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ORIGINAL ARTICLE

Scutellarin Ameliorated Ovalbumin-Induced Allergic Rhinitis in Experimental Mice through Potential Attenuation of the GATA3/p-STAT6 Pathway

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ABSTRACT

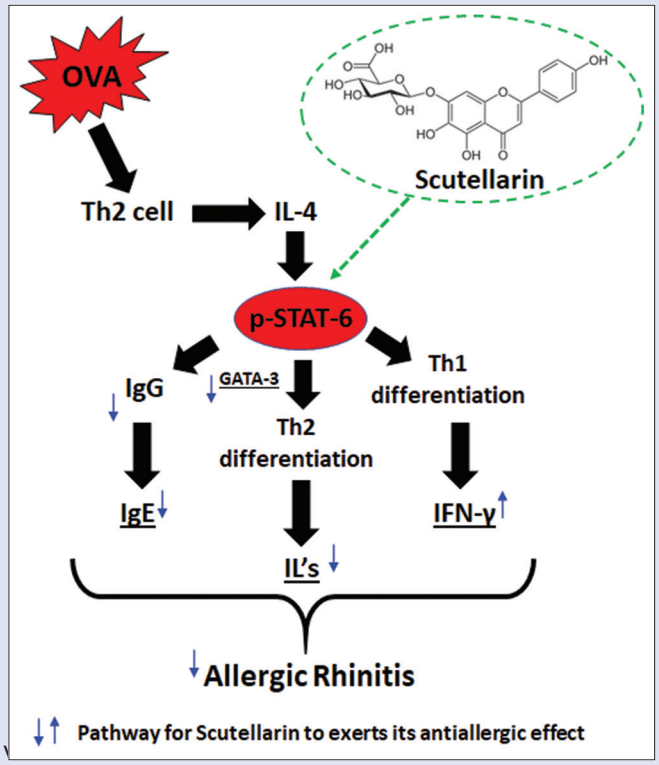
Background: Allergic rhinitis (AR) is an allergic ailment of the immune-inflammatory system affecting people's daily lives. Scutellarin (SCU) is known for its inhibitory potential against GATA binding protein 3 (GATA3), an important mediator of AR. **Aim:** To determine the effect of SCU against AR induced by Ovalbumin (OVA) in the experimental animal. **Materials and Methods:** Mice (BALB/c strain) were induced with AR by sensitizing them with OVA (for consecutively 13 days) followed by the intranasal challenge (on day 21). They were simultaneously treated with either vehicle or montelukast (10 mg/kg) or SCU (50, 100, and 200 mg/kg). **Results:** Administration of SCU (100 and 200 mg/kg) markedly ($P < 0.05$) inhibited elevated nasal rubbing, sneezing, and nasal discharge, OVA-specific and, total immunoglobulin (Ig) E, IgG1 in serum, total and differential cell count, interleukins (ILs) levels in nasal lavage fluid. The up-regulated GATA3 and p-signal transducer and activator of transcription-6 (STAT6) expression of mRNA in the spleen were effectively ($P < 0.05$) reduced by SCU. Additionally, SCU effectively ($P < 0.05$) reduced OVA-induced alteration in nasal histopathology. **Conclusion:** SCU demonstrated an antiallergic effect against OVA-induced AR in experimental mice by inhibiting the GATA3/p-STAT6 pathway, thus improving the Th1/Th2 imbalance.

Key words: Allergic rhinitis, GATA, ovalbumin, scutellarin, STAT6

SUMMARY

- The current work evaluated the antiallergic potential of SCU against OVA-induced AR in experimental mice. This study will contribute valuable insights to the existing body of literature regarding the treatment of AR, and be beneficial to both researchers and physicians alike. An array of biochemical and molecular evidence was evaluated which showed that SCU might be a new therapeutic approach for the management of AR. SCU exerts its antiallergic potential against OVA-induced AR to ameliorate nasal symptoms. SCU exerts its antiallergic potential against OVA-induced AR in experimental mice by inhibiting the GATA3/p-STAT6 pathway, thus improving the Th1/Th2 imbalance.

