INTRODUCTION

Skin tumors in humans account for about 30% of all new cancers reported annually; their incidence is expected to increase substantially because of increased recreational exposure to sunlight and depletion of the ozone layer (1). Ultraviolet (UV) radiation is the major etiologic factor for the non-melanoma skin cancer. In addition to its carcinogenic activity, UV also causes sunburns, immunosuppression and photoageing (2).

UVB radiation (290-320 nm) represents the most energetic, mutagenic and carcinogenic component of solar radiation because it is directly absorbed by DNA, giving rise to dimeric photoproducts between adjacent pyrimidine bases, cyclobutane pyrimidine dimers (CPDs) and pyrimidine (6-4) pyrimidone photoproducts (3). These types of DNA damage induce mutations of the p53 tumor suppressor gene, which are crucial for cell proliferation and/or apoptosis and enhance the risk of photocarcinogenesis (4). The UVB-damaged cells respond to these alterations by enhanced DNA repair and apoptotic death when the damage is too severe (5, 6).

Similar to many chemical tumor promoters, UV also elicits nuclear factor-kappa B (NF-κB)-mediated inflammation, epidermal hyperplasia and changes in the expression of numerous genes associated with cell proliferation and differentiation, as well as prostaglandin (PG) and cytokine production (7). Proinflammatory cytokines such as interleukin IL-1β, IL-6, IL-10 and tumor necrosis factor-alpha (TNF-α) impair the antigen presenting abilities of Langerhans cells (8) which may result in suppression of the immune response (9). UV-induced inflammation is considered an early event in tumor promotion and/or tumor development (10). The inflammatory cells produce reactive oxygen species (ROS) and IL-10 (11) which increase DNA oxidative damage and induce tumor promotion. UVB-generated ROS also affect regulation of the gene expression of signaling molecules such as mitogen activated protein kinases (MAPKs) and interrelated inflammatory cytokines as NF-κB and activator protein-1 (AP-1).

