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Inhibitory Effect of Persicaria perfoliata (L.) H. Gross on IgE Mediated Allergic Responses in RBL-2H3 Cells

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Abstract

Purpose : This study aimed to investigate the anti-allergic effect of Persicaria perfoliata water extract (PPWE) on IgE stimulated rat basophilic leukemia (RBL-2H3) cell line.

Methods : P. perfoliata (L.) H. Gross has been used in traditional medicine as an anti-allergic agent, antipyretic, and diuretic and for respiratory disorders. To analyze the anti-allergic activity of PPWE, release of β -hexosaminidase in RBL-2H3 cells was estimated by enzyme linked immunosorbant assay (ELISA). Also, the cytotoxic effect of PPWE was identified by WST assay, and nuclear factor (NF)-κ B and its upstream signaling molecules were assessed by western blot analysis.

Results : PPWE treatment significantly attenuated β -hexosaminidase release in a dose dependent manner without any cytotoxicity. PPWE inhibited β -hexosaminidase activity by 38.4±1.2, 36.6±0.6, 32.5±2.2 and 26.5±1.2 at 500, 250, 100, and 50 ㎍/㎖ of PPWE, respectively, compared with the control group. In addition, an analysis of the expression level of NF-κ B, an inflammation transcription factor, in RBL-2H3 cells upon IgE stimulation provided reults consistent with the results of β -hexosaminidase release. The phosphorylated status of upstream signaling molecules for transcription factor, mitogen activated protein kinases (MAPKs), was also analyzed. The results showed that PPWE treatment dose-dependently inhibited phosphorylation of extracellular regulatory kinase (ERK) and c-Jun N-terminal kinase (JNK). These results show that PPWE had a strong IgE-mediated degranulation inhibitory effect on RBL-2H3 cells.

Conclusion : P. perfoliata ameliorated IgE-mediated allergic reaction via the modulation of MAPK and NF-κ B signaling pathway in RBL-2H3 cells. These results indicate that P. perfoliata could be a potential candidate for a treatment strategy against various allergic disorders.

Key Words : antiallergic effect, mitogen activated protein kinase, nuclear factor-κ B, Persicaria perfoliata (L.) H. Gross, RBL-2H3

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Ⅰ. Introduction

Allergic response is known for abnormal reactions caused by hypersensitivity of the immune system to foreign bodies that can be allergens, such as drugs, foods, and plant pollens (Averbeck et al., 2007). Among numerous immune cells, mast cells play an important role in IgE-mediated hypersensitivity through the production of various inflammatory mediators including histamine, prostaglandins, and proinflammatory cytokines upon the binding of an antigen to the IgE receptor (Gilfillan & Tkaczyk, 2006). IgE-mediated allergic reaction is classified as Type I among four types of hypersensitivity and leads to allergic asthma, rhinitis, and atopic dermatitis (Jo et al., 2020). In addition, mast cells express affinity for the IgE receptor (Fcε RI) and are able to release several intragranular substances including serotonin, β -hexosaminidase, and histamine when specific allergens are crosslinked with the surface-bound IgE molecules (Hu et al., 2020; Yang et al., 2010). Histamine release from mast cells makes it possible for acute allergic response and inflammatory reactions such as swelling, redness, heat, and pain to be induced. Therefore, inhibiting degranulation could be an efficient strategy for alleviating allergic disorders. The rat basophil leukemia (RBL-2H3) cell line is usually applied to analyze allergic reactions because of its similarity to mast cells and basophils (Liu et al., 2017). For the treatment of allergic disorders, numerous natural resources have been analyzed to investigate their anti-allergic activity. Among them, Persicaria perfoliata (L.) H. Gross has been used as an anti-allergic agent, antipyretic, and diuretic in Korean and Chinese traditional medicine in order to treat numerous disorders, such as respiratory disorders (Branen, 1975). Therefore, the anti-allergic effect of P. perfoliata was analyzed by β -hexosaminidase release and the expression of inflammatory transcription factor, nuclear factor (NF)-κ B, in IgE stimulated RBL-2H3 cells. Based on the results of this study, we intend to use it as a composition for allergy relief and prevention of skin.

Ⅱ. Materials and Methods

1. Reagents

Mouse anti-dinitrophenol monoclonal IgE (anti-DNP IgE) and dinitrophenol-human serum albumin (DNP-HSA) were obtained from Sigma (St. Louis, MO, USA). Antibodies against phospho-p65, phospho-extracellular regulated kinase (p-ERK), ERK, phospho-c-Jun N-terminal kinase (p-JNK), JNK, phospho-p38, p38, actin, and the secondary antibody were purchased from Cell Signaling Technology (Boston, MA, USA).

2. Plant material

P. perfoliata water extract (PPWE) was obtained after adding ten times the amount of water and heating in a double boiler for 4 h. After cooling the extract, PPWE was lyophilized and applied for the experiments.

