**Polyphenol containing *Sargassum horneri* attenuated Th2 differentiation in splenocytes of ovalbumin-sensitised mice: involvement of the transcription factors GATA3/STAT5/NLRP3 in Th2 polarization**

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**ABSTRACT**

Context: *Sargassum horneri* (Turner) C. Agardh (Sargassaceae) is a brown marine alga used in oriental medicine to treat allergic conditions.

Objective: This study clarifies the effect of polyphenol-containing *S. horneri* ethanol extract (SHE) on T-helper type-2 (Th2) polarisation.

Materials and methods: All mice (BALB/c mice, n = 12) except in the healthy control group were first sensitised with an intraperitoneal injection of ovalbumin (OVA; 20 μg) and alum (2 mg) on Day 0 and Day 14. Similarly, phosphate-buffered saline (PBS) was injected according to the same schedule into the healthy control mice. After the final administration, splenocytes were obtained. OVA sensitised mice were challenged with OVA (100 μg/mL) in the absence or presence (62.5 and 125 μg/mL) of SHE while healthy control group remained untreated.

Results: SHE (0–1000 μg/mL) was not cytotoxic to splenocytes and demonstrated IC₅₀ values of 3.27 and 3.92 mg/mL, respectively, at 24 and 48 h of incubation. SHE suppressed cell proliferation at concentrations ≥62.5 μg/mL. SHE treatment (125 μg/mL) subdued (by 1.8-fold) the population expansion of CD3⁺ helper T cells induced by OVA challenge. SHE attenuated the OVA-induced activation of respective transcription factors GATA3 and NLRP3. Simultaneously, highly elevated levels of cytokines interleukin (IL)-4 and IL-5 caused by OVA stimulation were removed completely and IL-13 suppressed by 1.5-fold.

Conclusions: SHE exhibits Th2 immune suppression under OVA stimulation via GATA3- and NLRP3-dependent IL-4, IL-5, and IL-13 suppression. Therefore, SHE could be therapeutically useful for alleviating the symptoms of allergen-mediated immune diseases.

**Introduction**

T-helper (Th) cells differentiate to Th type-2 (Th2), which is clearly linked to the defense or pathogenesis of diseases and acts as a double-edged sword (Kool et al. 2012). Excessive Th2 cell immunity has been proven to be responsible for the pathogenesis of conditions, such as perennial allergic rhinitis, asthma, and atopy (Kool et al. 2012). As these conditions place a significant burden on the health of patients worldwide, a proper understanding of Th2 polarisation and candidates to attenuate Th2 polarisation move us closer to developing effective treatments against allergic diseases (Kool et al. 2012).

Interleukin (IL)-4, IL-5, and IL-13 from Th2 cells orchestrate protective responses via tissue repair and by targeting helminths (Walker and McKenzie 2018). B cells and T lymphocytes in the white pulp of the spleen attack the helminths, and IL-4 can further promote the IgE production which coats the helminths and initiates their destruction (Walker and McKenzie 2018). However, excessive Th2 responses result in prolonged inflammatory conditions, such as atopy, asthma, allergies, and hypersensitivity (Raphael et al. 2015; Walker and McKenzie 2018). The IL-4 mediated signal transducer and activator of transcription 6 (STAT6) pathway is mainly involved in Th2 differentiation; however, under certain circumstances, STAT6 knockout in mice also results in Th2 differentiation in CD4⁺ T cells (Zhu et al. 2001). In addition, STAT5 activation is essential for the production of Th2 cytokines via mediating GATA binding protein 3 (GATA3) expression (Zhu 2015).
STAT5 activation also drives the expression of NLR family pyrin domain containing 3 (NLRP3), a component of the inflammasome, and home to the nucleus promoting the transcription of genes encoding IL-4, IL-5, and IL-13 in T cells (Ting and Harton 2015). The transcriptional upregulation of STAT5, NLRP3, and GATA3 was revealed to be a key event in Th2 differentiation (Zhu 2015). Immunotherapy for certain conditions, such as atopy in children and perennial allergic rhinitis, typically works via the induction of tolerance or anergy to Th2 cells (Tanaka et al. 1998; Smart and Kemp 2002). In particular, natural sources to attenuate the function of pathogenic Th2 cells and their released cytokines have undoubtedly revolutionised the interest in immune therapy due to the cost effectiveness and absence of adverse effects (Li et al. 2000; Chitnis et al. 2004).

