



## Whitening Effect of Black Tea Water Extract on Brown Guinea Pig Skin

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To evaluate the whitening effect of black tea water extract (BT), BT was topically applied to artificially hyperpigmented spots on the back skins of brown guinea-pigs (weight: 450~500 g) induced by 1,500 mJ/cm<sup>2</sup> of ultraviolet B (UVB) irradiation. The test compounds of 30  $\mu$ l were applied twice a day, six days a week, for four weeks. The artificially hyperpigmented spots were divided into 5 groups: control (UVB + saline, C), vehicle control [UVB + propylene glycol: ethanol: water (5 : 3 : 2), VC], positive control (UVB + 2% hydroquinone, PC), experimental 1 (UVB + 1% BT), experimental 2 (UVB + 2% BT). After 4-week application, the spots were removed by biopsy punch under anesthetic condition and used as specimens for the histological examination. The total polyphenol and flavonoid contents of BT were 104 and 91 mg/g, respectively. The electron-donating ability of BT revealed a dose-dependent response, showing the excellent capacities of 86% at 800  $\mu$ g/ml. The artificially hyperpigmented spots treated with the PC and BT were obviously lightened compared to the C and VC groups. At the fourth week, the melanin indices for the PC and BT groups were significantly lower ( $p < 0.001$ ) than those of the C and VC groups. In histological examination, PC and BT groups were significantly reduced in the melanin pigmentation, the proliferation of melanocytes and the synthesis of melanosomes compared to the C and VC groups. It is found that BT inhibits the proliferation of melanocytes and synthesis of melanosomes *in vivo* using brown guinea pigs, thereby showing a definite skin whitening effect.

**Key words:** Black tea, Brown guinea pig, Melanin, Whitening effect

### INTRODUCTION

Oxidative stress initiated by reactive oxygen species (ROS) generation is an important factor modulating skin alterations, especially those caused by UV exposure and aging. Chronic exposure to solar UV radiation of mammalian skin induces a number of biological responses, including erythema, edema, sunburn cell formation, hyperpigmentation, photoaging and skin cancer development.

The total number of melanocytes in human skin decreases with age (Kurban and Bhaman, 1990), and aged skin often shows irregular pigmentation that is frequently associated with hyperpigmentation (Wulf *et al.*, 2004). Solar lentigos are considered as hallmarks of older skin and it has been postulated that skin color heterogeneity in ultraviolet (UV)-exposed areas is due to an uneven distribution of pigment cells (Haddad *et al.*, 1998), which have led to the develop-

ment of whitening cosmetics and medicines. Arbutin (AT), kojic acid (KA) and its derivatives were developed in 1990's (Chen *et al.*, 1991). However, the clinical effect of these materials is unsatisfactory. The hydroquinone (HQ) group compounds have been used as effective depigmenting agents for skin overpigmentation, but they are strongly irritable and exhibits cell toxicity (Maeda and Fukuda, 1991). Therefore, there is a large demand for newer whitening agents.

Tea leaves are rich sources of phenolic acids and flavonoids. The major polyphenols in fresh tea leaves are epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin gallate (ECG) and epicatechin (EC), which together may constitute 30% of the dry leaf weight (Graham, 1992). Green tea (GT) is steamed to avoid enzymatic oxidation and black tea (BT) is the most thoroughly enzymatically oxidized, which generates other distinct polyphenols such as theaflavins and thearubigins (Balentine *et al.*, 1997). Many studies have shown that GT and BT have antioxidant activity (Higdon and Frei, 2003; McKay and Blumberg, 2002). However, there have been no reports on whitening effects of BT using either *in vivo* or *in vitro* tests. The pigmented guinea-pig is a well-established animal model for human

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pigmentation because its skin contains active epidermal melanocytes and melanosomes in a similar pattern to human skin (Bologna *et al.*, 1990), and also similar reactions to ultraviolet radiation (Imokawa *et al.*, 1986).

This study investigated the depigmenting effect of black tea water extract on brown guinea-pig skin which was hyperpigmented by UVB irradiation. The depigmenting effect was evaluated by visual observation and quantitative color analysis of the skin-surface, and by morphological and numerical changes in epidermal melanocytes.

