Modulation of the UV-B-induced Oxidative Stress and Apoptosis in HaCaT Cell Line with *Calluna vulgaris* Extract

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Abstract

The reactive oxygen species (ROS) production due to ultraviolet B (UV-B) exposure is extremely harmful to the skin. It causes lesions of DNA, proteins and lipids and leads to cellular death. In the present study the UV-B-induced ROS and subsequent apoptosis in the human keratinocyte cell line (HaCaT) were counterbalanced by a plant extract with antioxidant capacity. Some molecules modulated by common heather (*Calluna vulgaris*) (CV) extract through which this may exert its photoprotective effects were also identified. The ROS were evaluated with CM-H$_2$DCFDA assay, while apoptosis and Bax-α/Bcl-xL molecules with ELISA. The extract was standardized according to its polyphenolic content and the most important biologically active compounds, such as hyperozid, quercetin, isoquercetin, kampferol were evidenced by high-performance liquid chromatography. The UV-B induced ROS production occurred at its highest level at 2 h after the exposure of the HaCaT cells, while apoptosis later, at 4 h. The most significant changes in Bax-α and Bcl-XL proteins induced by UV-B, as well as the highest effect of the extract on apoptosis, were both registered at 4 h. The CV extract decreased concentration- and time-dependently the UV-B-induced ROS production and prevented apoptosis. These effects of CV occurred, at least to a certain extent, due to the modulation of Bax-α/Bcl-X$_L$ proteins. These findings suggest that skin cells could be protected from some of the UV-B-induced harmful effects by the administration of the CV extract, which may be further exploited as a potential photoprotective agent.

Abbreviation list: Bak: a pro-apoptotic protein; Bcl-2 homologous antagonist/killer; Bax-α: a pro-apoptotic protein; Bcl-2 homologous antagonist/killer; Bcl-2: B-cell lymphoma 2; member of the Bcl-2 family of proteins, anti-apoptotic; Bcl-x: B-cell lymphoma extra large; member of the Bcl-2 family of proteins, anti-apoptotic; ccK18: apoptosis-associated caspase-cleaved keratin 18; C: control (non-irradiated) group; CM-H2DCFDA: 5-(and-6)-chloromethyl-2′,7′-dichlorodihydrofluorescein diacetate, acetyl ester; CV: *Calluna vulgaris*; CV1: *Calluna vulgaris* concentration 20 μg EqGA/ml; CV2: *Calluna vulgaris* concentration 40 μg EqGA/ml; DNA: deoxyribonucleic acid; DPPH: 2,2-diphenyl-1-picrylhydrazyl; EDTA: ethylenediaminetetraacetic acid; ELISA: enzyme-linked immunosorbent assay; EqGA: gallic acid equivalents; F.u.: Fluorescence units; HaCaT: human immortalized keratinocyte cell line; HRP: horseradish peroxidase; I: irradiated group; IC50: half maximal inhibitory concentration; K18Asp396 epitope: soluble caspase-cleaved fragments of the intermediate filament protein keratin 18 (K18) containing the M30 neo-epitope; KCs: Keratinocytes; M5: antibody, M5; M30: monoclonal antibody, M30; NAC: N-acetylcysteine; NP40: non-ionic polioxietilen-based surfactant solution; PBS: phosphate buffered saline; ROS: reactive oxygen species; SEM: standard error of the mean; U/L: Units/Liter; UV-B: ultraviolet B; UVR: ultraviolet radiation

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Introduction

Ultraviolet radiation (UVR), a high energy constituent of sunlight, is one of the most damaging environmental agents for the human skin. It causes alterations in the skin appearance, such as erythema, sunburn, hyper-pigmentation, photoaging or hyperplasia, immune suppression and DNA damage, which jointly can contribute to skin cancer induction (InYoung and Yu-Ying, 2014). UVR is a full carcinogen, being able to damage molecules (nucleic acids, proteins, lipids, saccharides) directly or indirectly through ROS production (Assefa *et al.*, 2005). However, neither the source nor the pathways of these molecules generation after UVR are clearly understood. Although ultraviolet B (UV-B) radiation represents a smaller fraction of the UVR (4-5%), it is harmful, causing sunburn and skin cancers (squamous and basal cell carcinoma) (Wu *et al.*, 2015). UV-B exerts its deleterious effects due to the production of cyclobutane pyrimidine dimers and ROS (hydroxyl and peroxyl radicals, superoxide anion, singlet oxygen, hydrogenperoxide and ozone) by the irradiated skin cells (Janovska *et al.*, 2015; Svobodova *et al.*, 2003).

