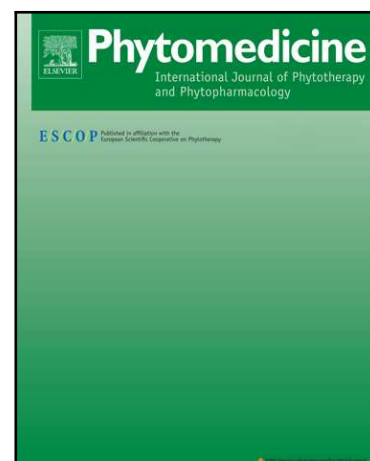


Accepted Manuscript

Anti-allergic actions of F-PASA, a novel herbal cocktail, in IgE/antigen-mediated allergic responses in RBL-2H3 cells and passive cutaneous anaphylaxis in mice

Jae-Myung Yoo , Kwang Il Park , Ju-Hye Yang , Won-Kyung Cho , Bohyoung Lee , Jin Yeul Ma

PII: S0944-7113(18)30222-8
DOI: <https://doi.org/10.1016/j.phymed.2018.06.034>
Reference: PHYMED 52555



To appear in: *Phytomedicine*

Received date: 19 April 2018
Revised date: 16 May 2018
Accepted date: 19 June 2018

Please cite this article as: Jae-Myung Yoo , Kwang Il Park , Ju-Hye Yang , Won-Kyung Cho , Bohyoung Lee , Jin Yeul Ma , Anti-allergic actions of F-PASA, a novel herbal cocktail, in IgE/antigen-mediated allergic responses in RBL-2H3 cells and passive cutaneous anaphylaxis in mice , *Phytomedicine* (2018), doi: <https://doi.org/10.1016/j.phymed.2018.06.034>

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Anti-allergic actions of F-PASA, a novel herbal cocktail, in IgE/antigen-mediated allergic responses in RBL-2H3 cells and passive cutaneous anaphylaxis in mice

Jae-Myung Yoo, Kwang Il Park*, Ju-Hye Yang, Won-Kyung Cho, Bohyoung Lee, Jin Yeul Ma*

Korean Medicine (KM)-Application Center, Korea Institute of Oriental Medicine (KIOM), 70 Cheomdan-ro, Dong-gu, Daegu 41062, Republic of Korea

* Corresponding authors

Kwang Il Park, KM-Application Center, KIOM, 70 Cheomdan-ro, Dong-gu, Daegu 41062, Republic of Korea

Tel.: +82-53-940-3877; fax: +82-53-940-3899.

E-mail address: kipark@kiom.re.kr (K.I. Park).

Jin Yeul Ma, KM-Application Center, KIOM, 70 Cheomdan-ro, Dong-gu, Daegu 41062, Republic of Korea

Tel.: +82-53-940-3810; fax: +82-53-940-3899.

E-mail address: jyma@kiom.re.kr (J.Y. Ma).

ABSTRACT

Background: The anti-inflammatory actions of *Polygonum cuspidatum*, *Angelica gigas*, *Sophora flavescens* and *Arctium fruit* are well known. Nonetheless, effects of herbal combination (PASA) or its fermentation by microorganisms (F-PASA) on the allergic response remain unknown.

Purpose: We investigated whether PASA or F-PASA could inhibit IgE/antigen complex (IgE/Ag)-mediated allergic responses.

Methods: To evaluate and compare anti-allergic actions of PASA and F-PASA, we performed cell viability, β -hexosaminidase activity, ELISA assays for cytokines and eicosanoids, immunoblot analysis, HPLC analysis and passive cutaneous anaphylaxis (PCA) models.

Results: F-PASA had stronger anti-degranulation actions (IC_{50} , 510.9 μ g/ml) than PASA (IC_{50} , 1,261 μ g/ml) without cytotoxicity until 2,000 μ g/ml in IgE/Ag-activated RBL-2H3 cells. Additionally, F-PASA inhibited formation of tumor necrosis factor- α (IC_{50} , 147.4 μ g/ml), interleukin-4 (IC_{50} , 213.4 μ g/ml), prostaglandin D₂ (IC_{50} , 42.40 μ g/ml) and leukotriene C₄ (IC_{50} , 157.9 μ g/ml). Moreover, F-PASA dose-dependently inhibited the phosphorylation and expression of proteins that are related to the Fc ϵ RI and arachidonate cascades. Consistent with *in vitro* studies, F-PASA from 25 to 100 mg/kg also suppressed IgE/Ag-induced PCA reaction more than PASA did in mice. In phytochemical analysis, using PASA and F-PASA, F-PASA showed a higher level of emodin-8-O- β -D-glucoside, whereas the level of arctiin, an artigenin glycoside, was reduced compared with that using PASA.

Conclusion: These findings indicate that F-PASA, including both artigenin and emodin-8-O- β -D-glucoside, possesses stronger anti-allergic properties. Therefore, F-PASA may be useful as a functional food or as a phytomedicine for allergic diseases.

Key Words: allergic response; Fc ϵ RI; F-PASA; inflammatory mediators; mast cells; passive cutaneous anaphylaxis

List of abbreviations

5-LO: 5-lipoxygenase

Akt: protein kinase B

COX-2: cyclooxygenase-2

cPLA₂: cytosolic phospholipase A₂

ERK: extracellular signal-regulated kinase 1/2

Fyn: feline yes-related protein

HSA: human serum albumin

IgE: Immunoglobulin E

IgE/Ag: IgE-antigen complex

IL-4: interleukin-4

JNK: c-Jun *N*-terminal kinase 1/2

LTC₄: leukotriene C₄

LTC₄S: leukotriene C₄ synthase

Lyn: Src family protein kinase

L-PGDS: lipocalin-type prostaglandin D synthase

F-PASA: PASA fermented by *L. rhamnosus*

PASA: aqueous extract of four medicinal plants

PCA, passive cutaneous anaphylaxis

PGD₂: prostaglandin D₂

PKCδ: protein kinase Cδ

PLCγ: phospholipase Cγ

Syk: spleen tyrosine kinase

TNF-α: tumor necrosis factor-α

1. Introduction

Allergic diseases such as allergic rhinitis, asthma and atopic dermatitis are known as obstinate chronic diseases, and patients with allergic diseases are widely distributed across all ages worldwide. The generation and progression of allergic diseases are correlated with anaphylactic shock. The anaphylactic shock, a type 1 allergic response class (Uzzaman and Cho, 2012), is particularly important clinically because it aggravates a patient's condition in a matter of minutes. Such anaphylactic shock is associated with the degranulation of immunoglobulin E/antigen complex (IgE/Ag)-stimulated mast cells (Gilfillan and Tkaczyk, 2006). When IgE-sensitized mast cells are activated by antigens, the cells rapidly liberate various inflammatory mediators, including histamine, cytokines, leukotrienes and prostaglandins (Church and Levi-Schaffer, 1997; Metcalfe et al., 1981). Consequently, the inflammatory mediators induce an acute inflammatory response in a wide region of tissues and organs (Theoharides and Kalogeromitros, 2006; Wedemeyer et al., 2000). Therefore, regulating the activation of IgE-sensitized mast cells is an important key point for the treatment or the prevention of allergic diseases.

The combination of some herbs has been reported to have synergistic effects in various biological events such as anti-cancer, anti-diabetes and anti-inflammation (Adams et al., 2006; Gao et al., 2009; Yang et al., 2014; Zheng et al., 2012). In addition, the fermentation process of herbal medicines by microorganisms produces their metabolites and/or converts glycosylated compounds into aglycones, which are more active forms than glycosides (Yoo et al., 2016). Indeed, the fermentation process improves the beneficial effects of herbal medicines on various diseases such as allergy, arteriosclerosis, cancer and diabetes (Kim et al., 2015; Lee et al., 2015; Lee et al.,

2014b; Yoo et al., 2016). Recently, our research group found that KIOM-MA containing *Polygonum cuspidatum*, *Angelica gigas*, *Sophora flavescens* and *Arctium fruit*, which possess anti-inflammatory actions in various tissues and organs (Bralley et al., 2008; Choi et al., 2000; Han and Wang, 2012; Knott et al., 2008; Lee et al., 2014a; Lee et al., 2012; Shin et al., 2010; Zhang et al., 2012; Zhang et al., 2015), and its fermentation, KIOM-MA128, exerted anti-allergic actions, such as anti-asthma and anti-atopic dermatitis (Chung et al., 2012; Kim et al., 2012; Park et al., 2016). However, the industrialization and quality control of KIOM-MA128 are very difficult because it is composed of 18 species of medicinal herbs. Therefore, we were interested in the optimization of KIOM-MA128 by the selection of some herbs having strong anti-allergic activity among the components of KIOM-MA128. Subsequently, we selected *Polygonum cuspidatum*, *Angelica gigas*, *Sophora flavescens* and *Arctium fruit* using IgE/Ag-activated RBL-2H3 cells. Furthermore, the effects of their combination (PASA) and its fermented form (F-PASA) on IgE/Ag-induced allergic reactions are still unknown.

