Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 8573 - 8585
Received 25 April 2021; Accepted 08 May 2021.

Protective Effect of Morin Hydrate in Humanepidermal Keratinocyte Cell Line (Hacat) Upon Exposure to Uvbirradiation Induced Oxidative Damage and Biochemicalalteration

Anjugam. C[1*] Ramani. G[2]

[1*]Department of Biochemistry, Vinayaka Mission's KirupanandaVariyar Medical College and Hospitals, Vinayaka Mission's Research Foundation (Deemed to be University) Salem, Tamilnadu, India.

[2] Department of Biochemistry, Vinayaka Mission's KirupanandaVariyar Medical College and Hospitals, Vinayaka Mission's Research Foundation (Deemed to be University) Salem, Tamilnadu, India.

***Corresponding Author**
Dr. C. ANJUGAM, M.Sc., M.Phil., Ph.D.
Assistant professor,
Department of Biochemistry,
Vinayaka Mission's KirupanandaVariyar Medical College and Hospitals,
Vinayaka Missions Research Foundation (Deemed to be University).
Chinnaseeragapadi, Salem– 636308,
Tamilnadu, India.
Mobile:+91-9789414899
Email ID: anjudeepini@gmail.com
OrcidID :0000-0002-3089-0791

ABSTRACT

Ultraviolet-B (UVB) 280–320 nm radiation penetrates epidermis and is completely absorbed in the upper dermis of the skin. It causes a several harmful effects which include basal and squamous cell carcinoma, cataracts, sunburn, melanoma, immunosuppression and photoaging of the skin. The incidence of skin cancers has been increasing worldwide in the last few decades. Thus, identification of more and appropriate potential drug targets is essential for the prevention of skin cancers. Hence, the present study was carried out to investigate the protective effects of morin in human epidermal keratinocyte cell line (HaCaT) upon exposure to UVB irradiation induced oxidative damage. HaCaT cells were pretreated with morin (50 μM) for 30 mins before UVB irradiation. Several cellular and oxidative end points parameters were analyzed. UVB irradiation pretreated with morin (50 μM) showed significant increase in the levels of antioxidants and lipid peroxidation, and DNA damage and apoptotic morphological changes were manifest drastically in HaCaT cells. The present study was demonstrating protective effect of morin against UVB radiation.

KEYWORDS: Morin; DNA damage; apoptosis; HaCaT cells; UVB.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 8573 - 8585
Received 25 April 2021; Accepted 08 May 2021.

INTRODUCTION

Genetic factors and environmentaldonate to the progress of skin cancers; Exposure to Ultra Violet Radiation (UVR) is acentral etiological agent for melanoma, non-melanomaskin cancers and approximately 1.3 million novel cases of skin cancers every year in the United States (Narayanan et al., 2010). Skin cancers are presently a main concern on health care and public health's expenditures. Greater than 90% of skin cancersare caused by exposure to UVR from the sun. Particularly, UVB radiation induces non - melanoma skin cancer by damage the DNA - mostly absorbs light of 260 nm (directly and indirectly) by increase the levels of reactive oxygen species (Cadet and Douki, 2018). UVB radiation is generally regulated by several pathways particularly signaling, which can initiate cell cycle arrest, DNA repairs, apoptosis, cell death (Veratti et al., 2011). UVB exposure causes either directly and indirectly adverse biological effects as well as, the foundation of pyrimidine photoproducts, trans- to cis- urocanic acid isomerization, initiation of ornithine decarboxylase activities, inspiration of DNA synthesis, generation of free radicals, cell cycle growth arrest, and photoaging (Afaq et al., 2002). It significantly decreases skin antioxidants levels, thereby free radicals generated impair the skins capacity to keep itself in opposition to the exposure to sunlight and lowers the skin's immune defense system (Trautinger, 2001). All these events lead to UVB radiation induced skin carcinogenesis.

Morin is a phenolic compound and has antioxidant properties, found in several fruits and vegetables such as osage orange, old fustic, fig, guava leaves, apple, and onion and in several beverages such as red wine, and tea. They are used as herbal medicines and several biological activities. The photoprotective effect of morin has already been reported in human keratinocyte stem cells (Lee et al., 2014). Therefore, antioxidants from a natural sourceseem to be an extreme capacity, their uses may be an effective strategy for decrease of prevalence of skin cancer and UVB induced oxidative damages (Chandrakesan et al., 2018). The human skin is the largest organ and straight exposed to UVB radiation.Hence, we have studied the preventive effect of morin on UVB radiation induced cytotoxicity, Reactive oxygen species generation, lipid peroxidation, antioxidants, DNA damage and apoptotic morphologic changesfrom human epidermal keratinocytecells (HaCaT cells).

MATERIAL ANDMETHODS

Chemicals and Reagents

Morin was obtained from Sigma Chemicals Co., HaCaT cells were procuredfrom Invitrogen Bioservices, India. All other chemicals and reagents were obtained fromMerck specialty Pvt. Ltd and Aromatic Limited,Chennai.

HaCaT cellsculture

Cultured HaCaT cells were incubated at 37°C in 5% air-95% CO2-5%, saturated culture cells incubator with added medium 106, low serum augmentation, with 2% FBS (Fetal bovine serum), 1μg/ml hydrocortisone, 3 ng/ml basic fibroblast growth factors, 10 ng/ml human epidermal growth factor, 109 μg/ml heparin and antibiotics. The cells were kept to grow for 7 days to reach the extreme confluence and were collected using 4ml of trypsin-EDTA solution; cells were then subculture and used for experiments.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 8573 - 8585
Received 25 April 2021; Accepted 08 May 2021.