The ROS-induced photolesions are normally repaired or removed by cellular death (Salucci *et al.*, 2014), according to the depth of the lesions and the repair capacity of the damaged cells. When the ROS production exceeds the skin's antioxidant defense capacity, a cascade of events is initiated resulting in inflammation, photoaging, abnormal gene expression and photocarcinogenesis (Sklar *et al.*, 2013). The balance between these processes varies according to the dose of UVR, the cell type and the involved molecules (Cu, Zn dismutases, catalase and heme oxygenase-1, gluthation S-transferase, inflammatory cytokines etc). Of great importance are the apoptosis regulatory proteins, such as anti-apoptotic Bcl-2/Bcl-X_L and pro-apoptotic Bax/Bak proteins, which exert their actions mostly at the level of mitochondria (Juba *et al.*, 2013).

A practical approach to protect the human skin from these undesirable effects of UV-B and to reduce the severity of UV-B-induced skin diseases is to use active photoprotective agents. Besides the well-known compounds (vitamins C, E, beta-carotene etc), phenolics with documented antioxidative properties gained great interest being added to diet or used for topical applications (Lorencini M *et al.*, 2014; Robbins, 2003). Biomaterials based on nanoparticles and natural extracts were tested recently as anti-inflammatory agents, beneficial in treating skin diseases (David *et al.*, 2014; Wu *et al.*, 2015).

Calluna vulgaris L. Hull (CV), common heather belongs to *Ericaceae* family, a low-growing perennial shrub found widely in Europe and Asia Minor on acidic soils, was selected for investigations in the present study. Some of the CV extract's biological activities were assessed earlier on SKH-1 nude mice (Olteanu *et al.*, 2012) and on umbilical vein endothelial cell line (Olteanu *et al.*, 2014). These studies demonstrated beneficial effects, i.e. apoptosis prevention, evaluated through sunburn cells formation and on caspase 3 activation, respectively. Another finding revealed that the CV extract prevents the UV-B-induced DNA lesions in HaCaT cells (Perde-Schrepler *et al.*, 2011).

The aim of the present study was to assess the potential protective effect of CV extract against the UV-B-induced ROS production and apoptosis in the human keratinocyte cell line HaCaT. A secondary objective was to identify some molecules involved in apoptosis and through which the CV extract may exert its beneficial effects.

Methods

Cell cultures

The spontaneously transformed human keratinocytes cell line (HaCaT) was purchased from the Cell Line Service of the German Cancer Research Centre in Heidelberg (Germany) (Boukamp *et al.*, 1998). Cells were cultured in Dulbecco's Modified Eagle's Medium with 4500 mg glucose. Media was supplemented with 10% Fetal Calf Serum and 1% Penicillin/Streptomycin. Cells were seeded in triplicate in 24-well plates at a cell population density of 125×10^3/well. A plate was maintained as control and the others were irradiated with UV-B or treated with the plant extract and afterward irradiated with UV-B.

UV-B irradiation of the cells

The cells were washed and overlaid with a thin layer of Phosphate Buffered Saline and irradiated with Waldmann Medizintechnik UVB 181 broadband device (Waldmann GmbH, Germany). This lamp, employed for therapeutical purposes, emits a continuous spectrum from 280 to 350 nm, with a peak emission at 320 nm, i.e preponderantly in UV-B (> 91%), this type of radiation ranging between 280-320 nm. The emitted radiation dose was measured with a Variocontrol radiometer (Waldmann GmbH, Germany) before each experiment. Irradiation doses were calculated using the formula:

$$\text{Dose (mJ/cm}^2) = \text{Exposure time (sec)} \times \text{Intensity (mW/cm}^2).$$

The UV-B's cytotoxicity on the HaCaT cells was previously determined (Perde-Schrepler *et al.*, 2011) and the half maximal inhibitory concentration (IC_{50}) (217 mJ/cm^2) was considered for the selection of the dose used in this study. Therefore, a dose within the physiological range of UVR exposure usually experienced by human skin and approximately equivalent to the minimal erythema dose (Pathak *et al.*, 1987), was chosen (100 mJ/cm^2). Therefore, a plate was maintained as control and the others were irradiated with 100 mJ/cm^2 UV-B or treated with the plant extract and afterward irradiated with 100 mJ/cm^2 UV-B.

