Contents lists available at ScienceDirect

Journal of Ginseng Research

Research article

Korean Red Ginseng affects ovalbumin-induced asthma by modulating IL-12, IL-4, and IL-6 levels and the NF-κB/COX-2 and PGE$_2$ pathways

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ARTICLE INFO

ABSTRACT

Background: Asthma is an incurable hyper-responsive disease of the pulmonary system that is caused by various allergens, including indoor and outdoor stimulators. According to the Global Asthma Network, 339 million people suffered from asthma in 2018, with particularly severe forms in children. Numerous treatments for asthma are available; however, they are frequently associated with adverse effects such as growth retardation, neurological disorders (e.g., catatonia, poor concentration, and insomnia), and physiological disorders (e.g., immunosuppression, hypertension, hyperglycemia, and osteoporosis).
Methods: Korean Red Ginseng has long been used to treat numerous diseases in many countries, and we investigated the anti-asthmatic effects and mechanisms of action of Korean Red Ginseng. Eighty-four BALB/c mice were assigned to 6 treatment groups: control, ovalbumin-induced asthma group, dexamethasone treatment group, and 3 groups treated with Korean Red Ginseng water extract (KRGWE) at 5, 25, or 50 mg/kg/day for 5 days. Anti-asthmatic effects of KRGWE were assessed based on biological changes, such as white blood cell counts and differential counts in the bronchoalveolar lavage fluid, serum IgE levels, and histopathological changes in the lungs, and by examining anti-asthmatic mechanisms, such as the cytokines associated with Th1, Th2, and Treg cells and inflammation pathways.
Results: KRGWE affected ovalbumin-induced changes, such as increased white blood cell counts, increased IgE levels, and morphological changes (mucous hypersecretion, epithelial cell hyperplasia, inflammatory cell infiltration) by downregulating cytokines such as IL-12, IL-4, and IL-6 via GATA-3 inactivation and suppression of inflammation via NF-κB/COX-2 and PGE$_2$ pathways.
Conclusion: KRGWE is a promising drug for asthma treatment.

1. Introduction

The Global Asthma Network reported that in 2018, 339 million people suffered from asthma, which is an important health disorder in children [1]. The main symptoms of asthma are very diverse; however, they are always associated with the pulmonary system and typically range from simple dyspnea, such as wheezing, irregular breathing, or coughing, to apnea [2], which, in severe

cases, may be fatal. Airflow obstruction is associated with morphological changes such as hypersecretion of mucous in bronchial duct, hyperplasia of epithelial cell in bronchial duct, infiltration of inflammation-related cells near bronchioalveolar ducts and vessels, and airway remodeling [3,4].

Asthma is a chronic pulmonary hyperresponsiveness [5] and can be caused by various allergens that consist of two classes: (1) indoor stimulators such as pet dander and house mite dust and (2) outdoor ones such as pollutants, chemicals, and cold temperatures, among others [6,7]. Allergens induce cytokine modulation, including that of Th1-related factors (IFN-γ and IL-12) [8,9] and Th2-related cytokines (IL-4, IL-5, and IL-13) [10−12] through Th17-related ones (IL-6 and TNF-α) [13,14]; asthma is then considered the result of an imbalance of Th1 and Th2 cells [15,16]. The physiological balance of Th1 and Th2 lymphocytes may be shifted due to asthma, which causes Th2-related factors such as IL-4, IL-5, and IL-13 to increase [17]. GATA-3 is a transcription factor for Th2 cells, and IL-4 modulates GATA-3 activation via positive feedback mechanisms [18].

In the initiation step, various cytokines can be released, which then induce inflammation in the respiratory system [19,20]. There are two main pathways of phlogogenesis, the leukotriene pathway and the prostaglandin pathway. In the prostaglandin pathway, NF-κB and COX-2 are important intermediate factors, and prostaglandin can be increased via the NF-κB/COX-2 pathway [21]. In patients suffered by asthma, this is one of the crucial strategies to control inflammation in order to reduce the occurrence of asthma and its severity.

According to global statistics, asthma occurs predominantly in the younger population [1], and although corticosteroids such as dexamethasone are typically used for treatment, they may exert several adverse effects such as growth retardation [22]. Asthma medication treatments are generally associated with numerous side effects such as catatonia, lack of concentration, insomnia, immunosuppression, hypertension, hyperglycemia, and osteoporosis, among others [23]. For this reason, the development of more effective and safer anti-asthmatic drugs has attracted considerable research attention.

Korean Red Ginseng (*Panax ginseng* Meyer) is one of important traditional medicines [24], and recent studies reported effects of ginseng compounds on physiological functions, including immunity [25], circulation [26], and inflammation [27], and diseases, such as allergies [28], cancer [29], metabolic diseases [30], and neurodegenerative diseases [31]. In the present study, therapeutic effects and mechanisms of action of Korean Red Ginseng were assessed using an ovalbumin (OVA)-induced asthma model.

