Anti-allergic and anti-inflammatory activity of *Phellinus linteus* grown on *Panax ginseng*

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Abstract *Panax ginseng* (PG) or *Phellinus linteus* (PL) have been widely used as traditional medicine owing to their many biological activities, including anti-inflammatory and anti-allergic activities. Previously, our group produced PL that was grown on PG media (PGP) to enhance anti-cancer activities of PGP. Here we studied the anti-allergic activity of PGP and its mechanism of action. The ethyl acetate fraction of PGP exhibited the anti-allergic activity by suppressing β -hexosaminidase release, a marker of degranulation, from antigen/immunoglobulin E (IgE)-stimulated RBL-2H3 cells. Exposure to PGP inhibited the level of antigen/IgE-induced TNF- α in RBL-2H3 cells. It markedly suppressed the phosphorylation of spleen associated tyrosine kinase, GRB2-associated-binding protein 2 (Gab2) and extracellular signal-regulated kinases proteins, which are required for the degranulation and production of pro-inflammatory cytokines. Its anti-inflammatory activity was observed in lipopolysaccharide-stimulated RAW 264.7 cells. In addition, PGP contained higher contents of Rg1 than PG. Our findings suggest that PGP might be developed as a therapeutic agent for IgE-mediated allergic diseases.

Keywords: PGP, anti-allergic activity, anti-inflammatory activity

Introduction

Allergic inflammation causes a myriad of diseases, including allergic asthma, atopic dermatitis, and allergic rhinitis, by altering several physiological features (1). The clinical symptoms of allergic inflammatory diseases are mainly due to the dysregulation of macrophage and mast cell function (1,2). The incidence of these diseases is drastically increasing in many developed countries (3). Currently, glucocorticoids are widely used as anti-inflammatory drugs. However, they are frequently associated with serious side effects such as liver damage, cancer, stroke, and stunted growth; therefore, physicians do not prescribe glucocorticoids for anti-inflammatory therapy (4).

Many research groups have reported that many traditional medicines derived from herbs, medicinal plants, and mushrooms exert anti-inflammatory, anti-allergic, and immunomodulatory activities (5,6). Nowadays, active medicinal components or extracts from natural products are gaining great attention because of their lower risk of side effects compared to current therapies (7). *Panax ginseng* (PG) C.A. Mey (Araliaceae) and *Phellinus linteus* (PL, Berkeley & M. A. Curtis) Teng (Hymenochaetaceae) have been used as traditional herbal medicine in East Asia. Many studies have reported that PG and PL possess biological activity, including anti-inflammatory and anti-allergic activity (8–13).

In our previous study, we produced PL by inoculating its starter

culture on PG media (PGP) to enhance its biological activity, and we also demonstrated the anti-cancer activity of PGP (14). However, the anti-allergic and anti-inflammatory activity of PGP has not been studied to date. Thus, we investigated the anti-allergic and anti-inflammatory activity of PL grown on PGP extract on IgE/antigen (Ag) complex-stimulated RBL-2H3 cells and lipopolysaccharide (LPS)-stimulated RAW 264.7 cells.

Materials and Methods

Preparation of extracts from PL grown on PGP PGP and PG were provided by the Cell Activation Research Institute (CARI, Seongnam, Korea). Authenticated voucher specimens of PG and PGP (kucari 0901 (PG) and kucari 1001 (PGP)) were stored in the herbarium at the College of Bioscience and Biotechnology, Konkuk University (Seoul, Korea). The commercial preparations were done similarly to the original method, as described previously (14). Dried 4-week-old PG (2.2 kg) and PGP (2.2 kg) were ground into a fine powder using a grinder. The powder was extracted with 80% ethanol (38 L) for 24 h at room temperature. After filtration, the extract (166 g) was fractionated sequentially by ethyl acetate (EtOAc) (3 L) and water (3 L) for 48 h at room temperature and then filtered again. The solvent was removed with a rotary evaporator under vacuum. Each

extract was stored at -20°C .

Cell culture Rat basophilic leukemia cells (RBL-2H3, catalog number KCLB no. 22256) were obtained from the ATCC Biological Resource Center (Manassas, VA, USA) and cultured in MEM supplemented with 15% fetal bovine serum (FBS) and 100 U/mL penicillin-streptomycin (Sigma-Aldrich, St. Louis, MO, USA). RAW 264.7 macrophages were purchased at the American Type Culture Collection (Rockville, MD, USA) and cultured in Dulbecco's modified Eagle's medium (DMEM; Invitrogen, Carlsbad, CA, USA) supplemented with 10% FBS and 100 U/mL penicillin-streptomycin (Sigma-Aldrich).