Morphology analysis

The morphological analysis of the cells was performed using a light microscope (Carl Zeiss MicroImaging GmbH, Gottingen, Germany), having digital photographic capability. The microscopic images of cells (non-irradiated; irradiated with UV-B; treated with the tested plant extract and afterward irradiated with UV-B) were compared with respect to morphological characteristics.

Evaluation of the ROS production with CM-H₂DCFDA

CM-H₂DCFDA, a chloromethyl derivative of 2′,7′-dichlorodihydrofluorescein diacetate is a general oxidative stress indicator. Inside the cells it is cleaved by esterases and its chloromethyl group reacts with glutathione and other thiols, resulting in a fluorescent adduct. In the present study, the cells were seeded in triplicate in 24-well plates at a cell population density of 125×10^3/well. After the cells' treatment CM-H₂DCFDA was added to the wells ($10 \mu M$) and the samples were incubated 30 min at $37°C$ and 5% CO_2 atmosphere. The fluorescence was recorded using Biotek Synergy 2, a multi-mode microplate reader (Winooski, USA), at 488 nm.
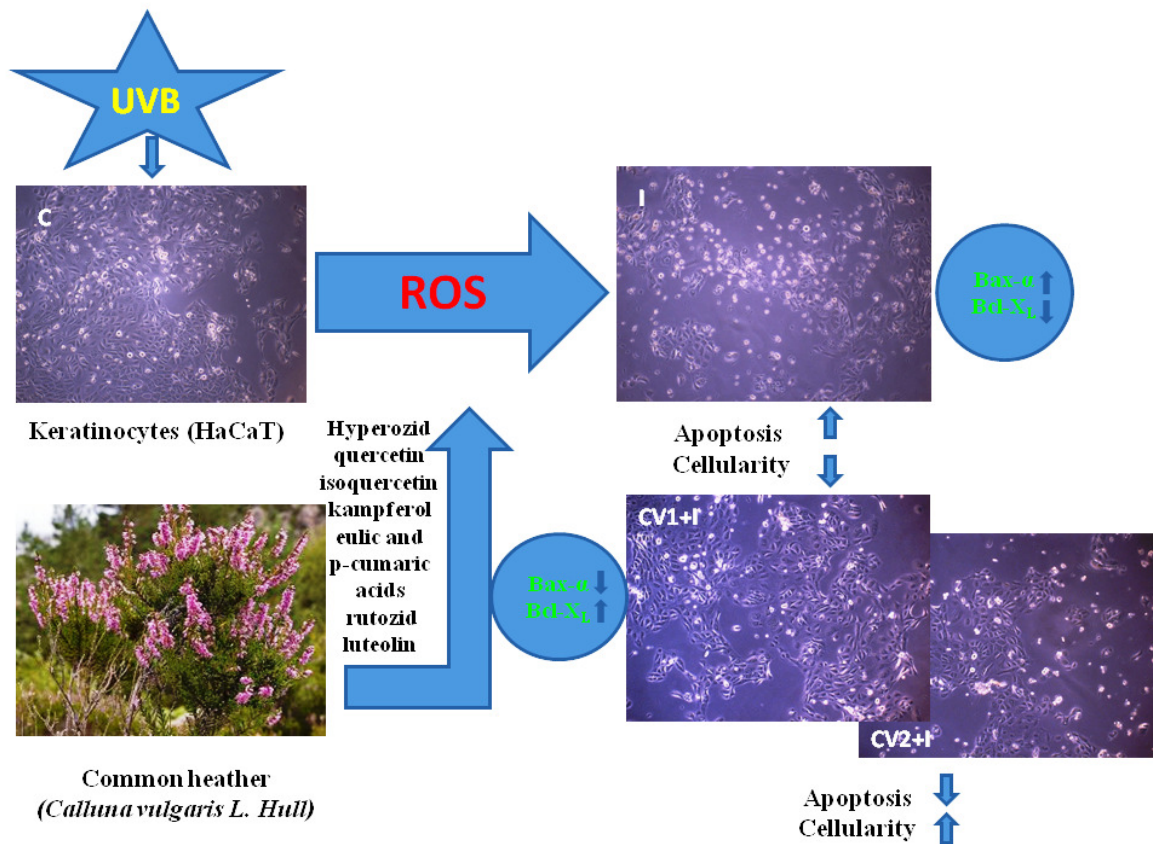
Fig. 1. Microscopic images of the control HaCaT cells (C), cells irradiated with UV-B dose of 100 mJ/cm² (I) and cells treated with: CV₁ (20 µEqGA/ml) or CV₂ (40 µEqGA/ml) and afterward irradiated with a UV-B dose of 100 mJ/cm² (CV₁+I; CV₂+I)