These topics emphasize the importance of developing additional strategies that could prevent and control UV-induced skin damage mainly nonmelanoma skin cancers (12). Chemoprevention by natural compounds represents a new concept in the attempt to control the carcinogenesis process, specifically prevention of tumour growth, delaying of the clinical evolution or even regression of an already existing tumor...
The hydroethanolic extract from grape seed (Vitis vinifera L.), variety Burgund Mare, was prepared as previously described from 1:20 w/v mixture of finely powdered dried seeds and water/ethanol 50/50 (v/v) (15). The Calluna vulgaris (CV) fluid extract (1:1) was obtained from 30 g dry plant and 300 ml 70% ethanol by maceration at room temperature, for 7 days with occasional stirring. Before the treatment, both solutions were concentrated, in vacuo, (10 fold), and the products were characterized by their total polyphenolic content (TPC), assessed by the Folin-Ciocalteu colorimetric reaction (16) and expressed in equivalents (Eq) galic acid (GA) per unit of volume. To study the chemical compounds found in fruits, vegetables, plants affect one or more stages of the carcinogenesis process by modulating the pathways of intercellular signals (14). Exploring the action mechanism of these compounds through in vivo studies aiming to identify the target molecules and signalling pathways is of critical importance in assessing their clinical applicability. The present study was designed to evaluate, in vivo, the effect of topical application of Calluna vulgaris (CV) and red grape seeds extract, Vitis Vinifera Burgund Mare variety (BM), on UVB-induced skin damage using a SKH-1 hairless mice skin model. The possible protective effects were estimated after a single dose of UVB irradiation by: immunohistochemical (IHC) and immunofluorescence (IF) expression profiles of CPDs, quantification of sunburn cells (apoptotic cells) and measurement of TNF-α and IL-6 levels in the skin. MATERIALS AND METHODS Reagents Monoclonal antibody to CPDs for IHC and IF (Mouse IgG2a, code number NMND001) were purchased from Cosmo Bio Co Ltd, Japan. A secondary antibody included in the kit LSAB+System HRP (Code K0679), Mayer's Haematoxylin and hydrogen peroxide were from Dako (Denmark). Rhodamine (Goat polyclonal to mouse Ig H&L, ab6786) was purchased from ABcam (UK) and DRAQ5 (Cat No. 4084) from Cell Signaling Technology Inc. (Danvers, MA). Sodium dodecylsulfate (SDS), Triton X-100, 2,2-diphenyl-l-picryl-hydrazyl (DPPH), 2,2’-azino-bis(3-ethylbenzothiazoline-6-sulfonate (ABTS), Bradford and Folin Ciocalteu reagents were purchased from Sigma-Aldrich Chemicals GmbH (Germany). ELISA tests to measure IL-6 and TNF-α level were obtained from R&D Systems (Minneapolis, MN, USA). All chemicals and reagents were of high grade of purity. Vegetal materials The free radical scavenging activity of polyphenols was previously measured by using the free radical 2,2-diphenyl-l-picryl-hydrazyl (DPPH) scavenging assay following the Brand-Williams method (17) with some modifications (18). Substrate concentrations that lower the initial DPPH-concentration by 50% (EC₅₀), the time required to reach EC₅₀ and redox reaction stoichiometry n were calculated (18). Antioxidant capacity was determined using ABTS test. The reagent containing ABTS⁺ was prepared from a solution of 7 mM ABTS containing 2.45 mM potassium persulfate (final concentration) left in the dark for 12 hours. This reagent was diluted with phosphate buffered saline pH 7.4 to obtain the working solution presenting absorption of 0.70±0.02. The readings were done at 4 min after the addition of the unknown sample. A concentration-response curve for % inhibition absorbance at 734 nm of ABTS⁺ solution as a function of standard Trolox solutions was used to evaluate the antioxidant capacity. Results are expressed as eq. mM Trolox (19). Experimental design and photoprotection protocol 1. Animal groups The SKH-1 hairless mouse is a well-established animal model to study UV-induced changes in skin (20). Forty SKH-1 female mice (Charles River Laboratory, Germany), 8 weeks old, weighing 25±3 g, kept on normocaloric standard diet (VRF 1) and water ad libitum, were used. The animals were housed (5 animals/cage) at room temperature (24±2°C), with a 12/12 hours light dark cycle. Four groups of 10 animals each, randomly divided, were treated as follows: group 1: control, received no treatment; group 2: only UVB irradiated; group 3: CV+UVB irradiated; group 4: BM+UVB irradiated. Both extracts were applied in a dose of 4 mg/40 µl/cm² of skin area 30 minutes before a single dose of UVB (240 mJ/cm²) exposure. Current doses of both extracts were selected relying upon prior studies when polyphenol treatments using the same doses resulted in a significant protection against photodamage (21). The irradiation dose of 240 mJ/cm² was established according to our previous results with Swiss mice (unpublished data). UVB irradiation was performed with a Waldmann UV 181 broadband UVB source, with 1.35 mW/cm² intensity at 7 cm distance from the source. The UVB emission was monitored before each exposure with a Variocontrol radiometer (Waldmann GmbH, Germany). Irradiation doses were established using the formula: dose (mJ/cm²)=exposure time (sec.) x intensity (mW/cm²). All the experiments were performed according to the approved animal-care protocols of the Ethical Committee on Animal Welfare of the "Iuliu Hatieganu" University of Medicine in accordance with the Romanian Ministry of Health and complied with the Guiding Principles in the Use of Animals in Toxicology. 2. Isolation of skin samples Before irradiation, the animals were anaesthetized with an i.p. injection of ketamine xylazine cocktail (90 mg/kg b.w. ketamine, 10 mg/kg b.w. xylazine). At 24 hours after UVB irradiation the animals were anaesthetised and sacrificed by cervical dislocation. Fragments of dorsal skin (approximately 2x2 cm) were excised from each mouse. Small fragments were used for the analysis of apoptotic sunburn cells and CPDs + cells and other fragments for the quantitative evaluation of cytokines levels. 3. Immunostaining of cyclobutane pyrimidine dimers 3.1. Immunohistochemistry Briefly, we used the following IHC protocol: after paraformaldehyde fixation, the samples were embedded in paraffin, then sectioned at 5 µm using a Leica RM 2125 RT microtome and mounted on slides embedded with APES (amino-propyl-tri-ethoxy-silane). For a better adherence, samples were incubated at 37°C for 24 hours. Deparaffinization and rehydration were followed by antigen retrieval in a sodium citrate solution (10 mM pH 6). Samples were brought to boiling temperature, and then kept 10 minutes at sub-boiling temperature and 30 minutes cooled on benchtop. Peroxidase blocking was done with 3% hydrogen peroxide. A monoclonal anti-cyclobutane pyrimidine dimers primary antibody (Cosmo Bio Co Ltd, Japan) and, for the visualisation, a secondary antibody included in the kit
LSAB+ System HRP were used. Nuclei were counterstained with Mayer's haematoxylin. Tissue sections stained exclusively with the secondary antibody were used as negative control.