## MATERIALS AND METHODS

**Reagents and apparatus.** UVB sunlamp (UVM-225D, Mineralight Lamp UVP, USA), UV-radiometer (HD 9021, Delta OHM, Italy) and mexameter (MX18, CK electronic GmbH, Germany) were used for UVB irradiation, UV measuring and melanin index measuring, respectively. Fluorescence microscope (Axio imager, Carl Zeiss, Germany) was used for histological observation, and i-solution (IMT i-solution ver. 8.0, Canada) was used for image analyzing.

**Materials.** Black tea (BT) was obtained from oriental medicinal herb market, Daegu, Korea. Six-hundred gram of BT with 6 l distilled water was boiled for 2 h in a heating extractor (COSMOS-660, Kyungseo Machine Co., Korea) and concentrated. Thereafter, the aqueous extract was lyophilized into powder. This specimen was dissolved into the vehicle [propylene glycol: ethanol: water (5 : 3 : 2)] for experimentation.

### Antioxidation ability experimentation.

**Total polyphenol contents:** Total polyphenolic contents of EGCG, AT, KA, HQ and BT were determined using the Folin-Denis assay (Folin and Denis, 1912). One ml of test agent dissolved in DMSO was introduced into test tubes, followed by 1 ml of folin-reagent, then the tubes were allowed to stand for 3 min. One ml of 10% Na<sub>2</sub>CO<sub>3</sub> was added and the mixture was shaken vigorously. The tubes were allowed to stand for 60 min before absorbance at 760 nm was measured. The standard curve was prepared using tannic acid.

**Total flavonoid contents:** Total flavonoid contents of EGCG, AT, KA, HQ and BT were determined using the modified method of Davis *et al.* (1980). One ml of test agent was introduced into test tubes, followed by 10 ml of diethylene glycol reagent, and 1 ml of 1 N NaOH. The mixtures were shaken vigorously, and were reacted in hot water at 37°C, for 60 min before absorbance at 420 nm was measured. The standard curve was prepared using rutin.

### Animal experimentation.

**Experimental animal:** Two brown guinea-pigs weighing about 450–550 g were obtained from Oriental Yeast Co.

Ltd, Japan (OYC). The animals were allowed to acclimatize to the laboratory environment for seven days. These animals were housed in individual cages with free access to food and water, with the environmental temperature maintained at 22 ± 1°C, and with a relative humidity of 50 ± 5%, and an alternating 12 h light/dark cycle. Both animal care and protocol for this study were in accordance with Institutional Animal Care and Use Committee (IACUC).

After 4-week application of test agent, the experimental animals were anaesthetized with ketamine hydrochloride, and the melanin pigmented areas were taken by biopsy punch (Ø 12 mm). These specimens were then fixed in neutral-buffered 10% formalin and embedded in paraffin for histological observation. The experimental groups were as follows:

Normal (N): non-treatment group, control (C): UVB irradiation + saline application group, vehicle control (VC): UVB irradiation + vehicle application group, positive control (HQ): UVB irradiation + 2% HQ application group, experimental 1 (BT1): UVB irradiation + 1% BT application group, and experimental 2 (BT2): UVB irradiation + 2% BT application group.

**UVB irradiation:** UVB-induced hyperpigmentation was elicited on the back skins of brown guinea-pigs using a modification of the method of Choi *et al.* (2004). The guinea pigs were anesthetized with ketamine hydrochloride (100 mg/BW) and 24 separate areas (Ø 12 mm) on the back of each animal covering with a leather were exposed to UVB radiation (302 nm sunlamp). These separate areas were irradiated with UVB once a week, 500 mJ/cm<sup>2</sup> each time, for three consecutive weeks, the total irradiation amounting to 1,500 mJ/cm<sup>2</sup>.

**Application of the test compounds:** Ten days after the last UVB irradiation, 2% HQ, 1% BT and 2% BT were topically applied to the hyperpigmented areas (9 areas per group) twice a day, five days a week for four weeks, 30 µl (1%: 0.53 mg/cm<sup>2</sup>/day, 2%: 1.06 mg/cm<sup>2</sup>/day) each with a micro-pipette. Also saline was applied as control, and propylene glycol: ethanol: water (5 : 3 : 2) as vehicle control.

**Gross observation of depigmentation:** The area of melanin pigmentation was observed once a week by gross observation, the test agent treated groups were compared with the control group in order to investigate their respective whitening effects. This comparison was repeated every week throughout the four weeks of the experiment, and photographs were taken on the surface of the skin every week.

