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RESEARCH ARTICLE

# Antiallergic effects of peiminine through the regulation of inflammatory mediators in HMC-1 cells

Bina Lee, Eun-Young Kim, Jae-Hyun Kim, Ju-Hee Min, Da-Won Jeong, Jae-Yun Jun, Chang-Young Cho, Youngjoo Sohn, and Hyuk-Sang Jung

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## Abstract

Peiminine is the main biologically active component derived from *Fritillaria ussuriensis*. Peiminine was investigated in various pulmonary diseases, but its antiallergic effect and the related mechanism have not been reported yet. The present study aimed to evaluate the effect of peiminine on mast cell-mediated allergic inflammation in HMC-1 cells. The pro-inflammatory cytokine production was measured using ELISA, reverse transcription-polymerase chain reaction and nuclear factor-kappaB (NF-κB), mitogen-activated protein kinases (MAPKs) pathway activation, as determined by Western blot analysis. Peiminine inhibits the production of the pro-inflammatory cytokine, such as interleukin (IL)-6, IL-8, tumor necrosis factor-alpha (TNF-α) and IL-1beta (IL-1β). It was shown to have inhibitory effects on MAPKs phosphorylation and NF-κB expression in human mast cells (HMC)-1 using Western blot. HMC-1 cells were observed for confirmation of histamine release. Passive cutaneous anaphylaxis (PCA) reactions were evaluated using an animal model and peiminine demonstrated inhibitory effects on IgE-dependent anaphylaxis. These results suggest that peiminine has regulatory potential for allergic inflammatory reactions mediated by HMC-1 cells.

## Keywords

Cytokine, inflammation, MAP kinases, nuclear factor-kappaB, peiminine

## History

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## Introduction

Peiminine is an alkaloid that is the main biologically active component of *Fritillaria ussuriensis* (FU, derived from the bulbs of various species of the genus *Fritillaria*, including *Fritillaria thunbergii* Miq.). FU has been used in traditional Korean medicine for various pulmonary diseases, such as relieving coughs, reducing heart rates and lowering blood pressure<sup>1,2</sup>. The effects of FU on various diseases are still emerging through clinical and basic studies. Our previous study showed that FU extract reduced the level of interleukin (IL)-6, IL-8, tumor necrosis factor (TNF)-α and phosphorylation of MAPKs in HMC-1 cells<sup>3</sup>. Peiminine isolated from FU was already reported to reduce cytokines, such as IL-4, TNF-α, interferon (IFN)-γ in sera, transforming growth factor beta (TGF-β), connective tissue growth factor, extracellular signal-regulated kinases (ERK)1/2 and NF-κB in tissues of leomycin-induced acute lung injury rat model<sup>4</sup>. However, peiminine as the main active component of FU has not studied in antiallergic effects and the related mechanism yet.

Allergic reactions are defined as symptomatic responses caused by environmental antigen<sup>5</sup>. An allergic reaction has the potential to develop into a fatal reaction known as

anaphylaxis in situation of allergen exposures<sup>6</sup>. Due to the environmental pollution and dietary changes, allergic diseases including allergic rhinitis, atopic dermatitis and some food allergies can affect people of all ages. The increased allergic diseases incidence, especially in children and young age groups, has caused concern<sup>7</sup>. Therefore, continuous study is important to improve the effective medicine of allergic diseases<sup>8</sup>.

Mast cells are effector cells that contribute to inflammatory reactions in the immune system<sup>9</sup>. Upon allergen provocation, mast cell release inflammatory mediators, such as histamines, cytokines and chemokines<sup>10,11</sup>. TNF-α, IL-6 and IL-8 are major cytokines in the inflammatory response and play important roles in chronic inflammation. These cytokines are released by mast cells and regulated by the activation of transcription factors, such as NF-κB<sup>12–14</sup>. NF-κB also controls the production receptors involved in the allergen recognition and contributes to increased proliferation and differentiation of lymphocytes<sup>15</sup>.

The aim of this study is to verify that peiminine as active compound of FU has antiallergic effect. The peiminine (Figure 1) was investigated on the expression of pro-inflammatory mediators induced by phorbol 12-myristate 13-acetate (PMA) and calcium ionophore A23187 (PMACI) treatment by suppressing the inhibitor of nuclear factor kappa B (IκB)-α/NF-κB signal pathways and reducing MAPKs phosphorylation as well as cytokine levels, such as IL-6, IL-8,

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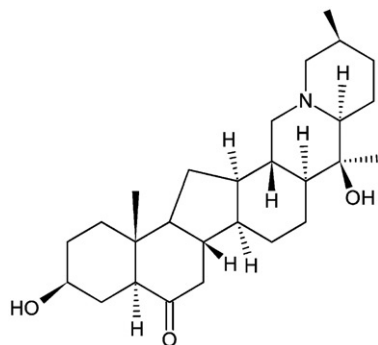


Figure 1. The chemical structure of peiminine.

TNF- $\alpha$  and IL-1 $\beta$  *in vitro*. We evaluated the antiallergic function of peiminine on compound 48/80-induced histamine release from HMC-1 cells. Peiminine was also investigated for the antiallergic effects on the IgE-dependent PCA reaction *in vivo*.

