Available online at www.tox.or.kr

MCT

Effects of EGb 761 and Korean Red Ginseng on Melanogenesis in B16F10 Melanoma Cells and Protection Against UVB Irradiation in Murine Skin

Seon Kyu Han[1], **Wook Hee Choi**[2,5], **Hyoung Soo Ann**[3], **Ryoung Me Ahn**[2] & **Seh Yoon Yi**[4]

[1]Bioanalysis and Biotransformation Research Center, Korea Institute of Science and Technology, Seoul 130-650, Korea
[2]Department of Health Science, Dongduk women's University, Seoul 136-714, Korea
[3]Department of Pharmacology, Dongduk women's University, Seoul 136-714, Korea
[4]College of Natural Science, Suwon University, Suwon 445-743, Korea
[5]Environmental Epidemiology Division, Environmental Health Research Department, National Institute of Environmental Research, Incheon 404-708, Korea
Correspondence and requests for materials should be addressed to W. H. Choi (belljin@me.go.kr)

Accepted 20 February 2008

Abstract

These days there is a constant possibility of exposure to UV radiation which can cause abnormal production of melanin and result in skin disease such as hyperpigmentation and melanoma. Many materials were investigated for skin whitening and protection against UV radiation. In this study, we assessed the melanogenesis inhibitory activities of Korean Red Ginseng (KRG, *Ginseng Radix Rubra*) and Ginkgo (EGb 761 *Ginkgo Biloba*) in an attempt to develop a new skin whitening agent derived from natural products. B16F10 melanoma cells were treated for 48 hr with KRG and EGb 761. The inhibitory effect on melanogenesis was measured and related cytokines and proteins expression were also investigated by RT-PCR and Western blotting. In addition, we also assessed the effects of these substances on the skin of C57BL/6 mice. Cell growth, melanin content and tyrosinase activity were inhibited effectively in B16F10 melanoma cells treated with KRG and EGb 761. Moreover, tyrosinase mRNA expression was inhibited clearly and melanogenesis related proteins (MRPs) containing tyrosinase, TRP1 and TRP2 were also reduced by KRG and EGb761, while cytokines such as IL-1β and IL-6 were induced. In the case of UV irradiated mice, we observed induction of cytokine mRNA levels and reduction of MRPs mRNA expression. In addition, a decrease in pigmentation from treatment with KRG and EGb 761 on the skin of mice was observed. These results indicate that KRG and EGb 761 inhibit melanogenesis in B16F10 cells and have display protective activities against UVB. Therefore, we suggest that KRG and EGb 761 are good candidates to be used as whitening agents and UVB protectors for the skin.

Keywords: Korea Red Ginseng (KRG), EGb 761, Melanogenesis, UVB

These days there is a constant possibility of exposure to UV radiation which can cause skin diseases such as hyperpigmentation and melanoma. Hyperpigmentation increases the amount of melanin in epidermis. Melanin synthesis is regulated by melanogenic enzymes such as tyrosinase, tyrosinase-related protein 1 (TRP-1), and tyrosinase-related protein 2 (TRP-2)[1]. Tyrosinase is a bifunctional enzyme which plays an essential role in the modulation of melanin production. First, it catalyzes the hydroxylation of tyrosine to DOPA, and second it catalyzes the oxidation of DOPA to DOPAquinone[2]. Moreover, TRP-2, which functions as DOPAchrome tautomerase, catalyzes the rearrangement of DOPAchrome to 5,6-dihydroxyindole-2-carboxylic acid (DHICA), and TRP1 oxidizes DHICA to carboxylated indole-quinone[3,4]. Both of these enzymes work at downstream points in the melanin biosynthetic pathway. Accordingly, regulation of tyrosinase may control melanin production and as a result tyrosinase inhibitors have become increasingly important in medicine and the cosmetic industry. Several tyrosinase inhibitors have been reported from natural and synthetic sources, and have been screened for their effect as skin-lightening compounds.