In this study, we first found that PASA possessed an anti-allergic effect on the IgE/Ag-mediated allergic reaction in RBL-2H3 cells. Furthermore, we hypothesized that the fermentation of PASA by *Lactobacillus rhamnosus* (*L. rhamnosus*) may enhance the anti-allergic action of PASA, and then examined the effects of F-PASA on IgE/Ag-mediated allergic responses in both *in vitro* and *in vivo* studies. To examine the anti-allergic roles of F-PASA in *in vitro* studies, degranulation was measured by β -hexosaminidase activity, a degranulation biomarker, and the amounts of proinflammatory mediators, such as interleukin-4 (IL-4), tumor necrosis factor- α (TNF- α), leukotriene C₄ (LTC₄) and prostaglandin D₂ (PGD₂), were measured using enzyme-

linked immunosorbent assay (ELISA) and enzyme immunoassay (EIA) kits. To investigate cellular signaling transduction for the FcεRI cascade, the expression or the phosphorylation of FcεRI cascade-related proteins were analyzed using immunoblot analysis. To compare and identify active phytochemicals for the anti-allergic properties of PASA and F-PASA, both extracts were analyzed using a high-performance liquid chromatography (HPLC) system. Finally, to demonstrate the anti-allergic effects of F-PASA on the *in vivo* system, the effects of PASA and F-PASA were evaluated using a passive cutaneous anaphylaxis (PCA) model, which is generally used as an allergic animal model (Inagaki et al., 1986). Herein, we report that F-PASA possesses anti-allergic activities by inhibiting the activation of both FcεRI and eicosanoid cascades in IgE/Ag-stimulated mast cells and demonstrated such effects of F-PASA on the PCA reaction in IgE/Ag-exposed mice. The effects of F-PASA may offer further information for the application of herbal medicine in patients with allergic diseases.

2. Materials and Methods

2.1. Reagents

MEM- α medium, penicillin, streptomycin, fetal bovine serum (FBS) and 1 $\times$ DPBS were obtained from GE Healthcare Life Sciences (HyCloneTM, Logan, UT, USA). A specific antibody against phospho-feline yes-related protein (Fyn; orb128087) was procured from Biorbyt Ltd. (Cambridge, UK). Specific antibodies against phospho-protein kinase B (Akt; #9271), phospho-cytosolic phospholipase A₂ (cPLA₂; #2831), phospho-extracellular signal-regulated kinase 1/2 (ERK; #9101), phospho-c-Jun N-terminal kinase 1/2 (JNK; #9251), phospho-Src family protein kinase (Lyn; #2731), phospho-p38 (#9211), phospho-protein kinase C δ (PKC δ ; #2055), phospho-phospholipase C γ 1/2 (PLC γ 1/2; #2821 and #3871, respectively), phospho-spleen tyrosine kinase (Syk; #2710) and cyclooxygenase-2 (COX-2; #4842) were purchased from Cell Signaling Technology, Inc. (Beverly, MA, USA). Specific antibodies against leukotriene C₄ synthase (LTC₄S; sc-22564-R) and β -actin (sc-47778) were obtained from Santa Cruz Biotechnology, Inc. (Dallas, TX, USA). Specific antibodies against lipocalin-type prostaglandin D synthase (L-PGDS; 160003) and phospho-5-lipoxygenase (5-LO; 10007820), and EIA kits for LTC₄ and PGD₂ were purchased from Cayman Chemical Co. (Ann Arbor, MI, USA). Arctiin, decursin, decursinol, emodin-8-O- β -D-glucoside, matairesinol and nodakenin were purchased from ChemFaces Co., Ltd. (Wuhan, China). ELISA kits for IL-4 and TNF- α were obtained from e-Bioscience, Inc. (Science Center Drive, San Diego, CA, USA). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), 4-Nitrophenyl *N*-acetyl- β -D-glucosaminide (p-NAG), dinitrophenyl-human serum albumin (DNP-HSA), DNP-IgE, and dexamethasone were purchased from Sigma-Aldrich Co. (St Louis, MO, USA). All

other chemicals were of analytical grade.

2.2. Preparation of extracts of PASA and F-PASA

PASA and F-PASA were prepared following a process reported previously (Yoo et al., 2016). The PASA mixture (1 kg) including *Polygonum cuspidatum*, *Angelica gigas*, *Sophora flavescens* and *Arctium fruit* (1, 1, 1, 1; w/w/w/w) were boiled in distilled water (10 liter) for approximately 3 h at 115°C. The aqueous extract was inoculated with *L. rhamnosus* ($1 \times 10^5 - 10^7$ CFU/ml; KFRI 128, KCTC 2182), after it had been adjusted to pH 7.0 with 1N NaOH and was autoclaved for 5 min, and then incubated for 48 h at 37°C. F-PASA was sifted through a nylon net filter (Aperture 60 µm; Millipore Co., Denver, MA, USA) and was then left. The supernatant was lyophilized, and then the dried pellet was kept in -20°C until use. The yields of PASA and F-PASA are 14.42 and 14.10%, respectively. PASA and F-PASA were dissolved in 10% dimethyl sulfoxide (DMSO) solution for *in vitro* studies.

2.3. High-performance liquid chromatography analysis

HPLC analysis was performed according to a modified process reported previously (Chung et al., 2012). To analyze the major compounds in PASA and F-PASA, HPLC analysis was performed using an Elite lachrom analytical HPLC PDA system equipped with an HPLC pump (L-2130), an autosampler (L-2200), a column oven (L-2350), a diode array UV/Vis detector (L-2455) and EZchrom Elite software (Hitachi High Technologies America, Inc., San Jose, CA, USA) with an OptimaPak C₁₈ column (4.6 mm × 250 mm, 5 µm; RS tech Co., Daejeon, Korea). The anti-allergic phytochemicals were eluted in a gradient system composed of solvent A (0.2% acetic acid in deionized

water) and solvent B (0.2% acetic acid in acetonitrile). The gradient was 10-50-10-10% of solvent B at the gradient time $t_G = 0-60-61-71$ min, respectively. The temperature of column oven was 40°C, the flow rate was 1.0 ml/min, and an injection volume of 5 μ l was applied. The UV/Vis detector was set at the wavelength range of 280 nm. The standards of arctiin, decursin, decursinol, emodin-8-*O*- β -D-glucoside, matairesinol and nodakenin, as well as the sample solutions (2.5 mg/ml), were dissolved and diluted in methanol. The relative standard deviation of the measured concentrations was used to assess precision. A comparison of the mean measured concentration versus the corresponding nominal concentration was used to assess accuracy.

2.4. Animals

ICR mice, Swiss CD-1 mice (Schwartz et al., 1974) (male, 5 weeks and 25–30 g), were purchased from SAMTAKO BIO KOREA Inc. (Osan, Korea) and were housed in cages (5 mice per cage) under specific pathogen-free conditions (21–24°C and 40–60% relative humidity) with a 12-h light/dark cycle and were given free access to standard rodent food (Orientbio Inc., Sungnam, Korea) and water. All animal experiments were approved by the Animal Care and Use Committee of the KIOM (Daejeon, Korea) with a reference number (#D-16-001). Experiments were carried out following the guidelines of the Animal Care and Use Committee at KIOM.

2.5. Passive cutaneous anaphylaxis

IgE/Ag-mediated PCA response was performed following the method reported previously (Yoo et al., 2014b). ICR mice were subcutaneously injected through the ears with anti-DNP-IgE (100 ng) diluted in 1× PBS using an insulin syringe. The next day,

IgE-sensitized mice were orally administered PASA or F-PASA (0–100 mg/kg), and then, 1 h later, they were intravenously injected with 100 µg of DNP-HSA in 1× PBS including 0.5% Evans blue. After 30 min, the mice were sacrificed by CO₂ gas and the ears were harvested and incubated with 1 ml of formamide for 2 h at 80°C. The extract was centrifuged for 10 min (17,000 ×*g*, 4°C). The absorbance at 620 nm was measured using a microplate reader (SpectraMax i3; Molecular Devices, Sunnyvale, CA, USA).

2.6. Cell culture

RBL-2H3 cells, a rat mast cell line (Siraganian et al., 1982), were purchased from the Korean Cell Line Bank (Seoul, Korea). The cells were cultured in MEM-α medium containing 10% (v/v) FBS and antibiotics at 37°C in a humidified atmosphere of 5% CO₂. All of the experiments used a vehicle control group containing 0.1% DMSO.

2.7. Cell viability assay

Cell viability was evaluated according to a modification of the method reported previously (Song et al., 2015). IgE-sensitized RBL-2H3 cells were preincubated with PASA or F-PASA (0–2,000 µg/ml) in MEM-α medium containing 1% FBS for 1 h and then were stimulated by 0.1 µg/ml DNP-HSA for 2 h. The above cells were further incubated with 200 µl of culture media containing 500 µg/ml MTT reagent for 2 h. The supernatant was aspirated, 100 µl of DMSO was added to each well, and then the 96-well plate was incubated for 15 min. The absorbance at 570 nm was measured using a microplate reader.