## Materials and methods

### Reagents

Peiminine was obtained from Ab cam, with a molecular weight of 429.64 g/mol (Cambridge, UK). Dexamethasone (DEX)-water soluble, anti-dinitrophenol (DNP) immunoglobulin (Ig)E, DNP-human serum albumin (HSA) and evans blue were purchased from Sigma (St. Louis, MO). Iscove's modified dulbecco's medium (IMDM) and fetal bovine serum (FBS) were from Gibco BRL (Grand Island, NY) and penicillin-streptomycin was from Invitrogen (Carlsbad, CA). Aqueous nonradioactive cell proliferation assay (MTS) was purchased from Promega (Madison, WI). Compound 48/80, PMA and calcium ionophore A23187 were purchased from Sigma (St. Louis, MO). Antibodies (Abs) to phosphorylation-ERK (P-ERK), ERK, P-P38, P38, P-c-Jun-NH2-terminal kinase (JNK), JNK, NF- $\kappa$ B and phosphorylation-I $\kappa$ B- $\alpha$  (p-I $\kappa$ B- $\alpha$ ) were purchased from Cell Signaling (Cell Signaling Technology, Danvers, MA).  $\beta$ -Actin was obtained from Santa Cruz (Santa Cruz Biotechnology, Santa Cruz, CA) and peroxidase IgG was from Jackson ImmunoResearch (Jackson ImmunoResearch, West Grove, PA). Recombinant IL-6, IL-8 and TNF- $\alpha$ , biotinylated IL-6, IL-8 and TNF- $\alpha$  and antihuman IL-6, IL-8 and TNF- $\alpha$  were from BD Biosciences (San Jose, CA). PCR primers were purchased from Genotech (Daejeon, Korea).

### Culture of HMC-1 cells

HMC-1 cells were provided by Prof. H. M. Kim (Department of Pharmacology, Kyung Hee University, Seoul, Republic of Korea). HMC-1 cells were incubated in IMDM at 37 °C, 5% CO<sub>2</sub> and 95% humidity with 100 units/mL penicillin, 100 mg/mL streptomycin and 10% heat-inactivated FBS.

### MTS assay for cell viability

Peiminine was dissolved in dimethyl sulfoxide (DMSO). HMC-1 cells ( $1 \times 10^5$  cells/well) were seeded and treated with 1, 10, 25, 50 and 100  $\mu$ g/mL concentrations of peiminine. Cells were also treated with 1, 10 and

100  $\mu$ g/mL concentrations of DEX as a positive control. The cells were incubated for 24 h in a 37 °C incubator. Cell viability was measured using the MTS colorimetric assay. MTS solution was added to each well. After 2 h, the optical density (OD) value of the 96-well culture plates was read at 490 nm with a ELISA microplate reader (VersaMax, Molecular Devices, Sunnyvale, CA). The formazan OD value of the untreated control cells was taken to represent 100% viability.

### Measurement of cytokines

The levels of IL-6, IL-8 and TNF- $\alpha$  were determined by enzyme-linked immunosorbent assay (ELISA) using a commercial cytokine/chemokine ELISA kit according to the manufacturer's recommendations (BD Biosciences, San Jose, CA). HMC-1 cells were pretreated with 10, 25 and 50  $\mu$ g/mL concentrations of peiminine 1 h and stimulated with PMACI (PMA 25 nM plus A23187 1  $\mu$ M) for 8 h. The cells were measured with a spectrophotometer (Molecular Devices). The effect of peiminine on allergic responses was calculated from the equation obtained with a standard curve plot for each cytokine standard solution in the kit.

### Reverse transcriptase-polymerase chain reaction (RT-PCR) analysis

HMC-1 cells were pretreated with various peiminine concentrations (10, 25 and 50  $\mu$ g/mL) for 1 h and stimulated with PMACI for 6 h. Total RNA was prepared using Trizol (TAKARA BIO, Otsu, Japan) and cDNAs were synthesized from 2  $\mu$ g of total RNA using SuperScript II reverse transcriptase (Invitrogen, Carlsbad, CA). RT-generated cDNAs encoding IL-6, IL-8, IL-1 $\beta$  and TNF- $\alpha$  were produced by PCR using selective primers as shown in Table 1. The cDNA samples were separated on a 1–1.2% agarose gel after the reaction, stained with SYBR staining solution (Invitrogen, Carlsbad, CA) and identified using a N $\alpha$ BI (Neoscience, Suwon, Korea).

### Western blot analysis

HMC-1 cells ( $5 \times 10^6$  cells/well) were pretreated with various peiminine concentrations (10, 25 and 50  $\mu$ g/mL) for 1 h. Then the cells were stimulated with PMACI 2 h for nuclear proteins, and 30 min for MAPKs. To obtain the cell extract, harvested cells were lysed using lysis buffer (HEPES, KCl, MgCl<sub>2</sub>, DTT, PMSF, NaCl glycerol, EDTA) for total protein lysis. The cells were washed with DPBS and lysed with NE-PER Nuclear and Cytoplasmic Extraction Reagents (Pierce, Rockford, IL) to obtain nuclear and cytosolic fractions. The proteins were centrifuged and heated at 95 °C for 5 min. Proteins were resolved by 10% SDS-polyacrylamide gel electrophoresis, then electro-transferred to nitrocellulose membranes (Whatman, Maidstone, Kent, UK). The membranes were blocked for 1 h with 5% skim milk and incubated with primary antibodies. The membranes were then incubated with peroxidase-conjugated secondary antibodies at room temperature and the antibody-specific proteins were visualized using a chemiluminescence (ECL) detection system

Table 1. Primer sets for RT-PCR.

| Name          | Forward primer                           | Reverse primer                           |
|---------------|------------------------------------------|------------------------------------------|
| IL-6          | 5' GAT GGC TGA AAA AGA TGG ATG C 3'      | 5' GTT TTG GGT CAG GGG TGG TT 3'         |
| IL-8          | 5' CGA TGT CAG TGC ATA AAG ACA 3'        | 5' TGA ATT CTC AGC CCT CTT CAA AAA 3'    |
| TNF- $\alpha$ | 5' CAC CAG CTG CGT TAT CTC TCA 3'        | 5' CGG GAC GTG GAG CTG GCC GAG GAG 3'    |
| IL-1 $\beta$  | 5' CCG GAT CCA TGG CAC CTG TAC GAT CA 3' | 5' GGG GTA CCT TAG GGA GAC ACA AAT TG 3' |
| GAPDH         | 5' CGT CTA GAA AAA CCT GCC AA 3'         | 5' TGA AGT CAA AGG AGA CCA CC 3'         |

according to the manufacturer's protocol (Santa Cruz Biotechnology, Santa Cruz, CA).