Preparations of morin and method of administration forHaCaT

Different concentrations of morin were dissolved in 0.5% dimethyl sulphoxide (DMSO). Working concentration of 25μM and 50μM morin was prepared from the stock solution and used for the cytotoxic assay.

Experimentaldesign

Cultured HaCaT cells were separated into five groups asfollows:

Group 1: HaCaT cells - without treatment

Group 2: HaCaT cells + Morin (50μM)

Group 3: HaCaT cells + UVBirradiated

Group 4: HaCaT cells + Morin (25μM) +UVB

Group 5: HaCaT cells + Morin (50μM) +UVB

Treatment of cellline

Two doses for test 25 μM, 50 μM of morin were additional to the grouped HaCaT cells 30 mins before UVB-exposure. The test,Trypan blue dye exclusion was carried out to check the suitability and toxicity of these concentration of morin for photoprotection studies. Before UVB - exposure, the HaCaT cells were washed once with PBS solution. Mock - irradiatedHaCaT showed not changes in viability over the 30 minsretro of incubation.

Radiation procedure for cellline

HaCaT cells were washed one time with PBS and UVB - irradiated in a tinny coating of medium. The culture medium waslaterdetachedand coveredwithaUVBpermeablemembranetostopcontamination.AbatteryofTL20W/20 fluorescent tubes was used as UVB- source (wavelength rangeo f 290-320 nm), Set at 312nm and intensity of 2.2mW/cm^2 for 9 mins. The totalUV- Bradiationwas20mJ/cm^{2}andanaveragevalueof1.52×10 - 3mJ/cells.Afterradiation,HaCaT cells were an allowed at room temperature for 4 hrs at 37°C in 5%CO^{2}then the HaCaTcells were cleaned and shift to sterilized tubes for investigations.

Cytotoxicityin HaCaT cells - MTT assay

The cytotoxic effect of UVB-irradiated HaCaT was determined by MTT assay based on the detection of mitochondrial dehydrogenase activity in living cells. Cultured HaCaT (1x106 cells/mL) were taken into a 96 well plate. Then the cells were pretreated with different concentration of morin (25, 50 μM). After 1 hr incubation with morin the cells were exposed to UVB - irradiation. Then the cells were incubated at 5 % CO_2 and 95 % O_2 environment at 37ºC for 24 hrs. MTT was added to the incubated cells and then further incubated for another 4 hrs. The cells were centrifuged for 10 mins and the supernatant were removed, 200 μL of DMSO were added into each tube. Absorbance was measured in a microplate reader at 540 nm. Images captured under a microscope and%of cytotoxicity was calculated.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 8573 - 8585
Received 25 April 2021; Accepted 08 May 2021.

$$\% \text{ Cytotoxicity} = \frac{\text{Test optical density}}{\text{Control optical density}} \times 100$$

ROSgeneration- Spectrofluorometer

The ROS level was assessed in the control,UVB - irradiatedplusmorin treated HaCaT cells. Briefly, an aliquot of the isolated cells was made up to a final volume of 2 ml in PBS. Then, 1ml of cell suspensions was taken, to which 10 µM DCFH-DA was added and incubated at 37°C for 30 mins. Then, morin pretreated and / or UVB irradiated HaCaT were incubated for 30 mins in 6 well plates with 10 µM of DCFH-DA in PBS. Finally, cells were washed three times with PBS and the fluorescence intensity was recorded using spectrofluorometer and the images were captured by fluorescence microscope (460 nm).

Lipid peroxidation and antioxidantstatus-Spectrophotometry

The concentration of TBARS and antioxidant status in the cell suspension were taken for estimations. The level of lipid peroxidation was determined by thiobarbituric acid reactive substances (TBARS) according to the procedure of Niehaus1968 (Niehaus and Samuelsson, 1968). The reactions of enzymatic antioxidants such as SOD, CAT,GPx were analyzed by the procedure of Kakkar1984, Sinha 1972 and Rotruck 1973 (Kakkar *et al.,* 1984, Sinha, 1972, Rotruck, 1973) respectively and non-enzymatic antioxidant like Reduced Glutathione (GSH) by the procedure of Ellman 1959 (Ellman, 1959).

DNAdamage-Comet Assay

Comet assay or Single cell gel electrophoresis (SCGE) used to evaluation DNA damage at the single cell level. Freshly suspended HaCaT cells (50 µL) were mixed with 200 µL in 0.8% Low - Melting Point Agarose (LMPA) was cast on to frosted microscopic slides and placed for 10 mins in icebox to solidify. Then, the cover slip was removed and a top layer of 100 µL of LMPA was added and the slides were cooled for 10 mins. The cells were then lysed by immersing the slides in the lysis buffer for 1hr at 4°C. After lysis, slides were placed in a horizontal electrophoresis tank. Filled with alkaline electrophoresis buffer above the slides. The cells were exposed to the alkaline electrophoresis buffer for 30 mins to allow DNA unwinding. Electrophoresis was conducted in a cold condition for 20 mins. After electrophoresis, the slides were placed horizontally and neutralized with Tris - HCl buffer. Finally, 50 µL of ethidium bromide was added to each slide and analysed using a fluorescence microscope. DNA damages were expressed as %.

