Ecklonia cava Extract and Dieckol Attenuate Cellular Lipid Peroxidation in Keratinocytes Exposed to PM10

Jeong-won Lee,1 Jin Kyung Seok,1 and Yong Chool Boo1,2

1Department of Molecular Medicine, Cell and Matrix Research Institute, BK21 Plus KNU Biomedical Convergence Program, School of Medicine, Kyungpook National University, 680 Gukchaebosang-ro, Jung-gu, Daegu 41944, Republic of Korea
2Ruby Crown Co., Ltd., 201 Kyungpook National University Business Incubation Center, 80 Daehak-ro, Buk-gu, Daegu 41566, Republic of Korea

Correspondence should be addressed to Yong Chool Boo; yboo@knu.ac.kr

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Airborneparticulatemattercancauseoxidativestress,inflammation, and premature skin aging. Marine plants such as Ecklonia cava Kjellman contain high amounts of polyphenolic antioxidants. The purpose of this study was to examine the antioxidative effects of E. cava extract in cultured keratinocytes exposed to airborne particulate matter with a diameter of <10 μm (PM10). After the exposure of cultured HaCaT keratinocytes to PM10 in the absence and presence of E. cava extract and its constituents, cell viability and cellular lipid peroxidation were assessed. The effects of eckol and dieckol on cellular lipid peroxidation and cytokine expression were examined in human epidermal keratinocytes exposed to PM10. The total phenolic content of E. cava extract was the highest among the 50 marine plant extracts examined. The exposure of HaCaT cells to PM10 decreased cell viability and increased lipid peroxidation. The PM10-induced cellular lipid peroxidation was attenuated by E. cava extract and its ethyl acetate fraction. Dieckol more effectively attenuated cellular lipid peroxidation than eckol in both HaCaT cells and human epidermal keratinocytes. Dieckol and eckol attenuated the expression of inflammatory cytokines such as tumor necrosis factor- (TNF-)α, interleukin- (IL-)β, IL-6, and IL-8 in human epidermal keratinocytes stimulated with PM10. This study suggested that the polyphenolic constituents of E. cava, such as dieckol, attenuated the oxidative and inflammatory reactions in skin cells exposed to airborne particulate matter.

1. Introduction

Air pollution of natural and artificial origins presents a significant concern. Particulate matter of less than 10 micrometers (PM10) suspended in the atmosphere has become a major threat to human health [1, 2]. Because PM10 is too small to be caught in the nasal cavity and bronchial cilia, it can deeply penetrate the lungs and cause inflammation, asthma, chronic bronchitis, and airway obstruction [3, 4]. PM10 can infiltrate blood vessels and then circulate throughout the body, which may cause cardiovascular and cerebrovascular diseases [5]. The particles can also attack eyes, causing various diseases such as allergic conjunctivitis and dry eye syndrome [6]. PM10 penetrates the skin through pores and other inflamed sites, aggravating skin diseases such as atopic dermatitis, acne, and psoriasis [7]. Furthermore, airborne particles are associated with premature skin aging [8].

The best strategy for the prevention and alleviation of airborne particle-induced diseases may be to reduce air pollution and the chance of exposure to air pollution. It is recommended that people minimize outdoor activity and wear a hat, mask, glasses, and long sleeves to protect the body from airborne particles. Although the skin is considered to be a physical barrier to the outer environment, the size and reactivity of PM10 are such that it can clog skin pores and induce inflammation. Therefore, dermatological and cosmetic approaches are needed to attenuate the oxidative and inflammatory reactions that arise from PM10 exposure.

Natural polyphenolic compounds may mitigate oxidative stress and inflammation as a result of exposure to PM10. In a previous study, chocolate reduced cardiac inflammation in mice exposed to urban air pollution [9]. The extract of Eucheuma cottonii attenuated inflammation and oxidative stress induced by chronic exposure to coal dust in rats [10].
Pomegranate peel extract and punicalagin attenuated the PM10-induced adhesion of THP-1 cells to endothelial cells, which was indicative of its anti-inflammatory activity against particulate matter [11].