Abbreviations used: AR: Allergic rhinitis; GATA: GATA binding protein 3 (i.e. Erythroid transcription factor); IFN-γ: Interferon-gamma; Ig: Immunoglobulin; ILs: Interleukins; MLT: Montelukast; NLF: Nasal Lavage Fluid; OVA: Ovalbumin; STAT6: Signal Transducer and Activator of Transcription-6; SCU: Scutellarin.



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INTRODUCTION

Allergic rhinitis (AR) is a disorder mankind is primarily categorized by nasal discharge, sneezing, redness, congestion, and lacrimation of the eyes with loss of taste and smell due to inflammation of the inner lining of nasal mucosal membranes.[1] AR is the fifth most common chronic disease, affecting about 10–30% of adults and 40% of children globally.[2] According to the National Bureau of Statistics of China, approximately 1.37 billion people in China were suffering from AR by 2014.[3] AR predominantly worsens QoL (quality of life) resulting in productivity

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loss and impaired social life. Additionally, studies estimated that the total societal cost for the management of AR in China is $17.49 billion per year,[4] establishing that AR is associated with a significant humanistic and economic burden.

Epidemiological studies suggested that an interaction between environmental conditions and genetic factors plays a vital role in developing AR.[1,5,6] Furthermore, chronic exposure to various indoor and outdoor allergens including pollens, dust mites, molds, and cockroaches induces an immune-inflammatory response in nasal mucosa via activation of immunoglobulin E (IgE) leading to activation of inflammatory cells.[5,7,8] This vicious cycle results in allergen-dependent type-2 T helper (Th2) cell activation, which induces overproduction of Th2 cytokines in the nasal mucosa.[5,7] Clinically, AR patients with elevated levels of Th2 cytokines have been linked with symptoms such as watery nose, sneezing, rubbing, lacrimation, and venous sinusoids.[9-11] In addition, numerous evidence have suggested that elevated production of IgE causes activation of mast cells through binding of IgE to FcεRI (high-affinity immunoglobulin Fc epsilon receptor I) present on a mast cell surface.[1,12] This interaction causes mast cell degranulation, releasing various allergic mediators such as histamine, chemokines, cytokines, prostaglandins, leukotrienes, β-hexosaminidase, and adhesion molecules.[13] These mediators are further implicated in the maintenance of allergic inflammatory reactions in the nasal mucosa. Thus, the counterbalance of Th1 and Th2 cells is an important strategy to mitigate the allergic response.[9,14]

Classical therapeutic modalities for the management of AR include antihistamines, anticholinergic, mast cell stabilizers, anti-leukotriene, anti-allergen, nasal decongestants, and topical corticosteroids.[7,9] However, these treatment regimens often fail to regulate allergic reactions and have been associated with several degrees of adverse reactions, including drying the mucous membrane, blurring of vision, urinary retention, constipation, sedation, and tachycardia.[7] Subsequently, evaluation of effective and safe therapy is urgently needed, and experimental animal models are a useful tool for identifying such therapeutic options. Among various animal models, Ovalbumin (OVA)-induced AR is an extensively used, inexpensive, and well-recognized murine model of AR.[7,15-17] Furthermore, systemic OVA installation is consequently followed by its challenge intranasally caused induction of IgE-mediated allergic airway disease, which reflects characteristic features such as congestion, sneezing, and nasal scratching, closely resembling clinical AR.[9,15,16]

