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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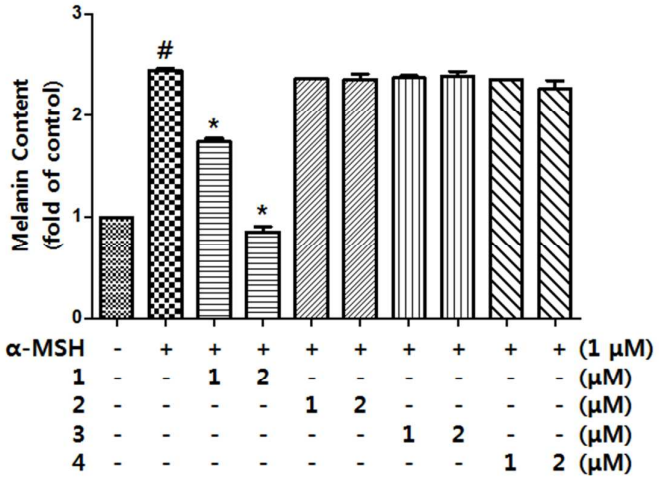
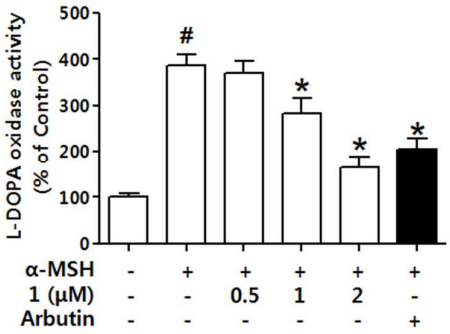


S1. Caffeic acid (**2**), phenethyl alcohol (**3**), and phenethyl cinnamate (**4**) did not significantly inhibit melanin synthesis. B16-F10 cells were cultured with α-MSH (1 μM) and/or **2**-**4** (at the indicated concentrations) for 72 h in phenol red-free DMEM. Caffeic acid phenethyl ester (**1**) 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 μM) and/or **1** (at the indicated concentrations) for 72 h.

The cells were lysed and equal amount (30 μg) of cell lysate was used for examining L-DOPA oxidase activity. Arbutin (100 μM) 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).

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.