Marine plants have emerged as a potential resource of bioactive compounds for the development of cosmeceutical ingredients [12]. Marine algae such as Ecklonia cava Kjellman have been shown to inhibit cellular melanin synthesis in murine melanoma B16/F10 cells [13, 14]. In our previous study, the extract of *Phyllospadix iwatesis* Makino was shown to inhibit human tyrosinase activity and melanin synthesis in human dermal melanocytes [15]. However, little is known about the antioxidant effects of marine plant extracts in skin cells that have been exposed to air pollution. In our preliminary experiments, 50 marine plants indigenous to Korea were analyzed to quantify the total phenol content (TPC). Among these plants, the *E. cava* extract was found to have the highest TPC. Therefore, we examined the antioxidant effects of *E. cava* extract in keratinocytes exposed to PM10.

2. Materials and Methods

2.1. Reagents. A standardized fine dust (PM$_{10}$-like) (European Reference Material ERM-CZ120) and gallic acid (purity > 98%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Eckol (purity > 98%) and dieckol (purity > 98%) were purchased from National Development Institute of Korean Medicine (Gyeongsangbuk-do, Republic of Korea).

2.2. Plant Extracts. The extracts of 50 different marine plants, extracted with 80% (v/v) aqueous ethanol, were purchased from Jeju Biodiversity Research Institute of Jeju Technopark (Jeju, Korea). The list of the plant extracts has been previously reported [15].

2.3. Determination of TPC. The TPC of each plant extract was determined using the Folin–Ciocalteau method. The extract was dissolved in 95% ethanol at 1.0 mg mL$^{-1}$, and 20 μL aliquots were added to a 96-well microplate. A calibration curve using gallic acid (100–500 μg mL$^{-1}$) was established. Folin–Ciocalteau reagent (Sigma-Aldrich) was diluted 10-fold in water and 100 μL was added to each well, which was reacted at 18–21°C (room temperature) for 5 min. Subsequently, 80 μL of 7.5% Na$_2$CO$_3$ solution was added to the mixture and then allowed to stand for 60 min in the dark. The absorbance of the mixture was measured at 765 nm using a SPECTROstar Nano microplate reader (BMG LABTECH GmbH, Ortenberg, Germany) and the calculated TPC value was expressed as μg gallic acid equivalent (GAE) per mg extract (μg GAE mg$^{-1}$ extract).

2.4. Preparation of *E. cava* Extracts and Fractions. The extract of *Ecklonia cava* Kjellman was prepared in our laboratory from the plant materials purchased from Jayeoncho (http://www.jherb.com) (Seoul, Korea). The dried *E. cava* (500 g) was extracted with 80% aqueous ethanol (2.5 L) for 7 days at room temperature. The extract solution was evaporated under reduced pressure to obtain the crude extract (71.5 g). The extract (4.2 g) was suspended in water (WT) (50 mL) and partitioned sequentially with an equal volume of methylene chloride (MC), ethyl acetate (EA), and n-butyl alcohol (BA). These fractions were evaporated under reduced pressure to yield four fractions: the MC (0.38 g), EA (0.72 g), BA (0.88 g), and WT fractions (2.2 g).

2.5. High-Performance Liquid Chromatography (HPLC). HPLC was performed by using a Gilson HPLC system (Gilson, Inc., Middleton, WI, USA) with a 321 pump and UV/VIS 151 detector. The separation was performed on a 5 μm Hector-M C$_{18}$ column (4.6 mm × 250 mm) (RS Tech Co., Daejeon, Korea) using a mobile phase of 0.1% phosphoric acid (A) and acetonitrile (B). The gradient program was set as follows: 0–30 min, a linear gradient from 0 to 100% B; 30–40 min, 100% B. The flow rate was 0.6 mL min$^{-1}$ and the detector was set at 280 nm.

2.6. Cell Culture. The cells were cultured in a closed incubator at 37°C in humidified air containing 5% CO$_2$. HaCaT cells (an immortalized human keratinocyte cell line) were grown in DMEM/F-12 medium (GIBCO-BRL, Grand Island, NY, USA) supplemented with 10% fetal bovine serum, antibiotics (100 U mL$^{-1}$ penicillin, 100 μg mL$^{-1}$ streptomycin, and 0.25 μg mL$^{-1}$ amphotericin B), and 10 μg mL$^{-1}$ hydrocortisone. Primary human epidermal keratinocytes from adult human donors (Invitrogen, Waltham, MA, USA) were cultured in EpiLife medium (Gibco BRL) supplemented with 10% EpiLife defined growth supplement (#S0125) and antibiotics.

