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Sesamol inhibits UVB-induced ROS generation and subsequent oxidative damage in cultured human skin dermal fibroblasts

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Abstract The exposure of cells to ultraviolet B radiation (UVB) can induce the production of reactive oxygen species (ROS) which damage cellular components. Free radical scavengers and antioxidants can interfere with the production of ROS. We studied cytotoxicity, intracellular ROS levels, lipid peroxidation, antioxidant status and oxidative DNA damage in cultured human skin dermal fibroblast adult cells (HDFa) exposed to UVB in the presence of sesamol, a natural phenolic compound. The levels of cytotoxicity, intracellular ROS, lipid peroxidation, oxidative DNA damage and apoptotic morphological changes were significantly increased in UVB irradiated HDFa cells. We also observed that the activities of enzymatic antioxidants (superoxide dismutase, catalase and glutathione peroxidase) and the levels of non-enzymatic antioxidant status (GSH) were significantly decreased in UVB irradiated cells. On the other hand, sesamol pretreatment significantly decreased cytotoxicity, intracellular ROS, lipid peroxidation, oxidative DNA damage and apoptotic morphological changes in sesamol-pretreated and UVB-irradiated HDFa cells. We have also observed increased enzymatic and non-enzymatic antioxidants status in sesamol plus UVB-irradiated cells. Among the different doses tested, 80 μM of sesamol shows maximum protection for UVB-induced oxidative damage. In conclusion, UVB-induced ROS formation, cell fatality, lipid peroxidation, antioxidant depletion and oxidative DNA damage in HDFa cells is inhibited by sesamol, which, probably through its ROS scavenging activity.

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Introduction

Ultraviolet radiation (UV), in particularly ultraviolet B (UVB) with a wave length range (290–320 nm), represents one of the most important environmental factor affecting human health [58]. UVB radiation is regarded as the "burning ray" and it makes up 4–5% of UV light, it is a minor but the most active constituent of solar light. The toxic effects of UVB from natural sunlight and therapeutic artificial lamps are a major concern for human health. An additional potential factor is thinning of the ozone layer which results in increased UVB exposure [38]. UVB is 1,000 times more capable of causing sunburn than UVA; it also causes more genotoxic than UVA [53]. Normal skin invariably suffers from the cytotoxic effects of UVB radiation; it produces both direct and indirect effects on the skin and subcutaneous tissues [24]. Many in vitro and in vivo studies on skin cells demonstrated that UV radiation can damage many molecules and structures [4]; this may result in changes of cellular functions. In recent years, accumulated evidence has demonstrated that UV-induced oxidative damage occurs through the formation of reactive oxygen species (ROS) and the induction of pyrimidine dimers and other photoproducts such as carbonyl derivatives [42]. UVB radiation-induced skin lesions involve immediate free radicals production and altered antioxidant defensive system [10]. A predominant role in UV-induced oxidation damage is played by singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), superoxide anion (O_2^-) and hydroxyl radical (HO·) [15].

Although skin possesses an extensive and effective network of antioxidant systems, many of the free radicals produced by UV radiation can escape this surveillance and induce substantial damage to cutaneous constituents, especially when skin defense mechanisms are overwhelmed [55]. The dermis contains predominantly collagen, elastin, proteoglycans, and fibronectin. Collagen fibrils and elastin are responsible for the strength and resiliency of skin, and their disarrangement during photoaging causes the skin to appear aged. Fibroblasts constitute a major element of bone marrow stroma, submucosal tissues and subcutaneous tissues, where they are important for repair of tissue injury. Some of the general events associated with the early phase of the oxidative stress response of the skin include: depletion of endogenous intra- and intercellular antioxidants [37]; enhancement of intracellular lipid peroxidation concentrations [35]; and the induction of specific signal transduction pathways that can modulates inflammatory, immunosuppressive, or apoptotic processes in the skin [26, 27]. Hence, there is a general need for a safe and effective antioxidant/skin protects to modulate the UV-induced redox (antioxidant/prooxidant) balance. Consequently, exogenous antioxidants that scavenge ROS and restore the normal redox state may be beneficial role [17]. Therefore, agents that can protect from UVB-induced cellular oxidation damage may be useful against photodamage.

The effect of phenolic compounds on oxidative DNA damage has been researched by many investigators, and phenolic phytochemicals have been shown to reduce oxidation damage induced by a variety of ROS generating systems [60]. Phenolic phytochemicals reduce oxidative damage by scavenging free radical species and by antioxidant activity [34]. Some studies have demonstrated the protection assured by natural polyphenols such as quercetin, epicatechin or polyphenols contained in green tea on UV-induced oxidation damage in skin cells [12, 24, 39]. Sesame, an important oilseed from *Sesamum indicum*, is the oldest oilseed known to man and is considered to have not only nutritional value, but also some medicinal effects. Sesame seeds and sesame oil have been known as traditional health foods and have been used in ancient Chinese medicine for a long time. However, the scientific evidence of their miraculous functions, especially in the prevention of aging as the food has often been prescribed for in traditional medicine, has not been well established. The high resistance of sesame oil to oxidative deterioration as compared with other vegetable oil is attributed to the phenolic compound sesamol, produced from sesamolin [33]. Sesamol is a potent phenolic antioxidant found mainly in roasted sesame or in processed sesame oil [25]. Sesamol exhibits powerful inhibitory effects on lipid peroxidation in rat liver microsomes [23]. Free radical

reactions and antioxidant activities of sesamol has been proved by pulse radiolytic and biochemical studies [19]. An in vitro study indicated that sesamol inhibited the mutagenicity in various tester stains of *Salmonella typhimurium* [32]. It has been proved that sesamol scavenges hydroxyl, lipid peroxyl and tryptophanyl radicals at a nanosecond time scales and prevents Fenton reaction-induced calf thymus DNA damage and inhibits UVB radiation-induced double strand DNA breaks [22, 23]. Previously, it has been shown that sesamol prevents gamma-radiation-induced chromosomal aberration and oxidative damage in cultured human lymphocytes [40]. In this study, we evaluated the protective effect of sesamol on UVB-induced oxidative damage in HDFa cells. HDFa cells are well suited for mechanistic and toxicological studies [1]. We studied the effect of sesamol on UVB-induced cytotoxicity, intracellular ROS levels, lipid peroxidation, antioxidant status, DNA damage and apoptotic morphological changes in cultured human skin dermal fibroblast.