Korean Red Ginseng (KRG, *Ginseng Radix Rubra*)

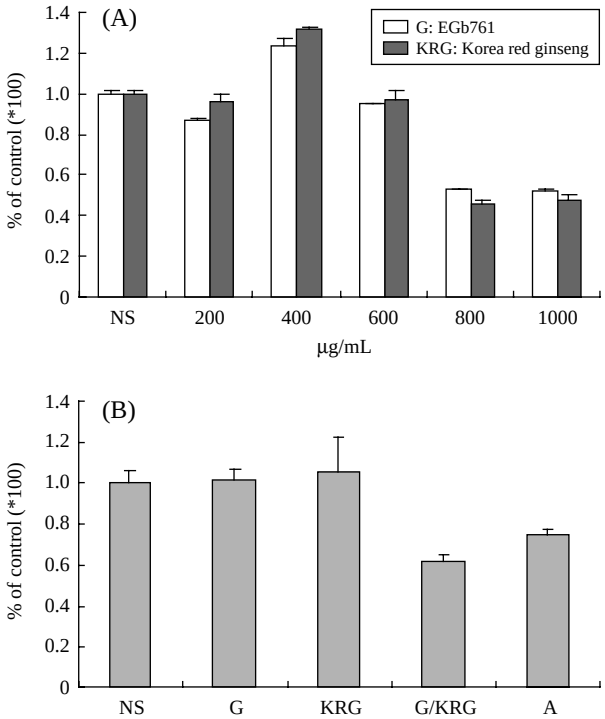

Figure 1. Effect of EGb761 and KRG on the cell proliferation. B16F10 cells were seeded at 5×10^3 cells/well. After 24 hr, the cells were treated with various concentrations of EGb761 and KRG for 48 hr. Cell proliferation was measured by MTT assay as described in Materials and Methods. (A) Each substance was treated with indicated concentrations. (B) EGb761 (300 μg/mL), KRG (300 μg/mL), and G/KRG (each 300 μg/mL) were treated for 48 hr. In all experiments, arbutin (100 μg/mL) was used as positive control. Data is presented as % of control and the values are presented as mean ± SE. of three independent experiments.

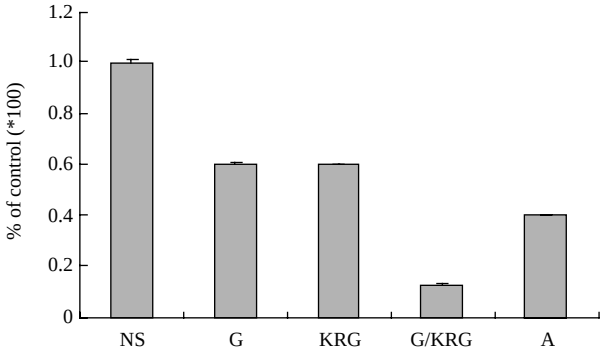

Figure 2. Effect of EGb761 and KRG on melanin content. B16F10 cells were treated with EGb761 (300 μg/mL), KRG (300 μg/mL), and G/KRG (each 300 μg/mL) for 72 hr. Data is presented as % of control and the values are presented as mean ± SE. of three independent experiments.

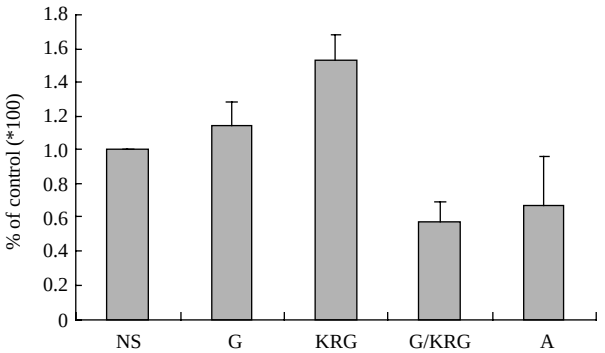

Figure 3. Effect of EGb761 and KRG on tyrosinase activity. B16F10 cells were treated with EGb761 (300 μg/mL), KRG (300 μg/mL), and G/KRG (each 300 μg/mL) for 96 hr. Data is presented as % of control and the values are presented as mean ± SE. of three independent experiments.

and Ginkgo extract (EGb761, *Ginkgo Biloba*) are well-known products, and their biological activities have been investigated extensively[5-7]. Furthermore, it was reported recently that red ginseng treatment inhibited B16 cell growth[8] and EGb 761 has an important effect both as a protective and therapeutic agent, in sunburn after UVB irradiation[9]. Consequently, the present study was undertaken to evaluate the melanogenesis inhibitory activity of KRG and EGb 761 both in B16F10 melanoma cells and on the skin of C57BL /6 mice. In addition, we assessed their protective effects against UVB irradiation.

