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Juglans regia L. protects against UVB induced apoptosis in human epidermal keratinocytes

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ABSTRACT

The present study was aimed to investigate the photoprotective effect of the male flower of *J. regia* L. (MEJR) against ultraviolet-B induced apoptosis in human skin cells. Human skin epidermal keratinocytes were pretreated with the MEJR (80 μg/ml, has been selected after MTT assay), prior to 30 min UVB-irradiation at a dose of 20 mJ/cm^2. Mitochondrial membrane potential was evaluated using Rhodamine-123 staining; the % apoptosis by Hoechst staining and acridine orange staining; DNA damage was measured by comet assay. The levels of p53, Bax, Bcl-xL, Bcl-2, Cytochrome c, Caspase-9 and Caspase-3 expression in HaCaT cells were analyzed by western blotting and RT-PCR. Pretreatment with MEJR 80 μg/ml prior to UVB-irradiation significantly prevents apoptotic characteristics, DNA damage and loss of mitochondrial membrane potential. Thus, MEJR protects UVB-mediated human skin cells, by modulating the expression of apoptotic markers and UVB-induced DNA damage in HaCaT cells.

1. Introduction

Sunlight is the main source of ultraviolet radiation, having three major components such as UVA (400–320 nm), UVB (320–280 nm) and UVC (280–100 nm). Among the three, UVB radiation is the most damaging component, reaching the surface of the earth. It mainly affects the epidermal layer of the skin [1] and is considered as a major risk factor responsible for the formation of free radicals, acute inflammation and increased risk of non-melanoma skin cancer. More than 90% of skin cancer caused to fair-skinned populations is due to their exposure to intensive UVB radiation [2]. Generally, people are exposed to 15 MED (Minimal Erythema Dose) of UVB in a day time [3]. In case of fair-skinned population, epidemiological studies have reported that individuals get erythema only after 20 min of exposure to sun light in mid-summer. This corresponds to 15–70 mJ/cm^2 of UVB and equals to 1 MED especially in case of fair-skinned population. Skin cancer is one of the most common cancers in the United States [4]. Current estimates show that one out of five, suffers from skin cancer in their lifetime and more than 8500 people in the United States, are diagnosed with skin cancer every day [5].

Apoptosis is an active suicidal machinery or programmed cell death, which participates in eliminating the unwanted or potentially harmful cells, under pathophysiological conditions. UVB radiation is a strong genotoxic agent, which may lead to apoptosis, characterized by membrane blebbing and nuclear fragmentation [6]. Exposure of skin cells to UVB irradiation may respond either by activating protective mechanisms or ultimately undergoing apoptosis. However, damaged cells when escapes apoptotic process, may lead to the tumorigenesis also. Therefore, apoptosis is an important approach for protecting normal cells from the UVB radiation [7]. UV radiation and other environmental toxicants induced apoptosis have two pathways; death receptor mediated apoptosis and mitochondria mediated apoptosis. The mitochondrial membrane permeability is mainly regulated by anti- and pro-apoptotic proteins belonging to the Bcl-2 family [8]. The anti-apoptotic proteins, including Bcl-2, Bcl-XL, neutralize apoptosis by interacting with some pro-apoptotic members on the mitochondrial membrane. Moreover, tumor suppressor protein p53, induced by DNA damage, can also lead to the onset of apoptosis [9]. UVB-induced damage to the keratinocytes, frequently promotes hot spot mutations in the p53 gene that ultimately may lead to a faulty trigger to apoptosis and can promote the non-melanoma skin cancers.

J. regia L. (Juglandaceae) is found primarily in temperate areas and commercially cultivated in the United States, Asia Minor and also in Central and Southern Europe [10]. It is one of the oldest cultivated species, for nuts, in history and is considered to be a source of sesquiterpene, alcohols, tocopherols, phospholipids, sphingolipids, sterols, hydrocarbons, unsaturated fatty acids, flavonoids, steroids, and terpenoids. It has also been used in traditional medicine for treatment of

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various disorders [11–13]. The extracts from *J. regia L.* inhibit oxidative damages [14,15], inflammation [16,17], tumor growth [18,19] and photo-aging [20]. The oil of *J. regia* nut is a constituent of dry skin creams, antiaging, and antiwrinkle products [21]. However, the effect of male flower of *J. regia* L. on UVB-induced apoptosis in human skin cells has not yet been investigated. The flower of this plant possesses numerous secondary metabolites like flavonoids, steroids, and terpenoids. Therefore, in this study, it is designed to investigate the effect of methanolic extract of a male flower of *J. regia* L. (MEJR) against UVB-irradiated apoptosis in skin cells.

2. Materials and methods

2.1. Chemicals and reagents

Acridine orange (AO), Hoechst stain, Ethidium Bromide and Rhodamine-123 (Rh-123) were purchased from Himedia, India. Cell culture chemicals such as heat inactivated fetal bovine serum (FBS), DMEM medium, glutamine, penicillin-streptomycin, EDTA, trypsin, phosphate buffered saline (PBS), low melting agarose, normal melting agarose; p53, Bcl-2, Bax, caspase-9, caspase-3 and Cytochrome c monoclonal antibodies were purchased from Sigma chemical Co., St. Louis, USA. All other chemicals and solvents were obtained from Fisher Inorg., Aromatic Limited Chennai and from S.D Fine Chemical Mumbai.

2.2. Preparation of plant extracts

Male flowers of *J. regia L.,* were collected from Pir Panjal (33.7167° N; 74.8333° E) area of Jammu and Kashmir, India. Plant identification was done at Department of Botany, Annamalai University with herbarium voucher No. ABH-2023. The methanolic extract of the flower of *J. regia* was obtained as described by Leite et al., 2006 [22]. The material was then shade dried, powdered and extracted with methanol at 1:3 volumes (1: material and 3: solvent). The supernatant was then filtered using whatman filter paper and then vacuum evaporated. The resulting extract of male flower of *J. regia,* (MEJR) was stored at 4 °C for future use.