3. Cell culture

RBL-2H3 cell line was obtained from the Korean Cell Line Bank (KCLB No. 22256, Seoul, Korea) and cultured in Dulbecco's modified Eagle's medium (DMEM, Cytiva, Marlborough, MA, USA) containing 10% fetal bovine serum (FBS, Cytiva) supplemented with penicillin (100 U/㎖) and streptomycin (100 ㎍/㎖).

4. Cell viability assay

Cytotoxic effect of PPWE was evaluated by a WST-1 cell proliferation assay (Daeil Lab. Science, Seoul, Korea). RBL-2H3 cell line was plated on a 96-well plate and treated with various concentrations of PPWE (0, 50, 100, 250, 500 ㎍/㎖) for 24 h. Then the cell line was incubated

with WST-1 for 1 h and absorbance was estimated at OD450 with a multiplate reader (Bio-Rad Laboratory, Hercules, CA, USA)

5. **Mast cell degranulation**

In order to measure β-hexosaminidase release, the RBL-2H3 cell line was cultured on a 12-well plate and then treated with anti-DNP-IgE for 24 h. IgE-sensitized RBL-2H3 cell line was treated with various concentrations of PPWE (0, 50, 100, 250, 500 ㎍/㎖) for 1 h, stimulated with DNP-HSA, and then incubated additionally for 4 h. The culture medium was transferred to new tubes and centrifuged at 13,000 × g for 10 min. Then 25 microliters of supernatant was mixed with 10 mM of 50 ㎕ p-NAG. The reaction was terminated by 0.1 M of sodium carbonate buffer after 1 h incubation at 37 °C. The β-hexosaminidase activity was determined by measurement at 405 nm (MyBioSource, San Diego, CA, USA).

6. **Western blot analysis**

RBL-2H3 cells were pre-incubated with and without various concentrations of PPWE (0, 50, 100, 250, 500 ㎍/㎖) for 2 h and additionally treated with anti-DNP-IgE for 18 h. Cells were washed twice with PBS solution and harvested with protein extract buffer (M-PER, Thermo Fisher Scientific, Waltham, MA, USA). Cells in lysis buffer were incubated for 10 min and centrifuged at 13,000 x g for 10 min to isolate the supernatant. Protein concentration was evaluated by the Bradford assay. Fifty micrograms of sample was loaded and run by an SDS-PAGE system and transferred to a PVDF membrane (Bio-Rad Laboratory). Membranes were blocked and the hybridization reaction was conducted for overnight at 4° C with each primary antibody. After the overnight reaction, horseradish peroxidase-conjugated anti-rabbit IgG was hybridized for 2 h at room temperature. Blots were developed with ECL solution (Thermo Fisher Scientific, Waltham, MA, USA) and the intensity of every target was quantified by the Gel Doc EQ System (Bio-Rad Laboratory).

7. **Statistical analysis**

Statistical significance was analyzed by SPSS ver. 25.0 (SPSS Institute, Chicago IL, USA). The values of the experimental results were presented as the mean value and the standard deviation, the difference between the groups was determined using one-way analysis of variance (ANOVA), and the post-hoc analysis was performed using Duncan's multiple range test. The statistical significance level was presented at $p<.05$.

Ⅲ. Results and Discussion

β-Hexosaminidase activity is commonly used as a marker of mast cell degranulation, which is stimulated by exposure to allegens. Degranulation assay by β-hexosaminidase activity in the RBL-2H3 cell line is applied widely for analysis of anti-allergic activity to study whether new molecules have any inhibitory effect on mast cell degranulation (Jin et al., 2019; Lee et al., 2020). Histamine is secreted with β-hexosaminidase from mast cells and can accelerate allergic inflammation. Recent studies have reported that several flavonoids and plant extracts exhibited an inhibitory effect on mast cell degranulation (Lee & Shim, 2020; Quan et al., 2013; Yoo et al., 2016). To identify the inhibitory effects of PPWE on IgE-mediated β-hexosaminidase release, cells were plated on a 6-well plate and various concentrations of PPWE were treated to investigate the anti-allergic activity. PPWE inhibited β-hexosaminidase activity by 38.4±1.2, 36.6±0.6, 32.5±2.2 and 26.5±1.2 at 500, 250, 100, and 50 ㎍/㎖ of PPWE, respectively, compared with the control group (Fig 1). No cytotoxic effect of PPWE was detected (Fig 2).

