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ORIGINAL ARTICLE

Protective Effect of *Viburnum grandiflorum* against Ultraviolet-B Radiation-induced Cellular and Molecular Changes in Human Epidermal Keratinocytes

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ABSTRACT

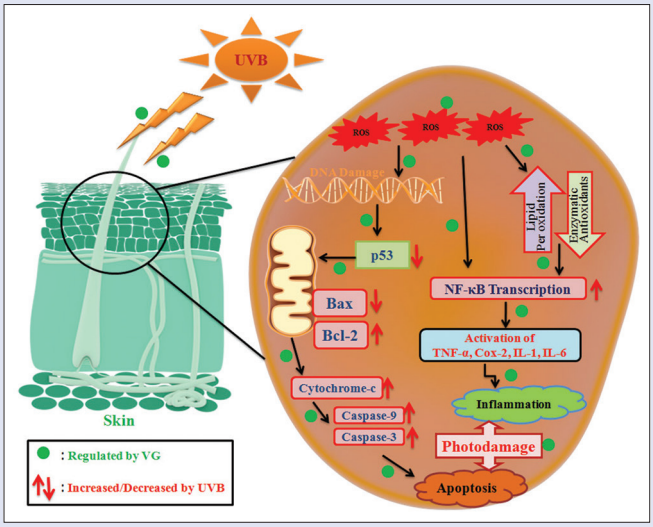
The aim of the present study was to evaluate the photoprotective potential of *Viburnum grandiflorum* (VG) against ultraviolet-B radiation-induced responses in HaCaT cells. The HaCaT cells were pretreated with VG prior ultraviolet-B (UVB)-radiation exposure and were further examined for lipid peroxidation, enzymatic antioxidant activity, % reactive oxygen species, DNA damage, mitochondrial membrane potential and for inflammatory, and apoptotic signaling markers such as tumor necrosis factor-alpha, nuclear factor kappa B, interleukin-1 (IL-1), IL-6, cyclooxygenase-2, p53, caspase-3/9, cytochrome-c, Bax, and Bcl-2. The VG pretreatment in UVB exposed cells shows significantly regulated both inflammatory as well as apoptotic signaling cascades. Our findings suggest that VG may be functional against UVB-induced photo-damages.

Key words: Apoptosis, inflammation, mitochondrial membrane potential, ultraviolet-B radiation, *Viburnum grandiflorum*

SUMMARY

- *Viburnum grandiflorum* (VG) is used as a diuretic, antispasmodic, and anti-sedative; it protects the liver and acts as anti-inflammatory medicine in traditional medicine. Nevertheless, the effect of VG on radiation-induced cellular damages in HaCaT has been explored against ultraviolet-B (UVB) encouraged photo-damages. The observation illustrated that VG offers protection against UVB-induced photo-damages by reducing the oxidative stress, modulation of lipid peroxidation, restoring the mitochondrial membrane potential, and regulating the inflammatory and apoptotic signaling cascades in skin epidermal cells. The findings suggest that VG might be the promising functional agent against UVB-induced photo-damages.

Abbreviations used: VG: *Viburnum grandiflorum*; UVB: Ultraviolet-B radiation; TBST: Tris-buffered saline (TBS) and Tween-20; EDTA: Ethylenediaminetetra acetic acid; Rh-123: Rodamine123; FBS: Fetal bovine serum; PBS: Phosphate-buffered saline; DMSO: Dimethyl sulfoxide; DMEM: Dulbecco's Modified Eagle Medium; ROS: Reactive oxygen species; AO: Acridine Orange; EtBr: Ethidium bromide; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, IC_{50}: The half-maximal inhibitory concentration; MED: Minimal erythema dose; MAPK: Mitogen-activated protein kinases; COX-2: Cyclooxygenase-2; DCFHDH: 2-7-diacetyl dichlorofluorescein diacetate; PMS: Phenazine methosulfate; DTNB: 5, 5-dithiobis 2-nitrobenzoic acid; TBARS: Thiobarbituric acid.



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INTRODUCTION

Ultraviolet radiation comes from the sunlight contains of three main components such as UVA (400-320 nm), ultraviolet-B (UVB) (320-280 nm), and UVC (280-100 nm). Among these three components, UVB is the most destructive module of sunlight, reaching the earth's surface. UVB has both, direct as well as indirect biological effects, including reactive oxygen species (ROS) production, DNA damage, oxidative imbalance, resulting in photo-aging, erythema, and inflammation.[1] UVB radiation leads to the induction of transitions (C to T) at dipyrimidine sites, resulting in the formation of typical photoproducts which are associated with DNA damage.[2] In a day's time, a person can receive 15 minimal erythema doses (MEDs)

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of UVB. Epidemiological studies on fair-skinned population have stated the incidence of erythema merely following 20 min of sunlight exposure, which is equivalent to 15–70 mJ/cm^2 (1MED) of UVB radiation dose.[3]