2.7. Treatments with PM10. The cells were plated onto 6-well culture plates (SPL Life Sciences, Pocheon, Korea) at 8 × 10$^4$ cells/well, cultured for 24 h, and then treated with PM10 at various concentrations (3–100 μg mL$^{-1}$) for 48 h. In some experiments, the cells were treated with a fixed concentration of PM10 (100 μg mL$^{-1}$) in the absence and presence of varied concentrations of a test material.

2.8. Cell Viability Assay. The cells were plated on 48-well culture plates at 1.6 × 10$^4$ cells/well and the cell viability was assessed by an MTT assay, which assesses the cellular metabolic activity of the reduction of 3-[4,5-dimethyldihalazon-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) to formazan dye. The cells were washed with phosphate-buffered saline (PBS) and incubated in culture medium (200 μL) containing 1 mg mL$^{-1}$ MTT (Amresco, Solon, OH, USA) for 2 h at room temperature. After the medium was removed, the formazan dye in the cells was solubilized in dimethyl sulfoxide (200 μL). The resulting dye solution was placed in a 96-well plate and the absorbance was determined at 595 nm by using a SPECTROstar Nano microplate reader.

2.9. Assay of Lipid Peroxidation. The cells were plated in 100 mm culture dishes at 5 × 10$^5$ cells/dish and the cellular lipid peroxidation was assessed through the measurement of 2-thiobarbituric acid-reactive substances (TBARS) [16]. The
cell extracts were prepared by using cell lysis buffer (20 mM Tris-Cl, 2.5 mM EDTA, 1.0% SDS, pH 7.5). The assay mixture consisted of cell extract (300 μg protein), 100 μL lysis buffer, 50 μL m-phosphoric acid, and 350 μL 0.9% 2-thiobarbituric acid (Sigma-Aldrich), which was then heated in a boiling water bath for 45 min. After cooling, 500 μL BA was added to the mixture, which was then vortex-mixed and centrifuged at 10,000 x g for 15 min to produce two separate layers. The fluorescence intensity of the BA layer (excitation at 540 nm and emission at 590 nm) was measured by using a Gemini EM fluorescence microplate reader (Molecular Devices).

2.10. Quantitative Reverse-Transcriptase Polymerase Chain Reaction (qRT-PCR) Analysis. The mRNA levels of TNF-α, IL-1β, IL-6, and IL-8 were determined by qRT-PCR. Cellular RNA was extracted with an RNaseasy kit (Qiagen, Valencia, CA, USA) and was used for the preparation of cDNA by using a High Capacity cDNA Archive Kit (Applied Biosystems, Foster City, CA, USA). Gene-specific primers were purchased from Macrogen (Seoul, Korea). The sequences of the primers were as follows: TNF-α (TNF, NM_000594.3), 5'-TGCTCC-TACCCCAACCAT-3' (forward) and 5'-GAGATAGTC-GGGCCGATTGA-3' (reverse); IL-1β (IL1B, NM_000576.2), 5'-CCTCTGCTGCTGCTTTGAAGA-3' (forward) and 5'-GTTGGTTGGGTTAAGAC-3' (reverse); IL-6 (IL6, NM_00138095.1, NM_000600.4), 5'-AAGCCAGAGTG-TGAGATGAGTA-3' (forward) and 5'-TGCTGGCA-GCCTGTGC-3' (reverse); IL-8 (IL8, NM_000584.3), 5'-CTGGGCAACAGAATTTA-3' (forward) and 5'-ACT-TCCTCAACAACCCTTCG-3' (reverse); glyceraldehyde 3-phosphate dehydrogenase (GAPDH, NM_002046.3), 5'-ATGGGGAAGTGTAAGGTCG-3' (forward) and 5'-GGG-GTATGTGGGAACAA-3' (reverse). The qRT-PCR was conducted with a StepOnePlus™ Real-Time PCR System (Applied Biosystems). The reaction mixture (20 μL) consisted of SYBR® Green PCR Master Mix (Applied Biosystems), cDNA (60 ng), and gene-specific primer sets (2 pmole). The thermal cycling parameters to be used for the PCR reactions were 50°C for 2 min, 95°C for 10 min, 40 amplification cycles of 95°C for 15 s and 60°C for 1 min, and a dissociation step. The mRNA levels of each gene were calculated relative to that of GAPDH by using the comparative threshold cycle method.