2.3. Cell line culture

The present work was carried out in human epidermal keratinocytes (HaCaT). HaCaT cells were purchased from natural centre for cell science (NCCS) Pune, India. The primary HaCaT cells devoid of any other cross contamination were grown as monolayer and were maintained in DMEM medium supplemented with 10% FBS, 1% glutamine and 100 U/ml penicillin streptomycin and were maintained at 37 °C in 5% CO2 atmosphere. Stocks were maintained in 25 cm^2 tissue culture flasks.

2.4. UVB-irradiation procedure

After washing with PBS, cells were exposed to UVB radiation in presence of PBS layer. A battery of TL 20 W/20 fluorescent tubes (Heber Scientific, Chennai) were used as UVB source, having a wavelength ranging from 290 to 320 nm (Peaking at 312 nm), with an intensity of 2.2 mW/cm^2 for 9 min, with total 20 mJ/cm^2 UVB. The UVB-irradiated and non-irradiated cells were kept at 37 °C in 5% CO_2 incubator for 6 h. Then cells were washed twice with PBS, then trypsinised and were transferred to sterile centrifuge tubes for further analysis.

2.5. Determination of the mitochondrial membrane potential (Δψm) by Rh-123

The alteration in mitochondrial membrane potential is considered as an indication of initial stage of apoptosis. For observing mitochondrial membrane potential (Δψm) in HaCaT, Rh-123 stain was used. After adding 5 μM of Rh-123, the cells were kept back in 5% CO_2 incubator for 15 min, as explained in the method of Bhosle et al. [23]. Then, the cells were washed with PBS and viewed under the fluorescence microscope using a green filter (450–490 nm). The fluorescence was measured at the intensity of 535 nm.

2.6. Detection of apoptotic nuclei

The apoptotic bodies were determined by Hoechst and Acridine orange (AO) staining in UVB plus MEJR treated HaCaT fallowed by the method of Darzynkiewiez et al. [24]. Hoechst stains nuclei and AO stain of both live and dead cells were visualized by a fluorescent microscopy at 440 nm. The cells were grown in 6-well culture plates and after washed with PBS, they were stained with different dyes (10 μg/ml) for 30 min. Then cells were observed under a fluorescence microscope with 330/380 nm excitation filter and 440 nm barrier filter under 40× magnification. In every sample, at least 400 cells were counted, and the cells having fragmented or condensed nuclei were expressed as percentage apoptotic cells.

2.7. Measurement of oxidative DNA damage by alkaline single-cell gel electrophoresis

In order to observe DNA damage, alkaline single cell gel electrophoresis (comet assay) was analyzed fallowed by the method of Singh et al., 1988 [25]. One percent (1%) normal melting agarose layer was prepared on slides for comet. Fifty (50) μl of UVB-irradiated cells were mixed with 120 μl of low melting agarose (0.5%). Then the suspension was pipetted on comet slides and immersed in lysis solution at pH 10 (100 mM Na2 EDTA, 10 mM Tris pH 10, 2.5 M NaCl, 1% Triton X-100, 10% DMSO) and kept at 4 °C for 60 min. For denaturation of DNA, the comet slides were kept in alkaline electrophoresis buffer at pH 13 for 20 min. Then slides were kept in electrophoresis tank, which was filled with alkaline electrophoresis buffer, then electrophoresis was performed at the field strength of 25 V for 27 min. Slides were then neutralized in 0.4 M Tris with pH 7.5 for 5 min, and then stained with 20 μg/ml EtBr for visualization of DNA damage. Observations were made using a 40× magnification in fluorescent microscope (Nikon, Eclipse TS100, Japan). Fifty to hundred comets were analyzed on slides. Images were captured with a camera (Nikon 4500 Coolpix, Japan) and analyzed by image analysis software, CASP.

2.8. Western blot analysis of apoptotic markers expression

The Western blot was carried out by the method of Towbin et al. [26]. Immunoblot analysis was carried out for p53, Bax, Bcl-2, Cytochrome c, Caspase-9 and Caspase 3 protein expressions. Cultured cells were washed with PBS solution and detached with trypsin. Cell suspensions were centrifuged at 1000 rpm for 10 min and the pellets were lysed with a protease inhibitor cocktail present in chilled lysis RIPA buffer for 30 min. Then by centrifugation for 10 min at 4 °C with 14,000 rpm, the lysate was cleared and the supernatant was taken for Western blotting analysis. The protein concentration of obtained supernatant was determined by Nanodrop (Thermo Scientific, USA). Cell extracts containing 50 μg of proteins were fractionated on SDS-PAGE gel electrophoresis (12%), and then the separated molecules were blotted onto a polyvinylidene fluoride (PVDF) membrane. After blocking, the respective primary antibody was added and was allowed to bind to the protein. It was followed by washing (which removes nonspecifically bound antibody) and secondary antibody was added, to detect the primary antibody. TBST was used for washing the membranes thrice, with 10 min interval, and the bands were analyzed using western blotting chemiluminescence substrate (LI-COR, USA). The density of band was analyzed by Image studio software (LI-COR, USA).

