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Rubus coreanus Unripe Fruits Inhibits Immediate-type Allergic Reaction and Inflammatory Cytokine Secretion

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Abstract – The immediate-type allergic reaction (anaphylaxis) is involved in many allergic diseases such as asthma, allergic rhinitis, and sinusitis. The discovery of drugs for the treatment of immediate-type allergic diseases is a very important subject in human health. In this study, we investigated the effect of *Rubus coreamus* Miq. (Rosaceae) unripe fruits (RCF) on mast cell-mediated allergic reaction and inflammatory cytokine secretion. RCF inhibited compound 48/80-induced systemic reactions in mice. RCF attenuated immunoglobulin (Ig) E-mediated local allergic reactions. In addition, RCF dependently reduced histamine release from rat peritoneal mast cells activated by compound 48/80 or IgE. Furthermore, RCF decreased the phorbol 12-myristate 13-acetate plus calcium ionophore A23187-stimulated tumor necrosis factor (TNF)-α and interleukin (IL)-6 secretion in human mast cells. Our findings provide evidence that RCF inhibits mast cell-derived immediate-type allergic reactions.
Keywords – *Rubus coreanus*, allergic reaction, mast cells, tumor necrosis factor (TNF)-α, interleukin (IL)-6

Introduction

The dried unripe fruits of *Rubus coreanus* Miq. (Rosaceae), well known as "Bok-bun-ja" in Korea, have been used for centuries as traditional medicine. This crude drug is used for the management of impotence, spermatorrhea, enuresis, asthma, allergic diseases and it also has been used as a stomachic and tonic in Korea (Lee, 1966; Perry, 1980; But *et al.*, 1997). Mast cells, which are constituents of virtually all organs and tissue, are important mediators of inflammatory responses such as allergy and anaphylaxis. Anaphylaxis, an acute systemic allergic reaction, is mediated by histamine released in response to the antigen cross-linking of immunoglobulin E (IgE) bound to FceRI on mast cells. Mast cell activation causes the process of degranulation that result in releasing of mediators, such as histamine and an array of inflammatory cytokines (Metacalf *et al.*, 1981; Church *et al.*, 1997; Miyajima *et al.*, 1997). Among the inflammatory substances released from mast cells, histamine remains the best-characterized and most potent vasoactive mediator implicated in the acute phase of immediate hypersensitivity (Petersen *et al.*, 1996). Mast cell degranulation also can be elicited by the synthetic compound 48/80, and it has been used as a direct and convenient reagent to study the mechanism of anaphylaxis (Ennis *et al.*, 1980). Activated mast cells can produce histamine, as well as a wide variety of other inflammatory mediators such as eicosanoids, proteoglycans, proteases, and several pro-inflammatory and chemotactic cytokines such as TNF-α, IL-6, IL-4, IL-13, and transforming growth factor-β (Burd *et al.*, 1989; Plaut *et al.*, 1989; Galli *et al.*, 1991; Bradding *et al.*, 1993). Therefore, modulation of secretion of these cytokines from mast cells can provide a useful therapeutic strategy for allergic inflammatory disease. Anal therapy is a kind of drug delivery system through the anus, which is utilized in the patients to whom oral administration is impossible. The drug absorbed in the rectum can avoid first-pass effect in the liver and circulate the whole-body directly. The absorption rate in the rectum is faster than that in the gastrointestinal tract. The absorption rate and total amount through the rectum have

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little difference with those of a venous administration. Thus, anal therapy can be expected to have good efficacy by the increased absorption rate and the strong medical action (Shin *et al.*, 2004). In this study, we evaluated the effect of RCF on compound 48/80-induced systemic reaction and anti-dinitrophenyl (DNP) IgE antibody-induced local allergic reaction by anal therapy and histamine release from rat peritoneal mast cells (RPMC). Additionally, the effect of RCF on phorbol 12-myristate 13-acetate (PMA) plus calcium ionophore A23187 (A23187)-induced TNF-α and IL-6 secretion in a human mast cell line (HMC-1) was also investigated.

Experimental

Culture of HMC-1 cells – HMC-1 cells, a human mast cell line were grown in Iscove's media supplemented with 10% FCS and 2 mM glutamine.