**Measurement of melanin index:** Using a mexameter, the change of melanin pigmentation before and after topical application of the test compounds was measured by a non-invasive method once a week and 3 measurements each. The degree of pigmentation was analyzed by the average of the values measured.

**Histological observation of skin tissue:** In an effort to observe the histological changes in the skin tissues by opti-

cal microscope, extracted skin tissues were fixed for 12 h in 10% neutral formalin solution and washed in running water. After dehydrating in 70, 80, 95, and 100% ethanol, clearing with xylene, embedding through paraffin infiltration, a specimen with 4  $\mu\text{m}$  thickness was prepared using a microtome.

**Hematoxylin and eosin staining:** A specimen was deparaffinized with xylene. Then the specimen was washed using running tap water. After moving to Harris hematoxylin solution and staining the nucleus for 5 min, the specimen was washed in running water, precipitated 3 times in 1% HCl alcohol solution and stained in blue with 1% ammonia solution. The cytoplasm was stained for 3 min in eosin solution and moved to 80, 95, 95, 100, and 100% alcohol for dehydration. After clearing process, the specimen was put in Canada balsam for microscopic observation.

**Fontana-Masson's silver staining:** A specimen was deparaffinized with xylene. By the method of Masson (1928) the specimen was stained in silver nitrate solution at 56°C for 60 min, washed, toned in 0.2% gold chloride solution, washed again and placed in 5% sodium thiosulfate solution for 5 min. Then the specimen was washed once more, contrast stained in nuclear fast red solution, and after dehydrating in 95, 95, 100, and 100% alcohol and clearing with xylene the specimen was put in Canada balsam for microscopic observation. The percentage area of melanin pigmentation was calculated using an image analysis software.

**Immunohistochemical staining:** In order to observe the degree of the production of melanocytes and melanosomes, extracted skin tissues were fixed for 12 h in 10% neutral formalin solution and washed in running tap water. After dehydration, clearing with xylene and infiltrating and embedding in paraffin, specimens were prepared with 4  $\mu\text{m}$  thickness. The specimen was attached to the coating slide and deparaffinized. Then BenchMark XT automated immunostainer (Ventana Medical Systems, USA) was used for staining. For automation process, the specimen was washed for 3 min using reaction buffer and placed in 3%  $\text{H}_2\text{O}_2$  for 3 min to suppress the activity of peroxidase. Primary antibodies for S-100 and HMB-45 were diluted at the ratio of 1 : 100 and reacted. The specimen was then washed using reaction buffer and allowed to react with biotin for 20 min and react with streptavidin for 25 min. After diaminobenzidine (DAB) (Ventana Detection kit, USA) coloring, the specimen was counter stained for optical microscopic observation. The percentage areas of S-100 and gp 100 protein expressions were calculated using an image analysis software to compare the degree of the production of melanocytes and melanosomes, respectively.

**Statistical analysis.** Differences of the values between the groups were evaluated statistically using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test for a post hoc comparison by SPSS (v17.0). Statistical significance was set at  $p < 0.05$ ,  $p < 0.01$ , and  $p < 0.001$ .

## RESULTS

### Antioxidation ability.

**Total polyphenol contents:** The total polyphenol contents of EGCG, AT, KA, HQ, and BT were shown to be 438, 22, 30, 98, and 104 mg/g, respectively, according to the standard curve using tannic acid.

**Total flavonoid contents:** The total flavonoid contents of EGCG, AT, KA, HQ, and BT were shown to be 193, 9, 10, 78, and 91 mg/g, respectively, according to the standard curve using rutin.

### Animal experimentation.

**Gross observation of depigmenting effect:** The melanin pigmentation of the guinea-pigs' skin irradiated with UVB was continually developed and no adverse clinical sign from the application of test compounds was observed throughout the experimental period. At the 2<sup>nd</sup> week of topical application in the PC and E groups, the hyperpigmented areas began to pale and the areas were remarkably depigmented at the 4<sup>th</sup> week.