β -Hexosaminidase (β -hex) release assay RBL-2H3 cells (2×10^5 cells/well) were cultured overnight in a medium containing 200 ng/mL of DNP-specific IgE. After washing with PIPES buffer (25 mM PIPES at pH 7.2, 119 mM NaCl, 5 mM KCl, 0.4 mM $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 1 mM CaCl_2 , 5.6 mM glucose, 40 mM NaOH, and 0.1% BSA), the cells were treated with or without PGP or PP2 at the indicated concentrations. After incubation for 30 min at 37°C , the cells were challenged with 200 ng/mL DNP-BSA for 15 min at 37°C . The extent of degranulation was determined by the measurement of the released β -hexosaminidase as previously described (15).

RNA isolation and real-time reverse transcriptase (RT)-PCR RT-PCR was performed as previously described (16). Total RNA was isolated from RBL-2H3 and RAW 264.7 cells using a First Strand cDNA Synthesis kit (Thermo Fisher Scientific, Waltham, MA, USA) and reverse-transcribed using Solg Taq DNA Polymerase (SolGent, Daejeon, Korea), respectively. PCR was performed at 95°C for 2 min, 95°C for 20 s (an elongation step), 58.5°C for 40 s, and 72°C for 45 s for 20 cycles, followed by a final elongation step at 72°C for 5 min (12,17). The following primers were used: rat TNF- α (forward 5'-CAC CAC GCT CTT CTG TCT ACT GAA C-3'); rat TNF- α (reverse 5'-CCG GAC TCC GTG ATG TCT AAG TAC T-3'); rat GAPDH (forward 5'-CAA CAC CAC CAT GGA GAA GGC TG-3'); rat GAPDH (reverse 5'-GAC CAC AGT CCA TGC CAT CAC TG-3'); mouse iNOS (forward 5'-CTT CAA CAC CAA GGT TGT CTG CA-3', reverse 5'-ATG TCA TGA GCA AAG GCG CAG AA-3'), mouse IL-1 β (forward 5'-TAC AAG GAG AAC CAA GCA ACG ACA-3', reverse 5'-TGT CGT TGC TTG GTT CTC CTT GTA-3'), mouse IL-6 (forward 5'-GCC AGA GTC CTT CAG AGA GAG ATA CAG-3', reverse 5'-CCC AAC ATT CAT ATT GTC AG-3'), mouse TNF- α (forward 5'-ATG AGC ACA GAA AGC ATG ATC CG-3', reverse 5'-CCA AAG TAG ACC TGC CCG GAC TC-3'), and mouse GAPDH (forward 5'-GCA AAG TGG AGA TTG TTG CCA TC-3', reverse 5'-CAT ATT TCT CGT GGT TCA CAC CC-3'), obtained from mouse (Cosmo Genetech, Seoul, Korea). iNOS, IL-1 β , IL-6, and TNF- α mRNA levels were normalized relative to the GAPDH mRNA level.

Western blot analysis Cells were lysed with ice-cold lysis buffer containing protease inhibitors (Sigma-Aldrich). The protein concentration was measured with the Bradford protein assay reagent (Bio-Rad

Laboratories, Hercules, CA, USA). Equal amounts of proteins were loaded into each well, separated by 10% SDS-PAGE, and blocked in 5% non-fat milk. Samples were probed with the following primary antibodies: phospho-spleen tyrosine kinase (Syk), phospho-Gab2, phospho-extracellular signal-related kinases (ERK1/2) (Santa Cruz Biotechnology, Santa Cruz, CA, USA), and β -actin (Santa Cruz Biotechnology). Secondary antibodies used were anti-goat IgG- or anti-rabbit IgG-peroxidase conjugates (Amersham, Little Chalfont, UK), and the membranes were incubated with horseradish peroxidase-labeled secondary antibody (Santa Cruz Biotechnology). The protein bands were analyzed by western blotting using LI-COR Odyssey (LI-COR Biosciences Inc., Lincoln, NE, USA).

