Blue Light–Induced Oxidative Stress in Human Corneal Epithelial Cells: Protective Effects of Ethanol Extracts of Various Medicinal Plant Mixtures

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Purpose. To investigate the effects of visible light on human corneal epithelial cells and the impact of natural antioxidants on oxidative stress produced by overexposure to light.

Methods. Light-emitting diodes with various wavelengths (410–830 nm) were used to irradiate human corneal epithelial cells, and cell viability was assessed. The production of reactive oxygen species (ROS) was analyzed using 2′,7′-dichlorodihydrofluorescein diacetate (DCF-DA). Ethyl alcohol (EtOH) extracts were prepared from mixtures of medicinal plants. After application of the EtOH extracts, the free radical scavenging activity was measured using a 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. The induction of antioxidant enzymes including heme oxygenase-1 (HO-1), peroxiredoxin-1 (Prx-1), catalase (CAT), and superoxide dismutase-2 (SOD-2) by the extracts was evaluated by reverse transcription-polymerase chain reaction and Western blotting. The ability of the extracts to inhibit ROS was also analyzed using DCF-DA.

Results. The viability of corneal epithelial cells was diminished after irradiation of blue light (above 10 J at 410 nm and 50 J at 480 nm). Reactive oxygen species production was induced by irradiation at 410 and 480 nm at doses of 5 J/cm^2 and higher. Ethyl alcohol extracts had potent radical scavenging activity. Application of the extracts not only increased the expression of HO-1, Prx-1, CAT, and SOD-2, but it also attenuated the ROS production induced by blue light in a dose-dependent manner.

Conclusions. Overexposure to blue light (410–480 nm) may have a harmful effect on human corneal epithelial cells compared with other visible light wavelengths. Medicinal plant extracts can have potent protective effects on blue light–induced oxidative stress.

Keywords: corneal epithelial cells, light emitting diode, visible light, reactive oxygen species, oxidative stress, medicinal plants

Light-emitting diodes (LEDs) are complex semiconductors that convert electrical current into incoherent narrow-spectrum light. These diodes are available at wavelengths ranging from UV to visible to near infrared bandwidths (247–1300 nm).[1] The visible spectrum is the portion of the electromagnetic spectrum that is visible to the human eye. A typical human eye responds to wavelengths ranging from approximately 400 to 700 nm.[2]

Oxidative stress leads to damage to lipids and DNA and the inhibition and deactivation of proteins with a consequent disruption of overall biological function.[3] Ocular UV radiation-induced DNA and protein damage has been associated with a number of eye pathologies, including photokeratitis, pterygium, eyelid malignancies (basal cell carcinoma and squamous cell carcinoma), cataract, and corneal and retinal degeneration.[4–9] It is well known that human corneal epithelial cells are responsive to UV light. Ultraviolet-induced corneal injury can result from oxidative stress by the localized generation of reactive oxygen species (ROS), including superoxide anion, hydrogen peroxide, and hydroxyl radical by corneal epithelial cells. Oxidative stress induced by UV-B light has been shown to upregulate the expression of proinflammatory cytokines, growth factors, and enzymes mediating prostaglandin and leukotriene biosynthesis, as well as antioxidant enzymes in corneal epithelial cells, suggesting that UV-B light can induce inflammation and tissue damage in the ocular surface epithelium.[10–16]

Many colors can be reproduced by current technologies, including smartphones, computers, and televisions. The spectrum of visible light as well as UV light may have an impact on normal naked eyes. However, although UV light has been demonstrated to produce harmful effects on eyes, it is uncertain whether a certain spectrum of visible light can also produce harmful effects on human eyes. We hypothesized that blue light with short wavelengths may also induce oxidative stress on the corneal epithelium. It is thought that medicinal plants may protect the eyes against oxidative stress induced by UV or visual light. In this study, ethyl alcohol (EtOH) extracts were prepared from a mixture of various medicinal plants (XERO-M; BM Biotechnology, Sunchon, South Korea) that have antioxidant and anti-inflammatory effects.

Table 1. Irradiance of LED Lamps With Various Wavelengths

LED Wavelength, nm	Irradiance, mW/cm²
850 ± 3	7.28
630 ± 8	17.34
595 ± 2	6.66
580 ± 4	8.4
525 ± 2	21.68
480 ± 7	25.6
410 ± 10	10.75

In the present study, we aimed to investigate which wavelengths of visible light from LEDs influenced human corneal epithelial cells by measuring cell viability and ROS production, and we also investigated whether EtOH extracts of various medicinal plants could reverse oxidative stress induced by visible light in human corneal epithelial cells in vitro.

Materials and Methods

LED Light Source

Seven LED lamps with various wavelengths, including 410, 480, 525, 580, 595, 630, and 850 nm, were used (Table 1). The irradiance of each LED was measured using a quantum photoradiometer (Delta OHM, Padova, Italy) connected to a visual probe (Sonda LP 9021 RAD; Delta OHM). The distance from the LED module to the cells was 5 cm.

Culture of Human Corneal Epithelial Cells and Cell Viability

Epithelial adenovirus 12-SV40 hybrid transformed human corneal epithelial cells (HCE-2) were received at passage 28 from American Type Culture Collection (Cat No. CRL-11135, Manassas, VA, USA) and were maintained in liquid tissue culture medium (EpiLife; Cascade Biologics, Inc., Portland, OR, USA) supplemented with human corneal growth supplement (Cascade Biologics, Inc.), 100 U/mL penicillin, and 100 μg/mL streptomycin at 37°C in a humidified incubator with 5% CO_2.

