

Crude polysaccharide from an anti-UVB cell clone of *Bupleurum scorzonerifolium* protect HaCaT cells against UVB-induced oxidative stress

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Abstract *Bupleurum scorzonerifolium* Willd has been found to have a wide range of immunopharmacologic functions. We isolated an anti-UVB *B. scorzonerifolium* cell clone and found elevated level of polysaccharides. In this study, we investigated the ability of crude polysaccharide (CP) from the anti-UVB *B. scorzonerifolium* cell clone to inhibit UVB-induced photodamage using a human skin keratinocyte cell line, HaCaT. Cells were UVB irradiated and then incubated in presence of different concentrations of CP. MTT assay showed that the CP did not induce cytotoxic effect under 10 mg/mL and after UVB irradiation, CP can inhibit UVB-induced HaCaT cell death. Decreased reactive oxygen species and lipid peroxidation and increased superoxide dismutase activity showed that CP can act as a free radical scavenger. Furthermore, CP had a strong protective ability against UVB-induced DNA damage. These

effects were compared to the crude polysaccharide (CP') from normal *B. scorzonerifolium* callus at concentration of 20 mg/mL. The portion of crude polysaccharide (CP) from the anti-UVB *B. scorzonerifolium* cell clone was more than 2.5-fold higher than crude polysaccharide (CP') from normal *B. scorzonerifolium* callus. Taken together, the protective mechanisms of crude polysaccharide from the anti-UVB *B. scorzonerifolium* cell clone against UVB-induced photodamage occur by the inhibition of UVB-induced reactive oxygen species production, lipid peroxidation and DNA damage.

Keywords Anti-UVB *Bupleurum scorzonerifolium* cell clone · Crude polysaccharide · Oxidative stress · Photodamage · UVB

Abbreviations

2,4-D	2,4-dichlorophenoxyacetic acid
CM-DCF	Chloromethyl 2',7'-dichlorodihydrofluorescein diacetate
CM-H ₂ DCFDA	5-(and-6)-chloromethyl-2',7'-dichlorodihydrofluorescein diacetate acetyl ester
CP	Crude polysaccharides from anti-UVB <i>B. scorzonerifolium</i> cell clone
CP'	Crude polysaccharides from normal callus of <i>B. scorzonerifolium</i>

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CP*	Crude polysaccharides from the aerial parts of Wild <i>B. scorzonerifolium</i>
DMEM	Dulbecco's modified Eagle medium
DMSO	Dimethylsulfoxide
FCS	Fetal calf serum
MDA	Malondialdehyde
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
ROS	Reactive oxygen species
SOD	Superoxide dismutase
UVB	Ultraviolet-B

Introduction

Ultraviolet (UV) radiation is one of the most harmful exogenous agents causing sunburn, immune suppression, cancer and photodamage. Based on wavelength, UV light can be categorized into UVA (320–400 nm), UVB (290–320 nm) and UVC (100–290 nm). Among these, UVA causes relatively weak cell damaging, whereas most UVC is absorbed by the ozone layer (Svobodová et al. 2006). Though UVB is a minor constituent of solar UV radiation, it is the most active one, which is 1,000 times more capable of causing photodamage than UVA (Matsumura and Ananthaswamy 2004). UVB acts mainly in the epidermal basal cell layer of the skin, inducing direct and indirect adverse biological effects, such as production of free radical and causing photoaging and photocarcinogenesis (photodamage), such as clinical sunburn, hyperpigmentation, erythema, plaque-like thickening, loss of skin tone, deep furrowing, and fine wrinkle formation (Svobodová et al. 2003).

UVB radiation can provoke oxidative stress through the formation of reactive oxygen species (ROS), such as hydroxy radical, superoxide anion radical and hydrogen peroxide, which will result in cell damage and DNA lesions (Nishigori et al. 2004). Therefore UVB is considered to be responsible for causing skin cancer due to DNA damage and skin aging due to accumulation of free radicals (Granstein and Matsui 2004; Ichihashi et al. 2003; Kulms and Schwarz 2002; Marrot and Meunier 2008). However, a number of protectors can be induced to cope with

adverse environmental stresses, such as stress-induced proteins and the antioxidant system (Sinha and Häder 2002; Sies and Stahl 2004).