**Changes in the melanin index:** At the first week all groups except VC and BT1 were significantly reduced ( $p < 0.05$ ) in melanin index. At the 2<sup>nd</sup> week the PC and all E groups showed a significant reduction ( $p < 0.001$ ) compared to the C group. At the 4<sup>th</sup> week the melanin indices of HQ,

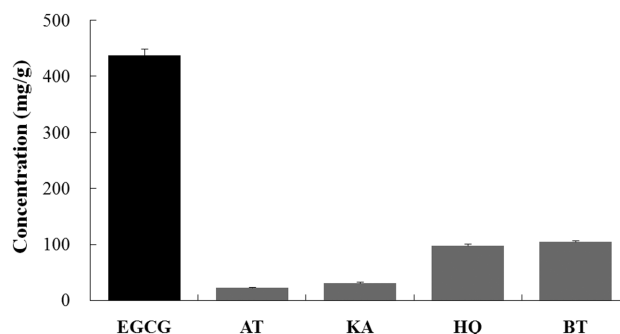


Fig. 1. Total polyphenol contents of test compounds. Values are mean  $\pm$  SD of 3 measurements.

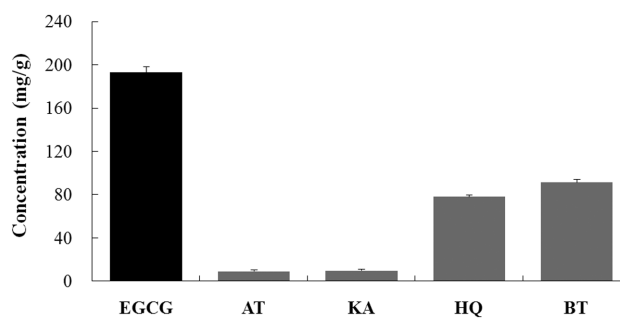
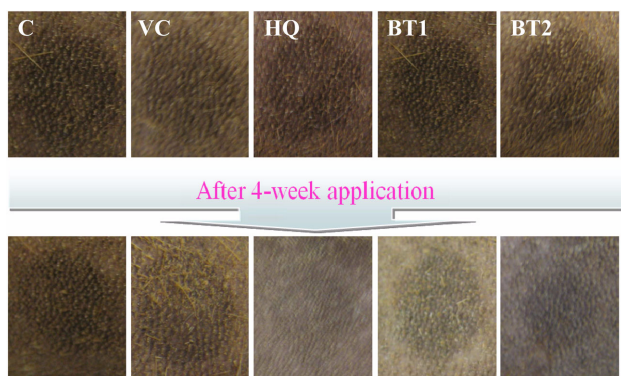
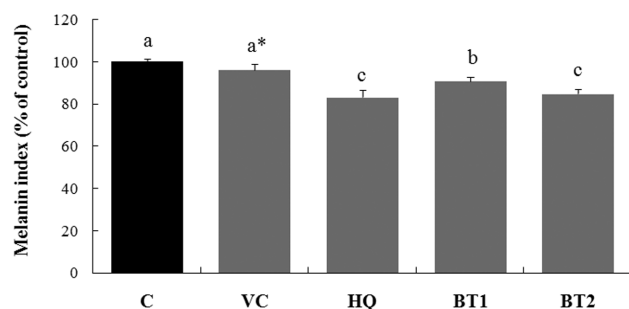


Fig. 2. Total flavonoid contents of test compounds. Values are mean  $\pm$  SD of 3 measurements.

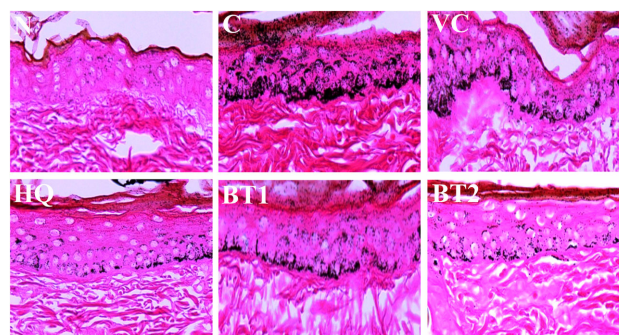


**Fig. 3.** Gross observation of depigmenting effect in brown guinea-pig skin applied with test compounds for 4 weeks. **C:** UVB irradiation group, **VC:** vehicle treatment group, **HQ:** 2% HQ treatment group, **BT1:** 1% black tea water extract treatment group, **BT2:** 2% black tea water extract treatment group. The HQ, BT1 and BT2 groups had a positive depigmenting effect compared to the C and VC groups.