### Histamine assay

The mast cells were pre-incubated with peiminine (final concentrations: 10, 25 and 50  $\mu\text{g/mL}$ ) for 10 min before incubation with compound 48/80 (5  $\mu\text{g/mL}$ ) for 30 min. The cell shapes were captured by light microscopy (Olympus BX51, Olympus Corporation, Tokyo, Japan), using a fitted camera (Olympus DP70 digital camera unit, Olympus Corporation, Tokyo, Japan). The histamine content was measured using log-logit curve with a Histamine EIA Kit (Oxford, MI) according to the manufacturer's manual. Absorbance was measured at 450 nm with a microplate spectrophotometer (Molecular Devices).

### Animals

Sprague-Dawley (SD) rats were purchased from Nara Biotech (Gangnam-gu, Seoul, Korea). All animal experiments were in accordance with the guidelines of the Kyung Hee University Institutional Animal Care and Use Committee (KHUASP(SE)-14-032). The rats were housed under controlled temperature ( $23 \pm 3^\circ\text{C}$ ) with a relative humidity of 40–60% and 12 h light/dark cycles. Food and water were provided *ad libitum*.

### PCA reaction

Anti-DNP IgE (Sigma-Aldrich Co., St. Louis, MO) was intradermally injected into three outlined dorsal skin sites in the SD rats, followed 48 h later by an injection of DNP-HSA containing 4% Evans blue into the dorsal vein of the rat's penis. DNP-HSA was diluted in phosphate buffered saline (PBS). One hour before the injection, DNP-HSA containing 4% Evans blue and peiminine was administered by peroral administration (p.o.) at two doses of 1 and 5 mg/kg. DEX was administered by p.o. at a dose of 1 mg/kg. Rats were sacrificed 30 min after the intravenous challenge. The dorsal skin of the rats was removed and dissolved with formamide in an incubator for 72 h. The amount of dye was then measured at 620 nm using a spectrophotometer (Molecular Devices).

### Statistical analysis

Data are presented as mean  $\pm$  standard error of the mean (SEM). Statistical analysis was performed using the Graph Pad PRISM Software (Graphpad Software Inc., San Diego, CA). One-way ANOVA was used to evaluate the treatment effect, followed by Dunnett's multiple comparison test. Values of  $p < 0.05$  were considered to be significant.

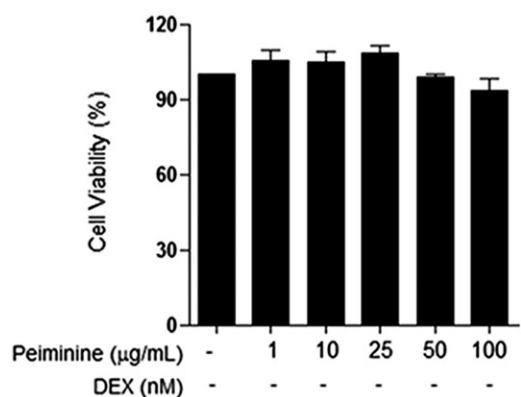


Figure 2. Cell viability of peiminine in HMC-1 cells. The optical density of the culture plates at 490 nm was measured with a spectrophotometer. The value represents the mean  $\pm$  SEM from three independent experiments.

## Results

### Effects of peiminine on the viability of HMC-1 cells

To determine the cell viability, MTS assay was performed. HMC-1 cells were treated with peiminine at concentrations of 1, 10, 25, 50 and 100  $\mu\text{g/mL}$ . DEX concentrations of 1, 10 and 100 nM were tested for cell cytotoxicity. DEX is an anti-inflammatory glucocorticoid that is commonly used worldwide<sup>16</sup>. According to previous reports, DEX reduced IL-6, IL-8, TNF- $\alpha$ , IL-3 and granulocyte-macrophage colony-stimulating factor (GM-CSF) expression in human leukemic mast cells<sup>17</sup>. In this study, 100 nM of DEX was used as a positive control. Neither peiminine nor DEX showed cell cytotoxicity (Figure 2). A 50  $\mu\text{g/mL}$  of peiminine was used as the optimal concentration based on viability results.

### Effects of peiminine on cytokine release from HMC-1 cells

To evaluate whether peiminine can inhibit PMACI-induced IL-6, IL-8 and TNF- $\alpha$  production, HMC-1 cells were pretreated with peiminine. The inhibition of PMACI-induced expression of IL-6, IL-8 and TNF- $\alpha$  in the mast cells was reduced following peiminine treatment (Figure 3). Especially, peiminine concentrations of 10, 25 and 50  $\mu\text{g/mL}$  as well as DEX significantly decreased IL-6 expression. Peiminine at 50  $\mu\text{g/mL}$  and DEX also significantly decreased IL-8, whereas peiminine at 10, 25, 50  $\mu\text{g/mL}$  and DEX significantly inhibited TNF- $\alpha$ . These results suggested that peiminine regulates the expression of the cytokines.

