Supporting information to:

**Mangiferin Inhibits Passive Cutaneous Anaphylaxis Reaction and Pruritus in Mice**

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**Isolation of mangiferin**
Artificial cultivated *Anemarrhena asphodeloides* Bunge (family Liliaceae) was collected at Taegu, Korea, and identified by Dr. N. J. Kim, East-West Medicine Research Institute, Kyung Hee University, Seoul, Korea. A voucher specimen was deposited at the Herbarium of the College of Pharmacy, Kyung Hee University, Seoul, Korea (KHOPS-06-017). The dried AA (3.5 kg) was extracted five times with MeOH in a boiling water bath. The MeOH extracts were combined and evaporated to dryness under reduced pressure to yield 846 g. This extract was partitioned with *n*-hexane and H$_2$O. Dried powder was extracted in a stepwise manner with CHCl$_3$ (71 g), ethyl acetate (20 g), and *n*-butanol (200 g). The butanol extract, which most potently inhibited the passive cutaneous anaphylaxis (PCA) reaction induced by IgE–antigen complex, was separated by column chromatography (14 × 110 cm) on silica gel
and eluted with a gradient of MeOH (10% → 20% → 40% → 60% → 70% → 100%) in CH₂Cl₂ (48 L). Six fractions (FB1–FB6, each 8 L) were collected on the basis of their thin-layer chromatography (TLC) profiles, and their inhibitory activity against PCA reaction was tested. FB5 (29 g), the most potent inhibitor, was separated by silica gel column chromatography (8 × 53 cm) and eluted with CH₂Cl₂–MeOH–H₂O (7:2:0.5) (6 L, each 1.2 L) to afford several subfractions (FB5-1–FB5-5), one of which (FB5-5, 5.9 g) was further subjected to semi-preparative HPLC (35% CH₃CN in 50% MeOH at a flow rate of 7 mL/min over 60 min, GS-320 column, 30 × 500 mm; Japan Analytical Instrument) to afford mangiferin (4.3 g). Its purity was measured by HPLC (Younglin Instrument): column, Develosil C30-UG-5 (250 × 4.6 mm, i.d. 5 μm, 100 Å; Nomura Chemical): mobile phase, 35% CH₃CN in 50% MeOH; flow rate, 1 mL/min; detector, 254 nm.

**Mangiferin:** Colorless, amorphous solid (purity >96%); ESI(−)-MS/MS: 421, 301 [M – Na]⁻.

**Assay of scratching behavior frequency**

The scratching behavior experiment was performed on male mice according to the method of Choo et al. [1]. Briefly, the mice were placed in acrylic cages (22 × 22 × 24 cm) and allowed to acclimatize for about 10 min. The rostral part of the skin on the back of the mice was clipped, and 50 μg/50 μL of compound 48/80 (saline alone in control group) was then intradermally injected into the BALB/c mice. Immediately after the intradermal injection, the mice (one animal/cage) were placed back in the same cages, and their scratching behavior was recorded using an 8-mm video camera (SV-K80; Samsung). Scratching with the hind paw at the injected sites was counted for 60 min. The test agents, given at doses of 5 and 20 mg/kg for intraperitoneal administration and 20 and 50 mg/kg for oral administration, and the positive control azelastine (10 mg/kg for intraperitoneal and oral administration) were administered 1 h prior to the scratching agent. Normal control animals were administered vehicle alone.

**References**