



Sargahydroquinoic acid isolated from *Sargassum serratifolium* as inhibitor of cellular basophils activation and passive cutaneous anaphylaxis in mice

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ABSTRACT

Basophils and mast cells are characteristic effector cells in allergic reactions. Sargahydroquinoic acid (SHQA), a compound isolated from *Sargassum serratifolium* (marine alga), possesses various biochemical properties, including potent antioxidant activities. The objective of the present study was to investigate inhibitory effects of SHQA on the activation of human basophilic KU812F cells induced by phorbol myristate acetate and A23187 (PMACI), a calcium ionophore. Furthermore, we confirmed the inhibitory effects of SHQA on the activation of rat basophilic leukemia (RBL)-2H3 cells induced by compound 48/80 (com 48/80), bone marrow-derived mast cells (BMCs) induced by anti-dinitrophenyl (DNP)-immunoglobulin E (IgE)/DNP-bovine serum albumin (BSA), DNP/IgE and on the reaction of passive cutaneous anaphylaxis (PCA) mediated by IgE. SHQA reduced PMACI-induced intracellular reactive oxygen species (ROS) and calcium levels. Western blot analysis revealed that SHQA downregulated the activation of ERK, p38, and NF- κ B in a dose-dependent manner. Moreover, SHQA suppressed the production and gene expression of various cytokines, including interleukin (IL)-1 β , IL-4, IL-6, and IL-8 in PMACI-induced KU812F cells and IL-4 and tumor necrosis factor (TNF)- α in com 48/80-induced RBL-2H3 cells. It also determined the inhibition of PMACI, com 48/80- and IgE/DNP-induced degranulation by reducing the release of β -hexosaminidase. Furthermore, it attenuated the IgE/DNP-induced PCA reaction in the ears of BALB/c mice. These results suggest that SHQA isolated from *S. serratifolium* is a potential therapeutic functional food material for inhibiting effector cell activation in allergic reactions and anaphylaxis in animal model.

1. Introduction

Allergies are immune responses to environmental factors. Allergens includes dust mites in the home pollen, food, insects and medications, and allergic reactions depend on the toxicity of these factors. Various immune cells, lymphocytes such as T and B cells, and granulocytes such as mast cells, eosinophils and basophils play a crucial roles in the induction, progression and alleviation of allergic reactions [1,2]. Among cells, mast cells and basophils are specialized as effector cells that have

been implicated in the expression of a wide variety of biological responses including immediate hypersensitivity, host defense against various pathogens and immunologically non-specific inflammatory signaling [1–3]. Basophils are granulocytes that play key role in specific immunity by interacting with multivalent antigens and various stimuli such as calcium ionophore, neuropeptides, compound 48/80, and anti-Fc ϵ RI α chain antibody in allergy. Activated effector cells in allergic reactions trigger the activation of mitogen-activated protein kinases (MAPK), which involve extracellular signal regulated kinase (ERK), c-

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jun, NH₂-terminal kinase (JNK), and p38 as an intracellular second messenger, and are associated with transcriptional regulation of allergic responses through activation of NF- κ B, a critical factor for regulating cytokines generation [7–11]. NF- κ B is an active complex found in the cytoplasm that stimulates increased translocation of free NF- κ B/p65 into the nucleus [12–14]. Activated MAPK then triggers degranulation, resulting in the release of various inflammatory mediators such as β -hexosaminidase, histamine, prostaglandins, and leukotrienes, which are known to cause allergic responses [12].

Marine algae are consumed by humans as vegetables and are also used as components of medicinal preparations because of their various biochemical properties and relative abundance as seaweeds. Brown algae are rich sources of bioactive components including meroterpenoids, phlorotannins, fucoxanthin, sterols, and glycolipids. *Sargassum serratifolium* is a perennial brown alga belonging to the Sargassaceae family. It is widely distributed on Korean and Japanese coasts, and has various physiological effects that are anti-inflammatory, antioxidant, anticancer, hepatoprotective, anti-melanogenesis, anti-diabetic, neuroprotective, and anti-Alzheimer [15–30]. However, the effect of sargahydroquinone acid (SHQA) from *S. serratifolium* through inhibiting basophils and mast cells activation, and anaphylaxis in mice have not yet been reported. Therefore, the objective of this study was to investigate the inhibitory effects of SHQA in allergic reaction, a potent antioxidant compound isolated from *S. serratifolium*, on the activation of human basophils, KU812F cells induced by phorbol myristate acetate and calcium ionophore, A23187 (PMACI), and rat basophilic leukemia (RBL)-2H3 induced by compound 48/80 (com 48/80). Furthermore, we determined the anti-allergic effects via inhibition of degranulation of SHQA in IgE-mediated bone marrow-derived cultured mast cells (BMCMCs) and passive cutaneous anaphylaxis (PCA) animal model.

2. Materials and methods

2.1. Samples

Authentic SHQA (Fig. 1) was isolated and purified from an ethanolic extract of *S. serratifolium* as previously described, [18], and was kindly provided by Prof. Taisun Shin (Chonnam National University, Gwangju, Korea). The structure and chromatogram of SHQA are shown in Fig. 1. ¹H and ¹³C NMR spectra were recorded on a JNM ECP-400 spectrometer (JEOL, Japan), using CD₃OD with tetramethylsilane as the internal standard. Using pulsed-field gradients, the heteronuclear multiple quantum correlation (HMQC) and heteronuclear multiple bond correlation (HMBC) spectra were recorded.

SHQA. C₂₇H₃₆O₄ (Mw 424) ¹H NMR (CD₃OD, 400 MHz); 3.25 (2H, d, *J* = 7.4 Hz, H-1), 5.29 (1H, dt, *J* = 7.3 and 1.4 Hz, H-2), 2.08 (2H, m, H-

4), 2.12 (2H, m, H-5), 5.14 (1H, t, *J* = 7.0 Hz, H-6), 2.08 (2H, m, H-8), 2.50 (2H, dt, *J* = 7.0 and 7.0, H-9), 5.83 (1H, t, *J* = 7.3 Hz, H-10), 2.21 (2H, t, *J* = 7.7 Hz, H-12), 2.12 (2H, m, H-13), 5.07 (1H, tt, *J* = 7.3 and 1.4 Hz, H-14), 1.65 (3H, s, CH₃-16), 1.56 (3H, s, CH₃-17), 1.59 (3H, s, CH₃-19), 1.70 (3H, s, H-20), 6.38 (2H, brs, H-3' and H-5'), 2.14 (3H, s, Aromatic-CH₃). ¹³C NMR (CD₃OD, 100 MHz); 29.6 (C-1), 124.1 (C-2), 136.8 (C-3), 40.9 (C-4), 27.6 (C-5), 125.96 (C-6), 135.5 (C-7), 40.3 (C-8), 28.9 (C-9), 142.7 (C-10), 132.9 (C-11), 36.0 (C-12), 29.0 (C-13), 124.8 (C-14), 133.3 (C-15), 25.9 (C-16), 17.8 (C-17), 171.7 (C-18), 15.96 (C-19), 16.2 (C-20), 146.5 (C-1'), 131.3 (C-2'), 114.5 (C-3'), 151.4 (C-4'), 115.7 (C-5'), 127.5 (C-6'), 16.9 (Aromatic-CH₃).