Animals – The original stock of male ICR mice and male Sprague-Dawley rats were purchased from Dae-Han Biolink Co. Ltd. (Chungbuk, Korea), and the animals were maintained in the College of Pharmacy, Woosuk University. The animals were housed five to ten per cage in a laminar air flow room maintained at a temperature of 22 ± 2 °C and relative humidity of 55 ± 5% throughout the study. The care and treatment of the mice were in accordance with the guidelines established by the Public Health Service Policy on the Humane Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee.

Reagents – Compound 48/80, anti-DNP IgE, DNP-human serum albumin (HSA), α-minimal essential medium (α-MEM), o-phthaldialdehyde, phorbol 12-myristate 13-acetate (PMA), calcium ionophore A23187 were purchased from Sigma Chemical Co. (St Louis, MO). rTNF-α and rIL-6 were purchased from R & D Systems Inc. (Minneapolis, MN).

Preparation of RCF – The fruits of *Rubus coreanus* were purchased from the oriental drug store, Bohwa Dang (Jeonju, Korea). A voucher specimen was deposited at the Herbarium of the College of Pharmacy, Woosuk University. The plant sample was extracted with purified water at 70 °C for 5 h. The extract was filtered, and lyophilized. The dried extract was dissolved in saline or Tyrode buffer A (10 mM HEPES, 130 mM NaCl, 5 mM KCl, 1.4 mM $CaCl_2$, 1 mM $MgCl_2$, 5.6 mM glucose, 0.1% bovine serum albumin) before use.

Compound 48/80-induced systemic reaction – Compound 48/80-induced systemic reaction was examined as previously described (Shin *et al.*, 2004). Mice were given an intraperitoneal injection of 0.008 g/kg body weight (BW) of the mast cell degranulator, compound 48/80. RCF was dissolved in saline and administered anally ranging from 0.001 to 1 g/kg BW 1 h before the injection of compound 48/80 (n = 10/group). In the time dependent experiment, RCF (1 g/kg) was administered anally at 5, 10, and 20 min after injection of compound 48/80 (n = 10/group). Mortality was monitored for 1 h after induction of anaphylactic shock.

PCA reaction – The mice were injected intradermally with 0.5 μg of anti-DNP IgE into each of 2 dorsal skin sites that had been shaved 48 h earlier. The sites were outlined with a water-insoluble red marker. After 48 h, each mouse was received an injection of 1 μg of DNP-HSA in PBS containing 4% Evans blue (1 : 4) via the tail vein. RCF (1 to 100 mg/kg BW) was anally administered 1 h before the challenge. Thirty minutes after the challenge, the mice were killed and the dorsal skin was removed for measurement of the pigment area. The amount of dye was determined colorimetrically after extraction with 1 ml of 1 M KOH and 9 ml of a mixture of acetone and phosphoric acid (5 : 13) based on the previous report (Katayama *et al.*, 1978). The absorbent intensity of the extraction was measured at 620 nm by using a spectrophotometer, and the amount of dye was calculated with Evans blue measuring-line.

Preparation of RPMC – RPMC were isolated as previously described (Kanemoto *et al.*, 1993). In brief, rats were anesthetized by ether and injected with 20 ml of Tyrode buffer B (137 mM NaCl, 5.6 mM glucose, 12 mM $NaHCO_3$, 2.7 mM KCl, 0.3 mM NaH_2PO_4 and 0.1% gelatin), into the peritoneal cavity and the abdomen was gently massaged for about 90 s. The peritoneal cavity was carefully opened, and the fluid containing peritoneal cells were aspirated by Pasteur pipette. The peritoneal cells were sedimented at 150 × *g* for 10 min at room temperature and resuspended in Tyrode buffer B. Mast cells were separated from the major components of rat peritoneal cells, i.e. macrophages and small lymphocytes. Peritoneal cells were suspended in 1 ml of Tyrode buffer B, layered on 2 ml of metrizamide (22.5 w/v%), and centrifuged at room temperature for 15 min at 400 × g. The cells remaining at the buffer-metrizamide interface were aspirated and discarded; the cells in the pellet were washed and resuspended in 1 ml of Tyrode buffer A. Mast cell preparations were about 95% pure as assessed by toluidine blue staining. More than 97% of the cells were viable as judged by trypane blue uptake.