Bupleurum scorzonerifolium Willd (Nan-Chai-Hu) is a common and important Chinese herb and commonly used to treat cold, influenza, fever, malaria and chronic liver disorders in China, Japan and many other places of Asia (Chinese Pharmacopoeia Commission 2005). In our previous study, we isolated a *B. scorzonerifolium* cell clone under UVB radiation and found that it can endure an UVB radiation of intensity of 91.2 mJ/cm² for 240 s (Li et al. 2011). This particular cell clone was named anti-UVB cell clone and had elevated higher levels of polysaccharide than the normal *B. scorzonerifolium* callus (Li et al. 2011). UVB irradiation damages the epidermal basal cell layer of the skin through direct and indirect adverse biological effects. So, the protection of the skin cells exposed to UVB irradiation-induced oxidative damage is very important. The aim of this study was to investigate the protective effect of the polysaccharide against UVB-induced photodamage by the use of human immortalized HaCaT keratinocytes.

Materials and methods

Chemicals

Dimethylsulfoxide (DMSO), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), 5-(and-6)-chloromethyl-2',7'-dichlorodihydrofluorescein diacetate acetyl ester (CM-H₂DCFDA), chloromethyl 2',7'-dichlorodihydrofluorescein diacetate (CM-DCF), and 2,4-dichlorophenoxyacetic acid (2,4-D) were purchased from Sigma-Aldrich (St Louis, MO, USA). Dulbecco's modified Eagle medium (DMEM), fetal calf serum (FCS), penicillin and streptomycin were purchased from Gibco (Grand Island, NY, USA). All other chemicals were of reagent grade and were used without further purification.

Culture of the anti-UVB *Bupleurum scorzonerifolium* cell clone

Protoplasts of *B. scorzonerifolium* and the aerial parts of *B. scorzonerifolium* Willd were provided by Prof. Xia Guangmin (School of life sciences, Shandong University, China). The anti-UVB *B. scorzonerifolium*

cell clone was obtained through continuous screening by UVB irradiation at the intensity of 91.2 mJ/cm² for 240 s and was maintained by subculture on solid MS medium (Murashige and Skoog 1962) supplied with 1.0 mg/L 2,4-D. The normal *B. scorzonerifolium* calli (without any UVB irradiation) were maintained by subculture on solid MS medium (Murashige and Skoog 1962) supplied with 1.0 mg/L 2,4-D.

Isolation of crude polysaccharides

Crude polysaccharides from anti-UVB *B. scorzonerifolium* cell clone (CP), normal callus of *B. scorzonerifolium* (CP') and the aerial parts of *B. scorzonerifolium* Willd (CP*) were extracted according to the methods described by Sun et al. (2010) with minor modifications. Approximately 50 g dry materials were extracted with 400 mL distilled water in a water bath at 90 °C for 2 h. Then the extracts were filtered and centrifuged to remove the contaminants. The supernatants were concentrated by evaporation under reduced pressure and precipitated with 95% ethanol for precipitation at 4 °C overnight. The precipitations were obtained by centrifugation, and dried under reduced pressure. The samples were dissolved in distilled water and centrifuged to remove insoluble materials. The supernatants were dialyzed to remove the small molecules. The dialyzed solutions were freeze-dried to yield crude polysaccharides.

HaCaT cell culture and treatment

The experiments were performed with the human skin keratinocyte cell line HaCaT. HaCaT cell line was purchased from the Kunming Medical College (Kunming, Yunnan Province, China). The cells were grown in DMEM medium (Gibco, Grand Island, NY, USA) containing 10% fetal calf serum (FCS), 10 U/mL penicillin and 10 µg/mL streptomycin, and incubated at 37 °C in a 5% CO₂ and 95% air humidified atmosphere. The medium was removed every 48 h and cells were subcultured every 7 days.

UVB radiation was carried out using a Spectrolinker XL-1,500 UV cross linker (Spectronics, USA), which emits most of its energy within the UVB range (280–320 nm) and peaking at 312 nm. The cells were washed with phosphate-buffered saline (PBS) before UV irradiation, and after UVB exposure the cells were washed twice in PBS. For each experiment, the cells

were exposed to UVB at the intensity of 60 mJ/cm² for 200 s, and then treated with different concentrations of crude polysaccharides for 24 h at 37 °C, respectively.

