed that apoptotic rate of HaCaT cells increased in UVB dose-dependent method. But there was a lower apoptotic rate in Ferulic acid treated group. The results on apoptosis may also be a possible mechanism of the photo-protective ability of Ferulic acid on the UVB-induced skin tumor development. Subsequently we further discussed whether Ferulic acid had a direct effect on the DNA damage repair process and the relevant mechanisms.

IL-6 and TNF-$\alpha$ are secreted by epidermic keratinocytes and play an important role in UV-mediated photobiology. IL-6 could cause fever and promote the synthesis of C reactive protein (CRP). There have been reports indicate that immediate adding TNF-$\alpha$ antibody after UV irradiation reduced cell debris formation and apoptosis remarkably (Obayashi et al., 2005; Kosmadaki et al., 2003). Our results confirmed that the levels of IL-6 and TNF-$\alpha$ increased after 30~90 mJ/cm$^2$ of UVB irradiation, which further implied the significance of cytokines in UV-induced skin injury. After Ferulic acid treatment, IL-6 and TNF-$\alpha$ secretion was apparently reduced ($P < 0.001$). These data showed the photo-protection of Ferulic acid may be associated with inhibiting the release of cytokines. P53 plays a key role in the cancerigenic process, and p21 it's an important downstream effectors gene. Over expression of p53/p21 may lead to cell cycle arrest and apoptosis (Melnikova and Ananthaswamy, 2005; Seo and Jung, 2004; Fotedar et al., 2004; Verschooten et al., 2006). NER is the major defensive mechanism against CPDs (De Lima-Bessa et al., 2008; Svejstrup, 2002). In NER pathway, p53 acts as the guardian of the genome and is crucial to the activation of many NER genes (Verschooten et al., 2006). RPA protein attends the formation of XPA-RPA complex which is responsible for DNA damage recognition (Lao et al., 2000; Vasquez et al., 2002; Maltseva et al., 2007). And in some reports, RPA can recognize a wide variety of DNA lesions by itself and plays an important role in initiation of DNA damage repair process (Reardon and Sancar, 2002). PCNA is able to bind to DNA and interacts with many other proteins involved in responses to DNA damage (Prakash et al., 2005). Therefore, we further investigated whether Ferulic acid could affect p53/p21, RPA and PCNA expression induced by UVB. Consistent with the inhibitory effect on apoptosis, the UVB induced elevation of p53 and p21 mRNA levels was inhibited by Ferulic acid. The changes of p53, RPA and PCNA protein levels were similar. These data showed that Ferulic acid might inhibit UVB-induced apoptosis through blocking of the p21 and p53 expression. And the resistance of Ferulic acid to UVB may contribute to the depression of p53, RPA and PCNA proteins. However, whether Ferulic acid could accelerate the DNA repairing processes need more discussions.

Cellular proliferation induced by UVB is also help to skin carcinogenesis. C-fos expression is critical to AP-1 activation, which is important for cell proliferation and tumor development (Young et al., 1999; Luo et al., 2007; Christmann et al., 2007; Lackinger and Kaina, 2000). As shown in the study, at 4 h after UVB irradiation c-fos gene expression level significantly increased, which demonstrated that c-fos gene expression might be one mechanism of carcinogenesis induced by UVB. Ferulic acid treatment suppressed the up regulation of c-fos mRNA level, which showed Ferulic acid had the ability of inhibiting cellular proliferation and tumor development.

In summary, these results indicated that Ferulic acid had a potent photo protective effect *in vitro* which results in inhibition of UVB-induced skin cytotoxicity, apoptosis, CPDs formation and blocking the expression of p53, p21, RPA, PCNA and c-fos genes. Ferulic acid may become a new agent against photo carcinogenesis.