Inhibition of histamine release – Purified RPMC were resuspended in Tyrode buffer A for the treatment with

compound 48/80. RPMC suspensions (2×10^5 cells/ml) were preincubated for 10 min at 37 °C before the addition of compound 48/80 (5 μg/ml). The cells were preincubated with the RCF (0.001 to 1 mg/ml) preparations, and then incubated for 10 min with compound 48/80. RPMC suspensions (2×10^5 cells/ml) were also sensitized with anti-DNP IgE (10 μg/ml) for 16 h. The cells were preincubated with the RCF (0.001 to 1 mg/ml) at 37 °C for 10 min prior to the challenge with DNP-HSA (1 μg/ml). The cells were separated from the released histamine by centrifugation at 400 × g for 5 min at 4 °C. The histamine content was measured by the o-phthaldialdehyde spectrofluorometric procedure (Shore *et al.*, 1959). The fluorescence intensity was measured at emission of 438 nm and excitation of 353 nm using a spectrofluorometer.

Assay of TNF-α and IL-6 secretion – TNF-α and IL-6 secretion was measured by modification of an enzyme-linked immunosorbent assay (ELISA) as described previously (Scuderi *et al.*, 1986). HMC-1 cells were cultured with α-MEM plus 10% FBS and resuspended in Tyrode buffer A. The cells were sensitized with PMA (20 nM) plus A23187 (1 μM) for 6 h in the absence or presence of RCF. The ELISA was performed by coating 96-well plates with 6.25 ng/well of monoclonal antibody with specificity for TNF-α and IL-6 respectively. Before use and between subsequent steps in the assay, the coated plates were washed twice with PBS containing 0.05% Tween-20 and twice with PBS alone. For the standard curve, rTNF-α and rIL-6 were added to serum previously determined to be negative to endogenous TNF-α and IL-6. After exposure to the medium, the assay plates were exposed sequentially to biotinylated anti-human TNF-α and IL-6, 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) tablets substrates. Optical density readings were made within 10 min of the addition of the substrate with a 405 nm filter.

Statistical analysis – Statistical analyses were performed using SAS statistical software (SAS Institute, Cary, NC). Treatment effects were analyzed using one-way ANOVA, followed by Duncan's multiple range tests. $P < 0.05$ was used to indicate significance.

Results

RCF inhibits compound 48/80-induced systemic reaction – Compound 48/80 (0.008 g/kg) was used as a model of induction of systemic fatal allergic reaction. After the intraperitoneal injection of compound 48/80, the mice were monitored for 1 h, after which the mortality rate was determined. Compound 48/80 induced fatal shock in 100% of animals. When RCF was administered anally at a concentrations ranging from 0.001 to 1 g/kg BW for 1 h, the mortality with compound 48/80 was dose-dependently reduced (Table 1). In addition, the mortality of mice administered with RCF (1 g/kg) 5, 10, and 20 min after compound 48/80 injection increased time-dependently (Table 2).

Table 1. Effect of RCF on compound 48/80-induced systemic allergic reaction

RCF treatment (g/kg BW)	compound 48/80 (0.008 g/kg BW)	mortality (%)
none (saline)	+	100
0.001	+	100
0.005	+	90
0.01	+	60
0.05	+	40
0.1	+	30
0.5	+	0
1	+	0
1	–	0

Groups of mice (n = 10/group) were anally pretreated with 200 μl of saline or RCF at various doses 1 h before the intraperitoneal injection of compound 48/80. Mortality (%) within 1 h following compound 48/80 injection is represented as the number of dead mice × 100/total number of experimental mice.

Table 2. Time-dependent effect of RCF on compound 48/80-induced systemic allergic reaction

RCF treatment (g/kg, BW)	time (min)	compound 48/80 (0.008 g/kg BW)	mortality (%)
none (saline)	0	+	100
1	5	+	0
	10	+	20
	20	+	100

Groups of mice (n = 10/group) were anally pretreated with 200 μl of saline or RCF. RCF (1 g/kg) was given at 5, 10, and 20 min after the intraperitoneal injection of compound 48/80. Mortality (%) within 1 h following compound 48/80 injection is represented as the number of dead mice × 100/total number of experimental mice.

RCF inhibits the IgE-mediated local allergic reaction – PCA is one of the most important *in vivo* models of anaphylaxis in local allergic reactions and, in part, mediated by histamine in the blood stream (Mican *et al.*, 1992). Local extravasation is induced by a local injection of anti-DNP IgE followed by an intravenous antigenic challenge. RCF dose-dependently inhibited PCA reaction (Fig. 1).

RCF inhibits compound 48/80- or anti-DNP IgE-mediated histamine release from RPMC – The inhibitory effect of RCF on compound 48/80- or anti-DNP IgE-mediated histamine release from RPMC are shown in Fig. 2.