2.8. β-Hexosaminidase activity assay

β -Hexosaminidase activity assay was performed following the previous method (Yoo et al., 2014a). IgE-sensitized RBL-2H3 cells were preincubated with PASA or F-PASA (0–2,000 μ g/ml) for 1 h before DNP-HSA challenge. After 4 h, the supernatant (25 μ l) was mixed with 50 μ l of p-NAG (10 mM) in 0.1 M sodium citrate buffer (pH 4.5) and then incubated for 1 h at 37°C. The enzyme reaction was terminated by 0.1 M sodium carbonate buffer (pH 10.0). The absorbance at 405 nm was measured using a microplate reader.

2.9. Enzyme-linked immunosorbent assay for IL-4 and TNF- α

To measure the levels of IL-4 and TNF- α in cultured media, all the cultured media were centrifuged (17,000 $\times g$ at 4°C) for 10 min and then kept in -80°C until use. IL-4 and TNF- α were determined using ELISA kits following the manufacturer's instructions.

2.10. Enzyme immunoassay analysis for LTC₄ and PGD₂

To determine the amounts of LTC₄ and PGD₂ in cultured media, all cultured media were centrifuged and stored at -80°C until use. LTC₄ and PGD₂ were measured using EIA kits according to the manufacturer's instructions.

2.11. Immunoblotting analysis

Immunoblotting analysis was performed according to the method reported previously (Yoo et al., 2016). PVDF membranes containing blotted proteins were visualized using a chemiluminescent reaction (ECL Plus kit, Bio-Rad, Hercules, CA, USA) and an imaging system (ChemiDoc Touch Imaging System, Bio-Rad, Hercules, CA, USA). The levels of target proteins were compared with the loading control proteins (β -actin)

and were measured using ImageJ software (version 1.50i for Windows; NIH, Bethesda, MD, USA). The results were expressed as a density ratio of each identified protein using a protein standard-size marker (BIOFACT, Daejeon, Korea).

2.12. Statistical analyses

The experimental results were reported as the mean $\pm$ SD and SEM. One-way analysis of variance (ANOVA) was used for multiple comparisons (GraphPad Prism version 5.03 for Windows, San Diego, CA, USA). The Dunnett test was applied for significant variations between the DNP-HSA-treated groups. Differences at the $*P<0.05$ and $**P<0.01$ levels were considered statistically significant.

3. Results

3.1. Inhibitory effects of PASA and F-PASA on the degranulation of IgE/Ag-mediated RBL-2H3 cells: comparison between PASA and F-PASA

To investigate the effects of PASA and F-PASA on the IgE/Ag-mediated allergic response in mast cells, IgE-sensitized RBL-2H3 cells were preincubated with various concentrations of PASA or F-PASA before antigen treatment (0.1 $\mu\text{g/ml}$ DNP-HSA). F-PASA (IC_{50} value, 510.9 $\mu\text{g/ml}$) showed stronger anti-degranulation action than PASA (IC_{50} value, 1,261 $\mu\text{g/ml}$), although both PASA and F-PASA dose-dependently inhibited degranulation in IgE/Ag-activated RBL-2H3 cells (Fig. 1a and 1b). In addition, PASA and F-PASA possessed no significant cytotoxicity at concentrations showing anti-degranulation action (Fig. 1c and 1d). These results indicated that F-PASA has a stronger anti-allergic action than PASA at non-cytotoxic concentrations. Therefore, the fermentation process for the above herbal medicine improves its inhibitory action on degranulation of IgE/Ag-activated mast cells.

3.2. Inhibitory effects of F-PASA on the formation of proinflammatory mediators

Because we showed more potent anti-degranulation action of F-PASA than of PASA, we further investigated the effects of F-PASA on the biosynthesis of proinflammatory mediators, such as cytokines, leukotrienes and prostaglandins, in IgE/Ag-activated mast cells. The proinflammatory mediators concerned with the initiation and/or progression of allergic diseases are produced and liberated from the above cells (Gilfillan and Tkaczyk, 2006). As shown in Fig. 2, F-PASA significantly reduced the production of $\text{TNF-}\alpha$ (IC_{50} value, 147.4 $\mu\text{g/ml}$), IL-4 (IC_{50} value, 213.4 $\mu\text{g/ml}$), PGD_2 (IC_{50} value, 42.40 $\mu\text{g/ml}$) and LTC_4 (IC_{50} value, 157.9 $\mu\text{g/ml}$). Taken together, these results

suggested that F-PASA can inhibit allergic inflammatory responses by suppressing the formation of proinflammatory cytokines and eicosanoids in IgE/Ag-activated mast cells. As a result, F-PASA may inhibit the initiation and progression of allergic diseases.

3.3. Regulatory effects of F-PASA on the activation of FcεRI and eicosanoid cascades

We further investigated the actions of F-PASA on the activation of FcεRI and eicosanoid cascades, after F-PASA reduced the formation of proinflammatory mediators such as IL-4, TNF-α, LTC₄ and PGD₂ in IgE/Ag-activated mast cells. The reason for this is that the formation of the proinflammatory mediators is implicated in the activation of the FcεRI signaling cascade (Gilfillan and Tkaczyk, 2006). In late phase (4 h), F-PASA significantly inhibited the phosphorylation of 5-LO and the expression of both L-PGDS and LTC₄S (Fig. 3). Meanwhile, it partially suppressed the phosphorylation of cPLA₂ and the expression of COX-2 (Fig. 3). In early phase (10 min), F-PASA significantly reduced the phosphorylation of Lyn, Syk (Fig. 4a), PLCγ1/2, PKCδ (Fig. 4b), JNK, ERK, p38 and Akt (Fig. 4c) but not that of Fyn (Fig. 4a). These findings suggested that F-PASA can regulate the activation of FcεRI signaling cascades by inhibiting Lyn, Syk, cPLA₂, COX-2, 5-LO, L-PGDS and LTC₄S in IgE/Ag-activated mast cells.

3.4. Inhibitory effects of PASA and F-PASA on the IgE/antigen-mediated PCA reaction in mice: comparison between PASA and F-PASA

We were interested in the anti-allergic action of F-PASA in the *in vivo* system because we revealed the anti-allergic mechanisms of F-PASA in IgE/Ag-activated mast

cells. Thus, we examined the anti-allergic actions of PASA and F-PASA in the PCA allergic animal model using mice (Inagaki et al., 1986). As shown in Fig. 5, when IgE-sensitized ICR mice were orally administered PASA or F-PASA at various concentrations (0–100 mg/kg) for 1 h prior to antigen challenge, PASA dose-dependently reduced the IgE/Ag-mediated PCA reaction in mice (Fig. 5a). Interestingly, F-PASA from 25 mg/kg suppressed IgE/Ag-mediated PCA reaction more than PASA did at a similar level of dexamethasone (Fig. 5b). This result indicated that F-PASA has stronger anti-allergic efficiency than PASA in the *in vivo* system. Therefore, it suggested that F-PASA has potentiality as an herbal medicine for the treatment of allergic diseases.

3.5. Identification of active compounds in F-PASA: comparison between PASA and F-PASA

To substantiate what is responsible for the anti-allergic actions of F-PASA, we analyzed and compared the phytochemical composition of PASA and F-PASA using an HPLC system. Arctiin, decursin, decursinol, emodin-8-*O*- β -D-glucoside, matairesinol and nodakenin are major compounds in *Polygonum cuspidatum* (Chu et al., 2005), *Angelica gigas* (Ahn et al., 2008), *Sophora flavescens* (Liu et al., 2011) and *Arctium fruit* (Yoo et al., 2016). Based on previous reports, we analyzed the amounts of phytochemicals in PASA and F-PASA. The retention times for the peaks of the nodakenin (1), decursinol (2), arctiin (3), matairesinol (4), emodin-8-*O*- β -D-glucoside (5), arctigenin (6) and decursin (7) standard were 16.63, 21.89, 24.62, 27.52, 28.02, 33.42 and 51.13 min on the HPLC chromatogram, respectively (Fig. 6a). The major peaks were arctiin and emodin-8-*O*- β -D-glucoside, although the peaks of other

compounds were determined on the HPLC chromatograms of PASA (Fig. 6b) and F-PASA (Fig. 6c). Thus, we further quantified the amounts of arctiin and emodin-8-*O*- β -*D*-glucoside in PASA and F-PASA. Table 1 summarizes the amounts of arctiin and emodin-8-*O*- β -*D*-glucoside in PASA and F-PASA. These results suggested that the fermentation process by *L. rhamnosus* elevates the levels of arctigenin and emodin-8-*O*- β -*D*-glucoside in F-PASA. Taken together, our findings indicated that the anti-allergic actions of F-PASA are associated with an increase in bioactive phytochemicals, such as arctigenin and emodin-8-*O*- β -*D*-glucoside, although some major unknown peaks remain. Therefore, F-PASA possesses the potential for use as a herbal medicine for the treatment or prevention of allergic diseases.

