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Full Length Research Paper

The enhancement effect of *Salvia miltiorrhiza* on melanin production of B16F10 melanoma cells

Shu-Hua Chiang[1], Yi-Shyan Chen[2], Ming-Shian Hung[2], Shu-Mei Lee[3*] and Chih-Chien Lin[2]*

[1]Department of Food and Beverage Management, Taiwan Hospitality and Tourism College, 268 Chong-Hsing St., Feng-Shan Village, Shou-Feng County, Hualien 974, Taiwan, Republic of China.
[2]Department of Cosmetic Science, Providence University, 200 Chung-Chi Road, Shalu, Taichung 43301, Taiwan, Republic of China.
[3]Department of Cosmetic Science and Management, Mackay Medicine, Nursing and Management College, 92 Shengjing Road, Beitou, Taipei 11260, Taiwan, Republic of China.

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Salvia miltiorrhiza **is a well-known and widely used medicinal plant for the treatment of angina pectoris, hyperlipidemia and acute ischemic stroke. However, no research has described the function of *S. miltiorrhiza* on melanogenesis. Therefore, in this study we analyzed the effect of *S. miltiorrhiza* leaf extract (SME) on the tyrosinase activity and the production of melanin of melanoma cells. Results indicated that SME at 0.1 mg/ml could enhance mushroom tyrosinase activity up to 132.14 ± 11.89% and increase melanin content up to 121.37 ± 16.57% in B16F10 melanoma cells. Besides, no cytotoxicity was observed when B16F10 cells were treated with SME. For this reason, we suggest that SME could be use for the treatment of hypopigmentation disorder and for development of gray hair preventing agents or self-tanning cosmetics.**

Key words: Danshen, melanin, melanogenesis, *Salvia miltiorrhiza*, tyrosinase.

INTRODUCTION

Salvia miltiorrhiza Bunge (Lamiaceae), generally called Danshen, is an important and widely used medicinal plant of Traditional Chinese Medicine (TCM) for thousands of years (Wang and Wu, 2010). According to the usage of TCM, the dried root of *S. miltiorrhiza* can be used to promote blood flow and to resolve blood stasis. Thus, it is also widely provided to patients for the treatment of angina pectoris, hyperlipidemia and acute ischemic stroke (Ho and Hong, 2011; Wang, 2010). The chemical components from *S. miltiorrhiza* root extract are classified into two major groups: water-soluble compounds (hydrophilic) and lipid-soluble diterpenoid quinines (lipophilic); these compounds of both categories have been mostly purified and identified (Han et al., 2008; Wang et al., 2011).

The functional constituents of *S. miltiorrhiza* root extract, including tanshinones, tanshinlactone, salvianolic acids and danshensu, play the potent activities of antioxidant, antimicrobial, anticancer and anti-inflammatory (Dong et al., 2011; Lee et al., 2008; Tang et al., 2011; Wang, 2010). Meanwhile, just a few researches have focused on the functions of *S. miltiorrhiza* leaf extract (Matkowski et al., 2008; Zhang et al., 2010b). These results indicated that the acetone and methanol extracts from S.miltiorrhiza leaves have potent antioxidant activity. Therefore, *S. miltiorrhiza* has the

*Corresponding author. E-mail: s107@eip.mkc.edu.tw Tel: +886-2-28584180. Fax: +886-2-28584183. or E-mail: chchlin@pu.edu.tw. Tel: +886-4-26328001 ext.15409. Fax: +886-4-26311167.

Abbreviations: SME, *Salvia miltiorrhiza* extract; **TCM,** Traditional Chinese Medicine; **DMSO,** dimethyl sulfoxide; **DMEM,** Dulbecco's modified Eagle's medium; **EDTA,** ethylenediaminetetraacetic acid; **FBS,** fetal bovine serum; **MTT**, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; **ddH_2O,** deionized distilled water; **α-MSH,** α-melanocyte stimulating hormone; **cAMP,** cyclic adenosine monophosphate.

potentials for application of numerous fields. Moreover, with the utilization of natural resources increasing, *S. miltiorrhiza* has been widely used in the industries of pharmaceutics, foods and cosmetics.