Marine algae have gained considerable research attention regarding their biological properties due to their abundant functional components (Wijesinghe and Jeon 2012a). Efforts to discover new metabolites from marine algae in previous years identified that several active compounds can be developed as cosmeceuticals and pharmaceuticals (Wijesinghe and Jeon 2012b). Sargassum horneri (Turner) C. Agardh (Sargassaceae) is an edible brown alga cultivated in Korea, China, and Japan that is rich in bioactive components such as polyphenols, polysaccharides, and chromenes (Motshakero et al. 2013). Studies on brown algae have reported that they contain a high amount of polyphenols compared to red and green algae (Generali et al. 2019). Moreover, the supercritical carbon dioxide extract was revealed to be rich in phenolic acids and flavonoids, which are responsible for their anti-inflammatory effects (Shipeng et al. 2015). We also revealed that it exhibits an immunosuppressive effect on the concanavalin A-stimulated splenocytes by downregulating the production of the IL-4, IL-5, and IL-13 (Th2) cytokines (Herath et al. 2019). Polyphenol-containing SHE attenuated the messenger ribonucleic acid (mRNA) expression of Th2 cytokines in mice lungs in a PM-exacerbated asthma mouse model (Herath et al. 2020).

Despite these efforts to establish S. horneri’s efficacy against inflammatory and anti-allergic reactions to exogenous stimuli, the understanding of its mechanisms with regard to Th2 polarisation, the lynchpin event in any antigen-induced immune response, is still incomplete. In this study, we investigated SHE’s effect on the Th2 immune response by focussing on specific Th2 transcription factors (STAT5, NLRP3, and GATA3), and their cytokine profiles in murine splenocytes sensitised with ovalbumin (OVA), a highly effective agent in provoking the CD4+ helper T cell response. Likewise, spleens containing vast immune cells were used as model systems for observing Th2 cell polarisation and cytokine profiles in OVA-immunised cells.

Materials and methods

Seaweed extract preparation

S. horneri was collected from the shores of Jeju Island, South Korea in 2020. The plant material was authenticated by Dr. Myung Sook Kim a voucher specimen is in Jeju National University, S. Korea (SJFC70180625, 201013). The 70% ethanol extract of S. horneri (SHE) was prepared as described previously (Herath et al. 2019). In brief, the collected seaweed was washed in pure water and dried in hot air. The dried seaweed was ground, passed through a 40-50 mesh using a Pin-mill, and dissolved in 70% ethanol (100 g/2L). After removing the residues, the solution was centrifuged (12,000 rpm), treated with 95% ethanol (to obtain purer supernatants), and concentrated to 20% of the solid concentration. The prepared samples were stored at −20 °C. We analysed the composition of SHE further and found that the main active component was polyphenol.

Animals

BALB/c mice (6-7 weeks and 20–25 g) were purchased from Orientbio, Inc. (Sungnam, Korea) and housed in a conventional animal facility in a well ventilated and temperature and humidity-controlled room upon arrival. Mice were fed ad libitum with a National Institute of Health (NIH) 07-approved diet and were provided with access to water in accordance with the regulations of the Animal Care and Use Committee, Jeju National University (Accreditation No: 2018–0021).

OVA immunisation and preparation of primary splenocytes

The mice (n = 12) were immunised intraperitoneally (i.p.) with 20 μg of ovalbumin (OVA) mixed with 2mg of aluminium hydroxide (alum) in 200 μL of phosphate-buffered saline (PBS). At 14 days after the first injection, the mice were given another booster injection with the same dose of the antigen. Similarly, PBS (vehicle) was injected according to the same schedule into the untreated control mice. At day 15, single-cell suspensions of murine splenocytes were obtained in Roswell Park Memorial Institute (RPMI) media containing 10% foetal bovine serum (FBS) purchased from Gibco Life Technologies (NY, USA) and 1% antibiotics (Gibco Life Technologies, NY, USA), as previously described (Herath et al. 2016).