4. Discussion

Polygonum cuspidatum (Bralley et al., 2008; Zhang et al., 2012), *Angelica gigas* (Choi et al., 2000; Shin et al., 2010; Zhang et al., 2015), *Sophora flavescens* (Han and Wang, 2012; Lee et al., 2014a) and *Arctium fruit* (Knott et al., 2008; Lee et al., 2012) are commonly used components of traditional medicine in Northeast Asia and have various beneficial properties, including anti-cancer, anti-inflammation, anti-microbial activity, and neuroprotection (Chan et al., 2011; He et al., 2015; Sarker and Nahar, 2004; Zhang et al., 2013). Additionally, our research group recently found that KIOM-MA128 containing the above four herbs exerted anti-allergic actions such as anti-asthma and anti-atopic dermatitis (Chung et al., 2012; Kim et al., 2012; Yoo et al., 2016). The optimization of KIOM-MA128 is necessary because the industrialization and quality control of KIOM-MA128 as the herbal medicine are very difficult. Additionally, the fermentation process enhances the beneficial effects of herbal medicines (Kim et al., 2015; Lee et al., 2015; Lee et al., 2014b; Yoo et al., 2016). Nonetheless, the effects of PASA and F-PASA on IgE/Ag-mediated allergic responses were not reported.

In this study, we observed that PASA exerted anti-allergic actions on IgE/Ag-activated mast cells. Furthermore, we hypothesized that F-PASA, the fermented form of PASA, may possess more potent anti-allergic properties than PASA, and we demonstrated stronger anti-allergic actions of F-PASA than of PASA in both *in vitro* and *in vivo* studies. In addition, we found that F-PASA had stronger anti-allergic effects than KIOM-MA128 (data not shown). Regarding the mechanisms responsible for the anti-allergic properties of F-PASA, one possible mechanism is related to the direct suppression of the FcεRI signaling cascade (Gilfillan and Tkaczyk, 2006). IgE/Ag-activated mast cells activate the degranulation process and production of

proinflammatory mediators by activating the FcεRI signaling cascade because IgE-sensitized mast cells are exposed to various antigens (Gilfillan and Tkaczyk, 2006). Moreover, the activation of Lyn or Syk as rate-limiting intermediate proteins in the FcεRI signaling cascade is closely correlated with the degranulation and formation of proinflammatory mediators in the cells (Gilfillan and Tkaczyk, 2006). Therefore, the regulation of Lyn or Syk activation is an important event for the anti-allergic actions of F-PASA. In support of this, F-PASA not only suppressed the phosphorylation of Lyn and Syk but also inhibited the phosphorylation of PLCγ1/2 and PKCδ, correlated with degranulation (Gilfillan and Tkaczyk, 2006), as well as that of Akt, ERK, JNK and p38, related to the formation of proinflammatory cytokines such as IL-4 and TNF-α (Jiang et al., 1998). Therefore, Lyn and/or Syk are important targets for the anti-allergic roles of F-PASA.

Another possible mechanism for the anti-allergic roles of F-PASA may be correlated with suppressing the activation of eicosanoid pathways in IgE/Ag-activated mast cells. IgE-sensitized mast cells stimulated by antigens can produce numerous proinflammatory eicosanoids, such as PGD₂ and LTC₄, and can then release them (Theoharides and Kalogeromitros, 2006). PGD₂ and LTC₄ in particular lead to chronic inflammation in allergic respiratory diseases such as allergic rhinitis and asthma (Theoharides and Kalogeromitros, 2006). Therefore, regulating the activation of the eicosanoid pathway in IgE/Ag-activated mast cells is another important event for the anti-allergic actions of F-PASA. In support of this, F-PASA not only reduced the formation of both PGD₂ and LTC₄ but also suppressed the activation of cPLA₂ and 5-LO, as well as the expression of COX-2, L-PGDS and LTC₄S. cPLA₂, 5-LO and COX-2 are known to be rate-limiting enzymes in eicosanoid biosynthesis (Theoharides and

Kalogeromitros, 2006). Moreover, L-PGDS and LTC₄S are known to be directly concerned in the biosynthesis of PGD₂ (Taketomi et al., 2013) and LTC₄ (Cai et al., 2003), respectively, in mast cells. Therefore, cPLA₂, COX-2, 5-LO, L-PGDS and LTC₄S are additional important targets for the anti-allergic actions of F-PASA.

Finally, such anti-allergic actions of F-PASA may originate from arctigenin and/or emodin-8-*O*- β -*D*-glucoside, which are major components of PASA and F-PASA, because the level of arctiin, a glycosylated form of arctigenin, was decreased in F-PASA by approximately 26.2% compared with that in PASA, whereas the level of emodin-8-*O*- β -*D*-glucoside was increased in F-PASA by approximately 139.9% compared with that in PASA. In support of this, artigenin and emodin-8-*O*- β -*D*-glucoside are known to have anti-allergic (Lee and Kim, 2010) and anti-inflammatory roles (Wang et al., 2007). Furthermore, when *Arctium fruit* is fermented by microorganisms, the artigenin level is elevated by the deglycosylation of arctiin (Yoo et al., 2016). Taken together, the improvement of the anti-allergic effects of F-PASA may be closely correlated with the higher levels of artigenin and emodin-8-*O*- β -*D*-glucoside in F-PASA than in PASA. In addition, the both chemicals may be useful to evaluate the quality of PASA and F-PASA.

5. Conclusions

The present study demonstrated that F-PASA has more potent anti-allergic actions than PASA in IgE/Ag-induced allergic reactions through strongly suppressing the biosynthesis of allergic inducers in *in vitro* and *in vivo* systems. The findings revealed a novel feature of F-PASA in IgE/Ag-induced allergic responses. The mechanisms for its anti-allergic properties may include various targets such as Lyn, Syk, PLC γ 1/2, PKC δ , Akt, ERK, JNK, p38, cPLA $_2$, 5-LO, COX-2, L-PGDS and LTC4S in IgE/Ag-stimulated mast cells. Such effects may be related to elevated active phytochemicals such as arctigenin and emodin-8-*O*- β -D-glucoside converted by *L. rhamnosus* in F-PASA and provide the information for the development of herbal medicine or functional food for patients of allergic diseases. Furthermore, other studies are necessary to identify other unknown compounds and to confirm the anti-allergic actions of F-PASA in other animal studies such as asthma and atopic dermatitis.

Conflict of Interests

The authors declare that they have no conflict of interest.

Acknowledgement

This work was supported by the Korea Institute of Oriental Medicine, Ministry of Education, Science and Technology (MEST), Republic of Korea. [Grant number K18100].

References

- Adams, L.S., Seeram, N.P., Hardy, M.L., Carpenter, C., Heber, D., 2006. Analysis of the interactions of botanical extract combinations against the viability of prostate cancer cell lines. *Evidence-based complementary and alternative medicine : eCAM* 3, 117-124.
- Ahn, M.J., Lee, M.K., Kim, Y.C., Sung, S.H., 2008. The simultaneous determination of coumarins in *Angelica gigas* root by high performance liquid chromatography-diode array detector coupled with electrospray ionization/mass spectrometry. *Journal of pharmaceutical and biomedical analysis* 46, 258-266.
- Bralley, E.E., Greenspan, P., Hargrove, J.L., Wicker, L., Hartle, D.K., 2008. Topical anti-inflammatory activity of *Polygonum cuspidatum* extract in the TPA model of mouse ear inflammation. *Journal of inflammation* 5, 1.
- Cai, Y., Bjerner, L., Halstensen, T.S., 2003. Bronchial mast cells are the dominating LTC₄S-expressing cells in aspirin-tolerant asthma. *American journal of respiratory cell and molecular biology* 29, 683-693.
- Chan, Y.S., Cheng, L.N., Wu, J.H., Chan, E., Kwan, Y.W., Lee, S.M., Leung, G. P., Yu, P.H., Chan, S.W., 2011. A review of the pharmacological effects of *Arctium lappa* (burdock). *Inflammopharmacology* 19, 245-254.
- Choi, Y.E., Ahn, H., Ryu, J.H., 2000. Polyacetylenes from *angelica gigas* and their inhibitory activity on nitric oxide synthesis in activated macrophages. *Biological & pharmaceutical bulletin* 23, 884-886.
- Chu, X., Sun, A., Liu, R., 2005. Preparative isolation and purification of five compounds from the Chinese medicinal herb *Polygonum cuspidatum* Sieb. et Zucc by high-speed counter-current chromatography. *Journal of chromatography. A* 1097, 33-39.
- Chung, T.H., Kang, T.J., Cho, W.K., Im, G.Y., Lee, G.S., Yang, M.C., Cho, C.W., Ma, J.Y., 2012. Effectiveness of the Novel Herbal Medicine, KIOM-MA, and Its Bioconversion Product, KIOM-MA128, on the Treatment of Atopic Dermatitis. *Evidence-based complementary and alternative medicine : eCAM* 2012, 762918.
- Church, M.K., Levi-Schaffer, F., 1997. The human mast cell. *The Journal of allergy and clinical immunology* 99, 155-160.
- Gao, J.L., He, T.C., Li, Y.B., Wang, Y.T., 2009. A traditional Chinese medicine formulation consisting of *Rhizoma Corydalis* and *Rhizoma Curcumae* exerts synergistic anti-tumor activity. *Oncology reports* 22, 1077-1083.
- Gilfillan, A.M., Tkaczyk, C., 2006. Integrated signalling pathways for mast-cell activation.

