Pearl extract protects HaCaT cells from UV radiation-induced apoptosis through mitochondrial pathway regulation

Fumin Cao, Jing WANG*, Anquan YANG, Lihua ZHANG

OSM Biology Co.，LTD, Wukang, China

*Correspondence: wangjing@osmbio.com

Abstract

Introduction: Previous studies demonstrated that pearl extract (PE) promotes wound healing and skin whitening. However, whether PE can inhibit ultraviolet (UV)-photodamage in HaCaT cells remains unclear. In this study, an *in vitro* photoaging cell model was established to observe the effect of PE on UV-induced damage and apoptosis of HaCaT cells. The aim was to provide a reference for future development of natural sunscreen agents.

Methods: HaCaT cells were cultured in RPMI-1640 medium supplemented with 10% foetal bovine serum and 1% penicillin and streptomycin in 5% CO_2 at 37℃. The cells were irradiated with 10 J/cm^2 UV, while control cells were sham-irradiated by covering with tin foil. Cell viability was assessed by the CCK8 assay. The cell suspensions were collected and assayed for ROS and MDA levels, and GSH-Px and SOD activities using assay kits in accordance with the manufacturer's instructions. Total RNA was isolated from HaCaT cells after treatment using the RNAiso Plus kit according to the manufacturer's guidelines. After 48 h of the indicated treatment,

the supernatant of each group of cells in the 6-well plate was collected. TNF-a and IL-10 were detected in accordance with the enzyme-linked immunosorbent assay kit instructions.

Results: PE concentrations of 0.1 and 1 μg/mL were considered as the most effective and safe concentrations. Compared to the control group, superoxide dismutase and glutathione peroxidase activities in the photoaging group were significantly reduced, while malondialdehyde and reactive oxygen species content, along with tumour necrosis factor-alpha (TNF-α) and interleukin (IL)-10 mRNA and protein levels were markedly increased. In contrast, Bcl-2 protein expression was significantly decreased, while caspase-3, caspase-9 and Bax protein expression levels were significantly increased. Compared to the photoaging group, HaCaT cell proliferation was significantly increased in the PE group. Both PE concentrations significantly increased superoxide dismutase and glutathione peroxidase activities in cells, reduced malondialdehyde and reactive oxygen species content, decreased TNF-α and IL-10 mRNA expression in cells, and reduced TNF-α and IL-10 protein levels in the supernatant. Additionally, Bcl-2 protein expression levels were significantly increased, while caspase-3, caspase-9, and Bax protein expression levels were significantly reduced by PE treatment.

Conclusions: PE can inhibit UV-induced apoptosis by inhibiting mitochondria-mediated apoptosis and regulating TNF-α and IL-10 expression.

Keywords: pearl extract; ultraviolet; human keratinocyte cell; apoptosis

Introduction

Human keratinocytes (HaCaT cells) are the main cellular constituent of the epidermis (outermost layer of skin), accounting for more than 90% of epidermal cells [1]. The keratinocyte cells prevent external physical, chemical and microbial damage and maintain the stability of the body's internal environment [2]. As well, they can protect the skin by absorbing 95% of ultraviolet (UV) radiation that reaches the skin [3]. Human keratinocytes participates in various cellular and biological processes, such as apoptosis and inflammation [4]. Damage caused by UV radiation to human epidermal keratinocytes occurs mainly because of the production of reactive oxygen species (ROS) [5], which induce DNA damage, enzyme activity and mitochondrial dysfunction, resulting in damage to various cell functions [6]. To maintain the normal function of human epidermal keratinocytes, the skin can be covered by clothing, which reduces UV radiation exposure and oxidative damage [7]. Additionally, antioxidants are effective in reducing oxidative damage [8]. Jian-min et al. found that the 50% ethanol macroporous resin elution site of *Eucommia ulmoides* effectively protected against UVA and UVB-induced photoaging in HaCaT cells [9]. Zhiwu et al. reported that rose water inhibited UV-induced apoptosis of HaCaT cells by regulating the nuclear factor-kappa B (NF-κB) nuclear transcription factor pathway [10]. Min et al. demonstrated that hesperidin antagonized the decreased antioxidant enzyme activity in HaCaT cells caused by UVB and showed photoprotective effects [11].

Pearl powder is used as a traditional Chinese medicine to moisturize the heart, liver and muscle [12] and retard skin aging [13]. Anti-inflammation and anti-apoptosis properties have also been described [14,15]. We previously reported that pearl extract (PE) effectively reduced the melanin content in cells by inhibiting the activity of intracellular tyrosinase, suggesting that PE has a whitening effect [16].

The inhibitory effect of PE on UV photodamage-induced HaCaT has not been reported. In this study, an *in vitro* photoaging cell model was established to evaluate the effect of PE on UV-induced damage and apoptosis of UV-irradiated HaCaT cells and explored the molecular mechanisms involved.

Materials and Methods

PE

PE containing 2.1% total protein was kindly provided by Zhejiang Osmum Biological Co., Ltd. (Huzhou, China). The main preparation method involves grinding of freshwater pearls to the nanometre scale (10–100 nm), followed by hydrolysis with neutral protease to obtain various amino acids, trace elements and polypeptides.

Cell Culture and Subgroups

HaCaT cells (purchased from Shanghai GeFan Biotechnology Co., Ltd., Shanghai, China) were cultured in RPMI-1640 medium supplemented with 10% foetal bovine serum and 1% penicillin and streptomycin in 5% CO_2 at 37℃. Under a biological-inverted microscope, round-shaped newly subcultured HaCaT cells were observed. After 4 h of culture, the cells began to adhere to the culture plate and after 24 h of growth the cells were completely adherent. The cells were collected during exponential growth for subsequent experiments and were divided into the following subgroups: control group, photoaging cell group (irradiated with 10 J/cm^2 UV) and PE + UV group (pre-treated with PE before irradiation with 10 J/cm^2 UV). According to our

84 previous study, PE was added to the culture medium for 48 h before UV irradiation at different
85 concentrations (0, 0.01, 0.1, 1 and 10 μg/mL).