Lactate dehydrogenase (LDH) assay

Murine splenocytes (1 × 10⁵ cells/well) were seeded on 96-well plates (Nunc) with varying concentrations of SHE (0–1000 μg/mL, suspended in PBS), with or without OVA (100 μg/mL in PBS). After incubation at 37 °C in 5% CO₂ for 24 and 48 h, the LDH concentration of the culture supernatant was measured using an LDH cytotoxicity detection kit (Takara Bio Inc., Japan) in accordance with the manufacturer’s instructions.

Cell proliferation assay

A ³H-thymidine incorporation assay was performed to determine whether SHE stimulated the proliferation of splenocytes. Splenocytes (5 × 10⁵ cells/well), in the presence or absence of OVA (100 μg/mL in PBS), were incubated on 96-well plates in triplicate with varying concentrations of SHE (0–1000 μg/mL in PBS) at 37 °C in 5% CO₂ for 72 h. At 54 h of incubation, 1 μCi of ³H-thymidine (Amersham, Arlington Heights, IL, USA) was added, followed by an additional 18 h incubation. The cells were harvested on glass fibre filters using a cell harvester, and the radioactivity of the splenocyte DNA was measured using liquid scintillation spectrometry (Wallac MicroBeta® TriLux, Perkin Elmer, Waltham, MA).

Flow cytometric analysis

Single-cell splenocyte suspensions were cultured with SHE (0, 62.5, and 125 μg/mL) in the presence or absence of OVA for 48 h. Then, the cells were incubated with 1% Fc Block (BD
Biosciences, San Jose, CA) in PBS for 15 min, before being stained with fluorescein isothiocyanate (FITC)-labelled anti-mouse CD3e (145-2C11), phycoerythrin (PE)-labelled CD4 (H129.19) and Gr-1 (RB6-8C5), and phycoerythrin (PC)5.5 labelled CD45 (30-F11) (BD Biosciences, San Jose, CA). After staining, the cells (20,000 cells per sample) were analysed using a CytoFLEX flow cytometer (Bio-Health Materials Core-Facility Centre, Jeju National University) and CytExpert 1.2 software (Beckman Coulter, Inc., Brea, CA, USA).

Enzyme-linked immunosorbent assay (ELISA) for cytokines

To evaluate the effect of SHE on the secretion of cytokines, an ELISA was performed. Murine splenocytes (3 × 10⁶ cells/well in 24 well plate) were cultured with SHE (0, 62.5, and 125 μg/mL in PBS) in the presence or absence of OVA (100 μg/mL in PBS) for 48 h at 37°C under 5% CO₂. For inhibitor experiments, CAS 285986-31-4 (Merk Millipore, Germany), a specific STAT5 inhibitor, was added 2 h prior to the OVA and SHE treatments. The cytokines produced in the culture supernatant (IL-4, IL-5, and IL-13) were measured using respective ELISA MAX deluxe set mouse kits (BioLegend, San Diego, CA), according to the manufacturer’s instructions.

Western blotting

Murine splenocytes were incubated with SHE (0, 62.5 and 125 μg/mL in PBS) in the presence or absence of OVA (100 μg/mL in PBS) for 24, 48, and 72 h. For inhibitor experiments, the STAT5 inhibitor (CAS 285986-31-4) suspended in PBS was added 2 h prior to the OVA and SHE treatments with a 24 h incubation period. Cytoplasmic and nucleic proteins were extracted from the cells by use of the NE-PA Neutral and Cytoplasmic Extraction Regent kit (Thermo Fisher Scientific, Walthman, MA). After measuring the protein concentration using a Bio-Rad Assay (Bio-Rad, Hercules, CA), the proteins (40 μg/mL) were separated using 7.5% SDS-PAGE and immunobotted on nitrocellulose membranes (Bio-Rad, Hercules, CA).