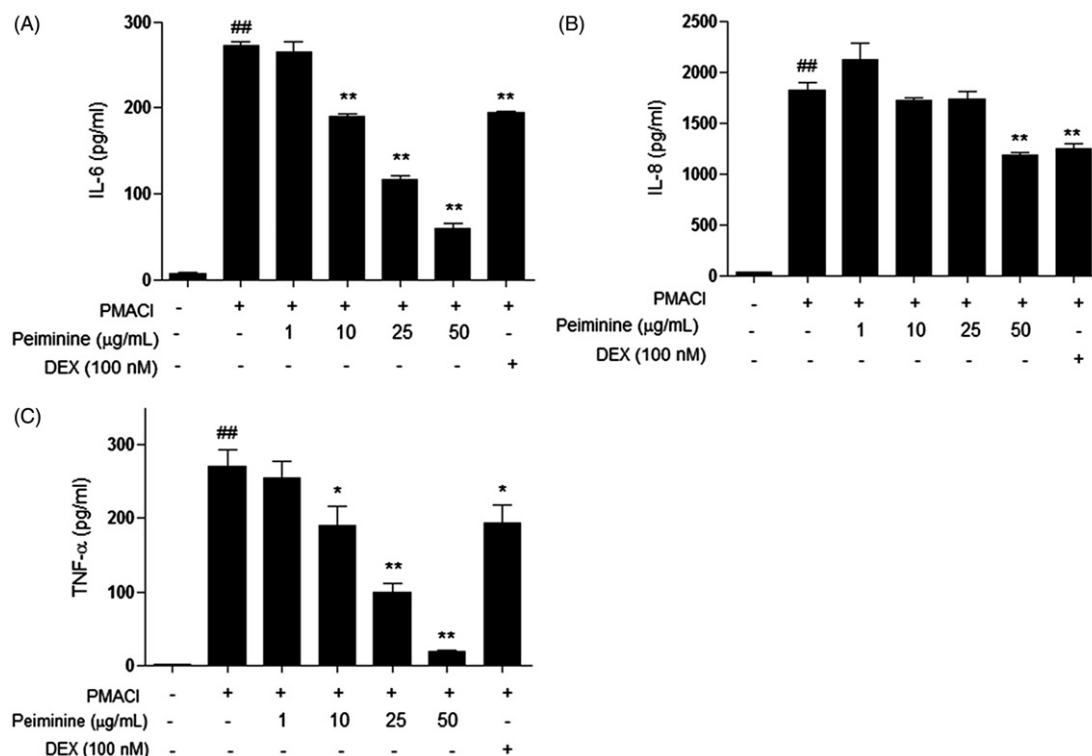


Figure 3. Effect of peiminine on production of the pro-inflammatory cytokines IL-6, IL-8 and TNF- $\alpha$  levels in PMACI-stimulated HMC-1 cells. HMC-1 cells were pretreated with peiminine (10, 25 and 50  $\mu$ g/mL) and DEX at 100 nM for 1 h before 8 h of PMACI stimulation. The protein production levels of (A) IL-6, (B) IL-8 and (C) TNF- $\alpha$  were measured in culture medium by ELISA. Columns and error bars represent the mean  $\pm$  SEM of three independent experiments. ## $p$  < 0.01 significantly different from the control group. \*\* $p$  < 0.01 and \* $p$  < 0.05 significantly different from PMACI alone.

### Effects of peiminine on cytokine mRNA expression in HMC-1 cells

To measure the effect of peiminine on the production of pro-inflammatory cytokines mRNA, we pretreated the cells with peiminine before stimulation with PMACI for 6 h and performed RT-PCR. The enhanced expressions of IL-6, IL-8, TNF- $\alpha$  and IL-1 $\beta$  mRNAs induced by PMACI were inhibited by pretreatment of the cells with peiminine (Figure 4). Peiminine at 50  $\mu$ g/mL and DEX significantly decreased the expressions of IL-6. Pretreatment with peiminine at concentrations of 25, 50  $\mu$ g/mL and DEX significantly inhibited the PMACI-induced gene expressions of IL-8. Peiminine at 50  $\mu$ g/mL and DEX significantly reduced the expression of IL-1 $\beta$ . Peiminine at 50  $\mu$ g/mL significantly decreased the expressions of TNF- $\alpha$  expression.

### Effects of peiminine on PMACI-stimulated MAPKs activation in HMC-1 cells

To determine the effects of peiminine, we examined the activation of MAPKs using Western blot analysis. The results showed that P-ERK and P-JNK were significantly inhibited by peiminine at 50  $\mu$ g/mL (Figure 5). Peiminine had no inhibitory effect on p-P38, whereas DEX significantly decreased the phosphorylations of P-ERK and P-JNK.

### Effect of peiminine on PMACI-stimulated activation of NF- $\kappa$ B in HMC-1 cells

To investigate NF- $\kappa$ B activation and I $\kappa$ B- $\alpha$  degradation in HMC-1 cells treated with peiminine, Western blotting

was performed. The results indicated that 25 and 50  $\mu$ g/mL peiminine inhibited the degradation of I $\kappa$ B- $\alpha$  in the cytosol in a concentration-dependent manner. DEX inhibited the degradation of I $\kappa$ B- $\alpha$  in the cytosol significantly. Moreover, 50  $\mu$ g/mL peiminine significantly reduced NF- $\kappa$ B activation (Figure 6).

### Effects of peiminine on histamine release from HMC-1 cells

To investigate the effects of peiminine on histamine release from human mast cells, we measured compound 48/80-induced mast cell activation. Peiminine inhibited compound 48/80-induced histamine release at 50  $\mu$ g/mL. DEX also decreased histamine release significantly compared with the compound 48/80 group (Figure 7).