## ACKNOWLEDGEMENT

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### REFERENCES

Barone E, Calabrese V, Mancuso C (2009). Ferulic acid and its

therapeutic potential as a hormetin for age-related diseases. Biogerontology 10(2): 97-108.
Bendesky A, Michel A, Sordo M, Calderón-Aranda ES, Acosta-Saavedra LC, Salazar AM, Podoswa N, Ostrosky-Wegman P. (2006). DNA damage, oxidative mutagen sensitivity, and repair of oxidative DNA damage in nonmelanoma skin cancer patients. Environ. Mol. Mutagen 47(7): 509-17.
Bickers DR, Athar M (2000). Novel approaches to chemoprevention of skin cancer. J. Dermatol. 27: 691-695.
Beattie PE, Finlan LE, Kernohan NM, Thomson G, Hupp TR, Ibbotson SH (2005). The effect of ultraviolet (UV) A1, UVB and solar-simulated radiation on p. 53 activation and p21. Br. J. Dermatol. 152(5): 1001-8.
Christmann M, Tomicic MT, Origer J, Aasland D, Kaina B (2006). c-Fos is required for excision repair of UV-light induced DNA lesions by triggering the re-synthesis of XPF. Nucleic Acids Res. 34(22): 6530-9.
Christmann M, Tomicic MT, Aasland D, Kaina B (2007). A role for UV-light-induced c-Fos: Stimulation of nucleotide excision repair and protection against sustained JNK activation and apoptosis. Carcinogenesis, 28(1): 183-90.
Darzynkiewicz Z, Smolewski P, Bedner E (2001). Use of flow and laser scanning cytometry to study mechanisms regulating cell cycle and controlling cell death. Clin. Lab. Med. 21(4): 857-73.
de Lima-Bessa KM, Armelini MG, Chiganças V, Jacysyn JF, Amarante-Mendes GP, Sarasin A, Menck CF (2008). CPDs and 6-4PPs play different roles in UV-induced cell death in normal and NER-deficient human cells. DNA Repair (Amst). 7(2): 303-12.
Dhanalakshmi S, Agarwal C, Singh RP, Agarwal R (2005). Silibinin up-regulates DNA-protein kinase-dependent p53 activation to enhance UVB-induced apoptosis in mouse epithelial JB6 cells. J. Biol. Chem. 280(21): 20375-83.
Fotedar R, Bendjennat M, Fotedar A. (2004). Role of p21WAF1 in the cellular response to UV. Cell Cycle 3(2): 134-7.
Fusenig NE, Boukamp P (1998). Multiple stages and genetic alterations in immortalization, malignant transformation, and tumor progression of human skin keratinocytes. Mol. Carcinog. 23(3): 144-58.
Grandjean-Laquerriere A, Le Naour R, Gangloff SC, Guenounou M (2003). Differential regulation of TNF-alpha, IL-6 and IL-10 in UVB-irradiated human keratinocytes via cyclic AMP/protein kinase A pathway. Cytokine 23(4-5): 138-49.
Gruijl FR, de HJ, van Kranen, LH Mullenders (2001). UV induced DNA damage, repair, mutations and oncogenic pathways in skin cancer. J Photochem. Photobiol B: Biol. 63: 19-27.
Hidema J, Taguchi T, Ono T, Teranishi M, Yamamoto K, Kumagai T (2007). Increase in CPD photolyase activity functions effectively to prevent growth inhibition caused by UVB radiation. Plant J. 50(1): 70-9.
Kaina B (2003). DNA damage-triggered apoptosis: critical role of DNA repair, double-strand breaks, cell proliferation and signaling. Biochem Pharmacol. 66(8): 1547-54.
Kawachi Y, Xu X, Taguchi S, Sakurai H, Nakamura Y, Ishii Y, Fujisawa Y, Furuta J, Takahashi T, Itoh K, Yamamoto M, Yamazaki F, Otsuka F (2008). Attenuation of UVB-induced sunburn reaction and oxidative DNA damage with no alterations in UVB-induced skin carcinogenesis in Nrf2 gene-deficient mice. J. Invest. Dermatol. 128(7): 1773-9.
Kosmadaki MG, Yaar M, Arble BL, Gilchrest BA. (2003). UV induces VEGF through a TNF-alpha independent pathway. FASEB J. 17(3): 446-8.
Krutmann J (2000). Ultraviolet A radiation-induced biological effects in human skin: relevance for photoaging and photodermatosis. J. Derm. Sci. 23(1): 22-26.
Lackinger D, Kaina B (2000). Primary mouse fibroblasts deficient for c-Fos, p53 or for both proteins are hypersensitive to UV light and alkylating agent-induced chromosomal breakage and apoptosis. Mutat Res. 457(1-2): 113-23.
Lao Y, Gomes XV, Ren Y, Taylor JS, Wold MS (2000). Replication protein A interactions with DNA. III. Molecular basis of recognition of
damaged DNA. Biochemistry. 39: 850–859.
Li M, Handa S, Ikeda Y, Goto S (2001). Specific inhibiting characteristics of tetramethylpyrazine, one of the active ingredients of the Chinese herbal medicine 'Chuanxiong,' on platelet thrombus formation under high shear rates. Thromb. Res. 104: 15-28.