Cell viability was measured using a colorimetric assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; Sigma-Aldrich, St. Louis, MO, USA) according to the manufacturer's instructions.[17] Human corneal epithelial cells (2×10^5 cells/well) were seeded in 35-mm tissue culture dishes and were irradiated with the LEDs. The cells were maintained in serum-free conditions for 24 hours. Two hundred microliters of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT: 5 mg/mL) was added to each dish. After 2 hours, 2 mL of dimethyl sulfoxide (DMSO) was used to dissolve the formazan, and the sample was aliquoted (100 μL) into 96-well plates and analyzed using an ELISA spectrophotometer (Molecular Devices, Sunnyvale, CA, USA) at a wavelength of 570 nm.

Measurement of ROS Production

The levels of intracellular and extracellular ROS were analyzed using 2′,7′-dichlorodihydrofluorescein diacetate (DCF-DA; Invitrogen, Carlsbad, CA, USA). Human corneal epithelial cells were washed with phosphate buffered saline and 10 μM DCF-DA and incubated for 20 minutes. To analyze the extracellular ROS production, after LED irradiation with various wavelengths (ranging from 410 to 850 nm) and doses (ranging from 0–50 J/cm²) and, the cell pellet was analyzed using a flow cytometer (FACSCalibur; BD Biosciences, Fullerton, CA, USA), with an excitation wavelength of 488 nm and emission wavelength of 530 nm. Data analysis was based on 10,000 detected events using flow and image cytometry analysis software (Cell Quest; BD Biosciences). Alternatively, changes in the fluorescence at 488/538 nm in the cells were quantified by a plate reader (SPECTRAmax Gemini; Molecular Devices).

To analyze the intracellular ROS production, after reacting with DCF-DA, the cell suspension was secured on a microscope slide and counter-stained with 4′,6′-diamidino-2-phenylindole (DAPI). Images were obtained using confocal microscopy with a laser scanning microscope (LMS 510; Carl Zeiss, Jena, Germany) and analyzed using the browser imaging software (LSM 5; Carl Zeiss).

Ethanol Extracts of Mixed Medicinal Plants

Mixed medicinal plants (BM Biotechnology), which consist of *Schizonepeta tenuifolia var. japonica Kitagawa, Angelica dahurica Bentham ET hooker, Rehmannia glutinosa Liboschitz var. purpurea Makino,* and *Cassia tora L.*, were used in this experiment. All plant samples were air-dried and ground into powder using a plant milling machine. A 100 g sample of the powder was extracted with 1000 mL of 95% aqueous EtOH under continuous shaking at room temperature for 12 hours and filtered through Whatman No. 4 qualitative filter paper. The extraction was repeated three times to ensure complete extraction of the plant material. The extracts were concentrated by evaporation under reduced pressure in a rotary evaporator at 40°C and freeze-dried.

2,2-Diphenyl-1-Picrylhydrazyl Radical Scavenging Assay

The free radical-scavenging activity of the mixed plant extracts was evaluated using a modification of a previously published method.[18] Aliquots of sample extracts at various concentrations were each mixed with ethanol and then with 100 μL of 2,2-diphenyl-1-picrylhydrazyl (DPPH) in EtOH to a final concentration of 100 μM. The mixture was vigorously shaken and left to stand at room temperature for 30 minutes in the dark. The absorbance of the reaction solution was spectrophotometrically measured at 517 nm with an ELISA reader (VERSAMAX; Molecular Devices). The percentages of DPPH decolorization of the samples were calculated according to the equation: % decolorization = $[1 - (ABS_{sample}/ABS_{control})] \times 100$. The IC_{50} value was the effective concentration at which 50% of the DPPH radicals were scavenged, and butylated hydroxytoluene (BHT) was used as a positive control. All tests were performed in triplicate.

RNA Isolation and Semiquantitative RT-PCR

Total mRNA was isolated from HCE-2 cells using an RNA purification kit (RNeasy Mini Kit; Qiagen, Valencia, CA, USA). The cDNA was obtained from 500 ng of total RNA using an RT kit (Omniscript; Qiagen). For semiquantitative PCR, a PCR-premixture kit (ELPIS, Taejeon, Korea) was used. Polymerase chain reaction products were analyzed using 1.5% agarose gel electrophoresis, stained with a gel stain buffer (Sybr Safe DNA; Invitrogen), and visualized by luminescence with a luminescent image analyzer (LAS 3000; Fujifilm, Tokyo, Japan). The primer sequences and thermal cycling conditions of the antioxidant enzymes, heme oxygenase-1 (HO-1), peroxiredoxin-1 (Prx-1), catalase (CAT), and superoxide dismutase 2 (SOD-2; Mn-SOD), are shown in Table 2. All quantities were normalized to the endogenous control glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

