Improvement of the Antiinflammatory and Antiallergic Activity of *Bidens pilosa* L. var. *radiata* SCHERFF Treated with Enzyme (Cellulosine)

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Bidens pilosa L. var. *radiata* SCHERFF (*Tachiawayukisendangusa*: Musashino Miyako Bidens Pilosa (MMBP)) has been reported to have antiinflammatory and antiallergic properties in the treatment of experimental diseases. We studied the antiinflammatory and anti-allergic effects of enzymatic digested MMBP (eMMBP), MMBP treated with Cellulosine, using an experimental inflammatory and allergic model. Oral administration of eMMBP suspension in carboxy-methyl-cellulose sodium solution inhibited the production of IgE 10 days after immunization with 2,4-dinitrophenylate (DNP)-ascaris in mice. Oral administration of eMMBP suspension inhibited dye exudation in rat skin induced by passive cutaneous anaphylaxis (PCA) and chemical mediators (histamine and substance P). The effects of eMMBP on the inhibition of histamine release from mast cells induced by compound 48/80 or antigen-antibody reaction was stronger than that of the hot-water extract (HWex). Caffeic acid and flavonoids (hyperin and isoquercitrin) in eMMBP are increased more than those in HWex of MMBP, as estimated using HPLC. These results suggest that eMMBP has antiinflammatory and antiallergic activity, and that enzymatic digestion enhances its antiallergic activity by the inhibition of histamine release from the mast cells due mainly to an increase in caffeic acid and flavonoids. The enzymatic digestion of MMBP might be useful in enhancing its antiinflammatory and antiallergic activities.

Key words —— enzymatic digestion, *Bidens pilosa*, antiinflammation, antiallergy, histamine

INTRODUCTION

Herbs, herbal compounds, Chinese medicines, food ingredients, and many other items contain physiologic factors that may prevent the incidence of disease and may promote human health, in addition to their functions as nutrients. In previous studies, we investigated the effects of *Bidens pilosa* L. var. *radiata* SCHERFF (*Tachiawayukisendangusa* in Japanese, Musashino Miyako Bidens Pilosa (MMBP)) which is used as a Kanpo tea for skin ulcers in summer.[1] We confirmed the antiallergic effects of MMBP in experimental treatment of diseases. However, the directions for the topical use of MMBP are inconvenient, because it is in the form of a hot-water extract. Therefore MMBP was improved by enzymatic digestion and commercialized, and we next investigated the antiinflammatory and antiallergic effects of enzymatic digested MMBP (eMMBP). It has been reported that intestinal absorption and metabolism of a soluble flavonoid, αD-rutin that attaches D-glucose to rutin (quercetin-3-*O*glucosyl-rhamnose), are rapid,[2] and the phenolic composition and radical-scavenging activity of sweet potato-derived shochu distillery by-products treated with koji are increased.[3] The processing of eMMBP in the present study is similar to the previous reports.

It was also confirmed that MMBP contains six caffeic acid derivatives and seven flavonoids.[3] It was reported that caffeic acid inhibits compound 48/80-induced allergic symptoms and flavonoids inhibit histamine release from mast cells.[4,5] In the present study, we clarified the antiinflammatory and antiallergic effects of eMMBP on inflammation of skin induced by chemical mediators such as histamine and substance P. Furthermore, we compared the effects of eMMBP and the hot-water extract (HWex) on histamine release from rat peritoneal mast cells and estimated the biologically active compounds in them using HPLC.

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MATERIALS AND METHODS

Animals —— Male Wistar/ST rats (200 g), male ddY mice (18–20 g), and male BALB/c mice (18–20 g) were purchased from Sankyo Japan SLC (Shizuoka, Japan). The animals were maintained in an air-conditioned room controlled for temperature ($24 \pm 1°C$) and humidity ($55 \pm 5\%$). The animals were given standard laboratory food (MF, Oriental Yeast, Tokyo, Japan) and water *ad libitum*. The present study was conducted in accordance with the Guiding Principles for the Care and Use of Laboratory Animals, as adopted by the Committee on Animal Research of Hoshi University, which is accredited by the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