2.2. Cell Culture, Treatment, and stimulation

Human basophilic KU812F and rat basophilic leukemia, RBL-2H3 cells were obtained from the American Type Culture Collection and Cell & Matrix Research Institute, Department of Pharmacology, Kyungpook National University School of Medicine, Daegu, Korea, respectively. KU812F cells were maintained in RPMI-1640 (Gibco BRL, Grand Island, NY, USA) and RBL-2H3 cells were maintained in Dulbecco modified Eagle medium (DMEM) (WELLGEBE Inc. Gyeongsangbuk-do, Korea), the media were supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Gibco), antibiotics and antimycotics (Gibco). Cells were cultured at 37 °C in a humidified atmosphere with 5% CO₂ and passaged every 3 ~ 4 days. Additionally, the cells were treated with various concentrations of SHQA for 1 h and stimulated with 40 nM of PMA (Sigma, Sigma-Aldrich, St. Louis, MO, USA) and 1 μ M of A23187 (Sigma) (PMACI) for KU812F cells and com 48/80 (Sigma) for RBL-2H3 cells during the indicated period under a serum-free conditions.

2.3. Cell viability assay

Cell viability was determined by 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfonylphenyl)-2H-tetrazolium (MTS) assay using a CellTiter96® Aqueous One Solution Cell Proliferation Assay Kit (Promega, Madison, WI, USA) according to the manufacturer's instructions. Briefly, cells (5 $\times$ 10⁴ cells/well) were pretreated with SHQA for 1 h and stimulated with or without PMACI and com 48/80 for 24 h in a serum-free medium. After 24 h of treatment, MTS solution (Promega) in a fresh medium was added to each well followed by incubation at 37 °C for 1 h according to the manufacturer's instructions. Absorbance was then measured at 490 nm using a microplate reader (Epoch, BioTek Instruments, Winooski, VT, USA).

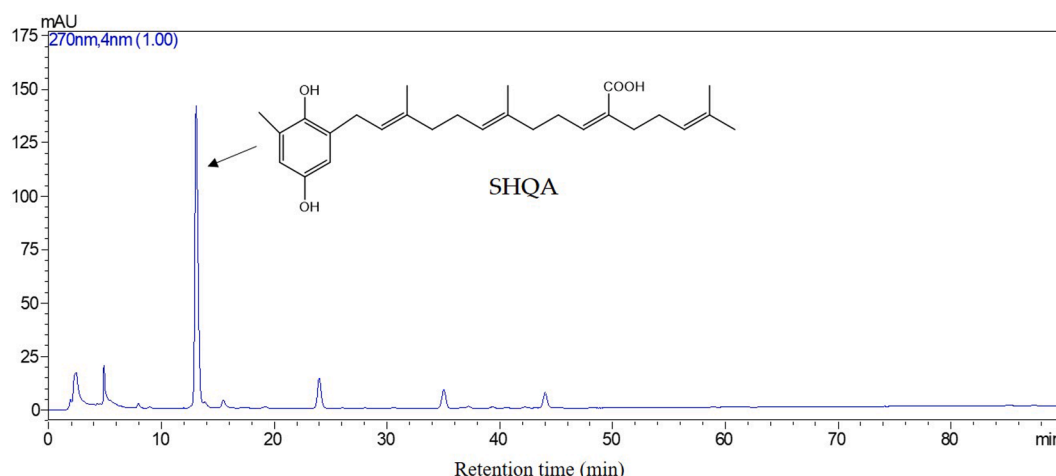


Fig. 1. HPLC chromatogram and chemical structure of SHQA isolated from *S. serratifolium*.

2.4. Intracellular ROS assay

Intracellular ROS production was assessed using dichlorofluorescein diacetate (DCFH-DA) (Sigma), an oxidant-sensitive fluorescent probe and DCF fluorescence was monitored by two methods using a fluorescence microplate reader and flow cytometry. Cells were seeded at a density of 1×10^4 cells/well and incubated with 0.01, 0.05, 0.10, and 0.50 μM of SHQA for 2 h; were then treated with PMACI for 2 h to induce ROS generation. Next, the cells were incubated with DCFH-DA (25 μM) in Hanks Balanced Salt Solution (HBSS) buffer (Sigma) for 30 min. Fluorescence intensities were measured at excitation and emission wavelengths of 485 and 538 nm, respectively, using a fluorescence microplate reader (Molecular Devices Co., San Jose, CA, USA). For flow cytometric analysis, the cells were pretreated with SHQA for 1 h and stimulated with PMACI for 30 min. Then, the cells were incubated with DCFH-DA in HBSS buffer for 1 h, washed, and resuspended in cold PBS. The cytoplasmic DCF fluorescence intensity was analyzed using a flow cytometer (Beckman Coulter, Brea, CA, USA) with a FITC barrier filter.

2.5. $[\text{Ca}^{2+}]_i$ concentration assay

The $[\text{Ca}^{2+}]_i$ concentration was measured using a calcium-reactive fluorescence probe, Fura 2-AM (Abcam, Danver, MA, USA). KU812F cells were pretreated with 0.01, 0.05, 0.10, and 0.50 μM of SHQA for 2 h, then stimulated with PMACI for 1 h under serum-free conditions. Cells were treated with 2 μM Fura 2-AM in Hanks Balanced Salt Solution (Sigma) for 1 h and Fura 2 fluorescence was monitored at excitation and emission wavelengths of 405 nm and 519 nm, respectively, using a fluorescence microplate reader (Molecular Devices).

2.6. Western blot analysis

Protein expression was assessed using western blot analysis. The pretreated and stimulated cells were washed with cold PBS and harvested. Whole-cell lysates were extracted using RIPA buffer (Thermo Fisher Scientific, Waltham, MA, USA) containing a protease inhibitor cocktail (Roche Diagnostics GmbH, Mannheim, Germany). Cytosolic and nuclear proteins were extracted from the cells using the NE-PER® Nuclear and Cytoplasmic Extraction kit (Thermo Scientific). The protein concentration was measured using a BCA™ Protein Assay Kit (Thermo Scientific) according to the manufacturer's instructions. Equal amounts of proteins were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride (PVDF) membranes (Millipore). Membranes were then blocked with 5% skim milk in a plain buffer (20 mM Tris pH 7.4 and 136 mM NaCl) at room temperature (RT) for 1 h and then incubated with primary antibodies (ERK1/2, p-ERK 1/2, p38, p-p38, JNK, and p-JNK) (Santa Cruz Biotech, Santa Cruz, CA, USA), NF- κB , and pNF- κB (Cell Signalling Technology, Danvers, MA, USA) at 4 °C overnight. These membranes were then incubated with a specific horseradish peroxidase (HRP) conjugated secondary antibody at RT for 1 h. Immunoreactive bands were visualized using an enhanced Super Signal West Femto Maximum Sensitivity Substrate Reagent Kit (Thermo Scientific) under the ChemiDoc™ Imaging System (Bio-Rad Laboratories, Inc. CA, USA) and the bands were analyzed using Image J software (US National Institutes of Health Bethesda, MD, USA).

