

Mangiferin Inhibits Passive Cutaneous Anaphylaxis Reaction and Pruritus in Mice

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Abstract

The antiallergic effect of mangiferin isolated from the rhizome of *Anemarrhena asphodeloides* Bunge (family Liliaceae) was measured *in vitro* and *in vivo*. Orally and intraperitoneally administered mangiferin potently inhibited passive cutaneous anaphylaxis (PCA) reaction induced by IgE-antigen complex as well as pruritus induced by compound 48/80 in mice. Mangiferin also inhibited the expression of the proinflammatory cytokine TNF- α and the IgE-switching cytokine IL-4 as well as transcription factor NF- κ B activation in RBL-2H3 cells stimulated by IgE-antigen complex. These findings suggest that mangiferin may improve PCA reaction and pruritus.

Key words

Anemarrhena asphodeloides · Liliaceae · mangiferin · passive cutaneous anaphylaxis · pruritus

Abbreviations

AA:	<i>Anemarrhena asphodeloides</i>
DNP-HSA:	dinitrophenol-human serum albumin
ICR:	institute of cancer research
IL:	interleukin
PCA:	passive cutaneous anaphylaxis
RBL:	rat basophilic leukemia
TNF:	tumor necrosis factor

Supporting information available online at <http://www.thieme-connect.de/ejournals/toc/plantamedica>

Allergies, such as asthma, rhinitis, and atopic dermatitis, are now the most prevalent and rapidly increasing chronic health problem in industrialized countries [1]. Mast cells and basophils are well known as critical participants in various biological processes, including allergic diseases [2]. These cells express on their surface membrane receptors with high affinity and specificity for IgE. The interaction of multivalent antigens with surface-bound IgE initiates a series of biochemical events that culminate in the release of histamine and the production of proinflammatory cytokines, which are stored within the cells' numerous cytoplasmic granules, and cause allergic reactions such as anaphylaxis, inflammation, and pruritus.

Anemarrhena asphodeloides Bunge (AA, family Liliaceae), which primarily contains mangiferin and timosaponins as the main constituents [3,4], has been used as an antioxidant, analgesic,

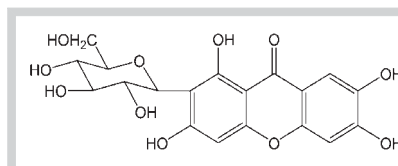


Fig. 1 Structure of mangiferin isolated from *Anemarrhena asphodeloides* Bunge.

anti-inflammatory, antiallergic, antidiabetic, and memory-enhancing agent in traditional medicine in China, Japan, and Korea [5–10]. In the preliminary experiment, AA inhibited passive cutaneous anaphylaxis (PCA) reaction induced by IgE-antigen complex in mice. Therefore, mangiferin (● Fig. 1) was isolated by active component-guided fractionation and its antiallergic effects in mice were investigated.

The inhibitory activity of five components isolated from the MeOH extract of AA against PCA reaction induced by IgE-antigen complex was measured. The most active component was determined to be mangiferin (purity >95%) through comparison of spectroscopic data with that of previously reported literature [3]. Orally and intraperitoneally administered mangiferin at a dose of 20 mg/kg potently inhibited PCA reaction (● Fig. 2). Orally administered mangiferin at a dose of 50 mg/kg was found to inhibit PCA reaction by 54.9% of the control group, comparable to the effects of azelastine at a dose of 10 mg/kg (63.5%). Orally and intraperitoneally administered mangiferin also potently inhibited compound 48/80-induced pruritic reaction. Particularly, orally administered mangiferin at a dose of 50 mg/kg was found to inhibit the pruritic reaction by 43.0% of the control group, having activity weaker than that of azelastine at a dose of 10 mg/kg (62.2%).

When the effect of mangiferin on cytokine mRNA expression in RBL-2H3 cells induced by IgE-antigen complex was investigated by using reverse transcription-polymerase chain reaction (RT-PCR) analysis, mangiferin at a dose of 100 μ M inhibited mRNA expression of IL-4 and TNF- α by 62.8% and 52.1%, respectively (● Fig. 3). However, mangiferin at a concentration of 200 μ M did not show cytotoxicity against RBL-2H3 cells. When its effect on cytokine production in RBL-2H3 cells induced by IgE-antigen complex was investigated by ELISA, mangiferin at a dose of 100 μ M also inhibited IL-4 and TNF- α production by 73.9% and 38.6%, respectively. Mangiferin also inhibited the activation of transcription factor NF- κ B in RBL-2H3 cells induced by IgE-antigen complex.