### Effects of peiminine on IgE-mediated PCA reaction in rats

To determine the effect of peiminine on allergic reactions *in vivo*, we established the PCA rat model. Peiminine concentrations of 1 and 5 mg/mL significantly inhibited the PCA reaction that both area and amount of dye (Figure 8). Additionally, 1 mg/mL DEX significantly decreased the area of PCA reaction and the amount of dye.

### Discussion

In the present study, we investigated the effect of peiminine isolated from *F. ussuriensis* on pro-inflammatory cytokines, mRNA expression level, activation of NF- $\kappa$ B,

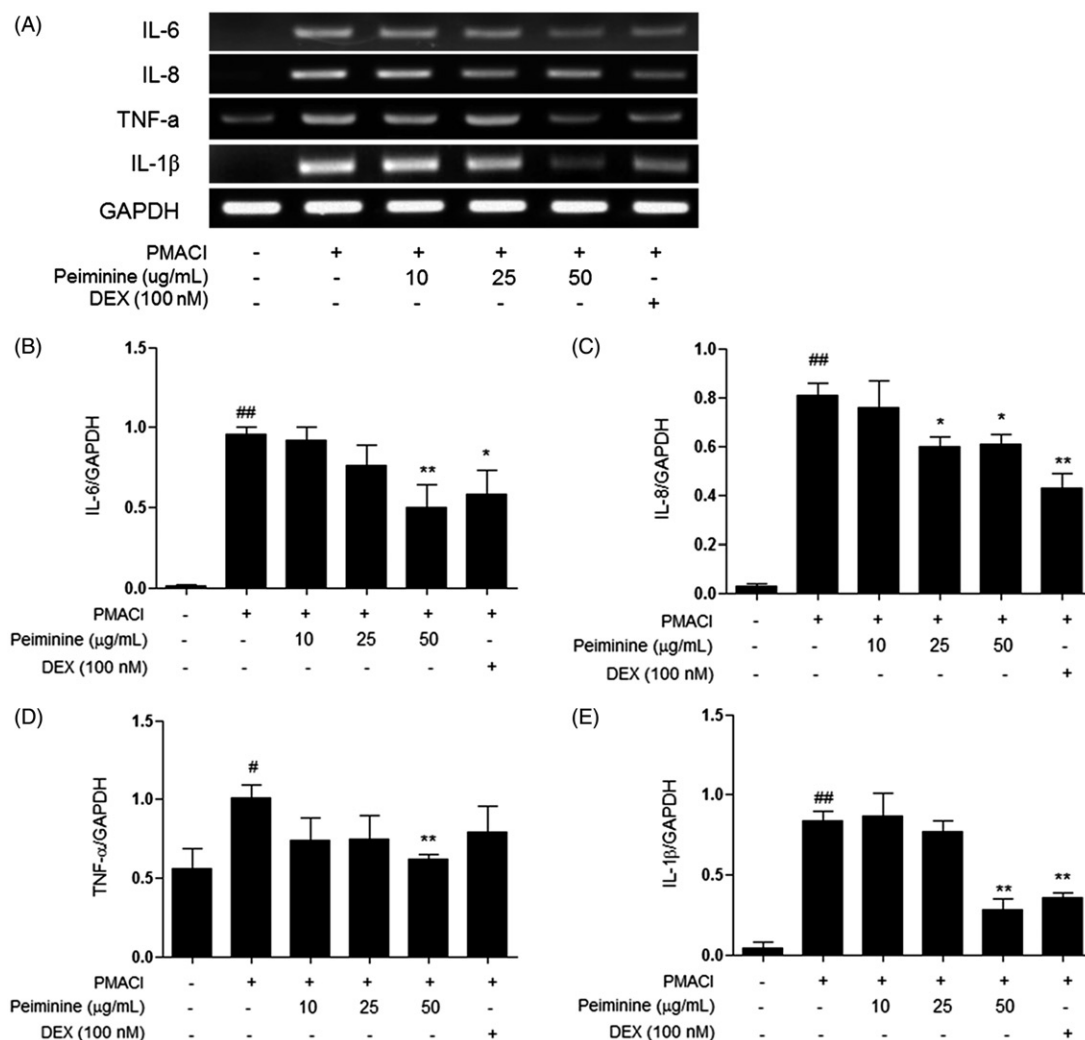


Figure 4. Effects of peiminine on PMACI-induced expression of inflammatory cytokines mRNA in HMC-1 cells (A). Cells were pretreated with peiminine (10, 25 and 50 μg/mL) and DEX at 100 nM for 1 h prior to PMACI stimulation for 6 h. The levels of (B) IL-6, (C) IL-8, (D) TNF-α and (E) IL-1β mRNA expression were measured using Image J. Columns and error bars represent the mean ± SEM for three independent experiments. ##*p* < 0.01 and #*p* < 0.05 significantly different from the control group. \*\**p* < 0.01 and \**p* < 0.05 significantly different from PMACI alone.

phosphorylation of MAPKs, histamine release *in vitro* and PCA reaction *in vivo*.

In this study, peiminine treatment significantly inhibited the production of IL-6, IL-8 and TNF-α in PMACI-stimulated HMC-1 cells. Human mast cells play key roles in the inflammatory reaction by mediating the production of inflammatory cytokines, such as IL-6 and IL-8<sup>18</sup>. IL-6 is released from mast cells and promote a systemic acute-phase reaction if produced in sufficient amounts<sup>19</sup>. TNF-α is released with histamine and other allergic mediators within minutes following allergen exposure and it activates mast cells as well as T cells. Furthermore, TNF-α induces the migration of neutrophils, resulting in the release of IL-8<sup>20</sup>. IL-8 is associated with acute allergic reactions and serves as a major chemotactic factor for lymphocytes and neutrophils<sup>21,22</sup>. In our study, peiminine reduced mRNA expression of IL-6, IL-8, IL-1β and TNF-α in PMACI-induced HMC-1 cells. IL-1β is a member of the IL-1 family and plays a key role in the activation of the cytokine network<sup>22</sup>. IL-1-induced inflammatory activities are involved in IL-6 expression<sup>23</sup>. The result of pro-inflammatory cytokine mRNA suggests that

peiminine has antiallergic effects through the inhibition of cytokine expression in HMC-1 cells.