MTT assay

The sensitivity of cells to crude polysaccharides were determined by a standard spectrophotometric 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Mosmann 1983). HaCaT cells were seeded at a density of 5×10^3 cells/well into 96-well plates and incubated for 24 h at 37 °C. Then the medium was removed and the cells were washed with PBS twice and the cells were incubated in FCS-free medium containing different concentrations of crude polysaccharides for 24 h at 37 °C, respectively. Wells treated only with vehicle were used as controls. Cell viability was evaluated by assaying for the ability of functional mitochondria to catalyze the reduction of MTT (5 mg/mL, 37 °C, 3 h) to form formazan salt by mitochondrial dehydrogenases, as determined by ELISA reader at 570 nm (Multiskan Spectrum; Thermo Electron Corporation, USA). The absorbance of untreated cells was considered as 100%. HaCaT cell viability after UVB irradiation was also evaluated by the same method. Assays were repeated at least three times, and plating was performed in triplicate for each dose. The cellular viability was expressed as the percentage of viable cells compared with mock-treated (untreated) cells.

Measurements of UVB-induced ROS

The formation of intracellular ROS was measured using an intracellular ROS fluorescent detection kit (Genmed Scientifics Inc., U.S.A.) according to the manufacturer's instructions. The fluorescence was detected at an excitation wavelength of 490 nm and an emission wavelength of 530 nm, using a spectrofluorometer. The data were expressed as a percentage of the mock-treated (untreated) cells.

SOD activity

Superoxide dismutase (SOD) activity in HaCaT cells was determined using a SOD detection kit (Nanjing Jiancheng Bioengineering Institute, China) according to the manufacturer's instructions. The data were expressed as a percentage of the mock-treated (untreated) cells.

Assay for lipid peroxidation

Lipid peroxidation was assayed according to the method described by Huang et al. (2010). The levels of MDA were measured by spectrometer using excitation and emission wavelength of 485–535 nm, respectively. The data were expressed as a percentage of the mock-treated (untreated) cells.

DNA damage quantification

DNA damage was counted as AP sites which were detected using a DNA Damage Quantification Kit (Dojindo Laboratory, Kumamoto, Japan) according to the manufacturer's instructions.

Measurement of the polysaccharides

Polysaccharides from anti-UVB *B. scorzonerifolium* cell clone, normal callus of and the aerial parts of *B. scorzonerifolium* Willd were expressed as glucan equivalents and estimated by phenol–sulfuric acid colorimetric method (Buyse and Merckx 1993).

Statistical analysis

Results presented are average means of at least three replicates each. The differences between mean data values obtained in this study were determined using the Student's *t* test ($p < 0.05$). Significant differences are marked with asterisks.

Results

Effect of UVB irradiation on cell viability and protection of HaCaT cells by crude polysaccharides

HaCaT cells were incubated with different concentrations of crude polysaccharides (1, 5, 10, 50, and 100 mg/mL). After 24 h-incubation, cytotoxicity was evaluated using standard MTT test. As shown in Fig. 1, comparing with negative control, crude polysaccharides from anti-UVB *B. scorzonerifolium* cell clone (CP), normal callus of *B. scorzonerifolium* (CP') and the aerial parts of *B. scorzonerifolium* Willd (CP*) did not cause cytotoxicity at concentrations inferior or equal to 10 mg/mL. In contrast, enhanced cell viability

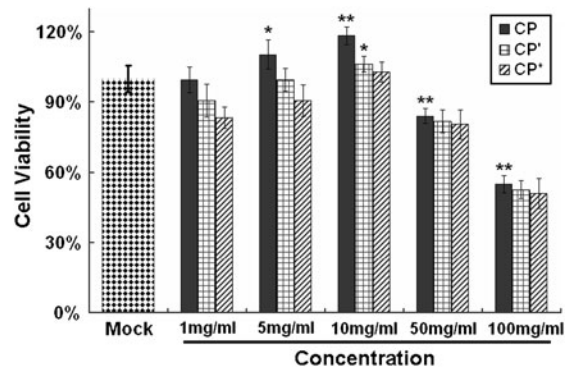


Fig. 1 Effect of crude polysaccharides on HaCaT cell viability. HaCaT cells were incubated with various concentration of crude polysaccharides from anti-UVB *B. scorzonerifolium* cell clone (CP), normal callus of *B. scorzonerifolium* (CP') and the aerial parts of *B. scorzonerifolium* Willd (CP*) for 24 h. Cell viability was measured with MTT assay. Results are expressed as percentage of the mock-treated cells (untreated negative control cells) as the mean \pm SD of triplicate independent experiments. A significant difference relative to untreated negative control cells is indicated with * $p < 0.05$ or ** $p < 0.01$