2. Materials and methods

2.1. Korean Red Ginseng water extract (KRGWE)

As previously reported, KRGWE (Korea Ginseng Corporation, Daejeon, South Korea) was prepared using the roots of *P. ginseng*, which contained compounds such as Rb1 (5.89 mg/g), -Rb2 (2.30 mg/g), -Rc (2.78 mg/g), -Rd (0.92 mg/g), -Re (1.16 mg/g), -Rf (1.00 mg/g), -Rg1 (0.96 mg/g), -Rg2s (1.42 mg/g), -Rg3r (1.16 mg/g), -Rg3s (2.41 mg/g), and -Rh1 (0.96 mg/g) [32].
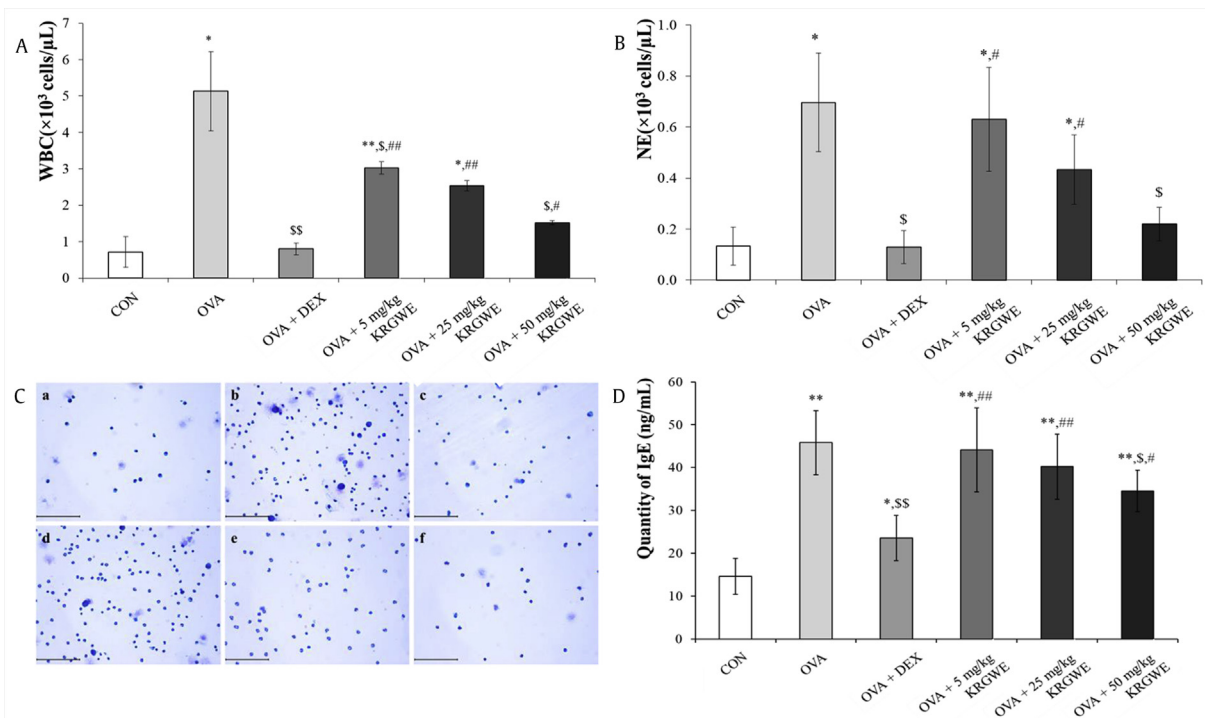