MAPKs, such as extracellular signal-regulated kinase (ERK), P38 and c-Jun N-terminal kinase (JNK), have been reported to play key roles in the signaling related to cytokine expression<sup>24</sup>. Transcription factors in the cytoplasm or nucleus are phosphorylated on activation of the MAPKs and trigger the expression of target genes, resulting in a biological response<sup>25</sup>. In the present study, peiminine significantly inhibited the expression of P-ERK and P-JNK, but did not affect P-P38. This means that peiminine decreased the inflammatory reaction in HMC-1 associated with the production of inflammatory cytokines by blocking the phosphorylation of ERK and JNK.

NF-κB plays a central role in the inflammation response by controlling the expression of cytokine genes, such as TNF-α, IL-6 and IL-8<sup>26</sup>. A transcription factor, NF-κB regulates the gene of cytokines in allergic inflammation<sup>2</sup>. Phosphorylation and proteolytic degradation of the inhibitory protein IκB-α are required for NF-κB activation<sup>27</sup>. Our results show that peiminine suppressed the nuclear translocation of

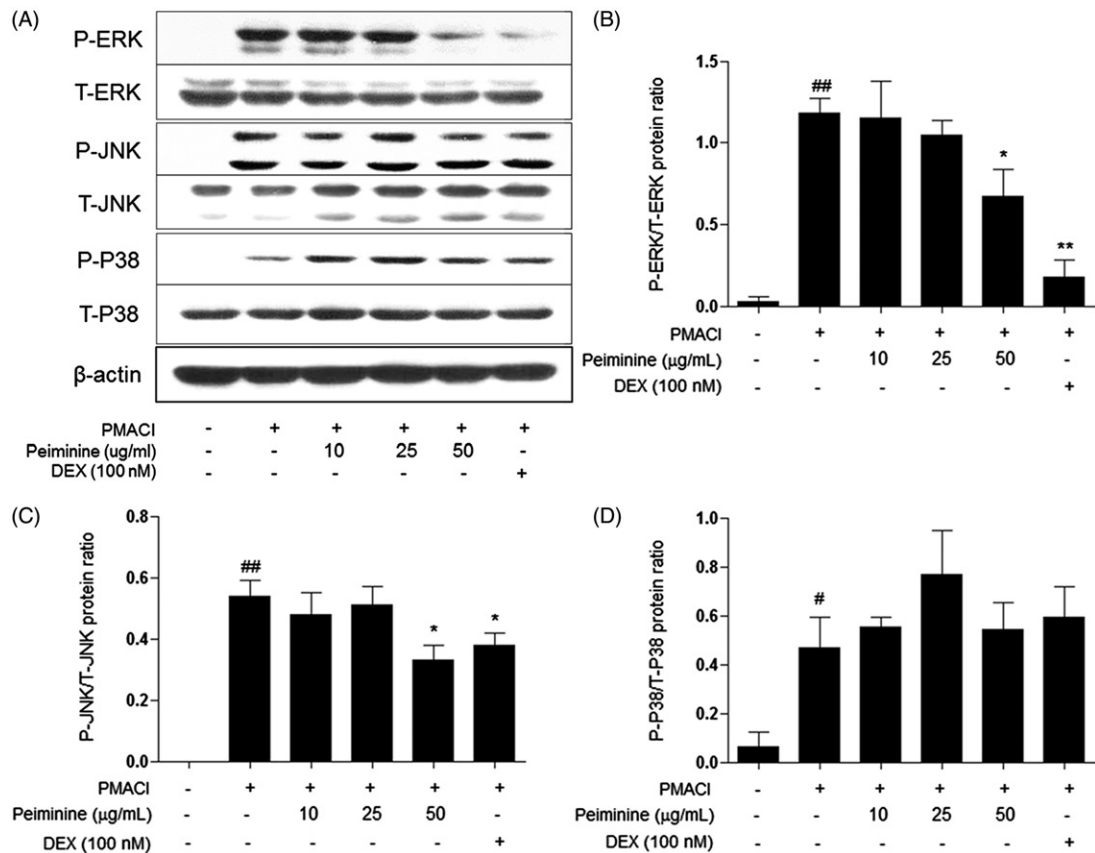


Figure 5. Effect of peiminine on PMACI-induced MAPKs activation in HMC-1 cells presented as protein levels (A). After pretreatment with peiminine for 1 h, HMC-1 cells were stimulated with PMACI for 30 min for MAPK activation. The phosphorylation of MAPKs was analyzed by Western blot. (B–D) The relative levels of expression of the MAPKs were measured using Image J. The columns and error bars represent the mean  $\pm$  SEM for three independent experiments.  $^{##}p < 0.01$  and  $^{\#}p < 0.05$  significantly different from the control group.  $^{**}p < 0.01$  and  $^{*}p < 0.05$  significantly different from PMACI alone.