Traditional Chinese medicine contains a wide range of herbal moieties comprising various phytoconstituents that can interact with multi-target combinations involved in the pathogenesis of allergic reactions.[18-21] Scutellarin (SCU) (4,5,6-trihydroxyflavone-7-glucuronide) is one such phytoconstituent available in several endemic Chinese plants, including *Erigeron breviscapus* (Vant.), *Scutellaria baicalensis, Scutellaria barbata,* and *Scutellaria lateriflora*. SCU has been recognized for its pharmacological properties against various diseases, including but not limited to cardiovascular, hypertension, angina pectoris, cerebral ischemia, platelet aggregation, hyperlipidemia, oxidative stress, cellular apoptosis, inflammation, diabetes, nephrotoxicity, and neurotoxicity.[22-27] Researchers have reported a wide range of safety profiles of SCU (100 and 500 mg/kg, for 28 days), where it did not produce any toxicity in experimental animals.[22] A study suggested that SCU exerts anti-inflammatory potential through attenuation of pro-inflammatory cytokines, such as interleukins (ILs) and tumor necrosis factor-alpha (TNF-α) during lung injury.[25] Furthermore, recent molecular network interaction studies have highlighted the inhibitory potential of SCU against GATA binding protein 3 (GATA3) protein.[18] However, the antiallergic potential of SCU has not been systematically studied against AR. Thus, this study aimed to determine the effect of SCU against AR induced by OVA in experimental mice.

MATERIALS AND METHODS

Study animals

BALB/c mice (18–22 g, male) were maintained in quarantine for a week in Yan'an University animal house at conditions: 24 ± 1°C (temperature), 45–55% (relative humidity), normal light/dark cycle, food (standard chaw pellet), and water *ad libitum*. Yan'an University animal ethical committee approved (No. YAU-0722) all the experimental protocols.

Experimental protocol

Induction of AR was carried out as previously described.[15] Briefly, mice were sensitized on each alternative day starting from day 1 to 13 using OVA (50 mg, Grade V, Sigma-Aldrich Co., St Louis, MO, USA) triturated in alumnium hydroxide (1000 mg, Sigma-Aldrich Co., St Louis, MO, USA) in saline (500 ml). On day 14, mice were divided randomly into groups (n = 18 mice/group) and treated orally with either distilled water (10 mg/kg) or montelukast (MLT) (10 mg/kg, Cipla Limited, Mumbai, India),[12] or SCU (purity ≥95%, 50, 100 and 200 mg/kg, p.o.),[23,24] for the next 7 days. A separate group of non-sensitized mice either received distilled water (10 mg/kg) or SCU (200 mg/kg) orally for 7 days. The mice were challenged with OVA intranasally on day 21 to record observations including sneezing, nasal rubbing, and discharge. On day 24, animals were challenged with histamine dihydrochloride and nasal symptoms were recorded for 10 min.[15] Rats were anesthetized with ether for blood withdrawal and then rats were sacrificed by cervical dislocation. Blood samples were analyzed for hematological and biochemical parameters. Nasal lavage fluid (NLF) was collected to determine total and differential cell count. The levels of total IgG1, OVA-specific, and total IgE were determined in the serum whereas levels of IL-4, IL-5, IL-17, and IFN-γ were evaluated in NLF using respective mouse ELISA quantitation kit (Bethyl Laboratories Inc., Montgomery, TX, USA). The splenic mRNA levels of GATA3 (up-stream: 5′-TCATTAAGCCCAAGCGAAGG-3′; down-stream: 5′-GTCATTGGCATTCCTC-3′; amplicon size: 327), and p-STAT6 (up-stream: 5 ′-CTCTGTGGGGCCTAATTT CCA-3′; down-stream: 5′-GCATCTGAACCGACCAGGAAC-3′; amplicon size: 119) were evaluated using reverse transcriptase polymerase chain reaction (RT-PCR) approach as per instructions provided by the manufacturer (MP Biomedicals India Private Limited, India). The histological analysis of nasal mucosa was evaluated using haematoxylin and eosin (H and E). The intensity of histological aberrations in the nasal tissue was graded as Grade 0 (not present or very slight); Grade 1 (mild); Grade 2 (moderate); and Grade 3 (severe). GraphPad Prism 5.0 software was used for statistical analyses to compare the difference between AR control animals and drug-treated groups. Statistical significance was achieved at $P < 0.05$.