86 **UV Irradiation of Cells**

87 Prior to UV irradiation, the cells were washed with phosphate-buffered saline (PBS) and
88 covered with a thin layer of PBS. The cells were irradiated on ice-cold plates to eliminate UV
89 thermal stimulation. Monolayers of HaCaT cells in a thin layer of PBS were irradiated with 10
90 J/cm^2 UV and incubated with culture medium contained PE for 24 h.

91 **CCK8 Assay of Cell Viability**

92 HaCaT cells (1 × 10^5/mL) were cultured in 96-well plates. Different concentrations of PE (0,
93 0.01, 0.1, 1 and 10 μg/mL) were added to the cell suspension and incubated for 48 h at 37°C in a
94 5% CO_2 incubator. The cells were irradiated with 10 J/cm^2 UV, while control cells were
95 sham-irradiated by covering with tin foil. Further, the cells were incubated for 24 h in a cell
96 incubator at 37°C in 5% CO_2. Cell viability was assessed by the CCK8 assay. After the indicated
97 treatment, 10 μL of CCK8 was added to each well for 4 h at 37°C, and the absorbance of each
98 well was measured with a plate reader at a test wavelength of 490 nm. Finally, the concentration
99 of PE showing a significant protective effect against UV radiation-induced cell damage was
100 selected for further experiments. The experiment was repeated five times.

101 **Assays of Cellular ROS, GSH-Px, SOD and MDA**

102 Cells were seeded into 6-well plates (2 × 10^4 cells/well) and treated with different
103 concentrations of PE (0.1 and 1 μg/mL) for 48 h prior to UV irradiation. Subsequently, the cell
104 suspensions were collected and assayed for ROS and MDA levels, and GSH-Px and SOD

activities using assay kits in accordance with the manufacturer's instructions (Jian Cheng Bioengineering Co., Nanjing, China).

Cytokine mRNA Levels

Total RNA was isolated from HaCaT cells after treatment using the RNAiso Plus kit (TaKaRa Bio, Shiga, Japan) according to the manufacturer's guidelines. For each RT-PCR sample, 1 μg of total RNA was added, and the purity of the RNA was determined as the ratio of the optical density reading at 260 nm to that at 280 nm. The ratio of the RNA used for RT-PCR was 1.8 to 2.0. TNF-α, IL-10 and β-actin mRNA levels were determined by real-time quantitative PCR using a SYBR® Premix Ex Taq™ Kit (TaKaRa Bio) according to the manufacturer's instructions. The cDNA amplification of a specific sequence of human TNF-α, IL-10 and β-actin was performed by PCR using the primer sequences shown in Table 1. PCR was conducted at 95°C for 30 s, followed by 40 cycles of 95°C for 5 s and 60°C for 34 s in the StepOne plus real-time PCR system (Applied Biosystems, Foster City, CA, USA). The qRT-PCR results were analysed and are expressed as relative mRNA expression of CT (threshold cycle) value, which was then converted to fold-changes. Quantitative real-time RT-PCR assay was performed to detect β-actin expression to normalise the amount of cDNA in each sample.

Table 1. Real-time PCR primers

Gene name	Primer squences (5′→3′)
TNF-α	Forwad：CTGCTGCACTTTGGAGTGAT
	Reverse：AGATGATCTGACTGCCTGGG
IL-10	Forwad：GAACCAAGACCCAGACATC
	Reverse：GCATTCTTCACCTGCTCCAC

Actin	Forwad：CATGTACGTTGCTATCCAGGC Reverse：CTCCTTAATGTCACGCACGAT

Cytokine Protein Expression

After 48 h of the indicated treatment, the supernatant of each group of cells in the 6-well plate was collected. TNF-α and IL-10 were detected in accordance with the enzyme-linked immunosorbent assay kit instructions (Jian Cheng Bioengineering Co.). The cytokine concentration was measured three times.

Western Blot Analysis

After appropriate treatment for 48 h, the HaCaT cells were collected using RIPA mixed with phenylmethylsulphonyl fluoride to extract protein (Solarbio Science & Technology Co., Ltd., Beijing, China). Protein levels were measured by bicinchoninic acid assay (Jian Cheng Bioengineering Co.). Briefly, 50 μg of protein was resolved by 12% sodium dodecyl sulphate-polyacrylamide gel electrophoresis for 60 min at 140 V, and the resolved proteins were transferred to a polyvinylidene fluoride membrane for 45 min at 60 V. The membrane was blocked with 5% fat-free dried milk powder in TBST (1×Tris buffered saline, 0.1% Tween-20) at room temperature for 2 h and incubated with primary antibody diluted 1:1000 in fresh blocking buffer overnight at 4°C with gentle shaking (rabbit anti-caspase-3, rabbit anti-caspase-9, rabbit anti-Bcl-2, rabbit anti-Bax and rabbit anti-actin antibodies were purchased from Cell Signaling Technology, Danvers, MA, USA). Goat anti-rabbit secondary antibodies (Abmart, Shanghai, China) were diluted at 1:8,000 in fresh blocking buffer and incubated for 1 h at room temperature. The membranes were washed five times for 10 min each in TBST, and the bands were detected using

the ECL Plus kit (Solarbio Science & Technology Co., Ltd.). The membranes were exposed to Tanon 5200 Multi (Tanon Science & Technology Co., Ltd., Shanghai, China), and TanonImage analysis software was used for quantitative analysis.