Fig. 1. **Dose-dependent effect of Korean Red Ginseng water extract (KRGWE) on white blood cells (WBCs), neutrophils, inflammatory cells in bronchoalveolar lavage fluid, and serum IgE** (A) Effect of KRGWE on WBC counts; (B) dose-dependent effect of KRGWE on the levels of neutrophils, which were increased by ovalbumin treatments; (C) KRGWE significantly downregulated inflammatory cells to levels similar to those in the DEX treatment group; (D) KRGWE dose-dependently decreased the levels of serum IgE; a: control group; b: ovalbumin-treated group; c: 1 mg/kg/day DEX treated group after ovalbumin treatment; d: 5 mg/kg/day KRGWE-treated group after ovalbumin treatment; e: 25 mg/kg/day KRGWE-treated group after ovalbumin treatment; f: 50 mg/kg/day KRGWE-treated group after ovalbumin treatment. N = 6. Magnification 400-fold. Scale bar indicates 100 μm. Statistical significance is indicated as follows: *$p < 0.05$ vs. control group; **$p < 0.01$ vs. control group; $^{\$} p < 0.05$ vs. ovalbumin-treated group; $^{\$\$} p < 0.01$ vs. ovalbumin-treated group; $^{\#}p < 0.05$ vs. DEX treated group; $^{\#\#}p < 0.01$ vs. DEX treated group
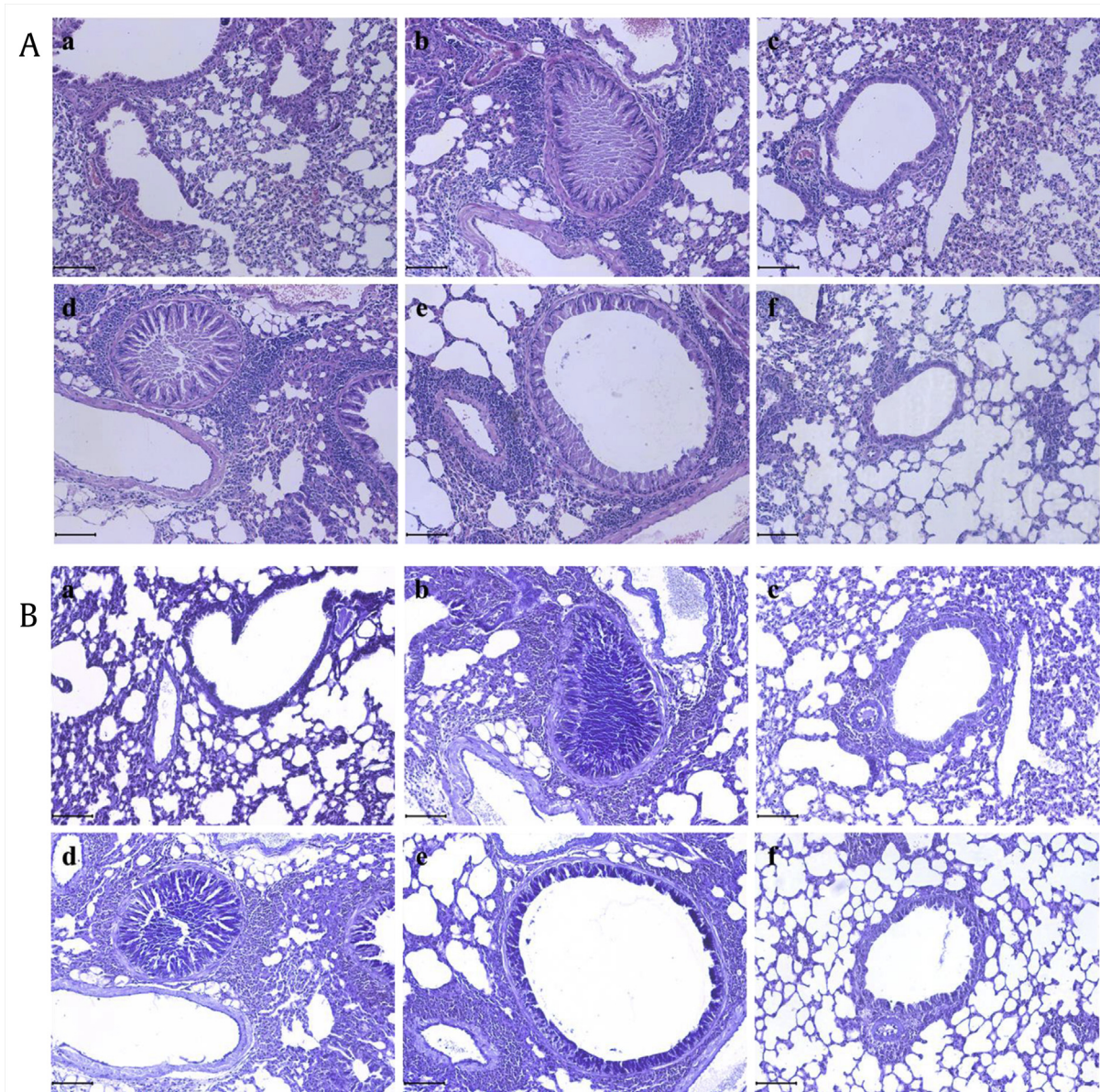
Fig. 2. Effect of Korean Red Ginseng water extract (KRGWE) on pulmonary morphological changes. (A) KRGWE blocked ovalbumin-induced morphological changes in the lungs such as mucous hypersecretion, epithelial cell hyperplasia, inflammatory cells infiltration, etc. (hematoxylin and eosin staining); (B) KRGWE markedly suppressed ovalbumin-induced mucous hypersecretion in the bronchioalveolar duct (periodic acid Schiff staining); a: control group; b: ovalbumin-treated group; c: 1 mg/kg/day DEX treated group after ovalbumin treatment; d: 5 mg/kg/day KRGWE-treated group after ovalbumin treatment; e: 25 mg/kg/day KRGWE-treated group after ovalbumin treatment; f: 50 mg/kg/day-KRGWE treated group after ovalbumin treatment. N = 8. Magnification 200-fold. Scale bar indicates 100 μm. Statistical significance is indicated as follows: $*p < 0.05$ vs. control group; $**p < 0.01$ vs. control group; $^{\$}p < 0.05$ vs. ovalbumin-treated group; $^{\$\$}p < 0.01$ vs. ovalbumin-treated group; $^{\#}p < 0.05$ vs. DEX treated group; $^{\#\#}p < 0.01$ vs. DEX treated group

2.2. Animal experiments

The animal experiments were conducted in two replicates, using 84 female BALB/c mice (Samtako Korea, Osan, South Korea). Animals were assigned to 6 treatment groups of 7 individuals, each: (1) control (tap water), (2) asthma group by ovalbumin-induction, (3) positive control receiving an asthma drug treatment (dexamethasone [DEX] 1 mg/kg/day, for 5 days) with ovalbumin treatment, and three ovalbumin-treated groups receiving KRGWE for 5 days at either 5 (4), 25 (5), or 50 mg/kg/day (6). After 7 days of acclimation, all mice apart from the control individuals were intraperitoneally injected with 20 μg ovalbumin (Sigma-Aldrich, St.

