

ORIGINAL ARTICLE

Suppressive effect of mangosteen rind extract on the spontaneous development of atopic dermatitis in NC/Tnd mice

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ABSTRACT

Atopic dermatitis (AD) is a chronic and relapsing skin disorder characterized by pruritic and dry skin lesions with allergic inflammation. Recent studies have revealed anti-inflammatory and anti-allergic effects of xanthenes in mangosteen through regulation of the nuclear factor (NF)- κ B signaling pathway. Activation of NF- κ B signals is responsible for allergic inflammation in AD. To develop a new preventive therapy for AD, we examined the effects of the natural medicine, mangosteen rind extract (ME), on AD in a murine model. ME (250 mg/kg per day) was administered to NC/Tnd mice, a model for human AD, for 6 weeks to evaluate its preventive effects on AD. We also confirmed the effects of ME on various immune cell functions. Oral administration of ME prevented the increase of clinical skin severity scores, plasma total immunoglobulin E levels, scratching behavior, transepidermal water loss and epidermal hyperplasia in NC/Tnd mice; moreover, no adverse effects were noted. We demonstrated that ME suppressed thymic stromal lymphopoietin and interferon- γ mRNA expression both *in vitro* and *in vivo*. Not only immunoglobulin E production from splenic B cells but also immunoglobulin E-mediated degranulation of bone marrow-derived cultured mast cells was significantly reduced by the addition of ME to the culture. In addition, mRNA expression levels of nerve growth factor were decreased in ME-administrated NC/Tnd mice compared with those of controls. Keratinocyte proliferation was well-controlled by ME application. Oral administration of ME exhibited its suppressive potential on the early development of AD by controlling inflammation, itch and epidermal barrier function.

Key words: α -mangostin, atopic dermatitis, mangosteen, rind, thymic stromal lymphopoietin.

INTRODUCTION

The number of patients with atopic dermatitis (AD) has been increasing steadily worldwide. In fact, the prevalence of AD has doubled or tripled during the past three decades, with 15–30% of children and 2–10% of adults being affected.¹ AD is a chronic and relapsing skin disorder characterized by pruritic and dry skin lesions with allergic inflammation and elevated levels of serum immunoglobulin (Ig)E. While the exact causes of AD are not understood, complex interactions of genetic and environmental factors result in immunological dysregulation and skin barrier dysfunction.²

T-helper (Th)2 immune responses play a crucial role in the pathogenic mechanism of AD in the early phase. After the invasion of antigens or irritants, keratinocytes produce thymic stromal lymphopoietin (TSLP),^{3–5} which activates Langerhans cells (LC). TSLP-activated LC prime naive CD4⁺ T cells to differentiate into Th2 cells, which produce cytokines such as interleukin (IL)-4, IL-5 and IL-13. TSLP-activated LC also produce Th2-attracting chemokines such as CCL17 (thymus and activation-regulated chemokine; TARC), CCL22 (macrophage-derived chemokine; MDC) and CCL24 (Eotaxin-2), causing an influx of Th2 lymphocytes and eosinophils into the skin and inducing inflammation.^{5–7} Therefore, controlling the early polarization to Th2-type immune responses may bring a favorable prognosis to patients with AD.

Itch is one of the most serious clinical symptoms of AD,^{8,9} which is induced by the extension of sensory nerve fibers to the epidermis and the release of chemical mediators from mast cells. Nerve fibers (C-fibers) possess free nerve endings at the

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boundary between the dermis and the epidermis; however, in patients with AD, C-fibers sprout in the epidermis,^{10,11} because nerve growth factor (NGF)^{12–14} released from keratinocytes induces their extension, and decreased expression of semaphorin 3a (Sema3a)^{15–17} weakens the retraction of C-fibers in the epidermis. Increasing density of C-fibers in the epidermis causes hypersensitivity to external stimuli. A Th2-dominated environment (in particular IL-4 and IL-13) induces hyperproduction of IgE and activation of mast cells, inducing itch sensation by chemical mediators from mast cells. Scratching aggravates the symptoms of AD by destruction of the skin barrier and induction of inflammation, leading to a remarkable decrease in the quality of life. So, alleviation of pruritus is the most desired outcome in the research and development of new reagents for AD. Affected lesions of AD display impaired epidermal barrier function through inherited and acquired factors. Dermatological aspects of AD including epidermal hyperplasia and barrier dysfunction accelerate penetration of environmental allergens and elevation of transepidermal water loss (TEWL).^{18,19} Repeated scratching behavior exacerbates barrier dysfunction leading to development of AD. To interrupt the progression of this syndrome, early approaches using natural resources are desired.

In the present study, we identified a new, natural preventive medicine for AD: mangosteen rind extract (ME). Mangosteen, *Garcinia mangostana* L., is a tropical plant grown in South-East Asia, the rind of which has been used as a traditional medicine for skin diseases and diarrhea.²⁰ In recent years, ME has been shown to contain xanthenes, α -mangostin and γ -mangostin, which are the most abundant compounds in the rind extract and possess the various activity such as anti-inflammatory,^{21–23} anti-allergic,^{21,24} antioxidant^{25,26} and antimicrobial activities.^{27–29} The anti-inflammatory mechanism of ME appears to be inhibition of the conversion of arachidonic acid to prostaglandin E₂ by cyclooxygenase and blocking of nuclear factor (NF)- κ B activity.²³ In an *in vivo* experiment, administration of α -mangostin and γ -mangostin attenuated ovalbumin (OVA)-induced allergic asthma, including inflammatory cell recruitment into the airway, and increased levels of Th2 cytokines and IgE.³⁰ Also, it was shown that these effects were associated with changes in the expression levels of signaling molecules from the Akt/NF- κ B pathway. Herein, we present that oral administration of ME for 6 weeks prevented AD development in NC/Tnd mice, a model for human AD.³¹ These observations indicate that ME will be an effective preventive reagent for AD, extensively affecting inflammation, itch and epidermal barrier function.

METHODS

Mice, cells and reagents

NC/Tnd mice were maintained in air-uncontrolled (conventional) or specific pathogen-free (SPF) conditions. The mice were provided with a pellet diet and water *ad libitum*. All experiments with animals complied with the standards specified in the guidelines of the University Animal Care and Use Committee of the Tokyo University of Agriculture and Technology. PAM212

keratinocytes were provided by Dr I. Katayama (Osaka University Graduate School of Medicine). P815 mast cells (JCRB0078), BCL1 B cells (JCRB9117) and BW5147 T cells (JCRB9002) were provided by the Japan Health Science Foundation.

Dried mangosteen rind was extracted using 70% ethanol at 80°C for 1 h. After extraction, ME was freeze-dried. The yield of ME from dried mangosteen rind was 27.0%, and ME contains 13.1% α -mangostin and 2.4% γ -mangostin. CRF-1 diet (Oriental Yeast, Tokyo, Japan) containing 0.25% freeze-dried ME was prepared. α -mangostin and γ -mangostin were obtained from ME as previously described.^{23,24} All chemicals used were purchased from Sigma-Aldrich (St Louis, MO, USA), unless otherwise indicated.