Table 2. Primer Sequences and Thermal Cycling Conditions Used for RT-PCR

Gene	Forward/Reverse (5′→3′)	Amplicon Size, bp	Annealing Temperature, °C	Cycle
GAPDH	TGACCACAGTCCATGCCATC/TTACTCCTTGGAGGCCATGT	485	60	28
HO-1	CAGGCAGAGAATGCTGAGTTC/GATGTTGAGCAGGAACGC AGT	555	58	35
CAT	AGATGCGGCGAGACTTTCCC/GCTCCAGGGCAGAAGGCTGT	518	60	30
Prx-1	ATGTCTTCAGGAAATGCTAAAAT/TCACTTCTGCTTGGAGAAATATTC	599	58	35
SOD-2	GCAGAAGCACAGCCTCCCCG/CCTTGGCCAACGCCTCCTGG	158	60	30

Western Blot Analysis

Whole cell extract was prepared by sonication in radioimmunoprecipitation assay buffer lysis buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 0.25% deoxycholic acid, 1% NP-40, 1 mM EDTA, pH 8.0, and protease inhibitor cocktail). After centrifugation (12,000*g*, 15 minutes at 4°C), the protein concentration of the supernatant was measured using a Coomassie plus protein assay reagent kit (Pierce Biotechnology, Rockford, IL, USA). The cell extracts (40 μg) were placed in a sodium dodecyl sulfate (SDS) sample buffer (50 mM Tris-HCl [pH: 6.8], 10% glycerol, 2% SDS, 0.1% bromophenol blue, and 5% β-mercaptoethanol), separated using 10% SDS-PAGE, and transferred to a polyvinylidene difluoride membrane (0.45 μm, Millipore Corp., Billerica, MA, USA). After incubation with blocking solution (5% skim milk in Tris-buffered saline with 0.1% Tween-20) for 1 hour at RT, the protein bands were probed with anti-HO-1 (SPA-896, 1:2000; Stressgen Bioreagents, Ann Arbor, MI, USA), anti-Prx-1 (#5499, 1:1000; Cell Signaling Technology, Danvers, MA, USA), anti-CAT (ab52477, 1:1000; Abcam, Cambridge, UK), anti-SOD-2 (#06-984, 1:1000; Millipore Corp.), or mouse monoclonal β-actin (1:1000, ab-6276; Abcam, Cambridgeshire, UK) antibody overnight at 4°C. After incubation with horseradish peroxidase-conjugated goat anti-rabbit IgG (1:3000; Jackson ImunoResearch, Inc., West Grove, PA, USA) or anti-mouse IgG (1:3000; Jackson ImunoResearch, Inc.), the immune complexes were visualized using an ECL kit (Millipore Corp.).

Antioxidant Enzyme Activities

Superoxide dismutase activity was measured using an assay kit (SOD Assay Kit-WST; Dojindo Laboratories, Kumamoto, Japan). Superoxide dismutase activity values were calculated using the following equation: SOD activity (inhibition rate %) = [(Ablank1 – Ablank3) – (Asample – Ablank2)]/(Ablank1 – Ablank3) × 100. Catalase activity was measured by monitoring the enzymatic decomposition of hydrogen peroxide spectrophotometrically at 540 nm, using a CAT assay kit (Cayman Chemical Company, Ann Arbor, MI, USA). Catalase activity values were calculated using the following equation: CAT activity = μmol of sample/20 min × sample dilution = nmol/min/mL.

Statistical Analysis

All experiments were performed in triplicate and the results are reported as the mean ± SD. The data were analyzed by one-way ANOVA with Duncan's multiple range tests. A P value < 0.05 was considered statistically significant.

Results

Cell Survival Assay

Human corneal epithelial cell viability was evaluated after irradiation with seven LED lamps with various wavelengths (410, 480, 525, 580, 595, 630, and 850 nm) and doses (1, 2.5, 5, 10, 25, 50, and 100 J/cm^2). Among these lamps, two wavelengths of 480 ± 7 and 410 ± 10 nm were found to influence cell viability. Survival of HCE-2 cells decreased at doses ≥50 J/cm^2 at 480 ± 7 nm and at doses ≥10 J/cm^2 at 410 ± 10 nm (Fig. 1), and the rate of decrease was directly proportional to the irradiation dose. Ethyl alcohol extracts of mixed medicinal plants did not affect the survival of HCE-2 cells at doses ≤125 μg/mL (data not shown).

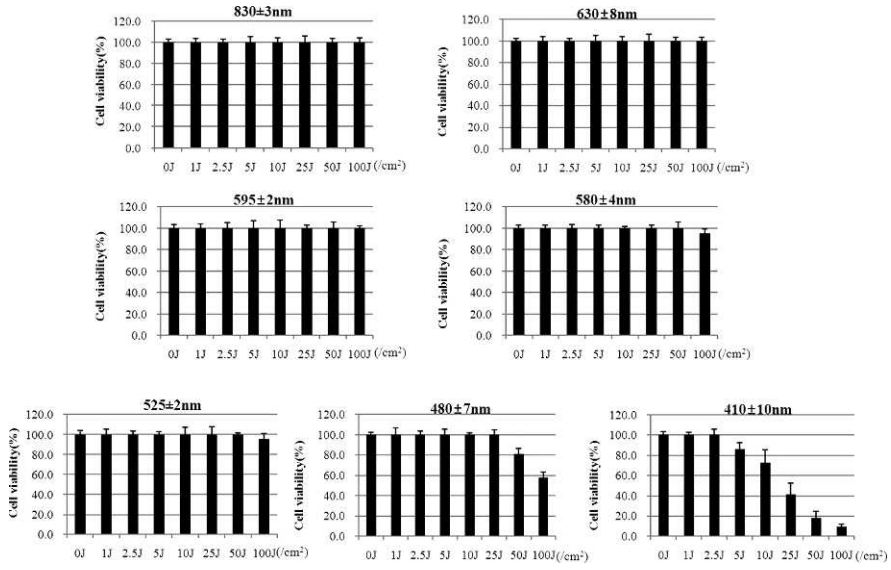

Figure 1. Viability of human corneal epithelial cells following irradiation of light emitting diodes with various wavelengths (410–830 nm), as determined by MTT assay.