Effects of EGb 761 and KRG in B16F10 Melanoma Cells

B16F10 murine melanoma cells were treated with predetermined concentrations of EGb 761 and/or KRG (Figure 1). Subsequently, the effect of each substance or mixture on cell proliferation was determined by MTT assay. Each substance showed inhibitory effect on cell proliferation, but in the case of mixture, cytotoxic effect was observed. Therefore, we selected the concentration of each substance at the point where each substance did not inhibit cell proliferation in order to assess the effect of mixture. Therefore, each substance could not inhibit cellular melanin content effectively, while the mixture inhibited effectively (Figure 2A). In order to induce melanin synthesis, we added α-MSH, which resulted in the 2-fold increase of cellular melanin content in control cell, and then cellular content of melanin decreased as a result of treatment with both test substances and also with the combination treatment of EGb 761 and KRG (G/KRG) (Figure 2B). Figure 3 shows that cellular tyro-

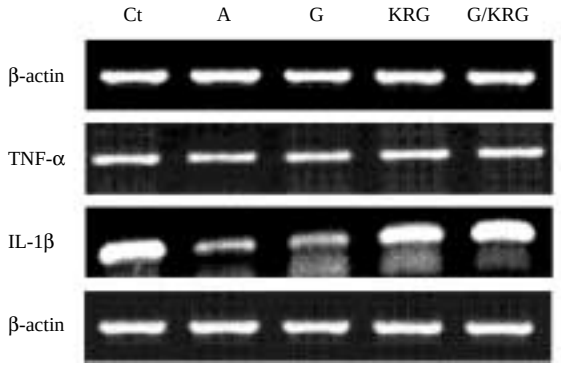

Figure 4. mRNA level of several cytokines following treatment with EGb761 and KRG in B16F10 melanoma cells. B16F10 cells were treated with EGb761 (300 μg/mL), KRG (300 μg/mL), and G/KRG (each 300 μg/mL) for 48 hr. Total RNA was extracted using TRIzol reagents as described in Materials and Methods. Two or three micrograms of intact total RNA was reversibly transcribed into first strand cDNA and amplified using PCR. The products were analyzed on 1 % agarose gel electrophoresis. The data shown is from one of the two independent experiments performed.

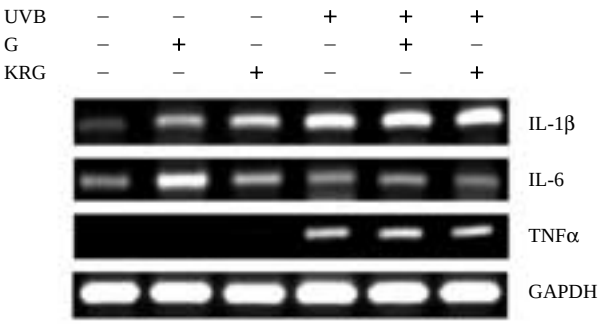

Figure 5. mRNA level of several cytokines following treatment with EGb761 and KRG or UVB on C57BL6 mice skin. The dorsal skin of C57BL/6 mice was shaved one day prior to UVB irradiation. EGb761 and KRG were dissolved in a PEG lotion at a concentration of 1% (w/v) and each lotion was applied to the shaved dorsal skin for 5 days. The skin was then exposed to 1MED UVB radiation for 6 days. Total RNA was extracted from the skin and RT-PCR was performed. The products were analyzed on 1% agarose gel electrophoresis. The data shown is from one of the two independent experiments performed.

sinase activity was increased by EGb 761 and KRG. Furthermore, treatment with G/KRG significantly inhibited cellular tyrosinase activity.

mRNA Level of Several Cytokines in B16F10 Melanoma Cells and in C57BL/6 Mice Skin

To define the effects of our substances on regulation of endogeneous cytokines such as IL-1β, IL-6 and TNF-α, we extracted total RNA from the B16F10 cells treated with EGb 761 or KRG and G/KRG for 48 hr. mRNA levels of each cytokines were then measured using semi quantitative RT-PCR method. In this study, induction of IL-1β was observed, but we were not able to detect IL-6 mRNA levels. IL-1β expression levels were lower than the untreated control, but IL-1β was induced by treatment with KRG, and treatment with G/KRG showed a synergic effect on the induction of IL-1β (Figure 4).