could be observed in a concentration-dependent manner. CP of 5 mg/mL increased viability by 10% compared with negative control ($p < 0.05$), while 10 mg/mL CP could increase viability by 18% compared with negative control ($p < 0.01$). At the same time, 10 mg/mL CP' could only increase viability by 6% compared with negative control ($p < 0.05$). At concentrations above 50 mg/mL, slightly cytotoxic effects could be observed for all three kinds of crude polysaccharides ($p < 0.01$). Thus, concentrations under 10 mg/mL (1, 5, 10 mg/mL) were used in the following experiments. Besides that, CP* increased slightly less the viability comparing with CP or CP' ($p < 0.05$), CP* was eliminated in the following experiments.

To determine the potential protective effects of crude polysaccharides on keratinocyte, we then performed MTT assay on HaCaT cell after UVB irradiation. As reported before, the cell viability of HaCaT cells decreased after exposed to UVB irradiation (Huang et al. 2010). HaCaT cells were exposed to UVB at the intensity of 60 mJ/cm² for 200 s, and then the cells were incubated with different concentrations of CP and CP' for 24 h at 37 °C, respectively. As shown in Fig. 2, cell viability of HaCaT cell decreased significantly after UVB exposure. However, the abatement could be reversed in a dose-dependent manner by the incubation with crude polysaccharides

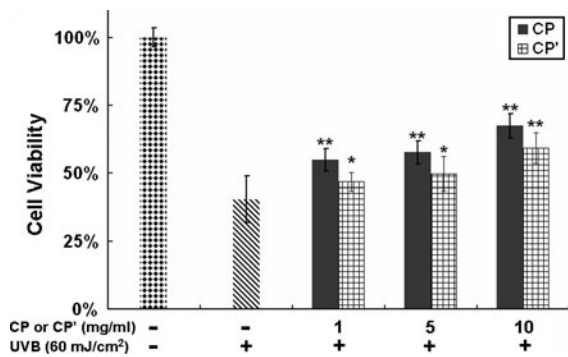


Fig. 2 Protective effects of crude polysaccharides on HaCaT cell under *UVB* exposure. HaCaT cells were exposed to *UVB* at an intensity of 60 mJ/cm² for 200 s, and then the cells were incubated with or without different concentrations of crude polysaccharides for 24 h. Cell viability was measured with the MTT assay. Results are expressed as percentage of the mock-treated cells (untreated negative control cells) as the mean \pm SD of triplicate independent experiments. A significant difference relative to *UVB* alone treated positive control cells is indicated with * p < 0.05 or ** p < 0.01

of HaCaT cells (Fig. 2). Moreover, crude polysaccharides had positive effects on the proliferation of HaCaT cells. CP at 10 mg/mL increased viability by 27% compared with positive control (*UVB*-irradiation alone) (p < 0.01), while 10 mg/mL CP' could increase viability by 19% compared with positive control (p < 0.01). Under the same concentration, CP had more positive effects comparing with CP'. For example, at the concentration of 1 mg/mL and 5 mg/mL, CP had a significantly positive effects (p < 0.01), while CP' had a relative slightly positive effects (p < 0.05).

This difference between CP and CP' was investigated by the measurement of the content of polysaccharides in anti-*UVB B. scorzonerifolium* cell clone and normal *B. scorzonerifolium* callus. Anti-*UVB B. scorzonerifolium* cell clone had 0.65 mg/g (dry weight) polysaccharides, which was 1.7-fold higher than normal *B. scorzonerifolium* callus and 2.9-fold higher than aerial parts of *B. scorzonerifolium* Willd (our unpublished data).