Plant Material —— MMBP was cultivated on Miyako Island, Okinawa prefecture, Japan, and the aerial parts were harvested and steamed. The dried powder in this study was provided by the Musashino Research Institute for Immunity. The dry powder of eMMBP was obtained from MMBP treated with the enzyme Cellulosine (HBI Inc., Hyogo, Japan) and also provided by the Musashino Research Institute for Immunity. The dry weight of HWex of MMBP, was approximately 30% of that of dry MMBP, and the dry weight of eMMBP was approximately 40% of that of dry MMBP. eMMBP is soluble in water, but was dissolved in 0.25% carboxy-methyl-cellulose sodium (CMC-Na) solution for reference with the effect of MMBP suspension in 0.25% CMC-Na in the previous report.[6] The supernatant of HWex (500 mg/kg, 100°C, 24 hr) was freeze-dried and dissolved in phosphate-buffered saline (PBS) for comparison with eMMBP and HWex. The animals were administered pharmacologic doses of eMMBP of 100, 250, and 500 mg/kg. The 500 mg/kg eMMBP dose was estimated to be 10-fold the medicinal dose of MMBP such as that contained in Kanpo tea.

Chemicals —— The chemicals used and their sources were as follows: histamine (Sigma Chemical, St. Louis, MO, U.S.A.), serotonin (5-hydroxytryptamine) (Sigma Chemical), substance P (Sigma Chemical), L-α-phosphatidyl-L-serine (Sigma Chemical), 2,4-dinitrophenylate (DNP)-ascaris (LSL Co., Tokyo, Japan), rat-anti-DNP-ascaris antiserum (LSL Co.), ketotifen (Sigma Chemical), cyclophosphamide (Sigma Chemical), and compound 48/80 (Sigma Chemical).

Production of Antibody (IgE) against DNP-Ascaris in Mice —— The IgE titer, which was induced by DNP-ascaris in the serum, was also determined using a mouse IgE ELISA Kit (Mouse IgE ELISA Kit, Shibayagi, Gunma, Japan). In this study, increasing IgE was regarded as increasing of anti-DNP-ascaris-IgE. To determine the titer of anti-DNP-ascaris-IgE, BALB/c mice were injected with DNP-ascaris (5 μg) in alum according to the method described in our previous report.[6] Ten days after injection, the IgE titer against DNP-ascaris in the serum was determined using ELISA, again according to our previous report.[6] Mice in the cyclophosphamide group received cyclophosphamide (20 mg/kg) p.o. for 5 days prior to immunization. The mice in the eMMBP group received the eMMBP suspension (100, 250, and 500 mg/kg, p.o.) in 0.25% CMC-Na for 5 days prior to immunization. Mice in the control group were provided 0.25% CMC-Na for 5 days prior to immunization, and the normal group no treatment.

DNP-Ascaris Antiserum-Induced PCA Reaction in Rats —— The test rats were then divided into six groups. The rats in the control group were administered 0.25%CMC-Na p.o. 1 hr before the injection of antigen and dye ($n = 10$). The rats in the pre-eMMBP group and pre-HWex group were given eMMBP (100, 250, and 500 mg/kg, p.o.) and HWex with the diet for 10 days before the antigen and dye injection ($n = 6$–8). The rats in the ketotifen group were administered ketotifen (5 mg/kg, p.o.) 1 hr before the injection of antigen and dye as a positive control ($n = 6$). Rats were passively sensitized on the back skin by an injection of anti-DNP-ascaris antiserum (0.1 ml/site, s.c.) according to the method described in our previous report.[6] After 48 hr, the animals were intravenously injected with a mixture of Evans blue dye (0.5%) and DNP-ascaris antigen (1 mg/ml). At 30 min after the antigen challenge, the leaked dye accumulated in the skin was determined colorimetrically at 620 nm.