Louis, MO, USA) and 1 mg aluminum hydroxide hydrate (Sigma-Aldrich) in 500 μL saline on day 1 and day 8. From day 21 to day 25, all animals were exposed to 5% ovalbumin for 0.5 h using NE-U17 (3 mL/min, OMRON Co. Ltd., Kyoto, Japan) on a daily basis in the mornings and afternoons.

2.3. Ethics statement

All animal experiments were conducted under approval from IACUC of Chonnam National University (CNU IACUC-YB-2017-04).

Table 1
Dose-dependent effects of Korean Red Ginseng water extract (KRGWE) on ovalbumin-induced morphological changes in lung tissue. KRGWE suppressed pulmonary morphological changes caused by ovalbumin treatments

	Mucous hypersecretion (0-3)	Epithelial cell hyperplasia (0-3)	Inflammatory cell infiltration (0-3)
CON	0.1 ± 0.14	0.3 ± 0.32	0.4 ± 0.29
OVA	$2.6 \pm 0.43^{**}$	$2.6 \pm 0.49^{**}$	$2.6 \pm 0.42^{**}$
OVA+DEX	$0.6 \pm 0.26^{*,\$\$}$	$0.8 \pm 0.50^{\$\$}$	$0.8 \pm 0.50^{\$}$
OVA+5 mg/kg KRGWE	$2.4 \pm 0.25^{**,\#\#}$	$2.4 \pm 0.38^{**}$	$2.5 \pm 0.46^{**,\#\#}$
OVA+25 mg/kg KRGWE	$1.9 \pm 0.26^{**,\$,\#\#}$	$1.7 \pm 0.44^{**,\$,\#}$	$1.3 \pm 0.42^{*,\$}$
OVA+50 mg/kg KRGWE	$0.7 \pm 0.42^{*,\$\$}$	$0.5 \pm 0.36^{\$\$}$	$0.8 \pm 0.25^{\$\$}$

The means $\pm$ standard deviation (N = 8) are shown; statistical significance is indicated as follows:$^{*}p < 0.05$ vs. control group; $^{**}p < 0.01$ vs. control group; $^{\$} p < 0.05$ vs. ovalbumin-treated group; $^{\$\$} p < 0.01$ vs. ovalbumin-treated group; $^{\#} p < 0.05$ vs. DEX treated group; $^{\#\#} p < 0.01$ vs. DEX treated group

2.4. Bronchoalveolar lavage fluid (BALF) and serum analysis

BALF and serum analyses were conducted as described previously [17]. Mice were anesthetized by 50 mg/kg Zoletil (Virbac, Carros, France) intraperitoneal injection, and a flexible plastic mouse feeding needle was cannulated into the trachea to apply 0.4 mL phosphate buffered saline to the lung. Fluid was collected 3 times and was then centrifuged for 5 min at 900 g (Sorvall Legend Micro 17R, Thermo Fisher Scientific, Waltham, MA, USA). To produce differential cell counts, cells were resuspended using 80 μL phosphate buffered saline. White blood cell (WBC) and differential cell counts were produced using a Hemavet Multi-Species Hematology System (Drew Scientific Inc., Waterbury, CT, USA). In order to analyze the change of inflammatory cells in the BALF collected fluid was centrifuged again and stained using the Kwick-Diff kit (Thermo Fisher Scientific). Serum concentrations of IgE were analyzed using an IgE enzyme-linked immunosorbent assay kit (ELISA; #555248; BD Bioscience, San Jose, CA, USA) according to the manufacturer's instructions.

2.5. Histopathological analyses

In order to evaluate the morphological changes in the lung histological evaluations were done as described previously [17]. Collected lung was fixed in 10% (v/v) formaldehyde, dehydrated using a gradient ethanol (80%, 85%, 90%, 95%, and 99.9%), xylene, and embedded in paraffin. Paraffin blocks were then cut longitudinally in 4-μm sections (LEICA RM2125 RTS, Leica Microsystems Inc. Buffalo Grove, IL, USA), and lung tissue was stained either by hematoxylin and eosin staining to assess morphological changes or by periodic acid Schiff (PAS) staining to examine glycoproteins. In order to stain with PAS paraffined sections were deparaffinized and hydrated. Sections were oxidized in 0.5% periodic acid solution, rinsed with distilled water, were immersed in Schiff reagent, and counterstained with hematoxylin. The pathological level was scored from 0 (none) to 3 (severe) based on the representative pulmonary changes such as mucous hypersecretion (0, none; 1, little mucous releasing; 2, half packed mucous in whole duct; 3, packed mucous), epithelial cell hyperplasia (0, none; 1, corrugated wall; 2, folded epithelium; 3, severe folded epithelium), and inflammatory cell infiltration (0, none; 1, few leukocytes; 2, moderate number of leukocytes; 3, large number of leukocytes).

