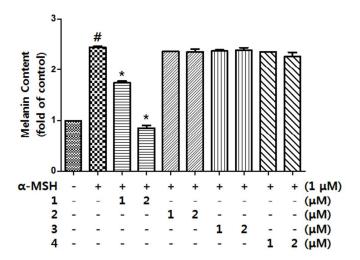
Caffeic Acid Phenethyl Ester Inhibits Alpha-Melanocyte Stimulating Hormone-Induced Melanin Synthesis through Suppressing Transactivation Activity of Microphthalmia Associated Transcription Factor

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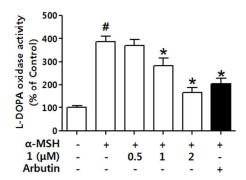
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Contents

- **S1.** Caffeic acid (2), phenethyl alcohol (3), and phenethyl cinnamate (4) did not significantly inhibit melanin synthesis.
- **S2.** Caffeic acid phenethyl ester (1) inhibits the intracellular activity of tyrosinase.
- **S3.** The sequences of promoter region of *TRP-1* and *TRP-2* genes were presented.



S1. Caffeic acid (2), phenethyl alcohol (3), and phenethyl cinnamate (4) did not significantly inhibit melanin synthesis. B16-F10 cells were cultured with α -MSH (1 μ M) and/or 2-4 (at the indicated concentrations) for 72 h in phenol red-free DMEM. Caffeic acid phenethyl ester (1) was used for comparison. The cells were lysed with 1 M NaOH and were analyzed by spectrophotometry at 400 nm for estimating melanin contents (# p <0.05 in comparison with the control group and * p <0.05 in comparison with the α -MSH group).



S2. Caffeic acid phenethyl ester (1) inhibits the intracellular activity of tyrosinase. B16-F10 cells were cultured with α -MSH (1 μ M) and/or 1 (at the indicated concentrations) for 72 h.

The cells were lysed and equal amount (30 μ g) of cell lysate was used for examining L-DOPA oxidase activity. Arbutin (100 μ M) was used as a positive control purpose (# p <0.05 in comparison with the control group and * p <0.05 in comparison with the α -MSH group).

A)
-180 TGGTATACAGATAAAGAAAAATAAAATCACTACAACGAAAGCAAAATCTCTTCAGCGTCT
CTAATACATCTTCCAAATCAGTGTGTCTGACCTTTTCTTAAGACTTTAACCATCACAAGG
Chip-trp1-F
AAACCAGTGGGGAGGGAGTCATGTGCTGCCTAGTAGTTAAAGGGCAGGAGAATTCACTGG
M-box

+1 TGTGAGAAGGGATTAGTGAGAGCTGGAAGAGAGACCAGCCCCTCCCAGTGTGAGGAATC
Chip-trp1-R
TGGCTTGGGATTTACTGTCTGGCAGAAAATCTCTTCGGGC

- B)
 -240 AGGTCACAAGCTTGGCTGGGACACATGAGCCCAATAAAGAGGCTGATTTTACCCTCCTGA

 ChIP-TRP2-F

 CACAAAGCCAGACACTTTATCAGCTTCATTGTGCACTTAGGGTCATGTGCTAACAAAGAG

 M-box
- **S3.** The sequences of promoter region of *TRP-1* and *TRP-2* genes were presented. (A) The sequence 180 bp upstream to 60 bp downstream of the mouse *TRP-1* transcription site (+1) was represented. (B) The sequence 240 bp upstream to 60 bp downstream of the mouse *TRP-2* transcription site (+1) was shown. Primer sequences used for ChIP assay of MITF are indicated by bolded characters. M-box, the MITF binding site, is boxed and labeled.