2.11. Enzyme-Linked Immunosorbent Assay (ELISA). The protein levels of TNF-α, IL-1β, IL-6, and IL-8 in the culture medium were determined using respective ELISA kits (K033131, K0331800, K033194, and K0331216; Koma Biotech, Seoul, Korea). Briefly, cell culture media or standard protein solutions (100 μL) were transferred to microplate wells containing immobilized antibodies for each protein, followed by the incubation for 2 h at room temperature. The wells were then washed and incubated for 2 h with biotinylated antibodies for each protein and incubated for 45 min with horseradish peroxidase-conjugated streptavidin. The wells were washed and 3,3',5,5'-tetramethylbenzidine substrate solution was added to initiate enzymatic color development. After 30 min, a stop solution was added to terminate the reaction and the absorbance was measured at 450 nm. The concentrations of cytokines were estimated from each standard curve.

2.12. Statistical Analysis. The data were presented as mean ± standard errors (SE) of three or more independent experiments. The significance of the differences between groups was determined by using Student’s t-test and value of p < 0.05 was considered statistically significant.

3. Results

When human HaCaT keratinocytes were treated with PM10 (3 to 100 μg mL⁻¹ for 48 h), their viability decreased in a dose-dependent manner. In addition, PM10 increased lipid peroxidation, as determined by the levels of TBARS (Figure 1(b)).

![Figure 1: The effects of PM10 on viability of and lipid peroxidation in cultured HaCaT keratinocytes. The cells were treated with PM10 at the indicated concentrations for 48 h, followed by the determination of the cell viability (a) and cellular lipid peroxidation (b). The data are presented as a percentage of the control values (mean ± SE, n = 3). *p < 0.05 versus control.](image-url)
Figure 2: The effects of *E. cava* extract on viability of and lipid peroxidation in HaCaT keratinocytes exposed to PM10. In (a), the cells were treated with *E. cava* extract at the indicated concentrations for 48 h. In (b) and (c), the cells were exposed to PM10 (100 μg mL⁻¹) for 48 h in the absence and presence of *E. cava* extract at the indicated concentrations. The cell viability (a, b) and cellular lipid peroxidation (c) were determined. The data are presented as the percentage of the control value (mean ± SE, *n* = 3). ∗* *P < 0.05 versus control and #* *P < 0.05 versus PM10 control.

Of the 50 plant extracts, the extract of *Ecklonia cava* Kjellman had the highest TPC (89 μg GAE mg⁻¹ extract), followed by *Distromium decumbens* (Okamura) Levring, *Acrosorium yendoi* Yamada, *Eisenia bicyclis* (Kjellman) Setchell, and *Martensia denticulata* Harvey, as shown in Table 1. Thus, further studies focused on *E. cava* extract.

*E. cava* extract was cytotoxic to HaCaT cells at concentrations above 100 μg mL⁻¹ (Figure 2a). In the following experiments, the cells were treated with *E. cava* extract at 25, 50, 75, or 100 μg mL⁻¹ and then exposed to 100 μg mL⁻¹ PM10 for 48 h. At 25–50 μg mL⁻¹, *E. cava* extract had no significant effect on the viability of HaCaT cells exposed to PM10 (Figure 2b), but significantly attenuated cellular lipid peroxidation (Figure 2c). At higher concentrations, *E. cava* extract attenuated cellular lipid peroxidation more effectively (Figure 2c) but it also decreased the cell viability (Figure 2b).

Cells were treated with various solvent fractions of *E. cava* extract at 100 μg mL⁻¹ and then exposed to 100 μg mL⁻¹ PM10 for 48 h. In the absence of PM10, the water and *n*-butyl alcohol fraction of *E. cava* extract showed significant cytotoxicities, whereas the ethyl acetate fraction had no effect on the cell viability in comparison with the control (Figure 3b). All solvent fractions had no effects on the viabilities of the cells exposed to PM10 (Figure 3b). In the absence of PM10, the ethyl acetate and *n*-butyl alcohol fraction decreased the basal levels of lipid peroxidation (Figure 3c). The ethyl acetate fraction attenuated the PM10-stimulated cellular lipid peroxidation more effectively compared with other solvent fractions (Figure 3c).

