Inhibitions of Histamine Release and Prostaglandin E_2 Synthesis by Mangosteen, a Thai Medicinal Plant

Keigo NAKATANI,[a] Masanori ATSUMI,[a] Tsutomu ARAKAWA,[a] Kenji OOSAWA,[c] Susumu SHIMURA,[c] Norimichi NAKAHATA,*[,a,b] and Yasushi OHIZUMI[a]

[a] *Department of Pharmaceutical Molecular Biology, Graduate School of Pharmaceutical Sciences, Tohoku University;* [b] *Department of Cellular Signaling, Graduate School of Pharmaceutical Sciences, Tohoku University; Aoba, Aramaki, Aoba-ku, Sendai 980–8578, Japan: and* [c] *Central Laboratory, Lotte Co., Ltd.; 3–1–1 Numakage, Saitama, Saitama 336–0027, Japan.* Received January 15, 2002; accepted May 24, 2002

The fruit hull of mangosteen, *Garcinia mangostana* L. has been used as a Thai indigenous medicine for many years. However, its mechanism of action as a medicine has not been elucidated. The present study was undertaken to examine the effects of mangosteen extracts (100% ethanol, 70% ethanol, 40% ethanol and water) on histamine release and prostaglandin E_2 synthesis. We found that the 40% ethanol extract of mangosteen inhibited IgE-mediated histamine release from RBL-2H3 cells with greater potency than the water extract of *Rubus suavissimus* that has been used as an anti-allergy crude drug in Japan. All extracts of mangosteen potently inhibited A23187-induced prostaglandin E_2 synthesis in C6 rat glioma cells, while the water extract of *Rubus suavissimus* had no effect. The 40% ethanol extract of mangosteen inhibited the prostaglandin E_2 synthesis in a concentration-dependent manner with relatively lower concentrations than the histamine release. In addition, passive cutaneous anaphylaxis (PCA) reactions in rats were significantly inhibited by this ethanol extract as well as by the water extract of *Rubus suavissimus*. These results suggest that the 40% ethanol extract of mangosteen has potent inhibitory activities of both histamine release and prostaglandin E_2 synthesis.

Key words mangosteen; histamine release; prostaglandin E_2; passive cutaneous anaphylaxis

Allergic inflammation is orchestrated by antigen-specific $CD4^+$ T cells, eosinophils and mast cells, and is a characteristic feature of bronchial asthma, rhinitis and atopic dermatitis.[1,2] Allergy is an immunological reaction to a foreign antigen (allergen) that causes tissue inflammation and organ dysfunction. Allergic reaction of types I, II and III is antibody mediated, and that of type IV is T-cell mediated.[3] Inflammation is often accompanied by tissue injury and the pathogenesis of many chronic disease states, including those of an autoimmune nature.[4] Regardless of etiology or localization, inflammation involves changes in vascular permeability, with concomitant recruitment of components of the immune system.[5] Edema, redness, pain and heat are the four cardinal symptoms of inflammation. The early-phase mediators of inflammation are histamine and serotonin, and the late-phase mediators are prostaglandins, lymphokines and monokines.

Rat basophilic leukemia (RBL-2H3) cells display properties of mucosal-type mast cells. The RBL-2H3 cells contain several hundred thousand IgE receptors on the membrane surface, and after sensitization with mouse monoclonal IgE, the cells respond to antigen and release histamine. Therefore, we used RBL-2H3 cells as a model cell line for histamine release.

Prostanoids, arachidonic acid metabolites produced from a variety of inflammatory cells upon stimulation, are thought to be involved in the pathogenesis of diseases. Prostanoid synthesis is regulated by two successive metabolic steps, the release of arachidonic acid from membrane phospholipids by phospholipase A_2 and its conversion to prostanoids by cyclooxygenase.[6,7] Prostaglandin E_2 is widely distributed in various organs and exerts an effect on various biological activities.[8] In inflammation processes, prostaglandin E_2 is believed to play crucial roles, since chemical mediators invoke prostaglandin E_2 synthesis in fibroblasts,[9] endothelial cells,[10] monocytes,[11] and neutrophils[12] at inflammation sites. Cyclooxygenase-2, which is rapidly induced in inflammatory states, may produce the prostanoids involved in immune and/or inflammatory responses.

