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RESEARCH ARTICLE

APIGENIN PREVENTS ULTRAVIOLET-B RADIATION INDUCED OXIDATIVE STRESS AND DNA DAMAGE IN HUMAN DERMAL FIBROBLASTS

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ABSTRACT

Background: The exposure of skin to ultraviolet-B radiations leads to deoxyribonucleic acid (DNA) damage and can induce production of free radicals which imbalance the redox status of the cell and lead to increased oxidative stress. The objective of the present study is to evaluate the protective effects of apigenin, against ultraviolet-B (290-320 nm) radiation induced cellular changes were investigated in human dermal fibroblasts (HDFa).

Materials and Methods: HDFa cells pretreated with increasing concentrations of apigenin (1, 5, 10, 15, 30 and 60 µg/ml) for 30 min, were UVB irradiated and different cellular end points were analyzed.

Results: The percentage of cytotoxicity, intracellular reactive oxygen species (ROS) levels, mitochondrial membrane potential (MMP), thiobarbituric acid reactive substances (TBARS), lipid hydroperoxides (LHP), morphological apoptotic cell death and DNA damage were significantly increased in 19.8 mJ/cm2 UVB exposed HDFa cells. Further, exposure to UVB causes significantly decreased antioxidants status in HDFa cells. Treatment of HDFa cells with apigenin before 30 min of UVB-irradiation significantly restored MMP, ROS levels, antioxidant status, apoptotic changes and DNA damage in HDFa cells.

Conclusion: The present findings indicate that apigenin prevents UVB induced cellular changes in human HDFa. Hence, it may be used as a constituent in sunscreen lotions.

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INTRODUCTION

Ultraviolet radiation (UV), in particularly UVB with a wave length range (280–320 nm), represents one of the most important environmental factor affecting human health (Wang and Kochevav, 2005). UVB exposure is a major health burden and in the human skin it causes etrythema, edema, inflammation, photoaging and cancer. Modern life style elicits decreased ozone layer which results in increased UVB-mediated pathological conditions (Pelle *et al*., 2003). UVB exposure directly induces cyclobutane pyrimidie dimer and indirectly induces reactive oxygen species in the cellular milieu. UVB-exposure generates singlet oxygen ($1O_2$), hydrogen peroxide (H_2O_2), superoxide anion ($O_2^{\bullet-}$) and hydroxyl radical ($^{\bullet}HO$) (Hou *et al*., 2009). Although skin possesses an extensive and effective network of antioxidant systems, many of ROS produced by UVB-radiation can escape this surveillance and induce substantial damage to cutaneous constituents, especially when skin defense mechanisms are overwhelmed (Stone *et al*., 2003). Acute UVB exposure causes depleted antioxidants system (Pattison and Davies 2006); enhancement of intracellular lipid peroxidation products (Ojima *et al*., 2007); and the induction of specific signal transduction pathways that can modulates inflammatory, immunosuppressive, or apoptotic processes in the skin (Levites *et al*., 2001). When the level of damage is too severe and/or failure of repair mechanisms, apoptosis is initiated. Cells with damaged DNA that is too extensive to be repaired are removed by apoptosis. Thus, a defect in this process contributes for many diseases, including cancer as well as ageing. Hence, there is a general need for safe and effective

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antioxidants to modulate the UVB-induced redox (antioxidant/pro-oxidant) balance. Consequently, exogenous antioxidants that scavenge ROS and restore the normal redox state may play a beneficial role against UVB-induced photodamages. The effect of flavonoids on oxidative DNA damage has been researched by many investigators and flavonoids have been shown to reduce oxidation damage induced by a variety of ROS generating agents (Begam and Prasad 2012). Dietary flavonoids have been investigated for their radioprotective activity. Silibinin, genistein, luteolin etc. exhibits radioprotective effects in different experimental models (Prabha *et al*., 2010; Landauer *et al*., 2003; Shimoi *et al*., 1996).

Apigenin is a dietary flavonoid present in fruits and vegetables like parsley, onions, oranges, tea, chamomile, wheat and sprouts. Apigenin exhibits various pharmacological actions including antioxidant, antinflammatory and antiproliferative effects (Begum and Prasad 2012; Gil-Saeng *et al*., 2009; Wei *et al*., 1990). Apigenin caused a significant decrease in lipid peroxidation (LPO) levels and improved the antioxidant status in liver and kidney of male Wister rats induced by DEN (N-nitrosodiethylamine), due to its antioxidant sparing property (Singh *et al*., 2004). Apigenin treatment prevented inflammatory markers such as matrix metalloproteinases (MMPs) and cyclooxygenase‑2 expression in UV exposed cellular models (Sim *et al*., 2007; Van Dross *et al*., 2007). Apigenin has been reported to prevent UVB induced cytogenetic and chromosomal alterations during gamma-radiation and hydrogen peroxide treatment (Begum and Prasad, 2012; Siddique and Afzal, 2009; Rithidech *et al*., 2005). However, its photoprotective effect against UVB induced cellular changes has not been thoroughly investigated. In the present study, we evaluated effect of apigenin against UVB-induced oxidative stress and DNA damage in human dermal fibroblasts (HDFa) cells.