Although, a few articles had demonstrated the cytotoxicity or anti-invasion activity of *S. miltiorrhiza* on melanoma cells (Fronza et al., 2011; Zhang et al., 2010a), however, no research had been done on the function of *S. miltiorrhiza* on melanogenesis of melanoma cells. Thus, in this study, we focused on the effect of leaf extract of *S. miltiorrhiza* on the tyrosinase activity and the production of melanin of melanoma cells.

MATERIALS AND METHODS

Chemicals

S. miltiorrhiza was provided by WinPower Technology CO., LTD. (Kaohsiung, Taiwan). Vitamin C (ascorbic acid), mushroom tyrosinase, *L*-tyrosine, dimethyl sulfoxide (DMSO) and other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA). Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), penicillin, streptomycin and trypsin-ethylenediaminetetraacetic acid (trypsin-EDTA) were purchased from Gibco BRL/Invitrogen (Carlsbad, CA, USA). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) was purchased from Affymetrix/USB (Cleveland, OH, USA). Deionized distilled water (ddH_2O) for solutions was obtained from the Milli-Q system (Millipore, Bedford, MA, USA).

Preparation of extracts

The leaf part of *S. miltiorrhiza* was cleaned with pure water. After cleaning, the prepared leafs were dried by airing. The dehydrated *S. miltiorrhiza* leafs were homogenized and then extracted by cold water at 25°C for 30 min. The collected supernatant was filtered by filter paper (0.45 µM) to remove debris. Finally, the filtered *S. miltiorrhiza* extract was freeze-dried and stored at 4°C prior to use. The extraction yield of *S. miltiorrhiza* extract (SME) was also calculated.

Tyrosinase activity assay

Tyrosinase activity assay was performed using a modified dopachrome method (Alam et al., 2011). For the assay, each 60 µL sample with different concentrations were mixed with 100 µL 1 mM L-tyrosine in phosphate buffer solution (pH 6.8), and then add 40 µL mushroom tyrosinase solution (100 units/ml) to the mixture for a 25 min incubation at 37°C. The spectrophotometric analysis was performed at 475 nm and the inhibition of dopachrome formation was calculated as inhibition percentage.

Cell line and cell culture

The B16F10 melanoma cells (BCRC 60031) was purchased from Food Industry Research and Development Institute (FIRDI, Hsinchu, Taiwan, R.O.C.). The B16F10 cells was cultured in DMEM medium supplemented with 10% FBS containing penicillin (100 units/ml) and streptomycin (100 µg/ml), and were placed at 37°C in a 5% CO_2 incubator. Cells grown in various culture dishes at 80% confluence were used in all experiments.

MTT Assay for cell viability

The B16F10 cells were seeded in 96-well plates for 24 h with 6×10^3 cells/well. The seeded cells were cultured in FBS-free medium for an additional 24 h. These cells were then treated with different concentration of samples for 48 h. When treatment was complete, the cells were added with 100 µL (0.5 mg/ml) solution of MTT at 37°C for 30 min after washing twice with PBS. The cells were lysed with 100 µL DMSO and the absorbance was measured spectrophotometrically at 540 nm using an ELISA reader (Lin et al., 2011).

Melanin content analysis

For melanin content analysis, vitamin C was used as the control agent. The B16F10 cells was seeded in the 6-well plates (2×10^5 cells/well) using the DMEM medium supplemented with 10% FBS for 24 h. The cells were subsequently treated with different concentration of samples for 24 h and detached by 0.05% trypsin-EDTA. The collected cell pellets were dissolved in 120 µL of 1 N NaOH for 1 h at 65°C and melanin contents were measured spectrophotometrically at 405 nm through an ELISA reader (Wang et al., 2011).