Materials and methods

Chemicals

Human skin dermal fibroblast adult (HDFa)-500K cells/vials were purchased from Invitrogen Bioservices, India (Catalogue No: C0135C). Medium 106 (Catalogue No: M-106-500), low serum growth supplement (LSGS; Catalogue No: S-003-10), fetal bovine serum, hydrocortisone, human epidermal growth factor, basic fibroblast growth factor, heparin, trypsin/EDTA solution (Cat # R-001-100) and trypsin neutralizer solution (cat. # R-002-100) were purchased from Casecade Biologics, Invitrogen cell culture, India. Sesamol, thiobarbituric acid (TBA), phenazine methosulphate (PMS) nitroblue tetrazolium (NBT), 5,5-dithiobis 2-nitrobenzoic acid (DTNB), 3-(4, 5-dimethyl-2-thiazozolyl)-2,5-diphenyl-2H tetrazolium bromide (MTT), 2′,7′-diacetyl dichlorofluorescein (DCFH-DA) and nicotinamide adenine dinucleotide (NAD) were purchased from Sigma chemical Co., St. Louis, USA. Low melting agarose (LMPA), normal melting agarose (NMPA), phosphate buffered saline (PBS) and reduced glutathione (GSH) were purchased from Himedia, Mumbai. All other chemicals, solvents and other analytical grades were obtained from S.D Fine Chemical, Mumbai and Fisher Inorganic and Aromatic Limited, Chennai.

Human skin dermal fibroblast adult cell culture

Human skin dermal fibroblast adult cells (HDFa) obtained from Invitrogen Bioservices were cultured at 37°C in 5% CO_2 in medium 106 (Casecade Biologics, Invitrogen cell

culture, India) supplemented with LSSG kit (Casecade Biologics, Invitrogen cell culture, India), (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 μg/mL and antibiotics). The cells were allowed to grow for 7 days to reach the maximum confluence. After reaching 80-90-% confluency the cells were subcultured and used for further experiments.

Experimental protocol

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

Group 1-control (sham UVB-irradiated fibroblasts);
Group 2-sham UVB irradiated fibroblasts and treated with 80 μM of sesamol;
Group 3-fibroblasts, UVB irradiated;
Group 4-fibroblasts, UVB irradiated and treated with 8 μM of sesamol;
Group 5-fibroblasts, UVB irradiated and treated with 40 μM of sesamol;
Group 6-fibroblasts, UVB irradiated and treated with 80 μM of sesamol;
Group 7-fibroblasts, UVB irradiated and treated with 160 μM of sesamol.

Treatment of the HDFa cells

Thirty minutes prior to irradiation, four test doses (8, 40, 80 and 160 μM) of sesamol were added to the grouped HDFa cells. Preliminary studies were carried out to ensure that whether this concentration had any toxic effect by trypan blue dye exclusion test. Before exposure to UV light, the cells were washed twice with PBS solution. Non-irradiated HDFa showed no decrease in viability over the 30-min period of incubation.

Irradiation procedure

Cultures of HDFa cells were washed once with PBS and exposed to UVB radiation in a thin layer of culture medium. The culture medium was later removed and covered with a UV permeable membrane to prevent contamination. A battery of TL 20 W/20 fluorescent tubes (Heber Scientific) served as UVB source, which had a wavelength range set 290–320 nm peaked at 312 nm and an intensity of 2.2 mW/cm^2 for 9 min. The total UVB irradiation was 19.8 mJ/cm^2, with an average value of 1.52×10^{-3} mJ/cell. After irradiation the HDFa cells were kept at room temperature for 30 min. Cells were then washed twice with PBS, scraped gently, and transferred to sterile tubes for further analysis.

MTT assay

The MTT assay is a colorimetric non-radioactive assay for measuring cell viability through increased metabolisation of tetrazolium salt [29]. Cultured fibroblasts in concentration of 1×10^6 cells/mL were taken into 96 well plat. Then the cells were pretreated with different concentration of sesamol (8, 40, 80 and 160 μM). After 30 min incubation with sesamol the cells were exposed to UVB irradiation. Then the cells were incubated in the presence of 5% CO_2 and 95% O_2 at 37°C for 24 h. MTT (0.5 mg/mL) was added to the incubated cells, then further incubated for another 4 h. The cells were centrifuged for 10 min and the supernatant was removed, 200 μL of DMSO were added into each tubes. Absorbance was measured in a microplate reader at 540 nm.

Quantification of intracellular ROS

The intracellular ROS levels were measured by 2′,7′-diacetyl dichlorofluorescein diacetate (DCFH-DA) method [13]. The diacetate group of DCFH-DA allows it to diffuse into the cell where intracellular esterase hydrolyze these, leaving DCFH to react with oxidants, upon oxidation, the probes become fluorescent and are thus amenable to quantify spectrofluoremetically. Sesamol-pretreated and/or UVB-treated fibroblasts in six well plates were incubated for 15 min with 10 μM/mL DCFH-DA in PBS, washed three times with PBS. Fluorescence was determined at 488/525 nm by spectrofluorimeter.