UVB is considered as a potent genotoxic agent; exposure to UVB radiation both burns the skin and drives the initiation, promotion, and progression of skin carcinogenesis. It leads toward inflammation, cell apoptosis, and photoaging.[4] The damages induced at the cellular level through UVB-exposure are also credited to a hike in intracellular ROS, which enhances the mitogen-activated protein kinases-mediated inflammatory responses[5] and upregulation of cyclooxygenase-2 (COX-2) expression.[6] UVB-mediated inductions of early genes responsible for inflammatory signaling could contribute toward the initiation of transcription factors leading to the initial stage of skin photo-damage and inflammation.[7]

Viburnum grandiflorum (VG) is a large deciduous precocious shrub belonging to the moschatel family (Adoxaceae), but previously, it went to Caprifoliaceae. It is a vast genus consisting of about 210 plant species. These species are largely found in Asia and northern hemisphere. VG grows wild from mid-Nepal east to Bhutan and southeast Tibet, where it grows in forests at an altitude from 2700 to 4300 m. It also encompasses westward into India (Kashmir) and Pakistan, where it is a dominant species. The flower of this plant is a spherical or somewhat flattened drupe, red to pink, black or blue, and contains a single seed; some are edible, but other species exhibit a mild poisonous nature. VG contains saponins, flavonoids, anthraquinones, and coumarins. It is used as a diuretic, anti-spasmodic, and anti-sedative. In traditional medicine, it defends the liver and acts as an anti-inflammatory medicine. It is also supportive for the gastric system, and it can be an anti-bacterial agent.[8]

The VG is renowned in folk remedy for various disorders such as anti-asthmatic, spasmolytic, and sedativeness with significant effects. VG has been used clearly to prevent complications such as menstrual cramps and post-partum bleeding.[9] Locally VG has been used to apply against slow abdominal pain,[10] and was given for antimalarial and diuretic complications.[11] Conventionally, VG is also applied for the treatment of wound healing,[12] effectively specified for stomachache,[9,13] anti-analgesic,[14] anti-typhoid, anti-toothache, and few respiratory disorders.[15] VG has also been reported with good antimicrobial activities.[16] However, the significance of VG on UVB encouraged damages on skin that have not been explored yet. In the present study, the impact of methanolic flower extract of VG against UVB-stimulated damages on the skin has been discovered.

MATERIALS AND METHODS

Chemicals and reagents

2-7-diacetyl dichlorofluorescein diacetate, phenazine methosulphate, 5, 5-dithiobis 2-nitrobenzoic acid, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), thiobarbituric acid (TBARS), Rodamine123 (Rh-123), and NBT were purchased from Himedia, China. Culture materials such as fetal bovine serum, Dulbecco's Modified Eagle Medium (DMEM), glutamine, ethylenediaminetetra acetic acid, penicillin-streptomycin, trypsin, phosphate-buffered saline (PBS), melting agarose and all monoclonal antibodies was purchased from Sigma Chemical Co., St. Louis, USA. Other used chemicals and the solvents used was purchased from Fisher Inorganic, China.

Preparation of plant extracts

VG flower was collected from Jinan, Shandong, China. The plant identification was performed at the Jinan Municipal Hospital of Traditional Chinese Medicine, Shandong, China. The VG extract (methanolic extract) was prepared with methanol (1: material and 3: solvent).[17] The supernatant was filtered, and followed by vacuum evaporation. The extract obtained was finally stored (4°C) for potential use.

Cell line culture

HaCaT cells were purchased from cell center, Kunming Yunnan, China. The cells were reserved in complete DMEM in 5% CO_2 and 37°C atmosphere.

Treatment of the HaCaT cells

Methanolic extract of VG (100 µg/mL, selected based on MTT assay) was added to the grouped HaCaT cells 30 min before UVB-exposure. Exclusion trypan blue dye test was conceded for the suitability and toxicity indication of VG (100 µg/mL) against photo-protection. Before UVB irradiation, cells were PBS washed.

Ultraviolet-B-irradiation procedure

The UVB irradiation was followed by Muzaffer *et al.*,[18] and was performed with a radiation source (Heber Scientific) (20 mJ/cm^2), in which 290–320 wavelength has been set for UVB, in PBS for 9 min. The non-irradiated controls and UVB-irradiated cells were then incubated at 37°C in carbon dioxide incubator for 6 h. After washing with PBS and trypsinization, further the analysis was performed.

Cell viability assay

The cell feasibility was determined by MTT assay with some minor modifications.[19] After treatment, cells (1 × 10^5/well) were plated in 96-well plates. The cells were washed thrice in order to remove the residual drug from each well, following the addition of MTT (50 µL) and incubation for 4 h. The formazan crystals were dissolved with 100 µL of dimethyl sulfoxide. Percent cell viability was determined by measuring the increase in the absorbance values (OD 570 nm). The half-maximal inhibitory concentration value was determined from the graph.