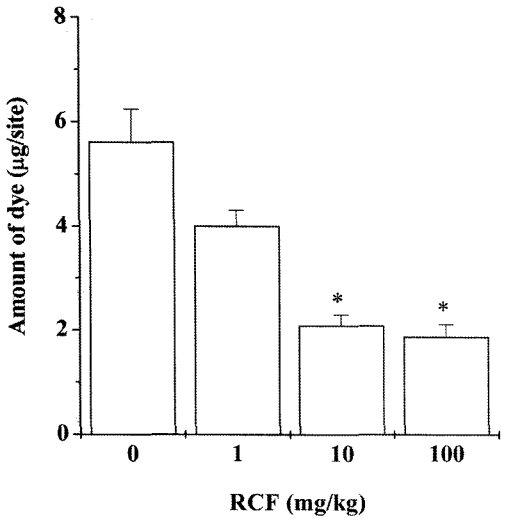

Fig. 1. Effect of RCF on the PCA reaction. RCF was anally administered 1 h prior to the challenge with antigen. Each date represents the mean ± SEM of three independent experiments. *p < 0.05; significantly different from the saline value.

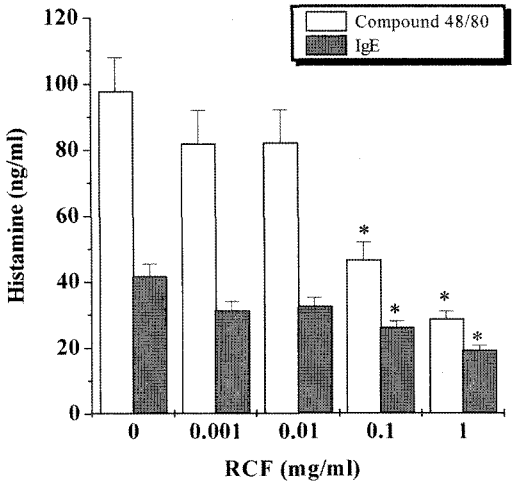

Fig 2. Effect of RCF on compound 48/80- or IgE-induced histamine release from RPMC. The cells (2×10^5 cells/ml) were preincubated with RCF at 37 °C for 10 min prior to incubation with compound 48/80 or DNP-HSA. Each data represents the mean ± SEM of three independent experiments. *$p < 0.05$; significantly different from the saline value.

Table 3. Effect of RCF on PMA plus A23187-stimulated pro-inflammatory cytokine secretion from HMC-1 cells

treatment	concentration (mg/ml)	content (ng/ml)	
		TNF-α	IL-6
none (saline)		0.382 ± 0.066	0.072 ± 0.010
PMA + A23187		1.039 ± 0.188	0.123 ± 0.023
PMA + A23187 + RCF	0.01	0.900 ± 0.235	0.106 ± 0.011
PMA + A23187 + RCF	0.1	0.646 ± 0.235*	0.095 ± 0.035*
PMA + A23187 + RCF	1	0.478 ± 0.053*	0.082 ± 0.021*

PMA plus A23187-stimulated HMC-1 cells were incubated for 8 h in the absence or presence of RCF. Pro-inflammatory cytokines secreted into the medium are presented as the mean ± SEM of three independent experiments. *p < 0.05; significantly different from the PMA + A23187 value.

RCF inhibited compound 48/80- or anti-DNP IgE-mediated histamine release from RPMC.

RCF inhibits pro-inflammatory cytokine secretion in HMC-1 cells – We examined whether RCF could regulate pro-inflammatory cytokines such as TNF-α and IL-6 in HMC-1 cells. HMC-1 cell line is a useful cell for studying cytokine activation pathway (Sillaber *et al.*, 1993). Stimulation of HMC-1 cells with PMA plus A23187-induced the secretion of both cytokines. However, pretreatment with RCF decreased PMA plus A23187-induced TNF-α and IL-6 secretion (Table 3).