We also attempted to define the effect of EGb 761 and KRG *in vivo*. To accomplish this we applied the test substances to the skin of C57BL/6 mice for 5 days. EGb 761 and KRG were dissolved in a PEG lotion at a concentration of 1% (w/v) and 200 μL of each lotion was applied to the shaved dorsal skin. IL-1β and IL-6 was effectively induced by treatment with EGb 761 and KRG, although we were unable to detect TNF-α mRNA expression (Figure 5). Unlike the *in vitro* experiments, both test substances displayed clear effects in the *in vivo* experiments. However, we did not observe any advantage as a result of the combination treatment (G/KRG).

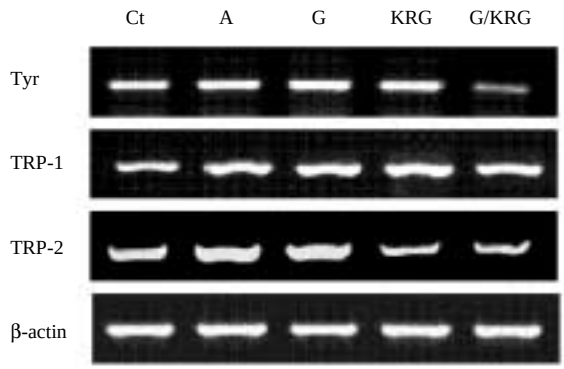

Figure 6. mRNA level of melanogenic enzymes following treatment with EGb761 and KRG in B16F10 melanoma cells. B16F10 cells were treated with EGb761 (300 μg/mL), KRG (300 μg/mL), and G/KRG (each 300 μg/mL) for 48 hr. Total RNA was extracted using TRIzol reagents as described in Materials and Methods. Two or three micrograms of intact total RNA was reversibly transcribed into first strand cDNA and amplified using PCR. The products were analyzed on 1 % agarose gel electrophoresis. The data shown is from one of the two independent experiments performed.

mRNA Level of Melanogenic Enzymes in B16F10 Melanoma Cells and in C57BL/6 Mice Skin

It is well known that several specific proteins are associated with melanogenesis. Melanogenic enzymes containing tyrosinase, tyrosinase-related protein 1 (TRP-1) and tyrosinase-related protein 2 (TRP-2) were examined[10-12]. We observed a reduction in mRNA

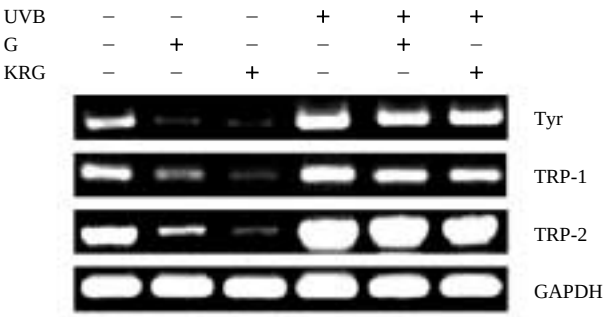

Figure 7. mRNA level of melanogenic enzymes following treatment with EGb761 and KRG or UVB on C57BL6 mice skin. The dorsal skin of C57BL/6 mice was shaved one day prior to UVB irradiation. EGb761 and KRG were dissolved in a PEG lotion at a concentration of 1% (w/v) and each lotion was applied to the shaved dorsal skin for 5 days. The shaved skin was then exposed to 1MED UVB radiation for 6 days. Total RNA was extracted from the skin and RT-PCR was performed. The products were analyzed on 1% agarose gel electrophoresis. The data shown is from one of the two independent experiments performed.