Detection of apoptosis with ELISA

Apoptosis was assessed with M30 Apotosense ELISA solid-phase sandwich enzyme immunoassay technique (PEVIVA, Bromma, Sweden), which quantitatively measures the apoptosis-associated caspase-cleaved keratin 18 (ccK18) in cell lysates, following the manufacturer's protocol. Briefly, cells were seeded in triplicate in 24-well plates at a cell population density of 125 x 10³/well, treated as described above and then incubated for 2, 4 and 8 h. The cells lysis was carried out with NP 40 10 %, a non-ionic polioxietilen-based surfactant solution. Cell lysates were added to a capture antibody M5 directed against ccK18. The secondary monoclonal antibody was represented by M30, directed against the K18Asp396 epitope. The absorbance was recorded at 450 nm with a microplate reader (Tecan Sunrise, Grödig/Salzburg, Austria).

Determination of the intracellular levels of Bax-α and Bcl-xL with ELISA

Bax-α and Bcl-xL levels from the cell lysates were determined using sandwich-ELISA technique (R&D Systems, Abingdon, UK), according to the manufacturer's indications. The cells were seeded in triplicate in 24-well plates at a cell population density of 125 x 10³/well and treated as described above. After 2, 4 and 8 h from UV-B exposure, the cells were lysed with a buffer based on 1 mM EDTA, 0.005% Tween 20, 0.5% Triton X-100, 10 µg/ml Leupeptin, 10 µg/ml Pepstatin, 100 µM PMSF and 3 µg/ml

Aprotinin in PBS, ph 7.2-7.4. Mouse anti-human and rat anti-mouse capture antibodies were used for Bax-α and Bcl-xL molecules, respectively. Biotinylated detection antibodies specific for these molecules were used in a standard streptavidin-HRP format. The fluorescence was recorded at 450 nm with a microplate reader (Tecan Sunrise, Grödig/Salzburg, Austria).

Plant material

The raw material - the aerial part of the plant *Calluna vulgaris* - was collected on August 2010 in Ciucea rural area, Cluj County, Romania. The species was identified by Professor Mircea Tămaş from the University of Medicine and Pharmacy, Cluj-Napoca. A voucher specimen was deposited in the Herbarium of the Botanical Department of the same University (index number: 825). The plant extract was prepared according to previous descriptions (Filip *et al.*, 2011; Postescu *et al.*, 2007). Briefly, a fluid extract (1:1) was obtained from 30 g dry vegetal material in 300 ml 70% ethanol, by maceration at room temperature for 1 week. The extract's total polyphenolic content was determined by Folin-Ciocalteu method (Postescu *et al.*, 2007) and was standardized as 19 mg gallic acid equivalents (GAE)/ml. Proanthocyanidins represented 3.15 mg/ml and monomer anthocyanins 5.81 mg/ml. The most important biologically active compounds were evidenced by high-performance liquid chromatography which indicated the presence of hyperozid, quercetin, isoquercetin, kampferol and
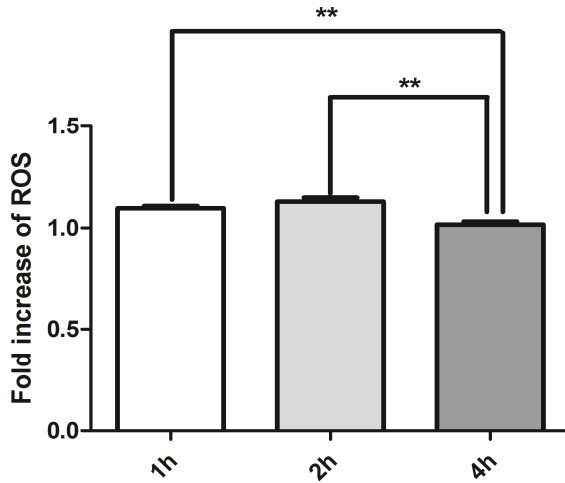
Fig. 2. Kinetics of ROS production in HaCaT cells exposed to UV-B radiation (at 1, 2 and 4 h after irradiation). Values are expressed as fold changes of ROS in irradiated *vs* control cells. Statistical comparisons between groups were made by Unpaired *t* test, **p<0.001 (Means ± SEM, n=3)
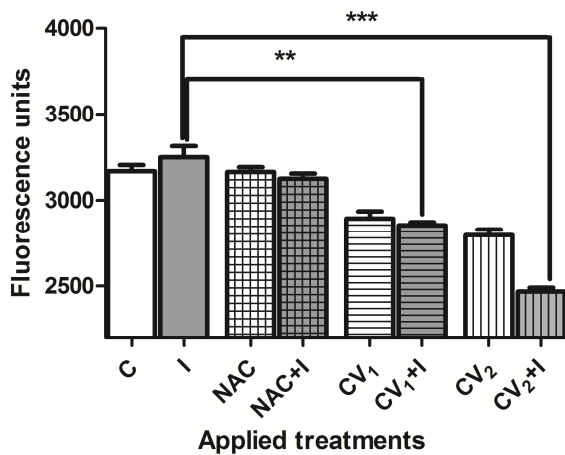