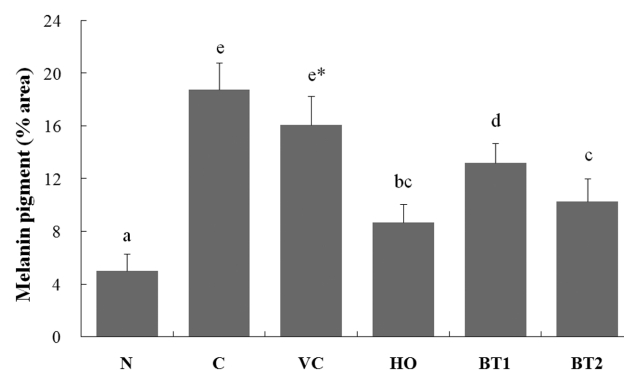


**Fig. 4.** Comparison of melanin index for artificial tanning spots of brown guinea-pig skin after 4 week-application of test compounds. Values are mean  $\pm$  SD of 9 samples. Unit: AU (Arbitrary Unit). Values with different superscript are significantly different ( $p < 0.001$ ) by ANOVA and Duncan's multiple range tests. \* $p < 0.05$  compared to the C group by ANOVA and Duncan's multiple range tests.

BT1, and BT2 groups were significantly reduced ( $p < 0.001$ ) by 17, 9, and 15% respectively, compared to the C group



**Fig. 5.** Histological observation of melanin pigmentation in brown guinea-pig skin epidermis after 4-week application of test compounds. F-M stain,  $\times 400$ . N: non-treatment (normal group). The HQ, BT1 and BT2 groups showed a definitely reduced melanin pigmentation in the skin epidermis as compared to the C and VC groups.



**Fig. 6.** Comparison of melanin pigmentation in brown guinea-pig skin epidermis after 4-week application of test compounds. Values are mean  $\pm$  SD of 9 samples. Values with different superscripts are significantly different ( $p < 0.001$ ) by ANOVA and Duncan's multiple range tests. \* $p < 0.05$  compared to the C group by ANOVA and Duncan's multiple range tests.

(Table 1, Fig. 4). VC group was reduced by 4% ( $p < 0.05$ ).

**Fontana-Masson's silver staining:** At the 4<sup>th</sup> week of experimentation, the granular melanins in the C and VC groups were much increased than the N group and distrib-

**Table 1.** Changes in melanin index of brown guinea-pig skin in 4-week experiment

Wk	C	VC	HQ	BT1	BT2
0	720.4 $\pm$ 12.7 <sup>a</sup>	718.5 $\pm$ 15.2 <sup>a</sup>	721.9 $\pm$ 18.6 <sup>a</sup>	723.0 $\pm$ 11.9 <sup>a</sup>	720.1 $\pm$ 12.0 <sup>a</sup>
1	717.3 $\pm$ 13.2 <sup>a</sup>	714.0 $\pm$ 14.8 <sup>a</sup>	700.2 $\pm$ 16.1 <sup>a*</sup>	708.6 $\pm$ 12.4 <sup>a</sup>	703.8 $\pm$ 11.7 <sup>a*</sup>
2	714.0 $\pm$ 11.7 <sup>a</sup>	703.4 $\pm$ 14.5 <sup>ab</sup>	665.3 $\pm$ 23.8 <sup>c</sup>	687.9 $\pm$ 13.2 <sup>bc</sup>	674.6 $\pm$ 17.6 <sup>c</sup>
3	705.1 $\pm$ 9.8 <sup>a</sup>	691.3 $\pm$ 13.9 <sup>a</sup>	628.6 $\pm$ 21.4 <sup>c</sup>	660.4 $\pm$ 12.1 <sup>b</sup>	639.2 $\pm$ 15.7 <sup>c</sup>
4	696.7 $\pm$ 10.3 <sup>a</sup>	670.2 $\pm$ 19.6 <sup>a*</sup>	579.5 $\pm$ 23.7 <sup>c</sup>	632.0 $\pm$ 14.9 <sup>b</sup>	591.0 $\pm$ 16.2 <sup>c</sup>

Values are mean  $\pm$  SD of 9 samples.

Unit: AU (Arbitrary Unit).

Values with different superscript in the same row are significantly different ( $p < 0.001$ ) by ANOVA and Duncan's multiple range tests. \* $p < 0.05$  compared to the C group by ANOVA and Duncan's multiple range tests.