Statistical analysis

Quantitative data of the present study were analyzed using Student's *t*-tests and presented as means ± S.D. for three independent experiments.

RESULTS AND DISCUSSION

In this study, we investigated the effects of *S. miltiorrhiza* extract (SME) on the melanin production of B16F10 melanoma cell. The extraction yield of prepared SME is 5.987%. Furthermore, the color appearance of SME is green to brown. Thus, result confirmed that the content of water-soluble components in *S. miltiorrhiza* leafs have about 6%. For tyrosinase activity assay, mushroom tyrosinase-catalyzed dopachrome formation was utilized to test the effect of SME. Besides, vitamin C (0.15 mM) was used as the control agent for this experiment and the results are shown in Figure 1. These results demonstrated that the SEM could help the activity of mushroom tyrosinase to increase the dopachrome formation. SME concentrations from 0.025 to 0.1 mg/ml raised the tyrosinase activity from 129.23 ± 7.8 to 132.14 ± 11.89% (Figure 1). Moreover, vitamin C at 0.15 mM displayed a potent inhibitory activity on mushroom tyrosinase (Figure 1).

To confirm the effect of SME on melanogenesis, we first examined the cytotoxicity of SME to B16F10 cells. Different concentrations of SME were used to treat the cells and the results of cell viability are shown in Figure 2. The SME have no cytotoxicity on B16 cells if SME concentrations are between 0.025 to 0.1 mg/ml. Besides, cell viabilities of SME treated B16F10 cells are higher than 100% when SEM concentrations are higher than 0.05 mg/ml. Cell viability of 0.05 and 0.1 mg/ml SME

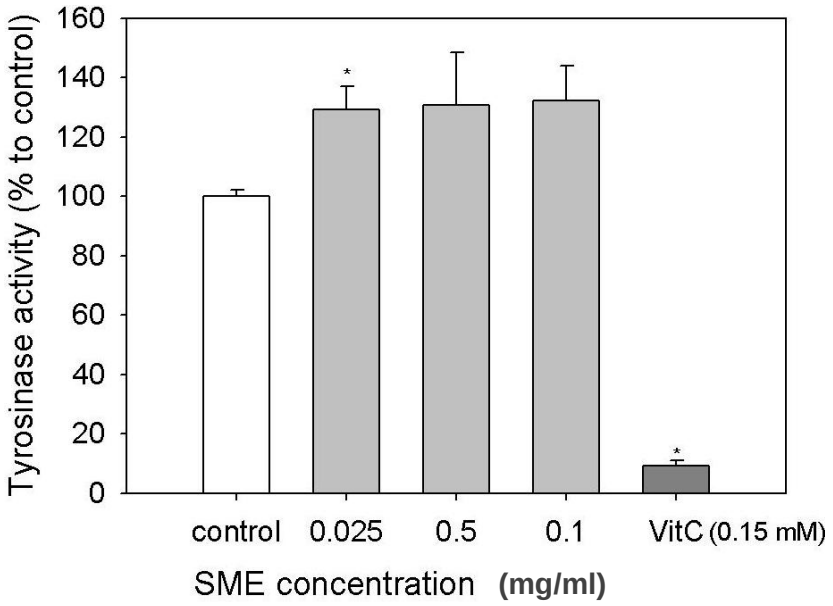

Figure 1. Effect of SME on tyrosinase activity. Vitamin C (VitC, 0.15 mM) was used as a control agent. Each value is expressed as mean ± S.E. (n = 3). *P < 0.05, compared with the control.

