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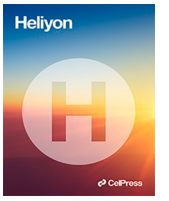

Research article

Anti-inflammatory and anti-allergic effects of Cheonwangbosim-dan water extract: An *in vitro* and *in vivo* study

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ABSTRACT

Ethnopharmacological relevance: Cheonwangbosim-dan is a traditional herbal prescription that is widely used to improve or treat physical and mental illnesses in East Asian countries.
Aim of the study: The aim of the present study was to investigate the preventive and protective effects of a Cheonwangbosim-dan water extract (CBDW) against allergic inflammation using *in vitro* and *in vivo* models.
Materials and methods: BEAS-2B and MC/9 cells were treated with various concentrations of CBDW and stimulated with different inducers of inflammatory mediators. The production of various inflammatory mediators was subsequently evaluated. BALB/c mice were sensitized and challenged by repeated application of ovalbumin (OVA). CBDW was administered by oral gavage once daily for 10 consecutive days. We assessed the number of inflammatory cells and production of Th2 cytokines in bronchoalveolar lavage fluid (BALF), the plasma levels of total and OVA-specific immunoglobulin E (IgE), and histological changes in lung tissue.
Results: Our findings showed that CBDW significantly decreased the levels of various inflammatory mediators (eotaxin-1, eotaxin-3, RANTES, LTC_4, TNF-α, MMP-9, 5-LO, ICAM-1, and VCAM-1) *in vitro*, significantly reduced the accumulation of total inflammatory cells, the production of Th2 cytokines (IL-5 and IL-13), the levels of IgE (total and OVA-specific) *in vivo*, and remarkably inhibited histological changes (infiltration of inflammatory cells and goblet cell hyperplasia) *in vivo*.
Conclusions: These results suggest that CBDW possesses anti-inflammatory and anti-allergic properties by lowering allergic inflammation.

1. Introduction

Allergic diseases, one of the most prevalent diseases worldwide, are a group of hypersensitivity disorders mediated by immunological reaction that can cause life-threatening reactions [1]. Allergic diseases, such as asthma, are characterized by inappropriate and excessive responses to allergens, leading to a T helper (Th)-2 biased immune environment. The immunological basis of allergic asthma is presented in 2 phases, namely, the development of memory T and B cell immune responses and production of immunoglobulin E

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Abbreviations

5-LO	5-lipoxygenase
ANOVA	analysis of variance
ATCC	American Type Culture Collection
BALF	bronchoalveolar lavage fluid
CBDW	Cheonwangbosim-dan water extract
CCK	cell counting kit
Dex	dexamethasone
DW	distilled water
ELISA	enzyme-linked immunosorbent assay
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
H&E	hematoxylin and eosin
ICAM	intercellular adhesion molecule
Ig	immunoglobulin
IL	interleukin
IT	IL-4 + TNF-α
LTC_4	leukotriene C_4
MCs	mast cells
MMP	matrix metallopeptidase
MMPs	matrix metalloproteinases
NC	normal control
OVA	ovalbumin
PA	PMA + A23187
PAS	periodic acid-Schiff
PBS	phosphate-buffered saline
PMA	phorbol 12-myristate 13-acetate
RANTES	regulated on activation, normal T-cell expressed and secreted
RT-PCR	reverse transcription-polymerase chain reaction
SEM	standard error of the mean
SPF	specific pathogen-free
Th	T helper
TNF	tumor necrosis factor
VCAM	vascular cell adhesion molecule

(IgE) and effector functions, which are related to the activation of several inflammatory cells and the production of multiple inflammatory mediators [2]. Thus, inhibition of these inflammatory mediators may be an effective therapy for inflammatory and allergic diseases, such as asthma.

Traditional herbal medicines have been used in many countries for therapeutic purposes because they are natural and cause fewer complications. Medical herbs are still in demand, and their acceptance is gradually increasing. Undoubtedly, various herbs play a significant role in ecosystem by providing fundamental services. Although many buyers recognize herbal medicines as a constituent of

Table 1
Cheonwangbosim-dan composition.

Latin name	Scientific name	Origin	Amount (g)
Rehmanniae Radix	*Rehmannia glutinosa*	Andong, Korea	15 (30.7%)
Coptidis Rhizoma	*Coptis japonica*	China	7.5 (15.4%)
Acori Gramineri Rhizoma	*Acorus gramineus*	Jeju, Korea	3.75 (7.7%)
Ginseng Radix Alba	*Panax ginseng*	Yeongju, Korea	1.875 (3.85%)
Angelicae Gigantis Radix	*Angelica gigas*	Bonghwa, Korea	1.875 (3.85%)
Schisandrae Fructus	*Schisandra chinensis*	Samcheok, Korea	1.875 (3.85%)
Asparagi Tuber	*Asparagus cochinchinensis*	China	1.875 (3.85%)
Liriopis Tuber	*Liriope platyphylla*	Miryang, Korea	1.875 (3.85%)
Thujae Semen	*Thuja orientalis*	China	1.875 (3.85%)
Zizyphi Semen	*Zizyphus jujuba*	China	1.875 (3.85%)
Scrophulariae Radix	*Scrophularia buergeriana*	Uiseong, Korea	1.875 (3.85%)
Hoelen	*Poria cocos*	Pyeongchang, Korea	1.875 (3.85%)
Salviae Miltiorrhizae Radix	*Salvia miltiorrhiza*	China	1.875 (3.85%)
Platycodonis Radix	*Platycodon grandiflorum*	Muju, Korea	1.875 (3.85%)
Polygalae Radix	*Polygala tenuifolia*	China	1.875 (3.85%)
Total amount			**48.75 (100%)**