3.2. Immunofluorescence

The protocol recommended by Cell Signaling Technology (22) was used. Paraffin embedded sections were reacted with monoclonal primary antibodies against cyclobutane pyrimidine dimers (CPDs) (Cosmo Bio Co Ltd, Japan) and a secondary antibody labelled with Rhodamine (ABcam, UK). Nuclei were stained with DRAQ5 (Cell Signaling Technology,Danvers MA).

3.3. Analysis of cyclobutane pyrimidine dimers positive cells

To evaluate the inhibitory effect of Cv and BM extracts on UVB-induced CPDs formation, CPDs positive cells from the epidermis and dermis compartments of the stained skin sections were counted from ten microscopic fields using an Olympus BX51 microscope equipped with Olympus cell B software at 400x magnification. The results were expressed as the mean percent of positive cells per field±S.D.

4. Sunburn cells

For the apoptotic sunburn cells counting, the skin samples were fixed with formalin 10% for 24-48 hours, dehydrated and embedded in paraffin. The sections made at 4 µm with a microtome Leica RM 2125 RT were deparaffinized, hydrated and stained with Trichrome-Masson and haematoxylin-eosin. Apoptotic cells were morphologically distinct, as having small, dense nuclei due to nuclear condensation and eosinophilic cytoplasm. Ten microscopic fields were counted for each sample. The results were expressed as the mean percent of positive cells per field±S.D.

5. Cytokine levels

After subcutaneous tissue removal, the skin tissue fragments were homogenized with a Polytron homogenizer (Brinkman Kinematica, Switzerland) for 3 min, on ice, in phosphate buffered saline (PBS) (pH 7.4), added at a ratio of 1:4 (w/v). The suspension was centrifuged for 5 min at 3000 x g and 4°C to prepare the cytosolic fraction. The proteins content in homogenates were measured with Bradford method (23). For the analysis of TNF-α and IL-6, ELISA assays were used according to the producer instructions. The IL-6 and TNF-α concentrations were expressed as pg/mg protein.

Statistical analysis

The data were expressed as means±standard deviation (S.D.). The groups treated with plant extracts were compared with the control group (untreated) and UVB irradiated group. All experiments were performed at least three times and were scored by two investigators. Statistical analysis was done by one-way ANOVA, followed by the Tukey’s multiple comparisons post test using GraphPad Prism 5.0 software (GraphPad, San Diego, Ca., USA). The statistical level of significance was p<0.05.

RESULTS

Spectra measurement of grape seeds and Calluna vulgaris extracts

For the both extracts the spectra were registered. The extracts were diluted 20x to fit the acceptable absorption range (Fig. 1). Both extracts have an abundance of flavonoid compounds which, due to their polyphenolic aromatic structures, strongly absorb UV radiation.