*E. cava* contains various polyphenolic compounds, including eckol and dieckol [17]. As shown in Figures 4a and 4b, *E. cava* extract contained eckol and dieckol; these compounds were enriched in the ethyl acetate fraction. When tested in HaCaT cells exposed to PM10, eckol rescued cell
| Marine plant name                      | Catalogue number | Parts used | TPC* (µg GAE mg⁻¹ extract) |
|---------------------------------------|------------------|------------|----------------------------|
| Acrosorium yendoi Yamada              | JBRI-20419       | Whole plants | 42.70                      |
| Agarum cribrosum Bory de Saint-Vincent| JBRI-20086       | Leaves     | 19.31                      |
| Amphiroa anepa (Lamarck) Decaisne      | JBRI-20173       | Whole plants | 8.16                       |
| Asparagopsis taxiformis (Delile) Trevisan| JBRI-20407      | Whole plants | 17.76                      |
| Bonnemaisonia hamifera Hariot         | JBRI-10278       | Leaves     | 7.18                       |
| Callophyllis japonica Okamura         | JBRI-20365       | Whole plants | 10.35                      |
| Callophyllis crispata Okamura         | JBRI-10353       | Leaves     | 3.28                       |
| Carpophelia affinis (Harvey) Okamura  | JBRI-10376       | Leaves     | 5.63                       |
| Chamaia parvula (C. Agardh) Harvey    | JBRI-20408       | Whole plants | 7.70                       |
| Chondracanthus tenellus (Harvey) Hommersand| JBRI-10270     | Leaves     | 18.79                      |
| Chondria crassicaulis Harvey          | JBRI-10264       | Leaves     | 3.74                       |
| Chondrus ocellatus Holmes             | JBRI-10267       | Leaves     | 5.81                       |
| Cladophora wrightiana Harvey          | JBRI-20396       | Whole plants | 16.67                      |
| Codium fragile (Suringar) Hariot      | JBRI-20092       | Leaves     | 1.95                       |
| Colpomenia sinuosa (Mertens ex Roth) Derbes et Solier| JBRI-10355 | Leaves     | 2.41                       |
| Costaria costata (C. Agardh) Saunders | JBRI-20020       | Leaves     | 2.76                       |
| Desmarestia tabacoides Okamura        | JBRI-20087       | Leaves     | 7.24                       |
| Dictyopteris dichotoma (Hudson) Lamouroux| JBRI-20096      | Leaves     | 6.95                       |
| Dictyota okamuriae (Dawson) Hötning, Schnetter, et Prud’homme van Reine| JBRI-20435 | Whole plants | 12.07                      |
| Distromium decumbens Okamura         | JBRI-20161       | Leaves     | 52.36                      |
| Ecklonia cava Kjellman               | JBRI-10282       | Leaves     | 89.43                      |
| Eisenia bicyclis (Kjellman) Sutchell | JBRI-20022       | Leaves     | 39.14                      |
| Galaxaura falcata Kjellman           | JBRI-20367       | Whole plants | 6.72                       |
| Gelidium amansii (Lam.) Lamouroux     | JBRI-10244       | Leaves     | 7.30                       |
| Gloiopeilitis complanata (Harvey) Yamada| JBRI-10527      | Leaves     | 33.10                      |
| Gracilaria verrucosa (Hudson) Papenfuss| JBRI-10698      | Leaves     | 5.23                       |
| Grateloupia crispata (Okamura) Lee    | JBRI-20366       | Whole plants | 5.29                       |
| Grateloupia elliptica Holmes          | JBRI-10263       | Leaves     | 3.22                       |
| Hydroclathrus clathratus (C. Agardh) Howe| JBRI-10366      | Leaves     | 0.29                       |
| Hypnea charoides Lamouroux           | JBRI-20378       | Whole plants | 9.31                       |
| Ishige okamurae Yendo                | JBRI-10276       | Leaves     | 29.20                      |
| Laurencia intermedi Lamouroux        | JBRI-10351       | Leaves     | 3.68                       |
| Leathesia difformis (Linnaeus) Areschoug| JBRI-20346      | Whole plants | 4.20                       |
| Lomentaria hakodatensis Yendo        | JBRI-10266       | Leaves     | 4.028                      |
| Martensia denticulata Harvey         | JBRI-20221       | Leaves     | 36.72                      |
| Myagropsis myagroides (Martens ex Turner) Fensholt| JBRI-20370  | Whole plants | 2.36                       |
| Myelophyclus simplex (Harvey) Papenfuss| JBRI-10262      | Leaves     | 2.30                       |
| Pachydictyon coriaceum (Holmes) Okamura| JBRI-20110      | Leaves     | 5.58                       |
| Padina arborescens Holmes            | JBRI-10172       | Leaves     | 5.00                       |
| Petalonia binghamiae (J. Agardh) Vinogradova| JBRI-10173   | Leaves     | 8.28                       |
| Phyllospadix iwatensis Makino         | JBRI-10700       | Leaves     | 23.79                      |
| Plocamium telfairiae (Harvey) Harvey  | JBRI-20039       | Leaves     | 11.67                      |
| Prioritis cornae (Okamura) Dawson     | JBRI-10272       | Leaves     | 5.12                       |
| Pterocladia capillacea (Gmelin) Bornet| JBRI-20027       | Leaves     | 6.38                       |
Table 1: Continued.