Statistical Analysis

All data are expressed as the mean ± SD. Experiments were independently repeated at least three times. $P < 0.05$ indicated significant differences between the experimental and control groups, which were analysed by one-way analysis of variance. Representative western blots from three independent experiments are shown.

Results

Effect of PE on HaCaT Cell Proliferation Rate

Compared to the blank group, 0.01 μg/mL PE significantly promoted cell proliferation ($P < 0.01$; Figure 1). PE concentrations of 0.1 and 1 μg/mL PE showed no obvious enhancement and there was no significant effect on the cell proliferation rate. In contrast, 10 μg/mL PE significantly inhibited cell proliferation ($P < 0.01$). Therefore, 0.1 and 1 μg/mL PE were used in subsequent experiments.

Effect of PE on Proliferation Rate of Photoaged HaCaT Cells

Compared to the blank group, the proliferation rate of the model group was significantly reduced ($P < 0.01$), suggesting that UV radiation inhibited cell proliferation (Figure 2). Compared to the model group, 0.1 and 1 μg/mL PE significantly promoted cell proliferation ($P < 0.01$),

suggesting that PE protects against photoaging in cells in a concentration-dependent manner.

Effect of PE on Cytokine mRNA Levels

Total cellular RNA was extracted after UV irradiation, and cDNA for β-actin was used as an internal control. The qRT-PCR results were converted to fold-changes. Significantly higher levels of tumour necrosis factor-alpha (TNF-α, Fig. 3A) and interleukin (IL)-10 (Fig. 3B) mRNA were evident in the photoaging group compared to the levels in the blank group ($P < 0.01$). Compared to the photoaging group, after the addition of 0.1 and 1 μg/mL PE, the TNF-α mRNA expression level was decreased significantly in a concentration-dependent manner ($P < 0.05$ and $P < 0.01$, respectively). Similar results were observed for IL-10 mRNA expression ($P < 0.05$ and $P < 0.01$, respectively), suggesting that PE downregulated TNF-α and IL-10 expression.

Effect of PE on Cytokine Protein Expression

The levels of IL-10 and TNF-α were measured by enzyme-linked immunosorbent assay. As shown in Figure 4, the protein expression levels of IL-10 and TNF-α in the photoaging group were significantly increased compared to in the control group (both $P < 0.01$). Compared to the photoaging group, the expression of TNF-α protein were significantly reduced in the presence of 0.1 and 1 μg/mL PE ($P < 0.05$ and $P < 0.01$, respectively), and the IL-10 protein level results were similar ($P < 0.05$ and $P < 0.01$, respectively), suggesting that PE decreased the inflammatory response in cells.

Effect of PE on Antioxidant Indices of UV-Irradiated HaCaT Cells

The effects of different concentrations of PE on the contents of ROS, superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and malondialdehyde (MDA) contents of UV-irradiated

HaCaT cells are shown in Figure 5. Statistical variance analysis revealed that the measurement indices between different groups were significant. Activities of GSH-Px and SOD in the photoaging group were significantly lower than those in the control group (both $P < 0.01$). In the 0.1 and 1 μg/mL PE treatment groups, SOD levels (both $P < 0.05$) and GSH-Px levels were higher in the photoaging group ($P < 0.05$ and $P < 0.01$, respectively). The ROS and MDA levels in the photoaging group were significantly increased compared to the levels in the control group ($P < 0.01$). Treatment with 0.1 and 1 μg/mL PE produced significant decreases compared to in the photoaging group ($P < 0.05$ and $P < 0.01$, respectively) in a concentration-dependent manner.

Effect of PE on Caspase-3, Caspase-9, Bax and Bcl-2 Protein Expression Levels in HaCaT Cells

The levels of caspase-3, caspase-9, Bax and Bcl-2 were measured by western blot analysis (Figure 6A). Compared to the control group, the Bcl-2 protein expression level in the photoaging group was significantly decreased ($P < 0.05$), while caspase-3, caspase-9 and Bax protein expression levels were significantly increased ($P < 0.01$, $P < 0.05$ and $P < 0.05$, respectively; Figure 6B). Compared to the photoaging group, Bcl-2 protein expression in the 1 μg/mL PE group was increased significantly ($P < 0.05$). In contrast, in the 0.1 and 1 μg/mL PE treated groups, there were significant decreases in caspase-3 ($P < 0.05$ and $P < 0.01$, respectively), caspase-9 ($P < 0.05$ and $P < 0.05$, respectively) and Bax protein levels (both $P < 0.01$).

Discussion

HaCaT cells reside in the most superficial layer of human skin and are the main target cells of UV radiation [17]. Long-term UV radiation can lead to skin photoaging and even cancer [18].

Apoptosis refers to the gene-regulated process of autonomous and orderly cell death to maintain a stable state in the internal environment [19]. Because of changes in the cellular internal and external environment, as well as stimulation of death signals, this process eliminates aging cells and other cells with potential abnormal growth to maintain a stable state in the cell population [20]. Apoptosis is mainly controlled by three pathways: mitochondrial signalling, death receptor-mediated signalling and endoplasmic reticulum-mediated signalling [21]. Apoptosis is closely related to changes in mitochondrial structure and function. Long-term UV radiation causes excessive accumulation of ROS in the cell, which damages DNA and the mitochondrial inner membrane [22], ultimately resulting in loss of mitochondrial function, lipid peroxidation, proteins and nucleic acids, and directly promotes apoptosis [23]. The level of MDA often reflects the degree of lipid peroxidation in the cell and indirectly reflects the degree of cell damage [24]. As such, MDA and SOD assays are often used interchangeably. GSH-PX is an enzyme that is widely found in cells. It catalyses the breakdown of hydrogen peroxide and protects the integrity and function of the cell membrane [25].