NO assay Cells were plated and treated with EtOAc fraction for 1 h, followed by treatment with LPS (1 $\mu\text{g/mL}$, Sigma-Aldrich). Supernatants were reacted with Griess solution (1% sulphanilamide in 5% phosphoric acid and 0.1% naphthylethylenediamine dihydrochloride in water). The absorbance at 540 nm was determined using an enzyme-linked immunosorbent assay plate reader (Bio-Tek, Winooski, VT, USA).

Cytokine profiling Levels of IL-6 and TNF- α cytokines in the culture supernatant were analyzed as previously described (18). Cytokines in cell culture supernatant (50 μL /well) were measured with Luminex multiplex assays (Life Technologies, Frederick, MD, USA) and then read on a Luminex 100 System (Panomics, Fremont, CA, USA). The median fluorescence intensity from at least 100 beads of each type (per sample) was used to determine the cytokine intensity levels. Standard curves were plotted and fitted using a 5-parameter logistic model (Bio-Plex 200; Bio-Rad Laboratories) and used to calculate the cytokine concentrations of the samples.

HPLC analysis HPLC analysis was conducted on an Agilent 1260 HPLC system (Agilent Technologies, Santa Clara, CA, USA). The separation was performed on a 250 mm $\times$ 4.6 mm i.d., 5 μm , Ultrasphere C18 column (Alltech, Deerfield, IL, USA). Distilled water (solvent A) and MeCN (solvent B) were used as the mobile phase, with a flow rate of 1.0 mL/min. Gradient elution began with 82% solvent A and 18% solvent B. Elution was then changed to 70% A for 20 min, 70% A for 10 min, 55% A for 18 min, 50% A for 25 min, and finally 82% A for 5 min. The detection wavelength was set to 203 nm. Regression equations of ginsenosides Rb1 and Rg1 were prepared using standard solutions with different concentrations. The sample solutions were prepared by dissolving 1 g of PGP or PG in 5 mL methanol. For the sample assay, 40 μL of 0.2 g/mL PGP or PG solution was injected, and the assay was repeated three times. All solutions were filtered through Millex 0.45-mm nylon membrane syringe filters (Millipore Co., Bedford, MA, USA) before use.

Statistical analysis Results are expressed as the mean $\pm$ standard deviation (SD). Statistical analyses were performed using Student's *t*-

test in the Excel program (Microsoft, Redmond, WA, USA). Significant differences were considered at $p < 0.05$.

Results and Discussion

PGP inhibits β -hexosaminidase release in IgE/Ag-induced RBL-2H3 cells Mast cells and basophils play a key role in IgE and Ag-mediated allergic reactions (1). To investigate the anti-allergic activity of PGP, we checked its effects on the degranulation of IgE/Ag-stimulated RBL-2H3 cells. The amount of β -hexosaminidase released reflects the degree of degranulation (19). As shown in Fig. 1A, exposure to PGP inhibited β -hex release from IgE/Ag-stimulated RBL-2H3 cells. Exposure to PGP did not affect cell viability (Fig. 1B).

PGP inhibits the expression of pro-inflammatory cytokines in IgE/Ag-stimulated RBL-2H3 cells Mast cells and basophils secrete pro-inflammatory cytokines such as TNF- α , thus mediating allergic reactions (16). TNF- α is a potent inflammatory cytokine (20). To further elucidate the inhibitory effect of PGP on IgE/Ag-stimulated mast cell function, we investigated whether PGP could affect the level of TNF- α mRNA expression in Ag-stimulated RBL-2H3 cells. Exposure to PGP significantly suppressed the TNF- α gene expression of IgE/Ag complex-stimulated RBL-2H3 cells (Fig. 2).

PGP reduces the level of signaling molecules responsible for mast cell activation Activation of Fc ϵ RI signaling events results in degranulation and production of pro-inflammatory cytokines within mast cells. Syk and Syk downstream molecules (e.g., Gab2, LAT, and PLC γ) are involved in Fc ϵ RI signaling pathways. PP2, a general Src-family kinase inhibitor, blocks the phosphorylation of Syk (20). Fc ϵ RI receptor signaling is dependent on the activation of Syk. Phosphorylated tyrosine residues on Syk act as docking sites for adaptor molecules, including Gab2, to propagate downstream signaling (21). Activated Syk-dependent signaling events rapidly induce intracellular calcium levels and promote mast cell degranulation. Therefore, Syk is a key target in developing anti-allergic drugs. To elucidate this mechanism,