Inflammatory Chemical Mediator-Induced Skin Reactions in Rats —— The test rats were divided into six groups. The rats in the eMMBP and HWex groups were administered a suspension of eMMBP (100, 250, and 500 mg/kg, p.o.) or HWex in 0.25% CMC-Na 1 hr before the dye injection ($n = 6$–8). The rats in the ketotifen group were administered ketotifen (5 mg/kg, p.o.) 1 hr before the injection of dye as a positive control ($n = 6$). The rats in the control group were administered 0.25% CMC-Na p.o. 1 hr before intravenous injection of the dye and chemical mediators or saline into the back skin ($n = 10$). We examined the changes in vascular per-

meability induced by the inflammatory mediators histamine and substance P according to the previously described method.[6] In this inflammatory model, vascular permeability was estimated by dye leakage in the dorsal skin of rats. The animals were intravenously injected with 50 mg/kg of pontamine sky blue 5 min after injection (0.1 ml/site, s.c.) of the chemical mediators histamine (10 nmol/site) and substance P (1 nmol/site) and saline which spontaneously leaked dye into the back skin. The animals were killed 1 hr after the injection of the inflammatory mediators, and the skin of each reaction locus was removed to determine whether the dye had leaked. The dye in the skin was extracted using a 0.6 N-phosphate solution : acetone (5 : 13). The dye in the extracts was measured colorimetrically at 590 nm.

Compound 48/80 or DNP-Ascaris-Induced Histamine Release from Isolated Rat Peritoneal Mast Cells —— Rat peritoneal mast cells were harvested from the abdominal cavity of the male rats according to the method described in our previous report.[6] The abdominal region was gently massaged for 90 s, and peritoneal fluid was collected and centrifuged for 1 min at $280 \times g$ at 4°C. After centrifugation, the precipitant was mixed in 20% Ficoll, and the mixture was loaded onto layers of 40% and 30% Ficoll, and then centrifuged for 20 min at $350 \times g$. The layer containing the mast cell pellet was then pooled and washed twice in PBS solution. Thereafter, eMMBP (2 mg/0.05 ml), HWex (2 mg/0.05 ml), or ketotifen (0.05 mg/0.05 ml) dissolved in PBS was added to equal numbers of mast cells (1×10^5 cells/0.2 ml) and was preincubated at 37°C for 10 min before the reaction. Compound 48/80 (0.2 μg/0.05 ml) was added to the tubes and then incubated at 37°C for 10 min. In the control group, only PBS was added instead of eMMBP or HWex. The reaction was then stopped by the addition of cool PBS (0.3 ml) to the tubes. The tubes were centrifuged for 1 min at $280 \times g$, and the histamine content in the supernatant was measured using ELISA (SPI Bio, Paris, France). In contrast, the histamine release from isolated rat peritoneal mast cells was also induced by the antibody [anti-DNP-ascaris, rat 48-hr passive cutaneous anaphylaxis (PCA); 1 : 512]. For the stimulus by the antigen (DNP-ascaris), spontaneous histamine release was examined in rat serum in PBS. Equal numbers of mast cells (2.0×10^6 cells/ml) were added to 0.1 ml of anti-DNP-ascaris antiserum and were incubated for 1 hr at 37°C. After the incubation, mast cells treated with the antiserum were separated in to an equal number of mast cells (1×10^5 cells/0.2 ml) and eMMBP (2 mg/0.05 ml), HWex (2 mg/0.05 ml), or ketotifen (0.05 mg/0.05 ml) were preincubated for 10 min at 37°C. L-α-phosphatidyl-L-serine (0.01 mg/0.05 ml) and DNP-ascaris (0.02 mg/0.05 ml) in PBS 0.05 ml were added to the tubes and incubated for 10 min at 37°C for antigen stimulus. In the controls, only PBS was added to equal numbers of mast cells (1×10^5 cells/0.2 ml), preincubated at 37°C for 10 min before the reaction, L-α-phosphatidyl-L-serine (0.01 mg/0.05 ml) and DNP-ascaris (0.02 mg/0.05 ml) in PBS were added, and then incubated. The reaction was stopped by the addition of cool PBS (0.3 ml). The tubes were centrifuged for 1 min at $280 \times g$, and the histamine content in the supernatant was measured using ELISA. Spontaneous histamine release was also calculated. Inhibition by eMMBP, HWex, or ketotifen was expressed as the percentage of histamine release compared with total histamine in the mast cells before the reaction.