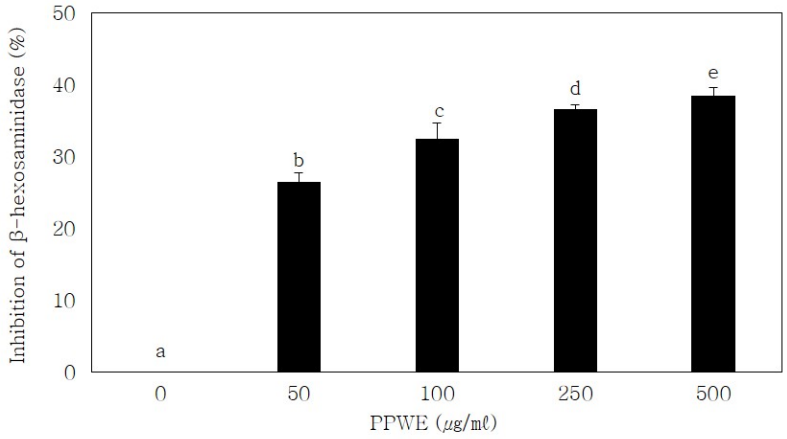

Fig 1. Effect of PPWE on inhibition of β-hexosaminidase production in IgE antigen complex-stimulated RBL-2H3 cell line. The data show the results of triplicate experiments as mean and standard deviation. Values with a different superscript show a significant difference at p<.05 in the results of post-hoc analysis (Duncan's test). PPWE, *P. perfoliata* (L.) H. Gross water extract.

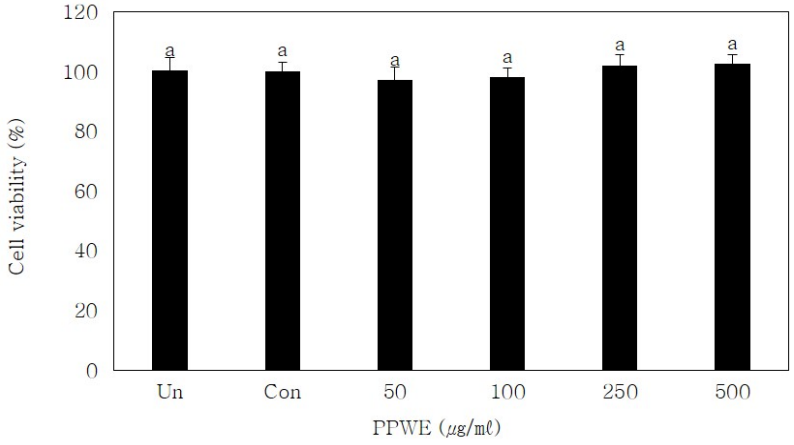

Fig 2. Effect of PPWE on cell viability in RBL-2H3 cell line. The data show the results of triplicate experiments as mean and standard deviation. Values with a different superscript show a significant difference at p<.05 in the results of post-hoc analysis (Duncan's test). PPWE, *P. perfoliata* (L.) H. Gross water extract.

Further investigation was conducted to analyze the effects of PPWE on activation of the enzymes involved in prostaglandin synthesis, including COX-2, in IgE-activated RBL-2H3 cells. PPWE sharply inhibited protein expression level of COX-2 at a concentration of 500 ㎍/㎖ (Fig 3).

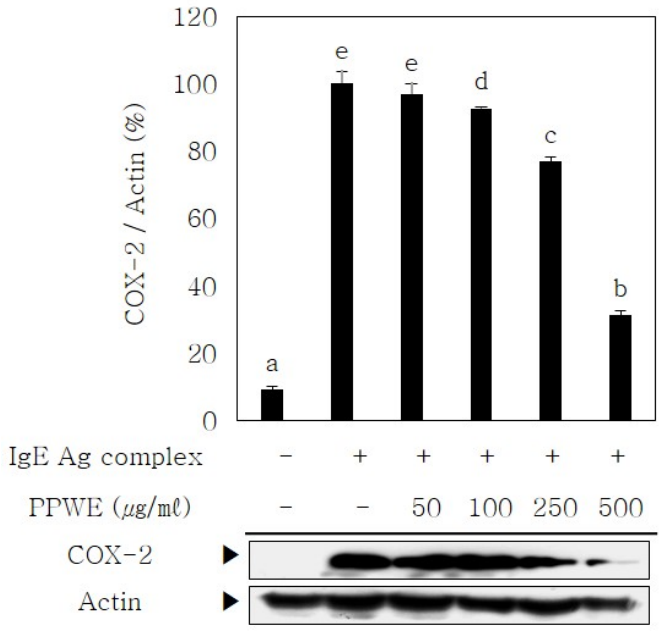

Fig 3. Effect of PPWE on protein expression level of COX-2 in IgE antigen complex-stimulated RBL-2H3 cell line. The protein expression level of COX-2 was measured at indicated concentrations of PWEE (50, 100, 250, 500 μg/mℓ) by western blot analysis. PPWE suppressed COX-2 expression stimulated by IgE antigen complex at a statistically significant level. For induction of COX-2, densitometrically quantified actin was used as an internal control group. The data show the results of triplicate experiments as mean and standard deviation. Values with a different superscript show a significant difference at $p<.05$ in the results of post-hoc analysis (Duncan's test). PPWE, *P. perfoliata* (L.) H. Gross water extract.