RESULTS

Change in body weight and spleen weight

OVA-challenged mice showed a noticeable ($P < 0.05$) decrease in body weight and relative spleen weight to body weight ratio in contrast to normal and *per se* treated mice. However, treatment with MLT significantly ($P < 0.05$) inhibited alterations induced in weight of the body, and spleen weight to body weight ratio in contrast with AR control mice. Similarly, SCU treatment (100 and 200 mg/kg) also significantly ($P < 0.05$)

elevated the weight of the body, and decreased spleen weight to body weight ratio as compared with AR control mice. Protection against alterations induced in body weight and spleen weight to body weight ratio post-OVA-challenge were more pronounced ($P < 0.05$) after MLT administration compared with SCU treatment [Table 1].

Alterations in nasal symptoms

When compared with normal and *per se* treated mice, nasal symptoms including nasal discharge, sneezing, and rubbing were effectively ($P < 0.05$) elevated in AR control mice post-OVA-challenge. However, MLT administration noticeably ($P < 0.05$) decreased nasal symptoms in contrast to AR control mice. Additionally, alterations in nasal symptoms post-OVA-challenge were prominently ($P < 0.05$) reduced by SCU treatment (100 and 200 mg/kg) compared to AR control mice. However, administration of MLT more notably ($P < 0.05$) reduced nasal symptoms compared to SCU treatment [Table 1].

Nasal hypersensitivity induced post histamine challenge

Histamine challenge induces hypersensitivity in mice reflected by effectively ($P < 0.05$) increased nasal rubbing and sneezing compared to normal and *per se* treated mice. However, MLT treatment significantly ($P < 0.05$) reduced histamine-induced nasal hypersensitivity in contrast to AR control mice. SCU treatment (100 and 200 mg/kg) also effectively ($P < 0.05$) lessened histamine-induced increased sneezing and nasal rubbing in contrast to AR control mice. Additionally, MLT administration more strikingly repressed ($P < 0.05$) histamine-induced nasal hypersensitivity in contrast to SCU treatment [Table 1].

Alterations in BALF cell count

A noticeable ($P < 0.05$) increase in BALF cells (eosinophils, neutrophils, lymphocytes, and macrophages) count was recorded post-OVA-challenge as compared with normal and *per se* treated mice. MLT treatment significantly ($P < 0.05$) decreased OVA-challenge-induced increased cell count in BALF compared to AR control mice. SCU treatment (100 and 200 mg/kg) also notably ($P < 0.05$) inhibited OVA-challenge-induced increased cell count in BALF, in contrast to AR control mice. However, elevated counts of eosinophils, neutrophils, lymphocytes, and macrophages in BALF were more noticeably decreased ($P < 0.05$) post-administration of MLT, confront to SCU treatment [Figure 1].

Alterations in serum IgE and IgG1 levels

Compared to normal and *per se* treated mice, IgE and IgG1 levels were efficiently ($P < 0.05$) elevated in the serum of AR control mice. Administration of MLT markedly ($P < 0.05$) reduced levels of serum IgG1 and IgE, in contrast to AR control mice. SCU treatment (100 and 200 mg/kg) also showed an effective ($P < 0.05$) reduction in levels of serum IgE and IgG1, confront to AR control mice. Administration of MLT more effectively ($P < 0.05$) reduced levels of serum IgE and IgG1, in contrast to SCU [Table 2].

Alterations in ILs and IFN-γ levels in NLF

OVA-challenge caused a noticeable ($P < 0.05$) increase in ILs in NLF, whereas a decrease in IFN-γ as compared to normal and *per se* group. Compared to AR control mice, administration of MLT noticeably ($P < 0.05$) ameliorated variations in levels of ILs and IFN-γ in NLF induced by the OVA-challenge. Treatment with SCU (100 and 200 mg/kg) also markedly ($P < 0.05$) reduced levels of ILs and increased levels of IFN-γ in NLF, in contrast to AR control mice. However, alterations induced in NLF ILs and IFN-γ levels post-OVA-challenge