Oral administration of ME to NC/Tnd mice

Conventional 6-week-old NC/Tnd mice were orally administered a CRF-1 diet containing or not containing ME daily for 6 weeks. The daily intake of ME was estimated to be 250 mg/kg per day. As a positive control, 100 mg of 0.1% FK506 (tacrolimus) ointment (0.1% Protopic, Fujisawa Pharmaceutical, Osaka, Japan) was applied to NC/Tnd mice. Clinical features of dermatitis were scored every week according to criteria described previously.³¹ Scratching behavior of mice was quantified at the beginning and the end of the oral administration course using a SCLABA-Real system (Noveltec, Kobe, Japan) according to the manufacturer's instructions.³² Blood was collected from the tail vein once every other week, using heparinized capillaries, and plasma was stored at –80°C until use. Total IgE levels in plasma samples were measured using a sandwich enzyme-linked immunoassay (ELISA). TEWL was measured on the back once every other week, using a multi-probe adapter (CK Electronic, Koln, Germany). At the end of the experiment, dorsal skin samples were obtained for real-time polymerase chain reaction (PCR) and histological analysis. Plasma levels of α -mangostin and γ -mangostin was analyzed by liquid chromatography mass spectrometry (LC-MS)/MS as previously described.³³

Histological analysis

Dorsal skin sections (6 μ m) were stained with hematoxylin-eosin (HE) for measurement of epidermal thickness, with acidic toluidine blue (pH 4.0) for counting mast cells, and with Congo red for counting eosinophils. Epidermal thickness was measured at five fields ($\times 200$) in each mouse by using HE specimens, and the average of five mice in each group was calculated. The total number of the mast cells or eosinophils in four fields ($\times 100$) in each specimen ($n = 7$) was counted under a microscope, and the mean number of cells in one field was calculated.

Real-time reverse transcription PCR

Total RNA obtained from each experiment (*in vivo* and *in vitro*) was reverse transcribed into cDNA using a TAKARA PrimeScript 1st strand cDNA Synthesis kit (TAKARA Bio, Shiga, Japan), according to the manufacturer's instructions. Reaction

mixtures were amplified with SYBR Premix Ex Taq II (TAKARA Bio) in the presence of 0.2 $\mu\text{mol/L}$ each of the sense and antisense primers for TSLP, IL-4, IL-5, IL-13, IL-12, interferon (IFN)- γ , TARC, MDC, eotaxin-2, NGF, Sema3a and β -actin mRNA. The sequences of primers are presented in Table 1. Fluorescence intensity was measured using an ABI Prism 7000 Sequence Detector (Applied Biosystems, Tokyo, Japan). Relative expression levels of the target gene, normalized to β -actin, were given by $2^{-\Delta\Delta\text{CT}}$.

Epidermal tissue culture

Skin samples (6-mm punch biopsies) were obtained from SPF NC/Tnd mice neonates (1–3 days after birth). After incubation with 0.25% trypsin ethylenediaminetetraacetic acid solution for 1 h, epidermal sheets were isolated from skin biopsies and pre-incubated in CnT-07 medium (CELLNTEC, Bern, Switzerland) containing various concentrations of ME for 1 h, followed by incubation with 25 ng/mL tumor necrosis factor (TNF)- α and 10 ng/mL IL-1 β (R&D Systems, Minneapolis, MN, USA) to induce TSLP mRNA expression. After 6 h, total RNA was extracted from the epidermis samples using a TAKARA RNA FastPure RNA kit (TAKARA Bio).

Cytokine production in lymphocytes

Single cells were obtained from axillary lymph nodes of conventional NC/Tnd mice with severe dermatitis, and suspended

in RPMI-1640 with 10% fetal calf serum (FCS) and antibiotics. Cells were adjusted to 2×10^6 cells/mL and cultured with or without 1 $\mu\text{g/mL}$ pokeweed mitogen (PWM) in the presence or absence of various concentrations of ME. After 24 h, total RNA was extracted using a TAKARA RNA FastPure RNA kit.

IgE production from splenic B cells

CD19⁺ B cells were isolated from spleens of SPF NC/Tnd mice using ROBOSEP (Stemcell Technologies, Vancouver, BC, Canada) and a CD19-positive selection kit (Stemcell Technologies). After cells were adjusted to 5×10^5 cells/mL, IgE production was induced with 500 U/mL rmlL-4 (R&D Systems) and 10 $\mu\text{g/mL}$ lipopolysaccharide (LPS) in the presence of various concentrations of ME. Nine days later, IgE concentrations in supernatants were measured by ELISA.

Bone marrow-derived cultured mast cells (BMCMC) degranulation

Bone marrow-derived cultured mast cells were generated from bone marrow in SPF NC/Tnd mice as previously reported.³⁴ In the presence of various concentrations of ME, IgE-mediated activation of BMCMC was induced using anti-dinitrophenyl (DNP) IgE and DNP bovine serum albumin (BSA). After 1 h, β -hexosaminidase activity in supernatants and cell lysates was measured using *p*-nitrophenyl-*N*-acetyl- β -D-glucosamine as a substrate. β -Hexosaminidase release (%) was calculated using the following formula: β -hexosaminidase release (%) = $\text{OD}_{405}[\text{supernatant}] / (\text{OD}_{405}[\text{supernatant}] + \text{OD}_{405}[\text{cell lysate}]) \times 100$

Cell proliferation assay

PAM212 cells (2×10^4 cells/mL) were incubated with Dulbecco's modified Eagle's medium (DMEM) containing 10% FCS and antibiotics, in the presence of various concentrations of ME. P815 (5×10^4 cells/mL) or BCL1 (2×10^5 cells/mL) cells were incubated with RPMI-1640 containing 10% FCS and antibiotics, and BW5147 cells (1.5×10^5 cells/mL) were incubated with DMEM containing 10% horse serum and antibiotics for 24, 48 and 72 h. Cells were collected and viable cell numbers were counted using a Trypan blue dye exclusion test.

Blood biochemistry

Biochemical examination of plasmas was performed using Vet-Scan VS2 (Abaxis, Union City, CA, USA), according to the manufacturer's instructions. Samples were collected from control or ME-treated mice at the end of the experiment.

Calculation for contribution ration

Mangosteen rind extract contains 13.1% α -mangostin and 2.4% γ -mangostin, with molecular weights of 410.5 and 396.4, respectively. So, the contribution ratios were calculated by the following formulas.