Liu CF, Lin CC, Ng LT, Lin SC (2002). Protection by tetramethylpyrazine in acute absolute ethanol-induced gastric lesions. J. Biomed. Sci. 9: 395-400.
Liu CF, Lin MH, Lin CC, Chang HW, Lin SC (2002). Protective effect of tetramethylpyrazine on absolute ethanol-induced renal toxicity in mice. J. Biomed. Sci. 9: 299-302.
Luo X, Puig O, Hyun J, Bohmann D, Jasper H (2007). Foxo and Fos regulate the decision between cell death and survival in response to UV irradiation. EMBO J 26(2): 380-90.
Maltseva EA, Rechkunova NI, Gillet LC, Petruseva IO, Scharer OD, Lavrik OI. (2007). Crosslinking of the NER damage recognition proteins XPC-HR23B, XPA and RPA to photoreactive probes that mimic DNA damages. Biochim. Biophys. Acta. 1770(5) : 781-9.
Melnikova VO, Ananthaswamy HN (2005). Cellular and molecular events leading to the development of skin cancer. Mutat. Res. 571(1-2): 91-106.
Mortusewicz O, Leonhardt H (2007). XRCC1 and PCNA are loading platforms with distinct kinetic properties and different capacities to respond to multiple DNA lesions. BMC Mol. Biol. 8- 81.
Ng TB, Liu F, Wang ZT (2000). Antioxidative activity of natural products from plants. Life Sci. 66: 709-723.
Obayashi K, Kurihara K, Okano Y, Masaki H, Yarosh DB (2005). L-Ergothioneine scavenges superoxide and singlet oxygen and suppresses TNF-alpha and MMP-1 expression in UV-irradiated human dermal fibroblasts. J Cosmet. Sci. 56(1): 17-27.
Prakash S, Johnson RE, Prakash L. (2005). Eukaryotic translesion synthesis DNA polymerases: Specificity of structure and function. Annu. Rev. Biochem. 74: 317-53.
Ravanat JL, T Douki, J Cadet (2001). Direct and indirect effects of UV radiation on DNA and its components. J. Photochem. Photobiol B: Biol. 63: 88-102.
Reardon JT, Sancar A (2002). Molecular anatomy of the human excision nuclease assembled at sites of DNA damage. Mol. Cell Biol. 22: 5938–5945.
Sander CS, Chang H, Hamm F, Elsner P, Thiele J (2004). Role of oxidative stress and the antioxidant network in cutaneous carcinogenesis. Int. J. Dermatol. 43: 326-335.
Schwarz A, Bhardwaj R, Aragane Y, Mahnke K, Riemann H, Metze D, Luger TA, Schwarz T (1995). Ultraviolet-B-induced apoptosis of keratinocytes: evidence for partial involvement of tumor necrosis factor-α in the formation of sunburn cells. J. Invest. Dermatol. 104: 922-927.
Seo YR, Jung HJ (2004). The potential roles of p53 tumor suppressor in nucleotide excision repair (NER) and base excision repair (BER). Exp. Mol. Med. 36(6): 505-9.
Stelter P, and Ulrich HD. (2003). Control of spontaneous and damage-induced mutagenesis by SUMO and ubiquitin conjugation. Nature. 425: 188-91.
Shieh DE, Lin LT, Lin CC. (2000). Antioxidant and free radical scavenging effects of baicalein, baicalin and wogonin. Anticancer Res. 20: 2861-2865.
Svedruzic ZM, Wang C, Kosmoski JV, Smerdon MJ (2005). Accommodation and repair of a UV photoproduct in DNA at different rotational settings on the nucleosome surface. J Biol Chem. 280(48): 40051-7.
Svejstrup JQ (2002). Transcription repair coupling factor: a very pushy enzyme. Mol. Cell. 9: 1151–1152.
Vasquez KM, Christensen J, Li L, Finch RA, Glazer PM (2002). Human XPA, RPA DNA repair proteins participate in specific recognition of triplex-induced helical distortions. Proc Natl Acad Sci USA. 99: 5848–5853.
Verschooten L, Declercq L, Garmyn M (2006). Adaptive response of the skin to UVB damage: role of the p53 protein. Int. J. Cosmet. Sci. 28: 1–7.
Vink AA, B Henegouwen, O Nikaido, RA Baan, L. Roza (1994). Removal of UV-induced DNA lesions in mouse epidermis soon after irradiation. J. Photochem. Photobiol. B: Biol. 24: 25-31.
Wozniacka A, Lesiak A, Boncela J, Smolarczyk K, McCauliffe DP, Sysa-Jedrzejowska A (2008). The influence of antimalarial treatment on IL-1beta, IL-6 and TNF-alpha mRNA expression on UVB-irradiated skin in systemic lupus erythematosus. Br. J. Dermatol. 159(5): 1124-30.
Young MR, JJ Li, M Rincon, RA Flavell, BK Sathyanarayana, R

Hunziker, N Colburn (1999). Transgenic mice demonstrate AP-1 (activator protein-1) transactivation is required for tumor promotion. Proc. Natl. Acad. Sci. USA. 96: 9827–9832.
Zhou S, Shao W, Zhang W (2000). Clinical study of Astragalus injection plus ligustrazine in protecting myocardial ischemia reperfusion injury. Zhongguo Zhongxiyi Jiehe Zazhi. 20: 504-507.