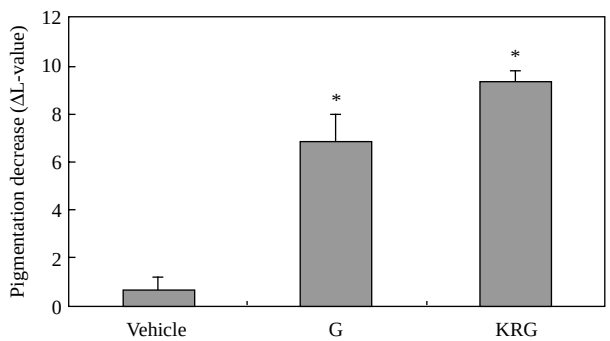

Figure 8. Level of protection against pigmentation by treatment with EGb 761 & KRG. The degree to which pigmentation decreased (ΔL-value) before, and 12 days after daily topical application of vehicle group, which was treated with PEG lotion without substances, and compounds treated group (1% EGb 761, KRG). The data are expressed as a mean ΔL-value ± SEM, t-test was used for the statistical analysis of the data ($P < 0.05$ vs. control).

levels of tyrosinase, and TRP-2 mRNA was reduced by treatment with G/KRG (Figure 6). mRNA expression of melanogenic enzymes such as tyrosinase, TRP -1 and TRP-2 was also reduced significantly by the test substances *in vivo* (Figure 7).

The dorsal skin of C57BL/6 mice was shaved one day prior to UVB irradiation. EGb 761 and KRG were dissolved in a PEG lotion at a concentration of 1% (w/v) and 200 μL of each lotion was applied to the skin for 5 days. The skin was then exposed to 1MED UVB radiation and immediately treated with the PEG lotion containing the test substances (1%) for 6 days. Total RNA was extracted from the skin and RT-PCR was performed. Inflammatory cytokines were induced by irradiation with a high level. Consequently, we were unable to observe changes in mRNA levels of cytokines by the test substances (Figure 5). However, tyrosinase and TRP-1 were slightly decreased by treatment with EGb 761 and KRG (Figure 7). In addition, the degree to which pigmentation decreased from treatment with EGb 761 and KRG was comparable to the vehicle group, which was treated with PEG lotion without substances, against UVB irradiation. The degree to which pigmentation decreased was significantly increased by treatment with EGb 761 and KRG (Figure 8).

Discussion

Recently, many studies have focused on the mechanism associated with melanogenesis in an attempt to develop new agents for skin-whitening. In this regard, agents which are able to inhibit tyrosinase activity and melanin production, such as arbutin, kojic acid, vitamin C and their derivatives and placenta extract have been used[8,13-16]. This study focused on the effects of Ginkgo (EGb 761) and Korean Red Ginseng (KRG) extracts on melanogenesis.

These substances displayed superior results to that of arbutin, which is normally used as a whitening agent. According to these results, we suggest that these substances and its mixture are good candidates to be used as whitening agents from.

We also attempted to explain the mechanism by which melanogenesis is suppressed by these substances. The epidermal melanin unit is dynamic and highly responsive to endogeneous and exogeneous stimuli containing multiple inflammatory mediators, cytokines, and growth factors that may alter constitutive melanocyte function. These inflammatory mediators and cytokines affect not only melanocyte pigment production, but also proliferation. However, the contribution of each to the eventual increase or decrease in melanocyte numbers remains unclear[17]. These cytokines are multifunctional cytokines which exhibit different biologic effects on various different cell types. In addition, they have an opposite effect to pigment production, although these cytokines are released during inflammation[18,19]. Nakajima S *et al.*[10] also reported that KRG is an effective inducer of IL-1β and accelerates endothelial cell proliferation and promotes physiological activities. TNF-α remained unchanged following treatment with the test substances (Figure 4), although it has been reported that TNF-α

inhibited melanogenesis in B16F10 melanoma cells[20]. Therefore, the whitening effect of G/KRG may be due to its ability to regulate mRNA of IL-1β and IL-1β induction which is also related to the inhibition of cell growth. This is consistent with a previous study which reported that IL-1β has an inhibitory effect on melanocyte functions such as pigmentation and cell growth[17-20].