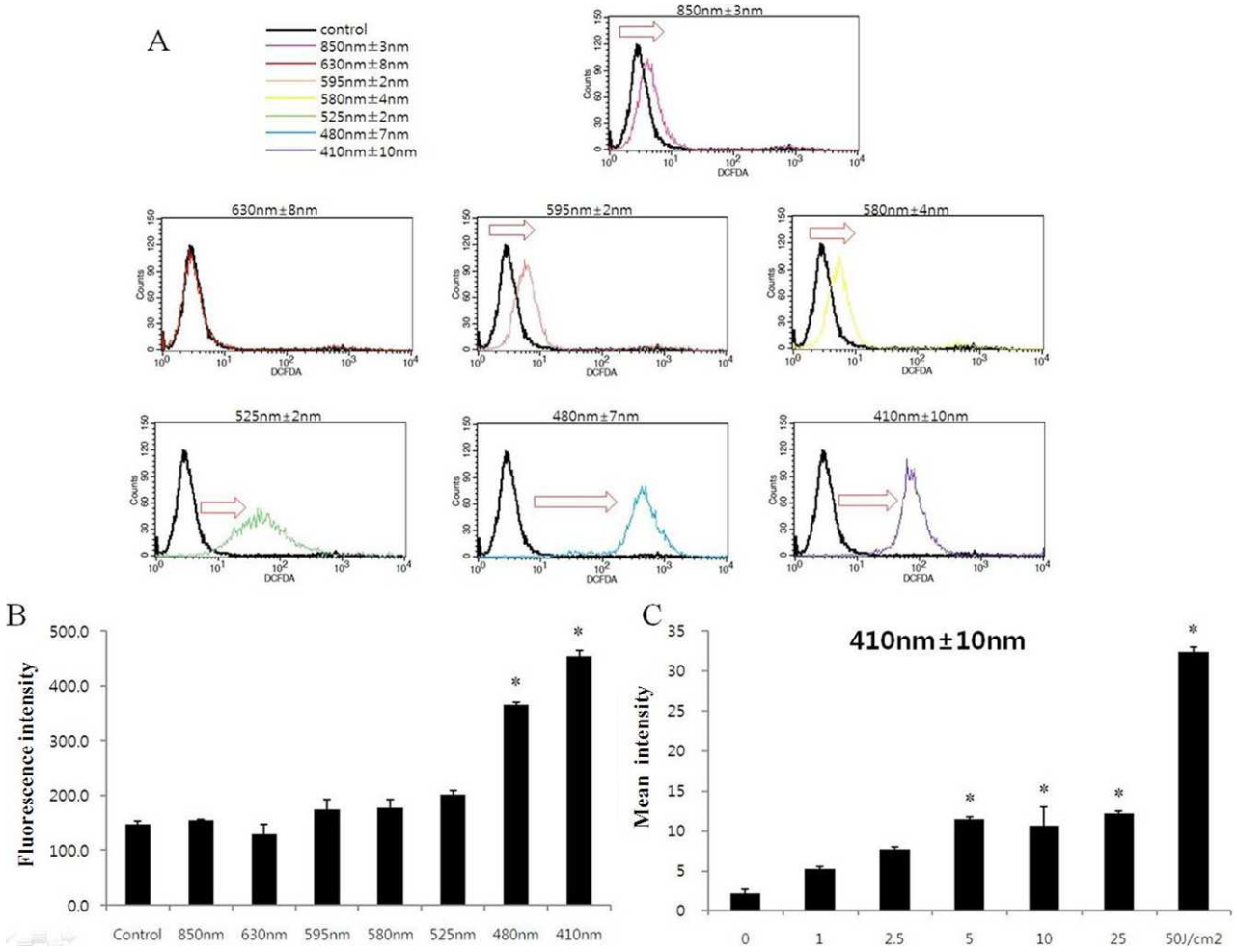

FIGURE 2. The levels of extracellular ROS measured by DCF-DA. (**A**, **B**) Representative figures (**A**) and the graph (**B**) showing ROS production by irradiation of LED with various wavelengths (410–830 nm) at a dose of 5 J/cm^2 in human corneal epithelial cells. (**C**) The graph showing ROS production by irradiation of 410 ± 10 nm LED with various doses (0–50 J/cm^2) in human corneal epithelial cells. *$P < 0.05$ compared with baseline.