Apoptotic morphological changes -AO/EtBrdual staining

Ethidium bromide is a membrane impermeable molecule that binds between the stacked base-pairs of relaxed DNAs. HaCaT cells were seeded in 6-well plate and incubated in CO_2 incubator for 24 hrs. The cells were fixed with methanol : glacial acetic acid (3:1) for 30 mins at room temperature. Then, the cells were washed in PBS and stained with (1:1) ratio of acridine orange / ethidium bromide (AO/EtBr). Stained cells were washed again with PBS and viewed

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 8573 - 8585
Received 25 April 2021; Accepted 08 May 2021.

under a fluorescence microscope. The number of cells showing features of apoptosis was counted as a function of the total number of cells present in the field.

STATISTICALANALYSIS

Allthevalueswereexpressedasmeansofsix(n=6)determinations.Thedatawerestatistically analyzedusingone-wayanalysisofvariance(ANOVA)onSPSS(statisticalpackageforsocial sciences)andthegroupmeanswerecomparedbyDuncan'sMultipleRangeTest(DMRT).The results were considered statistically significant if the $P < 0.05$ levels.

RESULTS

Effect of Morin on UVB induced cytotoxicity in HaCaT cells.

UVB -induced cytotoxicity shows a significant reduction in cell viabilities (38%) while compared with control and morin 50 µM treated cells (non-irradiated HaCaTcells) (Fig. 1). Morin pretreatment shows good improvement in UVB- induced cell deaths and restored cell viabilities in a concentration reliant manner. Concentrations of morin 25µM and 50µM was tested, 50 µM of morin restored (about 98%) cell viability when compared with 25µM in HaCaT cells.

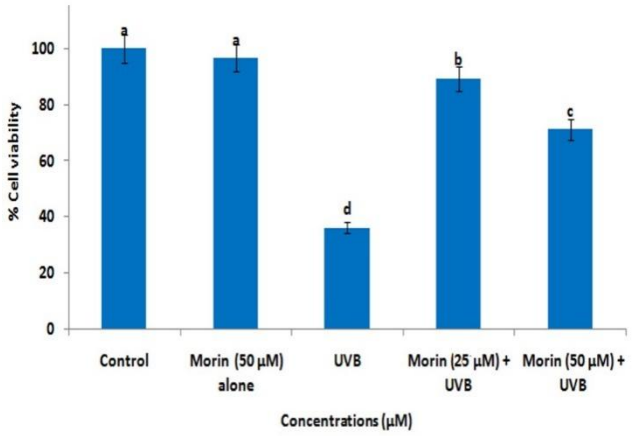

Fig: 1 Effect of morin on UV-B induced cytotoxicity in HaCaT by MTT assay. Values are given as means ± S.D. of six experiments in each group. Values not sharing a common marking (a, b, c & d) differ significantly at P<0.05 (DMRT).

Effect of Morin on UVB induced ROS generation in HaCaT cells

ROS produces a significantly higher in UVB irradiated HaCaT (C-776.66) which is compared to the non-irradiated HaCaT. Significantlygood reductionof ROS level was detected in morin (50 µM) plus UVB- irradiated HaCaT cellswhile compared withUVB- irradiated cells (Fig. 2ii). From the photomicrograph clearly evidence that of bright green fluorescence in UVB irradiated cells was shown (Fig. 2i C). Morin plus UVB - irradiated HaCaT cells exhibiteddwindled green fluorescence because ofreduced ROS generation (Fig. 2i D). No changes in non-irradiated HaCaT cell (Fig. 2i A, B).

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 8573 - 8585
Received 25 April 2021; Accepted 08 May 2021.

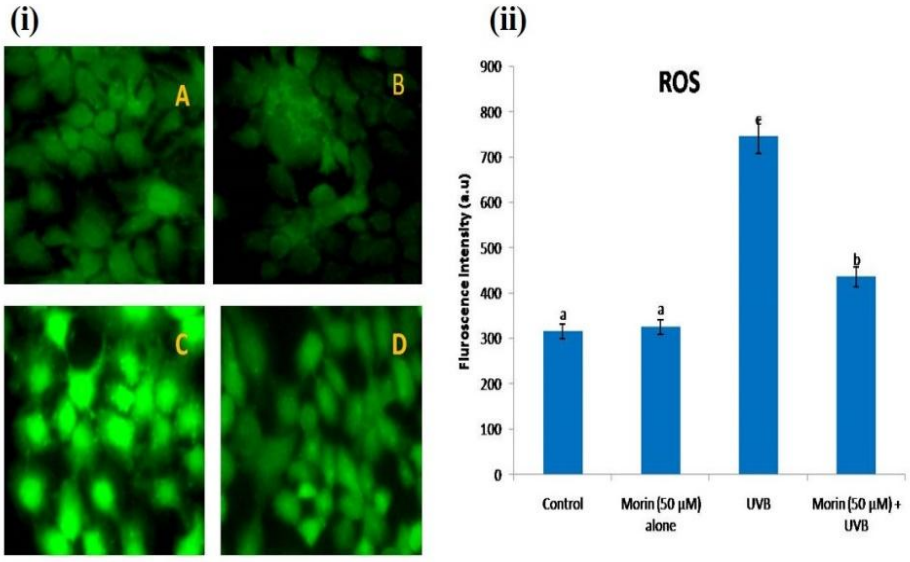

Fig. 2 Effect of morin on UV-B radiation induced ROS generation in HaCaT cells. **(i)** Photomicrographs showed (20x) enhanced green fluorescence in UV-B-exposed HaCaT cells; Morin pretreatment decreased UV-B-induced ROS generation in a dose dependent manner. (A) Control, (B) morin (50 µM), (C) UV-B, (D) UV-B + morin (50 µM). **(ii)** Spectrofluorometric

Morin inhibits on UVB- induced oxidative stress in HaCaT cells (Lipid peroxidation)

Levels of TBARS were significantly improved in UVB irradiated HaCaT when compared to non-irradiated HaCaT (Fig. 3). TBARS levels in morin (50µM) plusUVB- irradiated are significantly good reduction while compared toUVB irradiated HaCaT cells.