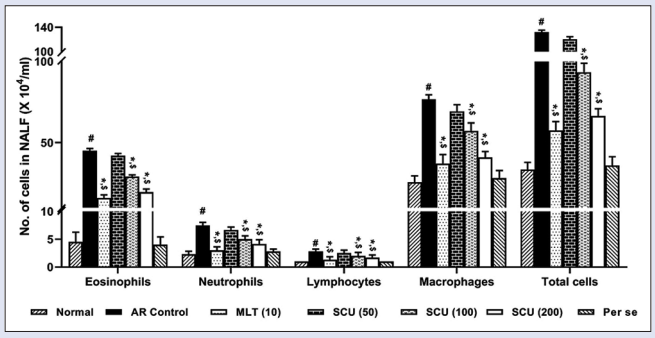

Figure 1: Effect of SCU treatment on OVA-induced alterations in total and differential cell count in NFL in AR mice. Data were represented as mean ± SEM ($n = 6$) and analyzed by one-way ANOVA followed by Tukey's multiple range test. $^{\#}P < 0.05$ as compared with normal group, $^{*}P < 0.05$ as compared with AR control group and $^{\$}P < 0.05$ as compared MLT with SCU. Figures in parentheses indicate oral dose in mg/kg. AR: allergic rhinitis; OVA: ovalbumin; MLT (10): montelukast (10 mg/kg) treated; SCU (50): scutellarin (50 mg/kg) treated; SCU (100): scutellarin (100 mg/kg) treated; SCU (200): scutellarin (200 mg/kg) treated

Table 1: Effect of SCU treatment on OVA-induced alterations in body weight, relative spleen weight, OVA-challenge-induced nasal rubbing, sneezing, and nasal discharge, as well as histamine challenge-induced nasal rubbing and sneezing in AR mice

Parameters	Treatment						
	Normal	AR control	MLT (10)	SCU (50)	SCU (100)	SCU (200)	Per se
Body weight (gm) on day 21	34.50±1.54	24.33±1.63$^{\#}$	29.17±1.74$^{*,\$}$	22.50±1.36	24.67±1.33$^{*,\$}$	28.83±1.76$^{*,\$}$	33.00±1.34
Spleen weight/body weight (mg/gm) (×10^{-3}) on day 21	3.24±0.12	5.55±0.37$^{\#}$	3.99±0.23$^{*,\$}$	5.93±0.39	5.15±0.38$^{*,\$}$	4.25±0.28$^{*,\$}$	3.38±0.15
OVA-challenge on day 21							
Rubbing (number)	16.67±1.12	71.17±1.30$^{\#}$	24.00±1.03$^{*,\$}$	65.00±1.10	45.50±1.29$^{*,\$}$	32.33±1.12$^{*,\$}$	16.83±0.87
Sneezing (number)	10.00±0.37	40.00±0.89$^{\#}$	14.33±0.71$^{*,\$}$	38.17±0.70	32.00±0.52$^{*,\$}$	22.17±0.79$^{*,\$}$	13.00±0.97
Discharge (score)	0.33±0.21	2.67±0.21$^{\#}$	0.67±0.21$^{*,\$}$	2.83±0.17	2.00±0.26$^{*,\$}$	1.50±0.22$^{*,\$}$	0.33±0.21
Histamine challenge on day 24							
Rubbing (number)	16.83±1.20	69.17±1.05$^{\#}$	25.33±1.45$^{*,\$}$	71.17±1.08	47.67±1.52$^{*,\$}$	31.17±1.30$^{*,\$}$	20.17±1.11
Sneezing (number)	9.83±1.20	53.00±1.37$^{\#}$	15.00±0.97$^{*,\$}$	45.67±0.95	33.67±1.09$^{*,\$}$	20.83±1.47$^{*,\$}$	14.83±0.87

Data were represented as mean±SEM (n=6). Data for body weight and relative spleen weight were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's multiple range test, whereas data of OVA and histamine challenge number and score were analyzed by non-parametric Kruskal–Wallis test ANOVA followed by Mann–Whitney's multiple comparison tests. $^{\#}P<0.05$ as compared with normal group, $^{*}P<0.05$ as compared with AR control group and $^{\$}P<0.05$ as compared MLT with SCU. Figures in parentheses indicate oral dose in mg/kg. AR: allergic rhinitis; OVA: ovalbumin; MLT (10): montelukast (10 mg/kg) treated; SCU (50): scutellarin (50 mg/kg) treated; SCU (100): scutellarin (100 mg/kg) treated; SCU (200): scutellarin (200 mg/kg) treated

were more effectively inhibited by MLT treatment than SCU treatment [Table 2].