Discussion

Immediate-type allergic reaction (anaphylaxis) is a life threatening syndrome induced by the sudden systemic release of inflammatory mediators, such as histamine, heparin and various cytokines from mast cells. Mast cells are located throughout the human body, and upon allergen exposure, they are stimulated via the IgE receptor (Kempo *et al.*, 2002). The results of this study demonstrated that RCF has anti-allergic properties. RCF inhibited compound 48/80-induced systemic reaction and anti-DNP IgE-mediated local allergic reaction. RCF attenuated compound 48/80-induced or anti-DNP IgE-mediated histamine release from RPMC. These results indicate that mast cell-mediated immediate-type allergic reactions are inhibited by RCF. Numerous reports established that stimulation of mast cells with compound 48/80 or IgE initiates the activation of signal-transduction pathway, which leads to histamine release. Several recent studies have shown that compound 48/80 and other polybasic compounds are able, apparently directly, to activate G-proteins (Mousli *et al.*, 1990a; Mousli *et al.*, 1990b; Chahdi *et al.*, 2000). Compound 48/80 increases the permeability of the lipid bilayer membrane by causing a perturbation in the mem-

brane. This result indicates that the increase in membrane permeability may be an essential trigger for the release of the mediator from mast cells. In this sense, anti-allergic agents having a membrane-stabilizing action may be desirable. RCF might stabilize the lipid bilayer membrane, thus preventing the perturbation being induced by compound 48/80. Additionally, the RCF administered mice are protected from anti-DNP IgE-mediated PCA, one of the most important *in vivo* models of anaphylaxis in local allergic reaction. This finding suggests that RCF might be useful in the treatment of allergic skin reactions. Mast cell-derived cytokines, especially TNF-α and IL-6 have a critical biological role in the allergic reaction. Mast cells are a principal source of TNF-α in human dermis, and degradation of mast cells in the dermal endothelium is abrogated by the anti-TNF-α antibody (Walsh *et al.*, 1991). IL-6 is also produced from mast cells and its local accumulation is associated with a PCA reaction (Mican *et al.*, 1992). These reports indicate that reduction of pro-inflammatory cytokines from mast cell is a one of the key indicator of reduced allergic symptom. In the present study, RCF inhibited the secretion of TNF-α and IL-6 in PMA plus A23187-stimulated HMC-1 cells. This result suggests that the anti-allergic effect of RCF results from its reduction of TNF-α and IL-6 release from mast cells.

In conclusion, the results obtained in the present study provide evidence that RCF contributes importantly to the prevention or treatment of mast cell-mediated allergic diseases. Also, it suggests that RCF may contain compounds with actions that inhibit mast cell-mediated allergic reactions *in vivo* and *in vitro*. Therefore, further investigation is necessary to clarify unknown anti-allergic constituents that may be more active than the RCF itself.

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References

Bradding, P., Feather, I.H., Wilson, S., Bardin, P.G., Heusser, C.H., Holgate, S.T., and Howarth, P.H., Immunolocalization of cytokines in the nasal mucosa of normal and perennial rhinitic subjects. The mast cell as a source of IL-4, IL-5, and IL-6 in human allergic mucosal inflammation. *J. Immunol.* **151**, 3853-3865 (1993).

Burd, P.R., Rogers, H.W., Gordon, J.R., Martin, C.A., Jayaraman, S., Wilson, S.D., Dvorak, A.M., Galli, S.J., and Dorf, M.E., Interleukin 3-dependent and -independent mast cells stimulated with IgE and antigen express multiple cytokines. *J. Exp. Med.* **170**, 245-257 (1989).

But, P.P.H., Kimura, T., Guo, J.X., Sung, C.K., and Han, B.H., *International Collation of Traditional and Folk Medicine (2)*, World Scientific, New Jersey, 1997, pp. 68-69.

Chahdi, A., Fraundorfer, P.F., and Beaven, M.A., Compound 48/80 activates mast cell phospholipase D via heterotrimetric GPT-binding proteins. *J. Pharmacol. Exp. Ther.* **292**, 122-130 (2000).

Church, M.K. and Levi-Schaffer, F., The human mast cell. *J. Allergy Clin. Immunol.* **99**, 155-160 (1997).

Ennis, M., Pearce, F.L., and Weston, P.M., Some studies on the release of histamine from mast cells stimulated with polylysine. *Br. J. Pharmacol.* **70**, 329-334 (1980).

Galli, S.J., Gordon, J.R., and Wershil, B.K., Cytokine production by mast cells and basophils. *Curr. Opin. Immunol.* **3**, 865-872 (1991).

Kanemoto, T.J., Kasugai, T., Yamatodani, A., Ushio, H., Mochizuki, T., Tohya, K., Kimura, M., Nishimura, M., and Kitamura, Y., Supernormal histamine release and normal cytotoxic activity of Biege rat mast cells with giant granules. *Int. Arch. Allergy Immuniol.* **100**, 99-106 (1993).