Sun protection factor

The UV absorbance for VG (200–400 nm) were calculated with the help of Nanodrop 2000 (Thermo Scientific).[20] 4-Aminobenzoic acid (PABA) has been used as standard (data not shown), while observing SPF of VG. The SPF was determined using the equation:

$$SPF = CF \times \sum_{290}^{320} EE \times I \times Abs$$

(CF = Correction factor = 10, Spectrum erythemal effect = EE, Solar intensity = I, and Absorbance = Abs) [Table 1].

Table 1: The sun protection factor value of *Viburnum grandiflorum*

Wavelength (nm)	EE × I	Abs	Abs × EE × I
290	0.0150	1.94	0.0291
295	0.0817	1.34	0.109478
300	0.2874	1.1	0.31614
305	0.3278	0.93	0.304854
310	0.1864	0.82	0.152848
315	0.0837	0.71	0.059427
320	0.0180	0.66	0.01188
		Total	0.983727
		Total × CF (10)	9.837
		SPF for VG	9.837

CF=10. SPF: Sun protection factor; VG: *Viburnum grandiflorum*; CF: Correction factor; EE: Erythemal effect; I: Solar intensity; Abs: Absorbance

Measurement of reactive oxygen species levels

The intensity of ROS generation was used to measure the consequence of VG on oxidative stress.[21] The evaluation is based on the oxidation of DCFH-DA by ROS. Cells were uncovered to UVB-irradiation (20 mJ/cm^2) after pre-treatment with VG. The cells were then preloaded with 5 μM fluorescent dye DCFH-DA in DMEM and incubated in the dark at 37°C for 1 h. The fluorescence intensity was taken from each group (Tecan, Austria). Cells were also visualized under a fluorescent microscope (Invitrogen).

Estimation of antioxidants and lipid peroxidation

The assessment of lipid peroxidation was carried out using TBARS reactive substances.[22] The anti-oxidant status (catalase [CAT], superoxide dismutase [SOD], GSH and glutathione peroxidase [GPx]) was determined to assess the effect of VG pretreatment prior and after the UVB exposure, following,[23-26] respectively.

Mitochondrial membrane potential (Δψm)

The treated and untreated cells were mixed with 5 μM of Rh-123 for 15 min to assess the Δψm,[27] following by the visualization under a fluorescence microscope (450–490 nm).

DNA damage (comet assay)

The comet assay has been applied to assess the damages in genetic material.[28] Briefly, cells (50 μL), mixed with low melting agarose (120 μL) were lysed by lysis solution following the incubation for 60 min at 4°C, to get unwinding of DNA in an alkaline buffer (pH 13 for 20 min). In the next step, neutralization and staining (5 μg/mL of ethidium bromide) were performed by following,[5] and further analysis of cellular DNA damage was measured using image analysis software, CASP after florescence microscopy.

Expression level of proteins

The cellular protein amount was calculated using Nanodrop. The protein samples (55 μg) from each treatment group were determined by using sodium dodecyl sulfate–polyacrylamide gel electrophoresis, followed by transferring of samples on nitrocellulose-membrane. The loaded membrane was then positioned in a solution for blocking for 12 h, followed by the addition of the primary and secondary antibody with time interval and washing with Tris-buffered saline (TBS) and Tween-20. The membrane development and visualization of protein bands were performed (LI-COR).

Statistical analysis

The analyses of all findings were prepared using ANOVA and DMRT, using SPSS as the statistical tool. $P < 0.05$ was considered statistically significant.

RESULTS

Effect of *Viburnum grandiflorum* on cell viability and its sun protection factor

The protective effect of VG against UVB-induced cytotoxic effects was analyzed using MTT. We observed a considerable decrease in cell viability in the UVB-exposed cells. VG pretreatment before UVB exposure considerably augmented the cell viability. We also detected the highest cell feasibility at 100 μg/mL of VG pretreatment. Conversely, higher concentrations (>120 μg/mL) of VG were toward decreasing viability [Figure 1a and b]. Therefore, we selected a non-toxic concentration (100 μg/mL) of VG for further experiments. Above and beyond, the VG is indication for the utmost absorption from 240 to 320 nm, which defines 9.837 SPF value [Table 1].