Free radical scavenging activity of Calluna vulgaris and grape seeds extracts using 2,2-diphenyl-l-picryl-hydrazyl (DPPH)

Table 1 shows the values of final state efficient concentration (EC50s), the time required to reach final state (TEC50s) and stoichiometric parameter (n) for Cv and BM extracts (18). V. Vinifera extract had higher antioxidant capacity than Cv extract. The time needed to reach the final state (TEC50s) had different rankings which indicated that the antioxidant redox kinetics are not the same for all plant extracts.

Table 1. EC50s, TEC50s and n values at the final state obtained with the exponential curves for the two extracts. TEAC (Trolox equivalent antioxidant capacity) for two extracts.

<table>
<thead>
<tr>
<th>Sample</th>
<th>EC50s (mmol/mmol DPPH)</th>
<th>TEC50s (min)</th>
<th>n</th>
<th>TEAC (mmol/mmol DPPH)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. vulgaris</td>
<td>0.211±0.08</td>
<td>11.3</td>
<td>2.4</td>
<td>38.92±0.01</td>
</tr>
<tr>
<td>V. Vinifera</td>
<td>0.072±0.002</td>
<td>7.5</td>
<td>6.9</td>
<td>52.89±0.02</td>
</tr>
</tbody>
</table>

DPPH: 2,2-diphenyl-l-picryl-hydrazyl; efficient concentration: EC50s; final state: EC50s; time required to reach final state: TEC50s; n: stoichiometric parameter; TEAC: Trolox equivalent antioxidant capacity.

For the both extracts the spectra were registered. The extracts were diluted 20x to fit the acceptable absorption range (Fig. 1). Both extracts have an abundance of flavonoid compounds which, due to their polyphenolic aromatic structures, strongly absorb UV radiation.

Fig. 1. The UV-Vis spectra of the BM (A) and Cv (B) extracts (diluted in ethanol at a ratio 1:20 (v/v) using Jasco V-530 UV/Vis spectrophotometer.

Table 1. EC50s, TEC50s and n values at the final state obtained with the exponential curves for the two extracts. TEAC (Trolox equivalent antioxidant capacity) for two extracts.
**Fig. 2.** Immunohistochemical and immunofluorescence distribution of CPDs+CELLS in mice skin exposed to a single dose of UVB (240 mJ/cm²). (A) For IHC, paraffin embedded sections were subjected to immunoperoxidase staining for CPDs+CELLS detection which are shown as dark brown. CPDs+CELLS are not detectable in the control (no UVB exposure) skin group. UVB-induced CPDs+ cellular staining is shown at 24 hours post UVB exposure with or without Cv and BM pretreatment. Original magnification x 400 (Scale bar=50 µm). Topical treatment of mice skin with BM extract inhibited UVB-induced expression of CPDs at 24 hours after treatment. (B) Paraffin embedded sections were used for reaction with monoclonal antibodies against CPDs and stained with rhodamine. Nuclei were stained with DRAQ5. At 24 hours after irradiation, CPDs+ distribution and hyperplasia (arrows) showed that especially epidermal cells are affected. (C) The results were expressed as the mean percent of positive cells per field ±S.D.