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 dithiothreital and centrifuged at $20,000 \times g$ 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 [34]. 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. [18]. Superoxide dismutase (SOD) activity was assayed by the method of Kakkar et al. [20], based on the inhibition of the formation of (NADH-PMS-NBT) complex. Catalase (CAT) activity was assayed by the procedure of Sinha [52], quantifying the hydrogen peroxide after reacting with dichromate in acetic acid. The activity of glutathione peroxidase (GPX) was assayed by method of Rotruck et al. [45], a known amount of enzyme preparation was allowed

to react with hydroperoxides (H$_2$O$_2$) and 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 [11]. This method was based on the development of a yellow color when 5,5-dithiobis 2-nitrobenzoic acid was added to compound containing sulphydryl groups.

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. [51]. 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$_2$EDTA, 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 1.33 V/cm 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 40× 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.

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 sesamol and UVB-irradiated cells [8]. The cells were fixed in 3:1 ratio of methanol and glacial acetic acid for 1 h at room temperature. 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 40× 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 condensed chromatin so called ethidium bromide positive nuclei were scored and the percentage apoptotic cells were calculated.

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.

Results

Effect of sesamol on UVB-induced cell viability by trypan blue dye exclusion test

Cell viability was determined before and immediately after UVB irradiation by trypan blue dye exclusion test. Figure 1
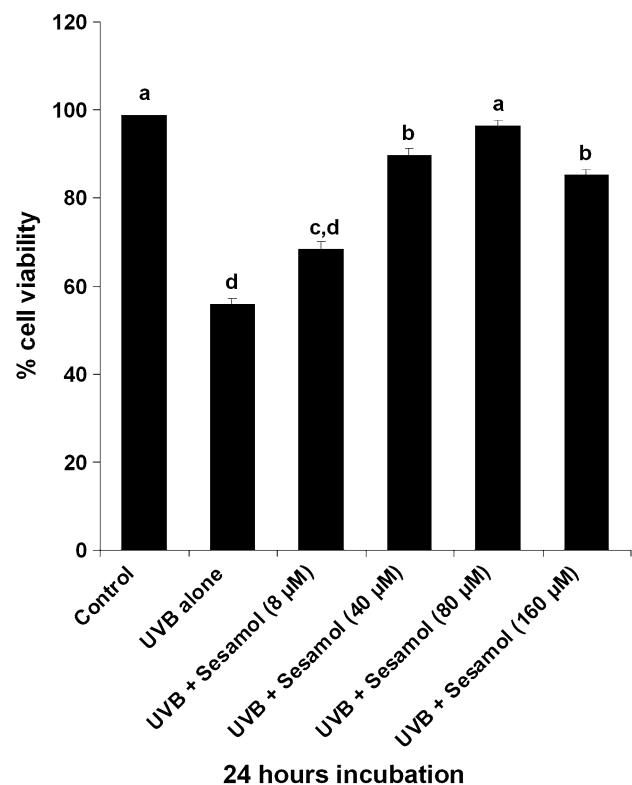

24 hours incubation

Fig. 1 Percentage cell viability was decreased in UVB irradiated HDFa cells. Sesamol pretreatment increased cell viability in a concentrations dependent manner. Values are given as mean ± SD of six experiments in each group. Values not sharing a common marking (*a*, *b*, *c*,…) differ significantly at *P* < 0.05 (DMRT)

Fig. 2 a UVB irradiation decreased HDFa cell viability. Cell ▶
viability was not affected by sesamol treatment at 80 μM. Reduced
cell viability of HDFa was observed with 160 μM of sesamol
treatment (compared with control group). The data are presented as
mean ± SD and all experiments were done in triplicate. **b** The image
represents after UVB-exposed HDFa incubated with 24 h. *A* Control,
B control + sesamol (160 μM), *C* UVB-irradiated fibroblasts, *D*
UVB + sesamol (8 μM), *E* UVB + sesamol (40 μM), *F* UVB + ses-
amol (80 μM), UVB + sesamol (160 μM)

shows the effect of UVB and/or sesamol pretreatment on
cell viability. UVB-irradiated groups show significantly
decreased cell viability than the normal cells. Pretreatment
with sesamol significantly restored the cell viability in a
concentration dependent manner.

Effect of sesamol on UVB-induced cytotoxicity

We assessed the effect of UVB radiation on cell viability
using MTT assays. Figure 2 shows the cytotoxicity obtained
by the MTT assay, which detects viable cells by assessing
the capability of cells to reduce MTT to a formazan product
by mitochondria. Our result shows that cytotoxicity was
greatly increased in UVB-irradiated cells. Cytotoxicity was
restored significantly in sesamol treated fibroblasts when
compared to the corresponding groups of irradiated fibro-
blasts. Both cell viability assays taken together demonstrate
that sesamol treatment reduces cell injury and protects cells
against UVB radiation-induced cytotoxicity.