Fig. 3. The level of ROS production at 4 h after the following treatments of the HaCaT cells: control (C); irradiated with 100 mJ/cm2 UV-B (I); treated with N-acetylcysteine (5 mM) (NAC); treated with N-acetylcysteine (5 mM) followed by irradiation with 100 mJ/cm2 UV-B (NAC+I); treated with CV1 (20 μEqGA/ml); treated with CV1 (20 μEqGA/ml) followed by irradiation with 100 mJ/cm2 UV-B (CV1+I); treated with CV2 (40 μEqGA/ml); treated with CV2 (40 μEqGA/ml) followed by irradiation with 100 mJ/cm2 UV-B (CV2+I). Statistical comparisons between groups were made by Unpaired t test, **p<0.001; ***p<0.0001 (Means ± SEM, n=3)

smaller amounts of eulic and p-cumaric acids, rutozid and luteolin (Perde-Schrepler *et al.*, 2011). The antioxidant activity of CV extract was established at 0.211 ± 0.08 mM/mM 2,2-diphenyl-1-picrylhydrazyl (DPPH), respectively 38.92 ± 0.01 mM Trolox eq (Filip *et al.*, 2011).

N-acetylcysteine (NAC), a well-known selective inhibitor of ROS (Sun, 2010), was used (5 mM) as positive control.
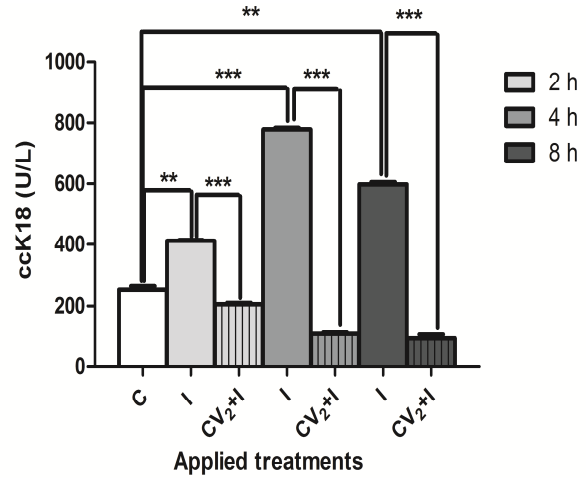

Fig. 4. Apoptosis production at 4 h after the following treatments of the HaCaT cells: control (C); irradiated with 100mJ/cm^2 UV-B (I); treated with CV$_1$ (20 μEqGA/ml); treated with CV$_1$ (20 μEqGA/ml) followed by irradiation with 100 mJ/cm^2 UV-B (CV$_1$+I); treated with CV$_2$ (40 μEqGA/ml); treated with CV$_2$ (40 μEqGA/ml) followed by irradiation with 100 mJ/cm^2 UV-B (CV$_2$+I). Statistical comparisons between groups were made by Unpaired *t* test, **p<0.001, ***p<0.0001 (Means ± SEM, n=2)