The membranes were incubated with 2% skim milk followed by another round of incubation with the primary antibodies: STAT5, p-STAT5, NLRP3 (Cell Signalling Technology, Inc.), and β-actin (Sigma, St. Louis, MO, USA) overnight. After washing, the membranes went through a final round of incubation with an appropriate horseradish peroxidase (HRP)-conjugated anti-mouse Immunoglobulin G (IgG) (Sigma, St. Louis, MO) or anti-rabbit IgG (Invitrogen, Carlsbad, CA, USA) for 45 min. The blots were developed with WestZol (iNtRON Biotechnology, Sungnam, Korea), and the densitometric analysis was performed with ImageJ software (version 1.47).

Statistical analysis

The numerical data are presented as the means ± standard error of the mean (SEM) for each group. The statistical analysis was performed by using one-way analysis of variance (ANOVA) followed by Tukey’s multiple comparison to compare the mean values among groups, and p < 0.05 was considered significant in this study.

Results

Effect of SHE on the proliferation of OVA-stimulated splenocytes

SHE was not cytotoxic to OVA (100 μg/mL)-challenged murine splenocytes at 24 (Figure 1(A)) and 48 h of incubation (Figure 1(B)). The 50% inhibitory concentrations (IC₅₀) of SHE were 3.27 and 3.92 mg/mL, respectively, at 24 and 48 h of incubation (Supplementary Figure 1).

We also investigated SHE’s effect on the proliferation of OVA-challenged murine splenocytes. As shown in Figure 2, OVA stimulation enhanced the cell proliferation by 4.0-fold compared with the untreated control, and SHE treatment (3.9–31.3 μg/mL) synergistically multiplied the proliferation of OVA-challenged murine splenocytes even compared to the OVA-only group. However, SHE concentrations ≥62.5 μg/mL decreased the proliferation of OVA-challenged splenocytes compared with the OVA-only group (by 2.2, 55.7, 243.4, 224.9, and 160.0-fold at 62.5, 125, 250, 500, and 1000 μg/mL, respectively). By combining the LDH and cell proliferation assay results, we determined that SHE concentrations ≥62.5 μg/mL had the potential to mitigate the harmful effects of OVA on splenic T cells without damaging the cell viability, and SHE concentrations of 62.5 and 125 μg/mL were used in subsequent experiments.

Figure 1. The effect of Sargassum horneri extract (SHE) on murine splenocyte cell cytotoxicity. Splenocytes were exposed to varying concentrations (0–1000 μg/mL) of SHE alone in the presence of ovalbumin (OVA) (100 μg/mL) for (A) 24 h and (B) 48 h, and the cell viability was measured by using a lactate dehydrogenase (LDH) assay. The data are represented as the mean ± SEM. The experiments were performed in triplicate, and the data are representative of three individual experiments. †(p < 0.05), ††(p < 0.005), †††(p < 0.0005) indicate significant decrease compared with the OVA-only group.
Effects of SHE on CD3\(^{+}\)CD4\(^{+}\) T cell populations of OVA-stimulated splenocytes

Flow cytometry analysis was performed to determine the effect of SHE on CD3\(^{+}\)CD4\(^{+}\) T cells and CD45\(^{+}\)Gr-1\(^{+}\) granulocyte populations of OVA-stimulated splenocytes from OVA-sensitised mice. As shown in Figure 3, OVA challenge increased the population of CD3\(^{+}\)CD4\(^{+}\) T cells by 1.5-fold \((p < 0.0005)\) compared with the untreated control; however, increasing concentrations of SHE decreased the population of T helper cells by 1.0- and 1.8-fold \((p < 0.0005)\), compared with the OVA-only group.

Effects of SHE on IL-4, IL-5, and IL-13 (Th2) cytokine secretion in OVA-stimulated splenocytes

We investigated SHE’s effect on the cytokine production of OVA challenged splenocytes from OVA-sensitised mice. Specifically, the protein levels of Th-2 cytokines IL-4 (Figure 4(A)), IL-5 (Figure 4(B)), and IL-13 (Figure 4(C)) in the cell culture supernatants were measured using ELISA. The splenocytes from OVA-sensitised mice cultured with OVA alone released significantly elevated levels of IL-4, IL-5, and IL-13 against the marginal (IL-5) or non-detectable (IL-4 and IL-13) background levels observed in the untreated control. By contrast, increasing concentrations of SHE declined the secretion of IL-5 (by 1.2- and 1.5-fold at 62.5 and 125 \(\mu\)g/mL, respectively) compared with the OVA-only group. Interestingly, the highly elevated levels of IL-4 and IL-13 caused by OVA stimulation were removed completely or diminished to non-detectable levels in the untreated control with SHE treatment. These outcomes suggest that SHE attenuates Th2-type cytokine production via the suppression of OVA-induced Th2 polarisation.

