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Inhibitory Effects of *Ulmus parvifolia* and *Liriope platyphylla Wang et Tang* on Histamine Release from Rat Peritoneal Mast Cells

Su Ok Yang[1] and Geun Eog Ji[1,2]*

[1]*Department of Food and Nutrition, Research Institute of Human Ecology, Seoul National University, Seoul 151-742, Korea*
[2]*Research Institute, Bifido Inc., Hongchon, Gangwon 250-804, Korea*

Abstract Twenty-four different natural food materials extracted with 80% methanol were used to investigate the inhibition of cell-mediated immediate type allergic reactions induced by compound 48/80 in rat peritoneal mast cells (RPMCs). Nine 80% methanol extracts screened at a concentration of 10 μg/mL inhibited histamine release from RPMCs induced by compound 48/80. Of these, two materials (*Ulmus parvifolia* and *Liriope platyphylla Wang et Tang*) were extracted and fractionated into four different solvent types (chloroform, ethylacetate, butanol, and water), and the fractions with major anti-allergic effects were assessed. The chloroform fraction of *U. parvifolia* (UP) at 5 μg/mL and the ethylacetate fraction of *L. platyphylla Wang et Tang* (LPWT) at 1 μg/mL showed the greatest inhibition of histamine release induced by compound 48/80. The chloroform fraction of UP and the ethylacetate fraction of LPWT in combination showed a greater inhibition of histamine release than either fraction alone. The cAMP levels in RPMCs treated with UP and LPWT were significantly greater than in cells treated with compound 48/80 alone. Our studies suggest that extracts from UP and LPWT may alleviate immediate type hypersensitivity reactions through the increase of cAMP levels in the mast cells.

Keywords: mast cell, histamine, *Ulmus parvifolia, Liriope platyphylla*

Introduction

The term 'allergy' was introduced in 1906 by von Pirquet to include both facets of the allergic response: the beneficial response was termed 'protective immunity' and the harmful response termed 'hypersensitivity reactions' (1). In the early 1960s, hypersensitivity reactions were further classified as Type I, II, III, and IV allergic reactions. One of these, type I hypersensitivity which is generally referred to as 'allergy', is a major clinical problem in humans. In recent years, the term allergy has also become synonymous with type I hypersensitivity or immediate hypersensitivity (2).

Mast cells are thought to play a major role in the development of many physiological changes during anaphylactic and allergic responses (3). During the degranulation of mast cells, histamine remains the most potent vasoactive mediator implicated in the acute phase of immediate hypersensitivity (4, 5). Because histamine is stored in granules, its biological effects are observed within minutes of mast-cell activation (6).

Many basic substances, cationic secretagogues such as the polymine compound 48/80 (7), mastoparan (8), and substance P (9) are able to release histamine from intact mast cells. Activation of mast cells and the initiation of a signal transduction cascade lead to the release of mediators such as histamine (6, 10). The basic secretagogues first bind to the plasma-membrane, most likely through sialic acid residues which are coupled to the G-protein cascade. Amphiphilic peptides such as basic secretagogues assume an alpha helical conformation in a hydrophobic medium. The combination of positive charges and hydrophobicity is sufficient for the insertion of the molecule into membrane lipids to activate the mast cell. Such a process would lead to the insertion of secretagogues into the plasma membrane and their translocation into the cytoplasm in order to reach trimeric G proteins located on the cytoplasmic face of the plasma membrane or other intracellular membranes (11-13). Cationic peptides directly stimulating G proteins coupled to phospholipase C induce a transient increase of inositol 1,4,5-triphosphate, which leads to calcium mobilization from intracellular stores (13). Ca^{2+} inhibits mast cell adenylate cyclase and modestly stimulates mast cell phosphodiesterase. Both actions rapidly lower mast cell cAMP concentrations, which is necessary for histamine release (14). The increase of Ca^{2+} also promotes the assembly of microtubules and the contraction of microfilaments, both of which are necessary for the movement of granules to the plasma membrane (6).

In this study, we investigated the active fractions of elm root (*U. parvifolia,* UP) and snake's beard (*L. platyphylla Wang et Tang*, LPWT) and 22 other natural food materials regarding their effects on compound 48/80-induced histamine release from rat peritoneal mast cells (RPMCs), and assessed the effects of the active fractions on intracellular cAMP levels in RPMCs. The results of this screening may provide useful information for the further investigation of functionally active compounds that alleviate the allergic response.