**Fig. 3.** (A) Epidermal hyperplasia, intracellular and intercellular oedema and induction of sunburn cells at 24 hours after UVB exposure in four mice experimental groups (arrows indicate representative sunburn cells). Cv and BM extracts were administered topically (4 mg polyphenols/cm²), 30 min before of UVB irradiation. Animals were sacrificed at 24 hours after UVB exposure; skin biopsies were sampled, preserved in 10% buffered formalin and processed for histopathological investigations. Representative photomicrographs of Trichrome-Masson and haematoxylin-eosin stain from each treatment group, n=10, with an original magnification x 400 (Scale bar=50 µm). (B) A total of ten microscopic fields were counted. The results were expressed as the mean percent of positive cells per field ±S.D.
a single dose of UVB irradiation (240 mJ/cm²). Pretreatment with BM extracts reduced the level of TNF-α significantly after a single dose of UVB irradiation (240 mJ/cm²). Pretreatment with BM extracts reduced the skin levels of TNF-α at 24 hours. Values are mean ±S.E., ***p<0.0001 vs. control group; **p<0.001 vs. irradiated group. (B) TNF-α secretion increased significantly after a single dose of UVB irradiation (240 mJ/cm²). Pretreatment with BM extracts reduced the skin levels of TNF-α at 24 hours. Values are mean ±S.E., ***p<0.0001 UVB-irradiated vs. control group; UVB-irradiated vs. treated groups.

Fig. 4. Quantitative evaluation of the IL-6 and TNF-α levels in SKH-1 skin mice after UVB exposure and pretreatment with natural extracts. (A) IL-6 secretion increased significantly after a single dose of UVB irradiation (240 mJ/cm²). Pretreatment with Cv and BM extracts reduced the skin levels of IL-6 at 24 hours. Values are mean ±S.E., ***p<0.0001 vs. control group; **p<0.001 vs. irradiated group. (B) TNF-α secretion increased significantly after a single dose of UVB irradiation (240 mJ/cm²). Pretreatment with BM extracts reduced the skin levels of TNF-α at 24 hours. Values are mean ±S.E., ***p<0.0001 UVB-irradiated vs. control group; UVB-irradiated vs. treated groups.

Induction of sunburn cells in a single dose ultraviolet B irradiated mouse skin

UVB-irradiated mouse skin was examined for the presence of sunburn cells 24 hours after irradiation (240 mJ/cm²). Representative photomicrographs of haematoxylin-eosin- and Trichrome-Masson stained sections obtained from unirradiated, UV-irradiated and treated skin are shown in Fig. 3. Examination revealed the presence of sunburn cells, which exhibited the classic patterns of apoptosis: pyknotic nuclei and condensed cytoplasm and intracellular oedema (Fig. 3). Haematoxylin and eosin stained sections of unirradiated mouse skin were used as controls and showed only few cells undergoing normal cell death, with a random distribution (1.50±0.57%). In the UV-irradiated skin, the number of sunburn cells peaked at 24 hours after irradiation (25.50±5.44%; p<0.0002). Some of the sunburn cells displayed contracted nuclei and clear cytoplasm and others had contracted nuclei and pink cytoplasm (probably directly linked to UVB induced damage) and they were mostly located in the lower epidermis. Formation of sunburn cells after UVB irradiation is primarily a consequence of DNA damage. Sunburn cells are keratinocytes that underwent apoptosis after receiving a high UVB dose that has severely and irreversibly damaged their DNA. Pretreatment with Cv extract insignificantly reduced the number of sunburn cells (19.20±12.64%; 25% inhibition; p>0.05), while BM extract administration determined an important reduction of apoptotic cells number (10.38±2.92%; 60% inhibition; p<0.002). Keratinocytes vacuolization and hydropic changes, characteristic for UVB irradiated mouse skin (Fig. 3, panel A) were less severe in the skin samples pretreated with Cv and BM extracts. In the dermis, the inflammatory reaction was scanty perivascular (Fig. 3).

The effects of ultraviolet B irradiation on cytokine levels in skin

IL-6 protein quantified by ELISA (pg/mg protein) showed that UVB exposure stimulated IL-6 protein synthesis in skin cells (113.7±36.60 compared with the control group 15.09±9.55; p<0.0001) (Fig. 4). Skin treatment with Cv and BM extracts before UVB exposure resulted in a significantly reduced IL-6 level in skin (20.76±2.68 respectively 21.33±13.95; p<0.0001) compared with UVB alone (81% inhibition). Both of the natural products conferred significant protection against UVB-induced rising of skin cytokines. The TNF-α level (pg/mg protein) in skin homogenates significantly increased at 24 hours after irradiation vs. unirradiated group (41.07±15.98 vs. 16.64±4.24; p<0.0001). Cv extract applied before irradiation significantly reduced skin TNF-α level (6.00±3.19; 85% inhibition, p<0.0001). The local treatment with BM extract also revealed a significant decline in TNF-α level (4.33±2.08; p<0.0001, 89% inhibition) (Fig. 4).
DISCUSSION