MATERIALS AND METHODS

Chemicals: Human dermal fibroblasts adult (HDFa) 500K cells/vials were purchased from Invitrogen Bioservices, India (Catalogue No: C0135C). Medium supplement (LSGS; Catalog No: S‑003‑10), fetal bovine serum, hydrocortisone, human epidermal growth factor, basic fibroblast growth factor, heparin, trypsin/ethylenediamine tetra acetic acid (EDTA) solution (Catalog No: R‑001‑100) and trypsin neutralizer solution (Catalogue No: R‑002-100) were purchased from Cascade Biologics, Invitrogen cell culture, India. Apigenin, 3‑(4, 5‑dimethyl‑2‑thiaozolyl)‑2, 5‑diphenyl‑2H tetrazolium bromide (MTT), 2,7‑diacetyl dichlorofluorescein (DCFH‑DA) and rhodamine 123 were purchased from Sigma Co., St. Louis, USA. Low melting agarose (LMPA), normal melting agarose (NMPA), phosphate‑buffered saline (PBS) and all other chemicals, solvents of analytical grades were obtained from S.D Fine Chemical, Mumbai and Fisher Inorganic and Aromatic Limited, Chennai.

Human skin dermal fibroblasts adult cell culture: Human dermal fibroblast adult cells (HDFa) obtained from Invitrogen Bioservices were cultured at 37 °C in 5% CO_2 in DMEMmedium supplemented with LSSG kit (fetal bovine serum 2% v/v, hydrocortisone 1 µg/ml, human epidermal growth factor 10 ng/ml, basic fibroblast growth factor 3 ng/ml, heparin 10 ng/ml and antibiotics). The cells were allowed to grow for 7 days to reach the maximum confluence. After reaching 80-90% confluences the cells were sub-cultured and used for further experiments.

Cultured human dermal fibroblasts were divided into four groups as follows:

- Group 1: Normal fibroblasts without any treatment
- Group 2: Normal fibroblasts with 15µM of Apigenin
- Group 3: UVB-irradiated fibroblasts
- Group 4: UVB-irradiated fibroblasts pretreated with 15µM of Apigenin

Treatment of the HDFa cells: Thirty minutes prior to irradiation, three test doses (1, 5, 10,15,30,60 µM) of apigenin were added to the grouped HDFa cells. Preliminary studies were carried out to check whether these concentrations had any toxic effect by conducting trypan blue dye exclusion test. Before exposure to UV light, the cells were washed twice with phosphate buffered saline (PBS) solution. Nonirradiated HDFa showed no decrease in viability over the 30 min period of incubation.

Irradiation procedure: HDFa cells were washed twice with PBS and UVB-irradiated in a thin layer of medium without FBS. A battery of TL 20 W/20 fluorescent tubes (Heber Scientific, Chennai, India) was used as UVB source, which possess a wavelength range of 290–320 nm, peaked at 312 nm, and with an intensity of 2.2 mW/ cm^2 for 9 min. The total UVB-radiation exposure was 19.8 mJ/ cm^2, with an average value of 1.52×10^3 mJ/cell. Immediately after UVB exposure, the HDFa cells were kept at 37 °C for 4 h at 5% CO_2 environment. Irradiated HDFa cells were then washed with PBS, and transferred to sterile centrifuge tubes for biochemical analysis (Ramachandran and Prasad 2012).

MTT based cytotoxicity assay: The MTT assay is a colorimetric nonradioactive assay for measuring cell viability through increased metabolism of tetrazolium salt (Moshmann, 1983). Cultured fibroblasts seeded at a density of 1×10^6 cells/ml was taken into 96 well plates. Then the cells were treated with different concentration of apigenin (1, 5, 10, 15, 30, 60 µM). After 30 min incubation with apigenin treatment, the cells were exposed to UVB‑irradiation. Then the cells were incubated in the presence of 5% CO_2 at 37°C for 24 h. The MTT (0.5 mg/ml) was added to the cells and then further incubated for another 4 h. The cells were centrifuged for 10 min and the supernatant was removed, 200 µl of dimethyl sulfoxide (DMSO) were added into each tubes. Absorbance was measured in a microplate reader at 560 nm.

Quantification of intracellular ROS: The intracellular ROS levels were measured by DCFH‑DA method (Hafer *et al*., 2008). The diacetate group of DCFH‑DA allows it to diffuse into the cells where the esterase react with oxidants, and upon oxidation, the probes become fluorescent and are thus

amenable to quantify spectrofluoremetically. Apigenin-pretreated and/or UVB-treated fibroblasts in six well plates were incubated for 15 min with 10 µM DCFH-DA in PBS, washed three times with PBS. Fluorescence was determined at 488/525 nm by spectrofluorometer.

Changes in mitochondrial transmembrane potential (Δψm): The changes in Δψm in different treatment groups were observed microscopically and determined spectrofluorometrically using fluorescent dye Rh-123. To the treated and control HDFa, 1µl of rhodamine-123 (5 mmol) was added and kept in the incubator for 30 min (Bhosle *et al*., 2005). The cells were then washed with PBS and observed with a fluorescence microscope using blue filter (450-490 nm). Polarized mitochondria emit orange-red fluorescence and depolarized mitochondria emit green fluorescence.