2.6. Immunofluorescence analyses

In order to evaluate activation of specific proteins, such as T helper cell transcription factors (T-bet for Th1 cells and GATA-3 for Th2 cells) and NF-κB/COX-2 for inflammation pathways, immunofluorescence analyses were conducted on individuals of four groups: control, OVA treatment, OVA-induced DEX treatment, and OVA-induced 50 mg/kg KRGWE treatment. Before the antibody binding step, the same materials detailed in the immunohistochemical analyses were used, in addition to T-bet (Biorbyt, orb7075, Cambridge, UK), GATA-3 (OriGene, TA305795, Rockville, MD, USA), NF-κB (ThermoFisher Scientific), or COX-2 (Invitrogen, PA1-9032, Carlsbad, CA, USA), which were used as primary antibodies for incubation at room temperature for 1 h. All of them were incubated with FITC-conjugated anti-rabbit IgG for 2 h (#315-095-003; Jackson Immunoresearch, West Grove, PA, USA) or Alexa Fluor 555-conjugated anti-goat IgG (ThermoFisher Scientific), and cells were counterstained using 4′,6-diamidino-2-phenylindole (DAPI; ThermoFisher Scientific).

Images were produced using a K1-Fluo confocal microscope (Nanoscope System, Daejeon, South Korea), and the fluorescence intensity was measured.

2.7. ELISA

ELISAs were conducted as described previously [18]. To analyze the levels of IL-12p40, IL-4, and IL-6 in lung tissue, OptEIA mouse ELISAs were purchased from BD Biosciences. All assays were conducted according to the manufacturer's instructions. Lung samples were incubated in lysis buffer containing a protease inhibitor cocktail and RIPA buffer (Thermo Fisher Scientific). Lung tissue aliquots from individuals of all groups were weighed and homogenized in lysis buffer, followed by centrifugation at 6,500 g for 15 min. Supernatants were collected and absorbance at 450 nm was measured using a microplate reader (EZ Read 400, Biochrom, Cambourne, UK).

2.8. Immunohistochemical (IHC) analyses

IHC analyses were conducted as previously described [33]. To remove endogenous peroxidases, tissue sections were placed in 3% hydrogen peroxide methanol for 10 min, and the antigen was retrieved in sodium citrate buffer (0.1M). To avoid unspecific binding, all slides were incubated with normal horse serum, were then incubated with rabbit anti-mouse PGE$_2$ primary antibodies (1:100, bs-2639R, Bioss, MA, USA) for 1 h and were incubated again for 10 min with biotinylated secondary antibodies (PK-7800; Vector Laboratories, Burlingame, CA, USA) and horseradish peroxidase-conjugated streptavidin. Measurements were conducted using 3,3-diaminobenzidine tetrahydrochloride substrate chromogen solution and counterstaining with Mayer's hematoxylin.

2.9. Statistical analyses

Results are shown as means $\pm$ standard deviation. Treatment effects were tested using one-way analyses of variance, followed by Dunnett's multiple comparison tests. Statistical significance is reported at $P < 0.05$.
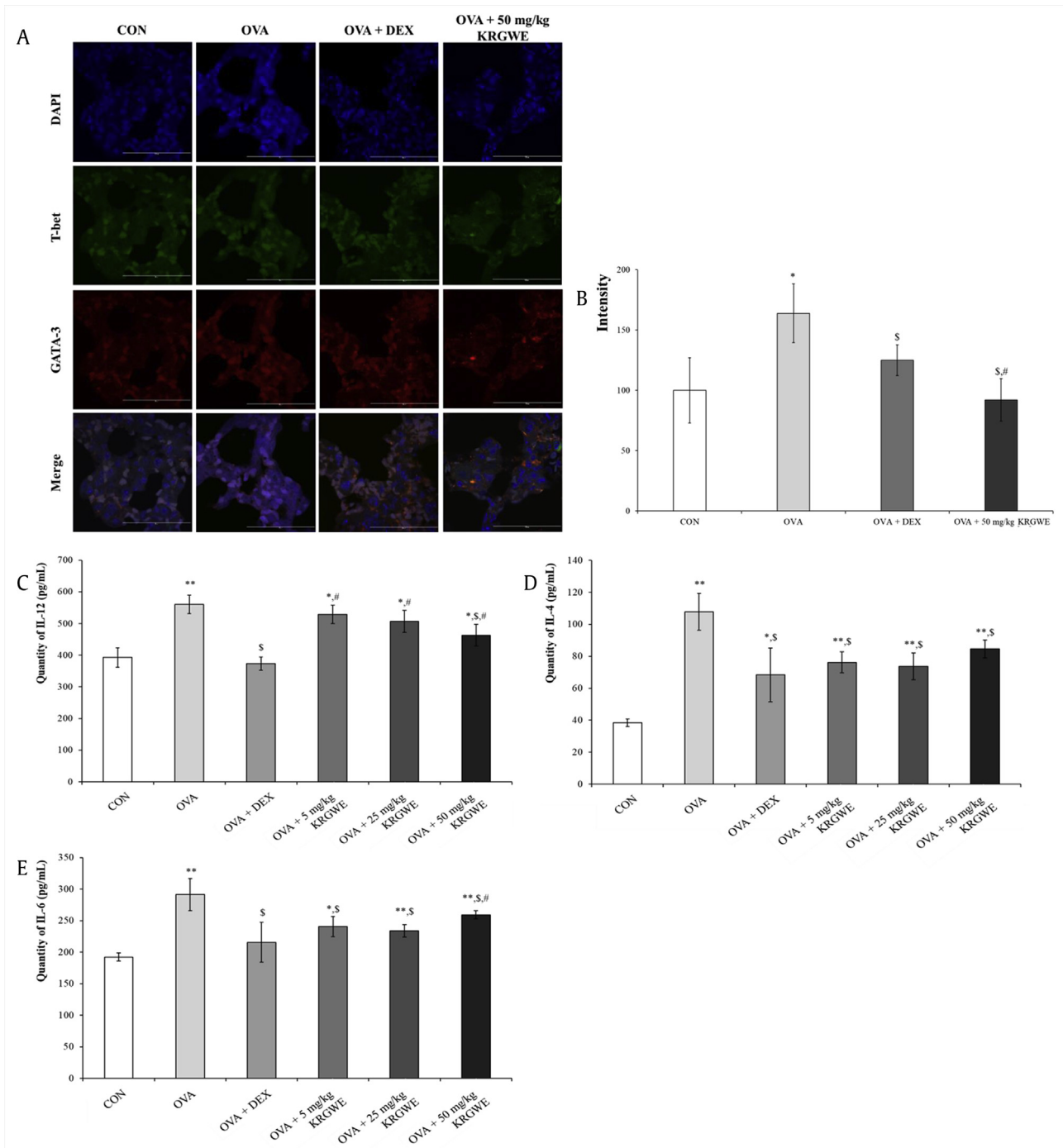
Fig. 3. Effect of Korean Red Ginseng water extract (KRGWE) on cytokines IL-12, IL-4, and IL-6. (A) KRGWE suppressed Th1 cell transcription factor T-bet and Th2 cell transcription factor GATA-3. KRGWE dose-dependently controlled the level of asthma-related cytokines such as (B) IL-12, (C) IL-4, and (D) IL-6, which were increased by ovalbumin treatment. N = 8. Magnification 1000-fold. Scale bar indicates 50 μm. Statistical significance is indicated as follows: *$p < 0.05$ vs. control group; **$p < 0.01$ vs. control group; $^$ $p < 0.05$ vs. ovalbumin-treated group; $^#$ $p < 0.05$ vs. DEX treated group