2.7. Reverse Transcriptase-Polymerase chain reaction (RT-PCR)

Gene expression was assessed using RT-PCR using specific primers. Cells were pretreated with SHQA for 1 h and then stimulated for 4 h. Total cellular RNA were extracted using TRI reagent (Molecular Research Center, Cincinnati, OH, USA) according to the manufacturer's protocol. Briefly, cells were resuspended in 1 mL of TRI reagent and incubated at RT for 5 min. After adding chloroform, the mixture was vortexed and incubated at RT for another 5 min. The aqueous phase

containing RNA was obtained after centrifugation at 12,000g for 30 min at 4 °C. Total RNA was precipitated using isopropyl alcohol. The RNA pellet was washed with ice-cold 70% ethanol and air-dried. For complementary DNA (cDNA) synthesis, 1 μg of total RNA was reverse-transcribed using an oligo dT₂₀ primer (Invitrogen, Frederick, MD, USA) and Moloney murine leukemia virus (MMLV) at RT (Promega). The resulting cDNA samples were amplified by PCR using specific sense and antisense primers. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal control. The primer sequences used in this study are listed in Table 1. PCR conditions were as follows: pre-denaturation at 94 °C for 5 min; 30 cycles of denaturation at 94 °C for 30 s, annealing at 55 ~ 60 °C for 30 s, and extension at 72 °C for 1 min. Amplified PCR products were visualized using 1.2 % agarose gel electrophoresis and HiQ Blue Mangodye staining (BioD, Gyeonggi-do, Korea) under UV transillumination (Vilber Lourmat, Marine la Vallée, France).

2.8. Enzyme-Linked immunosorbent assay (ELISA)

To investigate cytokine production, KU812F and RBL-2H3 cells were pretreated with 0.01, 0.05, 0.10 and 0.05 μM of SHQA for 1 h and then stimulated with PMACI and com 48/80 under serum-free conditions. Cells were harvested and centrifuged, cytokine production in the supernatant was measured using human ELISA kits (Invitrogen, Thermo Fisher Scientific) and rat ELISA kits (BD OptEIA, BD Biosciences, CA, USA) both in accordance with the manufacturer's instructions using a microplate reader (Epoch) at 450 nm.

2.9. Animal

C57BL/6 and BALB/c mice were used for BMCMCs preparation and PCA induction, respectively. C57BL/6 (6–12 weeks old) and BALB/c (8 weeks old) mice reared under specific pathogen-free conditions, were obtained from Orient Bio (Gwangju, Korea). These mice were housed under conditions that included a constant temperature of 23 ± 1.5 °C, humidity of $55 \pm 15\%$, and lighting following a 12 h on/ 12 h off cycle. The mice were provided with food and tap water *ad libitum*. All procedures performed on these animals were approved by the Institutional Animal Care and Use Committee of Chonnam National University (No. CNU IACUC-YS-2021–5).

2.10. Preparation and stimulation of BMCMCs

Bone marrow cells were obtained from 6 to 12 weeks old male C57BL/6 mice according to the method described in our previous publication [31]. BMCMCs were sensitized with 1 $\mu\text{g}/\text{mL}$ of monoclonal DNP/IgE (Sigma) for 4 h and challenged with the synthetic allergen, 100 ng/mL of DNP-bovine serum albumin (BSA) (LSL Japan Inc., Tokyo, Japan) for 1 h.

2.11. Degranulation assay

To assess the inhibitory effects of SHQA on degranulation, the contents of β -hexosaminidase released into the medium were measured. To examine the content of β -hexosaminidase released into the medium, KU812F, RBL-2H3 cells were pretreated with SHQA for 24 h, washed with a Sinagarian buffer (119 mM NaCl, 5 mM KCl, 5.6 mM glucose, 0.4 mM MgCl_2 , 1 mM CaCl_2 , 25 mM HEPES (Sigma), and 0.1% bovine serum albumin (Sigma), pH 7.2), and then stimulated with PMACI and com 48/80 for 1 h. The pretreated BMCMCs were treated with anti-DNP/IgE for 4 h and challenged with DNP-BSA for 1 h. Then, the cells were lysed with 0.5% Triton 100 in Tyrode's buffer and the supernatant was used for the assay. The supernatant was reacted with 1 mM p-nitrophenyl-N-acetyl- β -D-glucosaminide (NP-GlcNAc) (Sigma) at 37 °C for 1 h. The enzyme reaction was then stopped by adding an equal volume of 0.1 M sodium carbonate buffer (pH 10.0). The absorbance was measured at 405 nm

Table 1

Primer sequences used in this study.

Primers	Forward	Reverse
For KU812F cells		
hIL-1 β	5'-ATGGCAGAAGTACCTGAGCTCGC-3'	5'-CACAAATTGCATGGTGAAGTCAGTT-3'
hIL-4	5'-ATGGGTCTCACCTCCCACTGCT-3'	5'-GTTTCCAACGTACTCTGGTTGGC-3'
hIL-6	5'-AACCTGTCCACTGGGCACA-3'	5'-TCTGGCTCTGAAACAAAGGA-3'
hIL-8	5'-CAAACCTTTCCACCCCAAT-3'	5'-ATTGCATCTGGCAACCCTAC-3'
hSOD3	5'-ACTCAGAGCGCAAGAAGC-3'	5'-TCAGCAAAGGCGAAGGTG-3'
hGAPDH	5-GCTCAGACACCATGGGGAAGGT-3	5-GTGGTGCAGGAGGCATTGCTGA-3
For RBL-2H3 cells		
rIL-4	5'-ACCCTGTTCTGCTTTCTC-3'	5'-TCATTACGGTGCAGCTTCT-3'
rTNF- α	5'-GAAAGCATGATCCGAGATGTTG-3'	5'-TCATACCAGGGCTTGAGCTCA-3'
rGAPDH	5'-AACGGCACAGTCAAGGCTGA-3'	5'-ACGCCAGTAGACTCCACGACAT-3'

using a microplate reader (Epoch).

2.12. PCA test

First, 500 ng of anti-DNP-IgE (Sigma) was injected intradermally into the dorsal skin of both ears of BALB/c mice. SHQA was applied to the ears of the mice for 2 h before the anaphylaxis was induced using a 1 mL syringe. The mice were then treated intravenously with 30 μ L of 10 mg DNP-BSA saline solution containing 4% Evans blue dye (Sigma) [32]. Control mice were treated with saline instead of SHQA. After 30 min, the mice were euthanized by anaesthesia with isoflurane followed by cervical dislocation, and skin tissues were collected from the dorsal ear and soaked in 1 mL of formamide (Sigma) overnight at 64 °C. The absorbance at 620 nm was measured to determine the amount of extravasated dye needed for the quantitative detection of vascular permeability.