Figure 2. The effect of SHE on murine splenocyte proliferation. Splenocytes were treated with varying concentrations (0, 3.9, 7.8, 15.6, 31.3, 62.5, 125, 250, 500, and 1000 \(\mu\)g/mL) of SHE in the presence of OVA (100 \(\mu\)g/mL), and the \(^{3}\text{H}\) thymidine incorporation into splenocyte DNA was measured after 72 h incubation. The data are represented as the mean ± SEM. The experiments were performed in triplicate, and the data are representative of three individual experiments. \(*\#(p < 0.005)\) indicates statistically significant increase compared with the untreated control (empty bar), and \(**(p < 0.0005)\), \(***p < 0.0005)\) represent significant increase compared with the OVA only group (shaded bar). \(†††p < 0.0005)\) represent significant decrease compared with the OVA-only group (shaded bar).

Figure 3. The effect of SHE on T cell populations. Fluorescence-activated cell sorting (FACS) analysis was performed to evaluate the effect of SHE on the populations of OVA-stimulated splenocytes from OVA-sensitised mice. The mean values of splenic cell populations of (B) CD3\(^{+}\)CD4\(^{+}\) T cells in the absence or presence of OVA are shown. The data are represented as the mean ± SEM. \(\#\#(p < 0.005)\) represents significant increase compared with the untreated control (open bar), and \(\#\#\#(p < 0.0005)\) represents significant decrease compared with the OVA-only group (shaded bar).
Effects of SHE on CD4$^+$ T cell polarisation towards the Th2 immunophenotype in OVA-stimulated splenocytes

We then examined the effect of SHE on the expression level of GATA3, which orchestrates Th2 differentiation. GATA3 expression was also increased by 1.8-fold in the only OVA-stimulated cells compared with the untreated control (Figure 4(D)); however, SHE decreased the expression of GATA3 substantially compared with the OVA-only-stimulated cells (by 2.7- and 3.4-fold at 62.5 and 125 μg/mL, respectively) to a level comparable to that of the untreated control. These results suggest that SHE suppressed the GATA3 transcription factor, which is responsible for Th2 cell fate.

Effects of SHE on STAT5 phosphorylation and NLRP3 inflammasome protein expression and influence of STAT5 inhibitor on OVA-stimulated splenocytes

STAT5 activation plays a critical role in Th2 differentiation. To address the role of STAT5 in the observed Th2 cell differentiation in more detail, we assessed SHE’s role in STAT5 activation in the OVA-stimulated splenocytes of OVA-sensitised mice. As shown in Figures 5(A,C), OVA-only-stimulated cells (in particular when cultured for 48 and 72 h) revealed a markedly increased expression of p-STAT5 in both the cytoplasm and nucleus; however, SHE also dose-dependently decreased p-STAT5 in both the cytoplasm and nucleus. STAT5 activation drove the expression of NLRP3, a component of the inflammasome, in the OVA-only-stimulated cells compared with the untreated control (Figure 5(A)). However, SHE evidently reversed the expression of NLRP3 in OVA stimulated mice, confirming SHE’s potential to diminish Th2 polarisation.