Antihistamine agents such as azelastine and steroids such as betamethasone valerate are representative antiallergic drugs [11, 12]. Azelastine is an H1-receptor antagonist that also decreases mediator release from mast cells and basophils, while betamethasone valerate is a potent corticosteroid. Both of these agents have side effects. Therefore, new agents for clinical use have been developed from traditional Chinese medicines [13]. Mangiferin isolated from AA inhibited the proinflammatory cytokine TNF- α , as previously reported [14], as well as the IgE-switching cytokine IL-4. Mangiferin also inhibited proinflammatory cytokine-regulating transcription factor NF- κ B activation in RBL-2H3 cells induced by IgE-antigen complex, as previously reported [10,14]. In addition, mangiferin inhibited PCA reaction induced by IgE-antigen complex, as previously reported [7,8], as well as compound 48/80-induced scratching behavior. These results suggest that the anti-scratching behavioral effect of mangiferin may be due to the inhibition of IL-4 expression.

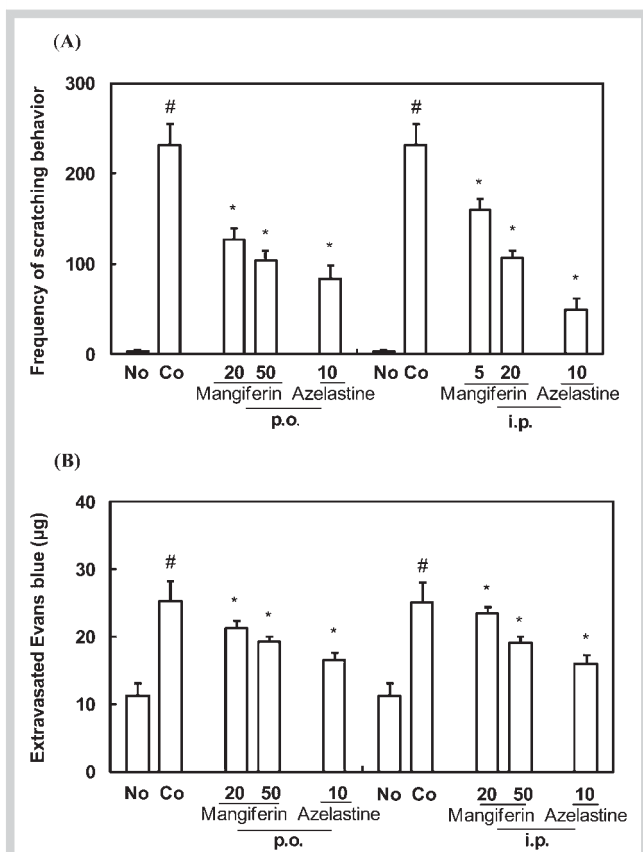


Fig. 2 Inhibitory effect of mangiferin and azelastine on PCA reaction induced by IgE-antigen complex in mice (A) and pruritus induced by compound 48/80 (B). PCA reaction in mice was induced by the intradermal injection of anti-DNP-HSA. Tested agents were orally or intraperitoneally administered 60 min prior to challenge with DNP-HSA antigen. The amounts of extravasated Evans blue from the dorsal skin (1 × 1 cm) of the control group (Co) stimulated with IgE-antigen complex and of the vehicle-treated group (No) were 25.3 ± 3.1 μg and 11.2 ± 1.9 μg, respectively. The frequency of scratching behavior, recorded over 1 h, of the normal control (No, treated with saline alone) and of the control group (Co, treated with compound 48/80) was 3 ± 1 and 232 ± 23, respectively. Inhibition values indicate the mean ± SD (n = 5). [#] Significantly different compared with normal group (p < 0.05). * Significantly different compared with control group (p < 0.05).

Based on these findings, mangiferin may improve IgE-mediated type I allergic reactions and inhibit allergic cytokine biosynthesis of mast cells and basophils via the regulation of transcription factor NF-κB activation.

Materials and Methods

Dinitrophenol-human serum albumin (DNP-HSA), histamine, and compound 48/80 were purchased from Sigma. Azelastine (purity > 98%) was purchased from Enzo Life Sciences. RNeasy Minikit was purchased from Qiagen.