Generation of ROS

To identify the mechanism of the impact of LED lights with various wavelengths on HCE-2, ROS production was analyzed after LED irradiation. At the irradiation dose of 5 J/cm^2, LED wavelengths of 850, 630, 595, 580, and 525 nm (mean fluorescence intensities of 155.7 ± 0.7, 129.5 ± 18.0, 174.4 ± 18.8, 177.5 ± 14.8 and 201.3 ± 8.3, respectively) did not induce extracellular ROS production compared with baseline (148.0 ± 5.1). In contrast, lights with wavelengths of 480 ± 7 nm (365.6 ± 3.8, $P < 0.05$ versus baseline) and 410 ± 10 nm (454.1 ± 10.4, $P < 0.05$ versus baseline) induced significant ROS production after irradiation (Figs. 2A, 2B). Additionally, LED irradiation at a wavelength of 410 ± 10 nm caused a significant increase in the generation of ROS at doses of 5 J/cm^2 or higher (Fig. 2C). The results of LED irradiation at 480 ± 7 nm were similar (data not shown). These results showed that the ocular surface could be damaged by the prolonged exposure to blue lights with wavelengths ranging from 410–480 nm.

DPPH Radical Scavenging Assay

To investigate the antioxidant activity of the mixed medicinal plant EtOH extracts, we evaluated their ability to scavenge the stable free radical DPPH. The scavenging activity of various concentrations of the extracts and the control compound, BHT, was analyzed, and the concentration required to inhibit each radical by 50% (IC_{50}) was measured. At concentrations of 50, 100, 250, and 500 μg/mL, the DPPH radical scavenging activity of the extracts reached 21.3%, 35.3%, 60.6%, and 69.3%, respectively. The IC_{50} values of BHT (the positive control) and the extracts associated with DPPH radical scavenging activity were 60.1 and 190.0 μg/mL, respectively. Among the individual EtOH extracts, the IC_{50} values of *Cassia tora L.* and *Schizonepeta tenuifolia var. japonica Kitagawa* were 400.5 and 780.0 μg/mL, respectively, while those of *Angelica daburica Bentham ET hooker* and *Rehmannia glutinosa Liboschitz var. purpurea Makino* showed minimal values, suggesting that the mixture was much more potent than the individual extracts (Fig. 3).

mRNA Expression and Protein Levels of Antioxidant Defense Enzymes

Heme oxygenase 1, Prx-1, CAT, and SOD-2 are antioxidant enzymes that protect against oxidative stress and tissue damage. All these enzymes are critical for defense against the harmful effects of ROS and free radicals on biological systems, including the eyes. The mRNA levels of these antioxidant enzymes in HCE-2 cells were increased by the EtOH extract mixture compared with the control level and increased in a

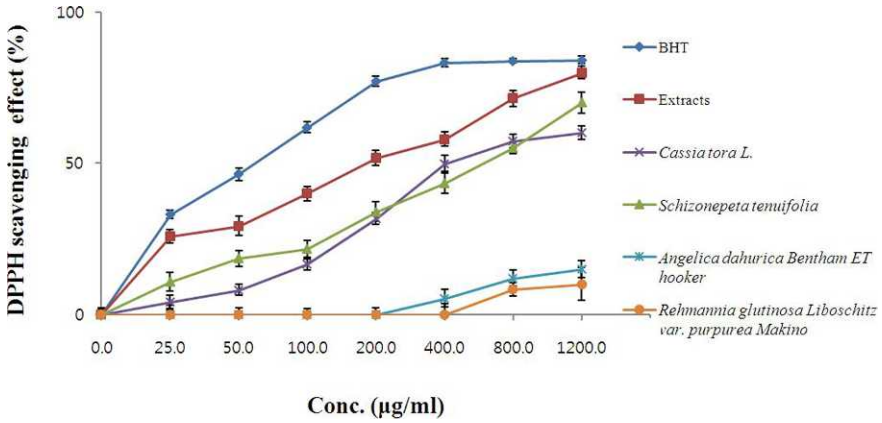

FIGURE 3. Radical scavenging assay of DPPH indicating percentages of antioxidant radical scavenging activity in the ethanol extracts of mixed medicinal plants and BHT.

dose-dependent manner (Supplementary Figs. S1A, S1B). Additionally, the protein levels of these antioxidants in HCE-2 cells were increased by the extracts and showed a pattern similar to that of mRNA expression (Figs. 4A, 4B).

SOD and CAT Activities

With regard to SOD activity, the inhibition rate of the control was 30.4%, whereas the inhibition rates of the extracts were 56.5% at 50 μg/mL ($P < 0.05$ versus the control) and 63.4% at 100 μg/mL ($P < 0.05$ versus the control), respectively. In addition, CAT activity also increased with the addition of the extracts (Figs. 5A, 5B).