primary health care, there is still a lot of concern about the efficacy of these herbal mixtures [3]. The traditional herbal medicine Cheonwangbosim-dan (known as Tianwang-buxin-dan in China and Tennou-hoshin-tan in Japan) consists of 15 different herbs and has been widely used for the treatment of insomnia, neurosis, cardiac malfunction-induced disease, and arterial or auricular flutter in East Asian countries [4]. Previous studies have demonstrated that Cheonwangbosim-dan exerts a variety of biological activities including anti-inflammatory [5], vasorelaxant, and hypotensive effects [6]. In order to identify and optimize potential herbal decoctions, we performed a therapeutic screening of various herbal medicines including Cheonwangbosim-dan. In this way, we hypothesized that Cheonwangbosim-dan water extract (CBDW) could have anti-inflammatory and anti-allergic effects *in vitro* and *in vivo*. Epithelial cell damage and an increase in intraepithelial mast cells (MCs) are characteristic features of allergic diseases, such as asthma [7]. Thus, considering that the immunomodulatory capacity is an important approach, the present study aimed to evaluate the efficacy of CBDW against allergic inflammation in a bronchial epithelial cell line, mast cell line, and ovalbumin (OVA)-induced asthmatic mouse model.

2. Materials and methods

2.1. Preparations of Cheonwangbosim-dan

The 15 raw medicinal herbs constituting Cheonwangbosim-dan (Table 1) were purchased from Kwangmyungdang Medicinal Herbs (Ulsan, Korea), and CBDW was prepared from dried herb mixtures in our laboratory. The mixing ratio of each herb is as follows: Rehmanniae Radix (8), Coptidis Rhizoma (4), Acori Gramineri Rhizoma (2), Ginseng Radix Alba (1), Angelicae Gigantis Radix (1), Schisandrae Fructus (1), Asparagi Tuber (1), Liriopis Tuber (1), Thujae Semen (1), Zizyphi Semen (1), Scrophulariae Radix (1), Hoelen (1), Salviae Miltiorrhizae Radix (1), Platycodonis Radix (1), and Polygalae Radix (1). CBDW was obtained by extraction in distilled water (DW) at 100 °C for 2 h. The water extract was evaporated to dryness and then frozen to a dry powder. Voucher specimens of CBDW (2012–KE34–1 to 2012–KE34–15) were deposited in a refrigerator refrigerated at the KM Science Research Division, Korea Institute of Oriental Medicine. In a previous study, our laboratory performed simultaneous determination and method validations of 7 bioactive components. The components investigated were; berberine and coptisine from Coptidis Rhizoma, β-asarone from Acori Gramineri Rhizoma, nodakenin from Angelicae Gigantis Radix, 5-hydroxymethyl-2-furaldehyde from Asparagi Tuber, and cinnamic acid and harpagoside from Scrophulariae Radix [8].

2.2. In vitro studies

2.2.1. Cell culture

The human bronchial epithelial cell line BEAS-2B, and murine mast cell line MC/9, were obtained from the American Type Culture Collection (ATCC; Rockville, MD, USA). BEAS-2B cells were cultured as previously described [9]. The MC/9 mast cells were cultured as previously described [10].

2.2.2. Cytotoxicity assay

BEAS-2B cells (6×10^3 cells/well) and MC/9 cells (3×10^3 cells/well) were incubated in 96-well plates with CBDW at various concentrations (125, 250, and 500 μg/mL in BEAS-2B cells; 25, 50, and 100 μg/mL in MC/9 cells) for 24 h. To measure cell viability, cytotoxicity assay was conducted as described previously using cell counting kit (CCK)-8 kit [9,10].

$$\text{Cell viability } (\%) = \frac{\text{Mean absorbance in test sample wells}}{\text{Mean absorbance in vehicle treated control wells}} * 100$$

2.2.3. Cell stimulation

BEAS-2B cells (5×10^5 cells/well) were stimulated as described previously using recombinant proteins (interleukin (IL)-4 + tumor necrosis factor (TNF)-α (IT; R&D Systems Inc., Minneapolis, MN, USA)) for 48 h [9].

MC/9 mast cells (5×10^4 cells/well) were cultured in 48-well plates and incubated in the absence or presence of phorbol 12-myristate 13-acetate (PMA, 50 nM; Sigma-Aldrich, St. Louis, MO, USA) and A23187 (1 μM; Sigma-Aldrich). The cells were stimulated with culture medium containing PMA + A23187 (PA) to produce leukotriene C_4 (LTC_4) and TNF-α for 24 h [10].

2.2.4. Measurement of inflammatory mediator production

Culture supernatants were used to measure the production of eotaxin-1, eotaxin-3, regulated on activation, normal T-cell expressed and secreted (RANTES), LTC_4, and TNF-α using an enzyme-linked immunosorbent assay (ELISA) protocol from R&D Systems Inc., Cayman Chemical Co. (Ann Arbor, MI, USA), and BD Bioscience (San Jose, CA, USA), according to the manufacturer's instructions. The absorbance was measured at 450 nm using a microplate reader. Triprolidine is an antagonist of the histamine H_1 receptor. In a previous study, our laboratory confirmed that it is also effective in inhibiting the production of TNF-α [11]. Zileuton (Sigma-Aldrich) and triprolidine (Sigma-Aldrich) were used as positive controls for LTC_4 and TNF-α, respectively [9,10].

2.2.5. Measurement of MMP-9 activity and 5-LO expression

Matrix metallopeptidase 9 (MMP-9) activity and 5-lipoxygenase (5-LO) expression was measured as described previously using gelatin zymography and Western blot, respectively [9,10]. Zileuton was used as a positive control for the inhibition of 5-LO overexpression.