Quantification of Caffeic Acid Using HPLC —— Caffeic acid, caffeoylquinic acid, and flavonoids were separated with HPLC. eMMBP or HWex was dissolved in water and filtered through a cellulose acetate membrane (0.45 μm, Advantec, Tokyo, Japan), and the filtrate was used as a sample. Portion (5 μl) of this flitrate was injected into an HPLC system consisting of two LC-10AD pumps, an SIL-10AXL autoinjector, a BENSIL 5 C_{18} column (4.6 × 150 mm) oven, and a Fasterertls UV-VIS (A_{254}) photodiode array (Shimadzu, Kyoto, Japan) at room temperature. The mobile phase consisted of MeOH/0.02 M KH_2PO_4 (30/70). The flow rate was 1 ml/min.

Statistical Analysis —— Data are expressed as mean ± S.D. of the number of animals described in the figure legends. Data were analyzed using two-way analysis of variance (ANOVA), followed by Dunnett's test or Bonferroni's test using StatView software. p values of less than 0.05 were considered significant.

RESULTS AND DISCUSSION

The prevalence of allergic disease, including asthma, atopic dermatitis, and allergic rhinitis has increased in Japan. In recent years, many herbs,

herb compounds, plants, Chinese medicines, food ingredients, and many other items have been shown to have anti-allergic activity, some of which are used as antiallergic supplements. Some nutrients in foods, drinks, or supplements have antiallergic or allergy-promoting activities, and many compounds in food have been reported to protect against disease. Alcaraz and Jimenez have reported flavonoids as anti-inflammatory agents,[7] and Inoue *et al*.[5] have reported the anti-allergic activity of flavonoid glycosides obtained from *Mentha piperita* L. Hossen *et al*. reported that Lo Han Kuo extract inhibits nasal rubbing and scratching behavior in mice,[8] while Osakabe *et al*. reported the antiallergic effects of rosmarinic acid.[9] In those studies, the effects of water-soluble solutions and alcohol extracts were examined. We reported that MMBP powder has antiallergic activity. In this study, we investigated the effects of eMMBP obtained from the enzymatic digestion of MMBP.

In the present study, eMMBP dose dependently lowered the serum IgE levels 10 days after immunization with DNP-ascaris, as did cyclophosphamide (used as a positive control) (Fig. 1). eMMBP markedly inhibited IgE production. It was previously reported that MMBP suspension in CMC-Na solution (100, 250, 500 mg/kg) inhibits IgE production due to the effects of flavonoids and caffeic acid. The effects of flavonoids and caffeic acid on various cytokines improve IgE levels in immunized animals.[10,11]

Figure 2 shows the effects of eMMBP and ketotifen on the exudation of dye in the dorsal skin induced by the PCA reaction in rats. Pretreatment with eMMBP (500 mg/kg) for 10 days suppressed the PCA reaction induced by the antigen (DNP-ascaris), although a single administration of ketotifen (5 mg/kg) also suppressed the PCA reaction.

Figures 3 and 4 show the effects of the oral administration of eMMBP suspension (100, 250, 500 mg/kg) in CMC-Na solution and ketotifen on the exudation of dye in the dorsal skin of rats treated with the chemical mediators. Capillary permeability at the local skin site induced by intradermal injection of the chemical mediators, as well as histamine and substance P increase, was inhibited by the treatment with eMMBP. These results suggest that eMMBP has an antiinflammatory effects against histamine and substance P as chemical mediators. eMMBP inhibited the capillary permeability induced by histamine or substance P, although the chemical mediator release from mast cells was

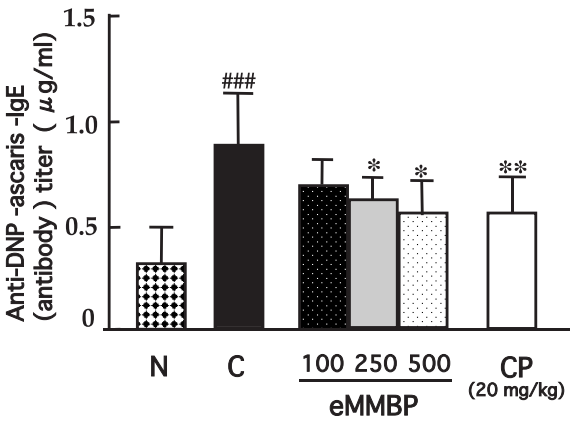

Fig. 1. Effects of eMMBP and Cyclophosphamide on Titer of Anti-DNP-Ascaris-IgE after Immunization