- ctivation. *Nature reviews. Immunology* 6, 218-230.
- Han, C.C., Wang, Y., 2012. Anti-inflammation effects of *Sophora flavescens* nano particles. *Inflammation* 35, 1262-1268.
- He, X., Fang, J., Huang, L., Wang, J., Huang, X., 2015. *Sophora flavescens* Ait. : Traditional usage, phytochemistry and pharmacology of an important traditional Chinese medicine. *Journal of ethnopharmacology* 172, 10-29.
- Inagaki, N., Goto, S., Nagai, H., Koda, A., 1986. Homologous passive cutaneous anaphylaxis in various strains of mice. *International archives of allergy and applied immunology* 81, 58-62.
- Jiang, A., Craxton, A., Kurosaki, T., Clark, E.A., 1998. Different protein tyrosine kinases are required for B cell antigen receptor-mediated activation of extracellular signal-regulated kinase, c-Jun NH₂-terminal kinase 1, and p38 mitogen-activated protein kinase. *The Journal of experimental medicine* 188, 1297-1306.
- Kim, A., Im, M., Hwang, Y.H., Yang, H.J., Ma, J.Y., 2015. *Jaeumganghwa-Tang* Induces Apoptosis via the Mitochondrial Pathway and *Lactobacillus* Fermentation Enhances Its Anti-Cancer Activity in HT1080 Human Fibrosarcoma Cells. *PloS one* 10, e0127898.
- Kim, D.S., Kim, S.H., Kim, B.K., Yang, M.C., Ma, J.Y., 2012. Antiasthmatic Effects of Herbal Complex MA and Its Fermented Product MA128. *Evidence-based complementary and alternative medicine : eCAM* 2012, 769508.
- Knott, A., Reuschlein, K., Mielke, H., Wensorra, U., Mummert, C., Koop, U., Kausch, M., Kolbe, L., Peters, N., Stab, F., Wenck, H., Gallinat, S., 2008. Natural *Arctium lappa* fruit extract improves the clinical signs of aging skin. *Journal of cosmetic dermatology* 7, 281-289.
- Lee, J.E., Kang, S.J., Choi, S.H., Song, C.H., Lee, Y.J., Ku, S.K., 2015. Fermentation of Green Tea with 2% *Aquilariae lignum* Increases the Anti-Diabetic Activity of Green Tea Aqueous Extracts in the High Fat-Fed Mouse. *Nutrients* 7, 9046-9078.
- Lee, J.H., Shin, H., Kim, Y.J., Paek, S.H., Jin, S., Ha, U.H., 2014a. *Pseudomonas aeruginosa*-induced IL-1 β production is inhibited by *Sophora flavescens* via the NF- κ B/inflammasome pathways. *Journal of microbiology* 52, 1044-1049.
- Lee, J.J., Kwon, H., Lee, J.H., Kim, D.G., Jung, S.H., Ma, J.Y., 2014b. Fermented *soshiho-tang* with *Lactobacillus plantarum* enhances the antiproliferative activity in vascular smooth muscle cell. *BMC complementary and alternative medicine* 14, 78.
- Lee, J.Y., Kim, C.J., 2010. Arctigenin, a phenylpropanoid dibenzylbutyrolactone li

- gnan, inhibits type I-IV allergic inflammation and pro-inflammatory enzymes. *Archives of pharmacal research* 33, 947-957.
- Lee, Y.J., Choi, D.H., Cho, G.H., Kim, J.S., Kang, D.G., Lee, H.S., 2012. *Arctium lappa* ameliorates endothelial dysfunction in rats fed with high fat/cholesterol diets. *BMC complementary and alternative medicine* 12, 116.
- Liu, G., Dong, J., Wang, H., Hashi, Y., Chen, S., 2011. Characterization of alkaloids in *Sophora flavescens* Ait. by high-performance liquid chromatography-electrospray ionization tandem mass spectrometry. *Journal of pharmaceutical and biomedical analysis* 54, 1065-1072.
- Metcalfe, D.D., Kaliner, M., Donlon, M.A., 1981. The mast cell. *Critical reviews in immunology* 3, 23-74.
- Park, K.I., Kim, D.G., Yoo, J.M., Ma, J.Y., 2016. The Herbal Medicine KIOM-M A128 Inhibits the Antigen/IgE-Mediated Allergic Response in Vitro and in Vivo. *Molecules* 21.
- Sarker, S.D., Nahar, L., 2004. Natural medicine: the genus *Angelica*. *Current medicinal chemistry* 11, 1479-1500.
- Schwartz, J.N., Daniels, C.A., Shivers, J.C., Klintworth, G.K., 1974. Experimental cytomegalovirus ophthalmitis. *The American journal of pathology* 77, 477-492.
- Shin, S., Joo, S.S., Park, D., Jeon, J.H., Kim, T.K., Kim, J.S., Park, S.K., Hwang, B.Y., Kim, Y.B., 2010. Ethanol extract of *Angelica gigas* inhibits croton oil-induced inflammation by suppressing the cyclooxygenase - prostaglandin pathway. *Journal of veterinary science* 11, 43-50.
- Siraganian, R.P., McGivney, A., Barsumian, E.L., Crews, F.T., Hirata, F., Axelrod, J., 1982. Variants of the rat basophilic leukemia cell line for the study of histamine release. *Federation proceedings* 41, 30-34.
- Song, M.Y., Kang, S.Y., Oh, T.W., Kumar, R.V., Jung, H.W., Park, Y.K., 2015. The Roots of *Atractylodes macrocephala* Koidzumi Enhanced Glucose and Lipid Metabolism in C2C12 Myotubes via Mitochondrial Regulation. *Evidence-based complementary and alternative medicine : eCAM* 2015, 643654.
- Taketomi, Y., Ueno, N., Kojima, T., Sato, H., Murase, R., Yamamoto, K., Tanaka, S., Sakanaka, M., Nakamura, M., Nishito, Y., Kawana, M., Kambe, N., Ikeda, K., Taguchi, R., Nakamizo, S., Kabashima, K., Gelb, M.H., Arita, M., Yokomizo, T., Nakamura, M., Watanabe, K., Hirai, H., Nakamura, M., Okayama, Y., Ra, C., Aritake, K., Urade, Y., Morimoto, K., Sugimoto, Y., Shimizu, T., Narumiya, S., Hara, S., Murakami, M., 2013. Mast cell maturation is driven via a group III phospholipase A2-prostaglandin D2-DP1 receptor paracrine axis. *Nature immunology*

gy 14, 554-563.

Theoharides, T.C., Kalogeromitros, D., 2006. The critical role of mast cells in allergy and inflammation. *Annals of the New York Academy of Sciences* 1088, 78-99.

Uzzaman, A., Cho, S.H., 2012. Chapter 28: Classification of hypersensitivity reactions. *Allergy and asthma proceedings : the official journal of regional and state allergy societies* 33 Suppl 1, S96-99.

Wang, C., Zhang, D., Ma, H., Liu, J., 2007. Neuroprotective effects of emodin-8-O-beta-D-glucoside in vivo and in vitro. *European journal of pharmacology* 577, 58-63.

Wedemeyer, J., Tsai, M., Galli, S.J., 2000. Roles of mast cells and basophils in innate and acquired immunity. *Current opinion in immunology* 12, 624-631.

Yang, Y., Zhang, Z., Li, S., Ye, X., Li, X., He, K., 2014. Synergy effects of herb extracts: pharmacokinetics and pharmacodynamic basis. *Fitoterapia* 92, 133-147.

Yoo, J.M., Kim, N.Y., Seo, J.M., Kim, S.J., Lee, S.Y., Kim, S.K., Kim, H.D., Lee, S.W., Kim, M.R., 2014a. Inhibitory effects of mulberry fruit extract in combination with naringinase on the allergic response in IgE-activated RBL-2H3 cells. *International journal of molecular medicine* 33, 469-477.

Yoo, J.M., Sok, D.E., Kim, M.R., 2014b. Anti-allergic action of aged black garlic extract in RBL-2H3 cells and passive cutaneous anaphylaxis reaction in mice. *Journal of medicinal food* 17, 92-102.

Yoo, J.M., Yang, J.H., Yang, H.J., Cho, W.K., Ma, J.Y., 2016. Inhibitory effect of fermented *Arctium lappa* fruit extract on the IgE-mediated allergic response in RBL2H3 cells. *International journal of molecular medicine* 37, 501-508.

Zhang, H., Li, C., Kwok, S.T., Zhang, Q.W., Chan, S.W., 2013. A Review of the Pharmacological Effects of the Dried Root of *Polygonum cuspidatum* (Hu Zhan g) and Its Constituents. *Evidence-based complementary and alternative medicine : eCAM* 2013, 208349.