Contribution ratio for ME activity of α -mangostin

$$= \{(\text{IC}_{50} \text{ of ME})(\mu\text{g/mL}) \times 0.131 \div 410.5 \times 1000\} / (\text{IC}_{50} \text{ of } \alpha\text{-mangostin})(\mu\text{mol/L}) \times 100$$

Table 1. Primer sequences for real-time polymerase chain reaction

mRNA target	Primer sequence
TSLP	5'-CGAGCAAATCGAGGACTGTGAG-3' 3'-CTTCGCGAGTTACTGCTGACG-5'
IL-4	5'-TCTCGAATGTACCAGGAGCCATATC-3' 3'-AGACATCCCGAAGGTTCCACGA-5'
IL-5	5'-AGCACAGTGGTGAAAGAGACCTT-3' 3'-TTTAGTGGTCGATACGTAACCT-5'
IL-13	5'-CAATTGCAATGCCATCTACAGGAC-3' 3'-AGTCGATGTGTTTCGTTGACAAAGC-5'
IL-12	5'-GGAAGCACGGCAGCAGATA-3' 3'-GGTAAGGATGAAGAGGGAGTTCAA-5'
IFN- γ	5'-CGGCACAGTCATTGAAAGCCTA-3' 3'-CTGTTAGTCCGGTAGTCGTTG-5'
TARC	5'-CCGAGAGTGCTCCCTGGATTA-3' 3'-AGACTGACAGGTCCCGTTTCA-5'
MDC	5'-GGCACCTATCCAGTGCCACA-3' 3'-CTCAAAGTCCGACCAGGTGGT-5'
Eotaxin-2	5'-AACATGGCGGGCTCTGCAAC-3' 3'-TCGTAGACAGGGTTCCGTCC-5'
NGF	5'-TGCCAAGGACGCAGCTTTC-3' 3'-GACTTCGGGTGACCTGATTTGAAGT-5'
Sema3a	5'-AGATGCTCCATTCCAGTTTGTTTAC-3' 3'-ATGTTCACTACGCCACCGAATACA-5'
β -Actin	5'-CATCCGTAAAGACCTCTATGCCAAC-3' 3'-ACACCTAGCCACCGAGGTA-5'

IFN, interferon; IL, interleukin; MDC, macrophage-derived chemokine; NGF, nerve growth factor; Sema3a, semaphorin 3a; TARC, thymus and activation-regulated chemokine; TSLP, thymic stromal lymphopoietin.

and

$$\begin{aligned} &\text{Contribution ratio for ME activity of } \gamma\text{-mangostin} \\ &= \{(\text{IC}_{50} \text{ of ME})(\mu\text{g/mL}) \times 0.024 \div 396.4 \times 1000\} \\ &\quad / (\text{IC}_{50} \text{ of } \gamma\text{-mangostin})(\mu\text{mol/L}) \times 100 \end{aligned}$$

Statistics

The multiple comparisons of Dunnett or two-tailed Student's *t*-tests were performed for statistical analyses of the data, and $P < 0.05$ was taken as the level of significance.

RESULTS

Preventive effects of ME on AD development in NC/Tnd mice

To evaluate the preventive effects of ME on AD development, 6-week-old NC/Tnd mice (before the onset of AD development) were administrated a CRF-1 diet containing ME (250 mg/kg/day) for 6 weeks. Positive controls were NC/Tnd mice to which 100 mg of 0.1% FK506 ointments were applied. Figure 1 shows the effects of ME administration on clinical skin severity scores, plasma total IgE levels, scratching behavior and TEWL in NC/Tnd mice. In control mice, clinical symptoms such as erythema, edema and dry skin were observed, and the clinical skin severity scores were increased steadily with aging (Fig. 1a). In contrast, the clinical skin severity scores in ME-administrated mice kept lower levels than in controls and FK506-treated mice during the experiments. ME-administrated mice had no obvious clinical symptoms. While plasma total IgE levels in controls were elevated at the end of the experiment (Fig. 1b), those in the ME-administrated group and FK506-treated group were significantly suppressed. Scratching behavior was recorded as frequency and duration of scratching per 20 min. Both frequency and duration of scratching were increased in controls but not in ME-administrated or FK506-treated mice over time (Fig. 1c,d). Furthermore, TEWL in ME-administrated mice remained at a lower level than in both controls and FK506-treated mice during the experiments (Fig. 1e). There was no statistical difference between ME-administrated mice and control mice in bodyweight (Fig. 1f). In addition, we performed blood biochemistry at the end of the experiment (Table 2). As a result, there was no statistical difference between ME-administrated mice and control mice in all of the evaluated items (albumin, alkaline phosphatase, alanine aminotransferase, amylase, total bilirubin, blood urea nitrogen, calcium, phosphorus, creatinine, glucose, sodium, potassium and total protein). Furthermore, we measured plasma levels of α -mangostin, the most abundant prenylated xanthone in mangosteen rind, in ME-administrated mice. α -Mangostin was detected in the average concentration of 0.3 $\mu\text{mol/L}$ in the plasma during the experiment (Fig. 1g), while γ -mangostin was below the detection limit (data not shown). As a result of histological analysis, hyperplasia of epidermis in ME-administrated mice was significantly suppressed compared to that in control mice (Fig. 2a). In addition, although there was no significant difference in the numbers of mast cells between the two groups, the numbers of eosinophils signifi-

cantly decreased in the skin of ME-administrated mice relative to controls (Fig. 2b,c). We also confirmed the dose dependency of ME, and ME-suppressed AD development in NC/Tnd mice in a dose-dependent manner (Fig. 3). Statistical significance was obtained in mice fed with 250 mg/kg per day ME when compared to mice fed the control diet.

Real-time PCR analysis in the skin of ME-administrated NC/Tnd mice

To determine the mechanism of the preventive effects of ME on AD development, we analyzed mRNA expression levels of various factors in the skin between ME-administrated and control mice by real-time PCR. mRNA expression levels of TSLP, IL-4, IFN- γ and Th2 chemokines such as MDC and eotaxin-2 in ME-administrated mice were significantly lower than in controls, the relative values were 0.26, 0.06, 0.58, 0.45 and 0.50, respectively (Fig. 4). Particularly, ME showed the strong suppressive effect on TSLP and IL-4 expressions. Of factors involved in itch, although there was no significant difference in Sema3a mRNA expression levels between the two groups, ME-administrated mice exhibited significantly lower expression levels of NGF mRNA than controls, the relative values were 0.51.

Direct effects of ME on TSLP and IFN- γ mRNA expression

To examine whether ME inhibited mRNA expression of TSLP and Th2/Th1 cytokine directly or indirectly, we conducted two different *in vitro* experiments. First, we investigated the effect of ME on TSLP mRNA expression in epidermal tissue culture. When epidermal sheets isolated from SPF NC/Tnd mice neonates were incubated with IL-1 β and TNF- α for 6 h, TSLP mRNA expression was increased (Fig. 5a). On the other hand, pretreatment of epidermal sheets with ME significantly reduced TSLP mRNA expression ($\text{IC}_{50} = 1.1 \mu\text{g/mL}$). We also confirmed the main active element of ME, α -mangostin, in the inhibitory effect of ME on TSLP mRNA expression ($\text{IC}_{50} = 0.4 \mu\text{mol/L}$).