Measurement of ROS levels in UVB- and/or sesamol-
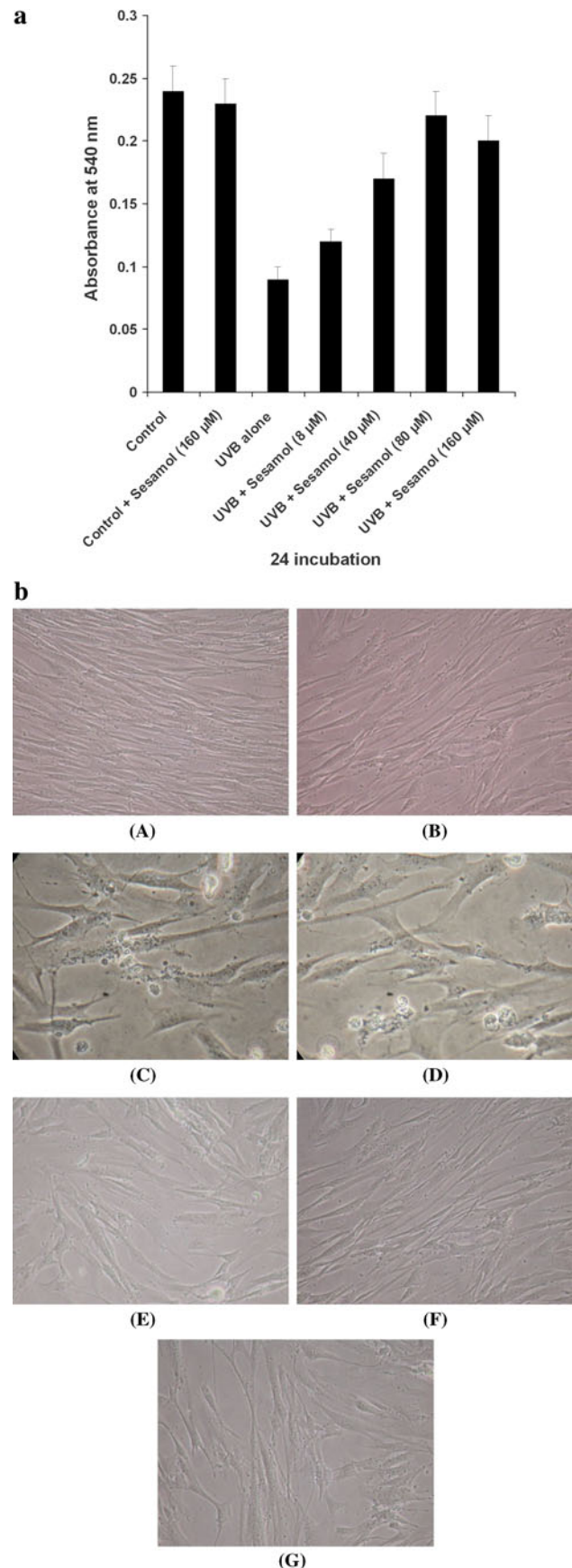pretreated cells

The DCFH-DA method measures intracellular generation
of hydrogen peroxide, an indirect procedure for estimating
ROS. In the present study (Fig. 3), the intracellular ROS
concentration was significantly higher in UVB irradiated
fibroblasts compared to the control and sesamol treated
groups. Sesamol pretreatment significantly inhibits the
intracellular ROS production in UVB-irradiated fibroblasts
in a dose-dependent manner.

Effect of sesamol on UVB-induced lipid peroxidation

The levels of TBARS and LPH were increased significantly
in UVB-irradiated HDFa cells (Fig. 4). Sesamol-pretreated
fibroblasts showed progressively decreased levels of
TBARS and LPH when compared with UVB-irradiated
cells.

Effect of sesamol on enzymatic antioxidant activity

Figure 5a shows the activities of SOD, CAT and GPX in
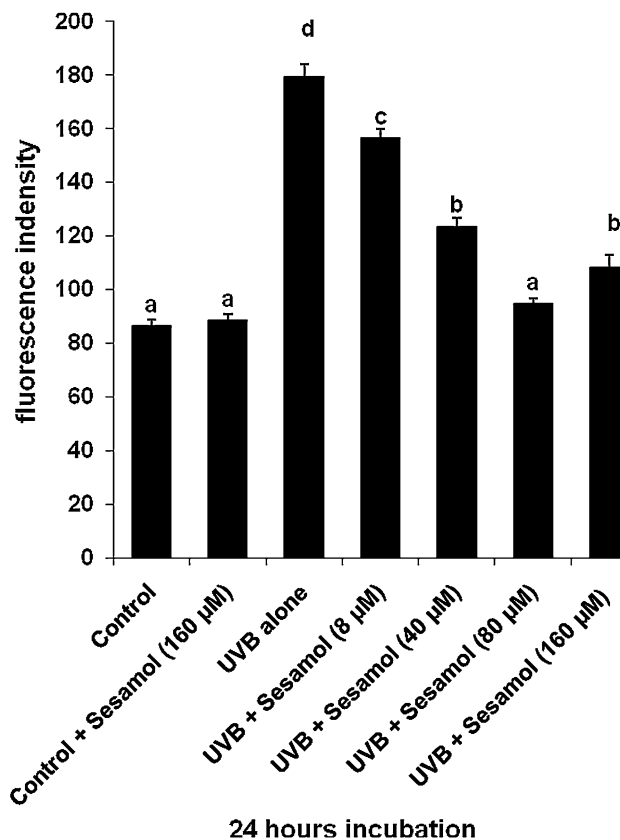normal, UVB-irradiated and sesamol-pretreated fibroblasts.

Fig. 3 Effect of sesamol on intracellular ROS in HDFa following UVB 30-min exposure. ROS production were decreased in sesamol-pretreated UVB irradiated HDFa in a concentration dependent manner. Values are given as mean ± SD of six experiments in each group. Values not sharing a common marking (a, b, c,…) differ significantly at $P < 0.05$ (DMRT)

Activities of these antioxidant enzymes were significantly decreased in UVB-irradiated fibroblasts. Pretreatment with sesamol significantly increased activities of SOD, CAT and GPX in a concentration dependent manner.