Fig. 1. Effect of PGP on degranulation and cell viability of IgE/Ag-stimulated RBL-2H3 cells. (A) Degranulation was determined by the amount of released β -hex in RBL-2H3 cells. The RBL-2H3 cells were treated in the presence or absence of PGP (* $p < 0.05$, ** $p < 0.01$ vs. negative control, and ## $p < 0.01$ vs. control). (B) RBL-2H3 cell viability was measured using the Cell Counting Kit-8 (CCK-8) assay. RBL-2H3 cells (2×10^4 cells/well) were treated in the presence or absence of PGP for 24 h. Each value represents the mean $\pm$ SD of three experiments.

we investigated whether PGP affects the levels of activated Syk, Gab2, and ERK1/2 proteins. The levels of activated Syk, Gab2, and

Fig. 2. Effect of PGP on pro-inflammatory cytokines in IgE/Ag-stimulated RBL-2H3 cells. IgE-primed RBL-2H3 cells were stimulated with 200 ng/mL of DNP-BSA (Ag). Total RNA was isolated and then reverse-transcribed. TNF- α mRNA levels were determined by quantitative RT-PCR. The representative images from three independent experiments are shown. Each value represents the mean $\pm$ SD of three experiments (* $p < 0.05$ vs. control, ** $p < 0.01$ vs. negative control).

Fig. 3. PGP inhibited the activation of Syk, Gab2, and ERK proteins in IgE/Ag-stimulated RBL-2H3 cells. Cell lysates were prepared as described in the materials and methods section. Total cell lysates were analyzed by immunoblotting with anti-phosphorylated Syk and anti-Syk, anti-phosphorylated Gab2 and anti-Gab2, anti-phosphorylated ERK and anti-ERK, and anti-actin antibodies, respectively. PP2 is a general Src-family kinase inhibitor. The representative images from three independent experiments are shown. Each value represents the mean ± SD of three experiments ([#] $p < 0.05$ vs. control and ^{*} $p < 0.05$ vs. negative control).

ERK1/2 proteins were significantly reduced by PGP in a dose-dependent manner (Fig. 3). These results indicate that PGP blocks FcεRI-mediated signaling pathway in IgE/Ag-complex-stimulated RBL-2H3 cells.

PGP inhibits the production of NO and pro-inflammatory cytokines in LPS-induced RAW 264.7 cells The macrophage response to internal or external stimuli and factors can exacerbate chronic inflammation and allergic symptoms (22). Excessive production of NO and pro-inflammatory cytokines can trigger an inflammatory reaction (18,23). The cytotoxic effects of PGP were tested in RAW 264.7 macrophages. As shown in Fig. 4B, PGP showed no cytotoxic effects in RAW 264.7 macrophages at concentrations of up to 200 μg/mL. Next, we tested the inhibitory activity of PGP on NO production. PGP inhibited NO production in LPS-induced RAW 264.7 macrophages (Fig. 4A). The anti-inflammatory effect of the PGP extract was examined by measuring the level of pro-inflammatory cytokine mRNA and its released amounts in the media. PGP

treatment reduced the expression of LPS-induced IL-6 and TNF-α mRNA (Fig. 4C). In ELISA experiments, pretreatment of PGP significantly inhibited the secreted amount of LPS-induced IL-6 and TNF-α cytokines in a dose-dependent manner (Fig. 4D).

Different composition profiles of PG and PGP total extract It was reported that Rg1 and Rb1 in PG have anti-inflammatory and immuno-stimulatory activity (24,25). The ginsenoside contents of PG and PGP were determined by HPLC. The content of Rg1 was greater in PGP than in PG (Table 1).

In conclusion, PGP suppressed degranulation and production of pro-inflammatory cytokines in IgE/Ag-stimulated RBL-2H3 cells and NO release in LPS-stimulated RAW 264.7 cells. PGP inhibited the activation of FcεRI receptor signaling molecules including Syk, Gab2, and ERK that led to allergic responses. Our findings suggest that PGP may be a potential therapeutic candidate against IgE-mediated allergic diseases.