To identify the direct effects of ME on Th2/Th1 cytokine expression, we examined mRNA expression levels of various cytokines in lymphocytes stimulated with PWM in the presence or absence of ME. IL-4, IL-5, IL-13 and IFN- γ mRNA expression levels in lymphocytes stimulated with PWM for 24 h were upregulated (Fig. 5b). FK506 significantly suppressed mRNA expression of IL-4, IL-5, IL-13 and IFN- γ , while the addition of ME significantly suppressed mRNA expression of IFN- γ ($\text{IC}_{50} = 6.2 \mu\text{g/mL}$) but not of Th2 cytokines, such as IL-4, IL-5 and IL-13. We also confirmed the effect of α -mangostin ($\text{IC}_{50} = 3.6 \mu\text{mol/L}$) and γ -mangostin ($\text{IC}_{50} = 3.6 \mu\text{mol/L}$) on mRNA expression of IFN- γ (Table 3).

Inhibitory effects of ME on various cell functions involved in allergic reaction

To clarify the mechanism involved in the inhibitory effects of ME on the increase of plasma total IgE levels and scratching behavior in NC/Tnd mice, we performed two different *in vitro* experiments. First, we examined *in vitro* IgE production from splenic B cells stimulated with IL-4 and LPS in the presence or absence of ME. In this experiment, CD19 $^{+}$ cells were isolated

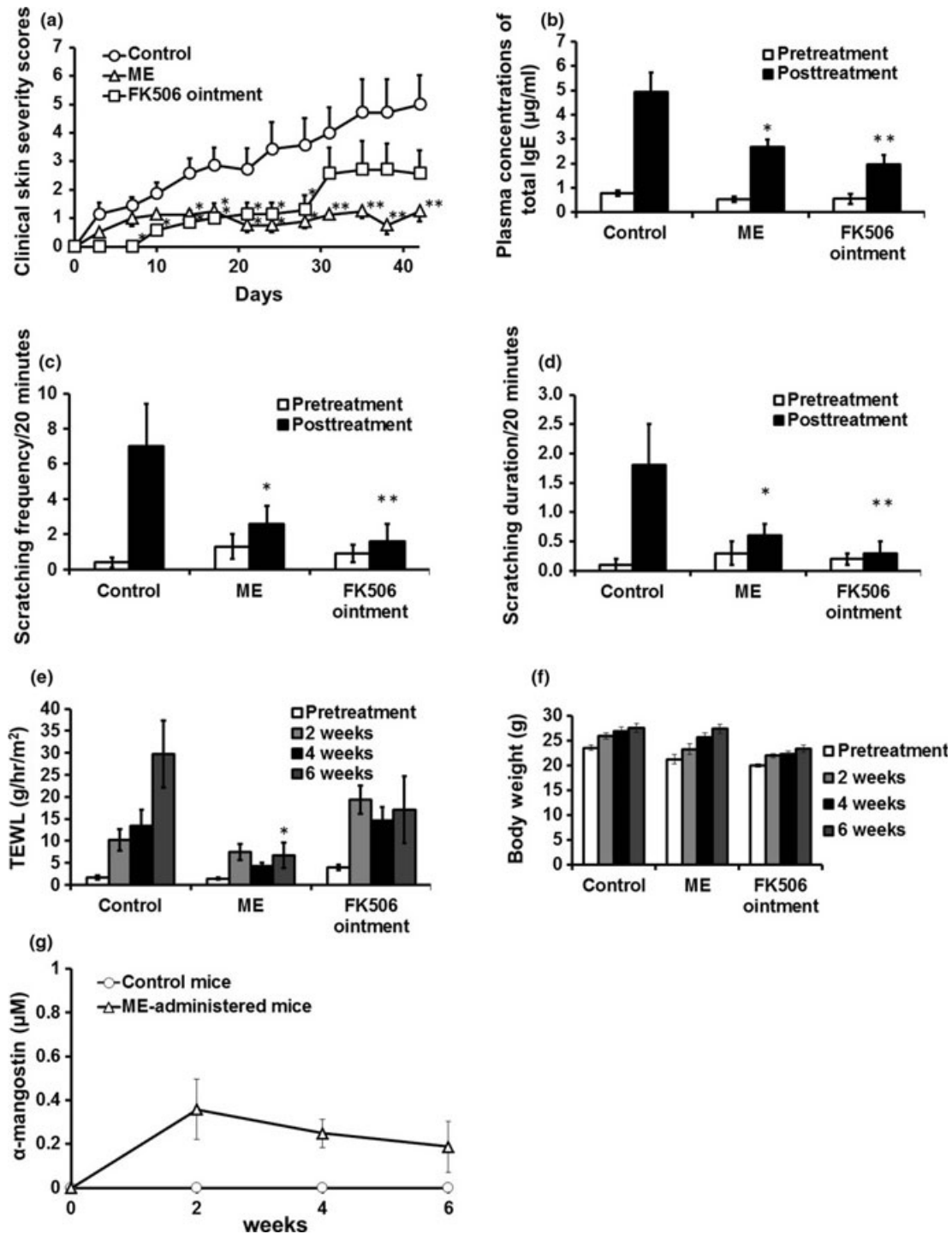


Figure 1. Preventive effects of mangosteen rind extract (ME) on the development of atopic dermatitis (AD) in NC/Tnd mice. (a) Clinical skin severity scores. (b) Plasma concentrations of total immunoglobulin (Ig)E. (c) Scratching frequency. (d) Scratching duration. (e) Transepidermal water loss (TEWL). (f) Bodyweight. (g) The concentration of α -mangostin in plasmas of ME-administrated NC/Tnd mice. Each point represents means $\pm$ standard errors of seven mice in each group. * $P < 0.05$ and ** $P < 0.01$ compared with control mice.