2.2.6. Measurement of adhesion molecules expression

Intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1 expression was measured as described previously using reverse transcription-polymerase chain reaction (RT-PCR) [9].

2.3. In vivo studies

2.3.1. Animal models of allergic asthma

Specific pathogen-free (SPF) BALB/c female mice (6 week-old) were purchased from Orientbio Inc. (Seongnam, Korea) and acclimated in an animal care facility for 7 days. Mice were maintained in environmentally controlled conditions (22 ± 3 °C, 12 h light/12 h dark cycle) and provided with water and standard chow *ad libitum*. All experimental procedures were approved by the Animal Experimental Ethics Committee of the Chungnam National University (Daejeon, Korea). The mice were randomly divided into the following 5 groups each containing 5 animals: normal control (NC; phosphate-buffered saline [PBS]-induction + oral gavage of PBS), OVA (OVA-induction + oral gavage of PBS), dexamethasone (Dex, Sigma-Aldrich; OVA-induction + oral gavage of Dex at 1 mg/kg), CBDW-200 (OVA-induction + oral gavage of CBDW at 200 mg/kg), and CBDW-400 (OVA-induction + oral gavage of CBDW at 400 mg/kg).

BALB/c mice were sensitized on days 1, 8, and 15 via an intraperitoneal injection of OVA (50 μg; Sigma-Aldrich) emulsified with 4 mg of Imject® alum adjuvant (Thermo Fisher Scientific Inc.) in PBS. Fourteen days after the third sensitization (on day 28), the mice were challenged by intranasal injection of OVA solution (1 mg/mL, in PBS) on days 28, 31, and 33. CBDW was administered by gavage to mice at doses of 200 or 400 mg/kg once daily from days 24–33. CBDW was dissolved in DW and prepared fresh daily before each treatment. The mice in the NC, OVA, or Dex groups were orally administered DW or Dex (1 mg/kg in DW), respectively. Dex was used as a positive control for the symptoms of allergic responses or inflammation. The mice were sacrificed 48 h after the last challenge by intraperitoneal injection of pentobarbital, and tracheostomy was performed to obtain the bronchoalveolar lavage fluid (BALF) as previously described [12]. The number of total inflammatory cells in BALF was assessed by counting at least four squares of a hemocytometer (C-Chip, NanoEnTek, Seoul, Korea). Plasma samples were obtained by cardiac puncture.

2.3.2. Measurement of Th2 cytokine and IgE levels

The levels of IL-5 and IL-13 (R&D Systems Inc.) in BALF and those of total and OVA-specific IgE (BioLegend, San Diego, CA, USA) in plasma were measured using ELISA kits according to the manufacturer's protocols.

2.3.3. Measurement of inflammatory cell infiltration and goblet cell hyperplasia

After plasma samples were obtained, lung tissue was fixed in 4% (v/v) paraformaldehyde (Biosesang, Seongnam, Korea). Tissues were embedded in paraffin, sectioned at a thickness of 4 μm, and stained using a hematoxylin and eosin (H&E) staining kit (Abcam, Cambridge, MA, USA) to assess inflammatory cell accumulation. A periodic acid-Schiff (PAS) staining kit (Abcam) was used to detect mucus-secreting goblet cells. Photomicrographs were taken using a Photometric Quantix digital camera, which was adapted to a microscope.

2.4. Statistical analyses

All data are presented as the mean ± standard error of the mean (SEM). Statistical significance was determined using analysis of variance (ANOVA) followed by Dunnett's multiple comparisons test (GraphPad Prism 8 software, San Diego, CA, USA). Differences were considered statistically significant at a P value of <0.05.

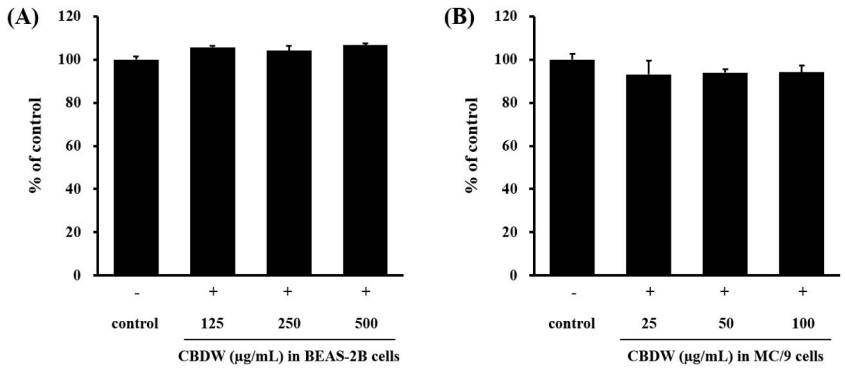

Fig. 1. Cytotoxicity effects of CBDW in BEAS-2B and MC/9 cells. BEAS-2B (A) and MC/9 (B) cells were seeded into 96-well plates and treated with various concentrations of CBDW for 24 h. Cell viability was assessed using a CCK-8 kit. The values are expressed as the mean ± SEM.

3. Results

3.1. In vitro studies

3.1.1. CBDW cytotoxicity in BEAS-2B and MC/9 cells

To determine the cytotoxicity of the test materials in BEAS-2B and MC/9 cells, the cells were exposed to various concentrations of CBDW for 24 h. Cell viability was then measured using the CCK-8 assay. CBDW did not produce any significant cytotoxic effects at any concentration. The nontoxic concentrations (at $\leq$ 500 μg/mL in BEAS-2B and $\leq$100 μg/mL in MC/9 cells) of the test materials were used for subsequent experiments (Fig. 1A and B).