**Table 2.** Comparison of melanin pigmentation in brown guinea-pig skin epidermis after 4-week application of test compounds

Area	N	C	VC	HQ	BT1	BT2
% of melanin pigment	5.0 ± 1.3 <sup>a</sup>	18.8 ± 2.0 <sup>c</sup>	16.1 ± 2.2 <sup>e*</sup>	8.7 ± 1.4 <sup>bc</sup>	13.2 ± 1.5 <sup>d</sup>	10.3 ± 1.7 <sup>c</sup>

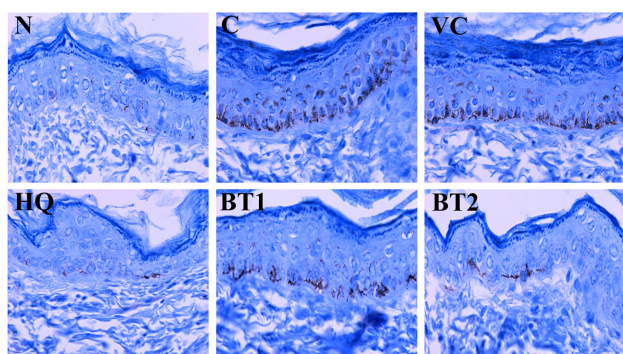
Values are mean ± SD of 9 samples.

Values with different superscripts in the same row are significantly different ( $p < 0.001$ ) by ANOVA and Duncan's multiple range tests.

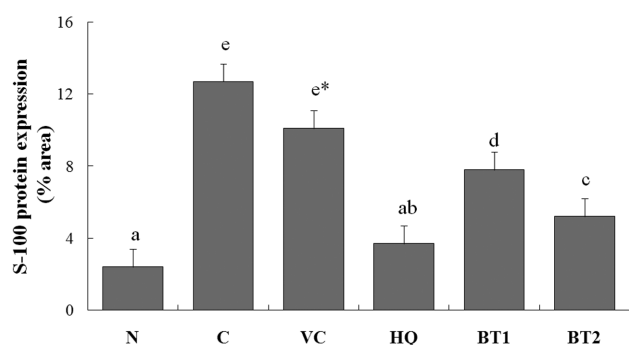
\* $p < 0.05$  compared to the C group by ANOVA and Duncan's multiple range tests.

uted throughout the epidermis. However, the melanin dispersion in the PC and E groups was distinguishably reduced and mostly restricted to the basal layer (Figs. 5~6).

The percentages of melanin pigmented area in the epidermis of C (19%) and VC (16%) groups were significantly higher ( $p < 0.001$ ) than the N group (5%). And those values in the HQ, BT1, and BT2 groups were significantly lowered ( $p < 0.001$ ) by 54, 30, and 45%, respectively, compared to the C group. VC group was also significantly low-



**Fig. 7.** Histological observation of S-100(+) melanocytes in brown guinea-pig skin epidermis after 4-week application of test compounds. S-100 immunohistochemical stain,  $\times 400$ . The HQ, BT1 and BT2 groups showed a definitely reduced S-100(+) melanocytes in the skin epidermis as compared to the C and VC groups.

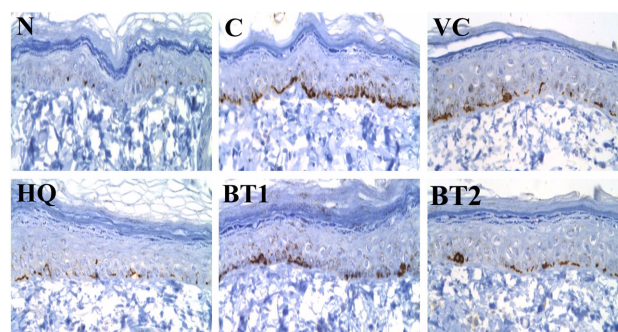


**Fig. 8.** Comparison of S-100 protein expression in brown guinea-pig skin epidermis after 4-week application of test compounds. Values are mean ± SD of 9 samples. Values with different superscripts are significantly different ( $p < 0.001$ ) by ANOVA and Duncan's multiple range tests. \* $p < 0.05$  compared to the C group by ANOVA and Duncan's multiple range tests.

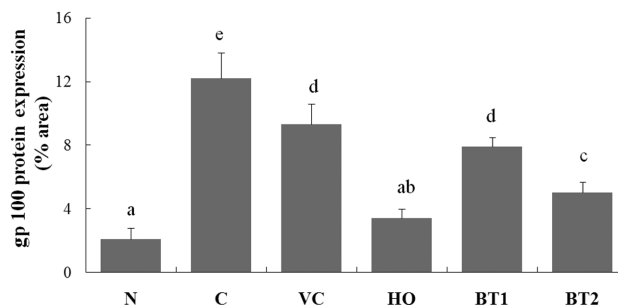
ered ( $p < 0.05$ ) by 14% (Table 2).