Mangosteen, *Garcinia mangostana* L. is a tree, which is fairly widespread in Thai, India, Sri Lanka and Myanmar. The fruit hull of mangosteen is used as a traditional medicine in Southeast Asia for anti-inflammatory, anti-diarrhoea, anti-ulcer and antiseptic purposes.[13,14] In this study, we examined the effects of extracts of this fruit hull (100% ethanol, 70% ethanol, 40% ethanol and water) on histamine release and prostaglandin E_2 synthesis. The results suggest that the 40% ethanol extract of the fruit hull has potent inhibitory activities on IgE-mediated histamine release and prostaglandin E_2 synthesis. Furthermore, the extract is effective in cutaneous anaphylaxis in rats *in vivo*.

MATERIALS AND METHODS

Materials Fetal bovine serum was obtained from the Cell Culture Laboratory (Cleveland, OH, U.S.A.). Horse serum was purchased from Dainippon Pharmaceutical Co., Ltd. (Osaka, Japan). The fruit hull of mangosteen was extracted by maceration in ethanol, 70% ethanol, 40% ethanol and water, respectively. The leaf of *Rubus suavissimus* was extracted by maceration in water. Each extract was filtered, and the filtrate was evaporated to dryness at room temperature. Eagle's minimum essential medium (EMEM) was purchased from Nissui Pharmaceutical Co., Ltd. (Tokyo, Japan). F-10 (Nutrient Mixture: ham) was obtained from GIBCO BRL (Grand Island, NY, U.S.A.). Mouse monoclonal anti-DNP-BSA IgE was purchased from Seikagaku Corporation (Tokyo), and DNP-BSA was obtained from LSL Co., Ltd. (Tokyo). *o*-Phthalaldehyde (OPT) was purchased from Wako Pure Chemical Industries, Ltd. (Tokyo). Prostaglandin E_2 was a generous gift from Ono Pharmaceuticals (Osaka).

* To whom correspondence should be addressed. e-mail: nakahata@mail.pharm.tohoku.ac.jp

Anti-prostaglandin E_2 antibody was obtained from Chemicon International Inc (Temecula, CA, U.S.A.). [^{3}H]-Prostaglandin E_2 (200 Ci/mmol) was from NEN/DuPont (Boston, MA, U.S.A.). A final concentration of dimethyl sulfoxide, a solvent for mangosteen extract, was kept at less than 0.5%. Other chemicals and drugs were of reagent grade or of the highest quality available.

Cell Culture RBL-2H3 cells were grown in EMEM containing 10% fetal bovine serum in a 37 °C humidified incubator in an atmosphere of 5% CO_2 in air. C6 rat glioma cells were grown in F-10 medium containing 15% horse serum and 2.5% fetal bovine serum in a 37 °C humidified incubator in an atmosphere of 5% CO_2 in air.

Assay of Histamine Release RBL-2H3 cells were seeded into 12 well plates at the density of 1.0×10^5 cells per well. The experiment was performed two days after cell seeding. The cells were washed twice with PIPES buffer (10 mM PIPES, 140 mM NaCl, 5 mM KCl, 5.5 mM glucose, 0.6 mM $MgCl_2$, 1 mM $CaCl_2$, pH 7.4) and were preincubated with mouse monoclonal anti-DNP-BSA IgE antibody (0.5 μg/ml) at 37 °C for 60 min. After sensitization, the cells were again washed twice with PIPES buffer and were incubated with or without test extracts for 5 min. Then, the cells were incubated with phosphatidylserine (10 μg/ml) for 5 min, and were stimulated with 0.1 μg/ml of DNP-BSA as antigen for 30 min. The medium (0.5 ml) was transferred to a tube containing 0.5 ml of $HClO_4$ (0.8 N), and cells in each well were used to measure histamine remaining in the cells by adding 1.0 ml of $HClO_4$ (0.4 N). To 1 ml of sample, a mixture of 125 μl of NaOH (5 N), 0.4 g of NaCl and 2.5 ml of *n*-butanol was added. Then, the samples were centrifuged at 1000 rpm for 3 min. The upper organic phase was transferred to tubes containing 2 ml of NaOH (0.1 N) saturated with NaCl, and the sample was centrifuged to remove contaminated materials from the organic phase; the procedure was then repeated. Next, the upper organic phase was transferred to tubes containing 1.8 ml HCl (0.1 N) and 7.6 ml *n*-heptane. To the 1 ml of the lower aqueous phase, 0.1 ml of NaOH (10 N) was added. The histamine-OPT reaction was carried out by incubation with 0.1 ml of OPT (10 mg/ml methanol) for 4 min at room temperature, and terminated by addition of 0.6 ml HCl (3 N). The fluorescence of the conjugate was assessed at 450 nm emission activated at 360 nm. Histamine content in the cells per well was 20 ng/well to 45 ng/well.