| Marine plant name                  | Catalogue number | Parts used | TPC* (µg GAE mg⁻¹ extract) |
|------------------------------------|------------------|------------|---------------------------|
| Sargassum fusiformis (Harvey) Okamura | JBRI-10495      | Leaves    | 2.93                      |
| Scytosiphon gracilis Kogame         | JBRI-10517      | Leaves    | 3.10                      |
| Ulothrix flaccia (Dillwyn) Thuret   | JBRI-20359      | Whole plants | 1.38                    |
| Ulva fasciata Delile                | JBRI-20025      | Leaves    | 4.37                      |
| Undaria pinnatifida (Harvey) Suringar | JBRI-20051     | Leaves    | 1.27                      |
| Zostera marina L.                   | JBRI-20093      | Leaves    | 4.43                      |

**Ecklonia cava**

70% aqueous ethanol

Ecklonia cava extract (4.2 g)

Methylene chloride (MC) fraction (0.38 g)

Ethyl acetate (EA) fraction (0.72 g)

\n
n-Butyl alcohol (BA) fraction (0.88 g)

Water (WT) fraction (2.2 g)

Figure 3: The effects of various solvent fractions of *E. cava* extract on viability of and lipid peroxidation in HaCaT keratinocytes exposed to PM10. In (a), *E. cava* extract was fractionated into the methylene chloride (MC), ethyl acetate (EA), \textit{n}-butyl alcohol (BA), and water (WT) fractions. In (b) and (c), the cells were exposed to PM10 (100 µg mL⁻¹) for 48 h in the absence and presence of each fraction (100 µg mL⁻¹). The cell viability (b) and cellular lipid peroxidation (c) were determined. The data are presented as the percentage of the control values (mean ± SE, *n* = 3). *p < 0.05 versus control and †p < 0.05 versus PM10 control.
viability at 3 μg mL⁻¹ and dieckol attenuated lipid peroxidation at 10 μg mL⁻¹ (Figure 5).

Human epidermal keratinocytes were also used to examine the antioxidant and anti-inflammatory effects of eckol and dieckol. Dieckol rescued cell viability and attenuated cellular lipid peroxidation in human epidermal keratinocytes exposed to PM10 (Figure 6). In the PM10-exposed human epidermal keratinocytes, dieckol attenuated the expression of inflammatory cytokines such as TNF-α, IL-1β, IL-6, and IL-8 at the mRNA and protein levels, more effectively than eckol (Figures 7 and 8).

4. Discussion

E. cava is a brown alga (Lessoniaceae) found in the coastal area of Korea and Japan and has been used as food and traditional medicine [18]. It is a rich source of phlorotannins and fucoidans that possesses a wide range of biological activities [17]. Phlorotannins are produced by the polymerization of phloroglucinol and are found only in marine brown algae. E. cava contains various phlorotannins, such as eckol, dieckol, 6,6'-dieckol, eckstolonol, and triphlorethol-A [19]. The biological effects of E. cava identified so far include antioxidant, anti-inflammatory, antibacterial, antidiabetic, and anticancer activities [20–25].