Assessment of the plant extract's cytotoxicity on the HaCaT cells

The cytotoxicity of the CV extract on HaCaT cells was determined earlier (Perde-Schrepler *et al.*, 2011), and the calculated IC$_{50}$ value (74.2 μg EqGA/ml) was considered as guide-mark for the tested concentrations. Aiming to identify protective effects concentrations in the non-toxic range were used, below the measured IC$_{50}$ values. Therefore, the following concentrations were considered for testing: CV$_1$ (20 μg EqGA/ml) and CV$_2$ (40 μg EqGA/ml). The cells were treated with the selected concentrations of the plant extract or with NAC (5 mM), used as positive control, for 30 min before 100 mJ/cm^2 UV-B irradiation.

Statistical analysis

Statistical processing of the experimental data was done using GraphPad Prism software program, version 5.0 (GraphPad, San Diego, CA, USA). Column statistics were performed, a method used with data entered on data tables formatted for Column data. The statistical comparisons between groups (two by two) were assessed by Unpaired *t* test, at p<0.05 statistical significance (control *vs* irradiated; irradiated with UV-B radiation *vs* treated with one of the concentration of the plant extract and afterward irradiated with UV-B radiation).

Results

Morphology analysis of HaCaT cells exposed to UV-B radiation and/or treated with plant extract

The microscopic analysis revealed distinct features for the control cells as compared to UV-B-irradiated ones and to those previously treated with the plant extract and afterward irradiated with UV-B (Fig. 1).
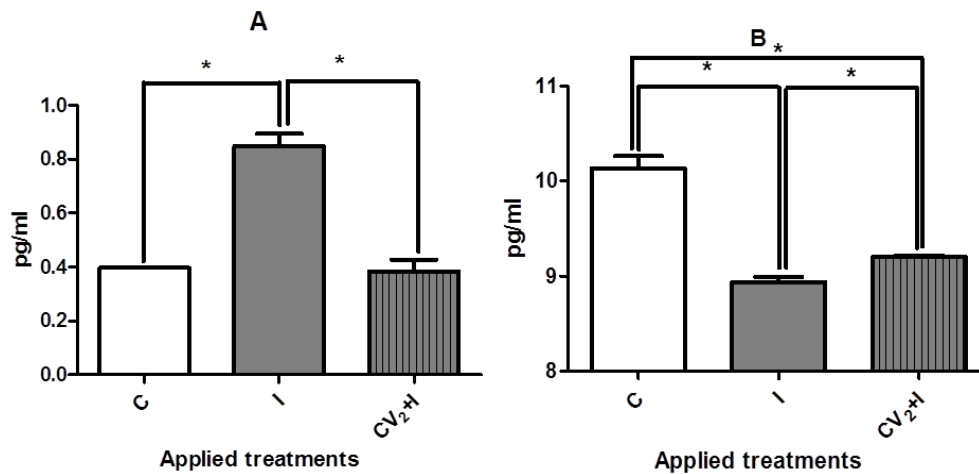
Fig. 5. Expression of Bax-α (A) and Bcl-xL (B) apoptosis regulatory proteins at 4 h after the following treatments of the HaCaT cells: control (C); irradiated with 100 mJ/cm² UV-B (I); treated with CV₂ (40 μEqGA/ml) followed by irradiation with 100 mJ/cm² UV-B (CV₂+I). Statistical comparisons between groups were made by Unpaired *t* test, *p<0.05 (Means ± SEM, n=2)

Thus, microscopy slides of HaCaT cells irradiated with UV-B showed decreased cellularity and elevated mass of apoptotic cells/total amount of cells, as compared to the controls. Conversely, slides representing cells treated with the plant extract and afterwards exposed to UV-B depicted higher cellularity and lower amount of apoptotic cells/total amount of cells, as compared to the solely irradiated ones. Their microscopic aspects i.e. cellularity and the occurrence of the apoptotic cells/total amount of cells, show similarity with the control cells.

The level of ROS produced in HaCaT cells exposed to UV-B radiation and/or treated with CV extract

The ROS production after exposing the cells to 100 mJ/cm² UV-B radiation was evaluated at several intervals (1, 2 and 4 h). The level of ROS was higher at early time points following UV-B exposure (1 h and 2 h) resulting in 1.1 fold increase *vs* control at 1 h and 1.13 at 2 h, respectively. The differences between irradiated and control cells showed decreasing tendency at 4 h. There were statistically significant differences between the magnitude of fold changes of ROS at 1 h and 2 h *vs* those determined at 4 h (p<0,001 in both cases) (Fig. 2).