2.13. Statistical analysis

Comparisons were made using one-way analysis of variance (ANOVA) and Duncan's multiple range tests using IBM SPSS Statistics

26. Values are reported as mean $\pm$ standard error (SE), and statistical significance was set at $p < 0.05$.

3. Results

3.1. Effects of SHQA on calcium influx and ROS production in PMACI-stimulated KU812F cells

Basophils and mast cells important roles as effector cells in allergic disorders [5,33,34]. KU812F cells, used in this study, were basophilic cells originally isolated from a human with chronic myelocytic leukemia. PMA and calcium ionophore, A23187 are potent inducers of allergic disorders. To investigate the non-toxic concentration of SHQA, its effect on the viability of human basophilic KU812F cells was assessed using an MTS assay. SHQA did not show any cytotoxic effects on PMA-stimulated KU812F cells at concentrations up to 0.50 μ M in comparison with control cells after treatment for 24 h (Fig. 2A). The activation of mast cells and basophils can increase the $[Ca^{2+}]_i$ concentration and ROS production. We evaluated the effects of SHQA on intracellular calcium and ROS levels in PMACI-induced KU812F cells using the specific probes

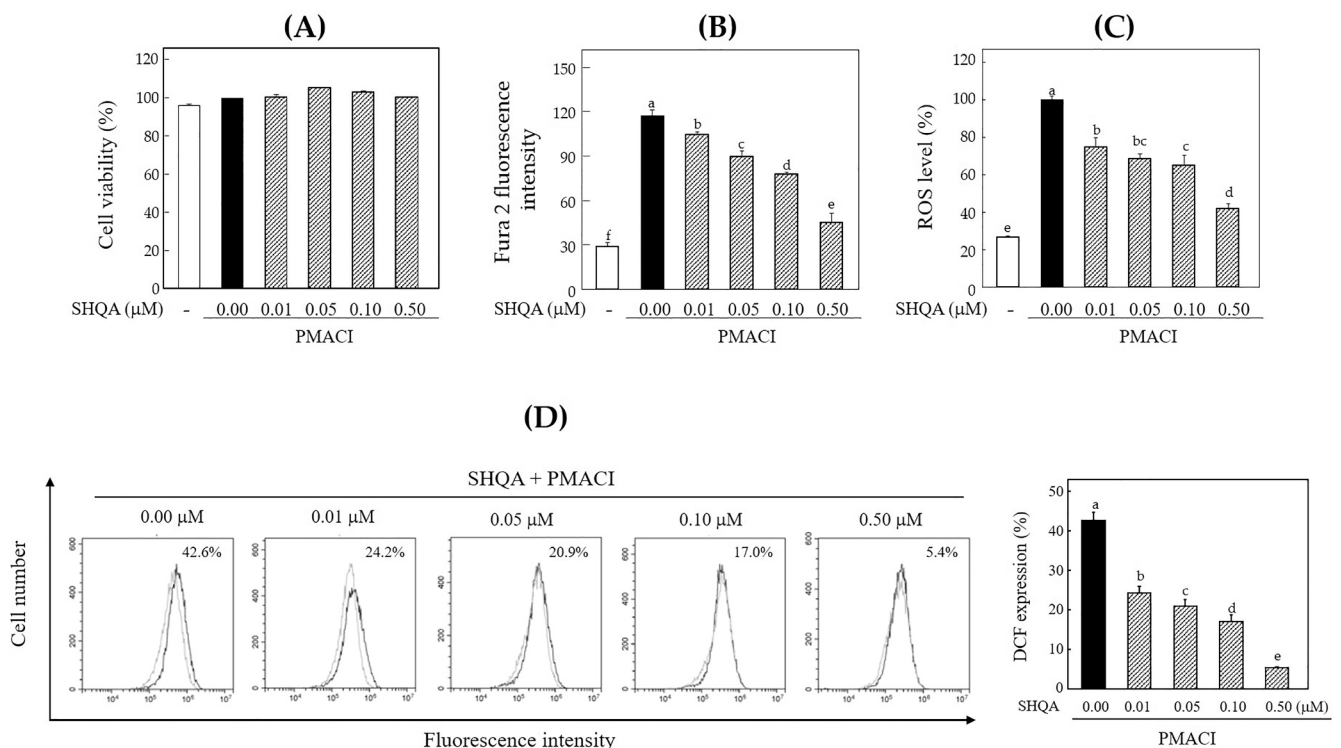


Fig. 2. Effects of SHQA on $[Ca^{2+}]_i$ level and ROS production in PMACI-stimulated KU812F cells. Cytotoxicity of SHQA (A), $[Ca^{2+}]_i$ level (B), intracellular ROS production (C), DCF expression (D) in PMACI-stimulated KU812F cells. Data are expressed as the means $\pm$ SD ($n = 3$) of three individual experiments. A value of p -value < 0.05 was considered statistically significant.

Fura 2-AM and DCFH-DA, respectively. SHQA pretreatment effectively inhibited PMACI-induced $[Ca^{2+}]_i$ levels (Fig. 2B) without causing cytotoxicity. DCF fluorescence was monitored using fluorescence microplate reader and flow cytometry. As shown in Fig. 2C, SHQA inhibited intracellular ROS production, as determined by fluorescence microplate reader, in a dose-dependent manner. Similarly, the intracellular DCF fluorescence intensity was lowered by pretreatment of SHQA, as determined by the flow cytometry analysis (Fig. 2D).

3.2. Effects of SHQA on MAPK and NF- κ B activation in PMACI-stimulated KU812F cells

PMACI can activate various transcriptional factors such as MAPKs (ERK, p38, and JNK) in basophils and mast cells [35]. We examined the effects of SHQA on transcriptional factors in PMACI-induced KU812F cells using western blot analysis and specific antibodies. The results showed that SHQA downregulated the activation of ERK 1/2, p38, and JNK (Fig. 3A) in a dose-dependent manner. NF- κ B is found in the cytoplasm in an inactive form, and is translocated to the nucleus by various stimulators such as PMACI, com 48/80 and anti-Fc ϵ RI antibody. SHQA downregulated NF- κ B activation in whole cell lysates, and NF- κ B translocation from the cytosol to the nucleus in PMACI-stimulated KU812F cells (Fig. 3B). Our results showed that SHQA downregulated the activation of NF- κ B, which is closely linked to the regulation of MAPK phosphorylation.

