The Effects of Bamboo Extract on Human Melanocytes and B16 Melanoma Cells *in vitro*

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ABSTRACT

To identify inhibitory effect of Bamboo extract on melanogenesis, the effect was compared with arbutin, ascorbic acid, hydroquinone, and kojic acid on the melanin biosynthesis in B16 mouse melanoma cells and cultured human melanocytes. The cell viability of the agent was tested on cultured human melanocytes. We also examined its free radical scavenging activity on 1,1-diphenyl-2-picrylhydrazyl (DPPH).

Bamboo extract showed considerable effect against melanin production and did not reduce cell viability at the concentration tested. It also showed potent free radical scavenging activity.

INTRODUCTION

Melanin is the main pigment found in skin, hair, and eyes. It is synthesized enzymatically from tyrosine within melanosomes, which are subsequently transferred to epidermal keratinocytes. Tyrosinase is a multifunctional enzyme responsible for this

melanin biosynthesis (1-3). Melanogenesis is a metabolic process that, in humans seems to have one main aim: to produce rapidly a light absorbing material to protect the body against harmful effects of ultraviolet light. Melanin pigmentation is largely responsible for normal skin color and protection against ultraviolet damage, inducing photocarcinogenesis (4).

However, on the other hand, hyperpigmentation of skin by increased melanogenesis induces cosmetic problems. UV-light, hormonal change, inflammation, and drugs can be etiologic or aggravating factors of hyperpigmentation. The problem with hyperpigmentation is especially severe to colored people compared with white people. This condition, particularly on the face, can be a source of cosmetic disability and mental distress and thus may require treatment.

Over several decades, considerable data have been gathered regarding depigmenting chemical agents. Only recently, however, have their action mechanisms been investigated. The whitening cosmetics are needed to solve this problem. There are many whitening components synthesized from natural extracts or other synthetic products, but these components are still in its early stage.

The objective of this study was to determine the effect of Bamboo extract (*Phyllostachys reticulata C. Koch.*) compared with arbutin, ascorbic acid, hydroquinone, kojic acid on the melanin biosynthesis of B16 mouse melanoma cells and cultured human melanocytes.

MATERIALS AND METHODS

Reagents

Arbutin, ascorbic acid, hydroquinone, and kojic acid were purchased from Sigma.

Preparation of Bamboo extract

Bamboo was purchased from a manufacturer of bamboo-related products in Damyang oriental pharmacy. We selected the inner films of Bamboo stem and then extracted with 80% aqueous ethanol or methanol, filtered and dried under reduced pressure. The extract was dissolved in ethanol or methanol and then used for each experiment.

Culture of B-16 melanoma cells

B-16 melanoma cells(ATCC CRL 6323) were cultured in RPMI supplemented with 10% fetal bovine serum and 10^{-7}M α-MSH in humidified incubator at 37°C under 5% CO_2. Medium changes performed twice a week.

Culture of human melanocytes

Neonatal foreskins obtained from circumcisions were used to culture human melanocytes. Melanocyte cultures were then incubated in F12 supplemented with 10% fetal bovine serum, 80nM TPA, 24ug/ml IBMX, 100ng/ml Cholera toxin, 6.5ug/ml BPE, and antibiotics at 37°C under 5% CO_2. Medium changes performed twice a week.

Assay on melanization of B16 mouse melanoma cells

We examined the inhibition of melanogenesis in B-16 melanoma cell by the modified method of Oikawa et al.(5). Cells were seeded into 100mm petridish at a density of $5x10^5$ cells per dish. After cells were attached, medium was replaced with fresh medium containing various agents. After 3 days, we washed the cells with phosphate buffer saline (PBS) and collected the cells by trypsinization and centrifugation. We dissolved the melanin in 1N NaOH solution. We determined the melanin contents with an

absorbance at 470 ㎚ and expressed as ㎍/4x10^6 cells. A standard curve for melanin determination was prepared using synthetic melanin (Sigma). The cell number was determined by the coulter counter (ZM, Coulter Co. Communications).

Assay on melanization of cultured human melanocytes

Human melanocytes were seeded into T-75 flask at a density of 5x10^5 cells per flask. After cells were attached, medium was replaced with fresh medium containing various agents. After 7 days, we washed the cells with phosphate buffer saline (PBS) and collected the cells by trypsinization and centrifugation. We dissolved the melanin in 1N NaOH solution. We determined the melanin contents with an absorbance at 470 ㎚ and expressed as ㎍/1x10^6 cells. A standard curve for melanin determination was prepared using synthetic melanin. The cell number was determined by the coulter counter.