3.1.2. Effect of CBDW on inflammatory mediator production

The effects of CBDW on eotaxin-1, eotaxin-3, and RANTES production were determined in IT-stimulated BEAS-2B cells. As shown in Fig. 2A–C, the production of chemokines, such as eotaxin-1 (40.12 pg/mL), eotaxin-3 (641.62 pg/mL), and RANTES (12.67 ng/mL) was significantly increased in IT-stimulated cells compared with that in vehicle-treated cells ($P < 0.01$). In contrast, CBDW significantly inhibited the concentration of these chemokines (eotaxin-1; <0.01 pg/mL in 125 μg/mL, <0.01 pg/mL in 250 μg/mL, and <0.01 pg/mL in 500 μg/mL eotaxin-3; 494.47 pg/mL in 125 μg/mL, 92.67 pg/mL in 250 μg/mL, and 52.35 pg/mL in 500 μg/mL. RANTES; 10.30 ng/mL in 125 μg/mL, 7.46 ng/mL in 250 μg/mL, and 6.16 ng/mL in 500 μg/mL) dose dependently ($P < 0.01$).

The effects of CBDW on LTC_4 and TNF-α release were assessed in PA-stimulated MC/9 cells. The release of LTC_4 and TNF-α was significantly increased in PA-stimulated cells compared to that in vehicle-treated cells ($P < 0.01$). However, CBDW significantly inhibited the release of LTC_4 in a dose-dependent manner ($P < 0.05$, $P < 0.01$) and significantly decreased the release of TNF-α in a dose-independent manner ($P < 0.01$). Zileuton and triprolidine, used as positive controls, significantly inhibited the release of LTC_4 and TNF-α in a dose-dependent manner ($P < 0.01$, Fig. 2D and E).

3.1.3. Effect of CBDW on MMP-9 and 5-LO activation

To investigate the regulator of inflammatory processes, we assessed the activities of MMP-9/MMP-2 in IT-stimulated BEAS-2B cells. MMP-9 activity was markedly increased by IT stimulus (Fig. 3A). However, CBDW gradually decreased MMP-9 activity. The relative ratio of MMP-9/MMP-2 was significantly higher in IT-stimulated cells compared with that in vehicle-treated cells ($P < 0.01$). However, CBDW exhibited a significantly attenuated relative ratio of MMP-9/MMP-2 in a dose-dependent manner compared with that in IT-treated cells ($P < 0.01$, Fig. 3B).

To examine the intracellular migration of the lipoxygenase gene into the nucleus, we determined the expression of 5-LO in PA-stimulated MC/9 cells. In the nucleus, the expression of 5-LO was markedly increased by treatment with PA (fold change: 2.39). As shown in Fig. 3C, zileuton and CBDW were shown to greatly decreased the nuclear 5-LO expression in a dose-independent manner (fold change in zileuton: 0.79, 0.68; fold change in CBDW: 0.68, 1.09, 1.26, 1.04). There were no differences between these groups in the cytoplasmic expression of 5-LO (fold change: 0.78 in PA; fold change in zileuton: 0.84, 0.71; fold change in CBDW: 0.86, 0.85, 0.81,

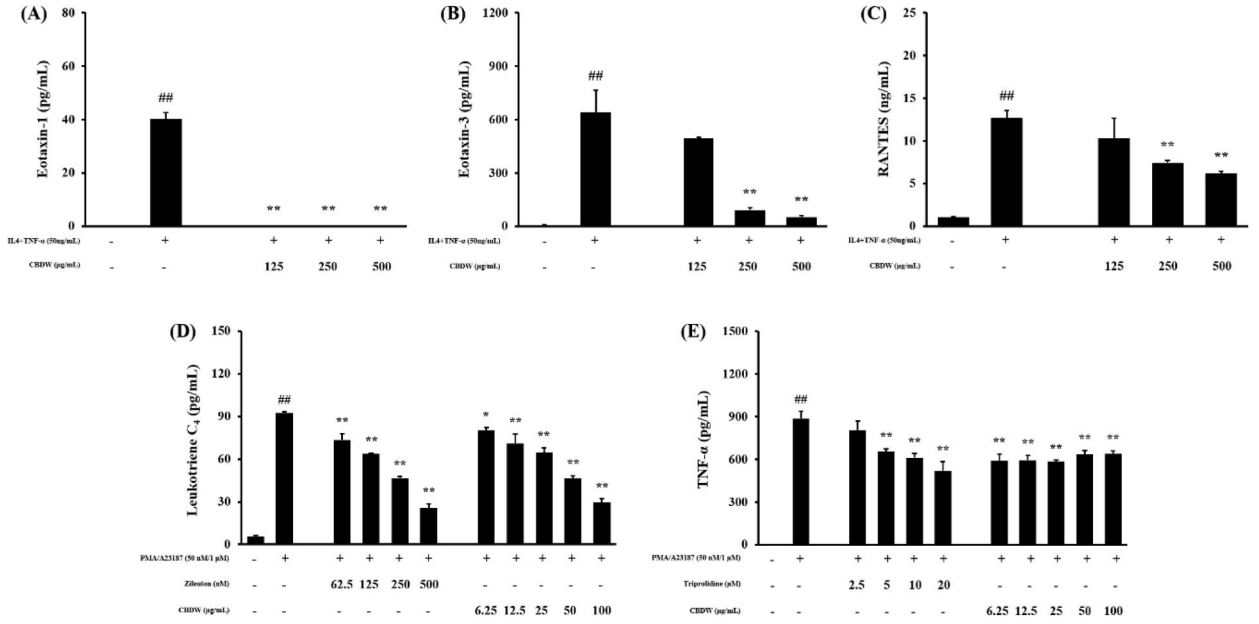

Fig. 2. Effects of CBDW on the production of inflammatory mediators in BEAS-2B and MC/9 cells. The levels of eotaxin-1 (A), eotaxin-3 (B), RANTES (C), LTC_4 (D), and TNF-α (E) released into the culture medium were assessed using commercially available ELISA kits. The values are expressed as the mean ± SEM. $^{\#\#}P < 0.01$ versus vehicle-treated cells; and * $P < 0.05$ or ** $P < 0.01$ versus IT/PA-stimulated cells, respectively.