To confirm SHE’s potential to mitigate the phosphorylation of STAT5, we investigated the effect of a specific STAT5 inhibitor, CAS 285986-31-4, on the OVA-stimulated splenocytes of OVA-sensitised mice (Figure 6). When used alone, CAS 285986-31-4 hindered STAT5 phosphorylation, resulting in a drastically reduced pSTAT5 level under OVA-only stimulation, as expected. Surprisingly, however, the combination treatment of CAS 285986-31-4 and SHE resulted in complicated crosstalk with regard to STAT5 phosphorylation. Under the combination treatment, STAT5 phosphorylation was always promoted compared with that observed for the CAS 285986-31-4 treatment alone ($p < 0.05$ in cytosol by 5.8- and 4.2-fold at 62.5 and 125 μg/mL of SHE (Figure 6(A,B)) and in the nucleus by 5.4-fold at 125 μg/mL of SHE (Figure 6(A,C)), respectively, but was still lower than or comparable to that observed for the SHE-only treatment at the same concentration, while the nucleic STAT5 phosphorylation at 125 μg/mL of SHE was higher than for SHE alone.

Effects of STAT5 inhibitor on secretion of IL-4, IL-5, and IL-13 in SHE-treated OVA-stimulated splenocytes

Confirming the suppressive effect of CAS 285986-31-4 on STAT5 phosphorylation, we investigated its role in the expression of the Th2 cytokines IL-4, IL-5, and IL-13. It significantly suppressed the secretion of IL-4, IL-5, and IL-13 when used...
alone, and the inhibitory effect was maintained even when SHE was simultaneously treated for IL-4 and IL-5 (data not shown). For IL-13 (Figure 7), however, the combination treatment produced a complex crosstalk profile, which was, again, similar to the one observed for nucleic STAT5 phosphorylation: the IL-13 secretion was always higher compared to CAS 285986-31-4 treatment alone [by 2.6-fold at $62.5\,\mu g/mL$ ($p=0.06$); and by 3.0-fold at $125\,\mu g/mL$ ($p<0.005$], but was lower than that of the SHE-only treatment at $62.5\,\mu g/mL$, despite being slightly higher at $125\,\mu g/mL$.

Discussion

Inhibitory or suppressive agents can be used to cure diseases that occur due to prolonged immune activation (Ito et al. 2013). In this regard, the utilisation of remedial plants to suppress undesired immunopotentiation prompted heightened interest in their use as an alternative to conventional therapies using synthetic drugs (Ilangkovan et al. 2015). Interestingly, several species of *Sargassum* have been identified for their anti-allergy effects (Jung et al. 2015; Kumar et al. 2016; Herath et al. 2019), but their mechanism and effect on the activation and differentiation of Th2 cells remained unclear. We characterised the effect of SHE on Th2 polarisation in view of the transcription factors and their cytokine profiles using OVA-sensitised splenocytes of OVA-immunised BALB/c mice in this study.

Previously, in OVA-specific immune responses in chickens, we observed that OVA immunisation was highly efficient in inducing a CD4$^+$ helper T cell response in a similar manner to a previous study (Pepper et al. 2004). Activated CD4$^+$ helper T cells and their produced cytokines and chemokines further enhance the activation of other immune organs to compose a panoply of immune responses (Zhu and Paul 2008). In addition to their main role as helper cells, CD4$^+$ helper T cells function as drivers of autoimmune diseases and allergies, including asthma (Hirahara and Nakayama 2016).

In this study, we revealed that SHE downregulated the population expansion of CD4$^+$ helper T cells, simultaneously suggesting its therapeutic potential. Based on the inhibitory function of the exhibited helper T cells, the suggested SHE-induced anti-allergy effects might be mediated through the disruption of Th2 cell polarisation. In this study, we investigated SHE’s effect on T cells in the spleen, a core organ in the immune system with the largest influence on immune function, and confirmed the reduction in splenic T cell proliferation without influencing the cell viability and the decreased secretion of IL-4, IL-5, and IL-13 (Th2) in SHE-treated murine splenocytes under OVA stimulation.

Unusual activation of Th2 cells through the ectopic production of IL-4, IL-5, and IL-13 cytokines is known to result in allergic diseases, such as asthma (Eigenmann 2002; Zhu and Paul 2008). Th2 cytokine production leads to eosinophil accumulation and mucus hypersecretion in the pathogenesis of the hyperresponsiveness of the airway in asthmatic conditions (Murdoch and Lloyd 2010). More importantly, the secreted IL-4 induced the secretion of immunoglobulin E, which stimulated the mast cells to release allergenic factors in order to create allergic responses (Poon et al. 2012).