**Immunohistochemical staining:** At the 4<sup>th</sup> week of experimentation, the S-100 and gp 100 protein expressions in the C and VC groups were much increased and clustered than the N group. However, those expressions in the PC and E groups were distinguishably reduced (Figs. 7~10).

The percentages of S-100 protein expressed area in the epidermis of C (13%) and VC (10%) groups were significantly higher ( $p < 0.001$ ) than the N group (2%). Those values in the HQ, BT1, and BT2 groups were significantly lowered ( $p < 0.001$ ) by 71, 39, and 59%, respectively, compared to the C group. VC group was also significantly low-



**Fig. 9.** Histological observation of gp 100(+) melanocytes in brown guinea-pig skin epidermis after 4-week application of test compounds. HMB-45 immunohistochemical stain,  $\times 400$ . The HQ, BT1 and BT2 groups showed a definitely reduced gp 100(+) melanocytes in the skin epidermis as compared to the C and VC groups.



**Fig. 10.** Comparison of gp 100 protein expression in brown guinea-pig skin epidermis after 4-week application of test compounds. Values are mean ± SD of 9 samples. Values with different superscripts are significantly different ( $p < 0.001$ ) by ANOVA and Duncan's multiple range tests.

**Table 3.** Comparison of S-100 protein expression in brown guinea-pig skin epidermis after 4-week application of test compounds

Area	N	C	VC	HQ	BT1	BT2
% of S-100 protein	2.4 ± 0.6 <sup>a</sup>	12.7 ± 1.5 <sup>c</sup>	10.1 ± 1.4 <sup>ce</sup>	3.7 ± 0.5 <sup>ab</sup>	7.8 ± 0.7 <sup>d</sup>	5.2 ± 1.2 <sup>c</sup>

Values are mean ± SD of 9 samples.

Values with different superscripts in the same row are significantly different ( $p < 0.001$ ) by ANOVA and Duncan's multiple range tests. \* $p < 0.05$  compared to the C group by ANOVA and Duncan's multiple range tests.

**Table 4.** Comparison of gp 100 protein expression in brown guinea-pig skin epidermis after 4-week application of test compounds

Area	N	C	VC	HQ	BT1	BT2
% of gp 100 protein	2.1 ± 0.7 <sup>a</sup>	12.2 ± 1.6 <sup>c</sup>	9.3 ± 1.3 <sup>d</sup>	3.4 ± 0.6 <sup>ab</sup>	7.9 ± 0.6 <sup>d</sup>	5.0 ± 0.7 <sup>c</sup>

Values are mean ± SD of 9 samples.

Values with different superscripts in the same row are significantly different ( $p < 0.001$ ) by ANOVA and Duncan's multiple range tests.

ered ( $p < 0.001$ ) by 20% (Table 3).

The percentages of gp 100 protein expressed area in the epidermis of C (12%) and VC (9%) groups were significantly higher ( $p < 0.001$ ) than the N group (2%). Those values in the HQ, BT1, and BT2 groups were significantly lowered ( $p < 0.001$ ) by 72, 35, and 59%, respectively, compared to the C group. VC group was also significantly lowered ( $p < 0.001$ ) by 24% (Table 4).

## DISCUSSIONS

Melanin, which is the major pigment of skin, plays an essential role in protection against UV injury under normal physiological conditions. Its formation beneath the skin proceeds through free radical mechanism. The accumulation of an abnormal melanin amount in specific parts of the skin as more pigmented patches (melasma, freckles, ephelide, senile lentiginos, etc.) might become an aesthetic problem (Solano *et al.*, 2006). The type and amount of melanin synthesized by the melanocytes and its distribution in the surrounding keratinocytes determine the actual colour of the skin (Kim and Uyama, 2005). A number of skin lightening agents which are derived from natural resources particularly plants, are already used in cosmetic products such as; HQ (isolated from *Cystoseria jabokae* and *C. adriatica*), azelaic acid (isolated from *Pityrosporum ovale*), KA (fungal metabolic product) and AT (a glycosylated hydroquinone). Despite the extensive researches on lightening agents and hyperpigmentation, the existing agents have got limitations in terms of high toxicity, low stability, poor skin-penetration, and insufficient activity.