Alterations in mRNA expressions of GATA3 and p-STAT6 in the spleen

The mRNA expressions of GATA3 and p-STAT6 in the spleen were markedly ($P < 0.05$) up-regulated in AR control mice post-challenge with OVA, in contrast to normal and *per se* treated mice. However, MLT and SCU treatment (100 and 200 mg/kg) effectively ($P < 0.05$) down-regulated mRNA expressions of GATA3 and p-STAT6 in the spleen, confront to AR control mice [Figure 2a and 2b].

Splenic GATA3 expression showed a positive correlation between OVA-challenge-induced sneezing ($R^2 = 0.8729$) and OVA-specific IgE ($R^2 = 0.8868$), depicting the importance of GATA3 expression during AR [Figure 2c and 2d].

Alterations in nasal mucosa histopathology

Figures 3a and 3f depict well organized nasal mucosa architecture from *per se* and normal mice. However, an intranasal challenge with OVA caused noticeable ($P < 0.05$) infiltration of eosinophils which resulted in hyperplasia, edema in AR control mice nasal mucosa. This causes a disturbance of mucosal epithelium [Figure 3b] compared to normal and *per se* treated mice. Conversely, MLT treatment effectively ($P < 0.05$) ameliorated OVA-challenge-induced aberrations in nasal histology compared to AR control mice [Figure 3c]. Similarly, SCU treatment (100 and 200 mg/kg) also noticeably ($P < 0.05$) reduced eosinophil infiltration,