Estimation of membrane lipid peroxidation and cellular antioxidants: Fibroblasts were suspended in 130 mM KCl plus 50 mM PBS containing 0.1 mL of 0.1 M dithiothreitol and centrifuged at 2,000 rpm for 15 min (4°C). The supernatant was taken for biochemical estimation. The level of lipid peroxidation was determined by analyzing TBA-reactive substance according to the protocol of Niehaus and Samuelsson (1968). The pink colored chromogen formed by the reaction of 2-TBA with breakdown products of lipid peroxidation was measured. The lipid hydroperoxides (LHP) levels were determined by analyzing BHT-reactive substance according to the protocol of (Jiang *et al*., 1992). Superoxide dismutase (SOD) activity was assayed by the method of Kakkar *et al*., (1984) based on the inhibition of the formation of NADH-PMS-NBT complex. Catalase (CAT) activity was assayed by the procedure of Sinha (1972), quantifying the hydrogen peroxide after reacting with dichromate in acetic acid. The activity of glutathione peroxidase (GPX) was assayed by the method of Rotruck *et al*. (1973) a known amount of enzyme preparation was allowed to react with hydroperoxides (H_2O_2) and glutathione (GSH) for a specified time period. Then, the GSH content remaining after the reaction was measured. The total GSH content was measured by the method of Ellman (1959). This method was based on the development of a yellow color when 5, 5-dithiobis 2-nitrobenzoic acid was added to compound containing sulfhydryl groups.

Detection of apoptotic nuclei by EB/AO staining: Ethidium bromide/acridine orange (EB/AO) staining was carried out to detect morphological evidence of apoptosis on the apigenin and UVB-irradiated cells (Darzynkiewiez *et al*., 1994).The cells were labeled with 1:1 ratio of AO (100 µg/ml) and EB (100 µg/ml) in PBS and incubated for 5 min then the excess unbinding dye was removed by washing with PBS.

Stained cells were visualized under UV illumination using the 20 × objective (Nikon fluorescence microscope) and the digitized images were captured. The apoptotic cells, with the shrunken, nuclear fragmentation, brightly fluorescent, apoptotic nuclei, were easily detected through their high fluorescence and the percentage apoptotic cells were calculated.

Alkaline single-cell gel electrophoresis (comet assay): DNA damage was estimated by alkaline single-cell gel electrophoresis (comet assay) according to the method of Singh *et al*. (1998). A layer of 1% NMPA was prepared on microscope slides. After UVB-irradiation, HDFa cells (50 µl) were mixed with 200 µl of 0.5% LMPA. The suspension was pipetted onto the precoated slides. Slides were immersed in cold lysis solution at pH 10 (2.5 M NaCl, 100 mM Na_2EDTA, 10 mM Tris pH 10, 1 % Triton X-100, 10 % DMSO) and kept at 4 °C for 60 min. To allow denaturation of DNA, the slides were placed in alkaline electrophoresis buffer at pH 13 and left for 25 min. Subsequently, slides were transferred to an electrophoresis tank with fresh alkaline electrophoresis buffer and electrophoresis was performed at field strength of 25 V for 25 min at 4 °C. Slides were neutralized in 0.4 M Tris (pH 7.5) for 5 min and stained with 20 µg/mL ethidium bromide. For visualization of DNA damage, observations were made using a 409 objective in an epifluorescent microscope equipped with an excitation filter of 510–560 nm and a barrier filter of 590 nm. One to two hundred comets on duplicated slides were analyzed. Images were captured with a digital camera with networking capability and analyzed by image analysis software, CASP. DNA damage was quantified by tail moment, tail length, olive tail moment (OTM). OTM is the product of the distance (in x direction) between the center of gravity of the head and the center of gravity of the tail and the percent tail DNA.

Statistical analysis: All the values were expressed as means of six (n = 6) determinations. The data were statistically analyzed using one-way analysis of variance (ANOVA) on statistical package for social sciences (SPSS) and the group means were compared by Duncan's multiple range test (DMRT). The results were considered statistically significant if the P value is the 0.5 levels. Values are given as means ± SD of six experiments in each group. Values not sharing a common marking (a, b, c.) differ significantly at $P < 0.05$ (DMRT).

RESULTS

Protective effect of apigenin against UVB-induced cytotoxicity in HDFa cells: In this study, one time UVB exposure significantly decreased HDFa cell viability. It has been found that only 30% of cell viability after 30 min UVB-exposure (Fig. 1). Conversely, apigenin pretreatment significantly prevented UVB-induced cell death and restored cell viability in a concentration-dependent manner. Among all the doses tested, 15 µg/ml of apigenin restored about 88.21% cell viability in HDFa cells.

Apigenin inhibits the ROS levels in UVB treated HDFa cells: The intracellular ROS production was significantly higher in UVB irradiated HDFa cells (FI 2400) compared to the control (FI 645) and apigenin alone treated cells (FI 650). Apigenin pretreatment (15 µg/ml) (FI 2950) significantly decreased the intracellular ROS production in UVB irradiated HDFa cells (Fig. 2).