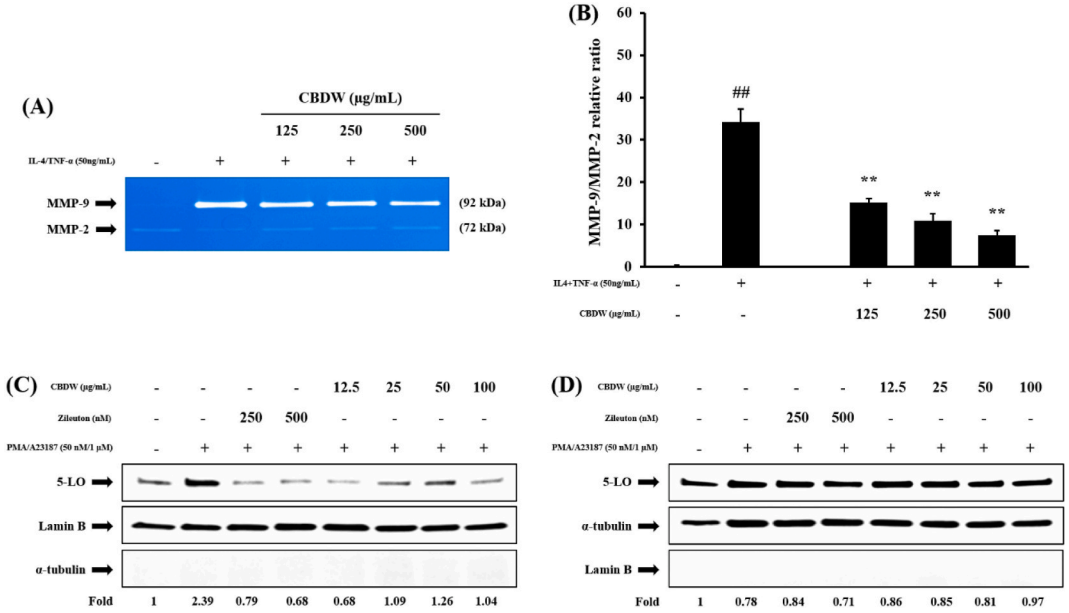

Fig. 3. Effects of CBDW on the activation of MMP-9 and 5-LO in BEAS-2B and MC/9 cells. Representative photographs of MMP-9 activity (A) and band intensities of MMP-9/MMP-2 (B). Representative photographs of 5-LO expression in the nucleus (C) and cytoplasm (D), respectively. Lamin B and α-tubulin were used as loading controls for the nucleus and cytoplasm, respectively. The values are expressed as the mean ± SEM. $^{\#\#}P < 0.01$ versus vehicle-treated cells; and $^{**}P < 0.01$ versus IT-stimulated cells.

0.97, Fig. 3D).

3.1.4. *Effect of CBDW on adhesion molecules expression*

To determine the indicator of inflammatory responses, we evaluated ICAM-1 and VCAM-1 expression in IT-stimulated BEAS-2B

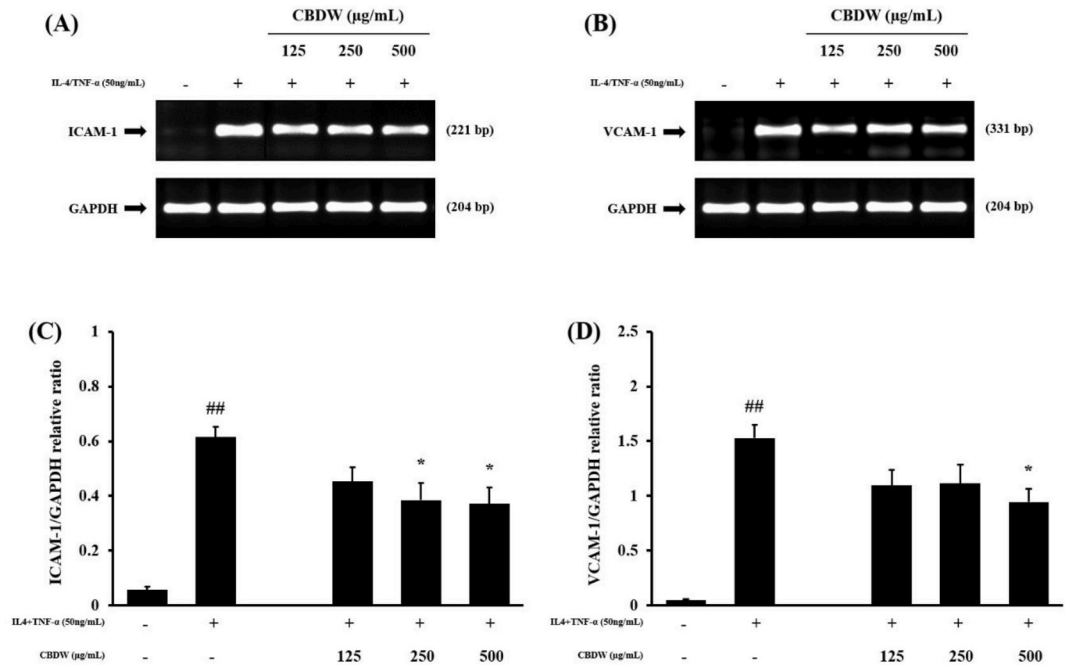

Fig. 4. Effects of CBDW on the expression of adhesion molecules in BEAS-2B cells. Representative photographs of the expression levels of ICAM-1 (A) or VCAM-1 (B), and band intensities of ICAM-1/GAPDH (C) or VCAM-1/GAPDH (D). The values are expressed as the mean ± SEM. $^{\#\#}P < 0.01$ versus vehicle-treated cells; and $^{*}P < 0.05$ versus IT-stimulated cells.