Cell viability assay

Melanocytes were seeded at a density of 2 x 10^4 cells / well in various culture media. After cells were attached, medium was replaced with fresh medium containing various agents. After 3 days, the cell viability in the presence of each agent was determined by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide, Sigma) assay, as described (6). Briefly, 15 ㎕ of MTT solution (5 ㎎/㎖ in PBS) was added to the medium and cells were incubated for 4 h at 37℃. The medium was gently removed from each well and 150 ㎕ of DMSO(dimethyl sulfoxide) was added. After 30 minutes, the plates were read on a microelisa reader, using a test wavelength of 540 ㎚, a reference wavelength of 650 ㎚.

Assay of scavenging effect on DPPH

To 900 μℓ of methanolic DPPH(200 μM) in a test tube was added 100 μℓ of the test compound(100 μg/mℓ) in ethanol. The decrease in DPPH absorption at 540 nm was measured after 30 min.

RESULTS AND DISCUSSIONS

Effect on melanization of B16 mouse melanoma cells (Figure 1)

We investigated the effect of Bamboo extract on the melanin content of B-16 melanoma cells. When treated with 50 μg/mℓ of Bamboo extract, there was 50.8% decrease in melanin contents of human melanocytes. These are significant decrease in melanin contents when compared with other melanogenic inhibitors (arbutin: 41.5%, kojic acid: 38.7%).

Effect on melanization of cultured human melanocytes (Figure 2)

We investigated the effect of Bamboo extract on the melanin content of human melanocytes. When treated with 50 μg/mℓ of Bamboo extract, there was 31.5% decrease in melanin contents of human melanocytes. These are significant decrease in melanin contents when compared with other melanogenic inhibitors (arbutin: 27%, ascorbic acid: 9.4%, hydroquinone: 38.4%, kojic acid: 12.5%).

Effect on cell viability of cultured human melanocytes (Figure 3)

The effects of Bamboo extract and other melanogenic inhibitors on the cell viability of cultured human melanocytes were determined with MTT assay. MTT assay showed

that Bamboo extract, arbutin, and kojic acid did not reduce cell viability at the concentrations of 50 ㎍/㎖, but hydroquinone did reduce cell viability at the concentration of 5 ㎍/㎖.

Effect of Bamboo extract on scavenging activities of DPPH (Figure 4)

The effects of Bamboo extract and other melanogenic inhibitors on the scavenging activities DPPH were determined with measuring the decrease in DPPH absorption. When treated with 10 ㎍/㎖ of Bamboo extract, there was 11.6% decrease in DPPH absorption. The scavenging effect of Bamboo extract are lower than those of ascorbic acid (89.9%) and hydroquinone (85.2%), but it is higher than those of arbutin (2.5%) and kojic acid (3.1%). According to the result, Bamboo extract seems to have the free radical scavenging effect slightly.

CONCLUSIONS

1. Bamboo extract (*Phyllostachys reticulata C. Koch.*) has considerable effect against melanin biosynthesis in B16 mouse melanoma cells and cultured human melanocytes.

2. Bamboo extract doesn't reduce cell viability in human melanocytes at the concentration tested.

3. Bamboo extract shows slightly free radical scavenging activity.

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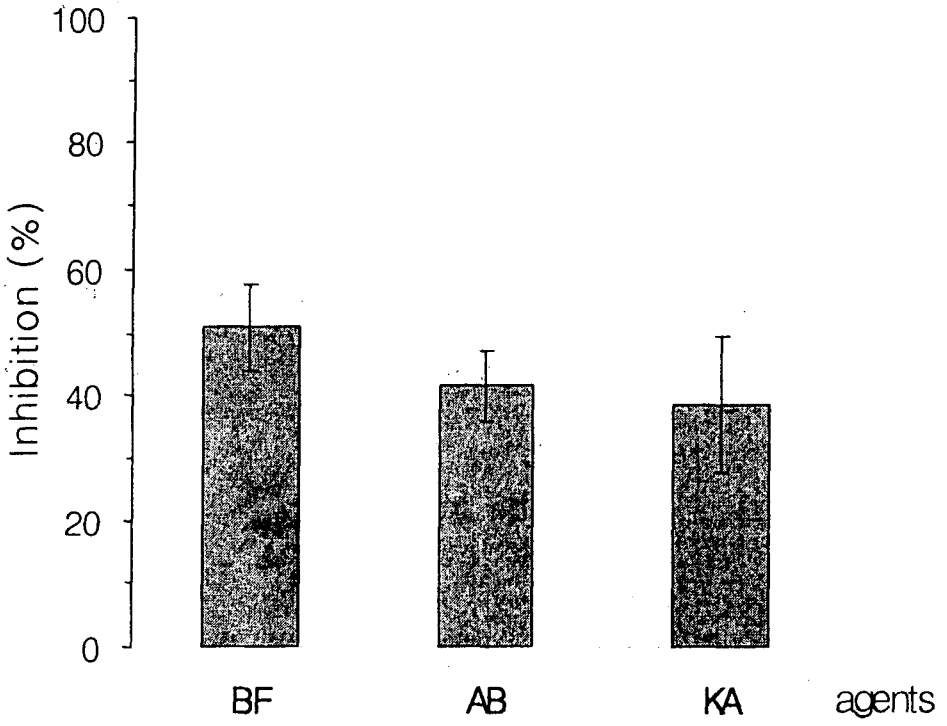