Consequently, these results confirm that these substances are good candidates to be used as whitening agents. Consistent with results from a previous study in which arbutin did not affect the expression of these proteins, the results of this study also showed that mRNA levels of these proteins remained unchanged by arbutin. Although arbutin did not affect mRNA expression of melanogenic enzymes, the test substances were able to regulate mRNA levels effectively. Therefore, these results suggest that these natural substances help to regulate the endogeneous cytokine and melanogenic enzymes expression at the transcription level and then mediate melanogenesis inhibitory activity.

We were also interested in hyperpigmentation, which abnormally increases the amount of melanin in the skin by UV irradiation, and wondered whether the regulatory activities of EGb 761 and KRG can mediate protection against UVB irradiation. Therefore, these substances were also effective in protecting against hyperpigmentation caused by UVB irradiation.

In conclusion, these results clearly demonstrate that these natural substances, EGb 761 and KRG, are potent inhibitors of melnogenesis in B16F10 melanoma cells and C57BL/6 mice skin. In addition, these substances may be useful for protection against UVB irradiation. We suggest that EGb 761 and KRG could be developed as ingredients of whitening or UV protective agents.

Methods

Cell Cultures and Treatment

The murine melanoma cells, B16F10 (provided by the Korean Cell line Bank, Seoul, Korea) were incubated in DMEM media supplemented with 10% fetal bovine serum, 50 μg/mL streptomycin, and 50 μg/mL of penicillin at 37˚C in 5% CO_2. The cells (1×10^5 cells/well) were seeded in 12-well or 6-well plates, and treatment with test substances (EGb 761, Korean red ginseng) began 24 hr after seeding.

Animal Treatment

Female C57BL/6 mice were purchased from Samtako Bio Korea Co., Ltd (Gyeonggi-do, Korea). The animals were 8 weeks of age and were housed in temperature-controlled (23 ± 1˚C), humidity-controlled (55 ± 5%) cages. There were four mice housed in each cage. They were fed water and a commercial diet (Sam31, SCF co., Ltd, Korea).

Reagents

EGb 761 extract (Yuyu industrial co., Ltd, Seoul, Korea), KRG extract (Cheong-Kwan-Jang, Korea Ginseng Corp. Seoul, Korea) were purchased from the companies mentioned above. For topical treatment, EGb 761 extract and KRG extract were dissolved in a PEG lotion at a concentration of 1% (w/v) and 200 μL of each lotion was applied to the shaved dorsal skin of the mice. The PEG lotion was prepared as outlined by Lee SK *et al*.[21]. Topical treatment was applied for 5 days pre-irradiation and immediately after irradiation for 6 or 12 days.

MTT Assay

The cells (1×10^5 cells/well) were seeded in 12-well plates, and then incubated in the supplemented DMEM medium which also contained the test substances at 37˚C in 5% CO_2 for 2 days. Next, 100 μL of the MTT solution (5 mg/mL) was added to each well, and the plates were then incubated for an additional 4 hr. Following the incubation period the supernatants (with MTT solution) were removed. The formazan crystals were resolved in 1.0 mL 0.04 N HCl in isopropanol for 5 min at room temperature. Optical density was determined at 570 nm using an ELISA reader (Molecular Devices, Sunnyvale, CA, USA).

Table 1. Oligonucleotide primers used in RT-PCR.

Oligo	Primer sequence
IL-1β	GCT ACC TGT GTC TTT CCC GTG G TTG TCG TTG CTT GGT TCT CCT TG
IL-6	TGG AGT GAG AGA AGG AGT GGC TAA G TCT GAC CAC AGT GAG GAA TGT CCA C
TNF-α	GGC ACC TCT ACT TTA GAG TCA TTG C ACA TTC GAG GCT CCA GTG AAT TCG G
Tyrosinase	TTC AAA GG GTG GAT GAC CG GAC ACC ATA GTA ATG CAT CC
TRP-1	GCT GCA GGA GCC TTC TTT CTC AAG ACG CTG CAC TGC TGG TCT
TRP-2	GGA TGA CCG TGA GCA ATG GCC CGG TTG TGA CCA ATG GGT GCC
β-actin	AGG CTG TGC TGT CCC TGT ATG C ACC CAA GAA GGA AGG CTG GAA A
GAPDH	GTC ATT GAG AGC AAT GCC AG GTG TTC CTA CCC CCA ATG TG