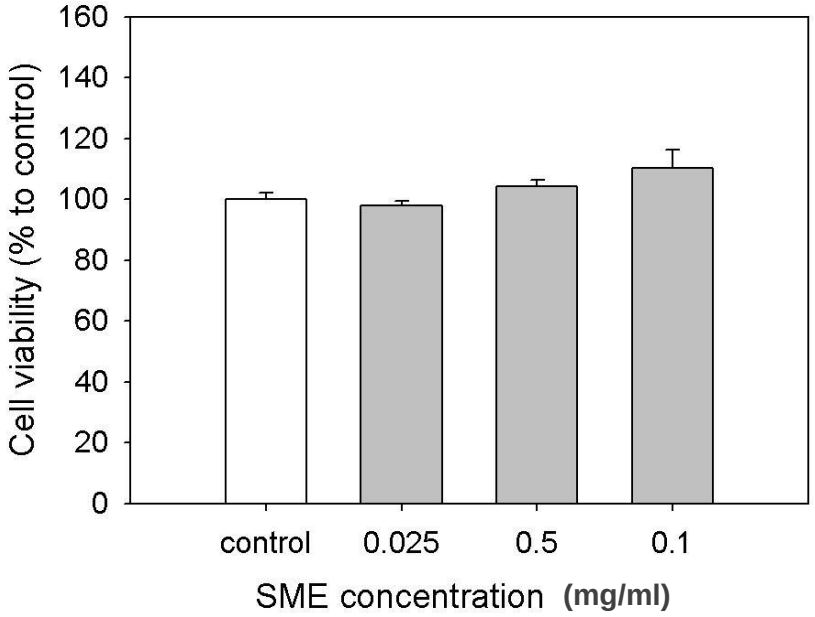

Figure 2. Cell viability of B16F10 melanoma cells treated with SME. Each value is expressed as mean ± S.E. (n = 3).

treated B16F10 cells is 104.38 ± 1.99 and 110.29 ± 5.98%, respectively (Figure 2). Therefore, we also suggested that SME have some ability to stimulate the cellular growth on B16F10 cells. Several studies have described the effects of *S. miltiorrhiza* on the inhibition of cancer cell growth (Lee et al., 2008; Wang, 2010; Zhang et al., 2010a). Therefore, anticancer activities are often associated with some functional compounds of the root of *S. miltiorrhiza*, such as salvianolic acid B for oral squamous carcinoma cells (Yang et al., 2011), tanshinone IIA for breast cancer cells (Lu et al., 2009) and tanshinone I for non-small cell lung cancer cells (Lee

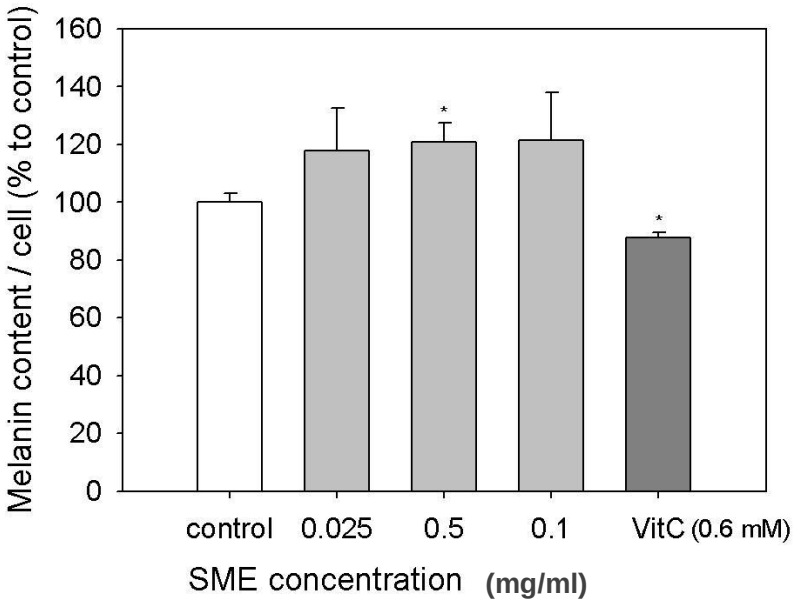

Figure 3. Melanin content of B16F10 melanoma cells treated with SME. Vitamin C (VitC, 0.6 mM) as control agent. Each value is expressed as mean ± S.E. (n = 3). *P < 0.05, compared with the control.

et al., 2008) and breast cancer cells (Nizamutdinova et al., 2008). However, our result indicated that the water-soluble extract from leaf of *S. miltiorrhiza* could enhance the cellular growth of B16F10 cells.