Table 2. Biochemical examination of plasmas

Evaluation item	Control	ME
ALB (g/dL)	1.2 ± 0.2	1.1 ± 0.1
ALP (U/L)	65.7 ± 7.2	60.6 ± 8.8
ALT (U/L)	31.1 ± 3.3	43.7 ± 10.5
AMY (U/L)	718.4 ± 32.3	780.7 ± 25.6
TBIL (mg/dL)	0.3 ± 0.0	0.2 ± 0.0
BUN (mg/dL)	24.0 ± 1.5	20.6 ± 1.4
Ca (mg/dL)	7.2 ± 0.2	7.2 ± 0.3
PHOS (mg/dL)	6.7 ± 0.8	6.7 ± 0.6
CRE (mg/dL)	0.2 ± 0.0	0.2 ± 0.0
Glu (mg/dL)	158.4 ± 6.6	158.1 ± 13.9
Na (mmol/L)	147.7 ± 1.4	150.1 ± 1.4
K (mmol/L)	3.8 ± 0.2	4.5 ± 0.3
TP (g/dL)	3.9 ± 0.1	3.9 ± 0.2

ALB, albumin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AMY, amylase; BUN, blood urea nitrogen; Ca, calcium; CRE, creatinine; Glu, glucose; K, potassium; ME, mangosteen rind extract; Na, sodium; PHOS, phosphorus; TBIL, total bilirubin; TP, total protein.

from spleens of SPF NC/Tnd mice. The purity of CD19⁺ cells was approximately 95%, as determined by flow cytometric analysis (data not shown). IgE levels in supernatants incubated with IL-4 and LPS for 9 days were increased (Fig. 6a); however, the addition of ME significantly suppressed IgE production ($IC_{50} = 4.8 \mu\text{g/mL}$) as well as FK506. In addition, we confirmed the effect of α -mangostin and γ -mangostin in suppression of IgE production (Table 3), with IC_{50} values of 2.5 and 1.6 $\mu\text{mol/L}$, respectively.

Next, we examined the effect of ME on IgE-mediated degranulation in BMCMC. β -Hexosaminidase release from BMCMC stimulated with anti-DNP IgE and DNP-BSA was increased (Fig. 6b). The addition of ME significantly suppressed β -hexosaminidase release from BMCMC.

Inhibitory effects of ME on proliferation of various cell lineages

In AD development, various cell types such as keratinocytes and lymphocytes proliferate and are involved in the exacerbation

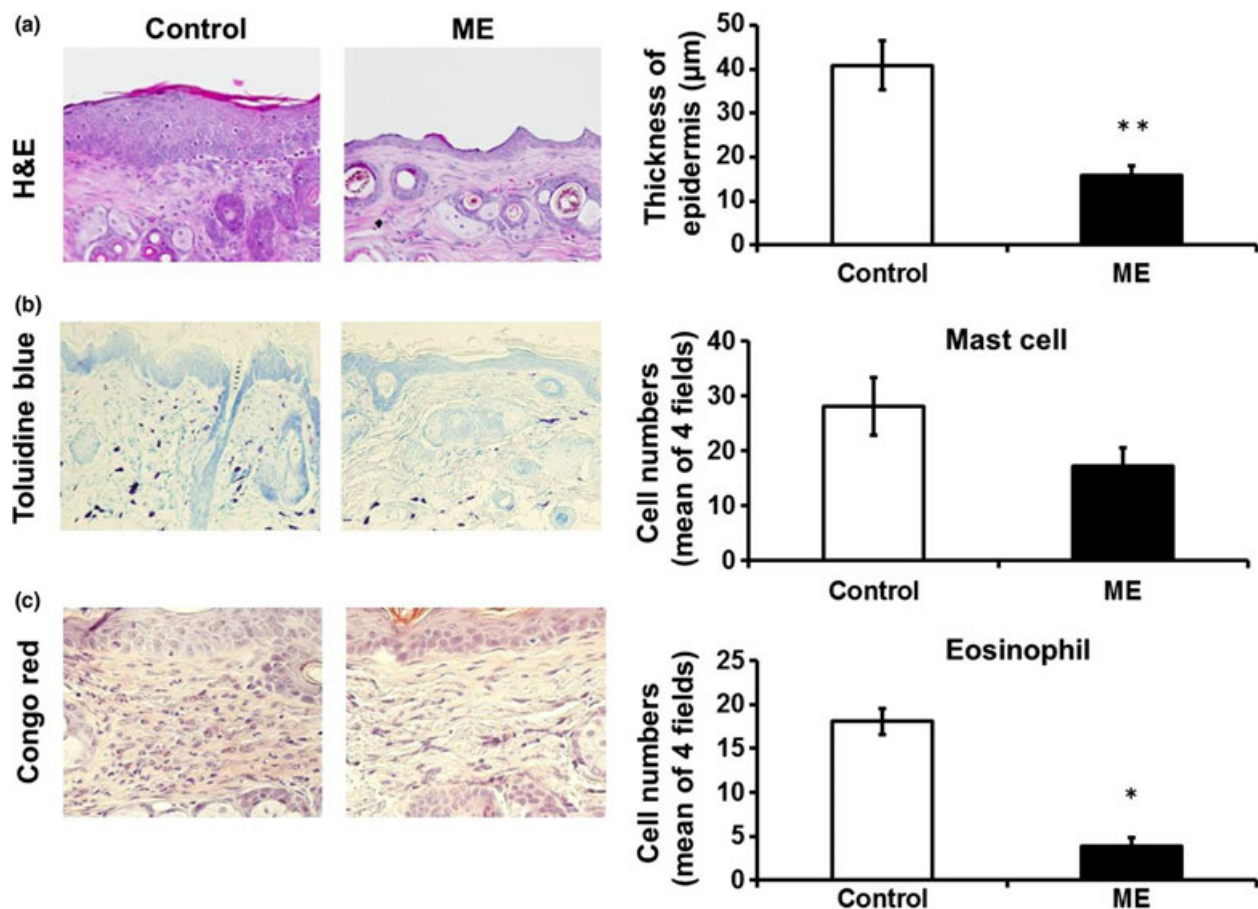


Figure 2. Histological analysis in mangosteen rind extract (ME)-administrated NC/Tnd mice. (a) (Left) Hematoxylin–eosin staining (original magnification $\times 200$). (Right) Epidermal thickness ($n = 5$). (b) (Left) Acidic toluidine blue staining ($\times 100$). (Right) The number of mast cells ($n = 7$). (c) (Left) Congo red staining ($\times 100$). (Right) The number of eosinophils ($n = 7$). Each column represents means $\pm$ standard errors. * $P < 0.05$ and ** $P < 0.01$ compared with control mice.