Inhibition of ROS Production in HCE-2 Cells In Vitro by Mixed Medicinal Plant EtOH Extracts

The inhibition of intracellular and extracellular ROS production in HCE-2 cells by mixed medicinal plant EtOH extracts was analyzed using DCF-DA fluorescence. After LED irradiation at a wavelength of 410 ± 10 nm and a dose of 5 J/cm^2, various concentrations of the extracts (50, 100, 250, and 500 μg/mL) were added to the cell cultures, followed by incubation for 40 minutes. In an extracellular ROS assay evaluated with flow cytometry, the mean intensities of fluorescence significantly increased after 410 ± 10 nm LED irradiation (128 ± 1.7) compared with the control (21.0 ± 2.0). The extracts significantly inhibited the amount of extracellular ROS produced after LED irradiation in a concentration-dependent manner (mean intensities of 65.2 ± 1.8, 64.1 ± 1.8, 32.4 ± 2.4, and 30.0 ± 1.8 in the 50, 100, 250, and 500 μg/mL extracts-treated groups, respectively), compared with that produced at 410 ± 10 nm LED and irradiation alone (Figs. 6A, 6B). In an intracellular ROS assay evaluated with confocal microscopy, fluorescence was noted in the group irradiated with the 410 ± 10 nm LED compared with that in the group irradiated with the 630 ± 8 nm LED and the control, whereas the fluorescence was attenuated by the extracts in a concentration-dependent manner (Figs. 7A–7F).

DISCUSSION

Today people live with many electric products such as smartphones, computers, and televisions and are exposed to artificial light such as white LEDs or fluorescence light at night. White LEDs are generated by mixing differently colored lights (red, green, and blue) or by coating LEDs of one color (mostly blue LEDs made of InGaN) with phosphors of different colors to form broad-spectrum white light.[19,20] It means that blue light is included in white light from LEDs. Although the quantity of light exposure of a day is not intense or abundant, prolonged exposure to the light for a long time may be harmful on naked eyes.

Although there is substantial evidence that blue light can participate in the generation of ROS in the lens and retinal pigment epithelium, very little is known about the effects of blue light on the ocular surface epithelium.[21,22] In the present study, we first evaluated the effects of visible light with various wavelengths on the viability of and ROS production in human

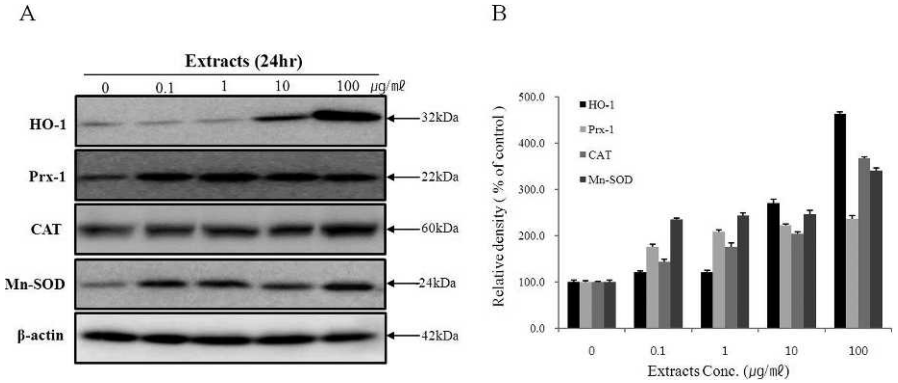

FIGURE 4. Western blot analysis (**A**) and the graph showing the relative expression (**B**) of HO-1, Prx1, CAT, and SOD-2 (Mn-SOD) proteins by the mixed plant extracts (0, 0.1, 1, 10, and 100 μg/mL), in human corneal epithelial cells.

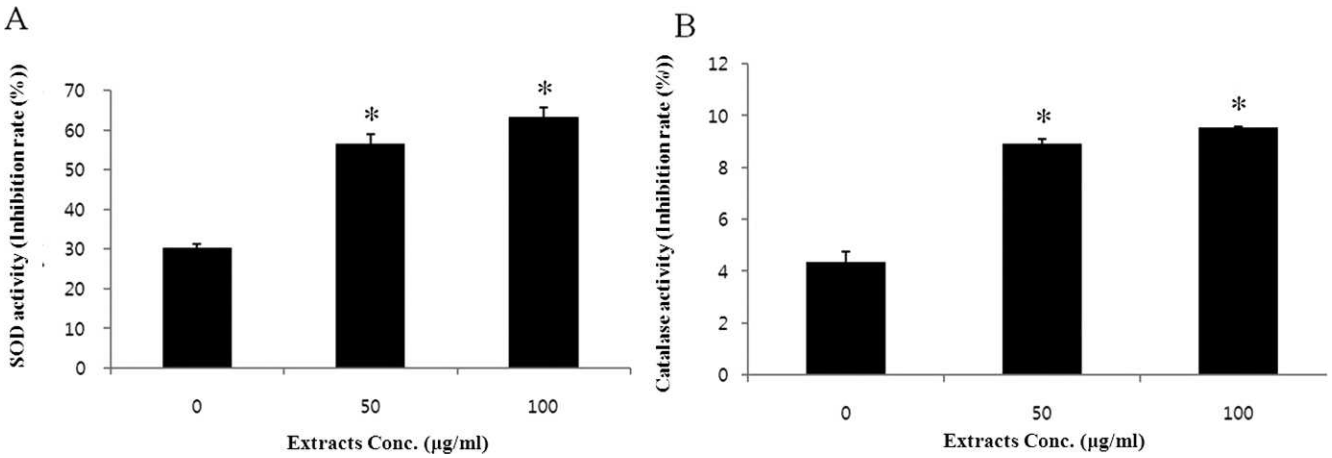

Figure 5. Activities of SOD (**A**) and CAT (**B**) enzymes after addition of the mixed plant extracts (50 and 100 μg/mL), as measured by SOD and CAT assay kits. *$P < 0.05$ compared with baseline.

corneal epithelial cells. Light-emitting diodes with wavelengths of 630, 595, 580, and 525 nm (visible light), as well as 850 nm (infrared), neither affected cellular viability nor induced ROS production in human corneal epithelial cells. In contrast, visible light with wavelengths of 480 and 410 nm decreased cellular viability and induced significant ROS production after irradiation. In addition, irradiation at 410 and 480 nm caused a significant increase in the generation of ROS at doses of 5 J/cm^2 or higher. These findings show that visible light, especially blue light, can also have a harmful impact on the ocular surface.