As a key pro-inflammatory factor widely distributed in the cell, TNF-α has a variety of biological effects, which include mediating inflammation, the immune response and apoptosis [26]. UV radiation causes an increase in secretory TNF-α protein and total TNF-α expression [27]. Increased TNF-α can accelerate the induction of keratinocyte apoptosis after binding to the corresponding receptor. Additionally, UV radiation causes increased secretion of TNF-α and IL-10 [28], which in turn promotes apoptosis of UV-induced keratinocytes, thus playing an important role in UV-induced skin radiation damage [29].

In the mitochondrial apoptosis pathway, the Bcl-2 protein family plays a very important role,

including pro-apoptotic and anti-apoptotic factors [30], which regulate apoptosis by controlling the permeability of the mitochondrial membrane [31]. The caspase family is a series of cysteine amino acid proteases with similar amino acid sequences and secondary protein structures. Caspase proteins can block the cell cycle, label apoptotic cells, break down structural proteins in the cytoskeleton and inactivate DNA repair enzymes, leading to apoptosis [32]. Under physiological conditions, apoptosis-promoting Bcl-2 family members are located in the cytoplasm; however, they activate apoptotic signals through external stimuli, which are transferred to mitochondria, leading to changes in mitochondrial membrane permeability [33]. These changes prompt transfer of cytochrome C (CytoC) from the mitochondrial inner membrane to the cytosol [34]. Caspase-9 acts as an apoptotic neutron that plays a key role in the mitochondrial apoptotic pathway [35]. Caspase-9 is located upstream of the cascade reaction, forming an active complex with apoptosis protease activator with CytoC [36]. Subsequently, the complex activates downstream apoptosis-inducing caspase-3, which hydrolyses apoptosis-inhibiting proteins and repair-related molecules, ultimately activating mitochondrial pathway-mediated apoptosis [37]. The anti-apoptotic factor Bcl-2 suppresses caspase activation in the aforementioned process, reducing CytoC release. In these processes, Bcl-2 can inhibit apoptosis [38].

In summary, after UV irradiation at 10 J/cm^2, SOD and GSH-Px activities in the photoaging group were decreased. In contrast, the contents of ROS and MDA and protein expression levels of IL-10 and TNF-α in the photoaging group were increased. However, after PE treatment, the activity of SOD and GSH-Px was increased, and the expression levels of ROS, MDA, IL-10 and TNF-α were decreased. These results suggest that PE can effectively improve the activity of SOD and GSH-Px, remove oxygen free radicals from the cell and inhibit lipid peroxidation. Further,

GSH-Px can lessen the effects of UV radiation in cells by reducing the production of inflammatory IL-10 and TNF-α. Additionally, the western blot results demonstrated that PE upregulated the expression level of anti-apoptotic factor Bcl-2 protein and downregulated caspase-3, caspase-9 and Bax. These results suggest that PE effectively protects HaCaT cells against photoaging damage through a mechanism that involves apoptotic mitochondrial pathways. Thus, PE may be utilized as a new natural sunscreen agent to prevent skin damage triggered by UV (Fig. 7).

Conclusion

Our study shows for the first time that PE can reduce HaCaT cell damage caused by UV radiation, which is mainly regulated by reducing ROS and MDA content, increasing the activity of SOD and GSH-Px, inhibiting inflammatory response and mitochondrial mediated apoptosis pathway. The findings indicate that PE can effectively prevent cell damage caused by UV.

Abbreviations

PE: pearl extract; UV: ultraviolet; SOD: superoxide dismutase; GSH-Px: glutathione peroxidase; MAD: malondialdehyde; ROS: reactive oxygen species; TNF-α: tumour necrosis factor-α; IL-10: interleukin-10

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and material

All data analysed during this study and material are included in this article

Competing interests

The authors declare that they have no competing interests

Funding

Not applicable

Authors' contributions

Not applicable

Acknowledgements

Not applicable

References

[1]Guo Q, Fang S, Zeng FQ, Li BY, Zhu XJ, Tan GZ. Establishment and optimization of two dimensional gel electrophoresis profiles of proteome from human keratinocytes.[J]. China Journal of Modern Medicine,2008,18(23):3453-3455+3459.

285 [2]Dou M, Chu X, Zhang J, Chen XH, Wang YJ, Shao BQ, Wang CB.Polypeptide from chlamys
286 farreri protect HaCaT cells from single UVA induced oxidative dam ages.[J].Chinese
287 Pharmacological Bulletin 2006 Apr;22(4):420-3.

288 [3]Wang HY, Zeng YY, Wang T, Xing FY, Zhao JX, Ji YH.Effects of ultraviolet on
289 mitochondrial functions and apoptosis in HaCaT cells.[J].Chinese Journal of Pathophysiology
290 2006, 22(5):1020 -1023.

291 [4] Chen F, Tang Y, Sun YJ, Priya VV, Surapaneni Krishna M, Cui CX . 6-shogaol, a active
292 constiuents of ginger prevents UVB radiation mediated inflammation and oxidative stress through
293 modulating NrF2 signaling in human epidermal keratinocytes (HaCaT cells).[J]. Journal of
294 Photochemistry & Photobiology, B: Biology,2019,197.

295 [5] Sreekanth N, Chapla ,Cynthia Tilley,Rajesh Agarwal. Silibinin is a Potent Sensitizer of UVA
296 Radiation‐induced Oxidative Stress and Apoptosis in Human Keratinocyte HaCaT Cells.[J].
297 Photochemistry and Photobiology,2012,88(5).

298 [6]Li JL, Liu N, Chen XH, Sun M, Wang CB. Inhibition of UVA-induced apoptotic signaling
299 pathway by polypeptide from Chlamys farreri in human HaCaT keratinocytes.[J]. Radiation and
300 Environmental Biophysics,2007,46(3).