Assay of Prostaglandin E_2 C6 cells were seeded into 12-well plates at the density of 1.0×10^5 cells per well. The experiment was performed two days after cell seeding. The cells were washed twice with EMEM buffered with 20 mM 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (HEPES), pH 7.35 (EMEM-HEPES) and were preincubated with or without test extracts for 10 min, then further incubated with or without A23187 for an additional 10 min. The medium was acidified to pH 4.0 by addition of 1 N HCl, and prostaglandin E_2 was extracted twice with ethyl acetate. After the ethyl acetate had been evaporated under a stream of N_2 gas, the sample was dissolved in 10 mM Tris–HCl (pH 7.6). Prostaglandin E_2 was determined by radioimmunoassay, as described previously.[15]

Passive Cutaneous Anaphylaxis (PCA) Reaction An IgE-dependent skin reaction was generated in rats by sensitizing the shaved skin with an intradermal injection of anti-DNP-BSA IgE antibody (0.1 mg/0.1 ml/rat) for 24 h and an intravenous injection of DNP-BSA (5 μg/0.5 ml/rat) from the tail vein. When antigen was injected, DNP-BSA containing 1% Evans blue was used to evaluate vascular permeability. Test extracts were administered intraperitoneally 30 min before antigen injection. The rats were sacrificed 30 min after the intravenous antigen challenge, and the dorsal skin was removed for measurement of the pigment area.

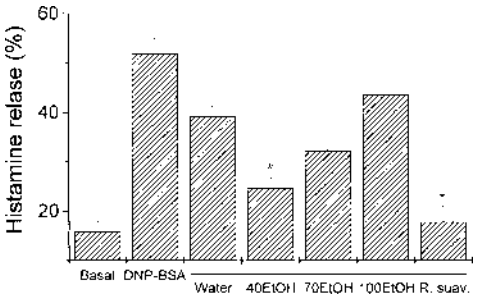

Fig. 1. Effects of Several Mangosteen Extracts and Water Extract of *Rubus suavissimus* on Histamine Release from RBL-2H3 Cells

The cells were preincubated with anti-DNP-BSA IgE antibody (0.5 μg/ml) for 60 min. After sensitization, they were incubated with 300 μg/ml of water extract (water), 40% ethanol extract (40EtOH), 70% ethanol extract (70EtOH), 100% ethanol extract (100EtOH) of mangosteen or water extract of *Rubus suavissimus* (R. suav.) for 5 min. Cells were then stimulated with antigen (DNP-BSA, 0.1 μg/ml) for 30 min. Results were expressed as % release of histamine (released histamine plus histamine remaining in the cells as 100%). Each column represents the mean with S.E. of three determinations. The data were representative of three independent experiments. * Significant difference from DNP-BSA alone ($p<0.05$).

Data Analysis The statistical differences ($p<0.05$) of values was determined by the analysis of variance (ANOVA).

RESULTS

Effects of Several Mangosteen Extracts on Histamine Release from RBL2H3 Cells Four kinds of extracts from mangosteen and water extract from *Rubus suavissimus* were examined for their inhibitory effects on histamine release from RBL-2H3 cells (Fig. 1). Histamine release from IgE-sensitized RBL-2H3 cells was induced by DNP-BSA as antigen stimulation. The 40% ethanol extract from mangosteen was found to inhibit the histamine release potently, like the water extract from *Rubus suavissimus*. The 100% and 70% ethanol extracts and water extract from mangosteen had a tendency to inhibit this release. We examined the concentration-dependence of the 40% ethanol extract from mangosteen and the water extract from *Rubus suavissimus* in inhibiting the IgE-mediated histamine release (Fig. 2). The 40% ethanol mangosteen extract (100, 300 μg/ml) showed more than 80% inhibition of the histamine release. In contrast, the water extract from *Rubus suavissimus* significantly inhibited the histamine release only at the concentration of 300 μg/ml. Major constituents of mangosteen α- and γ-mangostin had no effect on IgE-mediated histamine release (Fig. 3).