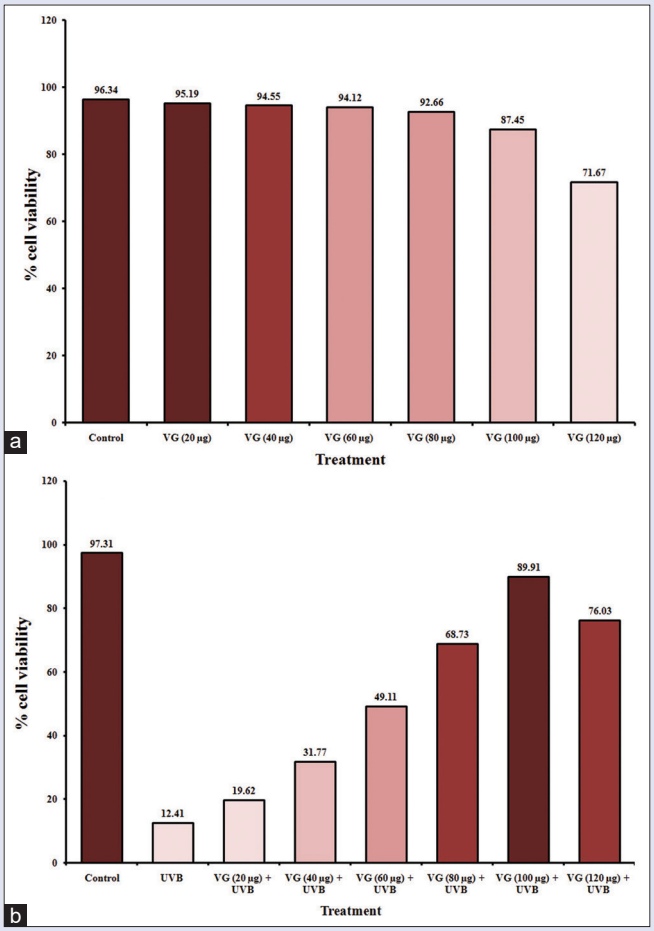

Figure 1: Effect of *Viburnum grandiflorum* on ultraviolet-B induced cell proliferation in HaCaT cells. (a) Treatment of different concentrations of *Viburnum grandiflorum* on cell viability of HaCaT cells, (b) post-treatment of *Viburnum grandiflorum* on ultraviolet-B induced cell toxicity was analysed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. Data were expressed as means ± standard deviation from three independent experiments

Effect of *Viburnum grandiflorum* on antioxidant levels and lipid-peroxidation

We observed a substantial increase in the lipid peroxidation levels, which is detected as TBARS when compared to control [Figure 2A]. However, VG pretreatment exhibited significantly low levels of TBARS in contrast to the UVB-group.

Endogenous antioxidants are measured to defend from free radicals. We perceived a striking reduce in SOD, CAT, and GPx levels by UVB-irradiation. Conversely, VG pretreatment appreciably maintained the anti-oxidant enzymes activity in the UVB exposed cells [Figure 2B].

Effect of *Viburnum grandiflorum* on reactive oxygen species generation

UVB irradiated cells illustrated augmented intracellular ROS levels in distinction to control. However, intracellular ROS levels were observed regulated toward normal in VG + UVB pretreated groups as well as in VG alone-treated cells [Figure 3].

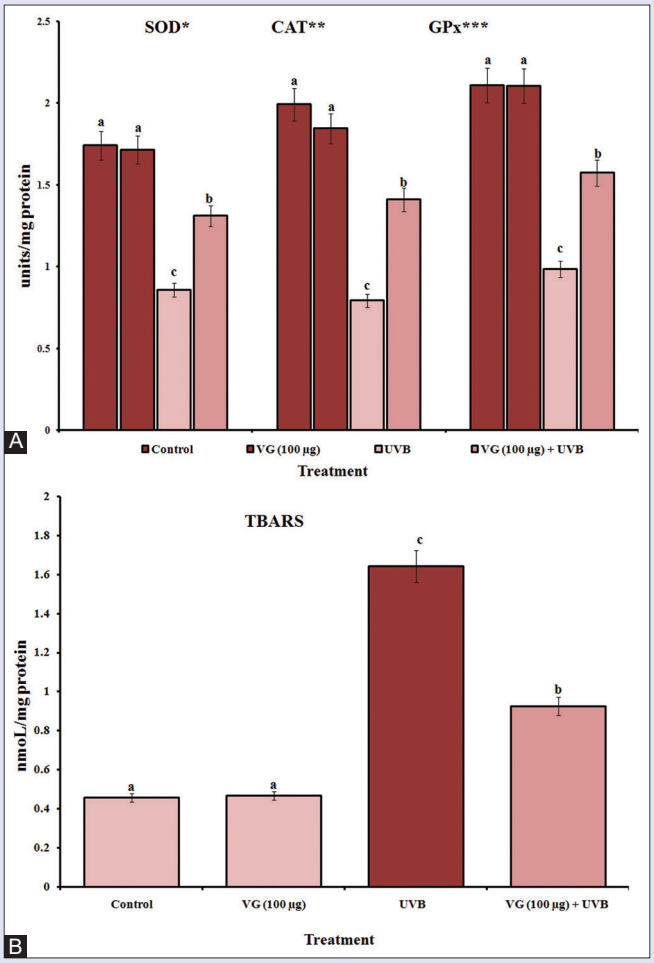

Figure 2: Effect of *Viburnum grandiflorum* on ultraviolet-B induced antioxidants and lipid peroxidation status in HaCaT cells. (A) Activities of superoxide dismutase, catalase and glutathione peroxidase in ultraviolet-B irradiated and *Viburnum grandiflorum* (100 µg/ml) pre-treated HaCaT cells. *Enzyme concentration required for 50% inhibition of nitroblue tetrazolium reduction in one minute. **µmol of hydrogen peroxide consumed per minute. ***µg of glutathione consumed per minute. (B) Lipid peroxidation status on control, *Viburnum grandiflorum*, ultraviolet-B irradiated and *Viburnum grandiflorum* pre-treated ultraviolet-B irradiated HaCaT cells. Lipid peroxidation expressed as mM/Protein. Values are given as means ± S.E. of six experiments in each group. Values not sharing a common superscript for a particular factor/group (a, b, c) differ significantly. [b]Significantly different from control ($P < 0.01$). Significantly different from the ultraviolet-B group ($P < 0.05$)