Crude polysaccharides inhibit *UVB*-induced free radicals production in HaCaT cells

UVB-irradiation induce the formation of photo-products directly through the products such as cyclobutane pyrimidine dimers and indirectly via the production of

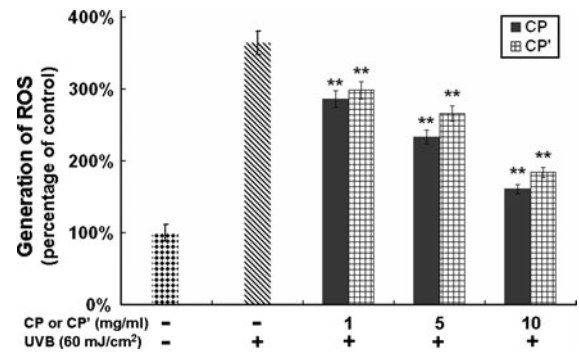


Fig. 3 Effect of crude polysaccharides on *UVB*-induced intracellular ROS generation in HaCaT cells. HaCaT cells were exposed to *UVB* (60 mJ/cm², 200 s), and then incubated with crude polysaccharides for 24 h. Results are expressed as percentage of the mock-treated cells (untreated negative control cells) as the mean \pm SD of triplicate independent experiments. A significant difference relative to *UVB* alone treated positive control cells is indicated with * p < 0.05 or ** p < 0.01

free radicals, which result in cell damages (Svobodová et al. 2006). Therefore, we investigated the potential protective effects of crude polysaccharides on the generation of ROS. CM-H₂DCFDA was used to detect the levels of intracellular ROS. Upon crossing the membrane, the compound undergoes deacetylation by intracellular esterases producing the non-fluorescent CM-H₂DCF, which quantitatively reacts with oxygen species inside the cell to produce the highly fluorescent dye CM-DCF (Bartosz 2006). As shown in Fig. 3, *UVB*-irradiation generated significant amounts of ROS in HaCaT cells, which were 3.65-fold higher than ROS generated from the negative control (untreated cells) (p < 0.001). However, incubation with both CP and CP' significantly inhibited intracellular ROS production (p < 0.01 compared to *UVB*-irradiated cells without any treatments) (Fig. 3). This observation indicated that crude polysaccharides may have scavenging activity by reducing the production of intracellular ROS under *UVB* exposure.

Superoxide dismutase (SOD) is one of the most important enzymes which maintain the prooxidant/antioxidant balance by rapid ROS elimination, resulting in cell and tissue stabilization (Fubini and Hubbard 2003). As we have observed reduced ROS production, we also tested the activity of SOD in HaCaT cells after *UVB* exposure. SOD activity decreased significantly to 61% in *UVB*-irradiated cells compared to the untreated negative control (Fig. 4). However, incubation with crude polysaccharides could enhance SOD

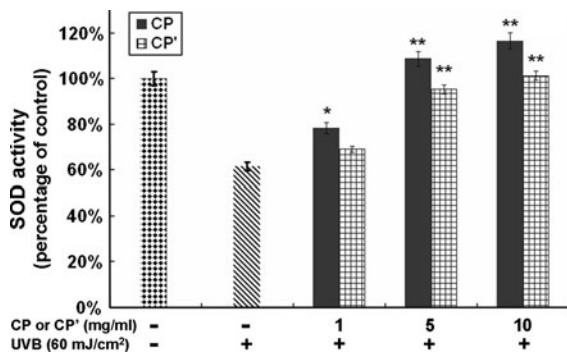


Fig. 4 Protective effect of crude polysaccharides on the decrease of SOD activity induced by UVB-irradiation in HaCaT cells. HaCaT cells were exposed to UVB (60 mJ/cm², 200 s), and then incubated with crude polysaccharides for 24 h. Results are expressed as percentage of the mock-treated cells (untreated negative control cells) as the mean \pm SD of triplicate independent experiments. A significant difference relative to UVB alone treated positive control cells is indicated with * $p < 0.05$ or ** $p < 0.01$

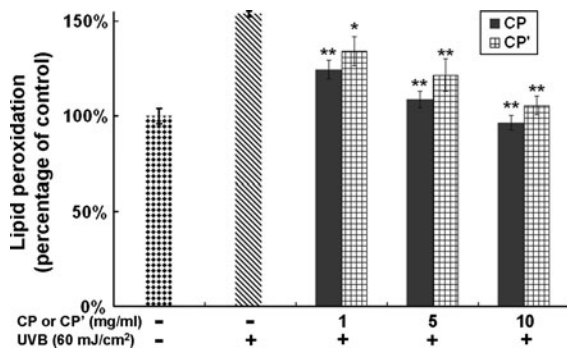


Fig. 5 Protection of HaCaT cells by crude polysaccharides against UVB-induced lipid peroxidation. HaCaT cells were exposed to UVB (60 mJ/cm², 200 s), and then incubated with crude polysaccharides for 24 h. Results are expressed as percentage of the mock-treated cells (untreated negative control cells) as the mean \pm SD of triplicate independent experiments. A significant difference relative to UVB alone treated positive control cells is indicated with * $p < 0.05$ or ** $p < 0.01$

activity in a dose-dependent manner. As shown in Fig. 4, 5 mg/mL and 10 mg/mL CP or CP' can significantly enhance SOD activity ($p < 0.01$), and 10 mg/mL CP can enhance SOD activity up to about 116.6% ($p < 0.01$).