Materials and Methods

Animals Seven-week-old male Wistar rats (200-250 g in weight) purchased from the Jungang Experimental Animal Center (Seoul, Korea) were kept in plastic cages and allowed free access to water and food. Temperature and humidity were maintained at 22-24°C and 45-65%,

*Corresponding author: Tel: 82-2-880-8749; Fax: 82-2-884-0305
E-mail: geji@bifido.com
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respectively. The mice were maintained on a 12:12 hr light:dark cycle in an environmentally-controlled chamber.

Materials Percoll was purchased from Pharmacia Fine Chemicals (Amersham, Uppsala, Sweden). Isotonic Percoll solutions were prepared by dissolving 9 parts of Percoll with 1 part 10-fold concentrated Hanks' solution (HS). Colour-markered beads obtained from Pharmacia Fine Chemicals (Piscataway, NJ, USA) covering the buoyant density range 1.016-1.178 g/mL were used for calibration of Percoll gradients and for checking equilibrium conditions. Compound 48/80, HS, and bovine serum albumin (BSA) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). A histamine enzyme-linked immunosorbent assay (ELISA) kit was purchased from Immunotech (Marseille Cedex 9, France), and a cAMP kit was purchased from R&D Systems (Minneapolis, MN, USA).

Preparation of each sample *Siberian ginseng, Angelica gigas,* UP, *Pueraria thomsonii, Zanthoxylum schinifolium, Cinnamomun cassia, Phellinus linteus, Asiasarum sieboldi,* LPWT, *Perilla folium, Ginko biloba leaves, Castanea crenata leaves, R. Akane Nakai,* and *Plantago asiatica* purchased from Kyung-Dong Market (Seoul, Korea) were powdered. *Mentha piperita* and *Rosemary* were purchased from Ilyeong Herbland (www.iyherb.co.kr). *Eucommia ulmoides, Gastrodia elata, Cudrania tricuspidata, Hovenis dulcis, Wasabia koreana, Cornus officinalis,* and *Panax notoginseng* were purchased from the Korea plant extract bank (www.pdrc.re.kr) (15). Fifty g of each dried and powdered sample was mixed with 500 mL of 80% methanol (MeOH) and active compounds were extracted from the solution after leaving it at room temperature for 24 hr and filtering through a Whatman 2 filter (Whatman Inc. Ltd., Maidstone, UK). The above procedure was repeated 2 times and the filtrates were combined. The 80% MeOH extracts were then concentrated in a vacuum rotary evaporator at 38-50°C (Lab Companion, Jeiotech, Korea) in order to remove the MeOH, and lyophilized in a freeze dryer (TFD5505; Ilshin Europe B.V). For analysis, the extracted samples were dissolved in dimethyl sulfoxide (DMSO) and further diluted with N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES) buffer.

Solvent fractionation of 80% MeOH extracts The 300 g plant samples (UP and LPWT) were extracted two times successively with 3 L of 80% MeOH at room temperature for 24 hr. The 80% MeOH extracts were filtered, evaporated at 40-50°C under vaccum and lyophilized. The aqueous layer of the 80% MeOH extract was extracted with chloroform ($CHCl_3$) followed by ethyl acetate (EtOH) and finally with butanol (BuOH) (5:3:2) (Fig. 1). The respective fractions and a residue fraction were evaporated at 50 under reduced pressure, and then the remaining aqueous fractions were lyophilized. The fractionated samples were dissolved in DMSO and further diluted with HEPES buffer. The respective extracts were then filtered through a 0.22 μm filter. All the above fractions were screened for anti-allergy activity *in vitro.*