301 [7]Cross Sheree E, Innes Brian, Roberts Michael S, Tsuzuki Takuya, Robertson Terry A,
302 McCormick Paul. Human skin penetration of sunscreen nanoparticles: in-vitro assessment of a
303 novel micronized zinc oxide formulation.[J]. Skin Pharmacology and Physiology,2007,20(3).

304 [8]Zhe H, Li Z, Cui QZ, Fu JJ, Wang YF . Apoptosis inhibition effect of Dihydromyricetin against
305 UVA-exposed human keratinocyte cell line.[J]. Journal of Photochemistry & Photobiology, B:

306 Biology,2016,161.

307 [9]LI JM, XU YM, Chen QY, Qi YH, Zhang N.Protective effects of UVA induced photoaging in
308 HaCaT cell by Eucommia ulmoides.[J].Chinese Journal of Aesthetic
309 Medicine.Sep.2010.Vol.19.No.9.

310 [10]Han ZW, Wang MZ, Wang LY, XU Long, Chen XH，Han YT. Study on Inhibitory Effects of
311 Rose Water on Ultraviolet-induced Apoptosis of HaCaT Cells.[J].China Pharmacy 2013 Vol. 24
312 No.7.

313 [11]Li M, Lin XF, Lu J, Zhou BR, Luo D. Hesperidin ameliorates UV radiation-induced skin
314 damage by abrogation of oxidative stress and inflammatory in HaCaT cells.[J]. Journal of
315 photochemistry and photobiology. B, Biology,2016,165.，

316 [12]Lin J, Wei MC， Mo MY. Analysis of the Clinical Application and Compatibility of
317 Pearl.[J].Journal of Basic Chinese Medicine December 2016 Vol.22. No.12

318 [13]Yang AQ, Shen YQ, Zhang LH, Mo JH, Chen ZX, Wang J. Study on the Skin Care Functions
319 of Active Ingredients in Pearl Extractive.[J].Flavour Fragrance Cosmetics Feb.2016,No.1.

320 [14]Yang YL, Chang CH, Huang CC, Liu HW. Anti-inflammation and anti-apoptosis effects of
321 pearl extract gel on UVB irradiation HaCaT cells.[J]. Bio-medical materials and
322 engineering,2015,26 Suppl 1.

323 [15]Zhou DX,, Wu SL. Anti- inflammation and Antioxidation of Pearl Water Extract.[J].Journal
324 Of Zhe Jiang College Of Tcm .Aug.2001 Vol.25. No.4.

[16]Yang AQ，Wang J，Zhang LH，Mo JH，Chen ZX，Shen YQ. Lighting Effect of Pearl Extract on Melanocytes in Vitro.[J].Pharmaceutical Biotechnology 2016，23(2) : 146 ～ 149.

[17]Ronald Marks. Seeing through the Stratum Corneum[J].The Keio Journal of Medicine,2009,49(2).

[18]Alena Svobodová, Adéla Zdařilová, Jana Mališková, Hana Mikulková, Daniela Walterová, Jitka Vostalová. Attenuation of UVA-induced damage to human keratinocytes by silymarin.[J]. Journal of Dermatological Science,2006,46(1).

[19]D'Arcy MarkSean. Cell Death. A review of the major forms of Apoptosis, Necrosis and Autophagy.[J]. Cell biology international,2019.

[20][1]László Virag, Rafael I. Jaén, Zsolt Regdon, Lisardo Boscá, Patricia Prieto. Self-defense of macrophages against oxidative injury: Fighting for their own survival.[J]. Redox Biology,2019.

[21]R. Takasawa, H. Nakamura, T. Mori, S. Tanuma. Differential apoptotic pathways in human keratinocyte HaCaT cells exposed to UVB and UVC.[J]. Apoptosis,2005,10(5).

[22]Svobodová A, Walterová D, Vostalova J. Ultraviolet light induced alteration to the skin. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub 2006;150:25—38.

[23]Brand Rhonda M, Wipf Peter, Durham Austin, Epperly Michael W,Greenberger Joel S, Falo Louis D. Targeting Mitochondrial Oxidative Stress to Mitigate UV-Induced Skin Damage.[J]. Frontiers in pharmacology,2018,9.

[24]Likidlilid Atip, Patchanans Natchai, Peerapatdit Thavatchai, Sriratanasathavorn Charn. Lipid peroxidation and antioxidant enzyme activities in erythrocytes of type 2 diabetic patients.[J].

345 Medical Association of Thailand. Journal,2010,93(6).

346 [25]Svobodová Alena Rajnochová, Galandáková Adéla, Sianská Jarmila, Doležal Dalibor,
347 Ulrichová Jitka, Vostalová Jitka. Acute exposure to solar simulated ultraviolet radiation affects
348 oxidative stress-related biomarkers in skin, liver and blood of hairless mice.[J]. Biological &
349 Pharmaceutical Bulletin,2011,34(4).

350 [26]Anne Valère Marionnet, Yvette Chardonnet, Jacqueline Viac, Daniel Schmitt. Differences in
351 responses of interleukin‐1 and tumour necrosis factor α production and secretion to
352 cyclosporin‐A and ultraviolet B‐irradiation by normal and transformed keratinocyte cultures.[J].
353 Experimental Dermatology,1997,6(1).

354 [27]Frank S, Kämpfer H, Wetzler C, Stallmeyer B,Pfeilschifter J. Large induction of the
355 chemotactic cytokine RANTES during cutaneous wound repair: a regulatory role for nitric oxide
356 in keratinocyte-derived RANTES expression.[J]. Biochemical Journal,2000,347 Pt 1.

357 [28]Di Girolamo Nick, Wakefield Denis, Coroneo Minas T. UVB-mediated induction of cytokines
358 and growth factors in pterygium epithelial cells involves cell surface receptors and intracellular
359 signaling.[J]. Investigative Ophthalmology & Visual Science,2006,47(6).