3.3. Effects of SHQA on production and gene expression of cytokine

As effector cells in allergic reactions, granulocytes such as basophils and mast cells, can release various cytokines involved in inflammatory responses [33,36,37]. We investigated the inhibitory effects of SHQA on

cytokine secretion in the media and gene expression of PMACI or com 48/80-stimulated cells using ELISA and RT-PCR, respectively. Stimulation of KU812F cells with PMACI induced remarkable increases in production (Fig. 4A) and gene expression (Fig. 4B) of IL-1 β , IL-4, IL-6, and IL-8. Com 48/80-stimulated RBL-2H3 cells increased production and gene expression of IL-4 and TNF- α . However, SHQA suppressed the production (Fig. 4C) and gene expression (Fig. 4D) of these cytokines in a dose-dependent manner. These results indicate that SHQA can efficiently inhibit PMACI-induced IL-1 β , IL-4, IL-6, and IL-8 production and com 48/80-induced IL-4 and TNF- α production, suggesting that SHQA can inhibit PMACI and com 48/80-induced basophil activation.

3.4. Effects of SHQA on degranulation

Activation of effector cells such as mast cells and basophils can release inflammatory mediators such as histamine, β -hexosaminidase, serotonin, prostaglandins, leukotrienes, which are essential for allergic reactions [29,34,37]. Among these inflammatory mediators, β -hexosaminidase is a potent marker of allergic disorders. To examine the inhibitory effects of SHQA on PMACI-, com 48/80-, and DNP-IgE-induced degranulation, media of pretreated and stimulated cells were used to examine β -hexosaminidase release. The results showed that SHQA inhibited the release of β -hexosaminidase in PMACI-induced KU812F (Fig. 5A) and com 48/80-induced RBL-2H3 (Fig. 5B) in a dose-dependent manner. Moreover, this compound inhibited the release of β -hexosaminidase in IgE/DNP-stimulated BMCMCs without cytotoxicity (Fig. 5C).

3.5. Effects of SHQA on DNP/IgE-Induced PCA in mice

To examine the *in vivo* antiallergic activity of SHQA, its effect on

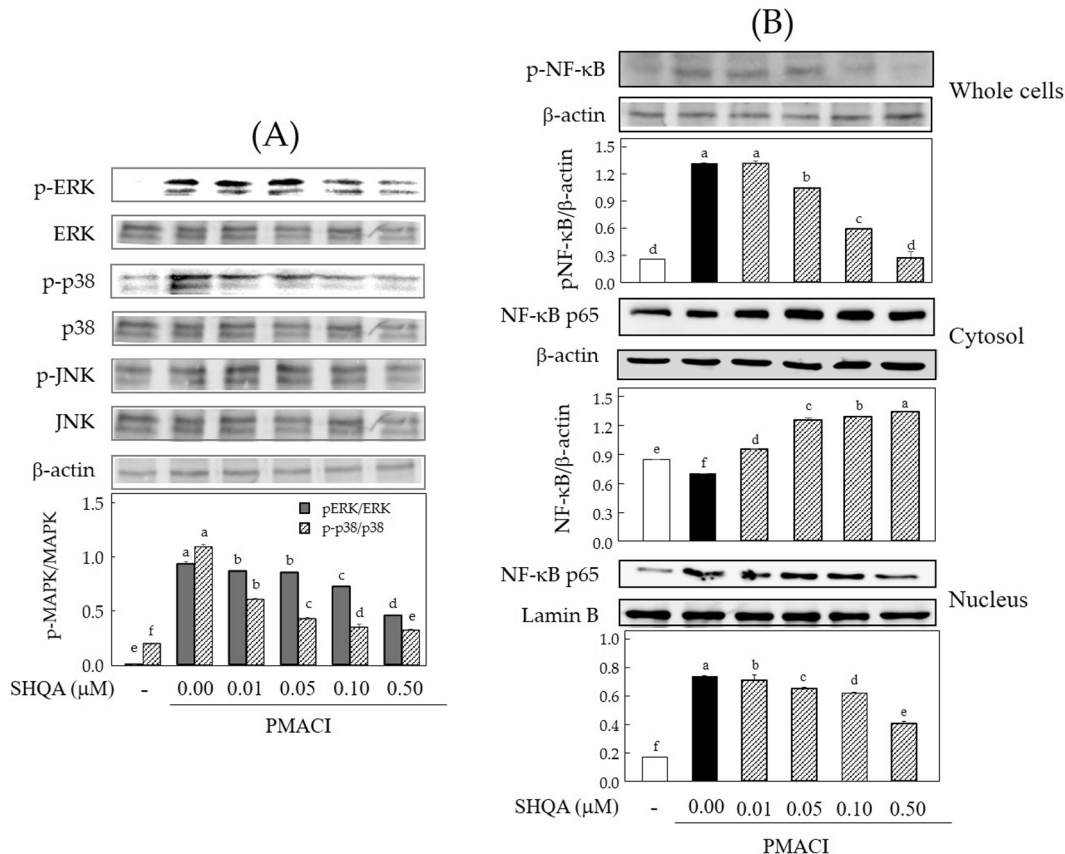


Fig. 3. Effects of SHQA on MAPK and NF- κ B activation in PMACI-stimulated KU812F cells. MAPK phosphorylation (A), NF- κ B activities (B) in PMACI-stimulated KU812F cells. Data are expressed as the means $\pm$ SD ($n = 3$) of three individual experiments. A value of p -value < 0.05 was considered statistically significant.

