Melanogenesis-Promoting Effects of *Sophora japonica* Methanol Extract in Mean-a Cells

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(Received May 15, 2015; Revised June 2, 2015; Accepted June 4, 2015)

**ABSTRACT**

The flowers and flower buds of *Sophora japonica* are well-known traditional Chinese medicinal herbs. Pharmacologic studies and cases from clinical practices have demonstrated that *S. japonica* has anti-tumor, anti-fertility, and anti-cancer activities. The active constituents of the herb are also known to be the flavonoid components. The melanogenic effects of *S. japonica* methanol extract (SJME) were evaluated in melan-a cells, which are immortalized cells derived from C57BL/6 mice. The maximum permissible level of SJME in melan-a cells was over 200 μg/mL. Additionally, SJME stimulated melanin synthesis, as well as tyrosinase activity, in a dose-dependent manner. Furthermore, the melanogenic effect of SJME was greater than that of IBMX, a well-known pigmenting agent. Lastly, SJME treatments at concentrations between 6.25 and 50 μg/mL increased tyrosinase messenger RNA expression. Collectively, these results suggested that SJME enhanced melanogenesis through an interaction with tyrosinase at the transcriptional level.

**Keywords**: Melan-a cells, Melanogenesis, *Sophora japonica*, Tyrosinase

**INTRODUCTION**

In patients with vitiligo, the white patches greatly affect their quality of life (Ongenae et al., 2006). Vitiligo may seem a minor disorder on first sight, but, people with severe depigmentation may have troubles in dating, self-esteem, or social activities psychologically (Kent & Al’Abadie, 1996; Papadopoulos et al., 1999). Therefore, modification of skin pigmentations with whitening agents and coloring agents is a great concern in the field of pharmacology and cosmeceuticals. Vitiligo is a depigmentation condition characterized by localized depigmented patches caused by loss of melanin in the epidermis or functional inability of melanocytes (Kemp et al., 2007). In order to explain the dysfunction of melanocytes in the epidermis, the autoimmune mechanism, the autotoxic mechanism, and the hypothesis that abnormal melanocytes nearby keratinocytes lose their function are presented (Moretti, 2002;...
Ongenae, 2003). Other causes of vitiligo include stress, infection, genetic factors, melatonin receptors, and migration and proliferation of damaged melanocytes (Kemp et al., 2007).

Melanin is secreted by melanocytes found in the basal layer of the epidermis. Upon biosynthesis by the melanocytes differentiating in the neural crest, melanin is transferred to the epidermis by keratinocytes (Yaar et al., 2006). The melanocyte has a specialized organelle called melanosome that regulates melanin production and contains various enzymes. Melanin plays important roles of protecting the skin from harmful effects via absorbing UV, removing reactive oxygen species, and scavenging toxic drugs and chemicals (Yaar et al., 2006). Melanin synthesis is initiated by the enzyme tyrosinase. Tyrosinase plays an important role in oxidizing tyrosine to 3,4-dihydroxyphenylalanine (DOPA) and DOPA to dopaquinone. Dopaquinone is spontaneously converted to dopachrome. The tyrosinase-related protein (TRP)-2 catalyzes the conversion of dopachrome to 5,6-dihydroxyindole carboxylic acid (DHICA), and TRP-1 catalyzes the oxidation of DHICA to indole-5,6-quinone-2-carboxylic acid (Kim & Uyama, 2005).

3-Isobutyl-1-methylxanthine (IBMX), which is a strong stimulant of melanin synthesis, increases cAMP content in cells via inhibiting cAMP phosphodiesterase, and dibutylryl cAMP increases tyrosinase activity and mRNA expression (Crow et al., 1994; Brown, 2001). Microphthalmia-associated transcription factor (MITF) is a master regulator of melanocyte development and melanin synthesis and regulates transcription of the major pigmentation enzymes including tyrosinase, TRP-1, and TRP-2 (Levy et al., 2006; Koo et al., 2008).

The flower and flower bud of *Sophora japonica* are well known as traditional medicinal herb in China and have anti-tumor, anti-sterilic and anti-cancer activities (Wang, 2001; Ma & Lou, 2006; Lo et al., 2009). The ingredients of *S. japonica* include flavonol triglucoside, isoflavonol, cumaronechromone, saponin, triterpene glucoside, phospholipid, alkaloid, amino acid, polysaccharide, and fatty acid (Grupp et al., 2001). *S. japonica* extract is usually used to treat hemorrhage-related disorders such as bloody excrement, rectal hemorrhage, uterine hemorrhage, and diarrhea (Zhao, 2004). This study was conducted to evaluate the effects of *S. japonica* methanol extract (SJME) on melanin synthesis and tyrosinase activity along with tyrosinase mRNA expression.

**MATERIALS AND METHODS**

1. Reagents and apparatus

Dimethyl sulfoxide (DMSO), 2,6-di-tert-butylated hydroxytoluene (BHT), 1,1-diphenyl-2-picryl hydrazyl (DPPH), IBMX, 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), tannic acid, L-tyrosine, ascorbic acid, and diethylene glycol were purchased from Sigma company (USA). Rutin was purchased from Acros company (USA). Cells were observed using an inverted microscope (CKX41, Olympus, Japan) and cultured in a CO2 incubator (MCO-15AC, Sanyo Electric, Japan).

2. Plant materials

Methanol extract from leaves and stems of *S. japonica* (Leguminosae: 019-100) was obtained from the Korea Plant Extract Bank (Daejeon, Korea). This specimen was dissolved into DMSO before use.

3. Antioxidant activity analysis

The total polyphenol content of SJME was determined with the Folin-Denis method (Folin & Denis, 1912). One milliliter of Folin’s reagent was added to 1 mL diluted sample. After allowing to be settled for 3 min, 1 mL of 10% Na2CO3 was added. The mixture was allowed to stand at room temperature for 1 h and absorbance at 760 nm was measured. A calibration curve was prepared using tannic acid.

Total flavonoid content of SJME was measured using the modified method of Davies et al. (AOAC, 1995). Ten milliliter of diethylene glycol and 1 mL of 1 N NaOH were added to 1 mL diluted sample. After mixing and allowing to be reacted in a water bath at 37°C for 1 h, absorbance at 420 nm was measured. A calibration curve was prepared using rutin.

Electron-donating ability was measured according to the method of Pérez et al. (2004). SJME was dissolved in DMSO to final concentrations of 100, 500, and 1,000 μg/mL. One milliliter of 4 M DPPH. The mixture was shaken and kept for 10 sec in a water bath at 60°C followed by stand at room temperature for 20 min. Absorbance at 525 nm was measured. The synthetic antioxidant BHT was used as positive control.

4. Cell culture

The melan-a cells used in this study were obtained from Dr. Dorothy Bennett (St. George’s Hospital, UK). The cells were grown in Roswell Park Memorial Institute medium (RPMI-1640) supplemented with 10% fetal bovine serum, 1% penicillin/streptomycin, and 200 nM 12-O-tetradecanoylphorbol-13-acetate at 37°C in an incubator with 10% CO2 for 72 h.