360 [29] Wang YQ，Chen QY，Li JM，Zhang N.Regulation of chlorogenic acid on expression of
361 tumour necrosis factor-α and interleukin-6 in HaCat cells damaged by ultroviolet.[J]. Chin J
362 Pharmacol Toxicol，Vol 28，No 1，Feb 2014.

363 [30]Tait Stephen W G, Green Douglas R. Mitochondria and cell death: outer membrane
364 permeabilization and beyond.[J]. Nature Reviews. Molecular Cell Biology,2010,11(9).

[31]Kaufmann Thomas, Schlipf Sarah, Sanz Javier, Neubert Karin, Stein Reuven, Borner Christoph. Characterization of the signal that directs Bcl-x(L), but not Bcl-2, to the mitochondrial outer membrane.[J]. The Journal of Cell Biology,2003,160(1).

[32]Chandra J, Samali A, Orrenius S. Triggering and modulation of apoptosis by oxidative stress.[J]. Free Radical Biology & Medicine,2000,29(3-4).

[33]B. Antonsson, Mitochondria and the Bcl-2 family proteins in apoptosis signaling pathways, Mol. Cell. Biochem. 256-257 (2004) 141–155.

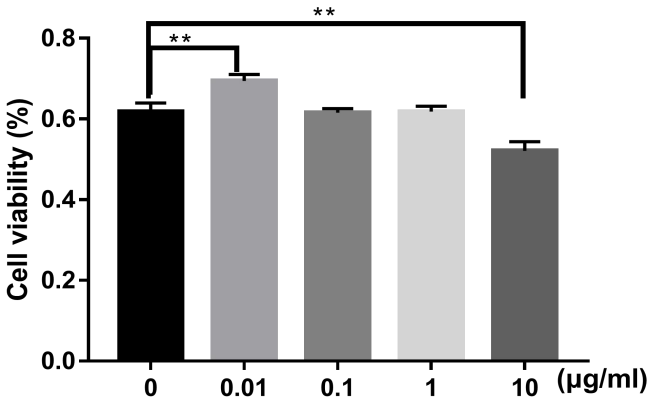
[34]Chang HY, Yang X. Proteases for cell suicide: functions and regulation of caspases.[J]. Microbiology and Molecular Biology Reviews,2000,64(4).

[35]Maximilian L, Würstle,Maike A, Laussmann,Markus Rehm. The central role of initiator caspase-9 in apoptosis signal transduction and the regulation of its activation and activity on the apoptosome.[J]. Experimental Cell Research,2012,318(11).

[36]Bhattacharya Sujoy, Ray Ramesh M, Johnson Leonard R. Cyclin-dependent kinases regulate apoptosis of intestinal epithelial cells.[J]. Apoptosis : an international journal on programmed cell death,2014,19(3).

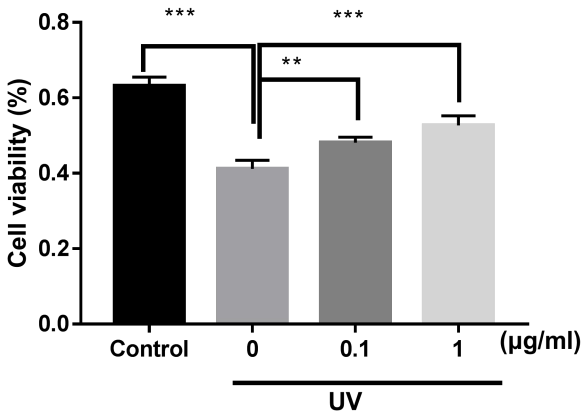
[37]K. Sinha, J. Das, P.B. Pal, P.C. Sil, Oxidative stress: the mitochondria-dependent and mitochondria-independent pathways of apoptosis, Arch. Toxicol. 87 (2013) 1157–1180

[38]Shimizu H, Banno Y, Sumi N, Naganawa T, Kitajima Y, Nozawa Y. Activation of p38 mitogen-activated protein kinase and caspases in UVB-induced apoptosis of human keratinocyte HaCaT cells.[J]. The Journal of investigative dermatology,1999,112(5).



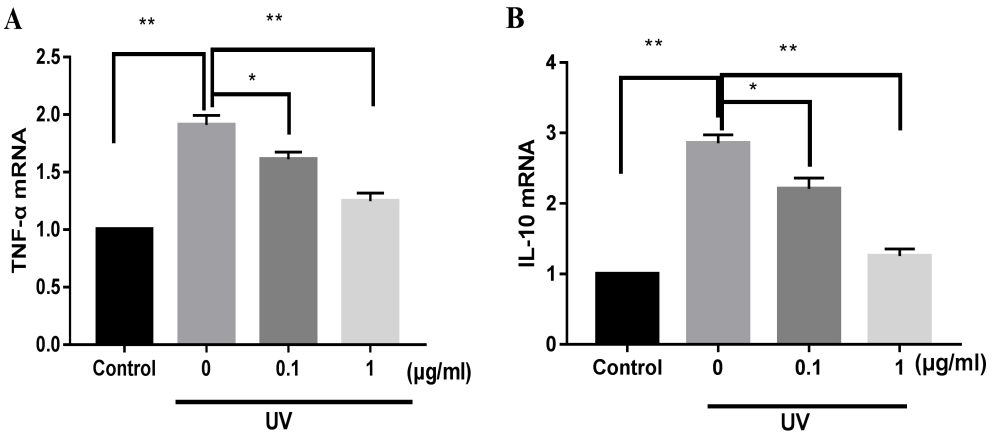
385

386 **Figure 1.** Effect of PE on proliferation rate of normal HaCaT cells. Cell viability was determined
387 by the CCK8 assay. HaCaT cells were treated with PE (0–10 μg/mL) for 48 h. Data are expressed
388 as the mean ± SD. The experiments were performed at least three times independently. **P < 0.01
389 indicates significant differences between experimental and control groups.