2.9. RNA preparation and reverse transcriptase (RT) PCR analysis

Cells were harvested and RNA was extracted by using RNA isolation kit (RNeasy-mini kit, Qiagen, Germany) according to manufacturer's protocol. The mRNA expression level was quantified for p53 (Forward: CGTTCACCGAGGACTGGAC; Reverse: AGATTCTCTTC CTTGTGCTG CCG.), Bcl-2 (Forward: 5′-CTGCACCTGACGCCCTTCACC-3′ Reverse: 5′-CA CATGACCCCACCGAACTCAAAGA-3′), Bax (forward: 5′- TGGAGCT GCAGAGGATG ATTG3′; reverse: 5′-GAAGTTGCCGTCAGAAAAC ATG3′), and Bcl-xL (Forward: 5′-CTGC ACCTGACGCCCTTCACC-3′; Reverse:5′ CACATGACCCCACCGAACTCAAAGA-3′). The extracted RNA was preserved at − 80 °C for further use. Complementary DNA (cDNA) was synthesized using 5 μg total RNA by superscript III reverse transcriptase enzyme (Invitrogen). cDNA was amplified in 20 μl total volume containing Taq polymerase enzyme, oligonucleotides, and respective forward and reverse primers. Cycling conditions were as follows: For cDNA synthesis; 25 °C for 10 min, 42 °C for 50 min, and 75 °C for 15 min. For DNA amplification, 2 min initial denaturation at 95 °C followed by 35 cycles with 15 s denaturation at 94 °C, 30 s primer annealing at 58 °C and 30 s of extension at 72 °C. For the sake of quantitation, the differences between groups and their treatments were examined by equating mRNA expression levels with the control GAPDH after normalization.

2.10. Statistical analysis of experimental data

The data was expressed as mean ± SD (n = 5). The statistical analysis of the experimental data was carried out by ANOVA (one-way analysis of variance) followed by DMRT, (Duncan's Multiple Range Test) using a statistical tool (SPSS). $P < 0.05$ be considered as statistically significant.

3. Results

3.1. Effect of MEJR on mitochondrial membrane potential (Δψm) in HaCaT

In this study, UVB-irradiated HaCaT cells showed decreased fluorescence intensity (160.08 ± 1.13). On the other hand, pretreatment with MEJR for 30 min prior to UVB-irradiation significantly increased mitochondrial membrane potential (321.04 ± 1.45) as evidenced by increased fluorescence Fig. 1. There was decreased fluorescence intensity in irradiated cells, when compared to control (468 ± 2.18) and MEJR alone treated cells (470 ± 1.15). MEJR treatment before UVB exposure significantly prevented UVB-induced loss of Δψm (Fig. 1).

3.2. Effect of MEJR on UVB-induced apoptotic morphological changes

The nuclear fragmentation of apoptosis was evaluated by Hoechst (Fig. 2A–B) and AO (Fig. 2C–D) staining. The observed condensed apoptotic bodies were 62.12 ± 1.43% and 84.29 ± 2.33% for Hoechst and AO staining respectively in UVB-irradiated HaCaT cells. The percentage of apoptotic cells was significantly decreased in MEJR plus UVB-treated HaCaT cells (27.51 ± 1.72% and 36.16 ± 2.13%) for Hoechst and AO staining respectively. Very few nuclear fragmentations were detected in control (2.3 ± 0.24% and 4.43 ± 0.33%) for Hoechst and AO staining respectively. MEJR alone treated HaCaT cells showed (1.8 ± 0.12% and 3.98 ± 0.72%) nuclear fragmentation for Hoechst and AO staining respectively.

3.3. Effect of MEJR on UVB induced DNA damage

Fluorescence microphotographs show distinct comet tails in UVB irradiated HaCaT (Fig. 3). While MEJR + UVB exposed cells showed diminished comet formation, non-irradiated control and MEJR alone treated cells showed intact round shaped nucleoid.

3.4. Effect of MEJR on UVB-induced activation of apoptotic markers in HaCaT

In order to understand the effect of MEJR on UVB mediated apoptosis in HaCaT cells, p53, Bcl-2, Bax, Caspase-9, Caspase-3 and Cytochrome c expressions were taken in to account (Fig. 4A–B). Pro-apoptotic proteins such as p53, Caspase-9, Caspase-3 and Bax was found to be overexpressed in UVB-irradiated cells. Conversely, MEJR plus UVB treated cells down-regulated p53, Cytochrome c, Bax, Caspase-9 and Caspase-3 expressions. While UVB-irradiation down-regulates the protein expression of Bcl-2 and is up regulated by MEJR pretreatment.

3.5. Effect of MEJR on UVB-induced activation of apoptotic genes in HaCaT

The results of the present study (Fig. 5A–B) show that while UVB-irradiation significantly increases the mRNA expression of p53 and Bax;

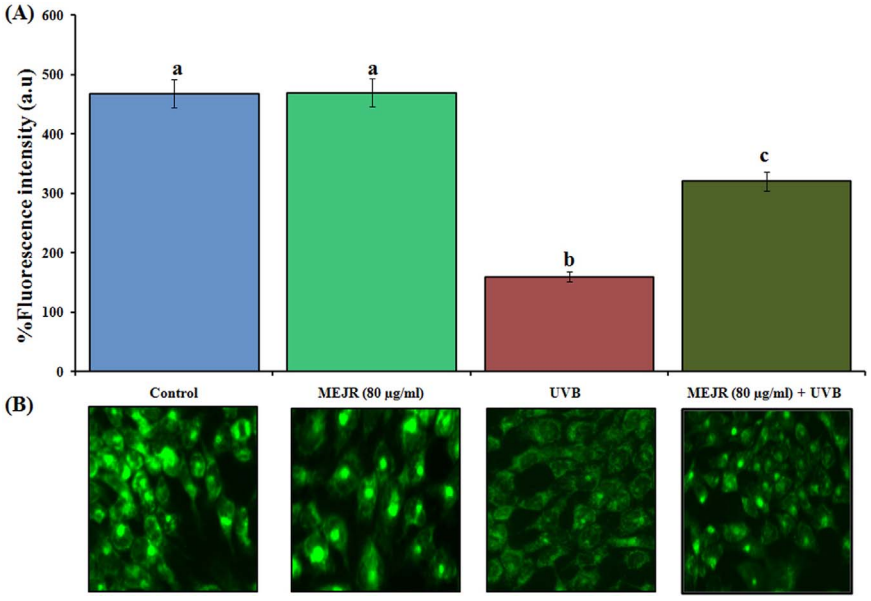

Fig. 1. Effect of MEJR and UVB irradiation on mitochondrial membrane potential (Δψm) using Rh-123 staining. (A) Fluorescence intensity as measured by spectroflurometric analysis. Values are given as means ± S.E. of five experiments in each group. Values not sharing a common superscript (a, b, c) differ significantly at $P < 0.05$ (DMRT). (B) Fluorescence microscopic images (40×) of normal, MEJR and/or UVB treated HaCaT cells.