Effect of Mangosteen Extracts on Prostaglandin E_2 Synthesis Stimulated by A23187 in C6 Cells A23187, a Ca^{2+} ionophore, is known to stimulate prostaglandin synthesis mediated through the activation of PLA_2 followed by arachidonic acid liberation in glial cells.[16] Four kinds of extracts from mangosteen and water extract from *Rubus suavissimus* were examined for their inhibitory effects on A23187-induced prostaglandin E_2 release from C6 rat glioma cells

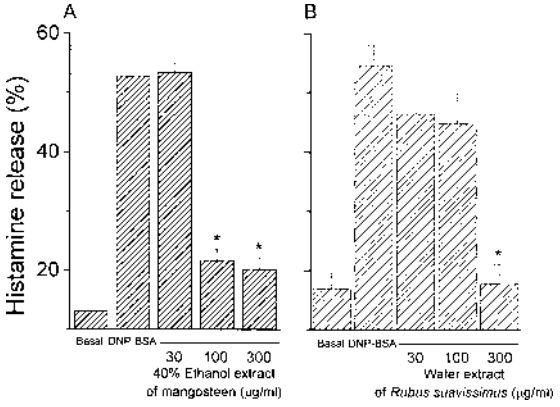

Fig. 2. Concentration-Dependence of 40% Ethanol Extract of Mangosteen and Water Extract of *Rubus suavissimus* on Histamine Release from RBL-2H3 Cells

The cells were sensitized as in Fig. 1, then incubated with 30—300 μg/ml of 40% ethanol mangosteen extract (A) or 30—300 μg/ml of *Rubus suavissimus* extract (B) for 5 min, and stimulated with antigen (DNP-BSA, 0.1 μg/ml) for 30 min. Each column represents the mean with S.E. of three determinations. The data were representative of three independent experiments. * Significant difference from DNP-BSA alone ($p<0.05$).

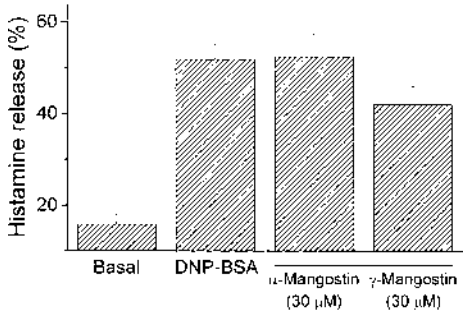

Fig. 3. Effects of α- and γ-Mangostin on Histamine Release from RBL-2H3 Cells

The cells were sensitized as in Fig. 1, then were incubated with 30 μM α-mangostin or 30 μM γ-mangostin for 5 min, and stimulated with antigen (DNP-BSA, 0.1 μg/ml) for 30 min. Each column represents the mean with S.E. of three determinations.

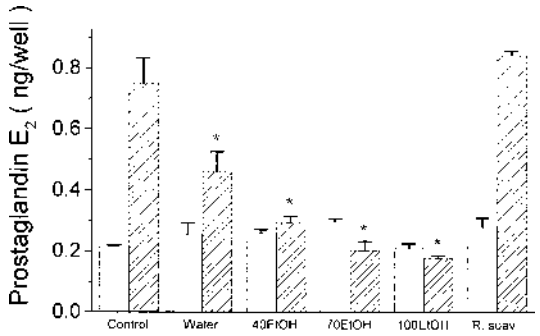

Fig. 4. Effects of Some Mangosteen Extracts and Water Extract of *Rubus suavissimus* on Prostaglandin E_2 Release from C6 Cells

The cells were preincubated with or without 30 μg/ml of water extract (water), 40% ethanol extract (40EtOH), 70% ethanol extract (70EtOH), 100% ethanol extract (100EtOH) of mangosteen or water extract of *Rubus suavissimus* (R. suav.) for 10 min, and then stimulated by 10 μM A23187 (hatched column) or vehicle (open column) for 10 min. The prostaglandin E_2 released in the medium was determined by radioimmunoassay. Each column represents the mean with S.E. of three determinations. The data were representative of three independent experiments. * Significant difference from A23187 alone ($p<0.05$).