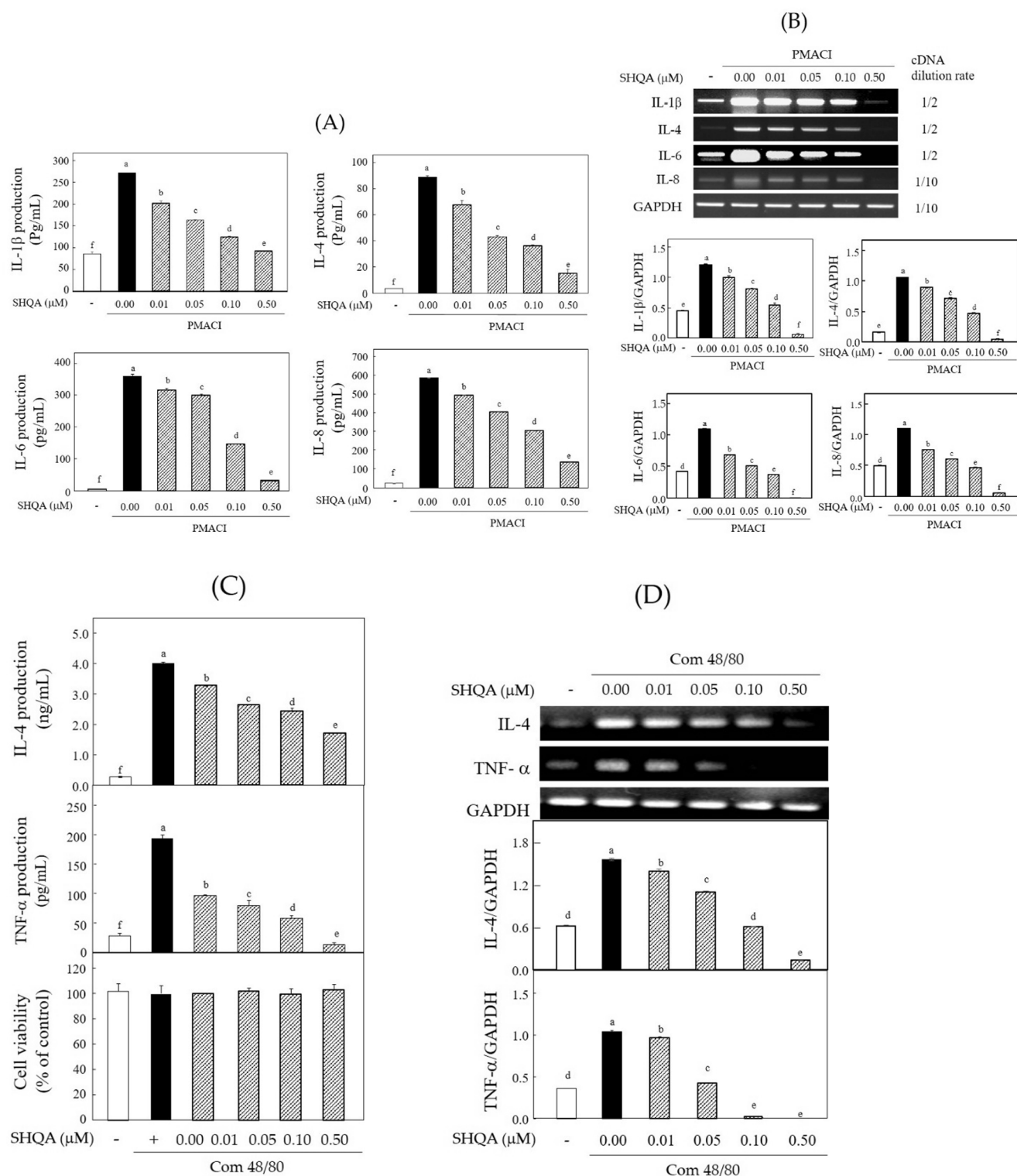


Fig. 4. Effects of SHQA on production and gene expression of cytokines in PMACI-stimulated KU812F and com 48/80-stimulated RBL-2H3 cells. Production of cytokines in PMACI-stimulated KU812F cells (A), mRNA levels of cytokines in PMACI-stimulated KU812F (B), Production of cytokines com48/80-stimulated RBL-2H3 cells (C), mRNA levels of cytokines in com48/80-stimulated RBL-2H3 cells (D). Data are expressed as the means $\pm$ SD ($n = 3$) of three individual experiments. A value of p -value < 0.05 was considered statistically significant.

DNP/IgE-induced PCA was assessed using a BALB/c mouse model. Evans blue dye enables the measurement of active, passive, and reverse PCA [23,28]. The ear subjected to anti-DNP-IgE stimulation should become dark blue 10 min after dye administration, unlike the ear that received the PBS control treatment. As expected and shown in Fig. 6A, DNP/IgE

stimulation increased the apparent blue color of the ear. However, in mice supplemented with SHQA, the intensity of the blue color decreased in a dose-dependent manner (Fig. 6A). Quantitative results for the extravasated dye from mouse skin tissues agreed with these qualitative observations (Fig. 6B).

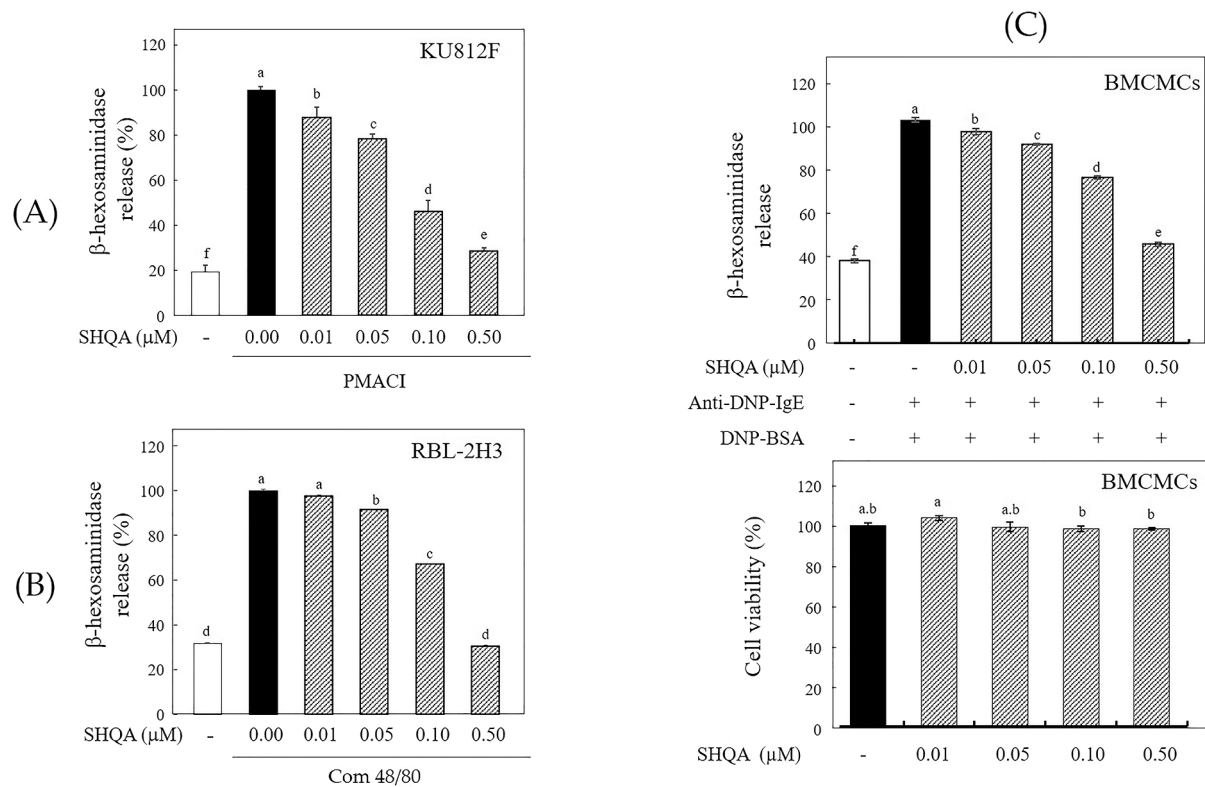


Fig. 5. Effect of SHQA on basophil degranulation. β -hexosaminidase release in PMACI-stimulated KU812F (A), β -hexosaminidase release in com 48/80-stimulated RBL-2H3 cells (B), β -hexosaminidase release in IgE/DNP-induced BMCMCs (C). Data are expressed as the means $\pm$ SD ($n = 3$) of three individual experiments. A value of p -value < 0.05 was considered statistically significant.