N, Normal group; C, control group that received CMC-Na p.o. for 5 days from the injection of antigen (DNP-ascaris, 5 μg); eMMBP, group that received eMMBP (100, 250, 500 mg/kg) p.o. for 5 days from the injection of antigen; CP, cyclophosphamide group that received cyclophosphamide (20 mg/kg) p.o. for 5 days from the injection of antigen. Each value represents mean ± S.D. of 6–9 mice. $^{*}p < 0.05$, $^{**}p < 0.01$, significantly different from the control group, $^{\#\#\#}p < 0.001$, significantly different from the normal group. Data were analyzed using two-way ANOVA, followed by Dunnett's test.

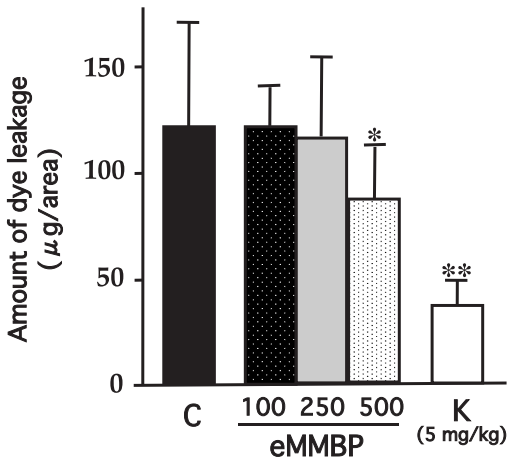

Fig. 2. Effects of eMMBP and Ketotifen on Dye Leakage Induced by DNP-Ascaris antiserum (1 mg/ml)

C, Control group that received 0.25% CMC-Na p.o. 1 hr before injection of antigen plus dye; pre-eMMBP, group that received eMMBP (100, 250, 500 mg/kg) with the diet for 10 days; K, ketotifen group that recived ketotifen (5 mg/kg) p.o. 1 hr before injection of antigen plus dye. Each value represents mean ± S.D. of 6–10 rats. $^{*}p < 0.05$, $^{**}p < 0.01$, significantly different from the control group. Data were analyzed using two-way ANOVA, followed by Dunnett's test.

induced in the rats by the intradermal administration of histamine and substance P. These results suggest that eMMBP has an effect of H_1-receptor blocking. The antiallergic effects of flavonoids are well known, and histamine release and antihistaminic action at the H_1-receptor of flavonoids have been reported.[5,12]

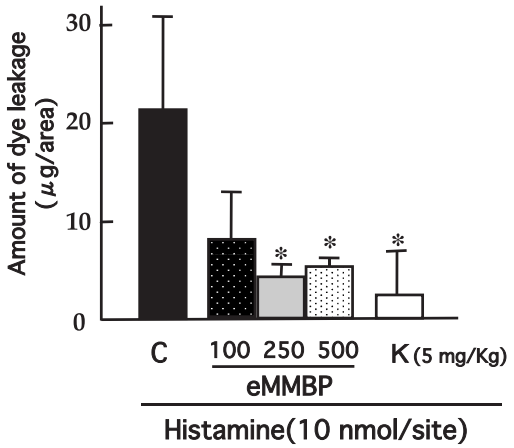

Fig. 3. Effects of eMMBP and Ketotifen on Dye Leakage Induced by Histamine (10 nmol)

C, Control group that received 0.25% CMC-Na p.o. 1 hr before injection of dye; eMMBP, group that received eMMBP (100, 250, 500 mg/kg) in 0.25% CMC-Na p.o. 1 hr before injection of histamine and dye; K, ketotifen group that received ketotifen (5 mg/kg) p.o. 1 hr before injection of histamine and dye. Each value represents mean ± S.D. of 6–10 rats. $^{*}p < 0.05$, significantly different from the control group. Data were analyzed using two-way ANOVA, followed by Dunnett's test.