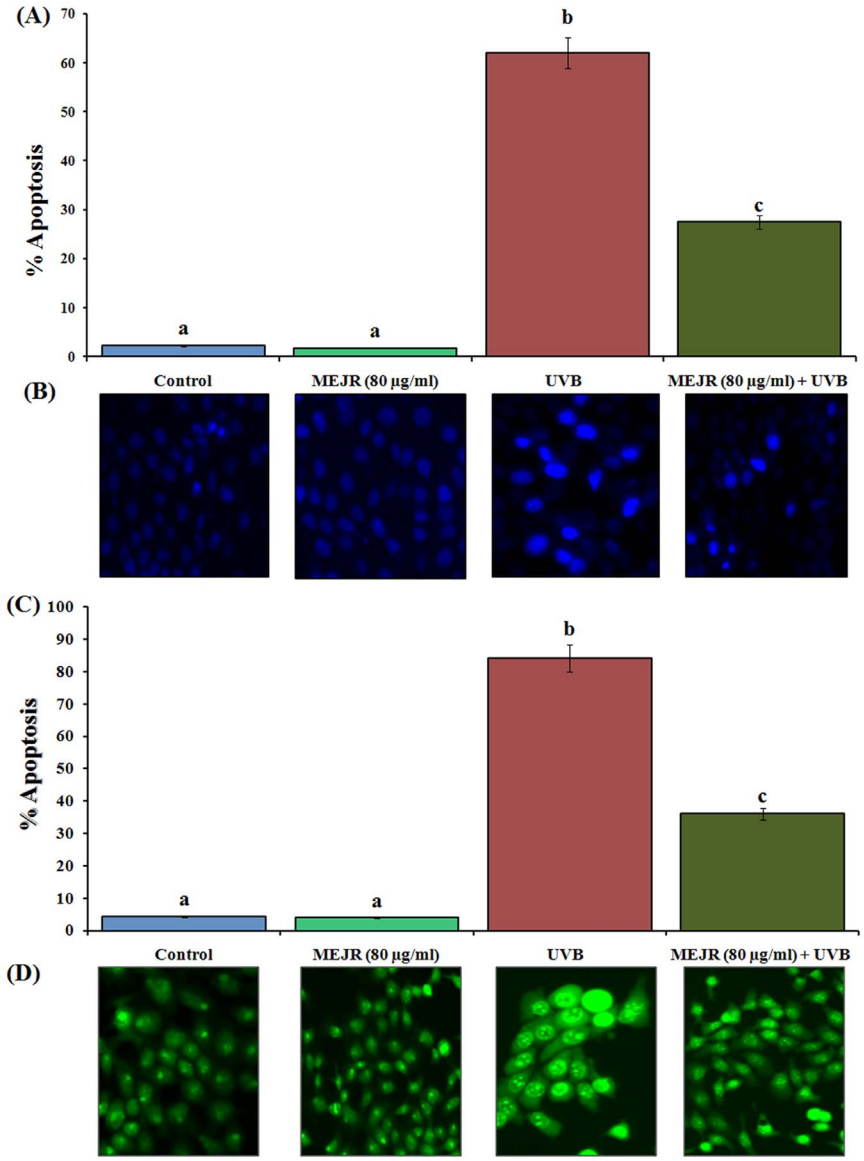

Fig. 2. Effect of MEJR and UVB radiation induced apoptotic morphological changes in HaCaT cells. (A&C) % apoptosis by Hoechst and AO staining on normal, MEJR and/or UVB treated HaCaT cells. (B&D) Fluorescence microscopic images (40× and 60× for UVB in (D)) of Hoechst (blue) and AO (green) stained cells. **Note:** Values are given as means ± S.E. of five experiments in each group. Values not sharing a common superscript (a, b, c) differ significantly at $P < 0.05$ (DMRT).

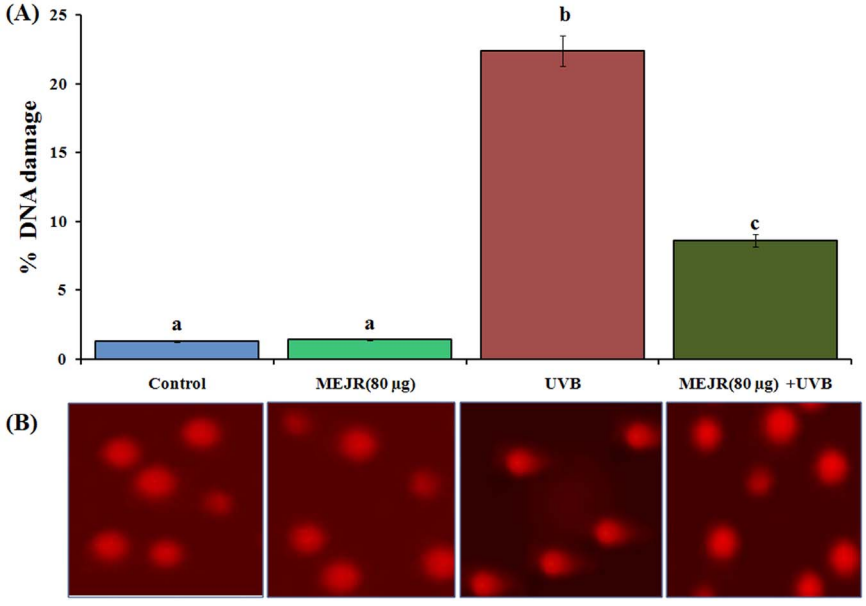

Fig. 3. Single cell gel electrophoresis showing effect of MEJR and UVB irradiation on DNA damage (Ethidium bromide) in HaCaT cells. Fluorescence microphotographs show intact nucleoid in control, MEJR alone treated group and tail DNA in UVB irradiated HaCaT cells. Values are given as means ± S.E., of five experiments in each group. Values not sharing a common superscript (a, b, c) differ significantly at $P < 0.05$ (DMRT).