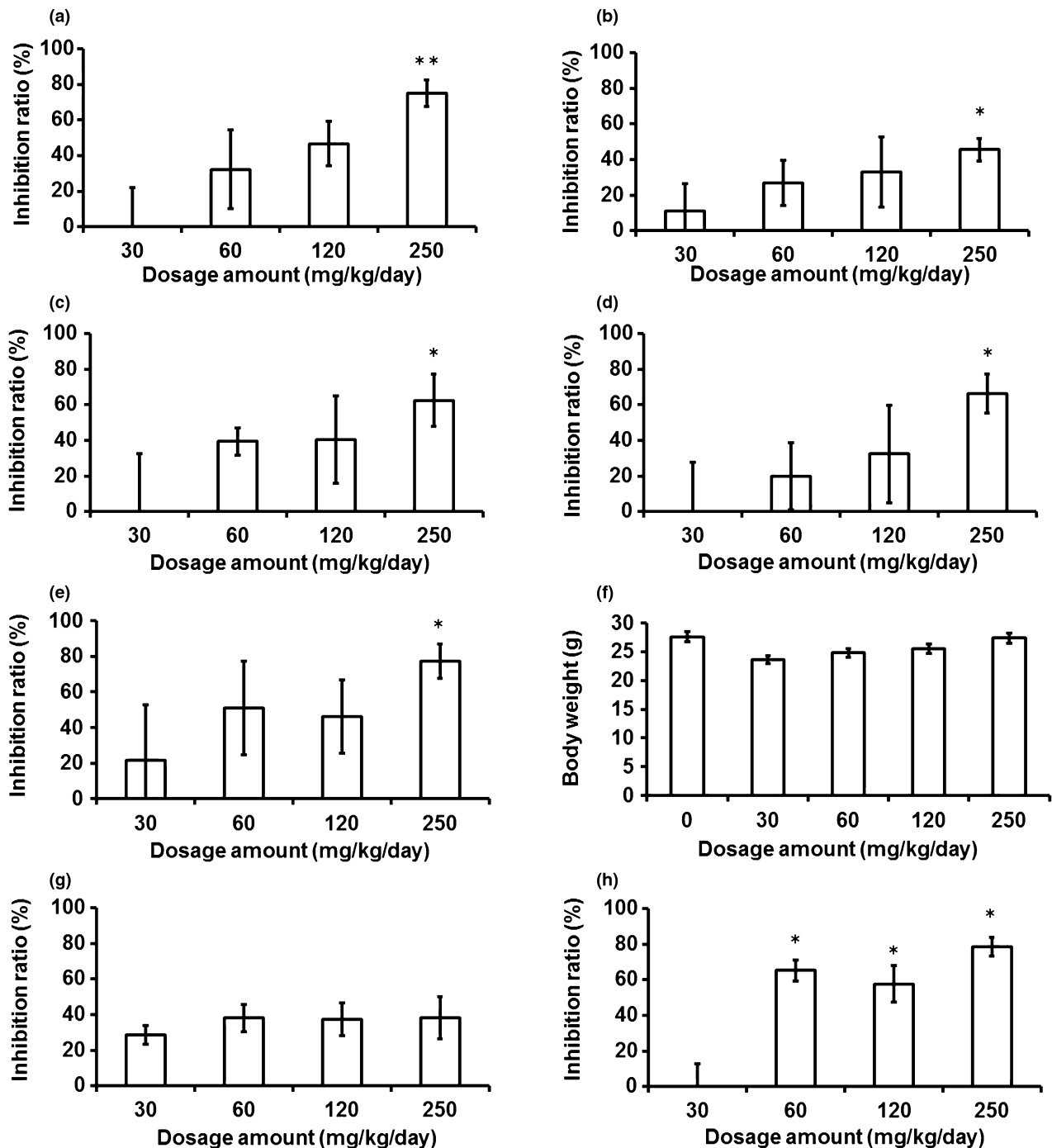


Figure 3. Dose-dependent effects of mangosteen rind extract (ME) on the development of atopic dermatitis (AD) in NC/Tnd mice. (a) Clinical skin severity scores. (b) Plasma concentrations of total immunoglobulin (Ig)E. (c) Scratching frequency. (d) Scratching duration. (e) Transepidermal water loss (TEWL). (f) Bodyweight. (g) The number of mast cells in the skin. (h) The number of eosinophils in the skin. Each value was presented as inhibition ratio (%) by comparing the value in each dose (30, 60, 120, 250 mg/kg per day) group with that of the control group at the end of the experiment, and data represent means $\pm$ standard errors of seven mice in each group. * $P < 0.05$ and ** $P < 0.01$ compared with control mice. Inhibition ratio was calculated by the following formula: Inhibition ratio = $\{1 - (\text{the value in each dose group}) / (\text{the value in control group})\} \times 100$.

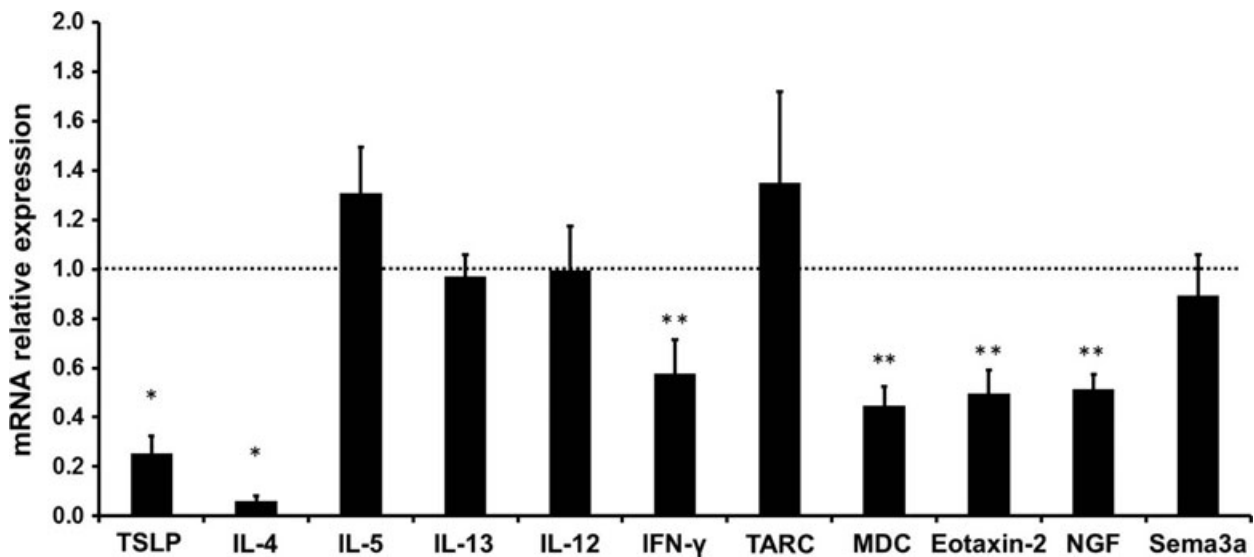


Figure 4. mRNA expression levels of several factors in the skin of mangosteen rind extract (ME)-administrated NC/Tnd mice. The amount of gene expression that was normalized to β -actin was given by $2^{-\Delta\Delta CT}$. Each value was presented as the expression value that was calculated control group as 1. Data represent means $\pm$ standard errors with seven mice. * $P < 0.05$ and ** $P < 0.01$ compared with control mice.

tion of AD symptoms. To examine the inhibitory effects of ME on the proliferation of cells involved in allergic inflammation, PAM212 (mouse keratinocytes), P815 (mouse mast cells), BCL1 (mouse B cells) and BW5147 (mouse T cells) cells were incubated with ME for 24, 48 and 72 h. Cell numbers at each time point were counted using a Trypan blue dye exclusion test. As indicated in Figure 7, ME significantly suppressed the proliferation of PAM212 ($IC_{50} = 5.9 \mu\text{g/mL}$), P815 ($IC_{50} = 7.7 \mu\text{g/mL}$) and BCL1 ($IC_{50} = 4.9 \mu\text{g/mL}$) but not of BW5147 cells, while FK506 only suppressed it of BCL1 (Fig. 7). Furthermore, α -mangostin and γ -mangostin suppressed the proliferation of PAM212 ($IC_{50} = 3.8$ and $1.6 \mu\text{mol/L}$), P815 ($IC_{50} = 3.0$ and $3.1 \mu\text{mol/L}$) and BCL1 ($IC_{50} = 2.2$ and $1.0 \mu\text{mol/L}$) cells (Table 3).