Acute UV irradiation of human and mouse skin causes a number of cellular and pathological changes, including DNA damage, cell-cycle arrest, repair through induction of the p53 protein, formation of sunburn cells (apoptosis), depletion of the antioxidant defense system, release of proinflammatory cytokines and immunosuppressive effects (5). UV radiation is known to exert its tumor initiating effects primarily through the formation of CPDs and 6-4 photoproducts (24) and formation of 8-oxo-7,8-dihydro-2′-deoxyguanosine (25) via ROS. CPDs have been reported to inhibit the progress of DNA polymerases and act as a block to DNA transcription and replication (26). Also, CPDs have been shown to be involved in the initiation of UV-induced immunosuppression (27). Some inflammatory mediators such as cytokines and proteoglycans are associated with carcinogenesis by stimulating cell proliferation, angiogenesis and inhibiting apoptosis (28).

An alternative to conventional sun protection methods is chemoprevention, which is defined as "a means of cancer control in which the occurrence of the disease can be entirely prevented, delayed or reversed by topical or oral administration of naturally occurring or synthetic compounds or their mixtures" (29). The disease can be entirely prevented, delayed or reversed by topical or oral administration of naturally occurring or synthetic compounds or their mixtures. Antioxidant, anti-inflammatory and immunomodulatory properties of botanical compounds in the diet or applied on skin could be a useful strategy to reduce the incidence of nonmelanoma skin cancer.

Calluna vulgaris is mainly used in folk medicine for its antiinflammatory properties. Several in vitro (30, 31) and in vivo studies (32) revealed antioxidant, antitumor and anti-inflammatory effects of Cv extract ascribed to polyphenols, triterpenes and ursoic acid. Grapes (Vitis Vinifera) are one of the most widely consumed fruits in the world. Grape seed are rich in polyphenols mainly flavan-3-ol derivatives (catechins) and oligomeric proanthocyanidins (29). Preliminary studies have shown that polyphenols from grape seeds have anti-inflammatory, antioxidant effects and inhibit the oxidative stress-mediated activation of MAPK and NF-κB involved in carcinogenesis pathways (33).

In this study we evaluated the protective efficiency of Cv and BM extracts against UVB-induced skin damage. Our results showed a strong suppression of UVB-induced damage after topical application of BM extract (but not Cv) via inhibition of DNA damage and apoptosis. Both BM and Cv extracts reduced the skin level of proinflammatory cytokines. BM extract had a higher antioxidant activity comparatively to Cv as revealed by DPPH and TEAC tests. Therefore it might be expected BM to have a greater efficiency in blocking the chain reactions produced by ROS in skin after UVB exposure. Thymine dimers in DNA are formed immediately following absorption of UVB energy and, hereby represent an early biomarker of UVB-induced DNA damage (3). Therefore, thymine dimers are used as biomarkers to study the protective effect of agents against UVB photodamage (34). Other authors reported that the number of CPDs in the epidermis increased after 1 hour but significantly decreased 3 days after UVB exposure (35). Accordingly, measuring the CPDs in skin at 24 hours following a single dose of UVB exposure, we found it strongly induced the formation of CPDs+CELLS (p<0.0001) compared to unirradiated group, both in epidermis and dermis.