Effect of apigenin on lipid peroxidation and antioxidant status in HDFa cells: Levels of TBARS were increased significantly in UVB-irradiated HDFa cells (Table 1).

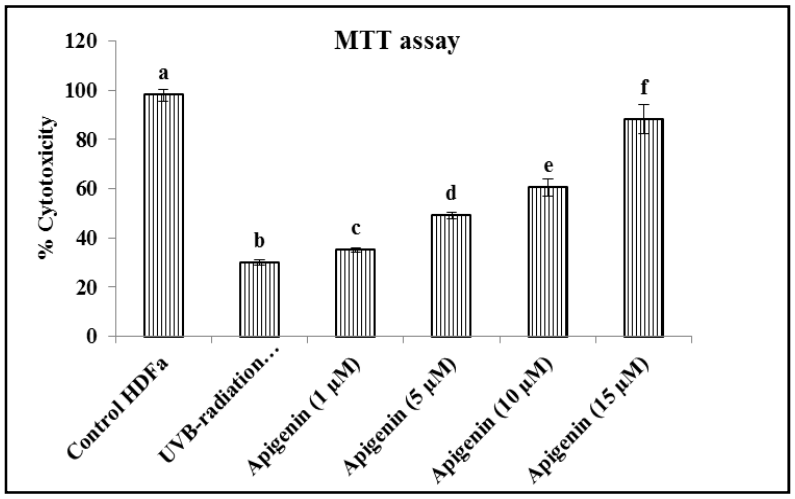
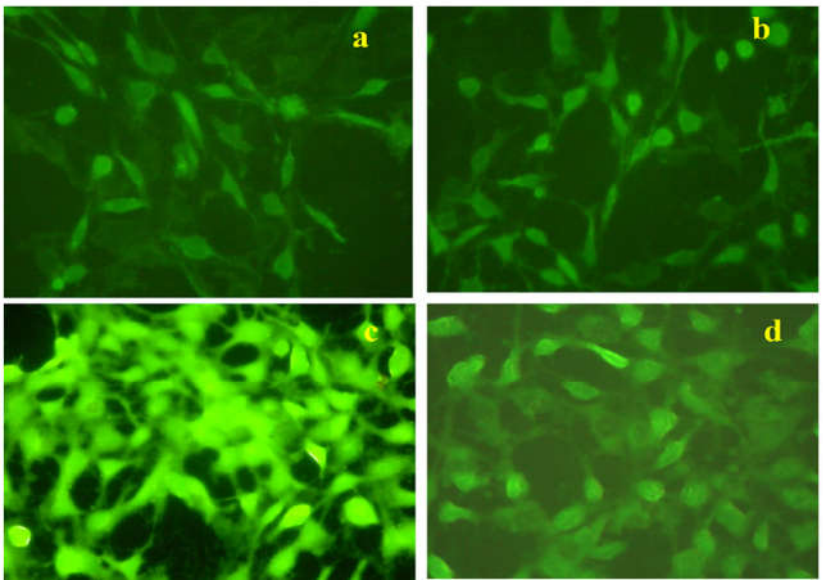
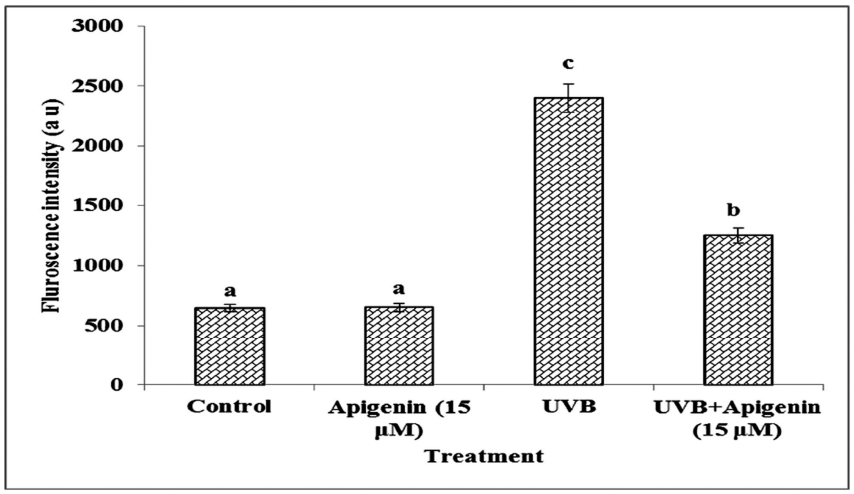


Fig. 1. Effect of apigenin on UVB-induced cytotoxicity in HDFa by MTT assay. Data were given as means ± SD of six experiments in each group. Values not sharing a common superscript differ significantly at P < 0.05 (DMRT)



A)



B)

Fig. 2. Effect of Apigenin acid on UVB induced intracellular ROS generation in HDFa. A) Photomicrographs showed (40x) enhanced green fluorescencein UVB-exposed HDFa. a.Control, b. Apigenin (15 µM), c. UVB Control, d. UVB+ Apigenin (15 µM). B) Bar diagram shows fluroscence intensity measured by spectrofluorometric readings of apigenin and/or UVB treated ROS generation in HDFa. Values are given as means ± SD of six experiments in each group. Values not sharing a common superscript differ significantly at P < 0.05 (DMRT)