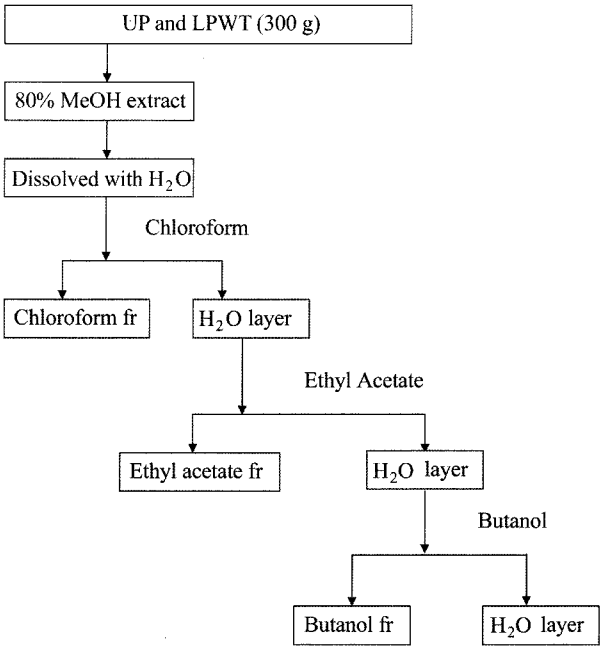

Fig. 1. Process of generating various solvent fractions from UP and LPWT.

Isolation and purification of rat peritoneal mast cells (RPMC) Male Wistar rats weighing 200-250 g were anesthetized by ether. Twenty mL of HEPES buffer (137 mM NaCl, 5.6 mM glucose, 10 mM HEPES, 2.7 mM KCl, 0.4 mM NaH_2PO_4, and 1 mM $CaCl_2$, pH 7.4) supplemented with 50 IU/mL of heparin was then injected into the abdominal cavity. The abdominal region was gently massaged for 90 sec, the peritoneal cavity was carefully opened, and the peritoneal fluid was collected using a pipette. Rat peritoneal mast cells (RPMC) were isolated and purified over a Percoll density gradient as previously described (16). It is recommended that red blood cells be removed prior to purifying mast cell suspensions. Consequently the mixed peritoneal cells were resuspended with 5 mL of 0.17 M ammonium chloride (NH_4Cl), which destroys red cells with minimal effects on other cells. The cells were incubated at 4°C for 5 min with occasional shaking. The cells were then centrifuged (400× g, 5 min, 4°C) and the lysis buffer was removed. The cells were resuspended in HEPES buffer and the pellets were washed twice by centrifugation (400×g, 5 min, 4°C). To the pellets, 1 mL of HEPES buffer supplemented with 1 mg/mL of bovine serum albumin was added. The cell suspension was mixed with 4 mL of 90% Percoll (isotonic Percoll) and 1 mL of bovine serum albumin supplemented HEPES buffer was then carefully layered over the Percoll-cell mixture. Purification was then performed by centrifugation (1900×g, 25 min, 4°C) which allowed cell separation and gradient formation simultaneously. The cell fraction (pellet) was then washed twice in HEPES buffer by centrifugation and finally resuspended at the desired cell density in HEPES buffer pre-warmed at 37°C.

Histamine release assay Mast cells pooled from three rats were resuspended at a cell density of 10^5 cells/mL and

warmed to 37°C for 10 min with HEPES buffer. One hundred mL of the equilibrated cells was seeded into 96-well plates (1×10^3 cells/well) and further incubated for 10 min at 37°C for stabilization. The peritoneal mast cells were incubated for 10 min with various concentrated anti-allergic compounds (0-1000 μg/mL) and then challenged for 10 min with compound 48/80 (1 μg/mL). The cells were separated from supernatant by centrifugation at 900×g for 5 min at 4°C. The histamine content of the supernatant was assayed using a Histamine ELISA kit (Immunotech, France).

cAMP assay Mast cells were transferred to 96-well culture plates (1×10^5 cells/100 μL /well) and pre-incubated for 10 min in HEPES buffer. The pre-incubated mast cells were then exposed to each effective fraction for 10 min. After incubation, cells were centrifuged at 600×g for 5 min at 4°C and the supernatant was removed. The reaction was allowed to proceed for specific time intervals and terminated by the addition of ice-cold 0.1 N HCl followed by incubation for 20 min. The supernatants and cell pellets were separated by centrifugation at 600×g for 5 min at 4°C. The supernatants were assayed immediately or stored at -80°C. cAMP content was assayed using a cAMP kit (R &D Syestems, Germany).

Statistical analysis Statistical significance was determined for the treated and the control cells by the Duncan's multiple range test. Results with a p value of <0.05 were considered statistically significant.