Effect of sesamol on GSH level

The levels of non-enzymatic antioxidant such as GSH (Fig. 5b) were found to be decreased in UVB-irradiated fibroblasts. Sesamol (8, 40 and 80 μM) pretreatment significantly restored the GSH level to the normal in a concentration dependent manner.

Effect of sesamol on UVB-induced DNA damage

Figure 6a–c represents the DNA damage in UVB-irradiated and/or sesamol-pretreated human fibroblasts. The extent of DNA damage was calculated by % head DNA, tail length, tail moment and OTM in normal, UVB-exposed and sesamol-pretreated fibroblasts. UVB irradiation significantly increased % head DNA, tail length, tail moment and OTM
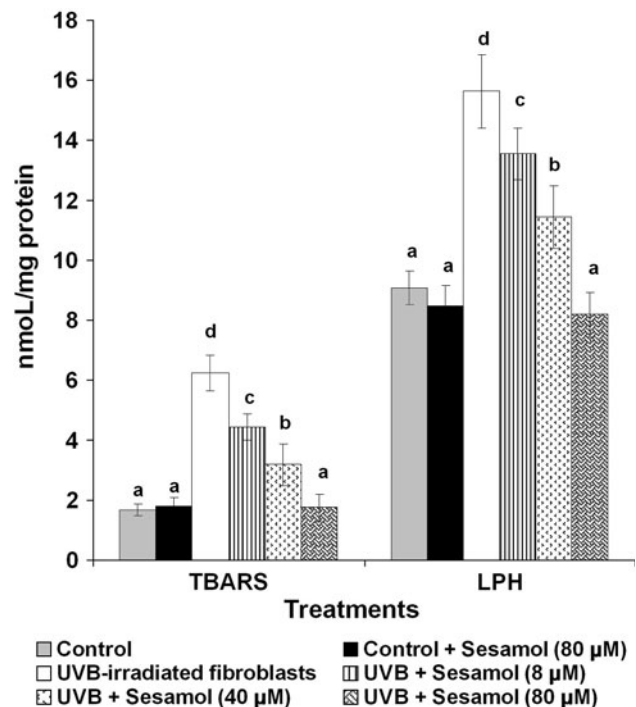

Fig. 4 Sesamol treatment decreased TBARS and LPH levels in HDFa. Increased TBARS and LPH were observed in HDFa with UVB exposure, while sesamol treatment at concentrations of 8, 40 and 80 μM significantly down-regulated the levels of TBARS and LPH (compared with control group, $P < 0.05$). Values are given as mean ± SD of six experiments in each group. Values not sharing a common marking (a, b, c,…) differ significantly at $P < 0.05$ (DMRT)

in cultured fibroblasts. Sesamol (8, 40 and 80 μM) pretreatment significantly decreased the levels DNA damage in a concentration dependant manner.

Sesamol inhibits nuclear condensation in UVB-radiated HDFa cells

In this study, we used fluorescent DNA binding dyes including acridine orange and ethidium bromide to differentiate cells that are in stages of apoptosis and necrosis (Fig. 7a, b). Early stage apoptotic cells take up acridine orange but not ethidium bromide. They are stained green, whereas non-viable cells take up both dyes and are stained orange. Because healthy cells also take up acridine orange, the cells undergoing apoptosis are identified by analysis of their chromosome condensation or by observation of fragmentation that did not occur in healthy cells. Acridine orange/ethidium bromide staining of fibroblast cells treated with UVB showed condensed nuclei, membrane blebbing and apoptotic bodies. Sesamol-pretreated cells showed decreased apoptotic morphological changes. In contrast the control cells showed intact nuclear architecture.
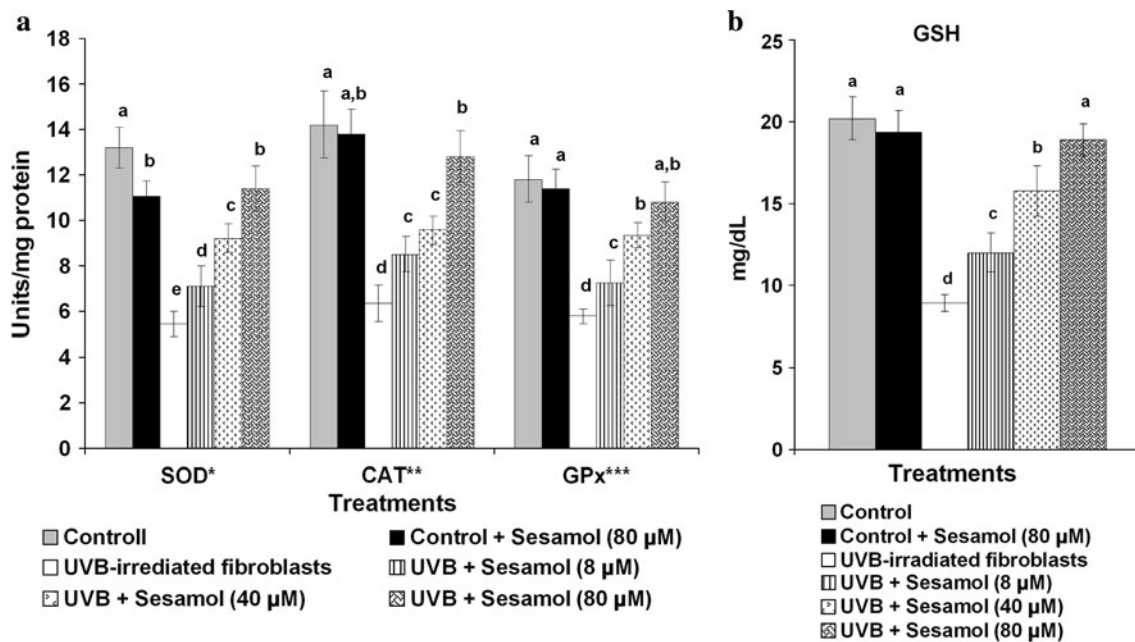
Fig. 5 a Sesamol treatment increases intracellular SOD, CAT and GPX activities in HDFa. Reduced enzymatic antioxidant activities were observed in HDFa with UVB exposure, while sesamol treatment at concentrations of 8, 40 and 80 μM significantly up-regulated the intracellular activity of enzymatic antioxidants (compared with 0 mg/mL control group, $P < 0.05$). Values are given as mean ± SD of six experiments in each group. Values not sharing a common marking (*a*, *b*, *c*,…) 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. **b** Effect of sesamol on endogenous GSH level in UVB irradiated HDFa. *Bars* represent mean ± SD of six separate experiments ($n = 6$ per group). Statistical analysis was performed by one-way ANOVA followed by DMRT at $P < 0.05$ compared to the non-irradiated control. Values not sharing a common marking (*a*, *b*, *c*,…) differ significantly at $P < 0.05$ (DMRT)