None of the selected concentrations of the plant extract exerted ROS decreasing effects at 1 or 2 h after UV-B irradiation (data not shown). Conversely, the extract concentrations-dependently reduced the levels of ROS at 4 h. For the CV₁ extract the level of ROS induced by UV-B was reduced from 3249±112.6 Fluorescence units (F.u.) to 2855±30.92 F.u. (p<0.001), corresponding to 1.13 fold reduction *vs* solely irradiated cells. CV₂ extract exerted a decrease in the level of ROS from 3249±112.6 F.u. to 2468±39.63 F.u. (p<0.0001), corresponding to 1.31 fold decrease *vs* control (Fig. 3.).

Compared to the results obtained with NAC (used as positive control), both concentrations of the plant extract had significantly superior ROS scavenging effects. NAC, a well-known selective ROS inhibitor (used as positive control in this study) determined a slight reduction in ROS level at 4 h, from 3249±112.6 to 3125±50.21 F.u. (Fig. 3), representing a 1.03 fold decrease *vs* solely irradiated cells.

The level of the apoptosis induced by UV-B irradiation and/or treatment with CV₂ extract in HaCaT cells

Apoptosis was evaluated in control cells, UV-B irradiated cells, and in cells previously treated with CV₁ or CV₂ and then irradiated, at three time points: 2 h, 4 h and 8 h. The intracellular level of ccK18 was assessed to quantify the apoptosis. At 2 h the ccK18 level increased from 253±16.43 U/L to 411.8±4.43 U/L in the solely irradiated cells (p<0.001), corresponding to 1.62 fold increase *vs* control. At 4 h, UV-B induced an important increase in the level of ccK18, to 778.4±7.6 U/L (p<0.0001), corresponding to 3.07 fold increase *vs* control. 8 h after irradiation the level of this protein was also increased but at a lower magnitude: 598.2±10.2 U/L (p<0.001), which represents 2.36 fold increase *vs* control. These data identify a peak level of apoptosis induction at 4 h after UV-B irradiation and a decreasing tendency after this time point (Fig. 4).

The highest ROS scavenging effect on UV-B irradiated HaCaT cells was produced by the higher concentration of the plant extract (CV₂ with 40 μg EqGA/ml concentration), therefore we tested this extract for its potential effects on apoptosis. CV₂ determined a significant decrease in ccK18 level when administered previously to UV-B radiation for all three time points. The highest effect was registered 4 h after irradiation, the level of this protein decreasing from 778.4±7.6 U/L to 107.7±5.9 U/L (p<0.0001), in the cells treated with this extract *vs* solely irradiated cells corresponding to a 7.22 fold change (Fig. 4)

The levels of Bax-α pro-apoptotic and Bcl-xL anti-apoptotic proteins in HaCaT cells after UV-B irradiation and/or treatment with CV₂ extract

Significant changes in the level of Bax-α (pro-apoptotic protein) in UV-B-exposed HaCaT cells were registered at 4 h after UV-B exposure, when the level of this molecule increased from 0.3965±0.0007 pg/ml to 0.8475±0.06 pg/ml (p<0.05), corresponding to a 2.13 fold increase as compared to control. Administration of CV₂ plant extract before irradiation significantly reduced the level of Bax-α protein: form 0.8475±0.06 pg/ml to 0.3825±0.06 pg/ml, (p<0.05), corresponding to 2.21 fold decrease *vs* solely irradiated cells (Fig. 5.A).

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Conversely, Bcl-xL (anti-apoptotic protein) decreased at 4 h after UV-B irradiation: from 10.13 ± 0.18 pg/ml to 8.93 ± 0.07 pg/ml (p<0.05), which represents 1.13 fold decrease *vs* control. When cells were treated previously with CV_2, Bcl-xL protein decreased at 4 h after irradiation from 10.13 ± 0.18 pg/ml to 9.19 ± 0.02 pg/ml (p<0.05), corresponding to 1.1 fold decrease *vs* control (Fig. 5.B). This fold change was less important than that induced by UV-B irradiation alone (9.19 ± 0.02 pg/ml *vs* 8.93 ± 0.07 pg/ml), corresponding to a 1.02 fold difference between Bcl-xL levels in cells treated or not with CV_2 extract.