Because of its critical role in the regulation of inflammatory cytokines, the phosphorylated status of mitogen activated protein kinases (MAPKs) was estimated by western blot analysis in IgE-stimulated RBL-2H3 cells. To identify whether PPWE can regulate allergic and inflammatory related signaling pathways, the phosphorylation of NF-κ B and MAPKs was analyzed in IgE-stimulated RBL-2H3 cells. In detail, the phosphorylation of p65, one subunit of NF-κ B, ERK, JNK, and p38 MAPK was analyzed in RBL-2H3 cells activated with anti-DNP IgE, and the results showed that PPWE treatment significantly attenuated the phosphorylation levels of NF-κ B, ERK, and JNK MAPK in a dose-dependent manner while there was no effect of PPWE on p38 (Fig 4, 5).

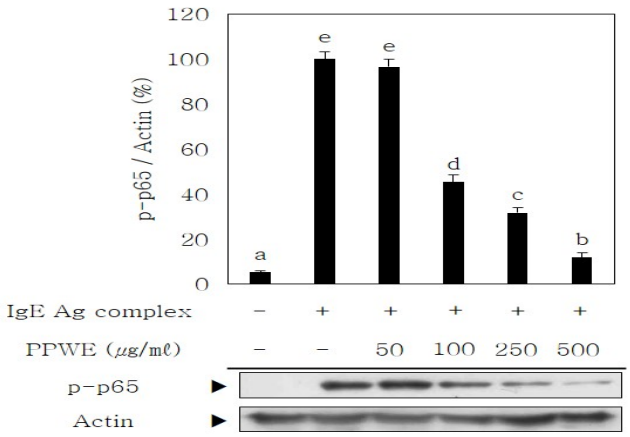

Fig 4. Effect of PPWE on phosphorylation of NF-κB in IgE antigen complex-stimulated RBL-2H3 cell line. The phosphorylation status of p65 was measured at indicated concentrations of PWEE by western blot analysis. The relative induction of p65 was quantified by densitometry. Actin was used as an internal control. The data show the results of triplicate experiments as mean and standard deviation. Values with a different superscript show a significant difference at $p<.05$ in the results of post-hoc analysis (Duncan's test). PPWE, *P. perfoliata* (L.) H. Gross water extract.

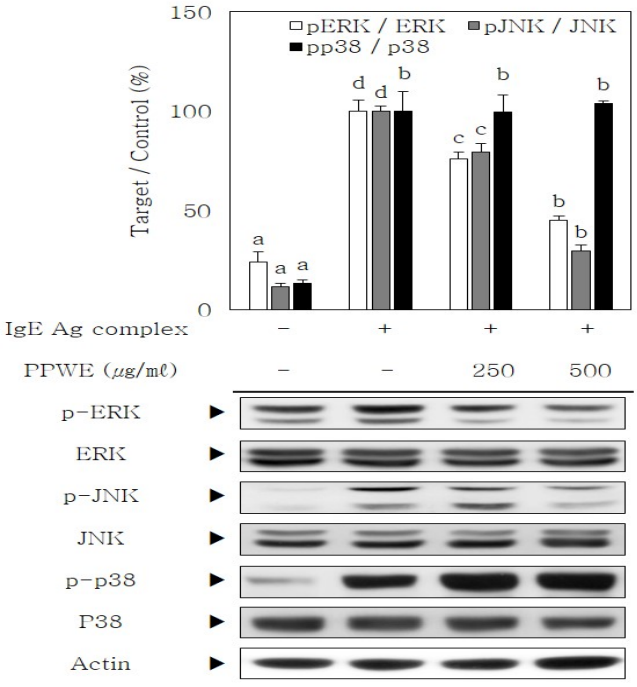

Fig 5. Effect of PPWE on phosphorylation of MAPKs in IgE antigen complex-stimulated RBL-2H3 cell line. The phosphorylation status of MAPKs was measured at indicated concentrations of PWEE by western blot analysis. The relative induction MAPKs was quantified by densitometry. Actin and each control for MAPKs were used as internal controls. The data show the results of triplicate experiments as mean and standard deviation. Values with a different superscript show a significant difference at $p<.05$ in the results of post-hoc analysis (Duncan's test). PPWE, *P. perfoliata* (L.) H. Gross water extract.

Ⅳ. Conclusion

P. perfoliata inhibited β -hexosaminidase activity through the regulation of NF-κ B and MAPKs expression in IgE antigen complex stimulated RBL-2H3 cells. These results indicate that PPWE could be a potential candidate for a treatment strategy against various allergic disorders.

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