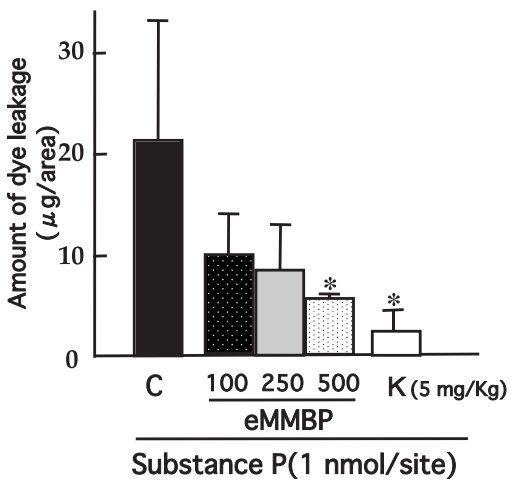

Fig. 4. Effects of eMMBP and Ketotifen on Dye Leakage Induced by Substance P (1 nmol)

C, Control group that received 0.25% CMC-Na p.o. 1 hr before injection of dye; eMMBP, group that received a suspension of eMMBP (100, 250, 500 mg/kg) in 0.25% CMC-Na p.o. 1 hr before injection of substance P and dye; K, ketotifen group that received ketotifen (5 mg/kg) p.o. 1 hr before injection of substance P and dye. Each value represents mean ± S.D. of 6–10 rats. $^{*}p < 0.05$, significantly different from control group. Data were analyzed using two-way ANOVA, followed by Dunnett's test.

Figures 5 and 6 show the effects of eMMBP and HWex on histamine release from rat peritoneal mast cells induced by compound 48/80 or the antigen-antibody reaction. Histamine release by compound 48/80 at a concentration of 0.2 μg/0.05 ml

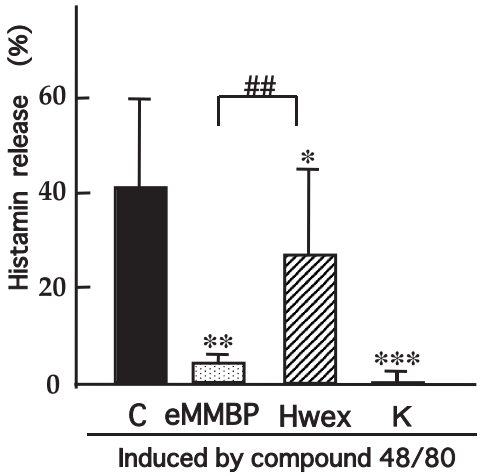

Fig. 5. Effects of eMMBP, HWex, and Ketotifen on Histamine Release from Rat Peritoneal Mast Cells Induced by Compound 48/80 (0.2 μg/0.05 ml)

C, Control group; eMMBP, group that received eMMBP (2 mg/0.05 ml) added to mast cells (1×10^5 cells/0.2 ml) in a tube; HWex, HWex (2 mg/0.05 ml) was added to mast cells (1×10^5 cells/0.2 ml) in a tube; K, ketotifen (0.05 mg/0.05 ml) was added to mast cells (1×10^5 cells/0.05 ml) in a tube. Each value (%) represents the mean ± S.D. of 5 experiment (5 rats were used). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, significantly different from each control group, $^{\#\#}p < 0.01$, eMMBP group significantly different from HWex group. Data were analyzed using two-way ANOVA, followed by Bonferroni's test.