DISCUSSION

Here, we found that oral administration of ME to NC/Tnd prevented AD development, including clinical skin severity scores, plasma total IgE levels, scratching behavior, TEWL and epidermal hyperplasia, giving rise to the possibility that ME may comprehensively affect inflammation, itch and epidermal barrier dysfunction.

Mangosteen rind extract-administrated mice exhibited significantly lower mRNA expression levels of Th2 polarizing factors in the skin than those in controls. However, IL-4 expression in PWM-stimulated lymphocytes was hardly suppressed by the addition of ME to the culture. The results suggest that oral administration of ME may indirectly suppress IL-4 production at the affected sites. On the other hand, ME suppressed TSLP production in the skin in both *in vivo* and *in*

vitro experiments. IC_{50} of α -mangostin, the main component of ME, on TSLP expression was extremely lower than that on other cytokines. Because the plasma concentration of α -mangostin after oral supplementation of ME was $0.3 \mu\text{mol/L}$, the most important target of ME may be TSLP in AD development. We have already reported that the inhibition of TSLP by administration of a peroxisome proliferator-activated receptor- γ agonist abrogated the early development of AD in NC/Tnd mice.³⁵ Therefore, the preventive effects of ME on AD in NC/Tnd mice may be mediated through inhibition of TSLP production in the skin, as well as following chemokine production and infiltration of both IL-4⁺ T cells and eosinophils into the skin lesions.

The expression levels of NGF mRNA were decreased in ME-administrated NC/Tnd mice compared to those in controls. In the skins of patients with AD, as well as NC/Tnd mice with AD, NGF production from keratinocytes and fibroblasts are increased,^{13,14} which induces an abnormal extension of sensory nerve fibers. By sinking into the epidermis, nerve fibers may induce skin hypersensitivity resulting in the vicious itch-scratch circle. ME may suppress scratching behavior of NC/Tnd mice by downregulating the NGF production and nerve extension. ME had potentials on suppression of both IgE production from B cells and IgE-mediated mast cell activation. Therefore, itch may be prevented by inhibition of the nerve extension simultaneously with the IgE-mediated allergic reaction by feeding with ME. Because ME inhibited the proliferation of keratinocytes, recovery of epidermal hyperplasia and TEWL in ME-administrated mice may be associated with a regulation of keratinocytes. In brief, ME may suppress the epidermal hyperplasia and NGF production from proliferating keratinocytes, resulting in a

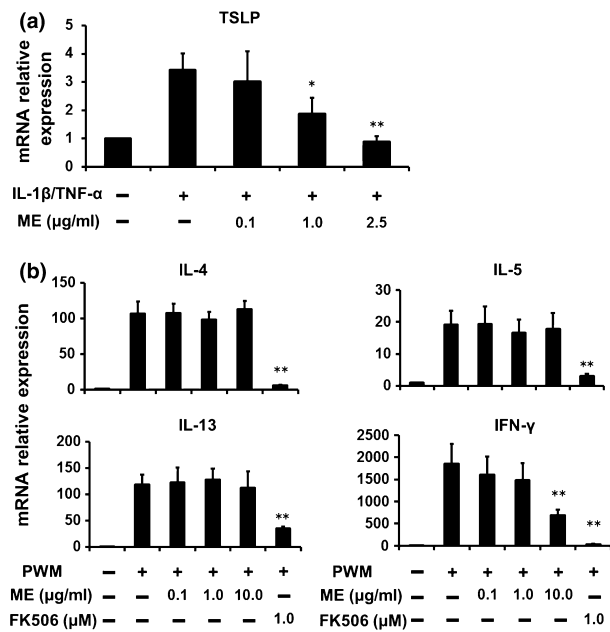


Figure 5. Inhibitory effects of mangosteen rind extract (ME) on mRNA expression of thymic stromal lymphopoietin (TSLP) and T-helper (Th)2/Th1 cytokines. (a) TSLP mRNA expression in the epidermis specimens cultured with ME or α -mangostin. Each value was presented as the expression value that was calculated with medium alone as 1. Data represent means $\pm$ standard errors of three different experiments with triplication. * $P < 0.05$ and ** $P < 0.01$ compared with cells applied with interleukin (IL)-1 β and tumor necrosis factor (TNF)- α . (b) Th2 and Th1 cytokine mRNA expression from lymphocytes cultured with ME. Each value was presented as the expression value that was calculated with medium alone as 1. Data represent means $\pm$ standard errors of three different experiments with triplication. * $P < 0.05$ and ** $P < 0.01$ compared with cells applied with pokeweed mitogen alone.

reduction of sensory nerve fiber invasion and itch sensation in NC/Tnd mice.

The main contributor in ME on suppression of AD development may be α -mangostin. As to the involvement of xanthones containing in ME in intracellular signaling, α -mangostin may exhibit these effects via the inhibition of mitogen-activated protein kinases (MAPK) and NF- κ B pathway. α -Mangostin attenuated LPS-induced expression of inflammatory genes in human