Effect of *Viburnum grandiflorum* on mitochondrial membrane potential (Δψm)

We observed a reduction in the fluorescence-intensity in the UVB-group. Conversely, a significant increase of the mitochondrial membrane potential, was found in cells pretreated with VG for 30 min before the UVB-irradiation. There was diminished fluorescence-intensity in the UVB group in contrast to control, and VG group. VG pretreatment noticeably barred the loss of Δψm [Figure 4].

Effect of *Viburnum grandiflorum* on DNA damage

Fluorescence-microphotographs demonstrated the clear comet-tails by UVB-irradiation. Conversely, VG pretreatment established a reduced comet. However, intact nucleoid was observed in the control and VG group [Figure 5].

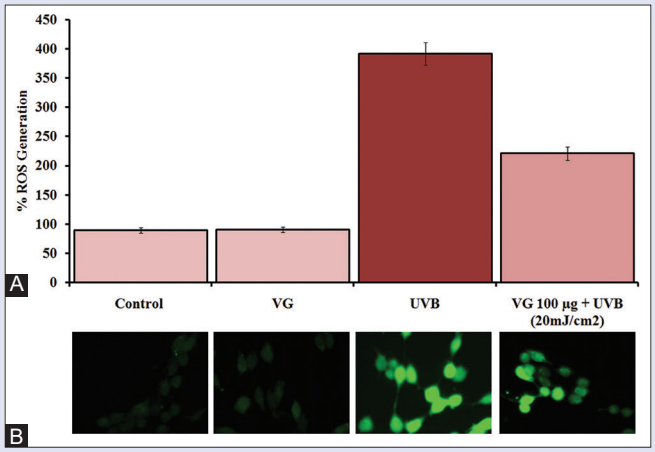

Figure 3: Effect of *Viburnum grandiflorum* and ultraviolet-B-irradiation on intracellular reactive oxygen species generation using DCFH-DA staining. (A) Fluorescence intensity as measured by spectrofluorometric analysis. Values are given as means ± S.D. of six experiments in each group. Values not sharing a common superscript (a, b, c) differ significantly. [b]Significantly different from control ($P < 0.01$). [c]Significantly different from ultraviolet-B group ($P < 0.05$). (B) Fluorescence microscopic images (×20) of control, *Viburnum grandiflorum* and/or ultraviolet-B treated HaCaT cells

Effect of *Viburnum grandiflorum* on apoptotic and inflammatory marker expressions

We observed a fold increase in the appearance of inflammatory markers such as tumor necrosis factor-alpha (TNF-α), nuclear factor kappa B (NF-kB), interleukin-1 (IL-1), IL-6, and COX-2 in the UVB-irradiated cells in contrast to the control group [Figure 6A and B]. Conversely, we noticed a fold inhibition in the expression of TNF-α, NF-kB, IL-1, IL-6, and COX-2 when the cells were pretreated with VG (100 µg/mL) before UVB exposure. The Western blotting results illustrated that the expression of apoptotic markers was increased in p53, caspase-3, caspase-9, Bax, cytochrome-c, and downregulated for Bcl-2 in UVB-irradiated cells when compared to control cells. Conversely, pretreatment with VG before UVB exposure significantly downregulated the expression of apoptotic marker such as p53, caspase-3, caspase-9, Bax, and cytochrome-c toward the normal and shows the up-regulation towards the normal in Bcl-2 protein expression level [Figure 7A and B].

DISCUSSION

UVB exposure causes some diseases such as erythema, inflammation, photoaging, and skin cancer. The present findings estimated the role of VG on UVB-induced damages in HaCaT. The curative measures retaining plant-derived compounds comprise about 60% of available anti-cancer drugs.[29] These phytocompounds exhibited a vital role in disturbing the mechanisms of cancer.