Discussion

The results in the present study showed that UV-B-induced cell death could be prevented by treating the cells with a plant extract having antioxidant properties. UV-B radiation influences a number of protein kinases, transcription factors and receptors, which may contribute to UVR-induced apoptosis (Kulms and Schwartz, 2002). Among these structures the central role is played by Bax/Bcl proteins exerting pro- or anti-apoptotic roles (Juba *et al.*, 2013).

In the present study, the outcome of exposing cells to UV-B radiation within the physiological range of UVR exposure and equivalent to the minimal erythema dose (100 mJ/cm²) was a significant ROS production. The peak level of ROS, registered at 2 h, is in accordance with other findings which demonstrated significantly higher rate of ROS production in the early time period after irradiation (0-2 h) (Dhumrongvaraporn and Chanvorachote, 2013; Masaki *et al.*, 2009). The maximum level of apoptosis occurred later (at 4 h), suggesting the implication of the radiation-induced ROS in this process. Other studies also demonstrated that ROS-mediated damages lead to lysosomal membrane rupture and consequent cell death in UV-B-irradiated keratinocytes (KCs) (Boya, 2003; Zhao *et al.*, 2000). However, neither the source of ROS generated in response to UV-B radiation nor the pathways by which they are generated in the skin are not entirely understood. Ryu et al. (2010) claim that a pathway linked to leukotriene B_4 receptor BLT2 and NADPH oxidase family protein Nox1 might have a crucial role. Others sustain that intracellular Ca^{2+} (Masaki *et al.*, 2009) or catalase enzyme in the skin (Heck *et al.*, 2003) might be the trigger for UV-B-induced ROS generation.

According to the present study's results, apoptosis in UV-B-exposed HaCaT cells occurred due to a significant increase in the Bax-α and to a decrease in Bcl-X_L proteins. Bcl-X_L protein is expressed in the upper layer of the epidermis (Krajevski, 1994) and it was shown to protect KCs against UV-B-induced cell death and against the formation of skin tumours in transgenic mice (Assefa *et al.*, 2005). Its overexpression in human KCs blocked the UV-B-induced apoptosis both *in vivo* and *in vitro* (Takahashi, 2001) and acted as antioxidant and inhibitor of ROS generation (Hockenbery *et al.*, 1993). In addition, this molecule was shown to prevent the disruption of the lysosomal and mitochondrial membranes, subsequently inhibiting Bax, the pro-apoptotic protein (Kane, 1993).

In the present study the treatment of the skin cells with the two concentrations of the CV extract prior to UV-B exposure diminished the level of ROS, as compared to the solely irradiated cells. Both apoptosis and the proteins related to this process (Bax-α and Bcl-X_L) were affected by the higher concentration of the extract (CV_2). The most pronounced effects on apoptosis and

regulatory proteins were registered at 4 h after UV-B irradiation. This finding might suggest that UV-B induced apoptosis was prevented through the modulation of these molecules, i.e. via reduction of Bax-α level and increase of Bcl-X_L protein, enlightening some of the potential underlying mechanisms of this extract's protective effects (Fig. 1.). The CV extract's protective effect against UV-B-induced oxidative stress and apoptosis, evaluated through sunburn cells formation (Olteanu *et al.*, 2012) and caspase 3 activity (Olteanu *et al.*, 2014), were demonstrated earlier on SKH-1 nude mice and on endothelial cells, respectively. Also, the extract's preventive effect on the UV-B-induced DNA lesions in HaCaT cells was previously established (Perde-Schrepler *et al.*, 2011).

Conclusion

The CV extract used in the present study exerted protective effects on HaCaT cells subjected to minimal erythema dose of UV-B radiation, consisting in significant reduction of ROS production and prevention of apoptosis. These effects are concentration-dependent and time-dependent and, at least to a certain extent, are due to the modulation of Bax-α and Bcl-X_L proteins. These results are in concordance to others in literature (Olteanu *et al.*, 2012; Olteanu *et al.*, 2014; Perde-Schrepler *et al.*, 2011) and support further studies to identify other molecules and pathways whereby the CV extract may exert its beneficial protective effects. These findings also suggest that the CV extract could be used as a potential photoprotective agent.

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