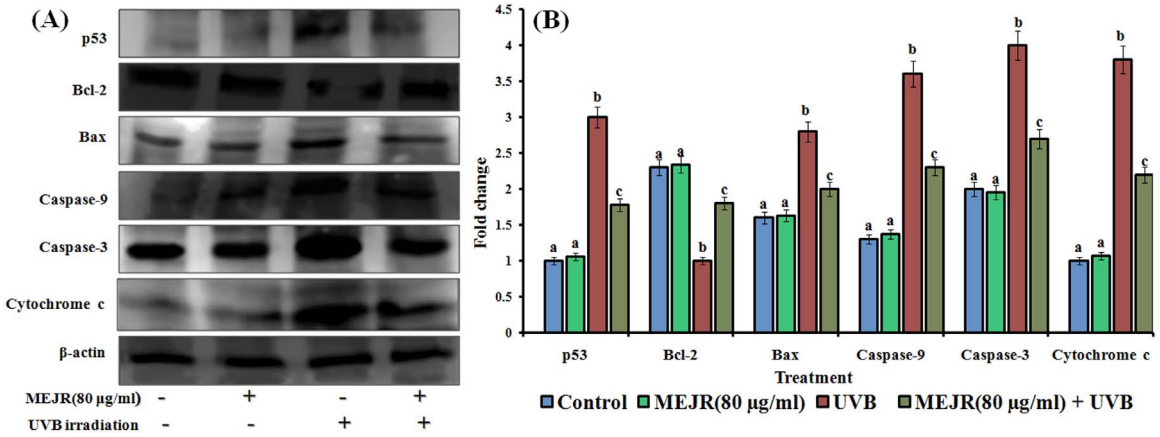

Fig. 4. Effect of MEJR on UVB mediated apoptotic marker expressions in HaCaT cells. (**A**) Western blotting analysis of p53, Bcl-2, Bax, Caspase-9, Caspase-3 and Cytochrome c expressions, normalized to β-actin. (**B**) Densitometric quantification of proteins of western blot using Image Studio software (LI-COR). Data are expressed as the mean of ratios of expressions of target genes to β-actin ± S.E. Vales not sharing a common superscript for a particular group/factor differ significantly at $p < 0.05$ (DMRT).

decreases expression of Bcl-2 and Bcl-xL. However, pretreatment with MEJR prior to UVB exposure significantly decreases the mRNA expression of p53 and Bax, and increases the mRNA expression of Bcl-2 and Bcl-xL in UVB-irradiated HaCaT.

4. Discussion

Remedial measures using plants played a significant role in dealing with cancer and plant derived compounds constitute around 60% of available cancer chemotherapeutic drugs worldwide [27]. *J. regia*, used as a dietary food, has been implicated traditionally for the treatment of diabetes, hypoxia, inflammation, skin diseases etc. The most abundant polyphenols in *J. regia* is the flavanone quercetin, gallic acid, rutin, L-ascorbic acid, glansrin, ellagic acid and valoneic acid [28–30]. It has been shown to exhibit antioxidant, anti-inflammatory and antitumor activities [31]. UVB-irradiations are associated with the ROS generation including singlet oxygen, superoxide anion, hydrogen peroxide, and hydroxyl radical [32], leading to activation of cell surface receptors and subsequent stimulation of kinase pathways resulting in mitochondria-mediated apoptosis [33]. *J. regia* oil has been attributed to have great free radical scavenging capacity due to presence of α-tocopherol and other compound of vitamin E family [34]. Previously we have observed that MEJR shows significant free radical scavenging activity as well as the good Sun protection factor as compared to available commercial products (*article in press*).

While mitochondrion plays a major role in the initiation of apoptosis, UVB is known to induce ROS resulting in oxidative stress subsequently leading to alteration in mitochondrial membrane potential. The permeability transition by oxidative stress in mitochondrial membrane potential, mitochondrial respiration, and an opening of the mitochondrial outer membrane channel allows Cytochrome c release into the cytosol. Cytochrome c release into the cytoplasm generally activates cascades of caspases which is responsible directly or indirectly for stimulating apoptosis [35,36]. The present study has demonstrated the alteration of mitochondrial membrane potential in UVB-irradiated HaCaT cells, indicating the possibility of UVB-irradiation induced loss of $\Delta\psi m$. It has been reported by Park et al. [37] that aqueous seed extract of *J. sinensis* L. cultivated in South Korea shows the preventive effect against loss of mitochondrial membrane potential. In the present study, which investigated the effect of MEJR pretreatment, has clearly confirmed the capacity of *J. regia* to prevent the loss of mitochondrial membrane potential in UVB irradiated HaCaT cells to a great extend. This might be associated with the presence of good amount of flavonoids, UV absorbance capability and its free radical scavenging property.