Figure 1. Effects of Bamboo extract, arbutin, and kojic acid on melanin content in B-16 melanoma cells. Cultures of 5 x 10^5 cells / petridish were incubated with these agents (50 ㎍/㎖) for three days. Results are expressed as inhibition percentage and represent mean ± standard error (n=4). BF : bamboo extract, AB : arbutin, KA : kojic acid.

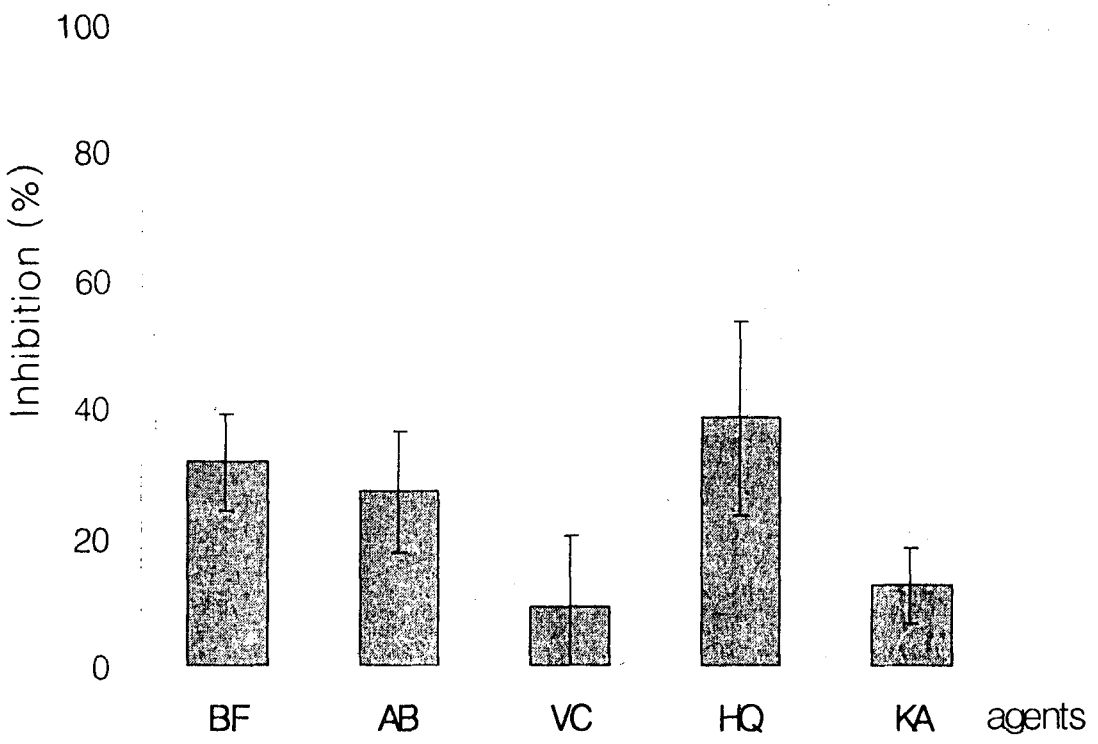

Figure 2. Effects of Bamboo extract, arbutin, ascorbic acid, hydroquinone, and kojic acid on melanin content in human melanocytes. Cultures of 5 x 10^5 cells / T-75 flask were incubated with these agents for seven days. Results are expressed as inhibition percentage and represent mean ± standard error (n=5). BF : bamboo extract, AB : arbutin, VC : ascorbic acid, HQ : hydroquinone, KA : kojic acid.