3. Results

3.1. Dose-dependent effects of KRGWE on populations of WBCs and inflammatory cells in BALF and on serum IgE

OVA treatments increased the numbers of WBCs (Fig. 1A), neutrophils (Fig. 1B), and inflammatory cells (Fig. 1C) in BALF and the concentrations of serum IgE (Fig. 1D). As shown in Fig. 1A and B, KRGWE significantly reduced the numbers of WBCs and

neutrophils in BALF, which were increased in the OVA treatment group. In order to compare changes in inflammatory cells, Kwick-Diff staining was conducted, and the population of inflammatory cells in the 50 mg/kg KRGWE treatment group was similar to that in the 1 mg/kg DEX treatment group (Fig. 1C). IgE is one of the most important biomarkers for testing hyperresponsiveness disorders such as asthma [34], and KRGWE treatment showed dose-dependent decrease of serum IgE concentrations, which were increased in the OVA treatment group (Fig. 1D).
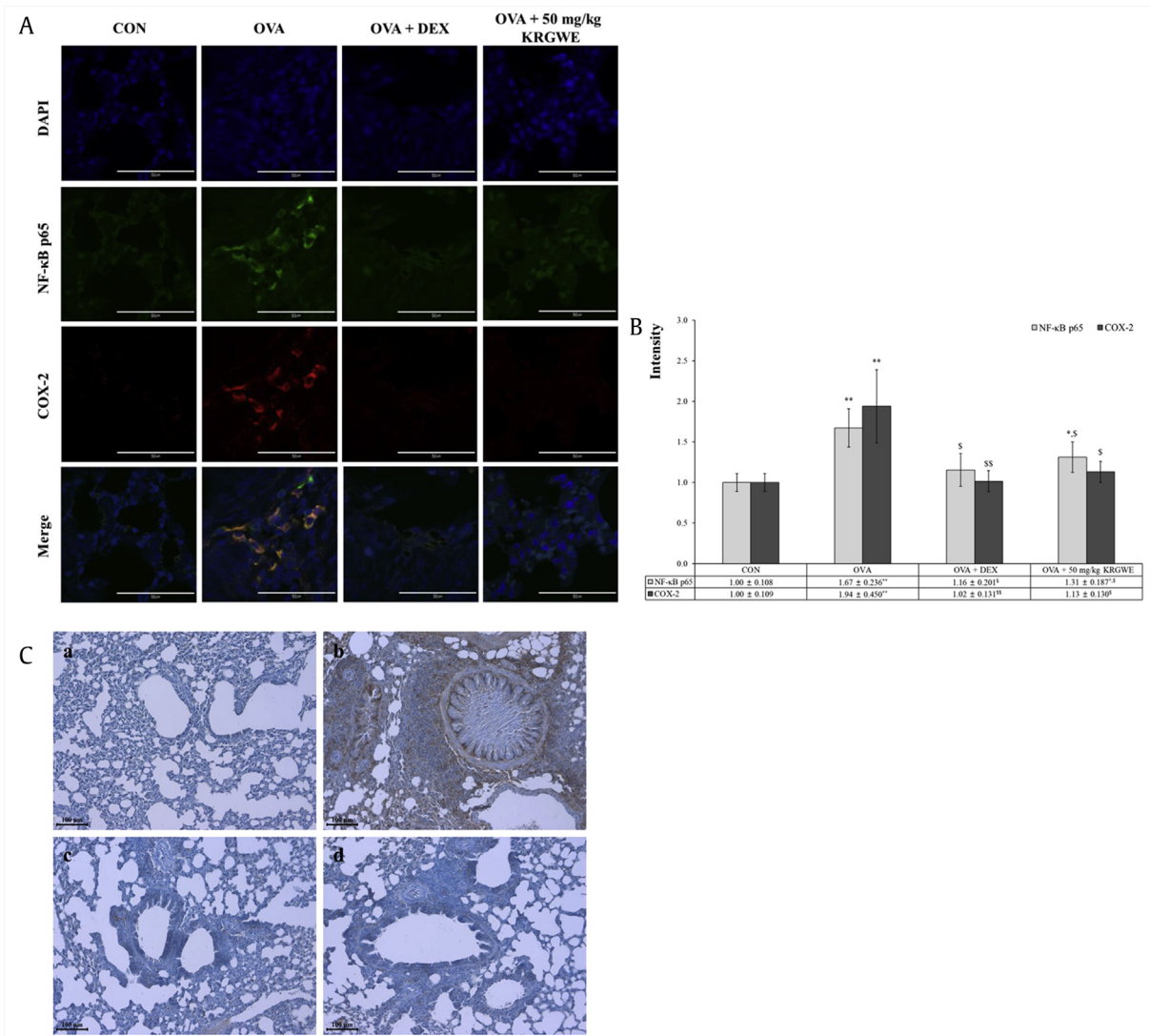
Fig. 4. Effect of Korean Red Ginseng water extract (KRGWE) on translocation of NF-κBp65 from the cytoplasm to the nucleus. (A) KRGWE significantly inhibited the level of COX-2 expression in the cytoplasm through NF-κB translocation from the cytoplasm to the nucleus where it would act as a transcription factor of COX-2. Magnification 1000-fold. Scale bar indicates 50 μm. (B) According to the respective fluorescence, KRGWE downregulated NF-κB translocation and COX-2 expression. (C) KRGWE significantly suppressed PGE_2 expression, which was elicited by ovalbumin treatments. Magnification 200-fold. Scale bar indicates 100 μm; a: control group; b: ovalbumin-treated group; c: 1 mg/kg/day DEX treated group after ovalbumin treatment; d: 50 mg/kg/day KRGWE-treated group after ovalbumin treatment. Statistical significance is indicated as follows: *$p < 0.05$ vs. control group; **$p < 0.01$ vs. control group; $^\$ p < 0.05$ vs. ovalbumin-treated group; $^{\$\$} p < 0.01$ vs. ovalbumin-treated group