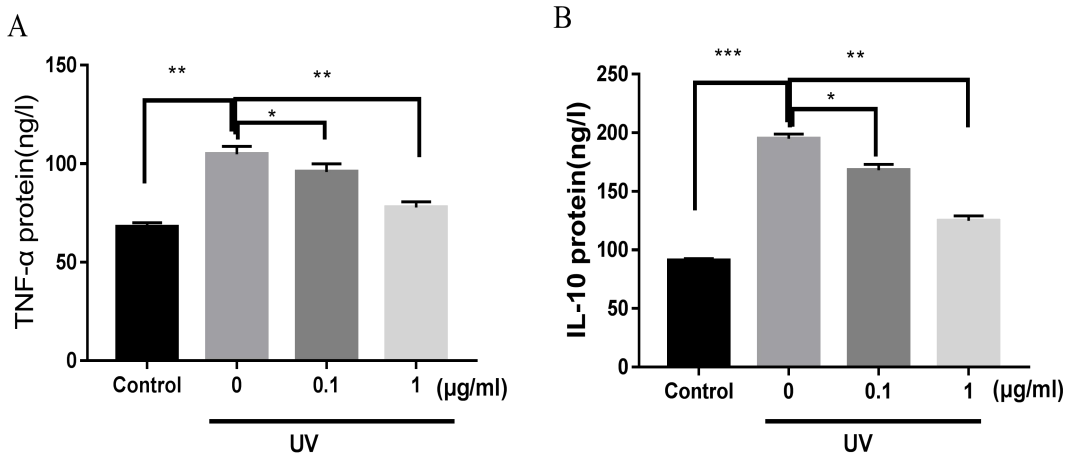
390

391 **Figure 2.** Effects of different treatment conditions on viability in UV-irradiated HaCaT cells. Cell
392 viability was determined by the CCK8 assay. HaCaT cells were treated with PE (0, 0.1 and 1
393 μg/mL) for 48 h. The cells were exposed to 10 J/cm^2 UV, and the control group was
394 sham-irradiated by covering with tin foil. Data are expressed as the mean ± SD. The experiments
395 were performed at least three times independently. **P < 0.01 and ***P < 0.01 indicate significant
396 differences between experimental and control groups.



397

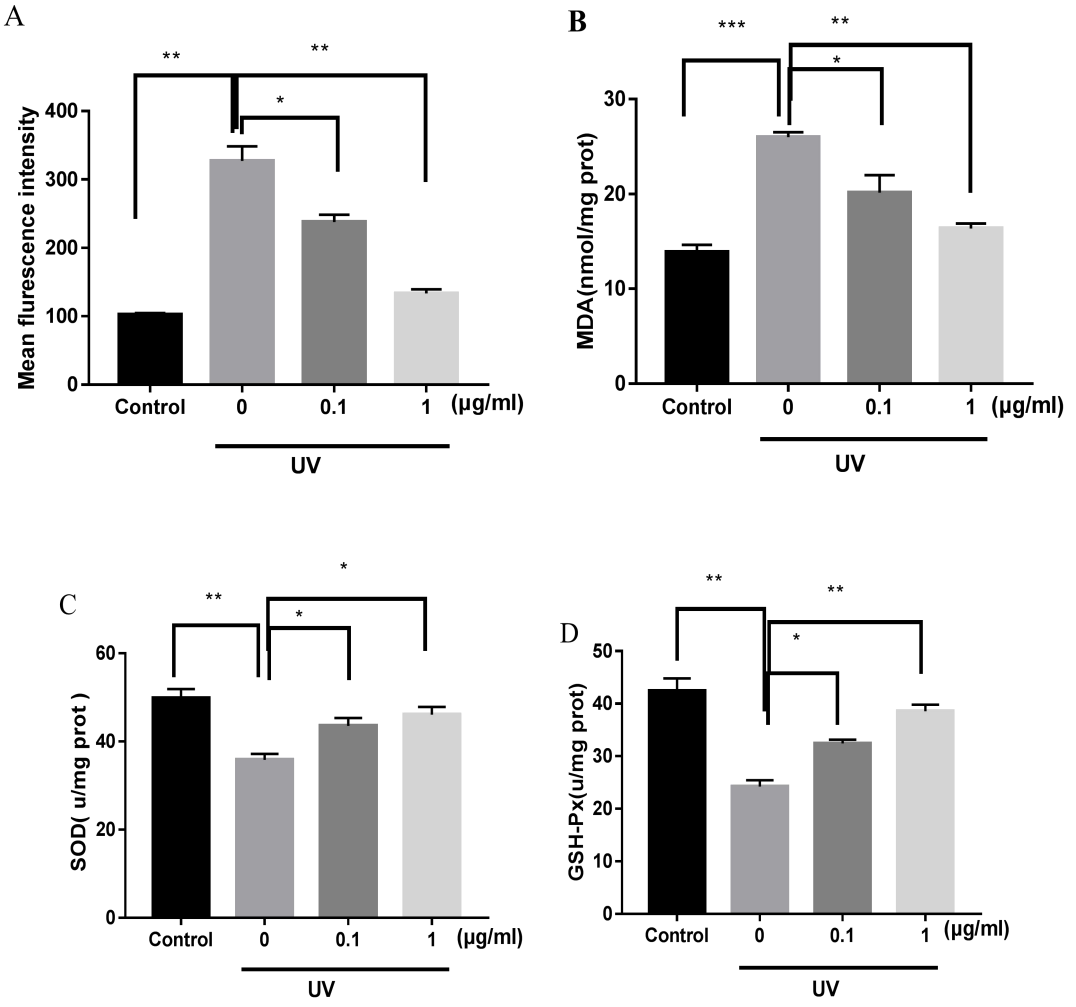
398 **Figure 3.** Effect of PE on mRNA expression. (A) TNF-α mRNA expression level. (B) IL-10
399 mRNA expression level. Data are expressed as the mean ± SD. The experiments were performed
400 at least three times independently. *P < 0.05 and **P < 0.01 indicate significant differences
401 between photoaging and other groups.