(Fig. 4). Although all extracts from mangosteen were found to strongly inhibit the prostaglandin E_2 release, the water extract from *Rubus suavissimus* had no effect. We examined the

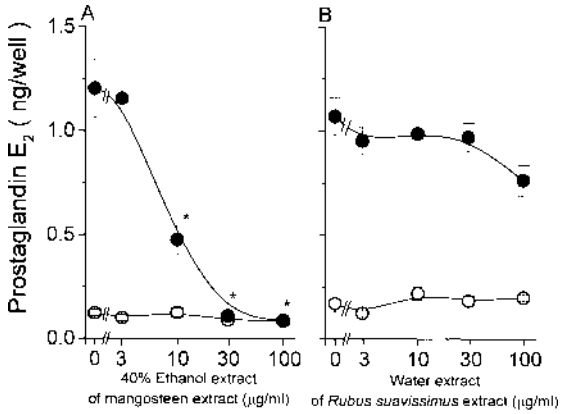

Fig. 5. Concentration-Dependence of 40% Ethanol Extract of Mangosteen (A) and Water Extract of *Rubus suavissimus* (B) on Prostaglandin E_2 Release from C6 Cells

The cells were preincubated with or without the crude drug for 10 min, and stimulated by 10 μM A23187 (●) or vehicle (○) for 10 min. The prostaglandin E_2 released in the medium was determined by radioimmunoassay. Each point represents the mean with S.E. of three determinations. The data were representative of three independent experiments. * Significant difference from without drugs ($p<0.05$).

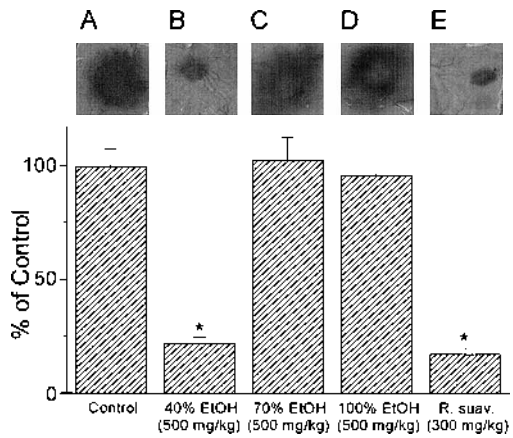

Fig. 6. Effects of Ethanol Extracts of Mangosteen and Water Extract of *Rubus suavissimus* on Passive Cutaneous Anaphylaxis

Rats were sensitized by an intradermal injection of anti-DNP-BSA IgE antibody (5 μg/kg) for 24 h. Thirty min after intraperitoneal injection of vehicle (A), 500 mg/kg of 40% ethanol (EtOH) extract of mangosteen (B), 500 mg/kg of 70% EtOH extract of mangosteen (C), 500 mg/kg of 100% EtOH extract of mangosteen (D) or 300 mg/kg of water extract from *Rubus suavissimus* (R. suav.) (E), allergic reaction was initiated by an injection of DNP-BSA (25 μg/kg) in PBS containing 1% Evans blue from the tail vein of rats. The animals were sacrificed 30 min after the intravenous challenge, and the dorsal skin was removed for measurement of the pigment area. Data were calculated as % of control (pigment area in vehicle-treated rat was taken as 100%). Each column represents the mean with S.E. of seven determinations. * Significant difference from control ($p<0.05$).

concentration-dependence of the 40% ethanol extract from mangosteen or the water extract from *Rubus suavissimus* in inhibiting this release (Fig. 5). Inhibition by the 40% ethanol extract from mangosteen was in a concentration-dependent manner, while other extracts from mangosteen showed similar effects on prostaglandin E_2 release (data not shown). The water extract from *Rubus suavissimus*, however, had no effect on this release.

Effects of Mangosteen Extracts on PCA Reaction

PCA is one of the most useful *in vivo* models for anaphylaxis in local allergic reactions.[17] Anti-DNP-BSA IgE antibody was injected into dorsal skin sites of rats, and DNP-BSA containing Evans blue dye was injected intravenously 24 h

thereafter. The PCA reaction was potently inhibited by intraperitoneal administration of the 40% ethanol extract from mangosteen (Fig. 6). Seventy and 100% ethanol extract showed only weak inhibition. The water extract from *Rubus suavissimus* also markedly inhibited PCA reaction.