Table 1. Effect of apigenin in UVB induced lipid peroxidative markers in HDFa cells. TBARS and LHP expressed in nmoles/mg protein. Values are given as mean ± SD of six experiments in each group. Values not sharing a common superscript differ significantly at *P* < 0.05 (DMRT)

Groups	TBARS (nmoles/mg protein)	LHP (nmoles/mg protein)
Control	2.48 ± 0.10[a]	9.61 ± 0.27[a]
Apigenin (15µM)	2.51 ± 0.12[a]	9.57 ± 0.52[a]
UVB-radiation	7.68 ± 0.39[c]	15.28 ± 1.19[c]
Apigenin (15µM) + UVB-radiation	3.18 ± 0.25[b]	10.20 ± 0.23[b]

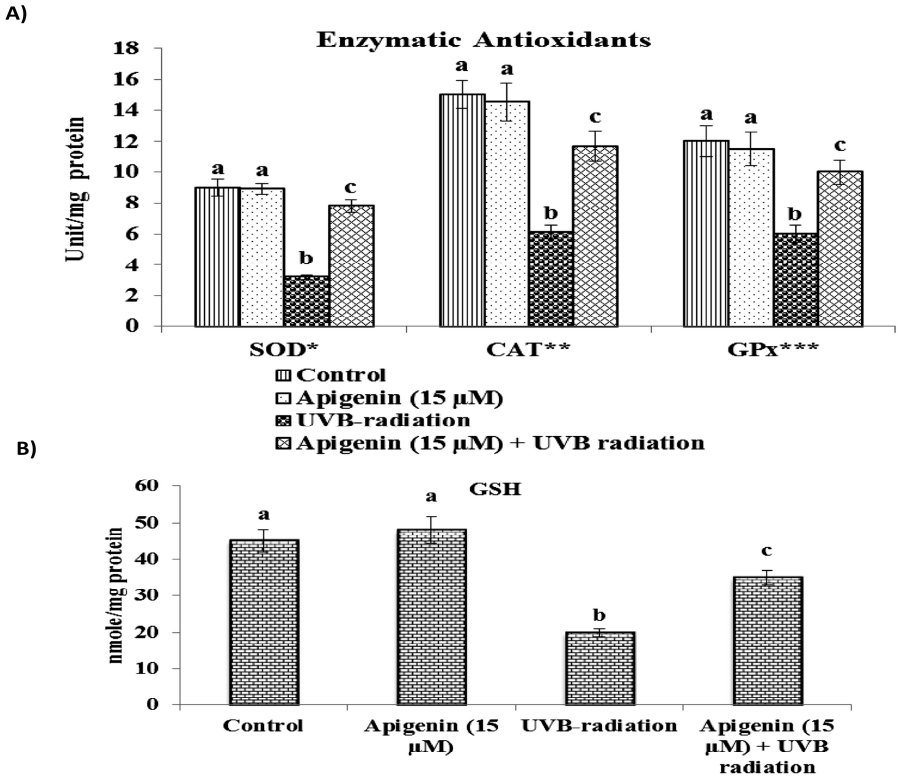


Fig. 3. A) Effect of apigenin on the activities of enzymatic antioxidants *SOD, **CAT and * GPX in normal, UVB-irradiated and apigenin pretreated HDFa cells. B) Effect of apigenin on radiation-induced GSH levels in HDFa cells. Values are given as mean ± SD of six experiments in each group. Values not sharing a common superscript differ significantly at P < 0.05 (DMRT). *Enzyme concentration required for 50% inhibition of nitroblue tetrazolium reduction in 1 min. *Micromoles of hydrogen peroxide consumed per minute. ***Micrograms of glutathione consumed per minute**