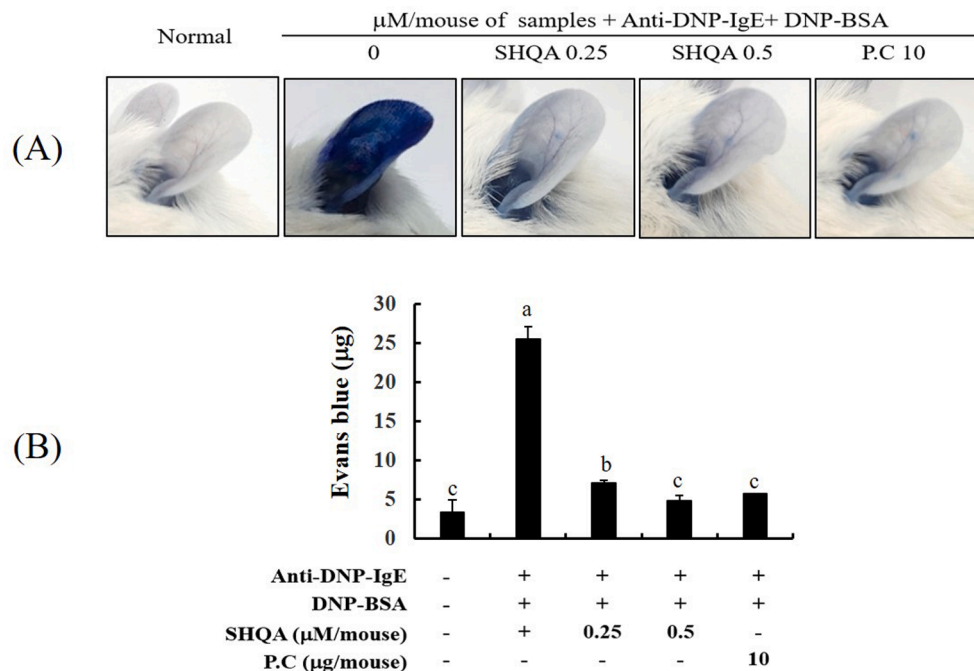


Fig. 6. Effect of SHQA on the IgE-mediated PCA reaction in mice. (A) Representative photographic images of ears, (B) Amount of extracted dye. Data are expressed as means $\pm$ SD ($n = 3$) of three individual experiments. Differences in mean values among groups were assessed by one-way analysis of variance followed by Duncan's test using PASW statistics 26.0. A value of $p < 0.05$ was considered statistically significant.

4. Discussion

Allergic diseases are rapidly increasing due to genetic and

immunological factors and various environmental factors such as fuel, cosmetics, pharmaceuticals, and food which can activate mast cells and basophils as multifunctional effector cells. These cells are granulocytes

that contain potent mediators such as histamine, β -hexosaminidase, and leukotrienes, which are involved in inflammation. Thus, they are commonly used as effector cells in allergy research. Basophils and mast cells can be activated by various stimuli such as immunoglobulin E, com 48/80, and PMACI. PMACI is known to activate immune cells via various pathways [2–4]. Recently, the search for biologically active antiallergic materials has garnered much attention. Among various materials, marine algae have been extensively studied as good sources of nutrients and functional materials for a wide range of food, supplement, pharmaceutical, and cosmetic industries. Sargassum, a genus of brown algae, contains approximately 400 species that have been used as food and in folk medicine in Korea and China for centuries. *S. serratifolium* is a marine brown alga that has been traditionally used to treat various diseases. It is known to contain high levels of meroterpenoids such as SHQA, sargaquinoic acid (SQA), and sargachromenol (SCM) with antioxidant activity [17–29]. Among these compounds, SHQA (Fig. 1) was evaluated for its inhibitory effects on cellular basophil activation and PCA reaction in mice. In response to various stimuli, effector cells can induce calcium influx and ROS generation. Our results showed that SHQA inhibited $[Ca^{2+}]_i$ levels (Fig. 2B) and ROS production (Fig. 2C, 2D) without showing any cytotoxicity (Fig. 2A) to PMACI-stimulated human basophilic KU812F cells. SHQA showed potent inhibition of calcium influx and ROS production. MAPK (ERK1/2, p38, JNK) plays a central role in regulating cellular signalling involving inflammation, allergic reactions, and apoptosis [7–11]. Phosphorylation of MAPKs such as ERK 1/2, p38, and JNK was down-regulated by SHQA in a dose-dependent manner (Fig. 3A). NF- κ B plays an important role in the pathophysiology of inflammatory diseases by regulating the expression of chemokines, cytokines, and cell adhesion molecules. It is regulated by the phosphorylation of cellular kinases such as MAPK in inflammatory responses. NF- κ B is a redox-sensitive transcription factor that functions in cytokine production [8,9,23,30] and is predominately found in the cytoplasm. NF- κ B signalling is redox mediated by the upregulation of adhesion molecules, chemokines, and inflammatory mediators. It is influenced by an imbalance between antioxidants and oxidants that occur in inflammatory diseases [8–11]. We observed an inhibitory effect of SHQA on the activation of p-NF- κ B and translocation of NF- κ B from the cytosol to the nucleus (Fig. 3B). Basophil and mast cell activation is related to various transcriptional factors that involve protein tyrosine kinases (Syk and Lyn) and the translocation of protein kinase C [35,38]. To better understand the inhibitory effects of SHQA on basophil and mast cell activation, more studies are needed on the regulation of its transcriptional signalling factors. Activation of NF- κ B and MAPK is essential for the upregulation of cytokines. IL-1 β , IL-6, and TNF- α are pro-inflammatory cytokines produced by activated effector cells upon stimulation by various stimulants such as PMACI, com 48/80, lipopolysaccharide, and Fc ϵ RI in many immune cells [5,6]. Moreover, IL-1 β can contribute to the development of various models in allergic diseases such as arthritis and skin inflammation [3,35,39]. IL-6 is considered a therapeutic target in certain autoimmune and inflammatory diseases [40]. IL-4 was the first cytokine proven to be made by activated basophils and mast cells. It plays an important role in stimulating type-2 immune responses and is produced after various immunological stimuli such as IL-1 and IL-6. IL-4 can maintain allergic responses along with IL-13, an important cytokine in type-2 immune responses. However, it is not induced in certain human mast cells [35,37,41]. IL-8, also known as CXCL8 and ‘neutrophil-attracting peptide’, is a chemokine involved in the recruitment of inflammatory cells. It is established that human IL-8 involved in recruiting neutrophils and basophils to sites of inflammation and is produced by multiple cell types including monocytes, endothelial cells, epithelial cells, fibroblasts, keratinocytes, synovial cells, and chondrocytes after stimulation with LPS, TNF, and IL-1 [42–44]. TNF- α is important for regulating immune cells and induces an inflammatory response with IL-1 β and IL-6 as pro-inflammatory cytokines. It can bind to human CXCR1 and CXCR2 chemokine receptors. This cytokine is