Zhang, H., Yu, C.H., Jiang, Y.P., Peng, C., He, K., Tang, J.Y., Xin, H.L., 2012. Protective effects of polydatin from *Polygonum cuspidatum* against carbon tetrachloride-induced liver injury in mice. *PloS one* 7, e46574.

Zhang, J., Wang, L., Zhang, Y., Li, L., Tang, S., Xing, C., Kim, S.H., Jiang, C., Lu, J., 2015. Chemopreventive effect of Korean *Angelica* root extract on TRAMP carcinogenesis and integrative "omic" profiling of affected neuroendocrine carcinomas. *Molecular carcinogenesis* 54, 1567-1583.

Zheng, K.Y., Choi, R.C., Guo, A.J., Bi, C.W., Zhu, K.Y., Du, C.Y., Zhang, Z.X.,

Lau, D.T., Dong, T.T., Tsim, K.W., 2012. The membrane permeability of Astragalus Radix-derived formononetin and calycosin is increased by Angelicae Sinensis Radix in Caco-2 cells: a synergistic action of an ancient herbal decoction Danggui Buxue Tang. *Journal of pharmaceutical and biomedical analysis* 70, 671-679.

ACCEPTED MANUSCRIPT

Table 1. Composition of two glycosides of PASA and F-PASA

Unit: mg/g dry weight

	Glycoside compounds	
	Arctiin	Emodin-8- <i>O</i> - β - <i>D</i> -glucoside
PASA	60.140 $\pm$ 6.014	4.302 $\pm$ 0.430
F-PASA	44.364 $\pm$ 4.436	6.018 $\pm$ 0.602

The data were expressed as the mean $\pm$ SD values of triple determinations.

Figure Legends

Figure 1. Effects of PASA and F-PASA on degranulation and cell viability in IgE/Ag-mediated RBL-2H3 cells.

RBL-2H3 cells were seeded on a 24-well plate (1×10^5 cells/well) or a 96-well plate (1×10^4 cells/well) in MEM- α with 10% FBS at 37°C overnight and were further incubated with DNP-IgE for 24 h. IgE-sensitized cells were preincubated with PASA or F-PASA (0–2,000 μ g/ml) for 1 h and were then stimulated with DNP-HSA (0.1 μ g/ml) for 4 h. β -Hexosaminidase activity and cell viability were determined as described in the Materials and Methods section. The data were expressed as the mean $\pm$ SD values of triple or octuplex determinations. ** $P < 0.01$ versus the DNP-HSA-treated group. A and B, β -Hexosaminidase; C and D, cell viability.

Figure 1

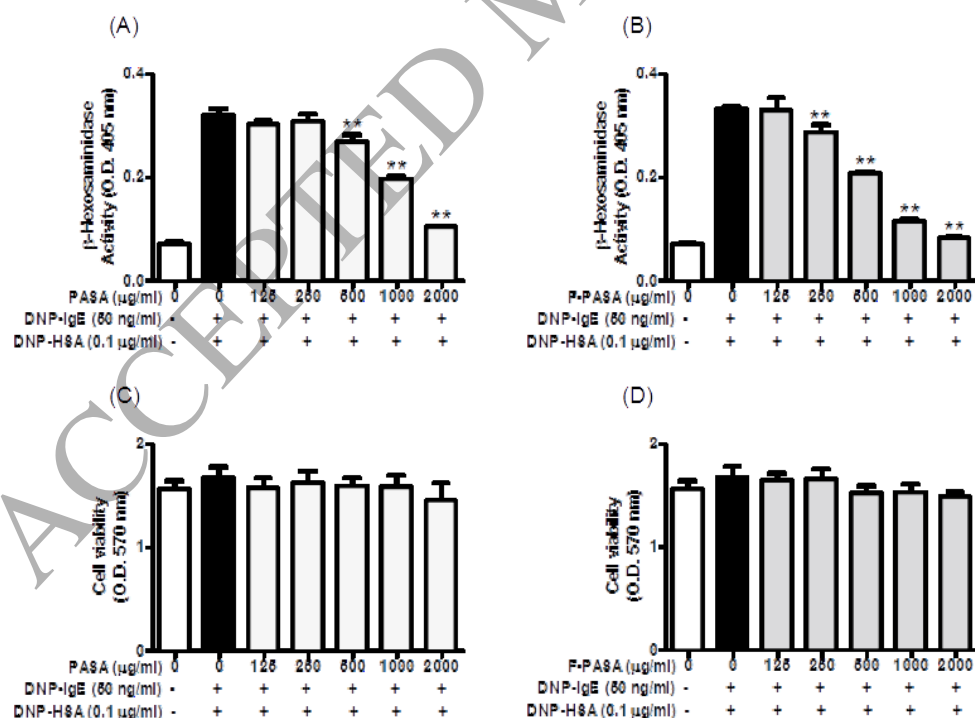


Figure 2. Inhibitory effects of F-PASA on the formation of proinflammatory mediators.

IgE-sensitized RBL-2H3 cells were preincubated with F-PASA for 1 h prior to antigen challenge. The amounts of TNF- α , IL-4, PGD₂ and LTC₄ were determined as described in the Materials and Methods section. The data were expressed as the mean $\pm$ SD values of triple determinations. ** $P < 0.01$ versus the DNP-HSA-treated group. A, TNF- α ; B, IL-4; C, PGD₂; D, LTC₄.

Figure 2

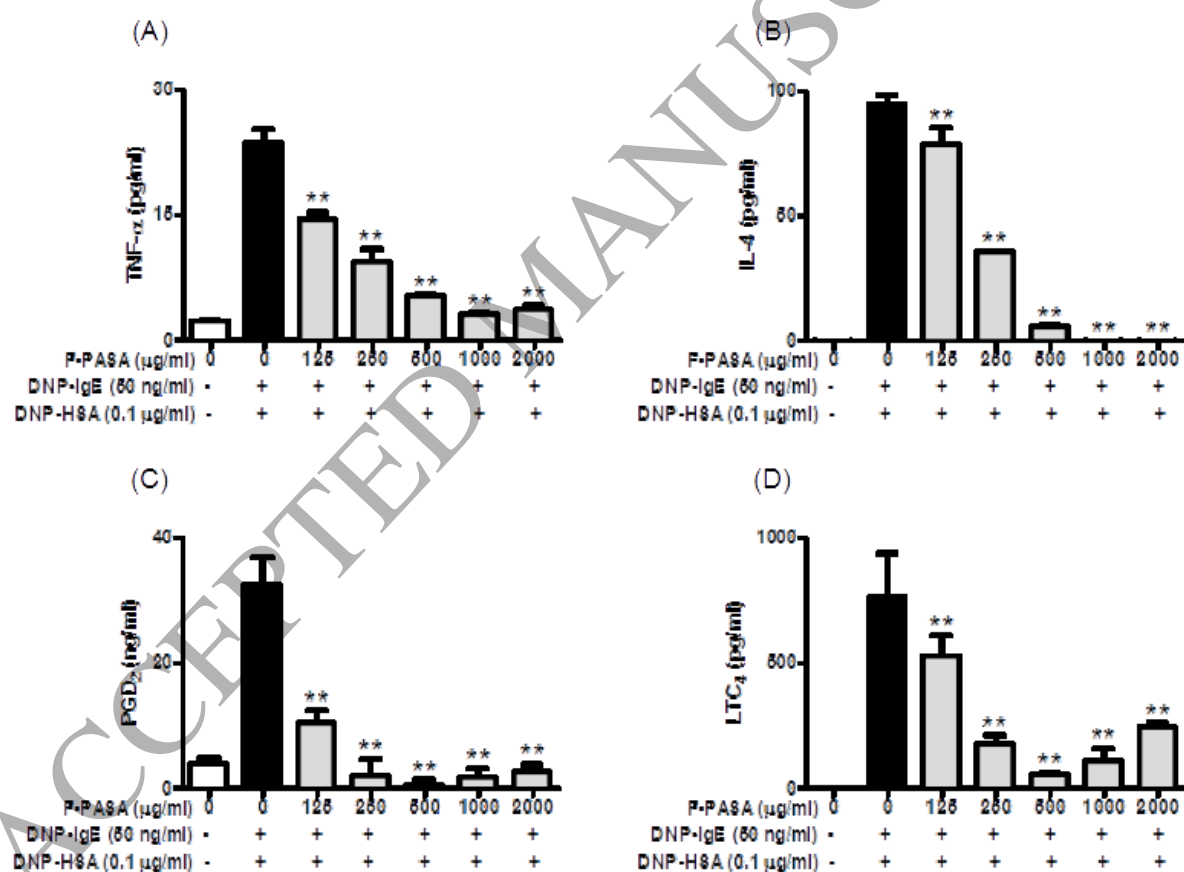


Figure 3. Effects of F-PASA on the phosphorylation or expression of rate-limiting enzymes in the arachidonate cascade.