UVB-irradiation is known for the formation of CPDs and the pyrimidine (6-4) pyrimidones (6-4PPs) in DNA. Such an UVB-induced oxidative DNA damage stimulates p53 pathway, which ultimately play an important role in the execution of apoptosis [38]. In the present study also, DNA damage by UVB-irradiation is clearly demonstrated. Muthaiyah et al. [39] reported that ethyl acetate extract of kernel of *J. regia* L. prevents DNA damage by amyloid beta peptide in PC12 Cells. More or less in the same line, MEJR pre-treatment of HaCaT shows a significant decrease in UVB-induced DNA strand break when compared to DNA tails in UVB alone exposed group. Further, results of the present study show that UVB alone exposed HaCaT cells were characterized by

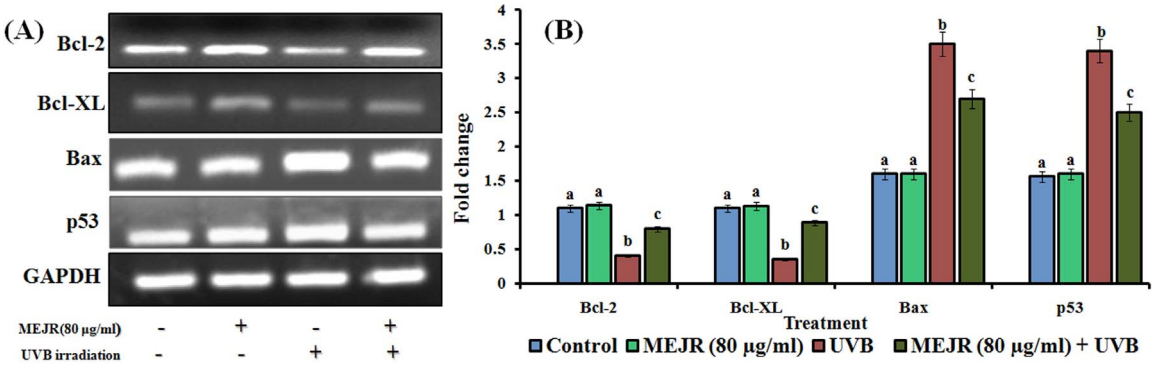

Fig. 5. Effect of MEJR on UVB mediated apoptotic gene expressions in HaCaT cells **(A)** mRNA expression levels of *Bcl-2, Bcl-xL, Bax* and *p53* by RT-PCR. (**B**) Densitometric quantification of mRNAs using Image Studio software (LI-COR). Data are expressed as the mean of ratios of target genes to GAPDH ± S.E. Vales not sharing a common superscript for a particular group/factor differ significantly at $p < 0.05$ (DMRT).

the formation of apoptotic bodies (membrane blebbing, nuclear and chromosomal condensation). However, pre-treatment of human epidermal Keratinocytes with MEJR significantly reduced the condensation and blebbing (Fig. 2). The molecular mechanisms underlying UVB-induced apoptosis are initiated by ROS generation and subsequent oxidative DNA damage resulting in the activation of apoptotic markers [40]. According to the results of the present study, UVB-exposed cells were characterized by the reduced cell viability, formation of apoptotic bodies, reduction of anti-apoptotic protein expression and induction of pro-apoptotic protein expression. Notably on the other hand, MEJR treatment protected keratinocytes from UVB-induced apoptosis more or less in a reverse order.

Skin is the inherent protective organ of the body directly absorbing UVB radiation, which is thought to damage the DNA of epidermal keratinocytes by the activation of the tumor suppressor gene p53 [41]. The p53 sense DNA damage caused by UVB radiation and subsequently arrests the cell in G1phase, until DNA repairs. If the damage is too serious and the cell fails to repair it, then p53 acts as an inducer of apoptosis [40,42]. Shen et al. [43], have reported the role of p53 in the regulation of DNA damage in arsenic induced apoptosis in HaCaT. The present study comparing pretreatment with MEJR to UVB-irradiated HaCaT cells provide evidence of regulation of p53 by MEJR when compared to UVB alone exposed group of HaCaT cells. In case of abortive reparation, p53 induces apoptosis via induction of Bax and the Caspase cascade [44]. Bax is capable of promoting apoptosis in the mitochondria-dependent cell death pathway, in which Cytochrome c and dATP dependent formation of the Apaf-1/Caspase-9 complex initiate an apoptotic protease cascade resulting in the cleavage of procaspases and activates downstream effecter caspases by activating Caspase-8. This suggests that p53, Bax and caspases contribute to the induction of apoptosis in human epidermal Keratinocytes via DNA damage due to UVB-irradiation [45,46]. In the present study, we found the up-regulation of p53, Bax, Cytochrome c, Caspases-9 and Caspases-3 (Figs. 4 and 5) and down-regulation of Bcl-2 and Bcl-xL (Figs. 4 and 5) by UVB-irradiation in HaCaT cells. Park et al., (2014) [47] also reported that aqueous seed extract of *J. sinensis* L. regulates the expression of Bax, Bcl-2 and Cytochrome c. The present study on the other hand found that pretreatment of HaCaT with MEJR significantly regulates the expression of p53, Bax, Cytochrome c, Bcl-2, Bcl-xL and Caspases-9, Caspases-3 towards normal against UVB-irradiation.

5. Conclusion

The present study illustrates that MEJR could offer protection against UVB-induced apoptotic responses by regulating mitochondrial membrane potential, modulating pro and anti apoptotic markers expression in skin epidermal cells. This property of protective effect against UVB-induced photodamage is probably through its antioxidant property, UV absorbance capability especially due to the presence of good amount of flavonoids.

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Appendix A. Transparency document

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bbrep.2018.01.004

References

[1] M. Zaid, F. Afaq, D.N. Syed, M.H. Dreher, Inhibition of UVB mediated oxidative stress markers of photoaging in immortalized HaCaT keratinocyte by pomegranate polyphenol extract POMx, Photochem. Photobiol. 83 (2007) 882–888.

[2] J. Chao, Y.L. Yang, Z. Yang, Y. Tu, L. Cheng, B. Chen, J.P. Xia, W.L. Sun, Z. Su, Perifosine sensitizes UVB-induced apoptosis in skin cells: new implication of skin cancer prevention? Cell. Signal. 24 (2012) 1781–1789.