produced by activated human basophils, mast cells, and monocytes after IgE cross-linking. It plays an important role in IgE-mediated allergic inflammation [2,3,5,37]. Our ELISA results showed that SHQA inhibited the production (Fig. 4A) and mRNA levels (Fig. 4B) of IL-1 β , IL-4, IL-6, and IL-8 in PMACI-induced KU812F cells and the production (Fig. 4C) and mRNA levels (Fig. 4D) of IL-4 and TNF- α in com 48/80-induced RBL-2H3 cells. Basophils and mast cells can secrete a wide array of performed and *de novo* synthesized and performed inflammatory cytokines and immunomodulatory mediators that play central roles in the pathogenesis of allergic diseases and inflammation upon stimulation by allergens or other stimuli. The immune response is mediated by various cytokines, chemokines, and growth factors secreted by activated immune cells such as macrophages and monocytes [33,34,37]. More studies are needed on cytokines, chemokines, and growth factors upon activating effector cells such as basophils and mast cells. Basophils and mast cells can be activated by various stimuli, resulting in the release of contents from their secretory granules. Such content may include inflammatory mediators such as β -hexosaminidase, histamine, proteases, cytokines, and chemokines. The levels of inflammatory mediators secreted in the medium can be utilized as markers of degranulation of effector cells [1,2,33,34,37]. To examine the effects of SHQA on degranulation, levels of released β -hexosaminidase in PMACI-stimulated KU812F cells (Fig. 5A), com 48/80-stimulated RBL-2H3 cells (Fig. 5B), and IgE/DNP-stimulated BMCMCs (Fig. 5C) were determined as were the inhibitory effects of SHQA on these levels. Finally, we examined the mechanism by which SHQA suppresses IgE/DNP-mediated PCA in mice. PCA is characterized by an immediate skin reaction by a localized IgE-mediated allergic response *in vivo*, resulting in increased vascular leakage in the skin which can be assessed by an intravenous injection of Evans blue [27]. SHQA treatment reduced the allergic inflammatory responses in PCA-induced mice (Fig. 6). For the first time, the results of this study showed that SHQA could suppress cellular basophils activation using PMACI-induced human-derived basophils, KU812F cells, com 48/80-induced rat-derived basophils, RBL-2H3 cells, and IgE/DNP-induced mouse-derived BMCMCs and reaction of PCA in mice (Fig. 7). Further study of the protective action of SHQA in the cellular molecular mechanism mediated by Fc ϵ RI is necessary to confirm the potential of functional food materials that use *S. serratifolium*, which contains potent antioxidant, SHQA to treat allergic disorders.

5. Conclusion

The results of this study demonstrated that SHQA isolated from *S. serratifolium* exhibited inhibitory effects on basophil activation and PCA reaction in mice. Our results showed, for the first time, that SHQA could suppress basophil activation in KU812F cells following exposure to PMACI, including ROS generation and calcium influx, degranulation and cytokine production. Furthermore, these protective SHQA effects were related to the downregulation of ERK, p38, and NF- κ B phosphorylation and translocation. We confirmed that SHQA inhibited degranulation and cytokine production of com 48/80-induced RBL-2H3 cells and degranulation of IgE/DNP-induced BMCMCs. Moreover, in our experiment on IgE/DNP-induced PCA mice, SHQA exerted an antiallergic effect, which may be due to the downregulation of signaling factor and inflammatory mediators. Altogether, these results suggest that *S. serratifolium* containing SHQA, potent antioxidant compound, is a potentially effective functional food and pharmaceutical agent.

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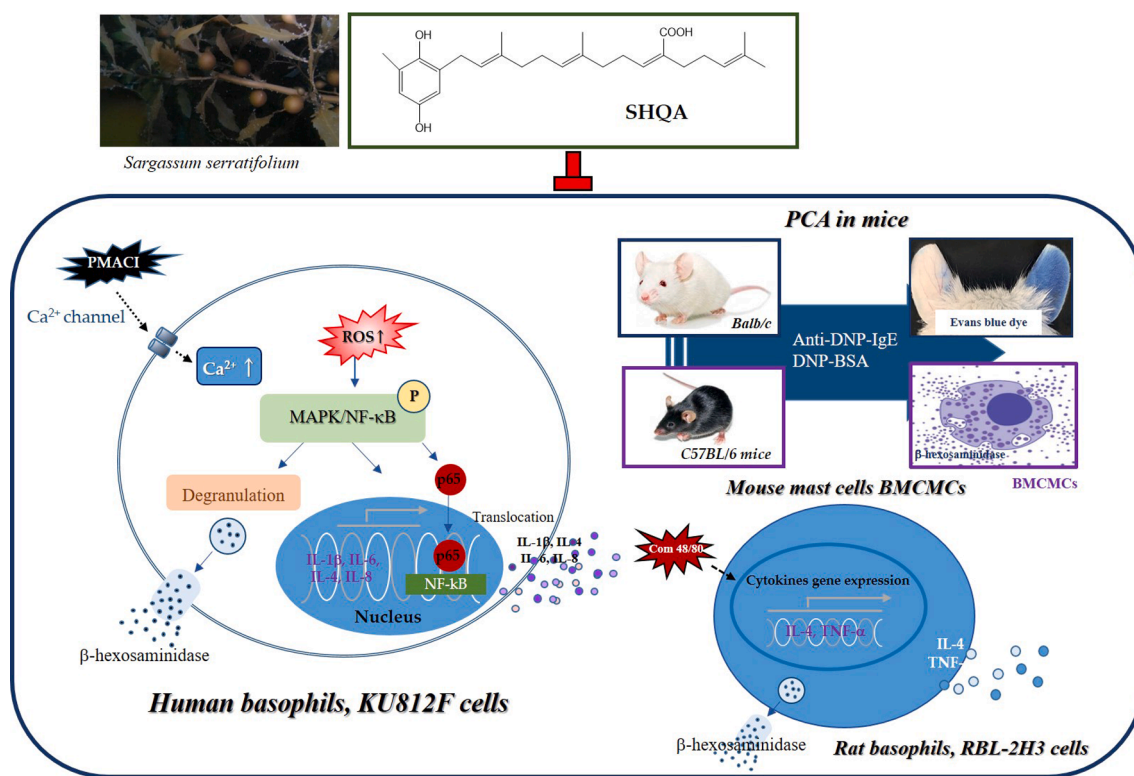


Fig. 7. Inhibitory mechanism of SHQA on cellular basophil activation and PCA in mice.

CRediT authorship contribution statement

Kap Seong Choi: Investigation, Writing – review & editing. **Taisun Shin:** Methodology. **Jiyeon Chun:** Writing – original draft. **Ginnae Ahn:** Investigation, Methodology. **Eui Jeong Han:** Investigation, Methodology. **Min-Jong Kim:** Investigation, Methodology. **Jung-Beom Kim:** Methodology. **Sang-Hyun Kim:** Investigation, Methodology. **Kang-Hee Kho:** Methodology. **Dae Heon Kim:** Methodology. **Sun-Yup Shim:** Writing – original draft, Writing – review & editing, Funding acquisition, Supervision, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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