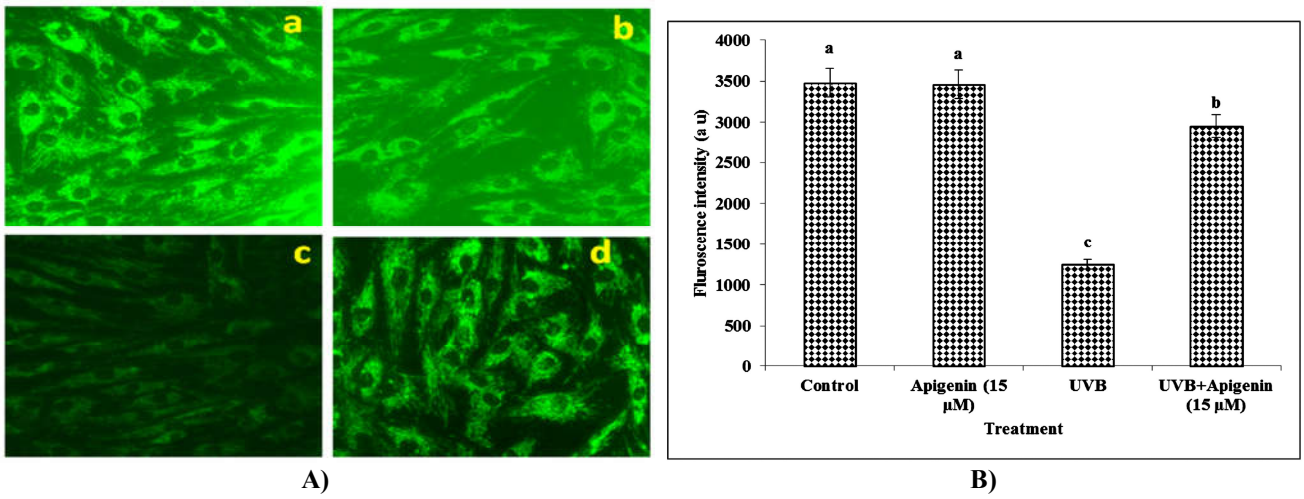


Fig. 4. Effect of Apigenin on UVB radiation induced mitochondrial membrane potential in HDFa cells. A) Photomicrographs showed (40x) Rh-123 staining on apigenin treated and/or UVB-exposed HDFa. *a.Control, b. Apigenin (15 µM), c. UVB Control, d. UVB+ Apigenin (15 µM).* B) Bar diagram represents fluroscence intensity measured by spectroflurimetry. Values are given as means ± SD of six experiments in each group. Values not sharing a common superscript differ significantly at P < 0.05 (DMRT)

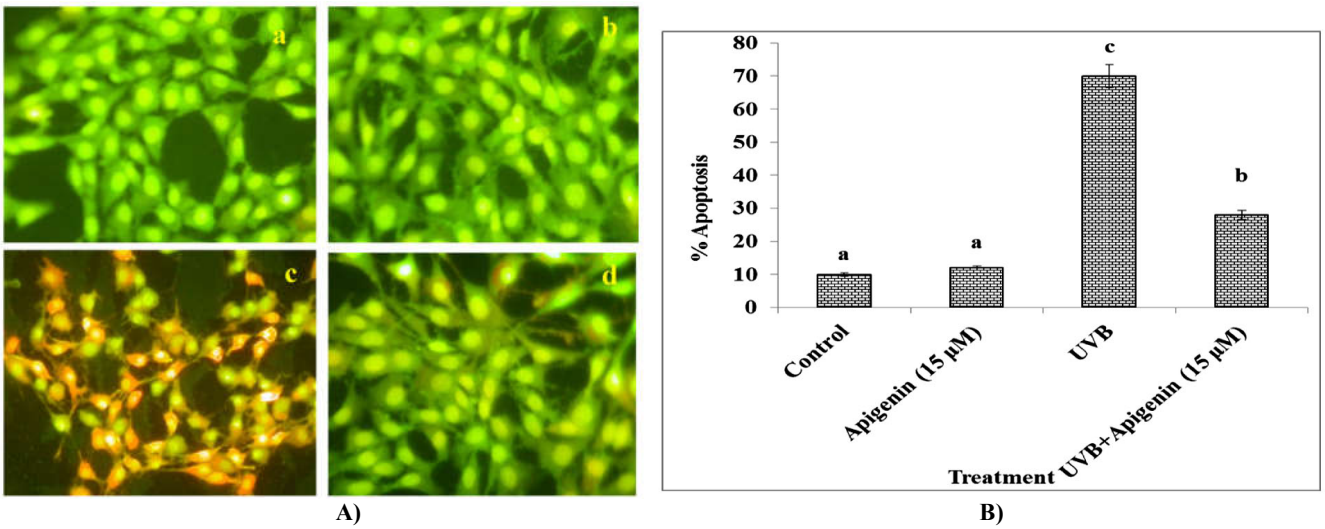


Fig. 5. Effect of apigenin on UVB radiation induced apoptotic morphological changes in HDFa cells. A) Cellular morphological changes were observed under a uorescence microscope using OA/EtBr staining (20x). *a. Control, b. Apigenin (15 µM), c. UVB Control, d. UVB+ Apigenin (15 µM).B)* Bar repreaents percentage of apoptosis on apigenin and/or UVB treated HDFa cells. Values are given as mean ± SD of six experiments in each group. Values not sharing a common superscript differ significantly at P < 0.05 (DMRT)