The ocular surface is composed of the tear film, conjunctiva, and cornea, which, together with the aqueous humor, form the first physical and biochemical barrier of the eye and play a pivotal role in combating free radicals. The main ocular surface pathology related to the failure of tear antioxidant defenses is dry eye disease, in which oxidative stress plays a significant role.[23–25] Lipid oxidative stress markers increased in the tear film and conjunctiva of dry eye patients with Sjögren syndrome and were correlated with disease severity. In addition, a close relationship was found between ROS production, lipid peroxidation–related membrane damage, and inflammation in dry eye disease.[26] Based on the role of oxidative stress in the pathogenesis of dry eye disease, many antioxidants, including sea buckthorn oil, green tea polyphenols, and omega-3 essential fatty acids, have been demonstrated to decrease inflammation in corneal epithelial cells and improve the tear film and ocular surface in experimental or clinical dry eye.[27–34]

In our study, we demonstrated the efficacy of a mixture of four natural plant EtOH extracts, including *Schizonepeta tenuifolia var. japonica Kitagawa, Angelica dahurica Bentham ET hooker, Rehmannia glutinosa Liboschitz var. purpurea Makino,* and *Cassia tora L.*, in protecting human corneal epithelial cells from oxidative stress induced by irradiation with LEDs with short wavelengths. Among the plant extracts evaluated, *Schizonepeta tenuifolia var. japonica*

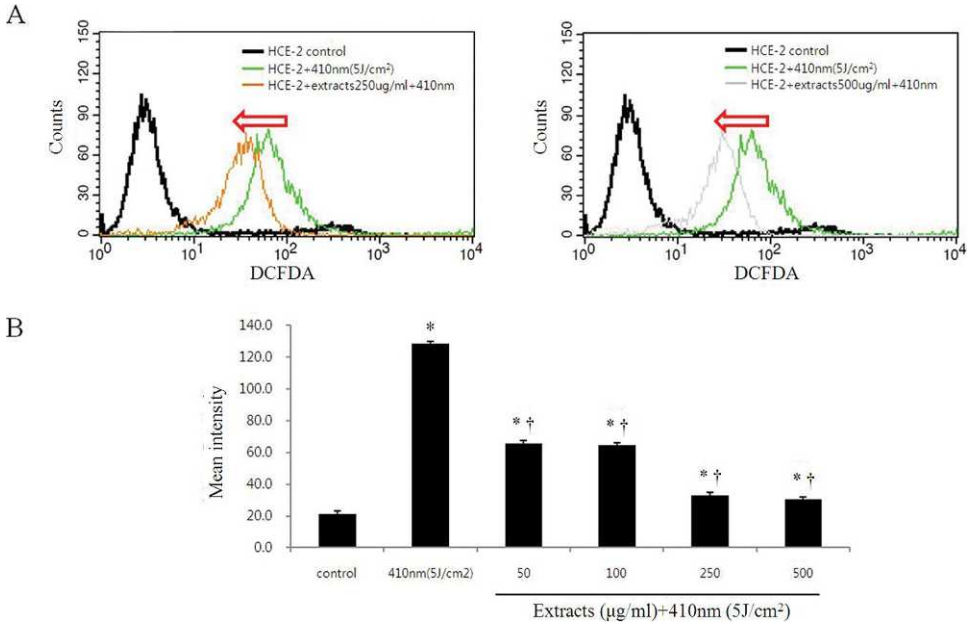

Figure 6. The levels of extracellular ROS measured by DCF-DA and flow cytometry. Representative figures (**A**) and the graph (**B**) showing the inhibition of ROS production by the mixed plant extracts with various concentrations (50–500 μg/mL) against irradiation of LED (410 nm and 5 J/cm^2) in HCEs. *$P < 0.05$ compared with the control. †$P < 0.05$ compared with LED irradiation alone.