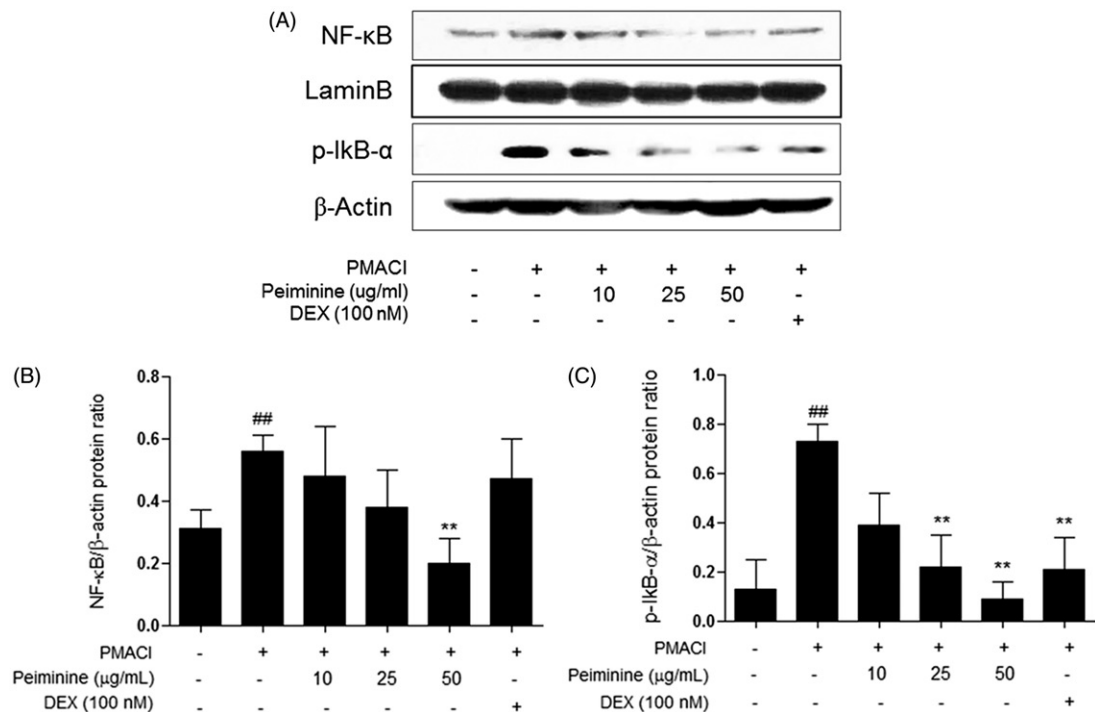


Figure 6. Effect of peiminine on NF- $\kappa$ B p65 activation in the nuclei of PMACI-induced HMC-1 cells. HMC-1 cells were pretreated with peiminine for 1 h and stimulated for 2 h by incubation with PMACI. The relative expression levels of p-I $\kappa$ B- $\alpha$  and NF- $\kappa$ B were measured using Image J (B–C). The columns and error bars represent the mean  $\pm$  SEM for three independent experiments.  $^{##}p < 0.01$  significantly different from the normal group,  $^{**}p < 0.01$  significantly different from PMACI alone.

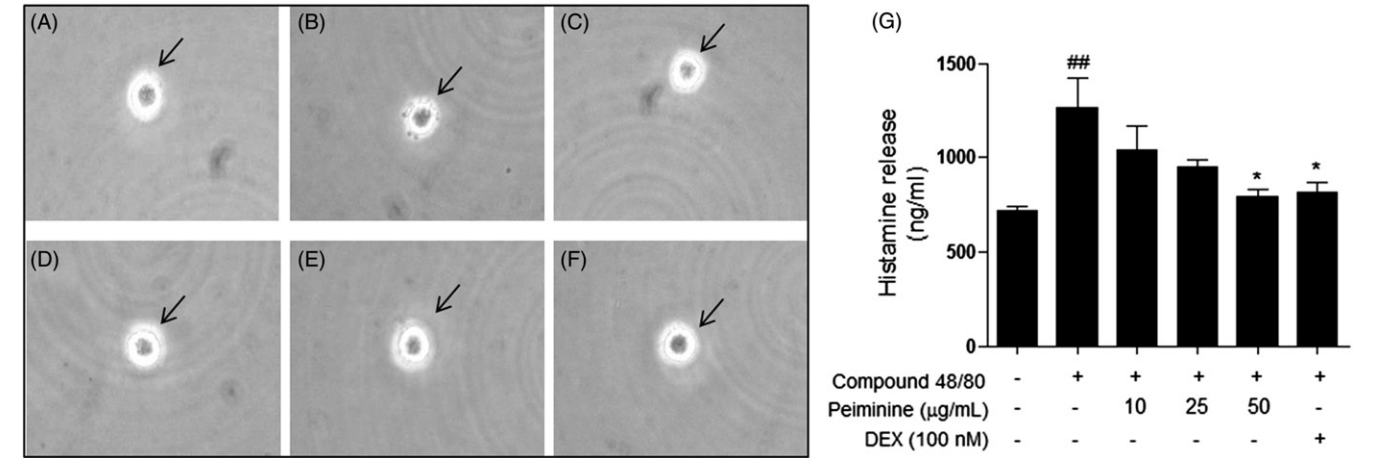


Figure 7. Effects of histamine in compound 48/80-induced HMC-1 cells. (A) Normal HMC-1 cells (arrow). (B) Degranulated HMC-1 cells after stimulation with compound 48/80 (magnification 400×). (C–E) HMC-1 cells with the addition of peiminine (10–50 µg/mL). (F) HMC-1 cells with the addition of 100 nM DEX. (G) Effect of peiminine on compound 48/80-induced histamine release from the degranulated HMC-1 cells. The value represents the mean ± SEM from three independent experiments. ##*p* < 0.01, significantly different from the normal group. \**p* < 0.05 significantly different from compound 48/80 alone.