The exposure of UVB-radiation are directly linked to ROS production,[30] eventually resulting in the consequent stimulation of the kinase pathways which further leads to inflammation, apoptosis, and photoaging.[18] On the other hand, phytochemicals and phytoextracts possess excellent antioxidant activity and can strongly absorb and later reduces UV transmission as a defense against UV radiation.[31] The photo-damaging effects are linked with long-term exposure to UVB radiation.[32] As a preventive measure, sunscreens

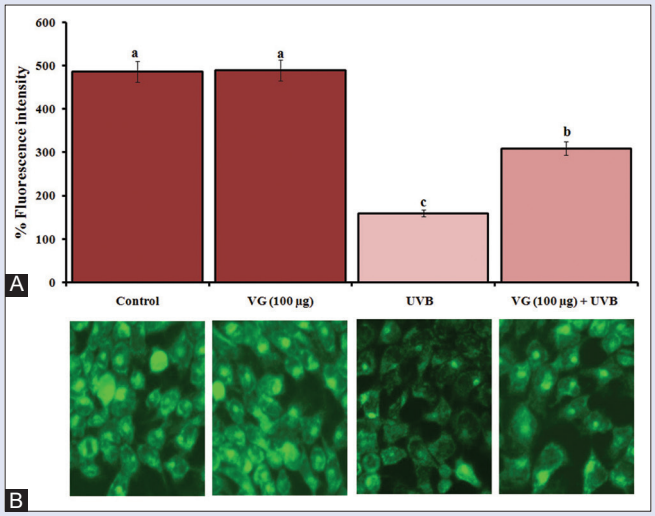

Figure 4: Effect of *Viburnum grandiflorum* and ultraviolet-B irradiation on mitochondrial membrane potential (Δψm) using Rodamine123 staining. (A) Fluorescence intensity as measured using spectrofluorometric analysis. Values are given as means ± S.E. of five experiments in each group. Values not sharing a common superscript (a, b, c) differ significantly at $P < 0.05$ (DMRT). (B) Fluorescence microscopic images (×40) of normal, *Viburnum grandiflorum* and/or ultraviolet-B treated HaCaT cells

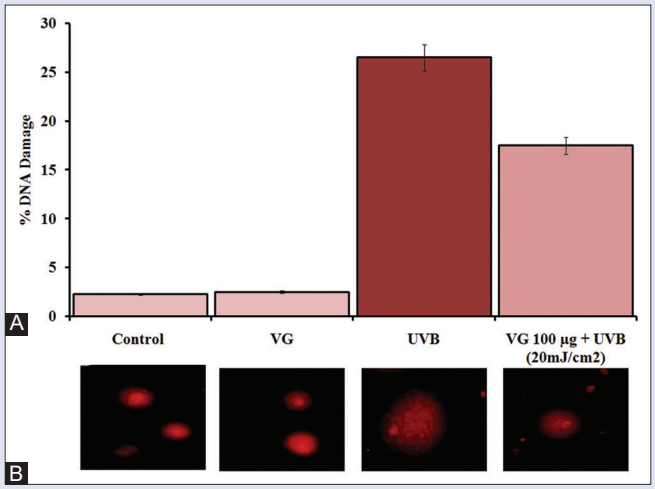

Figure 5: Single-cell gel electrophoresis showing effect of *Viburnum grandiflorum* and ultraviolet-B irradiation on DNA damage (ethidium bromide) in HaCaT cells. Fluorescence microphotographs show intact nucleoid in control, *Viburnum grandiflorum* alone treated group and tail DNA in ultraviolet-Birradiated 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)

are employed because they have the potential to absorb or reflect away the harmful and lethal portions of UV radiation. Although current sunscreen compounds in the market pose some severe effects on the skin are in use, their use is still a question.[33] Plant-derived bioactive molecules (secondary metabolites) have increased a lot of research attention. The findings of the present observation indicated that VG has a UV gripping property expressed as SPF of 9.837 [Table 1], which is relatively near to some commercially available sunscreens. The significant sunscreen property of VG may give the first line of defense against this photo-damage.

The sunscreen protection is time-limited, and after that, the defense against UVB radiation fades away, leading to oxidative damage. UVB-irradiation leads to ROS-mediated disturbance of the cellular antioxidant status.[34] Excessive cellular ROS that cause damage to diverse cellular machinery such as proteins, DNA, and membrane lipids. Human skin unsurprisingly encompasses a complex anti-oxidant protection organization to neutralize stress. However, the remarkably produced free-radicals can overcome the antioxidant defense.[35] Phytochemicals that show high antioxidant, but at the same time also carry a function of endogenous protective enzymes activation.[36] As far as the present observation is concerned the obtained results clarify that VG pretreatment also prevented the irradiation-induced decrease of antioxidants significantly and also hamper lipid peroxidation effectively. This oxidative stress may eventually result in cell apoptosis.[37] The observation also recorded the inhibition of UVB-mediated ROS generation by VG pretreatment.

The process of apoptosis is directly or indirectly controlled by mitochondrion; on the other hand, UVB mediated oxidative stress directly alters the potential of its membrane. This alteration effects on the respiration, potential, and gap in mitochondrion membrane results in the release of caspases and cytochrome c, which are known accountable for augmenting apoptosis.[38,39] The observation of the present finding has provided conclusive evidence validating the alteration of Δψm in UVB-exposed HaCaT cells. This also indicates the probability of Δψm loss through UVB-irradiation. This study investigated the capacity of VG to protect the Δψm in a stressed environment like UVB-irradiation to a significant level. This capacity or property might be interrelated to the being there of different flavonoids in VG, may be because of UV absorbance potential or maybe due to its scavenging ability.