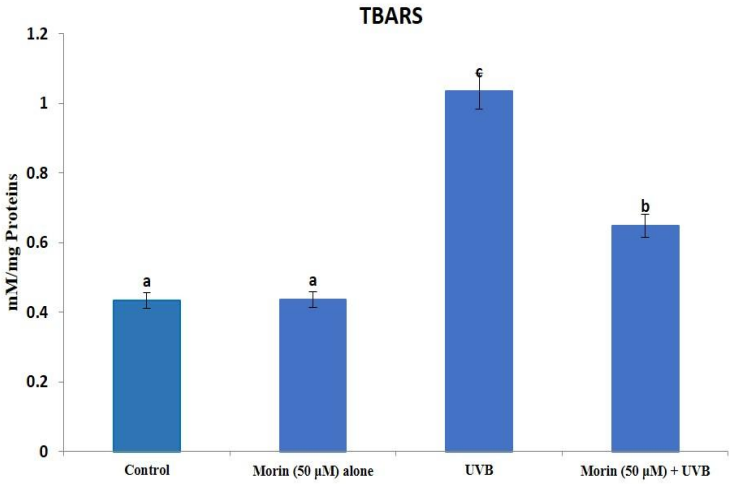

Fig. 3 Effect of morin on UV-B induced lipid peroxidation in HaCaT cells. Values are given as means ± S.D. of six experiments in each group. Values not sharing a common marking (a, b, c & d) differ significantly at P<0.05 (DMRT).

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 8573 - 8585
Received 25 April 2021; Accepted 08 May 2021.

Effect of morin on UVB-induced antioxidant status in HaCaTcells

Fig.4a shows the enzymatic antioxidant activities of SOD, GPx,CAT,non-enzymatic antioxidantactivitiesofGlutathione (GSH)(Fig.4b)weresignificantlydecreasedinUVB-exposedHaCaT when compared to non-irradiated HaCaT cells. Morin plus UVB - induced HaCaT cells shows significantly good improvement and bring back to the normalcy of SOD, CAT,GPx,GSHwhencomparedwith UVB- irradiatedHaCaT cells.

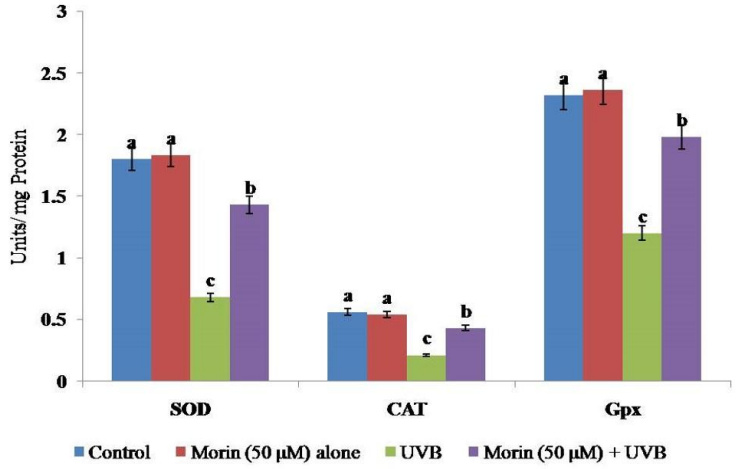

Fig. 4a Effect of morin and/or UV-B exposure on antioxidant enzymes activities (SOD, CAT and GPx) in HaCaT cells. Values are given as means ± S.D. of six experiments in each group. Values not sharing a common marking (a, b, c & d) differ significantly at P<0.05 (DMRT). SOD - Enzyme concentration required for 50% inhibition of nitroblue tetrazolium reduction in one minute. CAT - µmol of hydrogen peroxide consumed per minute. GPx - µg of glutathione consumed per minute.

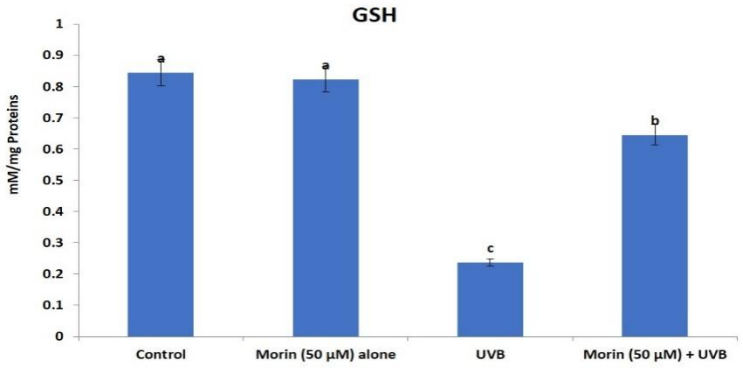

Fig. 4b Effect of morin on GSH levels in UV-B irradiated HaCaT cells. Bars represent means ± S.D. of six experiments in each group. Values not sharing a common marking (a, b, c & d) differ significantly at P<0.05 (DMRT).