For the effect of SME on melanogenesis, we investigated the melanin production of B16F10 cells if it were treated with different concentrations of SME, and the results are shown in Figure 3. Results revealed that the melanin production of B16F10 cells was increased. The melanin content could be enlarged to about 121.37 ± 16.57% when SME concentration is 0.1 mg/ml (Figure 3). In addition, the vitamin C treated B16F10 cells have only produced melanin at 87.91 ± 1.67%. Therefore, we suggested that SME could stimulate the production of melanin in B16F10 cells. Melanin synthesis in melanocytes is induced by numerous effectors, including α-melanocyte stimulating hormone (α-MSH), cyclic adenosine monophosphate (cAMP) elevating agents (such as forskolin and isobutylmethylxantine) and ultraviolet (UV) light (Ebanks et al., 2009; Hamid et al., 2011; Lee et al., 2010; Sato and Toriyama, 2009). Several types of hypopigmentation disorder are caused by the reduction of melanogenesis functions (Guan et al., 2008; Passeron et al., 2005). Thus, obtaining a melanin production enhancing agents is a possible approach to treat those melanin-related diseases. Furthermore, the melanin production enhancing agents can also be used to prevent gray hair occurrence and to enhance the skin color by tanning.

In summary, 0.1 mg/ml SME could enhance the tyrosinase activity up to 132.14 ± 11.89%. The SME at 0.1 mg/ml also increased melanin content up to 121.37 ± 16.57% in B16F10 cells. Moreover, the cell viabilities of SME treated B16F10 cells were higher than 100%. Therefore, these results suggest that *S. miltiorrhiza* leaf extract may be a potential candidate for the therapy of hypopigmentation disorder and for development of gray hair preventing agents or self-tanning cosmetics.

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REFERENCES

Alam N, Yoon KN, Lee TS (2011). Evaluation of the antioxidant and antityrosinase activities of three extracts from *Pleurotus nebrodensis* fruiting bodies. Afr. J. Biotechnol., 10: 2978-2986.

Dong Y, Nakagawa-Goto K, Lai CY, Morris-Natschke SL, Basto KF, Lee KH (2011). Antitumor agents 287. Substituted 4-amino-2H-pyran-2-one (APO) analogs reveal a new scaffold from neo-tanshinlactone with *in vitro* anticancer activity. Bioorg. Med. Chem. Lett., 21: 2341-2344.

Ebanks JP, Wickett RR, Boissy RE (2009). Mechanisms regulating skin pigmentation: the rise and fall of complexion coloration. Int. J. Mol. Sci., 10: 4066-4087.

Fronza M, Murillo R, Slusarczyk S, Adams M, Hamburger M, Heinzmann B, Laufer S, Merfort I (2011). *In vitro* cytotoxic activity of abietane diterpenes from *Peltodon longipes,* as well as *Salvia miltiorrhiza* and *Salvia sahendica*. Bioorg. Med. Chem., 19: 4876-4881.

Guan S, Su W, Wang N, Li P, Wang Y (2008). A potent tyrosinase activator from Radix *Polygoni multiflori* and its melanogenesis stimulatory effect in B16 melanoma cells. Phytother. Res., 22: 660-663.

Hamid MA, Sarmidi MR, Park CS (2011). Mangosteen leaf extract increases melanogenesis in B16F1 melanoma cells by stimulating tyrosinase activity *in vitro* and by up-regulating tyrosinase gene expression. Int. J. Mol. Med., 29: 209-217.

Han JY, Fan JY, Horie Y, Miura S, Cui DH, Ishii H, Hibi T, Tsuneki H, Kimura I (2008). Ameliorating effects of compounds derived from *Salvia miltiorrhiza* root extract on microcirculatory disturbance and target organ injury by ischemia and reperfusion. Pharmacol. Ther., 117: 280-295.