Discussion

UV radiation (200–400 nm), particularly UVA and UVB (290–400 nm) emitted from the sun permeates the atmosphere and penetrates deep into the epidermis and dermis layer, which then influence the immune system and lead to chronic skin cancers. UVB-induced ROS induces different hazardous effects on skin, including sunburn, photoaging and skin cancer [56]. Protection against sun-induced damage is therefore a highly desirable goal. Among many photochemoprotective agents, botanical antioxidants appear to be promise and their use may be an effective strategy for reduction of incidence of skin cancer and other UV-mediated oxidative damage.

UVB exposure generates ROS, which accounts for UVB-induced photocarcinogenesis [21]. Our 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 [9]. UVB are known to interact with cellular chromophores and photosensitizers, resulting in the generation of ROS [47]. Elevated ROS levels can induce severe tissue damage and can even lead to neoplastic transformation [7]. In this study, sesamol treatment significantly reduced

ROS generation in UVB-irradiated cells. This might be due to ROS scavenging property of sesamol, according to previous report, sesamol seems to be an ideal antioxidant because of several of its properties, including (a) the ability to scavenge ROS, including OH·, O_2^-, alkoxy radicals, and peroxide radicals; (b) the ability to regenerate other antioxidants such as SOD, CAT, GPX, vitamins E and C and GSH from their radical or inactive forms [6, 22, 36]. This might be the reasons for reduction of ROS in the UVB plus sesamol treated human skin fibroblasts.

If the ROS remain, without being scavenged in the biological system, they can induce biochemical alterations, including inflammation, oxidation of lipids, proteins, DNA damage and activation or inactivation of certain enzymes [41]. Lipid components in the membranes are highly susceptible to radiation damage [2]. We observed increased TBARS production in UVB-irradiated cells. Lipid peroxidation generated by oxidative stress of UVB light in the skin has been known to be potentially deleterious for cellular function, having cytotoxic effects, stimulatory or inhibitory effects on enzymes, and cell membrane damage and carcinogenic effects [49]. Our results demonstrate that sesamol prevents the formation of lipid peroxidation products generated by UVB irradiation. Sesamol is a
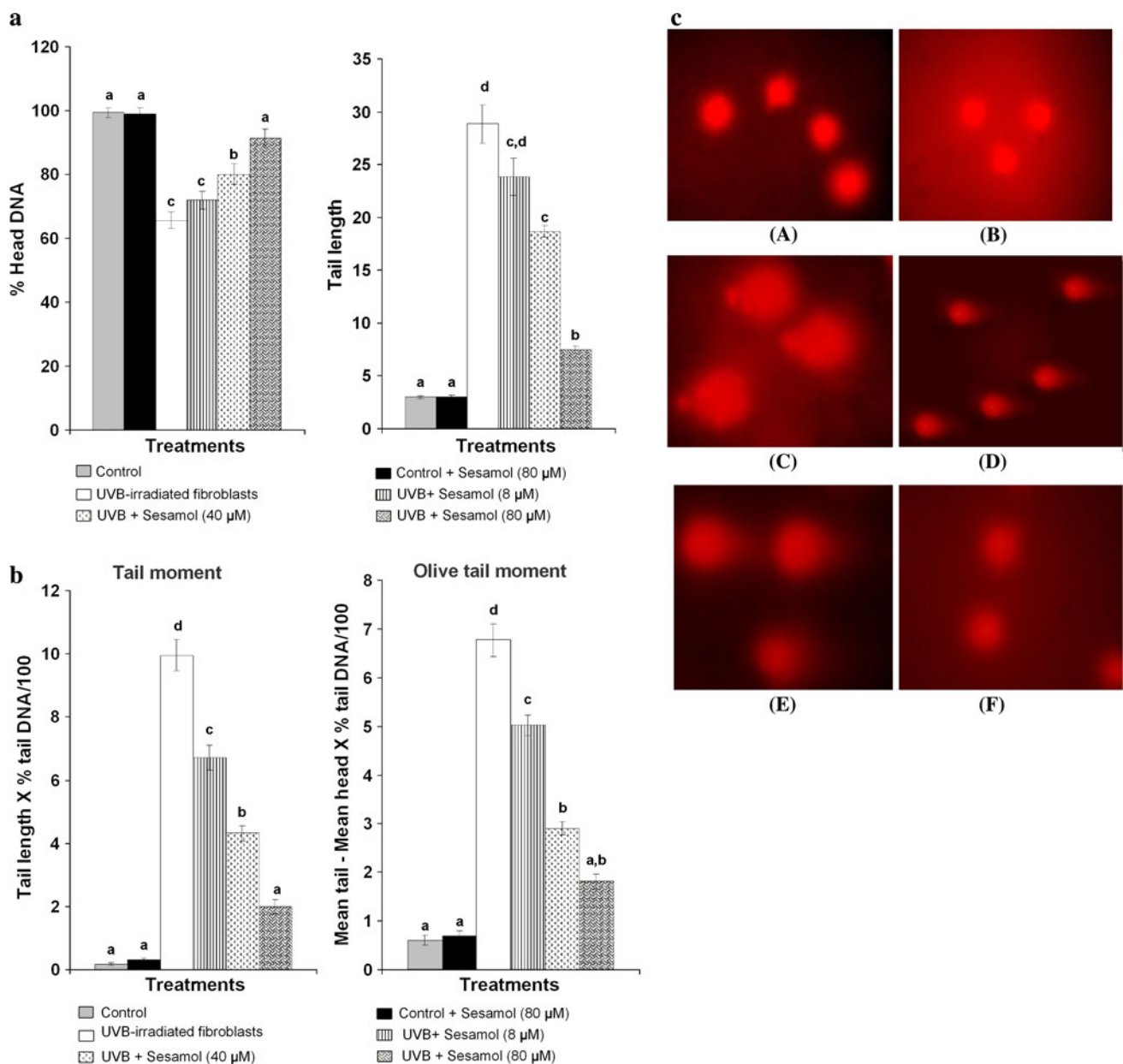
Fig. 6 a–c Effect of sesamol and UVB on DNA damage in human dermal fibroblasts. *Graphs* and *images* showing the % head DNA, tail length, tail moment and Olive tail moment levels. Comet attributes were increased in UVB-exposed fibroblasts. Its levels were decreased in sesamol-pretreated and UVB-exposed fibroblasts in a concentrations dependent manner. Values are given as mean ± SD of six experiments in each group. Values not sharing a common marking (*a*, *b*, *c*,…) differ significantly at $P < 0.05$ (DMRT). *A* Control, *B* control + sesamol (80 μM), *C* UVB-irradiated fibroblasts, *D* UVB + sesamol (8 μM), *E* UVB + sesamol (40 μM), *F* UVB + sesamol (80 μM)