402

403 **Figure 4.** Effect of PE on TNF-α and IL-10 content in HaCaT cell supernatant. TNF-α protein
404 expression (A) and IL-10 protein expression (B). Data are expressed as the mean ± SD. The
405 experiments were performed at least three times independently. *P < 0.05, **P < 0.01 and ***P <
406 0.01 indicate significant differences between photoaging and other groups.

407



408

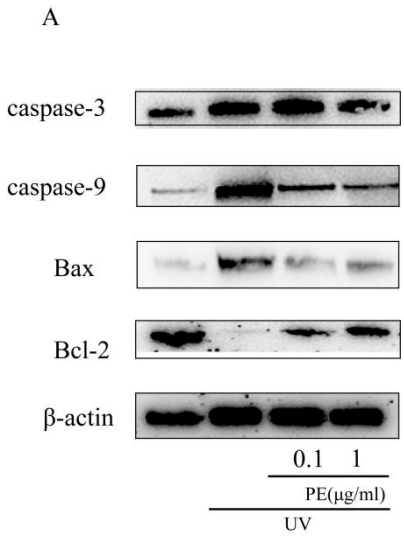
409

410 **Figure 5.** Effects of PE on antioxidant index in HaCaT cells. ROS levels (A), MDA levels (B),

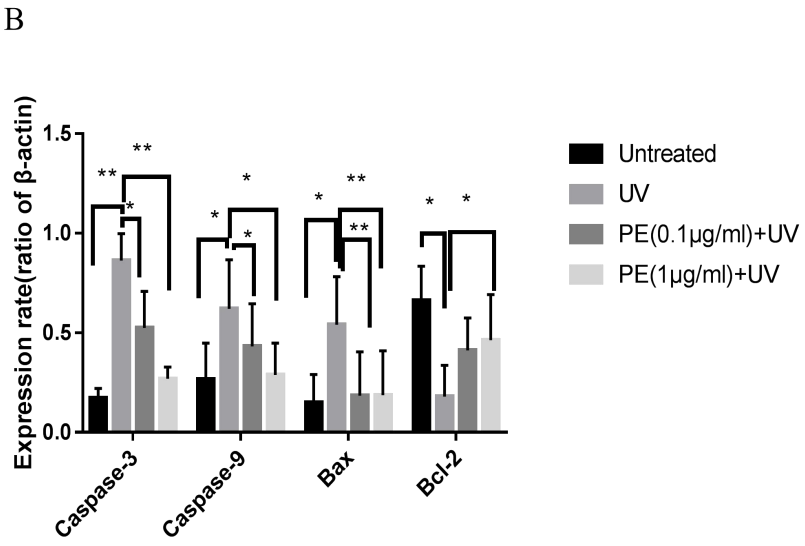
411 SOD activity (C) and GSH-Px activity (D). Data are expressed as the mean ± SD. The

412 experiments were performed at least three times independently. *P < 0.05, **P < 0.01 and ***P <

413 0.001 indicate significant differences between photoaging and other groups.



414



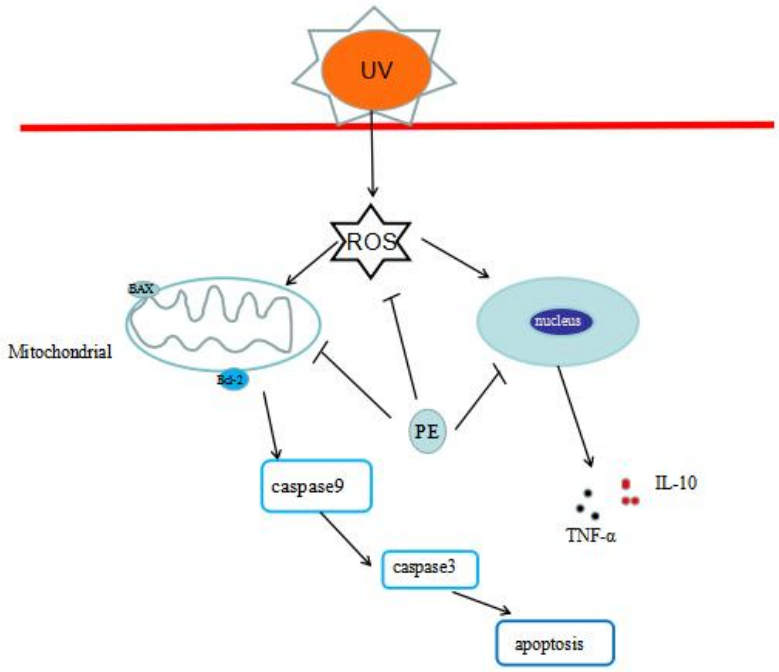
415

416 **Figure 6.** Effect of PE on UV-induced protein expression. Caspase-3, caspase-9, Bax and Bcl-2 in

417 HaCaT cells, as determined by western blotting (A) and band densitometry analysis (B). Data are

418 expressed as the mean ± SD. The experiments were performed at least three times independently.

419 *P < 0.05 and **P < 0.01: indicate significant differences between photoaging and other groups.



420

421 **Figure 7.** Schematic illustration of photoaging-protective properties of pearl extract.