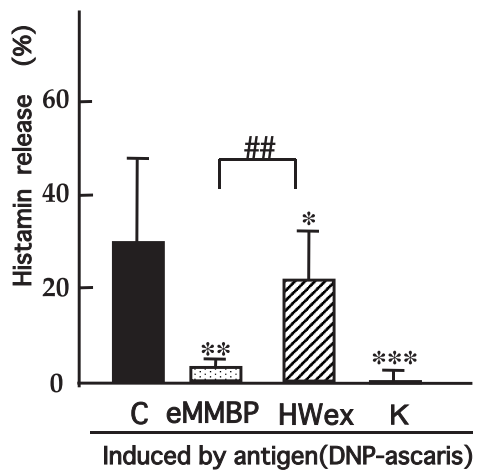

Fig. 6. Effects of eMMBP, HWex, and Ketotifen on Histamine Release from Rat Peritoneal Mast Cells Induced by Antigen (DNP-Ascaris) (0.02 mg/0.05 ml)

C, Control group; eMMBP, eMMBP (2 mg/0.05 ml) was added to mast cells (1×10^5 cells/0.2 ml) in a tube; HWex, HWex (2 mg/0.05 ml) was added to mast cells (1×10^5 cells/0.2 ml) in a tube; K, ketotifen (0.05 mg/0.05 ml) was added to mast cells (1×10^5 cells/0.2 ml) in a tube. Each value (%) represents the mean ± S.D. of 5 experiment (5 rats were used). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, significantly different from each control group, $^{\#\#}p < 0.01$, eMMBP group significantly different from HWex group. Data were analyzed using two-way ANOVA, followed by Bonferroni's test.

was 41.0 ± 13.0%, and that by antigen (DNP-ascaris) at a concentration of 0.02 mg/0.05 ml was 29.2 ± 18.8%. Spontaneous histamine release was 3.4 ± 0.9% and 19.3 ± 10.1%, respectively, showing that eMMBP (2 mg/0.05 ml) and HWex (2 mg/0.05 ml) significantly inhibited the histamine release induced by compound 48/80 or an antigen (DNP-ascaris). eMMBP inhibited the histamine release from rat peritoneal mast cells induced by compound 48/80 and antigen-induced histamine release from the mast cells. The spontaneous histamine release from rat peritoneal mast cells induced by the antigen was greater than that induced by compound 48/80 because the release occurred after incubation with rat control serum as the antigen (DNP-ascaris). eMMMB acted in the same manner as ketotifen, which stabilizes mast cells by inhibiting their release of histamine. eMMBP inhibition of histamine release from the mast cells by was statistically greater than that by HWex ($p < 0.01$, Figs. 5 and 6, histamine release %: stimulus by compound 48/80; 26.5 ± 9.7%, antigen; 5.4 ± 2.1%). It therefore seems likely that eMMBP inhibited not only the release of histamine from the mast cells induced by compound 48/80 but also suppressed that induced by the antigen-antibody reaction with the mast cells. Moreover, it has been suggested that treatment with enzymatically digested MMBP is a suitable method for inhibiting histamine release from mast cells because the inhibition of histamine release from the mast cells by eMMBP is approximately 5-fold more effective than that of HWex. We hypothesized that flavonoids and caffeic acid derivatives in MMBP powder are biologically active compounds in a previous report.[6] It may be that the amount of water-soluble compounds was increased by enzymatic digestion. In the present study, caffeic acid, caffeoylquinic acid, and flavonoids were analyzed with HPLC (Fig. 7). Peaks 1, 2, 3, and 4 (Fig. 7A) were, respectively, identified as caffeic acid, caffeoylquinic acid, hyperin, and isoquercitorin by chromatogrophy against their standards, which were isolated and purified from MMBP, and their structures analyzed by NMR.[13] It was clear that the caffeic acid in eMMBP (Fig. 7B) increased compared with that in HWex. It was suggested that caffeic acid and flavonoids in HWex were produced by hot-water extraction and enzymatic digestion. The volume of caffeic acid in eMMBP was calculated from the peak area, and it was found that there was approximately 5–7-fold more caffeic acid in HWex. The volumes of hyperin and isocerucitorin were calculated from the peak area, and there was approximately 3–5-fold and 2–4-fold more hyperin and isoquercitrin in HWex. Yoshimoto *et al*. identified caffeic acid as the dominant phenolic component in the shochu distillery by-products of the sweet potato, reporting that the phenolic component and its radical-scavenging activity were increased by adding an enzyme (koji and/or cellulosine T2).[3] In their next report, they described the enzymatic production of caffeic acid by koji from plant resources containing caffeoylquinic acid derivatives.[14] Hossen *et al*. reported that caffeic acid dose dependenty inhibited itch and edema in allergic dermatitis.[4] Caffeic acid is known to suppress histamine release from mast cells, and the increase in caffeic acid levels in response to enzymatic degestion may be connected with the suppression histamine release from mast cells. Moreover, caffeic acid is a bioactive compound that forms a diverse group of secondary metabolites universally present in higher plants.[3] Caffeic acid not only has inhibits histamine release but many also exert effects related to allergy or inflammation. It may thus protect the human body from the oxidative stress that is a risk factor for aging and cardiovascular diseases.[15–18] Caffeic acid is absorbed after oral administration and has been found to increase antioxidative capacity *in vivo*.[18–20] Furthermore, caffeic acid has been shown to exhibit such biologic and pharmacologic properties as antimicro-