[3] O. Engelsen, The relationship between ultraviolet radiation exposure and vitamin D status, Nutrients 2 (2010) 482–495.

[4] G.P. Guy, C.C. Thomas, T. Thompson, M. Watson, G.M. Massetti, L.C. Richardson, Vital signs: melanoma incidence and mortality trends and projections—United States, 1982–2030, MMWR Morb. Mortal. Wkly. Rep. 64 (2015) 591–596.

[5] American Cancer Society, Cancer Facts and Figures, American Cancer Society, Atlanta, 2016.

[6] Z. Su, Z. Yang, Y. Xu, Y. Chen, Q. Yu, Apoptosis, autophagy, necroptosis, and cancer metastasis, Mol. Cancer. 21 (2015) 14–48.

[7] S. Elmore, Apoptosis: a review of programmed cell death, Toxicol. Pathol. 35 (2007) 495–516.

[8] L. Duprez, E. Wirawan, B.T. Vanden, P. Vandenabeele, Major cell death pathways at a glance, Microbes Infect. 11 (2009) 1050–1062.

[9] G. Pistritto, D. Trisciuoglio, C. Ceci, A. Garufi, G. D'Orazi, Apoptosis as anticancer mechanism: function and dysfunction of its modulators and targeted therapeutic strategies, Aging 8 (2016) 603–619.

[10] M. Carvalho, P.J. Ferreira, V.S. Mendes, R. Silva, J.A. Pereira, C. Jeronimo, Human cancer cell antiproliferative and antioxidant activities of *Juglans regia L*, Food Chem. Toxicol. 48 (2010) 441–447.

[11] M.L. Martinez, D.O. Labuckas, A.L. Lamarque, D.M. Maestri, Walnut (*Juglans regia* L.): genetic resources, chemistry, by-products, J. Sci. Food Agric. 90 (2010) 1959–1967.

[12] M.A. Rather, B.A. Dar, M.Y. Dar, B.A. Wani, W.A. Shah, B.A. Bhat, Chemical composition, antioxidant and antibacterial activities of the leaf essential oil of *Juglans regia* L. and its constituents, Phyomedicine 19 (2012) 1185–1190.

[13] I.B. Abdallah, O. Baatour, K. Mechrgui, W. Herchi, A. Albouchi, A. Chalghoum, S. Boukhchina, Essential oil composition of walnut tree (Juglans regia L.)' leaves from Tunisia, J. Essent. Oil Res. 28 (2016) 545–550.

[14] M. Carvalho, P.J. Ferreira, V.S. Mendes, R. Silva, J.A. Pereira, C. Jerónimo, B.M. Silva, Human cancer cell antiproliferative and antioxidant activities of Juglans regia L, Food Chem. Toxicol. 48 (2010) 441–447.

[15] M.H. Zhao, Z.T. Jiang, T. Liu, R. Li, Flavonoids in Juglans regia L. leaves and evaluation of in vitro antioxidant activity via intracellular and chemical methods, Sci. World J. (2014) 303878.

[16] H. Hosseinzadeh, H. Zarei, E. Taghiabadi, Antinociceptive, anti-inflammatory and acute toxicity effects of Juglans regia L. Leaves in mice, Iran Red Crescent Med. J. 13 (2011) 27–33.

[17] Z. Papoutsi, E. Kassi, I. Chinou, M. Halabalaki, L.A. Skaltsounis, P. Moutsatsou, Walnut extract (Juglans regia L.) and its component ellagic acid exhibit anti-inflammatory activity in human aorta endothelial cells and osteoblastic activity in the cell line KS483, Br. J. Nutr. 99 (2008) 715–722.

[18] A.S. Negi, S. Luqman, S. Srivastava, V. Krishna, N. Gupta, M.P. Darokar, Antiproliferative and antioxidant activities of Juglans regia fruit extracts, Pharm. Biol. 49 (2011) 669–673.

[19] M. Carvalho, P.J. Ferreira, V.S. Mendes, R. Silva, J.A. Pereira, C. Jerónimo, B.M. Silva, Human cancer cell antiproliferative and antioxidant activities of Juglans regia L, Food Chem. Toxicol. 48 (2010) 441–447.

[20] D.S. Joshan, S.K. Singh, Investigational study of Juglans regia extract and quercetin against photoaging, Biomed. Aging Pathol. 3 (2013) 193–200.

[21] J.C. Espin, C. Soler-Rivas, H.J. Wichers, Characterization of the total free radical scavenger capacity of vegetable oils and oil fractions using 2, 2-di-phenyl-1-picrylhydrazyl radical, J. Agric. Food Chem. 48 (2000) 648–656.

[22] J.P.V. Leite, A.B. Oliveira, J.A. Lombardi, J.D.S. Filho, E. Chiari, Trypanocidal activity of triterpenes from Arrabidaea triplinervia and derivatives, Biol. Pharm. Bull. 29 (2006) 2307–2309.

[23] S.M. Bhosle, N.G. Huilgol, K.P. Mishra, Enhancement of radiation-induced oxidative stress and cytotoxicity in tumor cells by ellagic acid, Clin. Chim. Acta. 359 (2005) 89–100.

[24] Z. Darzynkiewicz, X. Huang, M. Okafuji, A.K. Malcolm, Cytometric methods to detect apoptosis, Methods Cell Biol. 75 (2004) 307–341.

[25] N.P. Singh, M.T. McCoy, R.R. Tice, E.L. Schneider, A simple technique for quantitation of low levels of DNA damage in individual cells, Exp. Cell Res. 175 (1988) 184–191.

[26] H. Towbin, T. Staehelin, J. Gordon, Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications, Proc. Natl. Acad. Sci. USA 76 (1979) 4350–4354.