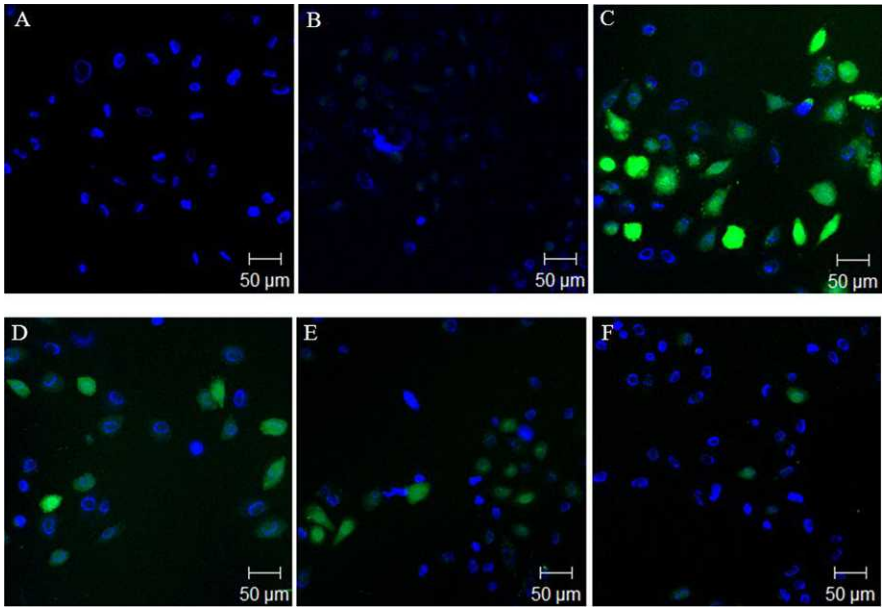

FIGURE 7. Confocal microscopic assay and DCF-DA showing the expression of intracellular reactive oxygen species (*green fluorescence*) after irradiation of LED with or without the mixed plant extracts. Nuclei were counterstained with propidium iodide (*blue fluorescence*). Representative images in human corneal epithelial cells in the control (**A**), 630 ± 8 nm LED (**B**), 410 ± 10 nm LED (**C**), 410 ± 10 nm LED + 50 μg/mL extracts (**D**), 410 ± 10 nm LED + 100 μg/mL extracts (**E**), and 410 ± 10 nm LED + 250 μg/mL extracts (**F**) groups.

Kitagawa is generally accepted to have antioxidant and anti-inflammatory effects, which may be related to the reduction of ROS and the increase in the activities of antioxidant enzymes.[35–39] *Angelica dahurica Bentham ET hooker* upregulates HO-1 expression and suppresses the NF-κB pathway, and it has been used to treat oxidative stress–related inflammatory diseases such as asthma.[40,41] In addition, *Rehmannia glutinosa* increases the activities of antioxidant enzymes and have potential as a preventive treatment for oxidative stress–mediated diseases.[42,43] Additionally, *Cassia tora L.* exhibited antioxidant properties in a model system that was dependent on the activities of reducing metal ions, scavenging hydroxyl radicals, and chelating ferrous ion.[44,45] We used a mixture of the four plant components to potentiate the antioxidant effects. Although the IC_{50} value of the extracts associated with DPPH radical scavenging activity was lower than that of BHT, the mixture of extracts had a higher level of the IC_{50} value compared with the individual EtOH extracts of *Schizonepeta tenuifolia var. japonica Kitagawa, Angelica dahurica Bentham ET hooker, Rehmannia glutinosa Liboschitz var. purpurea Makino,* and *Cassia tora L.* These findings suggest that additive effects might exist between these individual extracts, which could make the mixture more potent.

In our experiments, the increased mRNA and protein levels of the antioxidant enzymes HO-1, Prx-1, CAT, and SOD-2 in corneal epithelial cells were induced by EtOH extracts from the mixed medicinal plants and increased in a dose-dependent manner. The activities of the antioxidant enzymes SOD and CAT were also increased by the addition of the extracts. Interestingly, the mixed plant extracts could inhibit the amount of ROS produced after 410 ± 10 nm LED irradiation. In the intracellular ROS assay evaluated with confocal microscopy, fluorescence was noted to be increased in the 410 ± 10 nm LED irradiated group compared with the 630 ± 8 nm LED irradiated and control groups, while it was attenuated by the extracts in a concentration-dependent manner. These findings suggest that the mixed plant extracts used in this study can protect the ocular surface epithelium from blue light–induced oxidative stress through the antioxidant enzymatic defense system.

The present study was conducted only with EtOH extracts from the mixture of various medicinal plants. However, additional studies with more fractionated extracts should be performed to evaluate the details of the mixture of the medicinal plants. In addition, studies need to be conducted to identify and quantify the volatile compounds that contribute to naturally occurring flavors in the mixture of the various medicinal plants that are presumed to be protective agents against oxidative stress induced by blue and UV light in human corneal epithelial cells. Studies are also needed to compare the antioxidative activity of the mixture with that of other identified antioxidants.

Taken together, our results indicate that blue light with short wavelengths (410–480 nm) can induce oxidative damage to the corneal epithelium, and that medicinal plant mixtures are effective in decreasing ROS by inducing the production of antioxidant enzymes in epithelial cells. These findings can be extrapolated to help elucidate the pathogenesis of and develop treatments for human dry eye diseases. It is known that oxidative stress as well as inflammation in the lacrimal functional unit plays an important role in the pathogenesis of dry eye disease.[23–26] Several antioxidants such as green tea polyphenols and omega-3 fatty acids have both anti-inflammatory and antioxidative effects in human corneal epithelial cells, and topical or systemic use of these antioxidant agents can decrease clinical signs and inflammatory markers in the tear film, ocular surface, and lacrimal gland of dry eye.[28–34] Because mixed medicinal plant extracts exhibited potent efficacy in the treatment of blue light–induced oxidative stress in this study, they may also be effective in the treatment of oxidative stress–induced ocular surface diseases, including dry eye. Further studies are needed to validate anti-inflammatory and antioxidant effects of the extracts on the ocular surface.

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