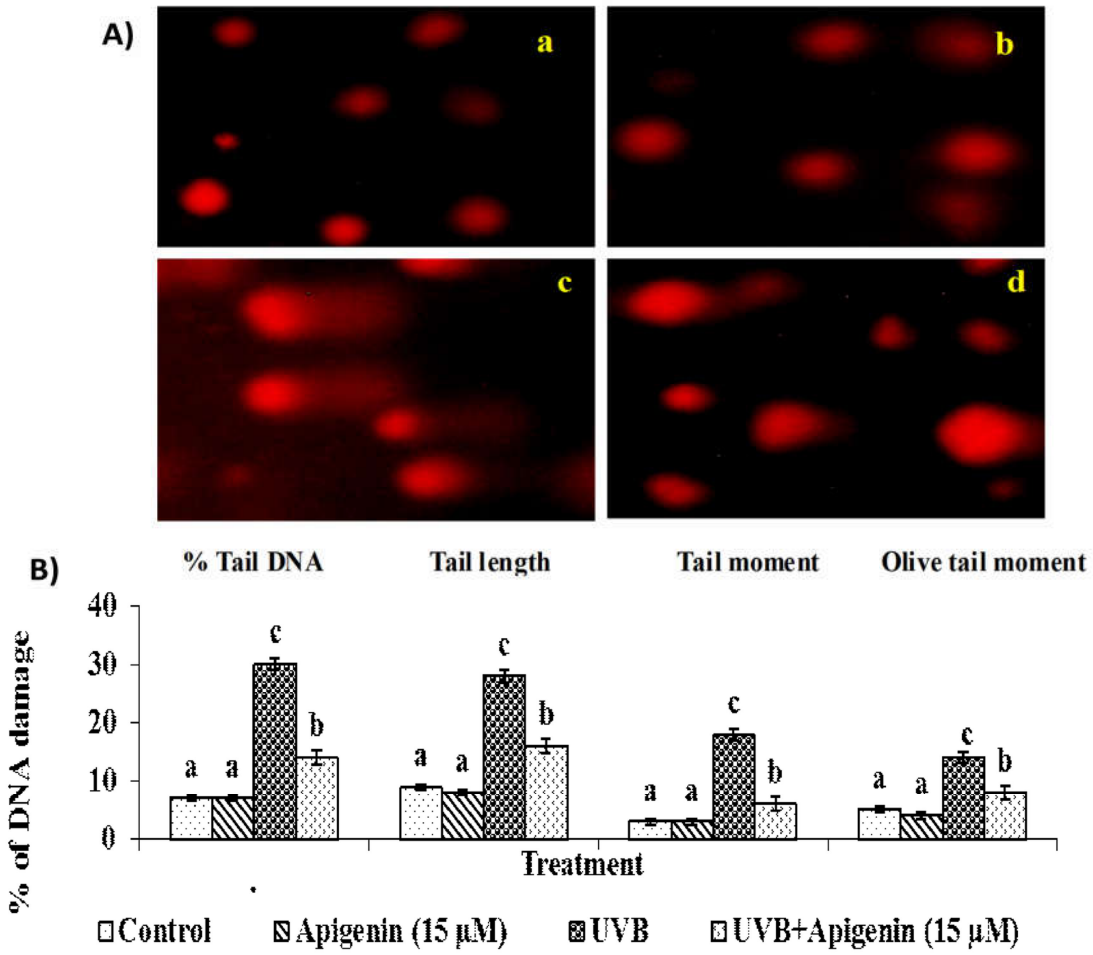


Fig. 6. Effect of Apigenin acid on UVB induced DNA damage in HDFa cells. A) Photomicrographs shows (20x) DNA damage on apigenin and/or UVB treated HDFa cells by comet assay. *a.Control, b. Apigenin (15 µM), c. UVB Control, d. UVB+ Apigenin (15 µM)*. B) Bar diagram represents DNA damage attributes (% tail DNA, tail length, tail moment and Olive tail moment) in normal, UVB-irradiated and apigenin pretreated HDFa cells. Values are given as means ± SD of six experiments in each group. Values not sharing a common superscript differ significantly at P < 0.05 (DMRT)

Apigenin pretreatment shows progressively decreased levels of TBARS and LHP when compared with UVB‑irradiated HDFa cells. Furthermore, Fig. 3A-B shows that UVB‑irradiation caused significant decrease in the activities of enzymatic antioxidants such as SOD, CAT, GPX) and levels of GSH in HDFa cells. Apigenin pretreatment prevented UVB induced loss of antioxidant status in HDFa.

Inhibitory effect of apigenin on UVB‑induced mitochondrial membrane potential: Fig.4 show the changes in the levels of mitochondrial membrane potential in the normal, UVB plus apigenin pretreated HDFa cells. UVB treatment significantly decreased the mitochondrial membrane potential levels (FI 259.5). Apigenin (15 µg/ml) significantly prevented loss of mitochondrial membrane potential.

Effect of apigenin on UVB‑induced apoptotic morphological changes: Fig. 5 shows the apoptotic morphological changes in normal, UVB plus apigenin pretreated HDFa cells. Apigenin treatment prevented UVB induced morphological changes and apoptotic features in irradiated HDFa.

Inhibitory effect of apigenin on UVB caused DNA damage (Comet assay): UVB‑irradiation significantly increased % tail DNA, tail length, tail moment and OTM in HDFa cells (Fig. 6). Apigenin (15 µM) pretreatment significantly prevented UVB induced DNA damage in HDFa.

DISCUSSION

Ultraviolet radiation elicits number of biological effects through reactive oxygen species and DNA damage. UVB-mediated cellular signaling induces numerous adverse reactions in the target tissues (Zhu and Bowden 2004). Cellular photosensitizers absorb UVB photons and generate ROS through one electron transfer reactions (Haralampus-Grynavisiki *et al*., 2002). In this study, apigenin pretreatment significantly prevented UVB‑induced cytotoxicity in HDFa (Fig. 1). UVB mediated ROS generation and DNA damage might be the reason for this UVB-mediated cell death. Apigenin probably through its antioxidant nature and its sunscreen nature prevents UVB mediated cytotoxicity in HDFa cells. Inhibitory activity of flavonoids against cytotoxicity has been well documented. Sasaki *et al*. (2005) recently proved the cytoprotective property of apigenin and other related flavonoids against amyloid-β peptide 42 induced cytotoxicity in PC-12 cells.