Effect of Morin on UVB - induced DNA damage in HaCaT cells

UVB- irradiation significantly increased comet attributes that is tail – DNA and tail - length in HaCaT when compare tonon-UVB irradiated HaCaT (Fig. 5ii). Morin plus UVB - irradiated shows significantly decreases the level DNA damage when compare with UVB - irradiated HaCaT. Fluorescence microphotograph shows distinguishable comet tail in UVB-irradiated HaCaT (Fig. 5i C). Morin plusUVB-exposed HaCaT showed dwindled comet formation (Fig. 5i D) and non-irradiated control HaCaTshowswhole round shaped nucleoid.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 8573 - 8585
Received 25 April 2021; Accepted 08 May 2021.

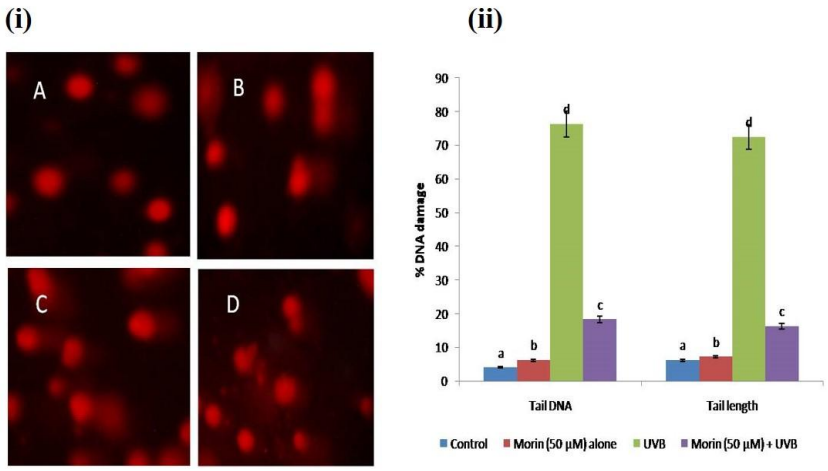

Fig. 5 Effect of morin on UV-B induced DNA damage attributes in HaCaT cells. **(i)** Fluorescence microphotograph shows enhanced comet tail in UV-B radiated HaCaT cells (C), morin pretreatment decreased UV-B induced comet formation (Figure 5D). **(ii)** Values are given as means ± S.D. of six experiments in each group. Values not sharing a common marking (a, b, c & d) differ significantly at P<0.05 (DMRT).

Effect of morin on UVB- induced apoptotic morphological changes in HaCaTcells

Inthisstudy,weusedAOandEtBrtodiscerncells that are apoptotic and/or viable (Fig. 6).UVB- irradiated HaCaTshowed condensed nuclei, membraneblubbingandapoptoticbodies.Incontrast,thecontrolcellsshowedintactnuclear architecture ,withonly7%apoptoticcells.MorinplusUVB -irradiatedcellsshowedreducedpercentageofapoptoticcellswhilecomparedwithUVB- exposure HaCaTcells.

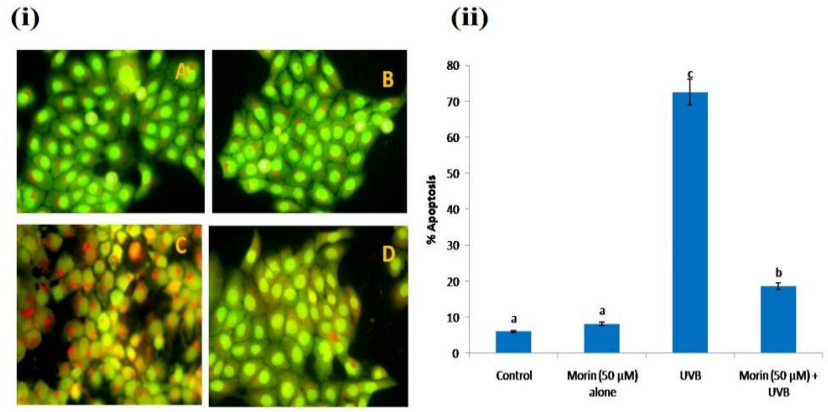

Fig. 6 Effect of morin on UV-B induced apoptotic morphological changes in HaCaT cells.**(i)** Cellular morphological changes were observed under a fluorescence microscope using OA/EtBr staining (20x). More number of apoptotic cells (red colour) was observed in UV-B exposed HaCaT (C), morin pretreatment decreased frequency of UV-B induced apoptotic cells (D). **(ii)** UV-B exposure increased percentage apoptotic cell death and DNA fragmentation in HaCaT. Morin treatment (50 µM) before UV-B exposure reduced percentage of apoptotic cells (AO stained cells). Values are given as means ± S.D. of six experiments in each group. Values not sharing a common marking (a, b, c & d) differ significantly at P<0.05 (DMRT).

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 8573 - 8585
Received 25 April 2021; Accepted 08 May 2021.

DISCUSSION

Skin is the biggest organ of the body and it divided into 2 primary layers, epidermis and dermis. Epidermis, of ectodermal origin, is the outmoststratum of skin and helps as the body's point of contact to the environments. UVB- radiation released from sun permeates the atmosphere and enter into the epidermis stratumof skin which then led to several pathological effects.