Results and Discussion

Screening for natural food materials that inhibit histamine release A total of 24 natural food materials shown in Table 1 were screened for their inhibitory effects

Table 1. The effects of methanol extracts (10 μg/mL) of natural food materials on compound 48/80-mediated histamine release from RPMC

Common name	Scientific name	Used part	Histamine conc.(nM)	Inhibition (%)
Compound 48/80	(1 μg/mL)	-	91.5	
DSCG	(10 μg/mL)	-	82.0	10.4
Cinnamon	*Cinnamomum cassia*	BT[1)]	82.4	9.9
Chestnut leaf	*Castanea crenata S. et Z*	L[2)]	103.2	-12.8
Cheoncho	*R. Akane Nakai*	F[3)]	92.5	-1.1
Cheonma	*Gastrodia elata*	WP[4)]	87.9	3.9
Danggui	*Angelica gigas Nakai*	PR[5)]	87.4	4.5
Duzhong	*Eucommia ulmoides*	BT	92.4	-1.0
Duzhong	*Eucommia ulmoides*	SH[6)]	92.6	-1.2
Elm (tree)	*Ulmus parvifolia var. japonica Nakaki*	PR	82.3	10.1
Gingko leaf	*Ginko biloba L.*	L	88.7	3.1
Gguzibbong tree	*Cudrania tricuspidata*	L	92.1	-0.1
Hutgae tree	*Hovenia dulcis*	L	92.3	-1.1
Hutgae tree	*Hovenia dulcis*	S[7)]	91.0	0.5
Hutgae tree	*Hovenia dulcis*	F	92.5	-1.1
Kochunaengi	*Wasabia koreana*	PR	92.3	-0.9
Kochunaengi	*Wasabia koreana*	WP	92.1	-0.7
Peppermint	*Mentha piperita*	L	101.3	-10.7
Plantain	*Plantago asiatica*	L	91.6	-0.1
Red perilla	*Perilla frutescens Var acuta*	L	90.2	1.4
Rosemary	*Rosmarinus officinalis*	L	103.5	-13.1
Sancho	*Zanthoxylum schinifolium S.et Z.*	F	92.2	-0.8
Samchil	*Panax notoginseng*	PR	90.7	0.9
Sansuyu	*Cornus officinalis*	F	88.5	3.3
Snake's beard	*Liriope platyphyllaWang et Tang*	PR	80.9	11.6
Wild-ginger plant	*Asiasarum sieboldi*	WP	90.0	1.6

RPMCs (1×10^3 cells/mL) were preincubated with natural food materials (10 μg/mL) at 37°C for 10 min prior to addition of compound 48/80 (1 μg/mL). Each data point represents the mean±SD of three experiments. Means with different superscripts are significantly different at $p<0.05$ by Duncan's multiple range test.
[1)]BT, the bark of the tree; [2)]L, leaf; [3)]F, fruit; [4)]WP, whole plant; [5)]PR, plant root; [6)]SH, stem heart; [7)]S, stem.

on compound 48/80-induced histamine release in rat peritoneal mast cells (RPMC). Compound 48/80 (c48/80) has been widely used as a histamine-releasing agent and its mechanism of action is commonly attributed to a direct, receptor-bypassing capacity to activate specific G proteins (17). To measure histamine release, RPMCs were preincubated with various 80% MeOH extracts (10 μg/mL) 10 min prior to the addition of compound 48/80 (1 μg/mL). The inhibitory effect of each extract on histamine release was compared to cells treated with disodium cromoglycate (DSCG) or the control treated with compound 48/80. The histamine release from RPMCs treated with natural food extracts is shown in Table 1. The extracts with inhibitory activity are as follows: *Gastrodia elata, Ginko biloba leaves, Cornus officinalis, Angelica gigas*, UP, LPWT, *Cinnamomun cassia, Asiasarum sieboldi,* and *Perilla folium* (Table 1).