cells. ICAM-1 and VCAM-1 expression was markedly higher by stimulus with IT (Fig. 4A and B). However, CBDW dramatically reduced ICAM-1 and VCAM-1 expression. As shown in Fig. 4C and D, the relative ratios of ICAM-1/glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and VCAM-1/GAPDH were significantly higher in the IT-stimulated cells compared with those of vehicle-treated cells ($P < 0.01$). In contrast, the relative ratio of ICAM-1/GAPDH and VCAM-1/GAPDH was significantly decreased in CBDW-treated cells compared with that in IT-stimulated cells ($P < 0.05$).

3.2. *In vivo studies*

3.2.1. *Effect of CBDW on the number of inflammatory cells and production of Th2 cytokines in BALF*

To examine whether GBDW influenced the recruitment of inflammatory cells and production of Th2 cytokines into BALF, we investigated the number of inflammatory cells and levels of Th2 cytokines in OVA-induced asthmatic mice. The number of inflammatory cells in BALF was significantly higher in the OVA-induced group than that in the NC group ($P < 0.01$). However, the OVA-induced mice administered CBDW (200 and 400 mg/kg, $P < 0.05$, respectively) and Dex ($P < 0.01$) had a significantly decreased total inflammatory cell number (Fig. 5A).

As shown in Fig. 5B and C, the production of IL-5 and IL-13 in BALF was markedly increased in the OVA-induced group compared with that in the NC group ($P < 0.05$). However, treatment with CBDW (200 and 400 mg/kg, $P < 0.05$, respectively) and Dex ($P < 0.01$) significantly attenuated the levels of Th2 cytokines, such as IL-5 and IL-13, compared with those of the OVA-induced group.

3.2.2. *Effect of CBDW on the plasma levels of total and OVA-specific IgE*

To investigate whether GBDW influenced the alterations in the adaptive immune response in the plasma, we evaluated the levels of total and OVA-specific IgE in OVA-induced asthmatic mice. As shown in Fig. 6A and B, the levels of total and OVA-specific IgE in the plasma were significantly increased in the OVA-induced group compared those in the with NC group ($P < 0.01$). However, the OVA-induced mice administered CBDW (200 and 400 mg/kg, $P < 0.01$, respectively) and Dex ($P < 0.01$) had significantly inhibited levels of total IgE. Moreover, treatment with CBDW (200 and 400 mg/kg, $P < 0.05$, respectively) and Dex ($P < 0.05$) markedly reduced the levels of OVA-specific IgE compared to those of the OVA-induced group.

3.2.3. *Effect of CBDW on histopathological changes in lung tissue*

To analyze whether GBDW influenced the alterations in the histological features of asthma into lung tissue, we stained lung sections using H&E and PAS staining kits. Inflammatory cell infiltration in the peribronchial/peribronchiolar lung tissue and mucus overproduction (caused by goblet cell hyperplasia), observed as a violet color in the bronchial airways, was markedly elevated in the OVA-induced group compared with that in the NC group. However, the OVA-induced mice administered CBDW (200 and 400 mg/kg) and Dex had remarkably decreased inflammatory cell infiltration (Fig. 7A) and goblet cells hyperplasia (Fig. 7B).

4. Discussion

Bronchial epithelial cells, closely associated with the progression of allergic diseases such as asthma, are essential for the primary immune defense, and mast cells are well known for their effector functions in Th2-biased inflammatory responses [7]. The BEAS-2B and MC/9 cell lines have been used to examine cytokine-, endotoxin- or activator-stimulated inflammatory responses that may consequentially induce allergic diseases [13–15]. Chemokines secreted by bronchial epithelial cells have also been reported to contribute to chronic airway inflammation by recruiting inflammatory cells [16]. Eotaxin, a member of the CC-chemokine family, is a potent attractant of eosinophils and thereby might contribute to airway inflammation. Eotaxins represent a group of chemokines consisting of three sets of subtypes: eotaxin-1, eotaxin-2, and eotaxin-3. Most importantly, eotaxin-3 is a more powerful chemoattractant than eotaxin-1 and eotaxin-2 for eosinophil recruitment [17]. RANTES is a chemotactic and activating factor for eosinophils as a candidate mediator in allergic asthma. This mediator has the ability to attract several types of inflammatory cells to the site of inflammation [18]. Matrix metalloproteinases (MMPs), such as MMP-9 and MMP-2, are enzymes that degrade the extracellular matrix