The treatment with BM extract before UVB exposure inhibited UVB-induced DNA damage as detected by CPDs quantification (p<0.001, accounting for 50% inhibition). This protective effect of BM could be due to an interference with UV absorption in epidermis - sunscreen effect (both physical and chemical) and interaction at molecular level in skin (6). Our preliminary in vivo studies (unpublished) revealed that BM and Cv administration subsequent UV-B irradiation (up to doses of 100 mJ/cm²) afforded protection in a dose-effect relationship.

Strong antioxidant properties of grape extract reported earlier (18, 21) suggested that suppression of oxidative stress by BM resulted in the activation of repair enzymes much earlier compared to UV alone. The same mechanisms have been suggested in the protective effects of different antioxidants on tumor cells (36). Indeed, several studies (37) have demonstrated the photoprotective effects of grape seeds polyphenols against UV-induced DNA damage and others suggest that phenols might be capable of acting in redox-sensitive signalling cascades to inhibit DNA damage (37). Resveratrol, an important antioxidant found in grapes, also regulates the gene expression of pro-oxidative and antioxidative enzymes showing protection (38).

Induction of apoptosis is an additional protective mechanism that eliminates cells which are unable to repair following the UVB-induced DNA damage (39). Our results demonstrated that exposure of skin SKH-1 mice to a single dose of UVB (240 mJ/cm²) caused an increased number of keratinocytes that undergo apoptosis. This suggests that the UVB-induced DNA damage exceeded the cells capacity to repair the DNA lesions leading to the activation of the apoptotic pathway. Disorders in the UVB-induced apoptosis could enhance the risk of photocarcinogenesis because it allows survival of the cells carrying DNA damage, which may give rise to mutations and finally to skin cancer. Pretreatment with BM extract protected the skin and inhibited cell sunburn formation (60% inhibition), whereas the Cv extract treatment did not offer significant protection against UVB-induced DNA damage.

UVB irradiation also stimulated the inflammatory response, causing erythema, oedema and an influx of inflammatory cells such as neutrophils and lymphocytes, as reported by others (40). Infiltrating macrophages into dermis and epidermis secrete the cytokines which exert immunosuppressive properties to help resolve the UVB-induced inflammatory response (40). When exposed to UVB radiation, keratinocytes produce cytokines IL-1α, IL-6, and TNF-α (9). These stimulate epidermal keratinocytes and dermal fibroblasts, respectively, up regulate the levels of metalloproteinases and degrade dermal collagen and elastic fibers, leading to wrinkle development (41). Our study showed that a single dose of UVB significantly (p<0.0001) increased the level of IL-6 and TNF-α in skin homogenates. Recent data showed that UVB irradiation induced TNF-α expression in both keratinocytes and dermal fibroblasts. TNF-α mRNA induction occurred earlier, at 1.5 hour after UVB (42). TNF-α is a pro-inflammatory cytokine; however it has been shown to be involved in UV induced immunosuppression and tumor growth signalling pathways (28). Topical application of the two natural extracts reduced the release of these cytokines from skin cells (p<0.0001).

Histological changes such as intercellular and intracellular oedema, perivascular swelling and dermal infiltration of inflammatory cells, epidermal hyperplasia occurred 24 hours following UVB irradiation. However, the lesions were less severe in the pretreated skin areas with both extracts, compared with the untreated ones.

In summary, the treatment with natural compounds, especially with the grape seeds extract (BM) inhibits sunburn cell formation in SKH-1 mice skin. The beneficial effect of grape seeds on the immunoreactivity expression profile of CPDs could play a significant role in the modification of UV-induced human skin cancers. These results suggest that the extract prevented the penetration of UVB into the skin by acting as a sunscreen, and/or modulated the inflammatory and apoptotic responses to UVB. BM seems to be more potent than Cv extract regarding antiapoptotic effects and DNA protection. More extensive studies are required in order to have a complete understanding of protective effects of these natural extracts in the skin.
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REFERENCES


3. Cadet J, Sage E, Douki T. Ultraviolet radiation-mediated damage to cellular DNA. Mutat Res 2005; 571: 3-17.


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