RBL-2H3 cells were seeded on a 6-well plate (5×10^5 cells/well) in MEM- α with 10% FBS at 37°C overnight and were further incubated with DNP-IgE for 24 h. IgE-sensitized RBL-2H3 cells were preincubated with F-PASA prior to antigen challenge. The above cells were washed with 1× DPBS and were lysed with cell lysis buffer. The expression of p-cPLA₂, COX-2, p-5-LO, L-PGDS, LTC₄S and β -actin was determined as described in the Materials and Methods section. Similar results were obtained in three independent experiments. * $P < 0.05$ and ** $P < 0.01$ versus the DNP-HSA-treated group.

Figure 3

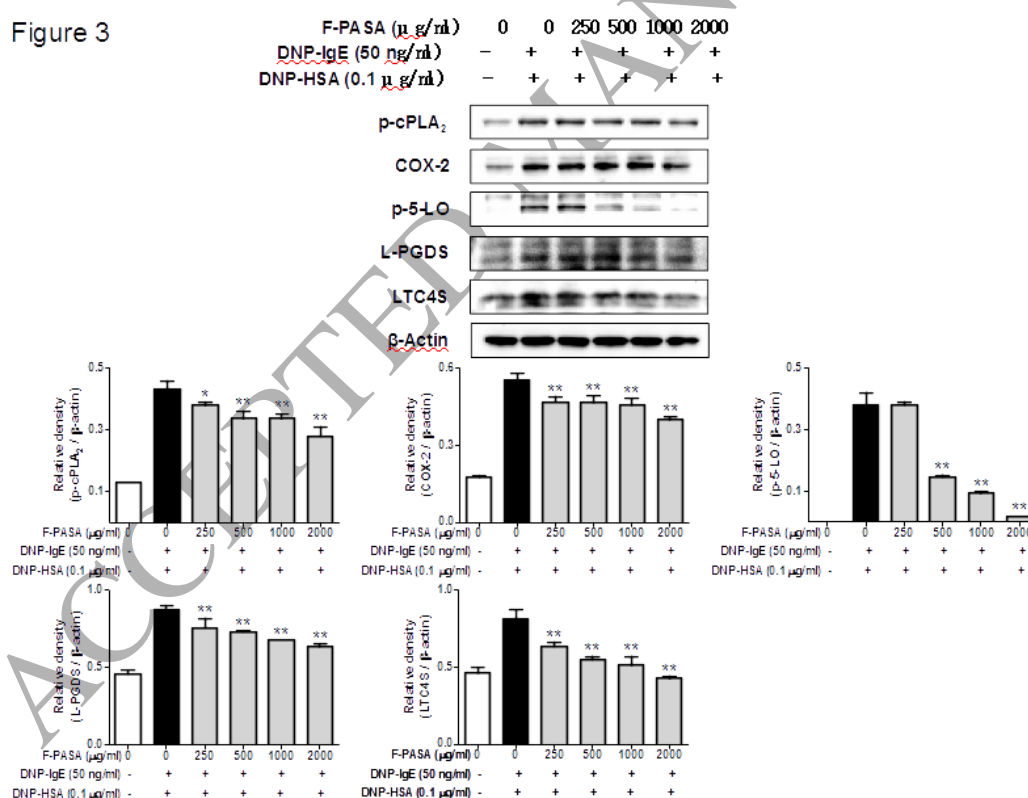


Figure 4. Effects of F-PASA on the phosphorylation of intermediate signal proteins

in the FcεRI signaling cascade.

IgE-sensitized RBL-2H3 cells were preincubated with F-PASA for 1 h and then stimulated with antigen for 10 min. The above cells were washed with 1× DPBS and lysed with cell lysis buffer. The expression of p-Syk, p-Fyn, p-Lyn, p-PLCγ1, p-PLCγ2, p-PKCδ, p-JNK, p-ERK, p-p38, p-Akt and β-actin was determined as described in the Materials and Methods section. Similar results were obtained in three independent experiments. * $P < 0.05$ and ** $P < 0.01$ versus the DNP-HSA-treated group. A, p-Syk, p-Fyn and p-Lyn; B, p-PLCγ1/2 and p-PKCδ; C, p-JNK, p-ERK, p-p38 and p-Akt.