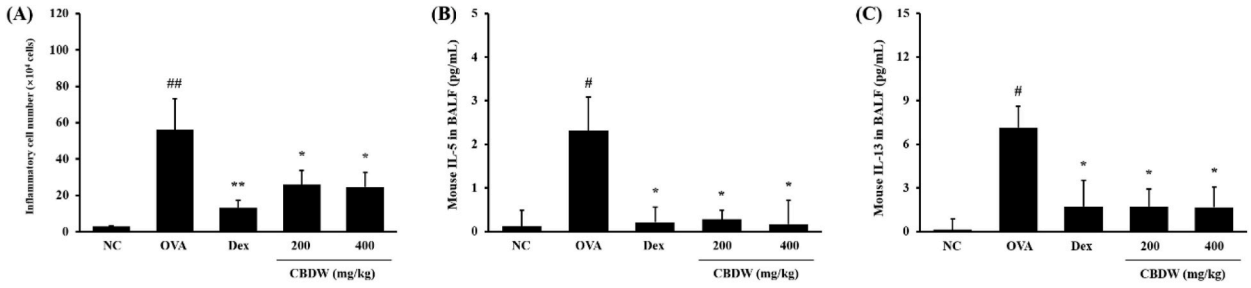

Fig. 5. Effects of CBDW on the number of inflammatory cells and production of Th2 cytokine in BALF from asthmatic mice. Number of inflammatory cells in BALF (A) counted using a light microscope with a hemocytometer. The levels of Th2 cytokine were determined using ELISA kits. Th2 cytokines: IL-5 (B) and IL-13 (C). The values are expressed as the mean ± SEM. $^{\#}P < 0.05$ or $^{\#\#}P < 0.01$ versus vehicle-induced mice, respectively; and * $P < 0.05$ or $^{**}P < 0.01$ versus OVA-induced mice, respectively.

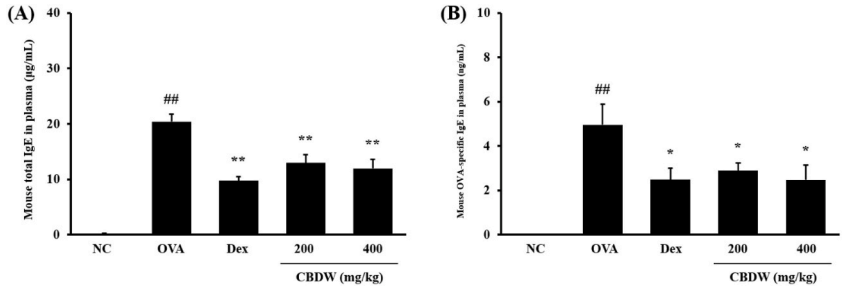

Fig. 6. Effect of CBDW on the levels of total and OVA-specific IgE in the plasma from asthmatic mice. The levels of IgE were determined using ELISA kits. IgE: total IgE (A) and OVA-specific IgE (B). The values are expressed as the mean ± SEM. $^{\#\#}P < 0.01$ versus vehicle-induced mice; and * $P <$ 0.05 or ** $P < 0.01$ versus OVA-induced mice, respectively.

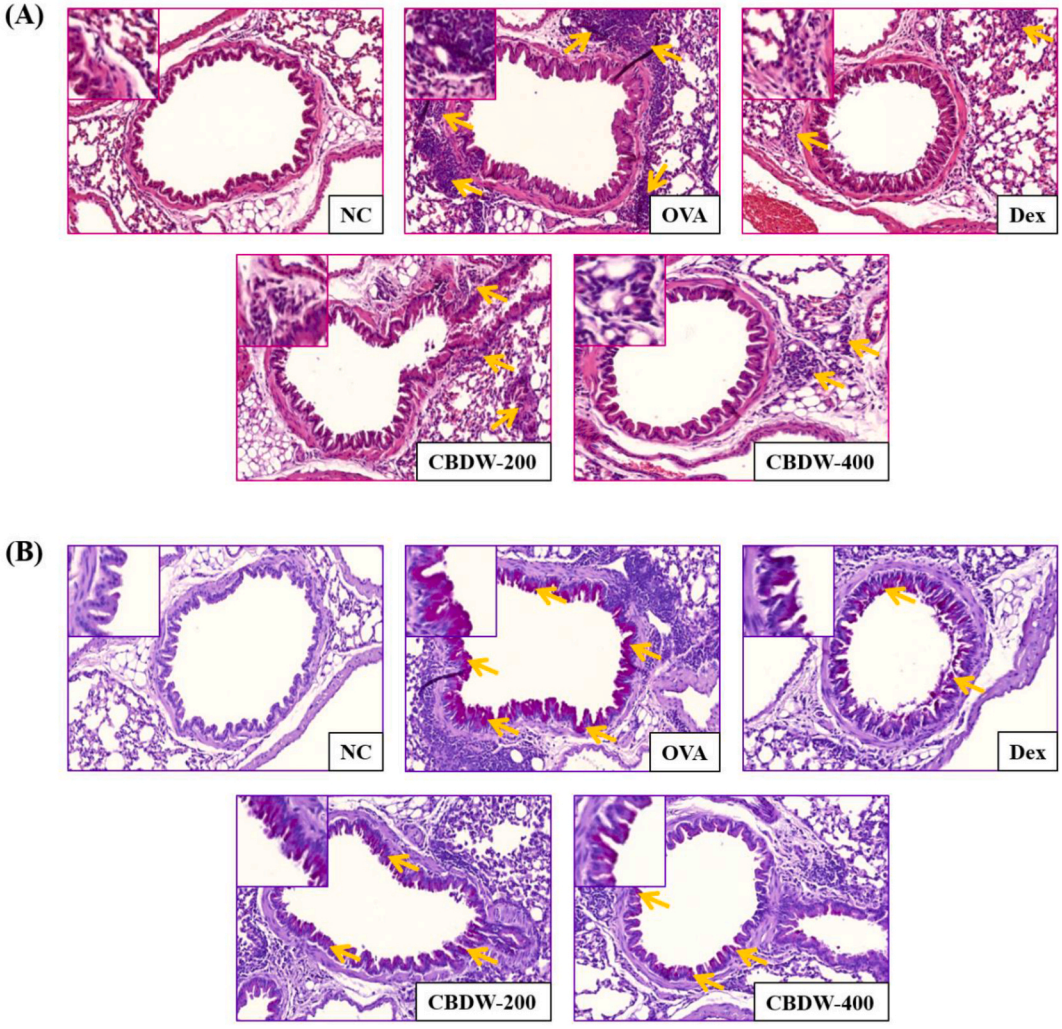

Fig. 7. Effect of CBDW on the infiltration of inflammatory cells and production of mucus caused by goblet cell hyperplasia in lung tissue from asthmatic mice. Lung tissues were stained using an H&E staining kit to assess inflammatory cells infiltration (A) and a PAS staining kit to detect mucus-secreting goblet cells (B). Representative photomicrographs of lung sections are shown (original magnification, × 200). Yellow arrows indicated as airway inflammation (A) and mucus production (B). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