However, in this study, we determined that SHE effectively inhibited the secretion of Th2-mediated cytokines (IL-4, IL-5, and IL-13) in OVA-induced allergic reactions in murine splenocytes. Therefore, SHE’s potential to suppress Th2 cytokines strengthens the probability of its utilisation as a natural therapeutic candidate in allergic inflammatory diseases and asthma. We assume that bioactive polyphenol components, such as oxyresveratrols and...
flavonoids, might be responsible for anti-allergic effects by inhibiting Th2 cytokines (Leyva-Lopez et al. 2016).

Th differentiation promotes cytokine secretion via several transcription factors, including GATA3, NLRP3, and STAT5 (Ma et al. 2010; Yagi et al. 2010). GATA3 is involved in IL-4, IL-5, and IL-13 (Th2) secretion (Kanhere et al. 2012). In this study, we observed that SHE mitigated the mRNA level of GATA3. We also identified that SHE attenuated the mRNA expression of GATA3 in the lungs of fine dust-exacerbated asthma mice (Herath et al. 2020). SHE’s potential to attenuate GATA3 expression might, in part, account for the observed reduction in IL-4, IL-5, and IL-13 (Th2) release in SHE-treated splenocytes sensitised with OVA. Treatment with a polyphenol component (4-hydroxy-3-methoxycinnamaldehyde [4H3MC]) was reported to attenuate Th2 cytokine production, as reflected by attenuated GATA3 transcriptional factors in atopic dermatitis-induced mice (Lee et al. 2015). We propose that other structurally similar polyphenol components, such as gallic acid in SHE, might also be involved in the observed attenuation of GATA-3 in murine splenocytes under OVA stimulation.

STAT5 also plays a crucial role in Th2 differentiation (Zhu et al. 2003); however, SHE inhibited the phosphorylation and translocation of STAT5 under OVA stimulation, as shown in the present study. This provides more evidence that SHE mitigates the Th2 immune response in OVA-sensitised murine splenocytes. SHE suppressed the OVA-induced phosphorylation of STAT5, confirming the Th2-dominant immune suppression of SHE. In a more detailed investigation with a STAT5 inhibitor, we also observed that a combination treatment of the STAT5 inhibitor and SHE abrogated the inhibitory effect of cytoplasmic and nucleic STAT5 phosphorylation as well as the cytokine secretion compared to treatments with the STAT5 inhibitor alone.

However, we also uncovered an intricate dose-dependent crosstalk in which STAT5 phosphorylation and cytokine secretions were still reduced compared with those of SHE alone at 62.5 μg/mL of SHE, while those in the combination treatment at 125 μg/mL of SHE were, instead elevated compared with those of SHE alone. These results confirm that SHE mitigated the OVA-
induced Th2 response; however, the signalling also involves pathways that differ from those affected by Th2-specific inhibitors.

NLRP3 plays an inflammasome-independent part in controlling the Th2 immune response (Bruchard et al. 2015). Bruchard et al. (2015) revealed that NLRP3 is a key Th2 transcription factor activated by STAT5 that promotes the production of Th2 cytokines, such as IL-4 and IL-5. They observed that NLRP3-deficient Th2 cells, as well as OVA-immunised NLRP3-deficient mice, exhibited attenuated IL-4 and IL-13 secretion (Bruchard et al. 2015). Polyphe nol components are well-known for their ability to attenuate NLRP3 activation, and we assume that the polyphenols in SHE effectively suppressed the NLRP3 activation in OVA-sensitised splenocytes. This may partially account for the observed suppression of the production of the Th2 cytokines IL-4 and IL-5.

In particular, we noticed that polyphenol-containing SHE exhibited Th2 immune suppression by inhibiting the transcription factors GATA3 and NLRP3, which resulted in the attenuated secretion of Th2 cytokines, such as IL-4, IL-5, and IL-13.

Conclusions
The present study shows that SHE exhibited Th2 immune suppression under OVA stimulation. These results suggest that SHE has the potential to be a cogent agent against allergic conditions via GATA3- and NLRP3-dependent IL-4, IL-5, and IL-13 suppression.

Disclosure statement
No potential conflict of interest was reported by the author(s).

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