Among these, we further investigated the effects of UP and LPWT on histamine release induced by compound 48/80 in RPMCs based on the results shown in Table 1. UP has been used as a natural food remedy in oriental medicine for stomatitis, wound healing and allergic rhinitis. Though UP has various bioactive substances, there are no reports of biological function corresponding to each bioactive substance. It is also unclear how it inhibits allergic responses and how effective it is in experimental models. LPWT is a medicinal herb that has been used for the treatment of asthma, lung inflammation, cough, and sputum in Korea. The active components of LP have been identified as homoisoflavonoids (ophiopogone, methyl-opiogonone, etc.), sterols (β-sistosterol etc.), and steroidal glycosides (ruscogenin, ophiopogonin, etc.), but the functions of each component have not been investigated. LPWT showed a remarkable effect on respiratory patterns and tracheal tissues in allergic asthma (18). We show that UP and LPWT have inhibitory effects on histamine release induced by compound 48/80 in RPMCs (Table 1).

We used DSCG, a mast-cell stabilizer, as a positive control for an inhibitory effect. This effect is thought to be based on the inhibition of mediator release from mast cells through cell membrane stabilization. The inhibitory effect of DSCG may be related to either the ability of the drug to increase intracellular cAMP levels or its preventing the entry of external Ca^{2+} into the mast cell, thus blocking exocytosis (19). One additional site of action for DSCG may be GTP-binding proteins, which relates to phospholipase C activation (20).

Dose-response of effective fractions We next assessed the effect of several solvent fractions (chloroform, ethylacetate, butanol, and water) from UP and LPWT to determine which fractions have the active anti-allergic components. The fractions containing high histamine inhibitory activity were as follows: the chloroform fraction of UP and the ethylacetate fraction of LPWT (Table 2).

To assess the effects of the chloroform fraction from UP and the ethylacetate fraction from LPWT on compound 48/80-induced histamine release, RPMCs were pre-treated with various concentrations (0.001-1000 μg/mL) of each extract for 10 min prior to compound 48/80 stimulation. A shown in Fig. 2, histamine release gradually decreased as the concentration of the chloroform fraction of UP increased from 0 to 5 μg/mL, but gradually increased as the concentration was further increased up to 1 mg/mL. At a concentration of 5 μg/mL, the inhibition rate was as high as 19.7%. In contrast, as shown in Fig. 2, histamine release gradually decreased as the concentration of ethylacetate fraction of LP increased from 0 to 1 μg/mL, but gradually increased as the concentration of ethylacetate fraction of LP was further increased up to 1 mg/mL. The degree of reduction of histamine release was significant at concentrations of 0.1-10 μg/mL. At a concentration of 1 μg/mL,

Table 2. The effects of different fractions (10 μg/mL) from UP and LPWT on histamine release induced by compound 48/80 (1 μg/mL)

Treatment	Fractions	Inhibition (%)
None	-	0
DSCG (10 μg/mL)	-	10.3
UP	Methanol	13.0
	Chloroform	19.6
	Ethyl acetate	11.3
	Butanol	10.7
	Water	15.5
LPWT	Methanol	7.0
	Chloroform	-3.7
	Ethyl acetate	14.4
	Butanol	-4.5
	Water	6.6

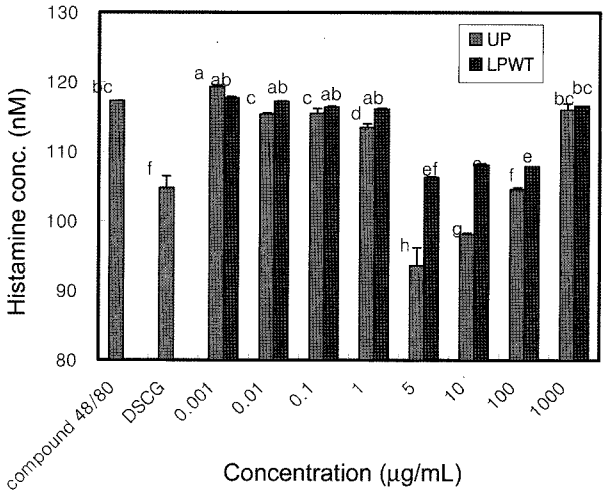

Fig. 2. The effects of UP chloroform fraction and LPWT ethyl acetate fraction on the histamine release induced by compound 48/80. RPMCs (1×10^3 cells/mL) were preincubated with chloroform or ethylacetate fractions (0.001-1000 μg/mL) at 37°C for 10 min prior to the addition of compound 48/80 (1 μg/mL). Each data point represents the mean±SD of three experiments. Means with different superscripts are significantly different at $p<0.05$ by Duncan's multiple range tests.