UVB-mediated ROS generation has been strongly advocated for UVB-induced photocarcinogenesis (Kang *et al*., 2003; Schafer *et al*., 2010). The current data clearly demonstrate that UVB radiation triggers the generation of intracellular ROS levels in HDFa cells. The generation of ROS during UVB exposure has been well documented in the literature (Debacq-Chainiaux *et al*., 2005). Elevated ROS levels can induce severe tissue damage and can even lead to neoplastic transformation. As radiation-induced, cellular lethality is attributed to the damages to DNA and membranes, protecting these cellular entities from radiation effects influence the fate of irradiated cells a great deal. Increased levels of LPO markers, i.e. TBARS and LHP were observed in UVB irradiated HDFa cells (Table. 1). Apigenin effectively brought back radiation-induced lipid peroxidation indices to near normal in HDFa. Protective effect of apigenin on the status of lipid peroxidation has been proved in carcinogen administered Wistar albino rats (Singh *et al*., 2004). In this study, it has been observed that there was an increased trend of lipid peroxidative markers in UVB irradiated HDFa is accompanied with the decreased activities of SOD, CAT and GPx. Pretreatment with apigenin increases the activities of SOD, CAT and GPx in UVB irradiated HDFa (Fig 3A). This indicates antioxidant sparing potential of apigenin. It has also been noticed that oxidative stress induced by UVB radiation resulted in an increased utilization of GSH and subsequent decreased level of GSH. Depletion of GSH in vitro and in vivo is known to increase LPO (Kojima *et al*., 2000). A similar correlation between the depletion of GSH and an increase of lipid peroxitative is observed in the present investigation. Apigenin treatment effectively restored the depleted levels of GSH in HDFa cells. Singh *et al.* (2004) have also shown that apigenin reduces the N-nitrosodiethylamine induced LPO and improved the GSH levels in the liver and kidney of experimental rats. For these reasons, it is plausible to hypothesize that the protective effect of apigenin against radiation-induced oxidative stress may be due to its antioxidative nature (Snyder and Gilies 2002).

Mitochondrial changes are critical for the inductive effect phase of apoptosis. The loss of $\Delta\psi m$ signifies metabolic cell death (Denning *et al*., 2002). In this study, rhodamine 123 was used to assess the effect of apigenin treatments on $\Delta\psi m$ in UVB‑irradiated HDFa cells. The changes in mitochondrial membrane potential have been strongly linked with apoptotic signaling envents (Denning *et al*., 2002; Fumelli *et al*., 2000). In this study, it has been observed that there was a significant loss of mitochondrial membrane potential during UVB exposure. Conversely, apigenin treatment prevented UVB-induced loss of mitochondrial membrane potential in HDFa cells (Fig. 4). These results suggest that protection of mitochondria by apigenin could be implicated due to its antiapoptotic property. Previous studies suggest that the 30 mJ/cm^2 UVB led to significant apoptosis of HaCaT cells, accompanied by a marked reduction of $\Delta\psi m$. Kanagalakshmi *et al*. (2014) reported that ferulic acid treatment before UVB‑exposure significantly prevented UVB‑induced loss of $\Delta\psi m$. UVB radiation is the primary environmental agent that leads to apoptosis in human cells. UVB‑irradiation of cells elicits a complex cellular response via cell surface receptor aggregation (Ji *et al*., 2012; Brash *et al*., 1991) and upon prolonged exposure; it induces apoptosis in mammalian cells including keratinocytes and lymphocytes (Kanimozhi *et al*., 2011). In agreement with this, the present results showed the protective effect of apigenin on UVB radiation‑induced apoptotic morphological changes.

Previous reports suggested that UVB generates ROS and it has been associated with oxidative DNA damage (Saitoh *et al*., 2011). This study showed that there was an increased frequency of DNA damage i.e. tail DNA, tail length, tail moment and OTM in UVB‑irradiated HDFa cells (Fig. 6A-B) increased comet attributes observed in this study might be due

to the DNA strand breaks induced during UVB-exposure. UVB-radiation has been known to induce pyrimidine dimer and (6-4) photoproducts (Sugimoto *et al*., 2009). Apigenin pretreatment decreased percentage of tail DNA, tail length, tail moment and OTM in HDFa cells. This clearly indicates DNA damage repairing capacity of apigenin in UVB-irradiated HDFa cells. It has been previously proved that caffeic acid pretreatment significantly prevented UVB-induced DNA damage in human blood lymphocytes (Prasad *et al*., 2009).

Conclusion

In this study, it has been observed that there was an increased trend of oxidative stress mediated damage in UVB-irradiated cells. Apigenin was effective in protecting against UVB radiation-induced cytotoxicity, lipid peroxidation, antioxidant depletion and DNA damage in HDFa. Consequently, apigenin, by virtue of its free radical scavenging capacity and replenishment of antioxidants stores, reduced the oxidative damage caused by UVB radiation. An, interventions with botanical antioxidants such as apigenin could be promising in the design and development of new treatment strategies aimed at limiting sun light-induced skin oxidative damage.

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