simple phenolic compound; phenolics are believed to be capable of acting in redox-sensitive cascades to inhibit lipid peroxidation reactions [46]. The most prominent phenolic compounds in green tea phenolics such as (−)-epigallocatechin, (−)-epigallocatechin-3-gallate, (−)-epi-catechin, (−)-epicatechin gallate, (+)-gallocatechin and (+)-catechin prevents adverse skin reactions following UV exposure, including skin damage, erythema and lipid per-oxidation [43]. Sesamol has potent inhibitory effect on the lipid peroxidation of liposomes induced by Fe^{2+}, on the lipid peroxidation of rat liver microsomes induced by CCl_4 or NAD phosphate, and on the lipid peroxidation of mitochondria induced by ascorbate/Fe^{2+} [57].

To protect against oxidative damage, skin cells have evolved a complex antioxidant defence system which includes enzymatic antioxidants, such as SOD, CAT and GPX [5] and several non-enzymatic antioxidants such as GSH [50]. Previous reports show that enhanced oxidative stress induced by UVB radiation is accompanied with decreases in activities of SOD, CAT and GPX [48, 54]. The

present investigation clearly shows that there was a significant decrease in the SOD, CAT and GPX activities in fibroblasts exposed to UVB irradiation. There are at least three ways of affecting antioxidant enzymes by UV irradiation, (1) direct absorbance of light, (2) interaction with ROS generated by UV light, and (3) antioxidant-recycling mechanisms, whereby one antioxidant can be spared at an expense of another [50]. Direct absorbance of UVB light by the heme group may be the reason for decreased CAT activity; interaction with the superoxide anion generated by UVB may be the reason for decreased SOD activity; and antioxidant-recycling mechanisms, to detoxify H_2O_2, may be the reason for decreased GPX activity. Pretreatment with sesamol increases the activities of antioxidant enzymes in UVB-irradiated fibroblasts, and thus, sesamol could exert a beneficial action against pathologic alterations caused by the UVB radiation. Because sesamol prevents the formation of ROS, the syntheses of these enzymes are not affected. Phenolics are powerful hydrogen-donating antioxidants and free radical scavengers in several in vitro systems [43] and in vivo models [15]. Hsu et al. [14] have reported that phenolic phytochemical of sesamol increased SOD, CAT and GPX activities in endotoxin-induced oxidative stress and multiple organ injury rats. GSH is considered to be a free radical-scavenger or a cofactor for protective enzymes, which plays a pivotal role in the cellular defence against oxidative damage [30]. UVB irradiation leads to decreased levels of GSH due to leakage and oxidation of GSH [28]. GSH depletion of cultured human skin cells make them sensitive to UVB-induced mutations and cell death [41]. Thus, the approaches countering ROS production may be useful for the prevention of photoaging and skin cancer.