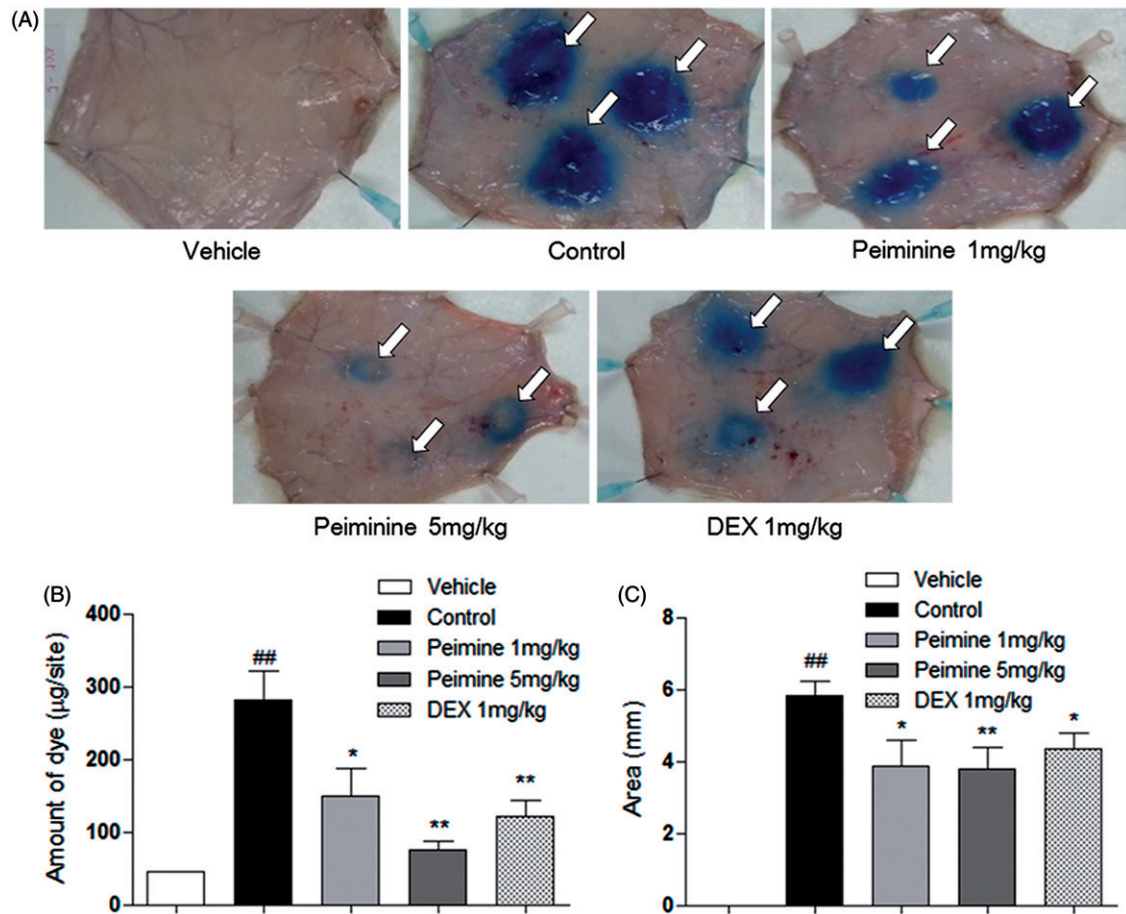


Figure 8. Antiallergic effects of peiminine on passive cutaneous anaphylaxis in rat model. Evans blue on internal surfaces of rat skins with formamide after 72 h. Evans blue-stained skin with peiminine 1 and 5 mg/kg, DEX 1 mg/kg groups appear less intensely stained than the control group. Independent experiments were performed (*n* = 8) and the columns and error bars represent the mean ± SEM. ##*p* < 0.01 significantly different from the normal group. \**p* < 0.05 and \*\**p* < 0.01 significantly different from the control.

NF-κB by inhibiting IκB-α degradation, which would inhibit the expression of TNF-α, IL-6, IL-1β and IL-8 in HMC-1 cells.

Acute allergic reaction is preceded by the release of histamine from mast cells<sup>28</sup> and the increase of capillary

permeability *in vivo*<sup>29</sup>. Histamine induces allergic events, such as anaphylaxis and atopic dermatitis<sup>30</sup>. Histamine is known to have remarkable capacity to control the expression and secretion of cytokines and chemokines<sup>30</sup> including IL-6 and IL-8<sup>31</sup>. The results of our present study suggest that

peiminine has antiallergic effects as it reduced histamine levels in compound 48/80-induced HMC-1 cells degranulation.

PCA reaction is a most useful experimental model for acute hypersensitivity, which can be used to measure anti-anaphylaxis ability *in vivo*<sup>32,33</sup>. Peiminine showed an antiallergic effect against IgE-mediated PCA reaction in rats after oral administration. This result may indicate that peiminine regulates IgE activity and reduces anaphylaxis *in vivo*.

## Conclusion

In conclusion, these results suggest an inhibitory effects for the antiallergic inflammatory activity of peiminine. Peiminine reduces histamine release as well as inflammatory cytokines, such as IL-6, IL-8 and TNF- $\alpha$ , by suppressing NF- $\kappa$ B and regulating MAPKs. Peiminine also had an effect on IgE-dependent anaphylaxis reaction. Especially, peiminine was better than DEX at suppressing the expression of inflammatory cytokines mediated by HMC-1 cells. There is evidence that peiminine could contribute to the protection or treatment of mast cell-associated allergic inflammatory diseases.

## Declaration of interest

The authors declare that there are no conflicts of interest.

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