Crude polysaccharides reduce lipid peroxidation in UVB-irradiation HaCaT cells

The oxidative stress caused by UVB-irradiation result in lipid peroxidation of bio-macromolecules such as

cell membrane, protein and DNA, which is strongly associated with cell death (Halliday 2005). MDA is the end product of the lipid peroxidation process induced by free radical or activated oxygen species when cells are exposed to oxidative stress, so we assessed lipid peroxidation of HaCaT cells by examining the levels of MDA. As shown in Fig. 5, exposure of HaCaT cells to UVB increased the production of MDA compared with the untreated control ($p < 0.001$), but the amount of MDA decreased in a concentration-dependent manner in the presence of CP or CP' obviously reduced the production of MDA at concentration of 5 mg/mL and 10 mg/mL compared with the UVB-irradiation positive control cells ($p < 0.01$).

The protective effect of crude polysaccharides against UVB-induced DNA damage

Oxidative attack by hydroxyl radical on the deoxyribose moiety will lead to the release of free bases from DNA, generating strand breaks with various sugar modifications and simple abasic sites (AP sites) (Besaratina et al. 2005). Here we used the amount of abasic sites (AP sites) to assess the protective effect of crude polysaccharides against UVB-induced DNA damage. Exposure of HaCaT cells to UVB-irradiation increased the AP sites, which were 2.6-fold higher than those from the untreated negative control cells (Fig. 6) ($p < 0.01$). UVB-irradiation-induced DNA damage in HaCaT cells was recorded as average 25.5 AP sites per 10⁶ bp, while for the untreated control cells an average of 9.8 AP sites per 10⁶ bp was detected. However, the DNA damage could be reduced by incubation with crude polysaccharides after UVB-irradiation. As shown in Fig. 6, AP sites decreased in a concentration-dependent manner for both CP and CP'. Concentration of 1 mg/mL CP can obviously reduce AP sites ($p < 0.01$), however, only a concentration above 5 mg/mL (crude polysaccharide from normal *B. scorzoniferolium* callus) can reduce AP sites.

Discussion

Nan-Chai-Hu, *B. scorzoniferolium* Willd, is an important Chinese herb which has been used for thousands of years in China, Japan and many other places of Asia (Chinese Pharmacopoeia Commission 2005). It has

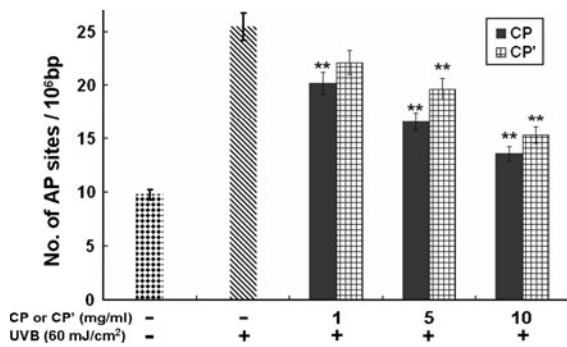


Fig. 6 Effects of crude polysaccharides on UVB-induced DNA damages in HaCaT cells. DNA damages are expressed as the amount of abasic sites (AP sites), which is generated from hydroxyl radical attack on the deoxyribose moiety of the DNA strand. HaCaT cells were exposed to UVB (60 mJ/cm², 200 s), and then incubated with crude polysaccharides for 24 h. AP sites were detected by ARP assay. Error bars indicate SD of three independent experiments. A significant difference relative to UVB alone treated positive control cells is indicated with * $p < 0.05$ or ** $p < 0.01$