We observed increased DNA damage in UVB irradiated HDFa cells (Fig. 6c). The mutagenic effects of UV radiation are primarily caused by photoreactions of DNA bases [4]. Adjacent pyrimidines can be dimerized by cyclo-addition reactions yielding mutagenic cyclobutane pyrimidine dimers and pyrimidine (6–4) pyriminone photoproducts, such as, cyclobutane thymine dimer and thymine (6–4) thyminone adduct. The latter products can be converted into the related Dewar valence isomer by UVB radiation [42]. Further consequences of UV radiation
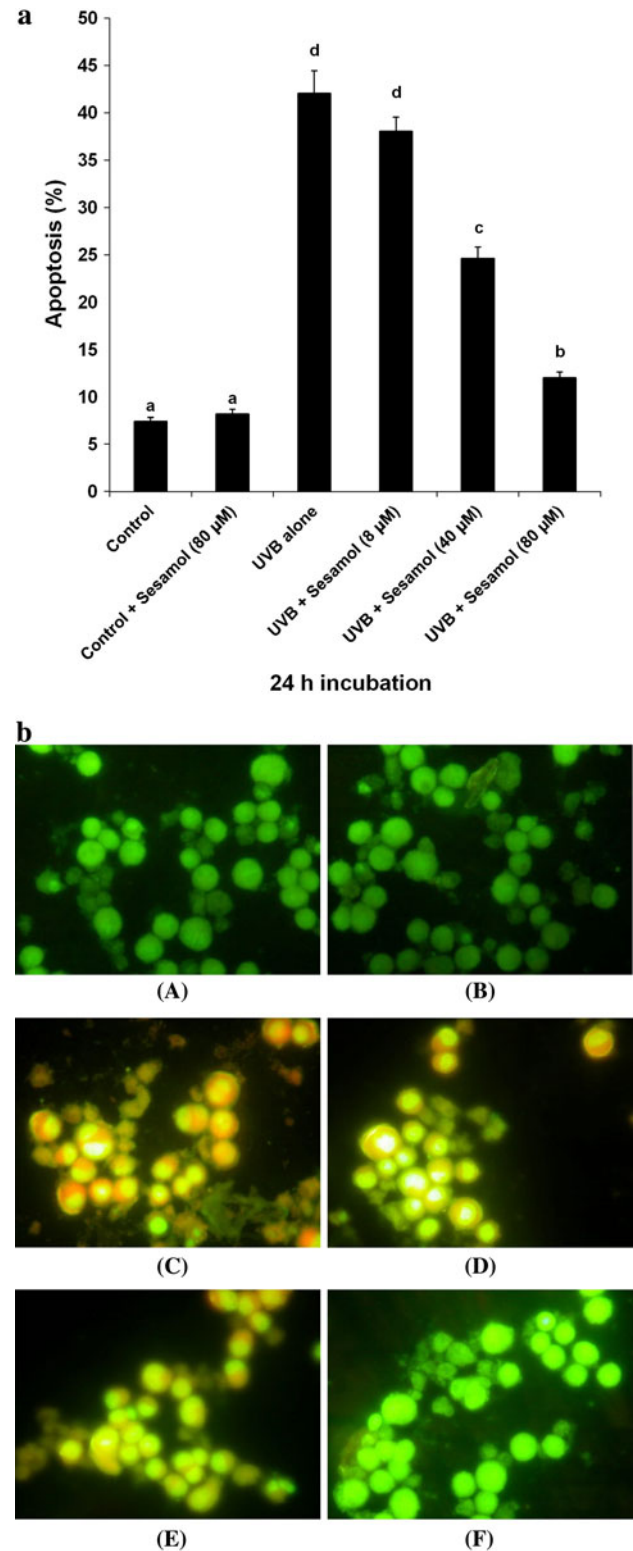
on the cells involve excitation of specific endogenous photosensitizers that ultimately photooxidize guanine residues leading to 8 oxo 7,8 dihydro 2-deoxyguanosine (8-oxodGuo). It appears that such a process is mediated by

singlet molecular oxygen and to a lesser extent by OH· radicals as oxidation inducers [3, 59]. Sesamol pretreatment significantly reduced UVB-induced DNA damage in HDFa cells. The antimutagenic activity of sesamol toward H_2O_2 and t-BOOH-induced mutagenesis in both TA100 and TA102 strains and especially in TA102 shows toward the scavenging of oxygen free radicals. Sesamol was, however, found to be ineffective in inhibiting NQNO-induced mutagenicity in TA98 strains of *S. typhimurium* suggesting its inability to modulate DNA replication or repair [16]. We observed the protective effect of sesamol on UVB radiation-induced apoptotic morphological change. The microscopic photograph (Fig. 7b) shows the control cells had intact nuclei, and the radiation-exposed cells exhibited significant nuclear fragmentation and destruction which is the characteristic of apoptosis (bright orange color) and necrosis (red color), respectively. However, the amount of fragmentation and destruction of irradiated cells were dramatically reduced when the cells were treated with sesamol. To further accentuate and support our postulation that sesamol acts as an antimutagen because of its free radical (OH·, O_2^{-}) scavenging activity, we previously performed certain in vitro colorimetric tests to demonstrate the O_2^{-} and OH· radical scavenging activity of sesamol [22].

Thus, the present study we observed increased oxidative stress mediated damage in UVB-irradiated cells. Sesamol was effective in protecting against UVB radiation-induced cytotoxicity, lipid peroxidation and antioxidant depletion, as well as scavenging of ROS. Consequently, sesamol, by virtue of its free radical scavenging capacity and replenishment of antioxidants stores, reduced the oxidative damage caused by UVB radiation. Consequently, interventions with botanical antioxidants such as sesamol could be promising in the design and development of new treatment strategies aimed at limiting sun light-induced skin oxidative damage.

Conflict of interest The authors have nothing to declare.

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