Katayama, S., Shionoya, H., and Ohtake, S., A new method for extraction of extravasated dye in the skin and the influence of fasting stress on passive cutaneous anaphylaxis in guinea pigs and rats. *Microbiol. Immunol.* **22**, 89-101 (1978).

Kemp, S.F. and Lockey, R.F., Anaphylaxis: a review of causes and mechanisms. *J. Allergy Clin. Immunol.* **110**, 341-348 (2002).

Lee, S.J., *Korean Folk Medicine*, Publishing Center of Seoul National University, Seoul, 1966, pp. 75.

Metcalfe, D.D., Kaliner, M., and Donlon, M.A., The mast cell. *Crit. Rev. Immunol.* **3**, 23-27 (1981).

Mican, J.A., Arora, N., Burd, P.R., and Metcalfe, D.D., Passive cutaneous anaphylaxis in mouse skin is associated with local accumulation of interleukin-6 mRNA and immunoreactive interleukin-6 protein. *J. Allergy Clin. Immunol.* **90**, 815-824 (1992).

Miyajima, I., Dombrowicz, D., Martin, T.R., Ravetch, J.V., Kinet, J.P., and Galli, S.J., Systemic anaphylaxis in the mouse can be mediated largely through IgG1 and Fc gammaRIII. Assessment of the cardiopulmonary changes, mast cell degranulation, and death associated with active or IgE- or IgG1-dependent passive anaphylaxis. *J. Clin. Invest.* **99**, 901-914 (1997).

Mousli, M.C., Bronner, C., Bockaert, J., Rouot, B., and Landry, Y., Interaction of substance P, compound 48/80 and mastoparan with a-subunit C-terminal of G protein. *Immunol. Lett.* **25**, 355-358 (1990a).

Mousli, M.C., Bronner, C., Landry, Y., Bockaert, J., and Rouot, B., Direct activation of GTP-binding regulatory proteins (G proteins) by substance P and compound 48/80. *FEBS Lett.* **259**, 260-262 (1990b).

Perry, L.M., *Medicinal Plants of East and Southeast Asia*, Attributed Properties and Uses. MIT Press, Cambridge, 1980, pp. 346.

Petersen, L.J., Mosbech, H., and Skov, P., Allergen-induced histamine release in intact human skin in vivo assessed by skin microdialysis technique: Characterization of factors influencing histamine releasability. *J. Allergy Clin. Immunol.* **97**, 672-679 (1996).

Plaut, M., Pierce, J.H., Watson, C.J., Hanley-Hyde, J., Nordan, R.P., and Paul, W.E., Mast cell lines produce lymphokines in response to cross-linkage of Fc epsilon RI or to calcium ionophores. *Nature*, **339**, 64-67 (1989).

Scuderi, P., Sterling, K.E., Lam, K.S., Finley, P.R., Ryan, K.J., Ray, C.G., Petersen, E., Slymen, D.J., and Salmon, S.E., Raised serum levels of tumor necrosis factor in parasitic infections. *Lancet* **13**, 1364-1365 (1986).

Shin, T.Y., Kim, S.H., Choi, C.H., Shin, H.Y., and Kim H.M., *Isodon japonicus* decreases immediate-type allergy reaction and tumor necrosis factor-α production. *Int. Arch. Allergy Immunol.* **135**, 17-23 (2004).

Shore, P.A., Burkhalter, A., and Cohn, V.H., A method for fluorometric assay of histamine in tissues. *J. Pharmacol. Exp. Ther.* **127**, 182-186

(1959).

Sillaber, C., Bevec, D., Butterfield, J.H., Heppner, C., Valenta, R., Scheiner, O., Kraft, D., Lechner, K., Bettelheim, P., and Valent, P., Tumor necrosis factor alpha and interleukin-1 beta mRNA expression in HMC-1 cells: differential regulation of gene product expression by recombinant interleukin-4. *Exp. Hematol.* **21**, 1271-1275 (1993).

Walsh, L.J., Trinchieri, G., Waldorf, H.A., Whitaker, D., and Murphy, G.F., Human dermal mast cells contain and release tumor necrosis factor alpha, which induces endothelial leukocyte adhesion molecule 1. *Proc. Natl. Acad. Sci.* **88**, 4220-4224 (1991).

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