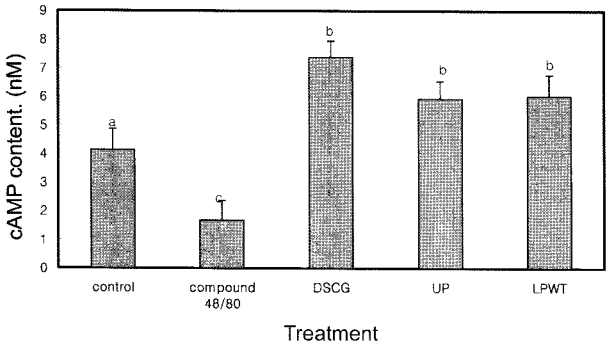

Fig. 3. The effects of UP and LPWT on cAMP levels from RPMCs. RPMCs (1×10^3 cells/mL) were preincubated with the chloroform fraction of UP (5 μg/mL) and the ethylacetate fraction of LPWT (1 μg/mL) at 37°C for 10 min. Each data point represents the mean±SD of three experiments. Means with different superscripts are significantly different at $p<0.05$ by Duncan's multiple range tests.

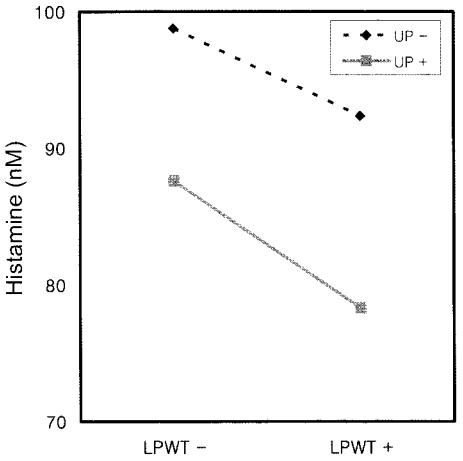

Fig. 4. The synergistic effect of UP+LPWT on histamine content. The analysis of the synergistic effect of UP and LPWT was significant at $\alpha=0.05$ by 2-way ANOVA.

the inhibition rate was as high as 16.2% and showed the most statistical significance. However, at 1 mg/mL concentrations of both UP and LPWT, histamine release was similar to that induced by compound 48/80.

cAMP assay We measured the concentration of cAMP in the mast cells to assess the mechanism by which the effective fractions inhibited histamine release from RPMCs (Fig. 3). When RPMCs were incubated with 5 μg/mL of the chloroform fraction of UP and 1 μg/mL of the ethylacetate fraction of LPWT, the cAMP content was high compared to that of control cells, and increased about 4-5 fold compared to that of compound 48/80 treated cells. Both UP and LPWT, therefore, significantly increased the cAMP content of RPMCs.

It has been suggested that the stimulation of mast cells with compound 48/80 initiates the activation of a signal transduction pathway leading to histamine release. There have been reports indicating that compound 48/80 is able to activate G proteins (9). Tasaka *et al.* (12) reported that the compound 48/80-induced increase in the permeability of the plasma membrane might be an essential trigger for the release of mediator from mast cells. The mechanism of this effect is presumed to be related to the activation of adenylate cyclase or the inhibition of cAMP phosphodiesterase, and a subsequent increase in intracellular cAMP which depresses the release of histamine. Since agents that prevent a fall in cAMP inhibit histamine release, it is apparent that cAMP is an important part of the regulation of histamine secretion (4). In this context, the mode of the action of natural food materials that inhibit histamine release might be related to the prevention of calcium release in mast cells due to elevated intracellular cAMP levels resulting from the inhibition of cAMP phosphodiesterase.

Synergistic effects of UP and LPWT on histamine release The effects of mixtures of the fractions showing the greatest inhibition of compound 48/80-induced histamine release from RPMCs are shown in Fig. 4. When RPMCs were incubated with a combination of the chloroform fraction of UP and the ethylacetate fraction of LPWT, the mixtures (UP+LPWT) significantly inhibited histamine release at $\alpha=0.05$ by 2-way ANOVA (Fig. 4) and caused an increase in cAMP levels (data not shown) compared to the compound 48/80 treated samples.

Further studies are required to characterize the effective components of the screened natural food materials and their mechanisms of action in order to utilize them in the development of functional foods with anti-allergy activity.

Acknowledgments

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