Ho JH, Hong CY (2011). Salvianolic acids: small compounds with multiple mechanisms for cardiovascular protection. J. Biomed. Sci., 18: 30.

Lee CY, Sher HF, Chen HW, Liu CC, Chen CH, Lin CS, Yang PC, Tsay HS, Chen JJ (2008). Anticancer effects of tanshinone I in human non-small cell lung cancer. Mol. Cancer Ther., 7: 3527-3538.

Lee J, Lee J, Roh K, Kim MO, Kim JD, Park D (2010). Conditions for the differentiation of melanocyte-precursor cells from human cord blood-derived mesenchymal stem cells. Afr. J. Biotechnol., 9: 5975-5977.

Lin CC, Wu PS, Woei-Ming Liang D, Kwan CC, Chen YS (2011). Quality, antioxidative ability, and cell proliferation-enhancing activity of fermented black soybean broths with various supplemental culture medium. J. Food Sci., 77: C95-C101.

Lu Q, Zhang P, Zhang X, Chen J (2009). Experimental study of the anti-cancer mechanism of tanshinone IIA against human breast cancer. Int. J. Mol. Med., 24: 773-780.

Matkowski A, Zielinska S, Oszmianski J, Lamer-Zarawska E (2008). Antioxidant activity of extracts from leaves and roots of *Salvia miltiorrhiza* Bunge, *S. przewalskii* Maxim., and *S. verticillata* L. Bioresour. Technol., 99: 7892-7896.

Nizamutdinova IT, Lee GW, Son KH, Jeon SJ, Kang SS, Kim YS, Lee JH, Seo HG, Chang KC, Kim HJ (2008). Tanshinone I effectively induces apoptosis in estrogen receptor-positive (MCF-7) and estrogen receptor-negative (MDA-MB-231) breast cancer cells. Int. J. Oncol., 33: 485-491.

Passeron T, Mantoux F, Ortonne JP (2005). Genetic disorders of pigmentation. Clin. Dermatol., 23: 56-67.

Sato K, Toriyama M (2009). Depigmenting effect of catechins. Molecules, 14: 4425-4432.

Tang Y, Wang M, Le X, Meng J, Huang L, Yu P, Chen J, Wu P (2011). Antioxidant and cardioprotective effects of Danshensu (3-(3, 4-dihydroxyphenyl)- 2-hydroxy-propanoic acid from *Salvia miltiorrhiza*) on isoproterenol-induced myocardial hypertrophy in rats. Phytomedicine, 18: 1024 -1030.

Wang BQ (2010). *Salvia miltiorrhiza*: Chemical and pharmacological review of a medicinal plant. J. Med. Plants Res., 4: 2813-2820.

Wang JW, Wu JY (2010). Tanshinone biosynthesis in *Salvia miltiorrhiza* and production in plant tissue cultures. Appl. Microbiol. Biotechnol., 88: 437-449.

Wang M, Li J, Zhang L, Yang RW, Ding CB, Zhou YH, Yin ZQ (2011). Genetic diversity among *Salvia miltiorrhiza* Bunge and related species using morphological traits and RAPD markers. J. Med. Plants Res., 5: 2687-2694.

Yang Y, Ge PJ, Jiang L, Li FL, Zhu QY (2011). Modulation of growth and angiogenic potential of oral squamous carcinoma cells *in vitro* using salvianolic acid B. BMC Complement. Altern. Med., 11: 54.

Zhang LJ, Chen L, Lu Y, Wu JM, Xu B, Sun ZG, Zheng SZ, Wang AY (2010a). Danshensu has anti-tumor activity in B16F10 melanoma by inhibiting angiogenesis and tumor cell invasion. Eur. J. Pharmacol., 643: 195-201.

Zhang Y, Li X, Wang Z (2010b). Antioxidant activities of leaf extract of *Salvia miltiorrhiza* Bunge and related phenolic constituents. Food Chem. Toxicol., 48: 2656-2662.

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