DISCUSSION

Mangosteen (*Garcinia mangostana* L.) is a tropical fruit tree species with promising value. The hull of the fruit has been widely used as an anti-inflammatory agent and in the treatment of skin infections, wounds and diarrhoea for many years in Southeast Asia.[18] The crude extract of the hull reportedly possesses several pharmacological activities: H_1-histamine receptor antagonistic activity,[19] anti-serotonin activity,[20] inhibition of eukaryote protein kinases and a cyclic nucleotide-binding phosphatase,[21] inhibitory activity against HIV-1 protease,[22] inhibition of the sarcoplasmic reticulum Ca^{2+}-pumping ATPase,[23] antibacterial activation against methicillin-resistant *Staphylococcus aureus*,[24] and anti-oxidant action.[25] The present study demonstrated that mangosteen extracts have anti-inflammatory or anti-allergy activity.

Histamine and prostaglandin E_2 are chemical mediators for inflammation and/or allergy. As far as we know, the present study is the first report that the 40% ethanol mangosteen extract has inhibitory activity of both histamine release and prostaglandin E_2 synthesis. Extract of *Rubus suavissimus*, known to have an anti-allergy action, potently inhibits histamine release, but has no effect on prostaglandin E_2 synthesis (Fig. 4). Therefore, the 40% ethanol extract of mangosteen may contain useful components beneficial for the treatment of allergy and inflammation.

We previously showed that γ-mangostin inhibited A23187-induced prostaglandin E_2 synthesis in C6 cells,[26] and in an *in vitro* experiment using purified enzymes COX-1 and COX-2, we clarified that γ-mangostin directly inhibits the activities of both these enzymes.[26] We also found that α-mangostin inhibits both COX activities *in vitro* at a similar concentration range to γ-mangostin (Nakatani *et al.*, unpublished observation). In HPLC analysis, 40% ethanol extract of mangosteen contained 10% α-mangostin and 12% γ-mangostin, 70% ethanol extract contained 7.7% α-mangostin and 39% γ-mangostin, and 100% ethanol extract contained 13% α-mangostin and 55% γ-mangostin, although the water extract of mangosteen contained neither α-mangostin nor γ-mangostin (Nakatani *et al.*, unpublished observation). Therefore, it seems likely that inhibition of the 40% ethanol mangosteen extract on prostaglandin E_2 synthesis is due to α- and γ-mangostin. Since the analysis indicated that other ethanol extracts of mangosteen also contained α- and γ-mangostin, their inhibitions of prostaglandin E_2 synthesis can be attributed to the constituents. However, water extract of mangosteen may contain unknown components having inhibitory potency of prostaglandin E_2 synthesis other than α- or γ-mangostin, because the extract that contained no α-mangostin or γ-mangostin also inhibited prostaglandin E_2 synthesis. On the other hand, inhibition of the 40% ethanol mangosteen extract on histamine release cannot be explained by α- and γ-mangostin, because these substances had no effect (Fig. 3). Therefore, further study is necessary to identify the constituent with the inhibitory activity of histamine release.

PCA reaction is one of the models most frequently used to evaluate anti-allergic drugs.[27] In the immediate phase of PCA, passively sensitized mast cells in skin are activated by intravenously administered specific antigen, followed by the release of vasoactive mediators such as histamine, which increase vascular permeability at the sensitized skin site.[28] The 40% ethanol extract of mangosteen significantly inhibited the PCA reaction as did the water extract of *Rubus suavissimus*. It has been shown that α-mangostin has H_1-histamine receptor blocking activity.[19] The 40% ethanol extract of mangosteen, which contained a similar amount of α-mangostin in the 70% or 100% ethanol extract, significantly inhibited the PCA reaction, although 70% or 100% extract showed only weak inhibition. Therefore, the potent inhibitory activity of the 40% ethanol extract of mangosteen in PCA reaction may be mainly due to the inhibition of histamine release.

In conclusion, the 40% ethanol extract of mangosteen has an anti-allergy/anti-inflammatory activity due to inhibitions of histamine release and prostaglandin E_2 synthesis. Mangosteen extract may be a useful crude drug for treatment of allergy and/or inflammation.

Acknowledgement This work was partly supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Science, Sports and Technology of Japan.