UVB- inducestheformation ofROSintheskinandprogressesoxidativestress,which potentially contributes to skin carcinogenesis (Ichihashi *et al.,* 2003). Hence, progress of"photoprotectors" particularlyfromnaturaloriginishighlydesirable target. Previous study suggested that phenolic compound (ferulic acid) like, an ideal antioxidant and ROSinfreeradicalscavengingsystems (Kanski *et al.,* 2002).UVB- radiationisknowntointeract tocellularporphyrins,cytochrome and flavonesresultingintheformationofROS.Inthecurrent study,wehaveexperiential that the exposure of UVB - induced in HaCaT cells reduced the percentage of cell viabilitywhile significantincreaseinthecell'sviabilitywasobservedinmorinwithUVB - irradiated HaCaT cells. Similarly, ROS generation was significantly decreased in HaCaT cells in the presence of morin. This indicates that morin exerts a protective effect on HaCaT cells upon exposure of UVB. Recently, some studies have authenticated the protective action of morin with respect to cell viability. Administrationofmorinincreasesthecellviabilityinprimaryrathepatocyteswhenexposedto highconcentrationofglucose (Surampalli *et al.,* 2015).Previous studyshows,the evidencethatprimary mechanismbywhichUVB- radiation instigates molecularresponsesinhumanskinisby formationofROS (Widel *et al.,* 2014).Inthis present study,morintreatmentsignificantlypreventedUVBinduced ROSgenerationandreducedsolarradiationinducedROSgenerationwithanetreductionof proteinoxidationinhumanepithermalkerotinocyte.Inaccordancewithourfindings,Domenicoreporte d that ferulic acid ethyl ester anti - oxidative stressmediated byUVB-irradiationinhumanepidermalmelanocytesandmorintreatedlungcancercellline (A549) (Tsai *et al.,* 2015)and alsoshoweddecreasedcellviability,colonyformationandmigration (Yao *et al.,* 2017).These evidences support that morin exerts an anti-tumor effect by *invitro*.

Lipidcomponentsinthemembranesareextremelypronetoradiationdamage (Bhattacharya *et al.,* 2009).We haveobservedthat,TBARSlevelsweresignificantlydecreasedinmorinplusUVB-irradiated cells while compared with the UVB- exposed group. TBARS is a reliable by product formedduring lipid peroxidation and it increases during oxidative stress. A decreased level of TBARS inmorin administered group suggests that morin employs an antioxidant action upon UVBexposure.The loss of antioxidants function which outcomes in the accumulation of ROS.Antioxidant enzymes likes SOD, GPx and CAT act in shows to protect cellular componentsfrom damagebyROS,whichembodiestheprimarylineofdefense (IghodaroandAkinloye, 2018).Earlierstudyhas proven that a polypeptide isolated from Chlamys farreririses the activitiesof antioxidative enzymes in UVB- irradiated HaCaT cells(Działo *et al.,* 2016). Phenolics are powerfulhydrogen-donatingantioxidantsandfreeradicalscavengersinmany*invitro*systemsand*invivo*models (Lala *et al.,* 2006).Inthe presentstudy,morinpre - treatmentsignificantlyimprovedtheactivitiesofSOD,GPx,CATin UVB -irradiatedHaCaTcells and thus, morin could wield a beneficial action against pathologic alterations caused by the

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 8573 - 8585
Received 25 April 2021; Accepted 08 May 2021.

UVB- radiation.GSHis a various protector and performs radioprotective function via-freeradical scavenging,restorationofthedamagedmoleculebyhydrogendonation,decreaseofperoxides and protection of protein thiols in the reduced state (Merwald *et al.*, 2005). Reduced levels of GSH duringUVB- exposuremaybeduetotheleakageandoxidationofGSH (Ali *et al.*, 2011).GSHdepletionofcultured skin cells make them sensitive to UVB- induced mutations and cell deaths (Punnonen *et al.*, 1991). UponUVB- irradiation,decreasedlevelsofGSHwereseeninHaCaTcellswhilemorinpretreatmenthave significantly increased GSHlevels.

Numerous evidences suggested that solar radiation cangive increase to cellular DNA - damage byindirect and directmechanismsfortheinductionofpyrimidinedimersandoxidativeDNA modifications (Cooke *et al.*, 2000). Previous evidence suggested that UVB generates ROS and it hasbeen associated with oxidative DNA damage. Oxidative DNA base damage via UV-rayscan probably donate to the induction of both melanoma and non-melanoma skin cancer (KvamandTyrrell, 1997).Singlecell gel electrophoresis (SCGE) or Comet assay has become oneof the common methodsforassessingDNAdamage,withapplicationingenotoxicitytestingaswellas fundamentalresearchinDNAdamageandrepair.Wehavenoticedimprovedfrequencyof DNAdamagei.e.,tail- DNAandtail- lengthinUVB- irradiatedHaCaT.Improvedcomet attributesobservedinthisstudymightbeduetotheDNAstrandbreaksinducedduringUVB- exposure. On the other side, morin pretreatment reduced percentage of tail - DNA and tail- length in HaCaT. Current study might be indicating that the DNA damage repairing capacity of morin inUVB- irradiatedHaCaT.Ithasbeen earlier proved that the protectiveeffectofmorin,on UVB- inducedDNAdamagewasanalyzedinkeratinocytestemcells (Lee *et al.*, 2014).Another study showed that caffeic acid is a phenolic compound on UVB- induced oxidative DNAdamage inculturedhumanlymphocytes(Prasad *et al.*, 2009).Basedontheoutcomes,phenoliccompoundcouldinhibitthe CPD formation and DNAdamage.