The present study demonstrated that the antioxidant effects of E. cava extract mitigated cellular lipid peroxidation in HaCaT keratinocytes exposed to PM10. In addition, one of its constituent compounds, dieckol, attenuated cellular lipid peroxidation and the expression of inflammatory cytokines in human epidermal keratinocytes exposed to PM10. Although the antioxidant and anti-inflammatory effects of E. cava extract have been reported in other experimental models, the present study was the first to describe the antioxidant
Figure 5: The effects of eckol and dieckol on viability of and lipid peroxidation in HaCaT keratinocytes exposed to PM10. The cells were exposed to PM10 (100 µg mL⁻¹) for 48h in the absence and presence of eckol or dieckol at different concentrations (1–10 µg mL⁻¹). The cell viability (a, b) and cellular lipid peroxidation (c, d) were determined. The data are presented as a percentage of the control values (mean ± SE, n = 3). #p < 0.05 versus PM10 control.

Figure 6: The effects of eckol and dieckol on viability of and lipid peroxidation in human epidermal keratinocytes exposed to PM10. The cells were exposed to PM10 (100 µg mL⁻¹) for 48 h in the absence and presence of eckol or dieckol (10 µg mL⁻¹). The cell viability (a) and cellular lipid peroxidation (b) were determined. The data are presented as the percentage of the control values (mean ± SE, n = 3). #p < 0.05 versus PM10 control.
Figure 7: The effects of eckol and dieckol on the expression of inflammatory cytokines in human epidermal keratinocytes exposed to PM10. The cells were exposed to PM10 (100 μg mL⁻¹) for 24 h in the absence and presence of eckol or dieckol (10 μg mL⁻¹). The mRNA levels of TNF-α (a), IL-1β (b), IL-6 (c), and IL-8 (d) were analyzed by qRT-PCR and normalized to those of GAPDH, a housekeeping gene. The data are presented as the percentage of the control value (mean ± SE, n = 3). *P < 0.05 versus PM10 control.

The composition of airborne PM10 varies depending on the locations and seasons. PM10 usually contains miscellaneous toxic compounds, including transition metals, endotoxins, and ultrafine components, and induces oxidative stress and inflammation in various organs [26, 27]. It stimulates cellular reactive oxygen (ROS) production [28–30] and the expression of inflammatory cytokines such as TNF-α and IL-1β [31, 32]. In the present study, it was confirmed that PM10 increased cellular lipid peroxidation and induced the gene expression of the inflammatory cytokines TNF-α, IL-1β, IL-6, and IL-8. In addition, the PM10-induced lipid peroxidation and cytokine expression were attenuated by dieckol and eckol in human epidermal keratinocytes.

Previous studies have used cell culture models and reconstructed human epidermis models to investigate the harmful effects of airborne pollution on the skin [33–35]. The keratinocytes culture model used in the present study...
Figure 8: The effects of eckol and dieckol on the expression of inflammatory cytokines in human epidermal keratinocytes exposed to PM10. The cells were exposed to PM10 (100 µg mL⁻¹) for 48 h in the absence and presence of eckol or dieckol (10 µg mL⁻¹). The protein levels of TNF-α, IL-1β, IL-6, and IL-8 in the culture medium were analyzed by ELISA and normalized to the total protein content of the cells. Data are presented as fold changes compared to the control value (mean ± SE, n = 3). *p < 0.05 versus PM10 control.

was useful in identifying potential antioxidants which might protect the skin exposed to air pollution. Further studies are needed to validate its antioxidative and anti-inflammatory effects against PM10 in vivo.

In conclusion, this study suggested that the polyphenolic constituents of E. cava, such as dieckol, attenuated the oxidative and inflammatory reactions in skin cells exposed to airborne particulate matter.

Disclosure

Parts of this study were presented as a poster at SfRBM’s 24th Annual Meeting in Baltimore, 2017, and the abstract was published in SfRBM’s 24th Annual Meeting Program and Abstracts (https://doi.org/10.1016/j.freeradbiomed.2017.10.029).

Conflicts of Interest

The authors have no conflicts of interest to declare with regard to the publication of this article.

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