The integument is a primary line of defense for the human body; its keratinocytes always remains a target for critical radiations like UVB. The irradiation process mostly activates a variety of pro-inflammatory markers such as interleukin-6 and transcription factors results in the commencement of NF-κB.[40] The NF-κB is a universal transcription factor of nucleolus, that leads for inflammation by responding to external stimuli.[41] The current study also confirmed the upregulation of COX-2, NF-κB, IL-6, and TNF-α by irradiation process. The results from earlier findings have stated that VG fruit extract significantly reduced the level of TNF-α, VEGF, and IL-6 expression prior and posttreatment in surgically-induced endometriosis in rat models.[9] The COX-2 has a noteworthy role to play in the activation of PGE2-mediated, an initiation of inflammation rooted by UVB-irradiation.[10] The observation of the present findings confirmed the authoritarian responsibility of VG on the induction of levels of NF-κB, COX-2, TNF-α, and IL-6 in irradiated cells, most likely through its sunscreen and antioxidant ability.

The inflammatory signaling molecules in our system are activated by continual radiation exposure.[10,11] The UVB radiation results in the commencement of TNF-α, IL-6, iNOS, COX-2 as well as the initiation of the intracellular signaling cascade, which ultimately results in the phosphorylation-mediated cleavage of IκB.[12] The IκBα degradation permits the NF-κB nuclear translocation, results in the initiation of inflammation and carcinogenesis.[13] Besides, we also noticed an increase in the NF-κB nuclear translocation, which confer its improved transcriptional movement. The presented findings proved the anti-inflammatory effect of VG pre-treatment against the over-expression of nuclear NF-κB.

UVB is notorious for oxidative stress following the configuration of CPDs in DNA, which results in the stimulation of the p53 pathway and, ultimately, in the implementation of apoptosis.[15] The present observation identified the damage in irradiated DNA and

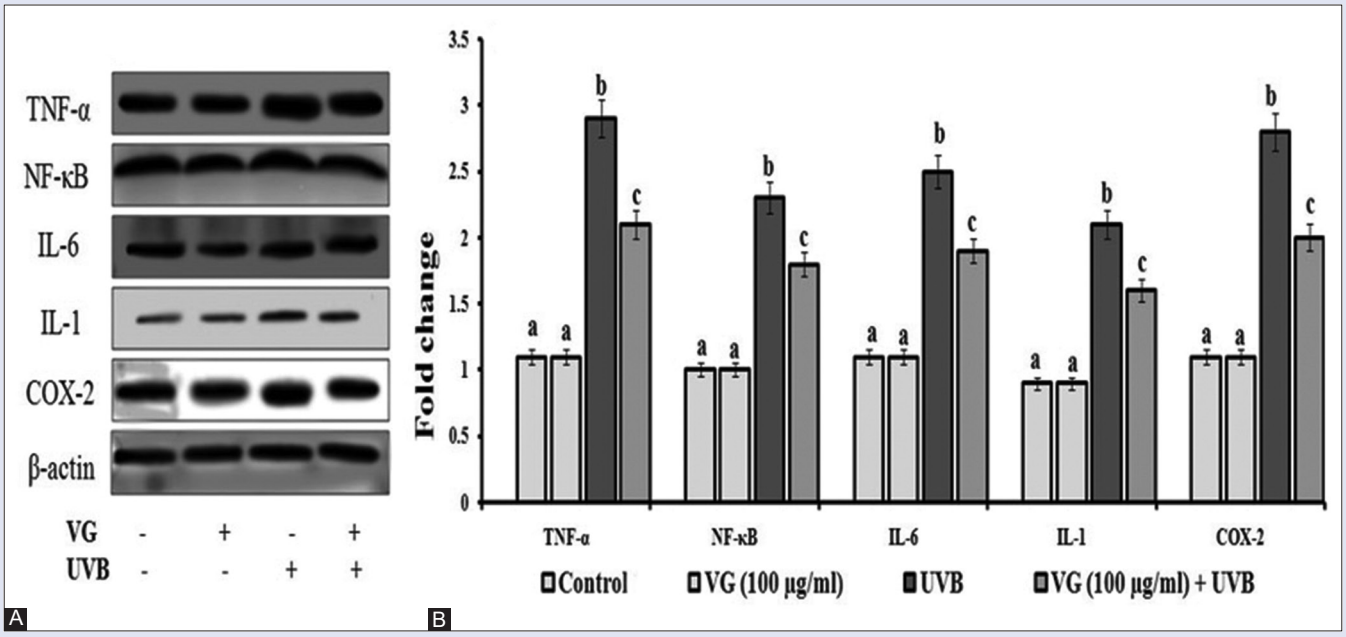

Figure 6: Effect of *Viburnum grandiflorum* on ultraviolet-B mediated protein expression levels in HaCaT cells. (A) Protein expression levels of tumor necrosis factor-α, nuclear factor kappa B, interleukin-1, interleukin-6 and cyclooxygenase-2 by Western blotting analysis, normalized to β-actin. (B) Quantification was performed by densitometric analysis using Image Studio software (LI-COR). Data are expressed as ratios of target proteins to β-actin as the means ± S.D. from three independent experiments. Values not sharing a common superscript for a particular pattern/factor differ significantly. [b]Significantly different from control ($P < 0.01$). [c]Significantly different from ultraviolet-B group ($P < 0.05$)