3.2. Effects of KRGWE on morphological changes

Morphological changes such as mucous hypersecretion, epithelial cell hyperplasia, or inflammatory cell infiltration occur in the respiratory system of asthma patients [35], and they are the most important biomarkers of asthma. Compared to lung tissues of controls (Fig. 2A.a and 2B.a), OVA-treated mice showed morphological changes such as mucous hypersecretion (2.6 ± 0.43; p < 0.01; Table 1), epithelial cell hyperplasia (2.6 ± 0.49; p < 0.01; Table 1), and inflammatory cell infiltration (2.6 ± 0.42; p < 0.01; Table 1) in proximity to bronchioalveolar ducts and vessels (Fig. 2A.b and 2B.b). KRGWE treatments reduced respiratory morphological changes caused by OVA treatment in a dose-dependent manner, including mucous hypersecretion (2.4 ± 0.25 → 0.7 ± 0.42), epithelial cell hyperplasia (2.4 ± 0.38 → 0.5 ± 0.36), and inflammatory cell infiltration (2.5 ± 0.46 → 0.8 ± 0.25; Fig. 2A.d–2A.f and Fig. 2B.d–2B.f; Table 1). No differences were observed in the morphological change

scores on epithelial cell hyperplasia and inflammatory cell infiltration between the control (0.3 ± 0.32 and 0.4 ± 0.29, respectively) and the 50 mg/kg KRGWE treatment group (0.5 ± 0.36 and 0.8 ± 0.25, respectively) (Table 1).

3.3. Effects of KRGWE on IL-12, IL-4, and IL-6 expression

Asthma is a hyperresponsive disease of the pulmonary tract and is caused by an imbalance of Th1-related factors and Th2-related factors [16,36]. In the OVA-induced asthma group, T-bet and GATA-3 occurred in the nucleus, whereas in the other groups, they were found in the cytoplasm, including the 50 mg/kg KRGWE treatment group (Fig. 3A). KRGWE treatment blocked T-bet and GATA-3 activation as transcription factors through translocation from the cytoplasm to the nucleus. KRGWE dose-dependently suppressed IL-12 expression (Fig. 3B) and reduced the levels of IL-4 and IL-6 in all KRGWE treatments (Fig. 3C and D).