Fig. 4. Effect of PGP on LPS-induced NO production and pro-inflammatory cytokines of RAW 264.7 cells. (A) Cells were pretreated with the indicated concentrations of PGP for 2 h and then stimulated with LPS (1 μg/mL) for 18 h. As an indicator of NO production, nitrite levels in the supernatant fraction were measured using the Griess reaction. Data are expressed as the mean±SD. (** p <0.01, *** p <0.001 vs. LPS, and ### p <0.001 vs. control). The iNOS mRNA level was determined by quantitative RT-PCR. Each value represents the mean±SD of three experiments ([#] p <0.05 vs. control and * p <0.05 vs. negative control). (B) RAW 264.7 cells (2×10^4 cells/well) were treated in the presence or absence of PGP for 24 h. Each value represents the mean±SD of three experiments (* p <0.05 vs. control). (C) TNF-α and IL-6 mRNA levels were determined by quantitative RT-PCR. The representative images from three independent experiments are shown. Each value represents the mean±SD of three experiments ([#] p <0.05 vs. control and ** p <0.01 vs. negative control). (D) The amounts of cytokines in culture media were assessed by multiplex ELISA technology. Each value represents the mean±SD of three experiments ([#] p <0.05 vs. control and * p <0.05 vs. negative control).

Table 1. Different composition profiles of PG and PGP total extract

Components	PG	PGP
Rg1	113±66.6 ppm ¹⁾	186±2 ppm
Rb1	1,983±16.8 ppm	27±3 ppm

¹⁾Data are presented as the mean±SD ($n=3$)