Figure 4

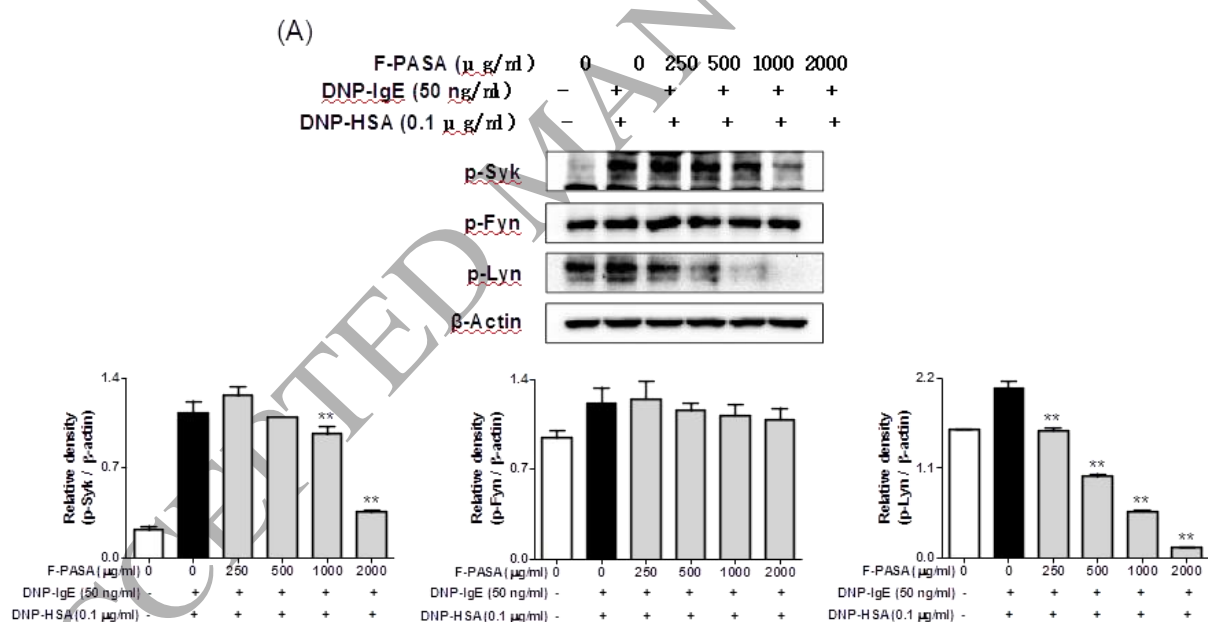


Figure 4

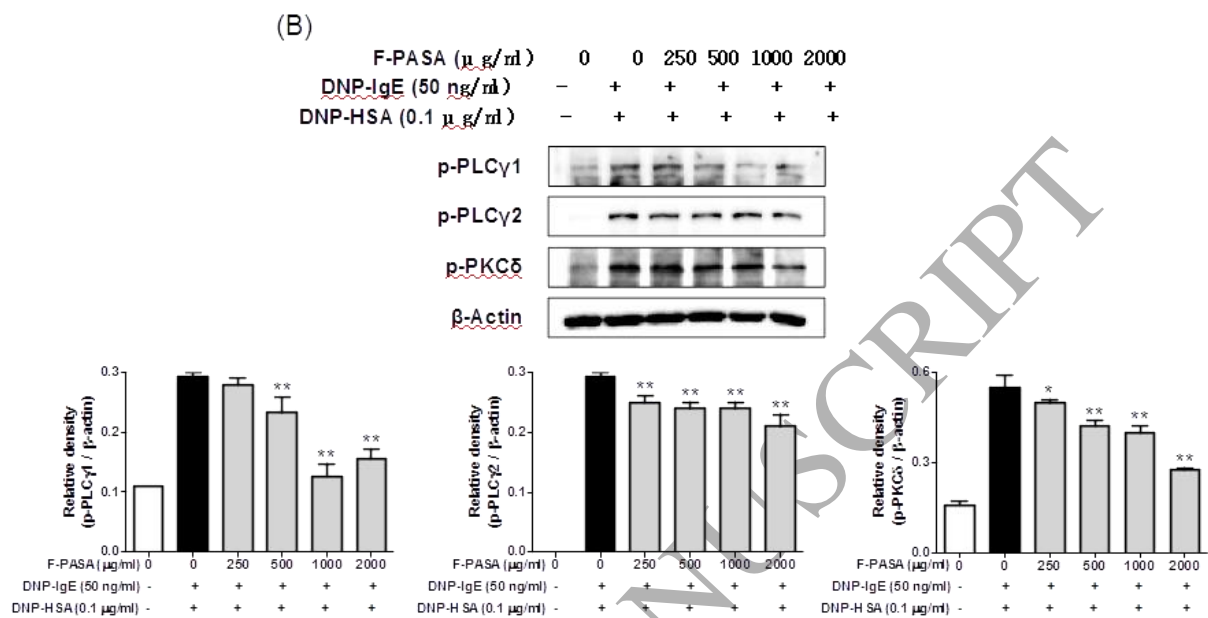


Figure 4

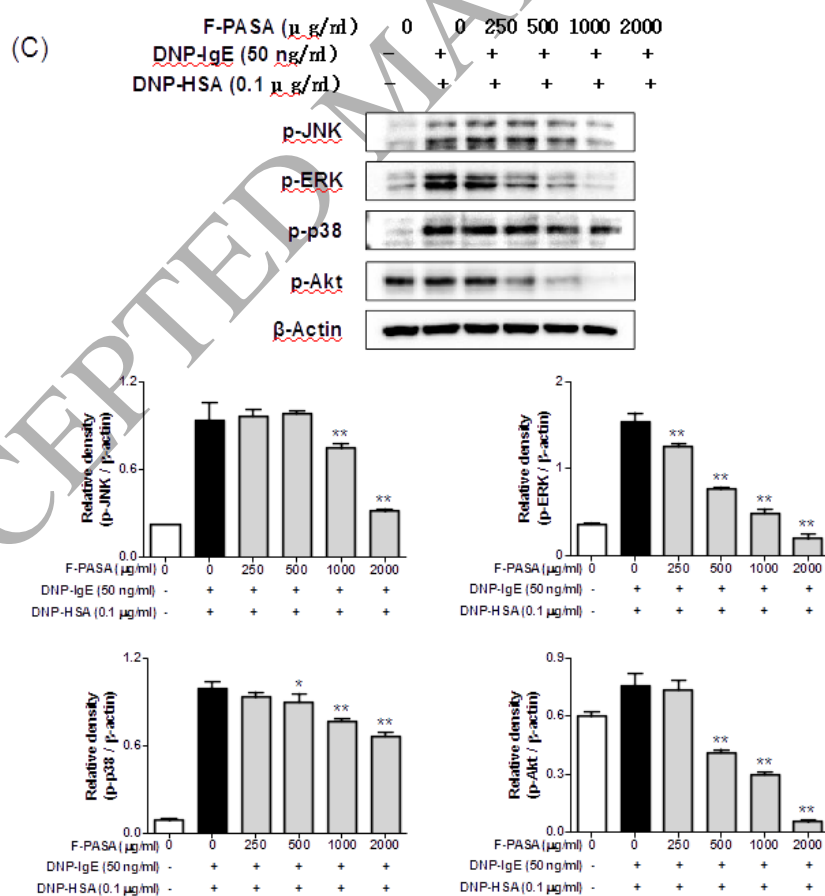


Figure 5. Inhibitory effects of PASA and F-PASA on IgE/Ag-mediated passive cutaneous anaphylaxis in mice.

IgE-sensitized mice were orally administered with PASA, F-PASA (0–100 mg/kg) or dexamethasone (Dexa; 10 mg/kg) for 1 h and then intravenously injected with 100 μ g DNP-HSA containing 0.5% Evans blue. After 30 min, the mice described above were euthanized, and then both ears of each mouse were excised. The extravasated dye in the ears was analyzed as described in the Materials and Methods section. The data were expressed as the mean $\pm$ SEM values of octuplex determinations. * P < 0.05 and ** P < 0.01 versus the DNP-HSA-treated group. A, PASA; B, F-PASA.

Figure 5

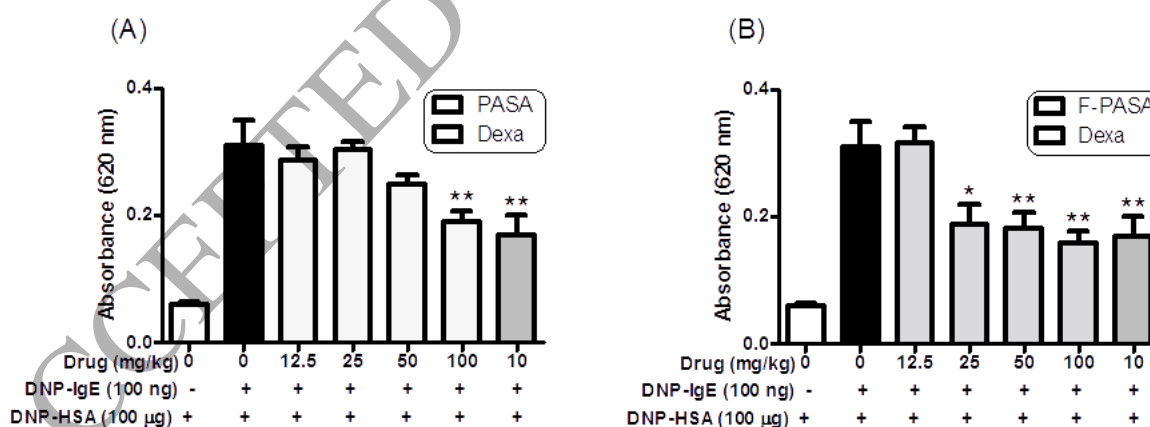
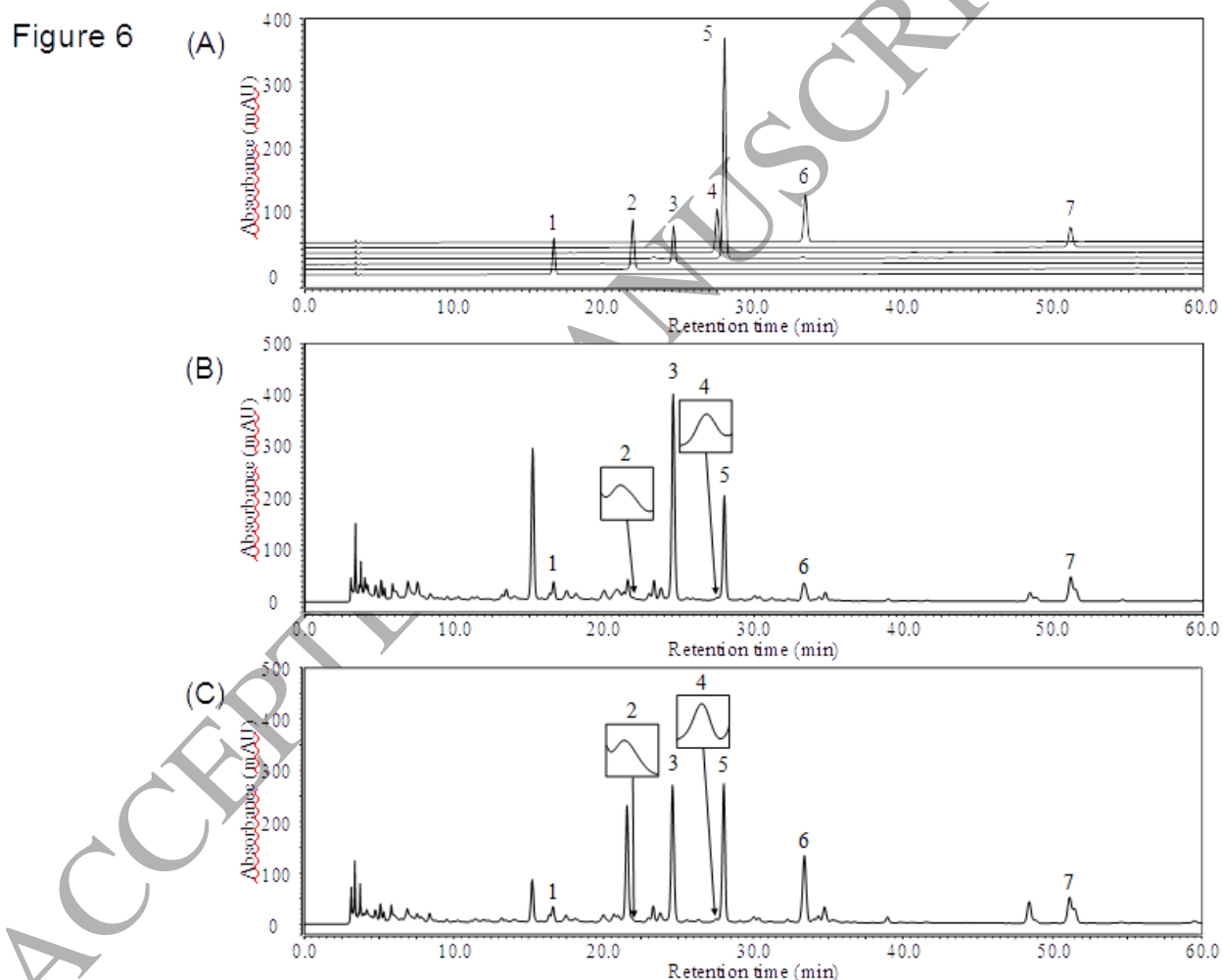


Figure 6. HPLC chromatograms of PASA and F-PASA. HPLC chromatograms of a standard mixture of seven standards in PASA or F-PASA at 280 nm. Nodakenin (1), decursinol (2), arctiin (3), matairesinol (4), emodin-8-*O*- β -*D*-glucoside (5), arcartigenin (6) and decursin (7) were identified. HPLC analysis was described in the Materials and Methods section. A, Standard chromatogram; B, PASA chromatogram; C, F-PASA chromatogram.



Graphical Abstract

Graphical abstract