There is growing interest in drinking tea all over the world, which could be connected with its polyphenol antioxidative activity, such as scavenging reactive oxygen species and free radicals through several proposed mechanisms, including depolarization of electrons, formation of intramolecular hydrogen bonds, and rearrangement of the molecular structure (Jovanovic *et al.*, 1994; Ostrowska *et al.*, 2001; Salah *et al.*, 1995). Probably the most important natural

phenolics are flavonoids because of their broad spectrum of chemical and biological activities. In fact, flavonoids have been reported as antioxidants, scavengers of a wide range of reactive oxygen species and inhibitors of lipid peroxidation (Kahkonen *et al.*, 1999; Williams *et al.*, 2004). The DPPH radical has been widely used to test the free radical scavenging ability of various natural products and has been accepted as a model compound for free radicals originating in lipids (Da Porto *et al.*, 2000). The total polyphenol and flavonoid contents of BT in the present study were much higher than AT and KA and slightly higher than HQ, ascertaining a high antioxidant activity. The DPPH radical scavenging activity of BT in the present study was much higher than AT and KA and slightly lower than HQ (data not shown). The degree of oxidation affects the polyphenol profile of the tea (Balentine *et al.*, 1997). Satoh *et al.* (2005) reported that the percent DPPH radical scavenging activities of various tea extracts were dose-dependent and decreased in the following order: green tea (not oxidized) > roasted tea (not-oxidized) > oolong tea (semi-oxidized) > black tea (fully oxidized).

Mechanism of antioxidant action can generally include suppressing ROS formation, either by inhibition of enzymes or by chelating trace elements involved in free-radical production, such as free copper and iron, scavenging reactive species, and upregulating or protecting antioxidant defences (Ammar *et al.*, 2009). Tyrosinase is known to be a key enzyme that catalyzes two major reactions of melanin synthesis: the hydroxylation of tyrosine to 3,4-dihydroxyphenylalanine (DOPA) and the oxidation of DOPA to dopaquinone. It is well known that tyrosinase (monophenol monooxygenase) or polyphenol oxidase (PPO), is a copper-containing monooxygenase. Because up to date, no *in vivo* research on the whitening effect of teas except for Oolong tea (Aoki *et al.*, 2007) have been found, in the present study we evaluated the whitening effect of BT using brown guinea pigs. The main physiological stimulus for human melanogenesis is UV radiation. Darkening of human skin due to increased melanin pigmentation after exposure to sunlight or to UV

from artificial sources is commonly known as tanning. This reaction results from a combination of immediate pigment darkening (IPD) caused by UVA, due to photooxidation of preformed melanins and delayed pigment darkening (DPD) occurring approximately 72 h after UV exposure, which is optimally stimulated by UVB and to a lesser extent by UVA and visible radiation. DPD is accompanied by increases in the number of DOPA-positive melanocytes, and in the synthesis of melanosomes and is associated with changes in the functional state of melanocytes (Ortonne, 1990). Histological methods, such as F-M stain for melanin pigment (Choi *et al.*, 2004) and immunohistochemical stains including both S-100 protein for melanocytes (Tobiishi *et al.*, 2004) and gp 100 protein for melanosome (Kikuchi *et al.*, 1996), have been widely used in pigmentation research. In the present study, BT have a little bit lower depigmenting efficacy than HQ in the evaluation through gross observation and histological examination. Melanin was seen throughout the epidermis and increased to a greater degree in the C and VC groups compared to the N group. However, HQ and BT groups showed significantly reduced ( $p < 0.001$ ) melanin, appearing mostly in the basal layer compared to the C or VC group. In addition, melanocytes and melanosomes are significantly decreased ( $p < 0.001$ ) in HQ and BT groups compared to the C or VC group. Depigmentation can be achieved by regulating one of following: the transcription and activity of melanogenic enzymes; the uptake and distribution of melanosomes in recipient keratinocytes; and melanin and melanosome degradation and turnover of pigmented keratinocytes (Briganti *et al.*, 2003). From these findings, it speculates that BT promotes the degradation of melanin and the turnover of pigmented keratinocytes, thereby results in the depigmenting effect.

Taken together, it is found that BT demonstrates a definite positive skin whitening effect on the delayed tanning spots induced by the UVB irradiation in brown guinea pigs by reducing melanin pigmentation, that is due to inhibiting the proliferation of melanocytes and the synthesis of melanosomes.

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