Mitochondrialchangesaredangerousfortheinductiveeffectphaseofapoptosis.UVB- radiation is the primary environmental agent that leads to apoptosis in human cells.UVB- irradiationofcellselicitsacomplexcellularresponsethroughcellsurfacereceptoraggregation (Brash *et al.*, 1991) anduponprolongedexposure;itinducesapoptosisinmammaliancellslike,keratinocytes andlymphocytes (KanimozhiandPrasad, 2009).Inthisstudy,wehavedetectedthepreventive effectofmorinonUVB- radiationinducedapoptoticmorphologicalchanges.Pre - treated with MorinplusUVB- irradiatedcells exhibitedreduced percentage of apoptotic cells while compared withUVB-exposure alone (Fig.6).

Dietarypolyphenolsbecauseoftheirantioxidantactivityandthecapacitytoscavengethefreeradi calsformedduringthepathologicalprocessalikecancerandanotherstudyshowed,to neutralizeROS,recovermitochondrialmembranepotential,andblockapoptoticpathways againstoxidativestressonUVB- inducedcancercelllines (Kanagalakshmi *et al.*, 2014).MorintreatmentreducedROSformation,bring backmitochondrialmembranepotentialandreducedcytochromeCrelease andintrinsicpathwayapoptosisactivationinducedbyoxidativestressmediatedapoptosisin primary rat hepatocytes (Kapoor andKakkar, 2012).

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 8573 - 8585
Received 25 April 2021; Accepted 08 May 2021.

CONCLUSION

The present study demonstrates that morin is playing a critical role against UVB- induced Oxidative stress, Lipid peroxidation, DNA damage and Apoptotic morphologicalchanges. Based on our study, we recommend that morin can be used as an actively protective agent forthe biochemical alterations caused by UVB- exposure.

CONFLICT OF INTEREST

There are no conflicts ofinterest

REFERENCES

1. Narayanan, D.L., Saladi, R.N., and Fox, J.L. 2010. Ultraviolet radiation and skin cancer.*International journal of dermatology*,49 (9):978-986.

2. Cadet,J.,Douki,T.2018. FormationofUVinducedDNAdamagecontributingtoskincancer development. *Photochemical and Photobiological Sciences*,17(12):1816-1841.

3. Veratti,E.,Rossi,T.,Giudice,S.,Benassi,L.,Bertazzoni, G., Morini, D., Azzoni, P., Bruni, E., Giannetti, A.,Magnoni, C. 2011. 18β-glycyrrhetinicacidandglabridinpreventoxidativeDNAfragmentationinUVB irradiated human keratinocyte cultures. *Anticancer research*, 31(6):2209-2215.

4. Afaq, F., Adhami, V.M., Ahmad, N.,Mukhtar, H. 2002. Botanical antioxidants forchemoprevention of photocarcinogenesis. *Front Biosci*,7:784-792.

5. Trautinger,F.2001. Mechanismsofphotodamageoftheskinanditsfunctionalconsequences for skin ageing. *Clinical and experimental dermatology*, 26(7):573-577.

6. Lee, J., Shin, Y.K., Song, J.Y.,Lee, K.W. 2014. Protective mechanism of morin against ultravioletB-inducedcellularsenescenceinhumankeratinocytestemcells.*Internationaljournalof radiation biology*, 90(1):20-28.

7. Chandrakesan,A.,Muruhan,S., Sayanam,R.R.2018. MorinInhibitingPhotocarcinogenesisby Targeting Ultraviolet-B Induced Oxidative Stress and InflammatoryCytokines Expression in Swiss Albino Mice. *International Journal of Nutrition,Pharmacology, Neurological Diseases*, 8(2):41-47.

8. Niehaus,W.G.,Samuelsson, B. 1968. Formation of malonaldehyde from phospholipid arachidonateduringmicrosomallipidperoxidation.*Europeanjournalofbiochemistry*, 6(1):126-130.

9. Kakkar,P.,Das,B.,Viswanathan,P.N.1984. Amodifiedspectrophotometricassayofsuperoxide dismutase.

10. Sinha, A.K. 1972. Colorimetric assay of catalase. *Analytical Biochemistry*,47(2):389-394.

11. Rotruck,J.T.,Pope,A.L.,Ganther,H.E.,Swanson,A.B.,Hafeman,D.G.,Hoekstra,W.G. 1980. Selenium:Biochemicalroleasacomponentofglutathioneperoxidase.*Nutrition Reviews*,

38(8):280-283.

12. Ellman, G.L. 1959. Tissue sulfhydryl groups. *Archives Biochemistry Biophysics*, 82(1):70-77.

13. Ichihashi, M., Ueda, M., Budiyanto, A., Bito, T., Oka, M., Fukunaga, M., Tsuru, K., Horikawa, T. 2003. UV induced skin damage. *Toxicology*, 189(1):21-39.

14. Kanski, J., Aksenova, M., Stoyanova, A.,Butterfield, D.A. 2002. Ferulic acid antioxidant protection against hydroxyl and peroxyl radical oxidation in synaptosomal and neuronal cellculture systems in vitro: structure-activity studies. *The Journal of nutritional biochemistry*,13(5):273-281.

15. Surampalli,G.,Nanjwade,B.K.,Patil,P.A.2015. Comprehensivecytotoxicevaluationofmorin,a bioflavonoid against verapamil on ratgastro intestinal epithelium for novel pharmaceutical application involving P-glycoprotein inhibition. *Journal of Pharmacyand Pharmacology*, 67(8):1083-1099.