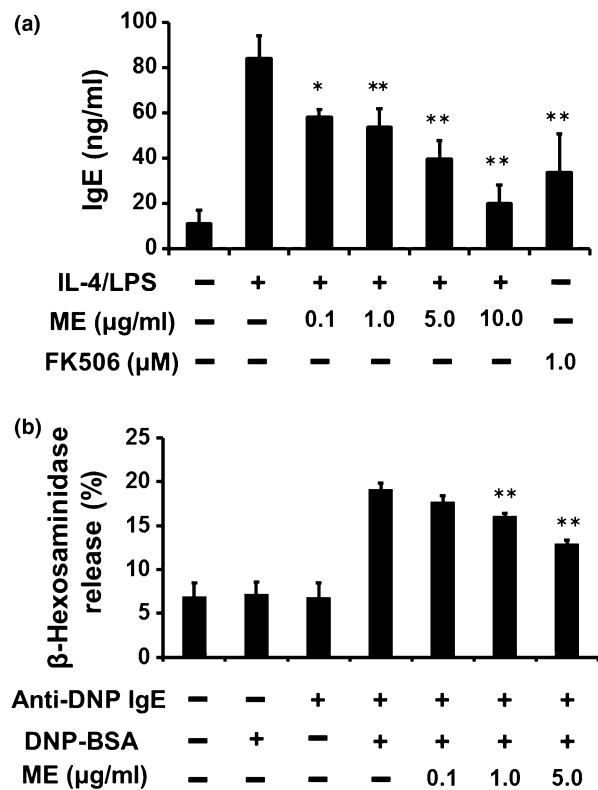


Figure 6. Inhibitory effects of mangosteen rind extract (ME) on immunoglobulin (Ig)E production and mast cell activation. (a) IgE levels in the culture supernatants with or without ME were measured. Data represent means $\pm$ standard errors of three different experiments with triplication. * $P < 0.05$, ** $P < 0.01$, when compared with cells applied with interleukin (IL)-4 and lipopolysaccharide (LPS). (b) β -Hexosaminidase released from bone marrow-derived cultured mast cells in the culture supernatants with or without ME was measured. Data represent means $\pm$ standard errors of three different experiments with triplication. * $P < 0.05$ and ** $P < 0.01$ compared with cells applied with anti-dinitrophenyl (DNP) IgE and DNP bovine serum albumin (BSA).

macrophage by preventing the activation of MAPK.³⁶ In addition, oral administration of α -mangostin reduced symptoms of allergic asthma by inhibition of NF- κ B pathway.³⁰ We previously demonstrated that an NF- κ B inhibitor improved AD

Table 3. IC₅₀ and contribution ratio for ME activity of α -mangostin and γ -mangostin

Constituent	IC ₅₀ (μmol/L)/contribution ratio (%)				
	Proliferation			IgE production	IFN-γ mRNA expression
	PAM212	P815	BCL1		
α -Mangostin	3.8/49.8	3.0/81.8	2.2/70.2	2.5/61.4	3.6/55.7
γ -Mangostin	1.6/22.7	3.1/14.8	1.0/30.7	1.6/17.2	3.6/10.5

IFN, interferon; Ig, immunoglobulin; ME, mangosteen rind extract.

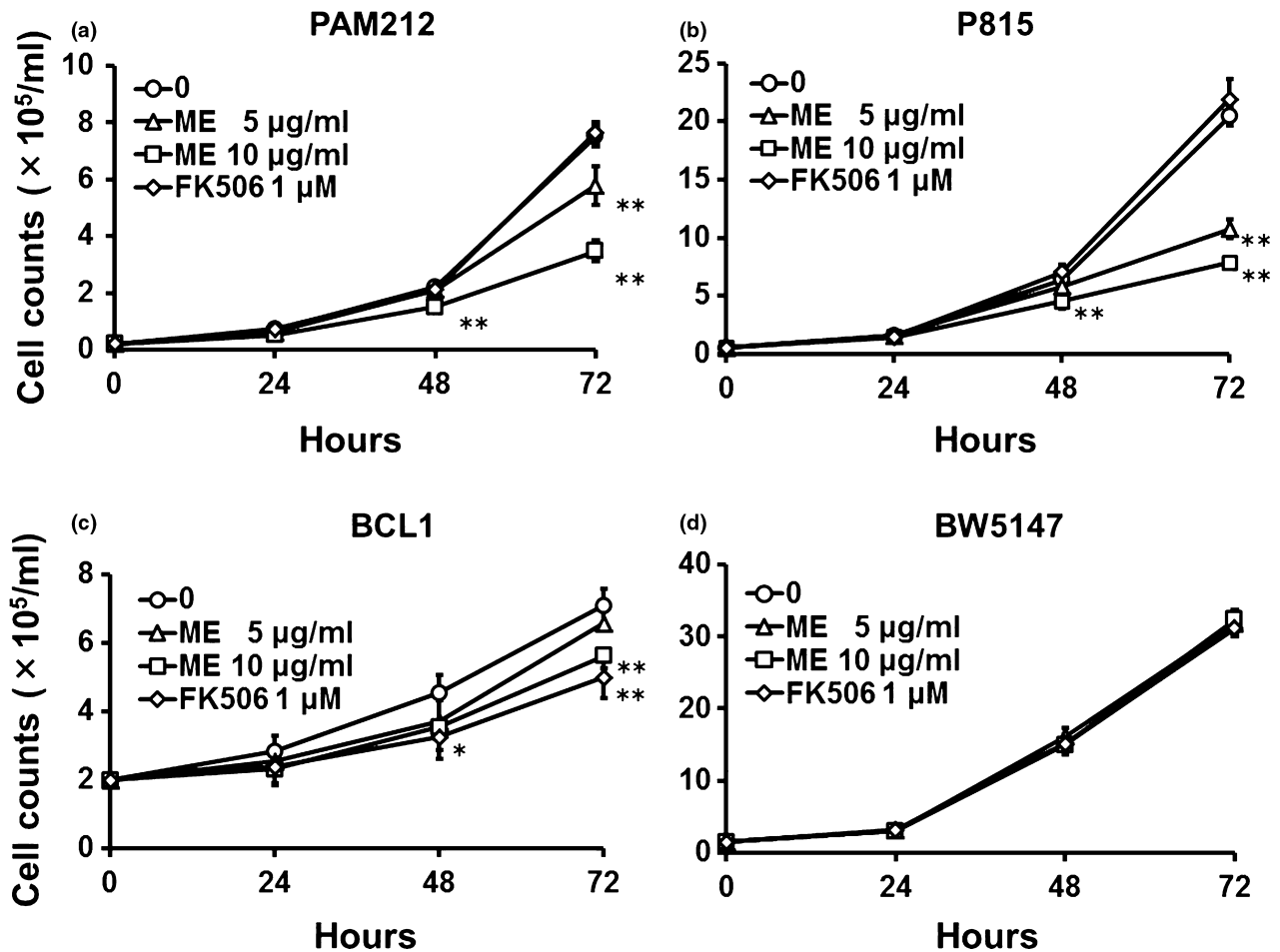


Figure 7. Inhibitory effects of mangosteen rind extract (ME) on proliferation of various cells. (a) PAM212. (b) P815. (c) BCL1. (d) BW5147. Data represent means $\pm$ standard errors of three different experiments with triplication. * $P < 0.05$ and ** $P < 0.01$ compared with cells cultured without ME.

symptoms in NC/Tnd mice.³⁷ Therefore, anti-AD effects of ME mainly may be due to the inhibition of NF- κ B pathway. Although additional experiments must take place, xanthenes in ME may be taken into a cell through the lipid bilayer membrane because of its hydrophobic nature, and may exhibit anti-AD effects via direct inhibition of NF- κ B pathway.

In conclusion, ME administration was shown to be effective in preventing AD in NC/Tnd mice without any adverse effects. Hence, a daily intake of ME may be a novel approach to the prevention of the early AD, allowing a reduction in the use of current immunosuppressive drugs.

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CONFLICT OF INTEREST

None declared.

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