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References

- Modena BD, Dazy K, White AA. Emerging concepts: Mast cell involvement in allergic diseases. *Transl. Res.* 174: 98-121 (2016)
- Chen Y, Xian Y, Lai Z, Loo S, Chan WY, Lin ZX. Anti-inflammatory and anti-allergic effects and underlying mechanisms of Huang-Lian-Jie-Du extract: Implication for atopic dermatitis treatment. *J. Ethnopharmacol.* 185: 41-52 (2016)
- Oh JY, Choi WS, Lee CH, Park HJ. The ethyl acetate extract of *Cordyceps militaris* inhibits IgE-mediated allergic responses in mast cells and passive cutaneous anaphylaxis reaction in mice. *J. Ethnopharmacol.* 135: 422-429 (2011)
- Chae HS, Song HH, Kim YM, Lee HK, Oh SR, Chin YW. *Euphorbia supina* inhibits inflammatory mediators in mouse bone marrow-derived mast cells and macrophages. *Int. Immunopharmacol.* 29: 966-973 (2015)
- Park DK, Park HJ. Ethanol extract of *Cordyceps militaris* grown on germinated soybeans attenuates dextran-sodium-sulfate- (DSS-) induced colitis by suppressing the expression of matrix metalloproteinases and inflammatory mediators. *BioMed. Res. Int.* 2013: 102918 (2013)
- Mollah ML, Park DK, Park HJ. *Cordyceps militaris* grown on germinated soybean induces G2/M cell cycle arrest through downregulation of Cyclin B1 and Cdc25c in human colon cancer HT-29 cells. *Evid-Based Compl. Alt.* 2012: 249217 (2012)
- Recio MC, Andujar I, Rios JL. Anti-inflammatory agents from plants: Progress and potential. *Curr. Med. Chem.* 19: 2088-2103 (2012)
- Lee EJ, Song MJ, Kwon HS, Ji GE, Sung MK. Oral administration of fermented red ginseng suppressed ovalbumin-induced allergic responses in female BALB/c mice. *Phytomedicine* 19: 896-903 (2012)
- Choi YH, Yan GH, Chai OH, Lim JM, Sung SY, Zhang X, Kim JH, Choi SH, Lee MS, Han EH, Kim HT, Song CH. Inhibition of anaphylaxis-like reaction and mast cell activation by water extract from the fruiting body of *Phellinus linteus*. *Biol. Pharm. Bull.* 29: 1360-1365 (2006)
- Jang KJ, Choi SH, Yu GJ, Hong SH, Chung YH, Kim CH, Yoon HM, Kim GY, Kim BW, Choi YH. Anti-inflammatory potential of total saponins derived from the roots of *Panax ginseng* in lipopolysaccharide-activated RAW 264.7 macrophages. *Exp. Ther. Med.* 11: 1109-1115 (2016)
- Huang SS, Deng JS, Lin JG, Lee CY, Huang GJ. Anti-inflammatory effects of trillinolein from *Panax notoginseng* through the suppression of NF-kappa B and MAPK expression and proinflammatory cytokine expression. *Am. J. Chinese Med.* 42: 1485-1506 (2014)
- Kim HG, Yoon DH, Lee WH, Han SK, Shrestha B, Kim CH, Lim MH, Chang W, Lim S, Choi S, Song WO, Sung JM, Hwang KC, Kim TW. *Phellinus linteus* inhibits inflammatory mediators by suppressing redox-based NF-kappa B and MAPKs activation in lipopolysaccharide-induced RAW 264.7 macrophage. *J. Ethnopharmacol.* 114: 307-315 (2007)
- Lin CJ, Lien HM, Chang HY, Huang CL, Liu JJ, Chang YC, Chen CC, Lai CH. Biological evaluation of *Phellinus linteus*-fermented broths as anti-inflammatory agents. *J. Biosci. Bioeng.* 118: 88-93 (2014)
- Park HJ, Han ES, Park DK. The ethyl acetate extract of PGP (*Phellinus linteus* grown on *Panax ginseng*) suppresses B16F10 melanoma cell proliferation through inducing cellular differentiation and apoptosis. *J. Ethnopharmacol.* 132: 115-121 (2010)
- Lee JH, Kim JW, Kim DK, Kim HS, Park HJ, Park DK, Kim AR, Kim BK, Beaven MA, Park KL, Kim YM, Choi WS. The Src-family kinase Fgr is critical for activation of mast cells and IgE-mediated anaphylaxis in mice. *J. Immunol.* 187: 1807-1815 (2011)
- Park DK, Choi WS, Park HJ. Antiallergic activity of novel isoflavone methylglycosides from *Cordyceps militaris* grown on germinated soybeans in antigen-stimulated mast cells. *J. Agr. Food Chem.* 60: 2309-2315 (2012)
- Debnath T, Hasnat MA, Pervin M, Lee SY, Park SR, Kim DH, Kweon HJ, Kim JM, Lim BO. Chaga mushroom (*Inonotus obliquus*) grown on germinated brown rice suppresses inflammation associated with colitis in mice. *Food Sci. Biotechnol.* 21: 1235-1241 (2012)
- Park HJ, Han ES, Park DK, Lee C, Lee KW. An extract of *Phellinus linteus* grown on germinated brown rice inhibits inflammation markers in RAW264.7 macrophages by suppressing inflammatory cytokines, chemokines, and mediators and up-regulating antioxidant activity. *J. Med. Food* 13: 1468-1477 (2010)
- Shimoda H, Tanaka J, Yamada E, Morikawa T, Kasajima N, Yoshikawa M. Anti type I allergic property of Japanese butterbur extract and its mast cell degranulation inhibitory ingredients. *J. Agr. Food Chem.* 54: 2915-2920 (2006)
- Klemm S, Ruland J. Inflammatory signal transduction from the Fc epsilon RI to NF-kappa B. *Immunobiology* 211: 815-820 (2006)
- Wong BR, Grossbard EB, Payan DG, Masuda ES. Targeting Syk as a treatment for allergic and autoimmune disorders. *Expert. Opin. Inv. Drug.* 13: 743-762 (2004)
- Hong JY, Chung YT, Steenrod J, Chen Q, Lei J, Comstock AT, Goldsmith AM, Bentley JK, Sajjan US, Hershenson MB. Macrophage activation state determines the response to rhinovirus infection in a mouse model of allergic asthma. *Respir. Res.* 15: 63 (2014)
- Bauer SM, Roy A, Emo J, Chapman TJ, Georas SN, Lawrence BP. The effects of maternal exposure to bisphenol A on allergic lung inflammation into adulthood. *Toxicol. Sci.* 130: 82-93 (2012)
- Gao Y, Chu SF, Li JW, Li JP, Zhang Z, Xia CY, Heng Y, Zhang MJ, Hu JF, Wei GN, Li YT, Chen NH. Anti-inflammatory function of ginsenoside Rg1 on alcoholic hepatitis through glucocorticoid receptor related nuclear factor-kappa B pathway. *J. Ethnopharmacol.* 73: 231-240 (2015)
- Takeda K, Okumura K. Interferon-gamma-mediated natural killer cell activation by an aqueous *Panax ginseng* extract. *Evid-Based Compl. Alt.* 2015: 603198 (2015)