16. Widel, M., Krzywon, A., Gajda, K., Skonieczna, M.,Rzeszowska-Wolny, J. 2014. Inductionof bystandereffectsbyUV-A,UVB,andUV-Cradiationinhumanfibroblastsandthe implicationofreactiveoxygenspecies.*FreeRadicalBiologyandMedicine*, 68(1):278-287.

17. Tsai, Y.C., Lee, Y.M., Hsu, C.H., Leu, S.Y., Chiang, H.Y., Yen, M.H, Cheng, P.Y. 2015. The effect offerulic acidethylesteronleptin-inducedproliferationandmigrationofaorticsmoothmuscle cells. *Experimental & molecular medicine*,47(8):180-187.

18. Yao, D., Cui, H., Zhou, S.,Guo,L. 2017. Morin inhibited lung cancer cells viability, growth,and migrationbysuppressingmiR-135bandinducingitstargetCCNG2.TumorBiology, 39(10):1-9.

19. Bhattacharya, S., Kamat, J.P., Bandyopadhyay, S.K., Chattopadhyay, S.2009. Comparative inhibitorypropertiesofsomeIndianmedicinalplantextractsagainstphotosensitization-induced lipid damage. *Food chemistry*,113(4):975-979.

20. Ighodaro,O.M., Akinloye,O.A.2018. Firstlinedefenseantioxidants-superoxidedismutase (SOD),catalase(CAT)andglutathioneperoxidase(GPX):Theirfundamentalroleinthe entire antioxidant defence grid. *Alexandria Journal of Medicine*,54(4):287-293.

21. Działo,M.,Mierziak,J.,Korzun,U.,Preisner,M.,Szopa,J.,Kulma,A. 2016.Thepotentialofplant phenolicsinpreventionandtherapyofskindisorders.*Internationaljournalofmolecular sciences*, 17(2):1-41.

22. Lala,G.,Malik,M.,Zhao,C.,He,J.,Kwon,Y.,Giusti, M.M., Magnuson, B.A. 2006.Anthocyanin-richextracts inhibitmultiplebiomarkersofcoloncancerinrats.*Nutritionandcancer*,54(1):84-93.

23. Merwald, H.,Klosner.G.,Kokesch,C.,Der-Petrossian,M.,Hönigsmann,H.,Trautinger,F. 2005. UVA-induced oxidative damage and cytotoxicity depend on the mode ofexposure. *Journal of Photochemistry and Photobiology B: Biology*,79(3):197-207.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 8573 - 8585
Received 25 April 2021; Accepted 08 May 2021.

24. Ali, D., Verma, A., Mujtaba, F., Dwivedi, A., Hans, R.K.,Ray, R.S. 2011. UVB-induced apoptosisand DNA damaging potential of chrysene via reactive oxygen species in human keratinocytes. *Toxicology letters*,204(2-3):199-207.

25. Punnonen,K.,Puntala,A.,Jansen,C.T.,Ahotupa,M.1991. UVBirradiationinduceslipid peroxidationandreducesantioxidantenzymeactivitiesinhumankeratinocytesinvitro. *Acta dermato-venereologica*,71(3):239-242.

26. Cooke,M.S.,Mistry,N.,Ladapo,A.,Herbert,K.E.,Lunec,J.2000. Immunochemicalquantitationof UV-inducedoxidativeanddimericDNAdamagetohumankeratinocytes.*Freeradical research*,33(4):369-381.

27. Kvam,E.,Tyrrell,R.M.1997. InductionofoxidativeDNAbasedamageinhumanskincellsby UV and near visible radiation. *Carcinogenesis*,18(12):2379-2384.

28. Lee, J., Shin, Y.K., Song, J.Y.,Lee, K.W. 2014. Protective mechanism of morin against ultravioletB-inducedcellularsenescenceinhumankeratinocytestemcells.*Internationaljournalof radiation biology*, 90(1):20-28.

29. Prasad,N.R.,Jeyanthimala,K.,Ramachandran,S.2009. Caffeicacidmodulatesultraviolet radiation-B induced oxidative damage in human blood lymphocytes. *Journalof Photochemistry and Photobiology B: Biology*,95(3):196-203.

30. Brash,D.E.,Rudolph,J.A.,Simon,J.A.,Lin,A.,McKenna,G.J.,Baden,H.P., Halperin A.J.,Ponten, J. 1991. A role for sunlight in skin cancer: UV induced p53 mutations in squamouscellcarcinoma. *Proceedings of the National Academy of Sciences*,88(22):10124-10128.

31. Kanimozhi,P.,Prasad,N.R. 2009.Antioxidantpotentialofsesamolanditsroleonradiation-inducedDNAdamageinwhole-bodyirradiatedSwissalbinomice.*Environmental Toxicology and Pharmacology*,28(2):192-197.

32. Kanagalakshmi,A.,Agilan,B.,Mohana,S.,Ananthakrishnan,D.,Velmurugan,D., Karthikeyan, R., Ganesan, M., Srithar, G., Prasad, N.R.2014. Ferulicacidmodulatesultraviolet-Bradiationmediated inflammatory signaling in human dermal fibroblasts. *J. Res. Biol*,4(1):1505-1515.

33. Kapoor, R.,Kakkar, P. 2012. Protective role of morin, a flavonoid, against high glucose induced oxidative stress mediated apoptosis in primary rat hepatocytes. *PLoS One*, 7(8):1-11.