Measurement of Melanin Contents

Melanin content in the cultured B16F10 cells was measured using a previously reported method[22]. Briefly, the B16F10 cells (5×10^5 cells/well) were seeded in 6-well plates and incubated in the supplemented DMEM medium which also contained the test substances for 3 days. Cells were washed twice with phosphate-buffered saline (PBS) and lysed with 1 N NaOH for 30 min at 100˚C. Relative melanin content was determined by absorbance at 405 nm using an ELISA reader.

Measurement of Tyrosinase Activity

Tyrosinase activity was measured as described previously[23]. The B16F10 cells (5×10^5 cells/well) were seeded in 6-well plates and incubated in the supplemented DMEM medium which also contained the test substances for 3 days. The cells were washed twice with PBS, and lysed with 1% Triton X-100/PBS (pH 6.8). After freezing and thawing, the extracts and 30 μL of 10 mM L-Dopa were incubated at 37˚C for 1 hr or 3 hr. Absorbance was measured at 475 nm. Mushroom tyrosinase was used the standard.

Semi-Quantitative Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)

Total RNA was extracted from the B16F10 cells and treated with the test substances using TRI-zol solution according to the manufacturer's instructions. RNA concentrations were determined spectrophotometrically from absorbance at 260 nm. One microgram of total RNA was reversibly transcribed into first strand cDNA, which was then amplified using PCR. The PCR primers were purchased or synthesized from Bioneer Co. (Seoul, Korea). RT reactions were performed at 42˚C, for 1 hr and then PCR was performed in same tube using RT-PCR pre-mix according to the manufacturer's manual. The oligo-nucleotide primers used for PCR are shown in Table 1. The reaction was cycled 25-30 times through 1 min at 95˚C, 1 min at 55-60˚C and 1 min at 72˚C. The products were analyzed by electrophoresis on 1% agarose gels and stained with ethidium bromide. Each experiment was performed a minimum of three times.

UVB Irradiation

The UVB source was a bank of four T-15M lamps (Vilber lourmat, Germany). The UVB irradiance was measured using a VLX-3W radiometer (6501-54, France). The dorsal skin of mice was shaved with clipper one day prior to UVB irradiation. On the following day, the shaved skin was exposed to 1MED UVB radiation for 6 or 12 days. The minimal erythemal dose (MED) was determined by a statistically significant increase in mid-dorsal skin fold thickness at 24 hr post-irradiation, to be 1.4 KJ/m^2 UVB under our laboratory conditions.

UVB-Induced Pigmentation in C57BL/6 Mice

The mice were divided into six groups with each group containing eight mice. The shaved skin was exposed to 1MED (1.4 KJ/m^2) UVB radiation for 12 days. The irradiated skin was treated immediately after irradiation with lotion containing EGb 761 and KRG. The degree of pigmentation was assessed as the *L*-value measured from a chromameter (Spectron Tech color measurement system).