[27] M.S. Stankovic, M.G. Curcic, J.B. Zizic, M.D. Topuzovic, S.R. Solujic, S.D. Markovic, Teucriumplant species as natural sources of novel anticancer compounds: anti proliferative, pro apoptotic and antioxidant properties, Int. J. Mol. Sci. 12 (2011) 4190–4205.

[28] T. Fukuda, H. Ito, T. Yoshida, Antioxidative polyphenols from walnuts (Juglans regia L.), Phytochemistry 63 (2003) 795–801.

[29] L. Li, R. Tsao, R. Yang, C. Liu, H. Zhu, J.C. Young, Polyphenolic profiles and antioxidant activities of heartnut (Juglans ailanthifolia Var. cordiformis) and Persian walnut (Juglans regia L.), J. Agric. Food Chem. 54 (2006) 8033–8040.

[30] R. Tsao, Chemistry and biochemistry of dietary polyphenols, Nutrients 2 (2010) 1231–1246.

[31] D.S. Joshan, S.K. Singh, Investigational study of *Juglans regia* extract and quercetin against photoaging, Biomed. Aging Pathol. 3 (2013) 193–200.

[32] M.J. Piao, W.J. Yoon, H.K. Kang, E.S. Yoo, Y.S. Koh, D.S. Kim, N.H. Lee, J.W. Hyun, Protective effect of the ethyl acetate fraction of *sargassum muticum* against ultraviolet B-irradiated damage in human keratinocytes, Int. J. Mol. Sci. 12 (2011) 8146–8160.

[33] G. Park, H.G. Kim, S.P. Hong, S.Y. Kim, Walnuts (Seeds of Juglandis sinensis L) protect human epidermal keratinocytes against UVB-induced mitochondria-mediated apoptosis through upregulation of ROS elimination pathways, Skin Pharmacol. Physiol. 27 (2014) 132–140.
[34] G. Raja, A.S. Ivvala, S. Inampudi, A. Swaminathan, S.S. Basha, S.B. Kondaveeti, Study of antioxidant potential in leaves, stems, nuts of *Juglans regia* L, Int. J. Bioassays 01 (2012) 79–85.
[35] J. Wen, Y. Kyung-Ran, L. So-Youn, S. Chang-Ho, K. Dae-Ghon, Oxidative stress-mediated apoptosis, J. Biol. Chem. 277 (2002) 38954–38964.
[36] Z. Assefa, M. Garmyn, A. Vantieghem, W. Declercq, P. Vandenabeele, J. Vandenheede, Ultraviolet B radiation-induced apoptosis in human keratinocytes: cytosolic activation of procaspase-8 and the role of Bcl-2, FEBS J. 540 (2003) 125–132.
[37] G. Park, H.G. Kim, S.P. Hong, S.Y. Kim, M.S. Oh, Walnuts (seeds of Juglandis sinensis L.) protect human epidermal keratinocytes against UVB-induced mitochondria-mediated apoptosis through upregulation of ROS elimination pathways, Skin Pharmacol. Physiol. 27 (2014) 132–140.
[38] B.G. Childs, D.J. Baker, J.L. Kirkland, J.V. Campisi, J.M. Deursen, Senescence and apoptosis: dueling or complementary cell fates? EMBO Rep. 15 (2014) 1139–1153.
[39] B. Muthaiyah, M.M. Essa, V. Chauhan, A. Chauhan, Protective effects of walnut extract against amyloid beta peptide-induced cell death and oxidative stress in PC12 cells, Neurochem. Res. 36 (2011) 2096–2103.
[40] P.M.D.J. Fernando, M.J. Piao, S.R.K.M. Hewagea, H.K. Kang, E.S. Yoo, Y.S. Koh, M.H. Ko, C.S. Ko, S.H. Byeon, S.R. Munc, N.H. Lee, J.W. Hyun, Photo-protective effect of sargachromenol against UVB radiation-induced damage through modulating cellular antioxidant systems and apoptosis in human keratinocytes, Environ. Toxicol. Pharmacol. 43 (2016) 112–119.
[41] P. Maß, K. Hoffmann, T. Gambichler, P. Altmeyer, H.G. Mannherz, Premature keratinocyte death and expression of marker proteins of apoptosis in human skin after UVB exposure, Arch. Dermatol. Res. 295 (2003) 71–79.
[42] M.H. Zhao, Z.T. Jiang, T. Liu, R. Li, Flavonoids in Juglans regia L. leaves and evaluation of in vitro antioxidant activity via intracellular and chemical methods, Sci. World J. (2014) 303878.
[43] S.C. Shen, W.R. Lee, L.Y. Yang, H.H. Tsai, L.L. Yang, Y.C. Chen, Quercetin enhancement of arsenic-induced apoptosis via stimulating ROS-dependent p53 protein ubiquitination in human HaCaT keratinocytes, Exp. Dermatol. 21 (2012) 370–375.
[44] D.J. Leffell, The scientific basis of skin cancer, J. Am. Acad. Dermatol. 42 (2000) 18–22.
[45] L. Zhuang, B. Wang, D.N. Sauder, Molecular mechanism of ultraviolet-induced keratinocyte apoptosis, J. Interferon Cytokine Res. 20 (2000) 445–454.
[46] K.H. Park, H.O. Choi, D.D. Jang, Y.I. Park, K.C. Park, Down regulation of Bcl-2 and activation of caspase-8 in the UVB-induced apoptosis of a cultured human melanoma cell line, Photodermatol. Photoimmunol. Photomed. 17 (2001) 218–222.
[47] G. Park, H.G. Kim, S.P. Hong, S.Y. Kim, Walnuts (Seeds of Juglandis sinensis L) protect human epidermal keratinocytes against UVB-induced mitochondria-mediated apoptosis through upregulation of ROS elimination pathways, Skin Pharmacol. Physiol. 27 (2014) 132–140.