RBL-2H3 cells were prepared by the method of Choo et al. [15]. Briefly, the previously cultured RBL-2H3 cells (5 × 10⁵ cells) were treated with 0.5 μg/mL of mouse monoclonal IgE for sensitization of cells. The cells (1.8 mL) were exposed to 0.2 mL of test agents for 20 min (for RT-PCR), 1 h (for immunoblot), or 4 h [for enzyme-linked immunoassay (ELISA)], followed by treatment with

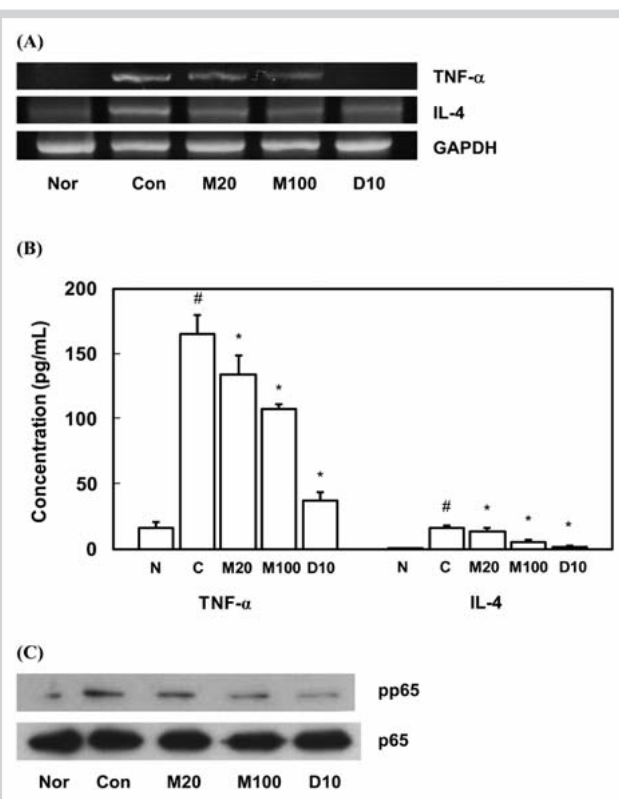


Fig. 3 Effect of mangiferin on some cytokine mRNA and protein expression levels of RBL-2H3 cells induced by IgE. RBL-2H3 cells (5 × 10⁵ cells) were treated with 0.5 μg/mL of mouse monoclonal IgE and then exposed to 0.2 mL of agent (C: vehicle alone; M20: 20 μM mangiferin; M100: 100 μM mangiferin; D10: 10 μM dexamethasone) for 20 min (for RT-PCR) or 4 h (for ELISA), followed by treatment with 0.2 mL DNP-HSA (1 μg/mL) for 40 min (mRNA expression) or 4 h (protein expression) at 37 °C. Finally, RT-PCR for IL-4, TNF-α, and GAPDH (A); ELISA for IL-4 and TNF-α (B); and immunoblot for NF-κB (pp65 and p65) (C) were performed. The normal group (N) was treated with vehicle alone instead of agent and IgE-antigen complex. Values represent the mean ± SD for triplicate experiments. [#] Significantly different compared with normal group (p < 0.05). * Significantly different compared with control group (p < 0.05).

0.2 mL DNP-HSA (1 μg/mL) for 40 min at 37 °C. Total RNA was extracted by using the RNeasy Minikit, and then RT-PCR for IL-4, TNF-α, and GAPDH was performed. The immunoblot for NF-κB (pp65 and p65) was performed according to the method of Lee et al. [16]. The supernatant (50 μL) was transferred into 96-well (ELISA) plates, and then IL-4 and TNF-α concentrations were determined using commercial ELISA kits (Pierce Biotechnology). The cytotoxicity of mangiferin against RBL-2H3 cells was assayed by the SRB method [17].

The male ICR and BALB/c mice (5 weeks old, 20–25 g) were supplied by Orient Experimental Animal Breeding Center. All animals were housed in wire cages at 20–22 °C and 50 ± 10% humidity, fed standard laboratory chow (Orient Experimental Animal Breeding Center), and allowed water *ad libitum*. All procedures relating to animals and their care conformed to the international guidelines Principles of Laboratory Animal Care (NIH publication no. 85–23, revised 1985).

PCA reaction induced by IgE-antigen complex and pruritus induced by compound 48/80 were measured according to the previous method of Choo et al. [15].

All data were expressed as the mean $\pm$ standard deviation, and statistical significance was analyzed by one-way ANOVA followed by the Student-Newman-Keuls test.

Supporting information

Descriptions of the isolation of mangiferin from AA and of the assay of scratching behavior frequency are available as Supporting Information.

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