REFERENCES

1) Drazen J. M., Arm J. P., Austen K. F., *J. Exp. Med.*, **183**, 1—5 (1996).
2) Wills-Karp M., *Annu. Rev. Immunol.*, **17**, 255—281 (1999).
3) Kelley D. S., *Nutrition*, **17**, 669—673 (2001).
4) Dinarello C. A., *Curr. Opinion Immunol.*, **3**, 941—948 (1991).
5) Payan D. G., Levine S. D., Goetzl E. J., *J. Immunol.*, **132**, 1601—1604 (1984).
6) Flynn J. T., Henry J. M., Perkowski S., *Can. J. Physiol. Pharmacol.*, **59**, 1268—1273 (1981).
7) Rosen G. D., Birkenmeier T. M., Raz A., Holtzman M. J., *Biochem. Biophys. Res. Commun.*, **164**, 1358—1365 (1989).
8) Shimizu T., Wolfe L. S., *J. Neurochem.*, **55**, 1—15 (1990).
9) Unemori E. N., Ehsani N., Wang M., Lee S., McGuire J., Amento E. P., *Exp. Cell Res.*, **210**, 166—171 (1994).
10) Bottoms G. D., Johnson M. A., Lamar C. H., Fessler J. F., Turek J. J., *Circ. Shock*, **15**, 155—162 (1985).
11) Nichols F. C., Schenkein H. A., Rutherford R. B., *Biochim. Biophys. Acta*, **927**, 149—157 (1987).
12) Doerfler M. E., Danner R. L., Shelhamer J. H., Parrillo J. E., *J. Clin. Invest.*, **83**, 970—977 (1989).
13) Harborne J. B., Baxter H., "Phytochemical Dictionary, A Handbook of Bioactive Compounds from Plants," Taylor and Francis, London, 1993.
14) Chairungsrilerd N., Takeuchi K., Ohizumi Y., Nozoe S., Ohta T., *Phytochemistry*, **43**, 1099—1102 (1996).
15) Nakahata N., Imata K., Okawa T., Watanabe Y., Ishimoto H., Ono T., Ohizumi Y., Nakanishi H., *Biochim. Biophys. Acta*, **1310**, 60—66 (1996).
16) Overstreet D. H., Lee Y. W., Rezvani A. H., Pei Y. H., Criswell H. E., Janowsky D. S., *Alcohol Clin. Exp. Res.*, **20**, 221—227 (1996).
17) Wershil B. K., Mekori Y. A., Murakami T., Galli S. J., *J. Immunol.*, **139**, 2605—2614 (1987).
18) Mahabusarakam W., Iriyachitra P., Taylor W. C., *J. Nat. Prod.*, **50**, 474—478 (1987).
19) Chairungsrilerd N., Furukawa K.-I., Ohta T., Nozoe S., Ohizumi Y., *Eur. J. Pharmacol.*, **314**, 351—356 (1996).
20) Chairungsrilerd N., Furukawa K.-I., Ohta T., Nozoe S., Ohizumi Y., *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **342**, 9—16 (1998).
21) Lu Z. X., Hasmeda M., Mahabusarakam W., Ternai B., Ternai P. C., Polya G. M., *Chem.-Biol. Interact.*, **114**, 121—140 (1998).

22) Chen S.-X., Wan M., Loh B.-N., *Planta Med.*, **62**, 381—382 (1996).
23) Furukawa K.-I., Shibusawa K., Chairungsrilerd N., Ohta T., Nozoe S., Ohizumi Y., *Jpn. J. Pharmacol.*, **71**, 337—340 (1996).
24) Iinuma M., Tosa H., Tanaka T., Asai F., Kobayashi Y., Shimano R., Miyauchi K.-I., *J. Pharm. Pharmacol.*, **48**, 861—865 (1996).
25) Yoshikawa M., Harada E., Miki A., Tsukamoto K., Si Q. L., Yamahara J., Murakami N., *Yakugaku Zasshi*, **114**, 129—133 (1994).
26) Nakatani K., Nakahata N., Arakawa T., Yasuda H., Ohizumi Y., *Biochem. Pharmacol.* **63**, 73—79 (2002).
27) Church M. K., Miller P., *Br. J. Pharmacol.*, **62**, 481—486 (1978).
28) Inagaki N., Nakamura N., Nagao M., Musoh K., Kawasaki H., Nagai H., *Eur. J. Pharmacol.*, **367**, 361—371 (1999).