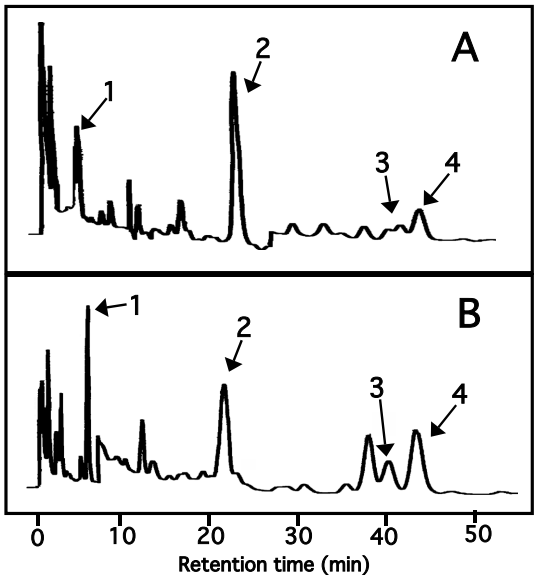

Fig. 7. HPLC Chromatograms of (A) HWex and (B) eMMBP
Peak 1, Caffeic acid; peak 2, caffeoylquinic acid; peak 3, hyperin; peak 4, isoquercitorin. Chromatographic conditions are detailed in Materials and Methods.

bial activity, inhibition of histamine release,[21] and antimutagenic activity, suggesting that it may also be useful as a physiologically functional substance. Thus there are numerous possible applications of eMMMB.

On the other hand, higher levels of flavonoids such as hyperin and isoquercitrin were present in eMMBP compared with HWex, as estimated using HPLC. It is hypothesized that flavonoids in the eMMBP solution would be effective in treating allergy. Alcaraz and Jimenez noted that flavonoids are present in food products and in plants that have been used since ancient times to treat a variety of human diseases and that they affect inflammatory responses.[7] It has been found that apple polyphenols affect allergy in mice, and flavonoids have antioxidant activity. Moreover, the flavonoids present in vegetables, fruit, and teas have been used to treat a variety of human diseases since ancient times. These have been shown to have antiinflammatory and antiallergic activities such as inhibition of histamine release, synthesis of IL-4 and IL-3, and CD40 ligand expression by basophils and mast cells.[22] Tea catechin, a flavonoid with antiallergic activity, is known to suppress FcεRI expression in human basophilic cells.[23,24] In another report, it was indicated that the flavonoid narirutin can reduce airway inflammation in ovalbumin (OVA)-sensitized rats and IL-4 and IgE in a murine model of allergy.[25] Furthermore, it was reported that caffeic acid has radical-scavenging activity and prevents chemical mediator release from mast cells. It is clear that MMBP has radical-scavenging activity, and two caffeioylquinic acids and four flavonoids in MMBP have been found to have antioxidant activity.[13] Therefore the radical-scavenging activity of MMBP may affect the stability of mast cells. The observed antiallergic activity may depend on the blocking of superoxide generation by caffeoylquinic acid and flavonoids.

In conclusion, it was clarified that eMMBP has antiinflammatory and antiallergic effects, and the inhibition of histamine release from mast cells was greater than that of HWex. The present results suggest that the levels of caffeic acid and flavonoids are increased by enzymatic digestion and that they have positive effects against allergy.

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