been found to have a wide range of immunopharmacologic functions, such as anti-inflammatory, mitogenic, or antiviral activities (Bermejo et al. 2002), however, to our knowledge, there was no record on *B. scorzonerifolium* for treatment or prevention of photo-damage. Like many other traditional herbal medicines, the underlying mechanisms of the medicinal effect of *Bupleurum scorzonerifolium* were intensively studied in the past decades. Natural compounds extracted from the plants were proven to possess various biological activities (Chang et al. 2003). Polysaccharides as important natural compound derived mainly from plant sources, become more interesting because of their essential role in many molecular processes. The mechanisms of pharmaceutical effects of bioactive polysaccharides on diseases have been extensively studied and more and more kinds of natural polysaccharides with different curative effects have been tested and even applied in therapies (Liao et al. 2005; Singla and Chawla 2001). Although used in various diseases, it has been suspected that most curative effects of polysaccharides were based on their antioxidant activity. Certain type of antioxidative reaction in certain types of cells leads of treatment to specific disease (Li et al. 2009; Tomida et al. 2009).

In our previous study, we isolated an anti-UVB *B. scorzonerifolium* cell clone, which was tolerant to high dose of UVB radiation, 8 times of the dose lethal to

most cells. Chemical analysis showed that the cells were remarkably rich in polysaccharides. We speculated that the high content of polysaccharides was the reason of its high tolerance to UVB. Here in this study, it was proven that supplement of crude polysaccharides from this *B. scorzonerifolium* cell clone could protect HaCaT cells from UVB irradiation.

Keratinocytes are the main target of UV, and play a central role in several responses of photo damage after UV exposure (Portugal et al. 2007). In present work, human immortalized HaCaT keratinocytes cells were irradiated with UVB (60 mJ/cm²) and then incubated with crude polysaccharides from anti-UVB *B. scorzonerifolium* cell clone (CP), normal callus of *B. scorzonerifolium* (CP') and the aerial parts of *B. scorzonerifolium* Willd (CP*). To evaluate the protective role of crude polysaccharides against UVB insult to cells, two parameters have been evaluated. First, cell viability assessment was measured by the MTT assay. The second parameter investigated is the level of lipid peroxidation by measuring MDA in the culture medium under UVB irradiation. MDA is the product of the lipid peroxidation process induced by free radical or activated oxygen species when cells are exposed to oxidative stress. The results showed cell viability and lipid peroxidation are inversely correlated when cells are exposed to UVB (Figs. 2, 5).

Human skin is equipped with a network of antioxidant enzymes and the antioxidant enzymes play an important role in the balance of the intracellular level of ROS. Catalase, SOD, glutathione-peroxidase, glutathione-reductase and intracellular-reduced glutathione work corporately, so that changes in one component can affect the state of balance of ROS. Over-generation of ROS in the biological system can cause cellular damage and biochemical alterations, such as oxidation of proteins and lipids, inflammation, DNA damages, and activation and inactivation of enzymes (Shindo et al. 1993). Therefore, the prevention of the production of ROS after UVB irradiation in HaCaT cells (Fig. 3) prevents the attenuation of antioxidant enzymes (e.g., SOD) (Fig. 4) by the treatment with crude polysaccharides might be an important strategy for protection against UVB-induced skin damage.

UVB is also a well-known genotoxic agent that is able to induce oxidatively damaged DNA including DNA strand breakage and base modification (Van Laethem et al. 2009; de Gruijl 2002). UVB-induced

oxidatively damaged DNA was tested as AP sites. Incubation with crude polysaccharides after UVB-irradiation can reduce the generation of AP sites (Fig. 6). Since the increase of intracellular ROS formation induced by UVB exposure can be prevented by the treatment of crude polysaccharides (Fig. 3), the result is in line with the hypothesis that crude polysaccharides play an important role in the UVB-induced oxidative damage.

Crude polysaccharides from anti-UVB *B. scorzonerifolium* cell clone (CP) and normal callus of *B. scorzonerifolium* (CP') both displayed significant protective capability against UVB-induced oxidative damage, but CP has a better effect than CP' at the same concentration. Therefore, the major component in CP and CP', which directly modulates the activity of antioxidant system, needs further investigation.

Conclusion

In the present study, we found that crude polysaccharides could prevent keratinocytes HaCaT cell death from UVB-induced damage. We suggest that the inhibitory effects of lipid peroxidation and ROS production by crude polysaccharides after UVB exposure may contribute to the protective effects against UVB. Finally, we investigated whether UVB-induced DNA damage was prevented by pretreatment with crude polysaccharides. Crude polysaccharides from anti-UVB *B. scorzonerifolium* cell clone may have an important place among cosmeceuticals and can be used as cosmetic formulations.

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