3.4. Effects of KRGWE on NF-κB/COX-2 and PGE₂ pathways

Asthma is strongly associated with allergic inflammation [20], and reducing the occurrence of inflammation may also reduce asthma severity. OVA treatment increased NF-κB translocation from the cytoplasm to the nucleus, where it is activated as a transcription factor for COX-2, compared to the control, and it enhanced COX-2 synthesis in the cytoplasm (Fig. 4A). However, the 50 mg/kg KRGWE treatment reduced NF-κB translocation and prevented COX-2 expression. As shown in Fig. 4B, the 50 mg/kg KRGWE treatment prevented COX-2 synthesis (1.13 ± 0.130), compared to OVA treatment (1.94 ± 0.450; $p < 0.05$), and no difference in COX-2 expression between the 50 mg/kg KRGWE treatment and the control was observed. Compared to the OVA treatment group, the 50 mg/kg KRGWE treatment suppressed PGE₂ expression (Fig. 4C).

4. Discussion

Asthma is an incurable chronic pulmonary disease [37], and most medications only alleviate the symptoms. Drugs for asthma treatment have a lot of adverse effects such as growth retardation [22], neurological disorders (e.g., catatonia, lack of concentration, and insomnia), and physiological disorders (e.g., immunosuppression, hypertension, hyperglycemia, and osteoporosis) [23]. Therefore, numerous studies have been conducted to identify safer and more effective anti-asthma drugs [4,8,13,17,18,28]. Korean Red Ginseng is known to affect various aspects of physiology, such as immunity [25], circulation [26], and inflammation [27], and diseases, such as allergy [28], cancer [29], metabolic diseases [30], and neurodegenerative diseases [31]. In asthma patient severe airway blockage was observed and this symptom was caused by airway remodeling which is related with epithelial cell and goblet cell hyperplasia, mucous hypersecretion by goblet cells, etc [3,4].

Numerous hypotheses regarding the pathogenesis of asthma have been suggested; however, the exact mechanism remain unclear. An imbalance in Th1 and Th2 cells is considered one of the most important causes [16,36]. T helper cells can be categorized as Th1, Th2, and T17 cells [38], and in asthma patients, Th2-related factors are increased but Th1-related factors are not [16,35]. IFN-γ and IL-12 are associated with Th1-related cytokines, and IL-4, IL-5, and IL-13 belong to Th2-related cytokines. The function of T17 cell-related factors such as TNF-α and IL-6 is to modulate expression of Th1-/Th2-related factors [39] and to gather chemo-attractive neutrophils and eosinophils [40]. IFN-γ is the important cytokine affecting asthma as it exerts a positive feedback control function on T-bet [41] and downregulation of IgE [42]. IL-12 can contribute to regulating asthma by inhibiting Th2-related factor proliferation and by promoting IFN-γ production [43,44]. IL-4 is a regulator of the positive feedback effects of GATA-3 and increases the levels of IgE and eosinophil proliferation [45−47]. IL-5 is strongly associated with the modulation of the eosinophil life cycle [44], and IL-13 induces morphological changes such as airway remodeling in the pulmonary system of asthma-suffered patients [12]. IL-6 stimulates IgE expression and T helper cell modulation [48], and TNF-α modulates airway hyperresponsiveness through interactions of mast cells and smooth muscle [49].

Phylogonesis is associated with leukotriene and prostaglandin pathways, and asthma occurrence and severity is correlated with inflammatory processes in the pulmonary system. NF-κB and COX-2 belong to one of the major pathways of prostaglandin synthesis [21], and blocking NF-κB and COX-2 pathways is one of the important strategies to control inflammation. NF-κB is the transcription factor for COX-2 protein synthesis, and in order to act as a transcription factor, it must be translocated from the cytoplasm to

the nucleus. Synthesized COX-2 protein stimulates the release of prostaglandin, which may lead to severe inflammation.

We evaluated the anti-asthmatic effects of Korean Red Ginseng and investigated the underlying mechanisms. We measured the regulatory effects of Korean Red Ginseng on OVA-induced morphological changes in the pulmonary system. KRGWE prevented morphological changes induced by OVA treatments, and the respiratory system of the 50 mg/kg KRGWE treatment group was similar to that in control group (Table 1, Fig. 2). And we evaluated the modulatory effect of KRGWE on Th1-/Th2−Th17-related cytokines, and noted that it suppressed IL-12 expression and controlled the expression of IL-4 and IL-6 in a dose-dependent manner (Fig. 3B−D) through inhibiting T-bet and GATA-3 (Fig. 3A). KRGWE inhibited GATA-3 translocation from the cytoplasm to the nucleus where it would act as a Th2 cell transcription factor and then control Th2-related cytokine IL-4, and it restored the balance of Th1- and Th2-related factors via Treg-related cytokine IL-6. Especially IL-6 modulated the balance of Th1/Th2 and down-regulated the level of IgE. KRGWE effectively blocked NF-κB translocation, which was induced by the OVA treatment and then controlled COX-2 expression (Fig. 4A and B). Regarding the results of NF-κB/COX-2 pathway control by KRGWE, PGE₂ synthesis was entirely suppressed (Fig. 4C).

5. Conclusions

KRGWE prevented OVA-induced morphological changes in the pulmonary system, such as mucous hypersecretion, epithelial cell hyperplasia, and inflammatory cell infiltration, by restoring the balance of Th1-/Th2-related cytokines including transcription factors such as T-bet and GATA-3, and IL-12, IL-4, and IL-6, and it blocked inflammatory pathways such as NF-κB/COX-2 and PGE₂.

Data availability

All data will be made available upon reasonable request.

Conflicts of interest

The authors declare that there is no conflict of interest.

Acknowledgement

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jgr.2020.10.001.

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