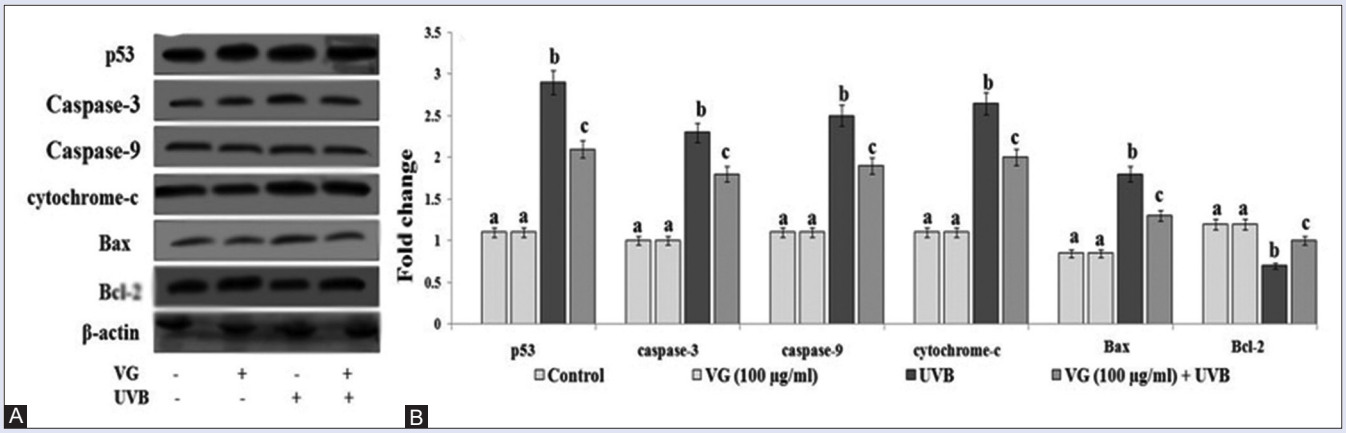

Figure 7: Effect of *Viburnum grandiflorum* on ultraviolet-B mediated apoptotic marker expressions in HaCaT cells. (A) Western blotting analysis of p53, Caspase-9, Caspase-3, Cytochrome c, Bax and Bcl-2, expressions, normalized to β-actin. (B) Densitometric quantification of proteins of Western blot 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. [b]Significantly different from control ($P < 0.01$). [c]Significantly different from ultraviolet-B group ($P < 0.05$)

found significant regulation of tail formation toward normal, when pre-treated with VG. DNA damage in epidermal keratinocytes is mainly connected with the commencement of p53, which is known as TSG.[14] Following this event the p53 consequently arrests G_1phase, and waiting for repairing of DNA, if not p53 proceeds for apoptosis.[16,18] The findings compare pretreatment with VG and irradiation, which gave confirmation of regulating p53 by VG in comparison with control. The p53 mediates apoptosis through the commencement of caspases and Bax. Bax is proficient in encouraging the mitochondrial-reliant cell-death, following the configuration of Apaf-1/Caspase-9 complex, resulting in the cleavage of pro-caspases and prompts downstream caspases by triggering Caspase-8 and hence, initiate the progression of apoptosis via DNA damage. The opinion of this study elucidates the upregulated Caspases-9, p53, Bax, Cytochrome c, and Caspases-3, with downregulated Bcl-2 [Figure 7] by irradiation. However, pretreatment with VG notably normalizes the appearance level of p53, Cytochrome c, Bax, Bcl-2, Caspases-9, and Caspases-3 toward regular. These findings may suggest that the VG can prevent the cell from UVB-related stress and hence may protect HaCaT from photo-damage. However, there is a need to suggest phytochemistry of VG and assess the main constituents against stress-mediated photo-damage.

CONCLUSION

Our findings demonstrate that VG can bid defense aligned with the radiation-induced reaction of decreasing the oxidative stress, modulation of lipid peroxidation, restoring the mitochondrial membrane potential, and regulating the inflammatory signaling cascades in HaCaT. This normalizing effect of VG against radiation-induced photo-damages may be due to the survival of a fine quantity of phytoconstituents or may be due to its antioxidant capability or by having UV absorbance capability.

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Conflicts of interest

There are no conflicts of interest.

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