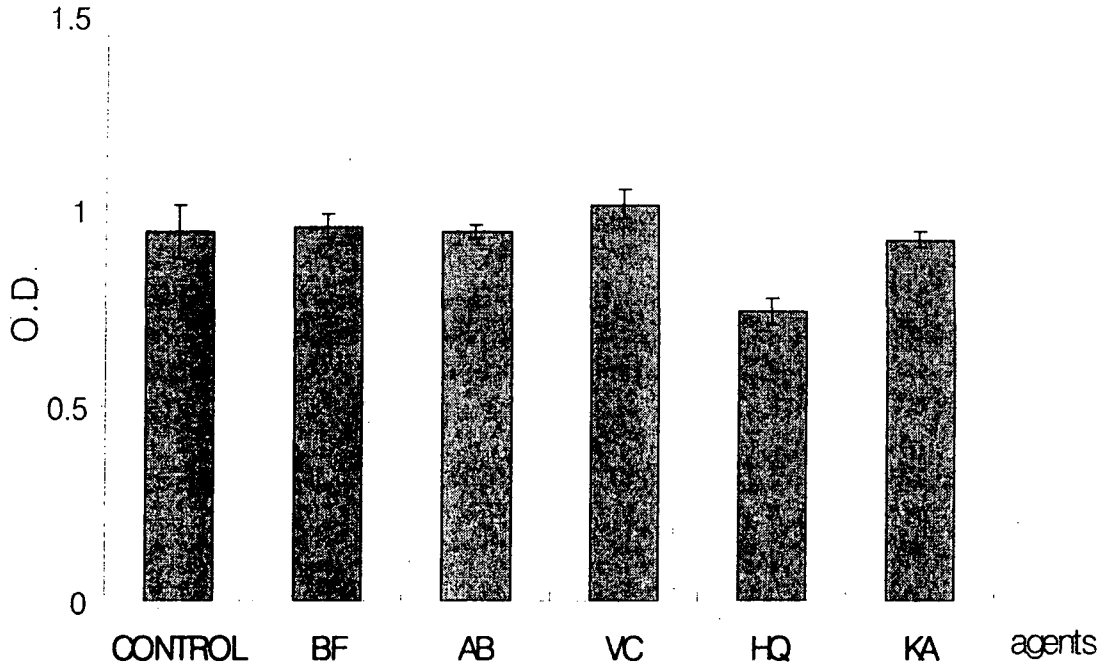

Figure 3. Effects of Bamboo extract, arbutin, ascorbic acid, hydroquinone, and kojic acid on cell viability in human melanocytes. Cultures of 2 x 10^4 cells / well were incubated with these agents for three days. Results are expressed as inhibition percentage and represent mean $\pm$ standard error (n=3). BF : bamboo extract, AB : arbutin, VC : ascorbic acid, HQ : hydroquinone, KA : kojic acid.

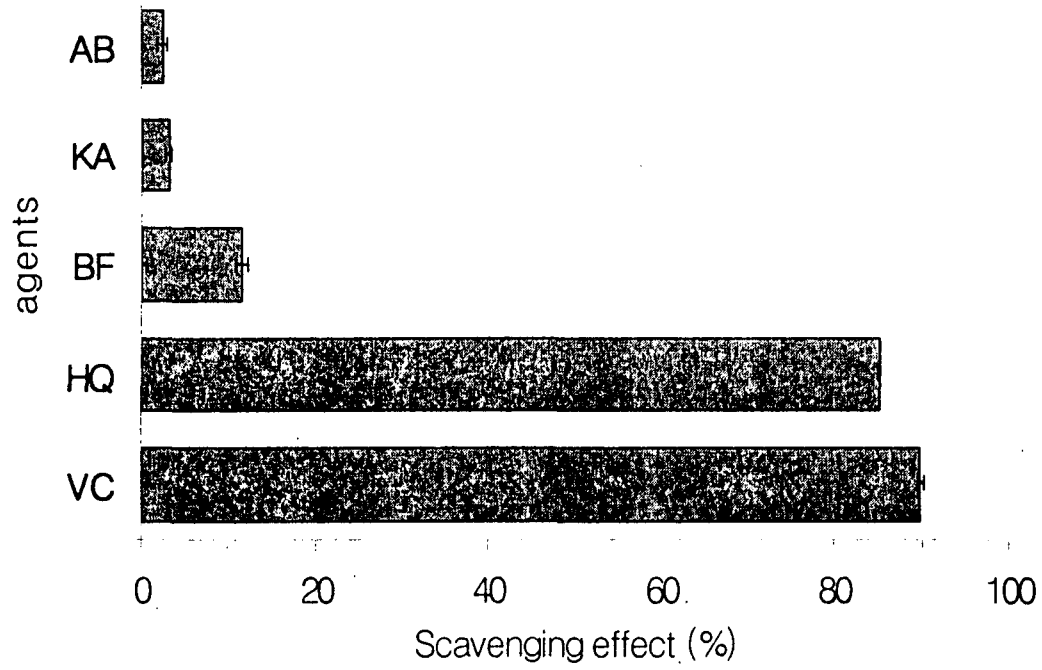

Figure 4. Effects of Bamboo extract, arbutin, ascorbic acid, hydroquinone, and kojic acid on free radical scavenging activity in DPPH. The free radical, DPPH was treated with 10 $\mu g/m\ell$ agents for 30 minutes. Results are expressed as inhibition percentage and represent mean $\pm$ standard error (n=3). BF : bamboo extract, AB : arbutin, VC : ascorbic acid, HQ : hydroquinone, KA : kojic acid.