References

1. Hearing, V. J. Biochemical control of melanogenesis and mealnosomal organization. *J Investigative Dermatol Symp Proc* **4**:24-28 (1999).
2. Hearing, V. J. & Jimenez, M. Mammalian tyrosinase-the critical regulatory control point in melanocyte pigmentation. *Int J Biochem* **19**:1141-1147 (1987).
3. Kobayashi, T. *et al.* Tyrosinase related protein 1 (TRP -1) functions as a DHICA oxidase in melanin biosynthesis. *EMBO J* **13**:5818-5825 (1994).
4. Yokoyama, K., Yasumoto, K., Suzuki, H. & Shibahara, S. Cloning of the human DOPAchrome tautomerase/tyrosinase-related protein 2 gene andidentification of two regulatory regions required for its pigment cell -specific expression. *J Biol Chem* **269**:27080-27087 (1994).
5. Matsunaga, H. *et al*. Studies on the panaxytriol of Panax ginseng C. A. Meyer.; Isolation, determination and antitumor activity. *Chem Pham Bull* **37**:1279-1281 (1989).
6. Lyn, S. Y. & Chnag, H. P. Induction of superoxide dismutase and catalase activity in different rat tissues and protection from UVB irradiation after topical application of Ginkgo biloba extracts. *Methods Find Exp Clin Pharmacol* **19**:367-371 (1997).
7. Kwak, W. J. *et al.* Effects of Ginkgetin from Ginkgo biloba leaves on cyclooxygenases and *in vivo* skin inflammation. *Planta med* **68**:316-321 (2002).
8. Im, S. J. *et al*. Effect of radix ginseng and radix trichosanthis on the melanogenesis. *Biol Pharm Bull* **26**:849-853 (2003).
9. Ozkur, M. K. *et al*. The effects of EGb 761 on lipid peroxide levels and superoxide dismutase activity in sunburn. *Photodermatol Photoimmunol Photomed* **18**:117-120 (2002).
10. Kameyama, K. *et al*. Pigment production in murine melanoma cells is regulated by tyrosinase, tyrosinase-related protein 1 (TRP1), DOPAchrome tautomerase (TRP2), and a melanogenic inhibitor. *J Invest Dermatol* **100**:126-131 (1993).
11. Boissy, R. E. & Nordlund, J. J. Molecular basis of

congenital hypopigmentary disorders in humans: a review. *Pigment Cell Res* **10**:12-24 (1997).
12. Mallick, S. *et al*. Human placentallipid induces melanogenesis by increasing the expression of tyrosinase and its related proteins *in vitro*. *Pigment Cell Res* **18**: 25-33 (2005).
13. Jimbow, K., Obata, H., Pathak, M. A. & Fitzpatrick, T. B. Mechanism of depigmentation by hydroquinone. *J Invest Dermatol* **62**:436-449 (1974).
14. Imokawa, G. & Mishinma, Y. Loss of melanogenic properties in tyrosinases induced by glucosylation inhibitors within malignant melanoma cells. *Cancer Res* **42**:1994-2002 (1982).
15. Maeda, K. & Fukuda, M. Arbutin: mechanism of its depigmenting action in human melanocyte culture. *J Pharmacol Exp Ther* **276**:765-769 (1996).
16. Roh, J. S., Han, J. Y., Kim, J. H. & Hwang, J. K. Inhibitory effects of active compounds isolated from safflower (Carthamus tinctorius L.) seeds for melanogenesis. *Biol Pharm Bull* **27**:1976-1978 (2004).
17. Morelli, J. G. & Norris, D. A. Influence of inflammatory mediators and cytokines on human melanocyte function. *J Invest Dermatol* **100**:191S-195S (1993).
18. Swope, V. B., Abdel-Malek, Z., Kassem, L. M. & Nordlund, J. J. Interleukin 1α and 6 and tumor necrosis factor-α are paracrine inhibitors of human melaocyte proliferation and melanogenesis. *J Invest Dermatol* **96**:180-185 (1991).
19. Nakajima, S. *et al*. The effects of ginseng radix rubra on human vascular endothelial cells. *Am J Chin Med* **26**:365-373 (1998).
20. Martinez-Esparza, M. *et al*. Mechanisms of melanogenesis inhibition by tumor necrosis factor-alpha in B16/F10 mouse melanoma cells. *Eur J Biochem* **255**: 139-146 (1998).
21. Lee, S. K. *et al*. Prevention of ultraviolet radiation-induced suppression of contact hypersensitivity by Aloe vera gel components. *International Journal of immunopharmacology* **21**:303-310 (1999).
22. Curto, E. V. *et al*. Inhibitors of mammalian melanocyte tyrosinase: *In vitro* comparisons of alkyl esters of gentisic acid with other putative inhibitors. *Biochem Pahrmacol* **57**:663-672 (1999).
23. Tomita, Y., Maeda, K. & Tagami, H. Melanocyte-stimulating properties of arachidonic acid metabolites: possible role in postinflammatory pigmentation. *Pigment Cell Res* **5**:357-361 (1992).