and basement membrane, infiltrate inflammatory cells, and consequently participate in tissue remodeling [19]. Moreover, the upregulation of adhesion molecules on the surfaces of respiratory epithelial cells is responsible for the development of allergic diseases. Members of the endothelial adhesion molecule family, ICAM-1 and VCAM-1, play an important role in inflammatory cell infiltration into the inflamed site [20]. Our findings demonstrate that CBDW significantly decreased the increased production of CC-chemokines, including eotaxin-1, eotaxin-3, and RANTES, increased MMP-9 activity and the expression of adhesion molecules, including ICAM-1 and VCAM-1, in IT-stimulated BEAS-2B cells. These results suggest that CBDW exerts a therapeutic effect on IT-stimulated epithelial damage, perhaps due to its anti-inflammatory properties. LTC_4 secreted from mast cells activates bronchoconstriction and mucus production [21]. TNF-α induces abnormal production of pro-inflammatory cytokines and specific chemokines, which can play a role in the infiltration of inflammatory cells into the inflamed site during the development of allergic diseases [15]. In the present study, CBDW significantly decreased the increased release of LTC_4 and TNF-α and the activation of 5-LO in PA-stimulated MC/9 cells. Therefore, CBDW may have therapeutic potential against inflammatory responses via the suppression of inflammatory mediators and the 5-LO pathway as demonstrated in the *in vitro* studies.

The OVA-induced mouse is an established asthma animal model that was developed to study the potential therapeutic efficacy of asthma drugs and the underlying mechanisms. Asthmatic mice frequently have patterns and symptoms that are similar to those found in patients with allergic asthma, such as infiltration of inflammatory cells, such as eosinophils into the BALF and immediate asthma reactions [22,23]. In the pathogenesis of allergic asthma, inflammatory cell influx and inflammatory cytokines are closely involved in the development of Th2-driven inflammation. Thus, a dominant Th2 response induces several of the characteristic features of asthma, including inflammatory cell recruitment, overproduction of Th2 cytokines (such as IL-5 and IL-13), elevation of IgE levels, and goblet cell hyperplasia-caused mucus overproduction [24]. Previous studies have demonstrated that the anti-inflammatory effects observed in allergic asthma models are exerted through the inhibition of their infiltration and production [25,26]. In the *in vivo* experiments, we found that CBDW treatment had strong anti-inflammatory and anti-allergic effects by inhibiting the accumulation of inflammatory cells and production of Th2 cytokines in BALF, lowering the plasma levels of total and OVA-specific IgE, and the reducing the inflammatory cell infiltration and goblet cell hyperplasia-caused mucus production in lung tissue. These results suggest that CBDW administration may contribute to suppressing the OVA-induced asthmatic response as an effective therapeutic agent.

Medicinal herbs have been traditionally used for disease treatment because of their specific properties, including synergistic actions and fewer adverse effects. The herb constituents can interact with each other, and this interaction may be beneficial for both or adverse to either of them, or negate the effects of both. Herb-derived components are also characterized by their ability to prevent the progression and development of certain diseases [3]. Cheonwangbosim-dan is a traditional herbal prescription extracted from 15 medicinal herbs (Rehmanniae Radix, Coptidis Rhizoma, Acori Gramineri Rhizoma, Ginseng Radix Alba, Angelicae Gigantis Radix, Schisandrae Fructus, Asparagi Tuber, Liriopis Tuber, Thujae Semen, Zizyphi Semen, Scrophulariae Radix, Hoelen, Salviae Miltiorrhizae Radix, Platycodonis Radix, and Polygalae Radix). In our laboratory, a high-performance liquid chromatography-photodiode array detector method was established for the simultaneous determination of 7 components in CBDW [8]. Many experimental studies have demonstrated a wide range of pharmacological effects of the crude herbs. Therefore, we expected CBDW to exert pharmacological effects, such as anti-inflammatory and anti-allergic effects, based on the interactions between its constituent herbs, in an *in vitro* and *in vivo* experimental model. As a result, CBDW has been proven to have anti-inflammatory and anti-allergic properties, although further studies are needed to identify the effects of the active compounds including berberine, coptisine, β-asarone, nodakenin, 5-hydroxymethyl-2-furaldehyde, cinnamic acid, and harpagoside.

5. Conclusions

Taken together, these findings demonstrate that CBDW treatment has anti-inflammatory and anti-allergic activity, at least via downregulation of inflammatory mediators, resulting in inhibited airway inflammation in the OVA-induced asthma model.

Additional information

Supplementary content related to this article has been published online at [URL].

Author contribution statement

Mee-Young Lee: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.
Woo-Young Jeon: Seong Eun Jin: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.
Kyuhyung Jo: Eunjin Sohn: Performed the experiments.
Hyekyung Ha: Analyzed and interpreted the data.
Hyeun-Kyoo Shin: Contributed reagents, materials, analysis tools or data.

Data availability statement

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e16172.

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