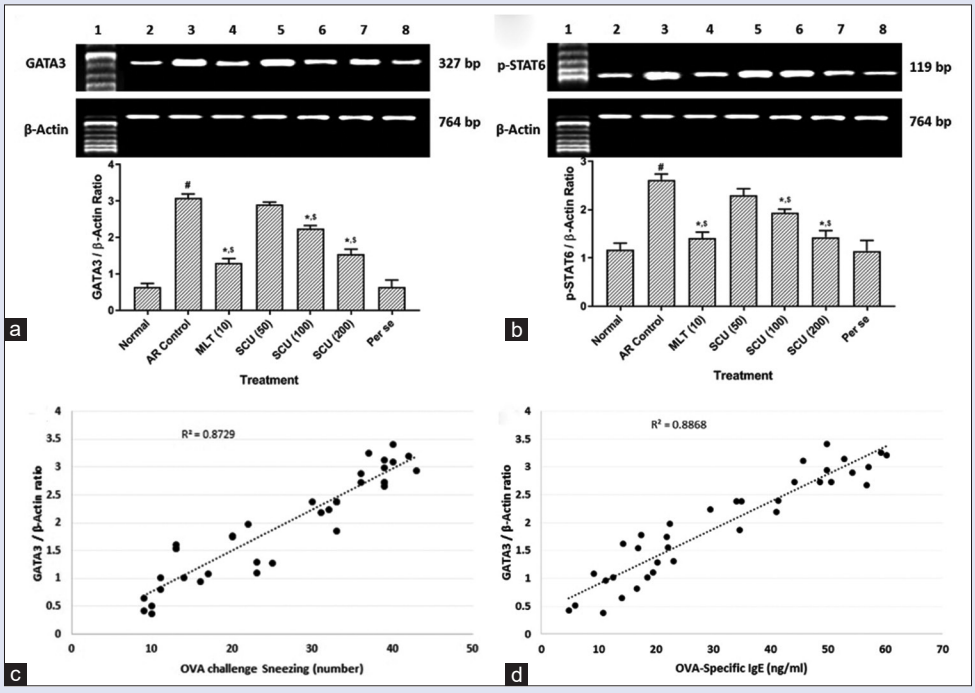

Figure 2: Effect of SCU treatment on OVA-induced alterations in spleen GATA3 (a) and p-STAT6 (b) mRNA expressions in AR mice. The plot depicts correlation of GATA3 expression against OVA-challenge-induced nasal sneezing (c) and OVA-specific IgE (d) in AR mice. Data were represented as mean ± SEM ($n = 6$) and analyzed by one-way ANOVA followed by Tukey's multiple range test. $^{\#}P < 0.05$ as compared with normal group, $^{*}P < 0.05$ as compared with AR control group and $^{\$}P < 0.05$ as compared MLT with SCU. Figures in parentheses indicate oral dose in mg/kg. AR: allergic rhinitis; OVA: ovalbumin; MLT (10): montelukast (10 mg/kg) treated; SCU (50): scutellarin (50 mg/kg) treated; SCU (100): scutellarin (100 mg/kg) treated; SCU (200): scutellarin (200 mg/kg) treated; GATA3: GATA binding protein 3; STAT6: signal transducer and activator of transcription-6

Table 2: Effect of SCU treatment on OVA-induced alterations in OVA-specific IgE, total IgE and IgG1 in serum, and IL-4, IL-5, IL-17, and IFN-γ levels in NLF in AR mice

Parameters	Treatment						
	Normal	AR control	MLT (10)	SCU (50)	SCU (100)	SCU (200)	Per se
OVA-specific IgE (ng/ml)	11.80±2.30	55.51±1.87$^{\#}$	14.36±1.88$^{*,\$}$	49.36±1.63	35.91±1.84$^{*,\$}$	20.74±0.87$^{*,\$}$	18.31±1.52
Total IgE (ng/ml)	109.00±6.03	407.60±6.22$^{\#}$	141.70±8.31$^{*,\$}$	400.30±10.17	323.30±8.06$^{*,\$}$	223.50±8.91$^{*,\$}$	108.10±9.43
Total IgG1 level (ng/ml)	0.308±0.018	0.658±0.032$^{\#}$	0.347±0.021$^{*,\$}$	0.608±0.029	0.495±0.016$^{*,\$}$	0.443±0.013$^{*,\$}$	0.317±0.021
IL-4 (pg/ml)	43.79±2.88	149.40±4.51$^{\#}$	61.44±4.63$^{*,\$}$	138.20±5.12	116.20±4.81*	73.22±0.80$^{*,\$}$	71.87±2.29
IL-5 (pg/ml)	40.30±3.24	89.57±3.31$^{\#}$	42.39±2.32$^{*,\$}$	86.45±3.97	70.17±3.02$^{*,\$}$	56.14±2.81$^{*,\$}$	45.45±1.81
IL-17 (pg/ml)	1.70±0.42	38.17±0.91$^{\#}$	7.71±0.78$^{*,\$}$	37.03±2.50	22.94±0.82$^{*,\$}$	11.14±0.64$^{*,\$}$	3.93±0.54
IFN-γ (pg/ml)	72.08±3.83	38.58±4.67$^{\#}$	54.34±6.01$^{*,\$}$	41.18±3.69	45.25±4.16	58.63±4.67$^{*,\$}$	66.97±3.39
IL-4/IFN-γ ratio	0.61±0.03	4.20±0.58$^{\#}$	1.21±0.16$^{*,\$}$	3.51±0.36	2.68±0.26*	1.28±0.09$^{*,\$}$	1.090±0.07

Data were represented as mean±SEM (n=6) and analyzed by one-way ANOVA followed by Tukey's multiple range test. $^{\#}P$ <0.05 as compared with normal group, $^{*}P$ <0.05 as compared with AR control group and $^{\$}$P <0.05 as compared MLT with SCU. Figures in parentheses indicate oral dose in mg/kg. AR: allergic rhinitis; OVA: ovalbumin; MLT (10): montelukast (10 mg/kg) treated; SCU (50): scutellarin (50 mg/kg) treated; SCU (100): scutellarin (100 mg/kg) treated; SCU (200): scutellarin (200 mg/kg) treated; Ig: immunoglobulin; ILs: interleukins; IFN-γ: interferon gamma; NLF: nasal lavage fluid.

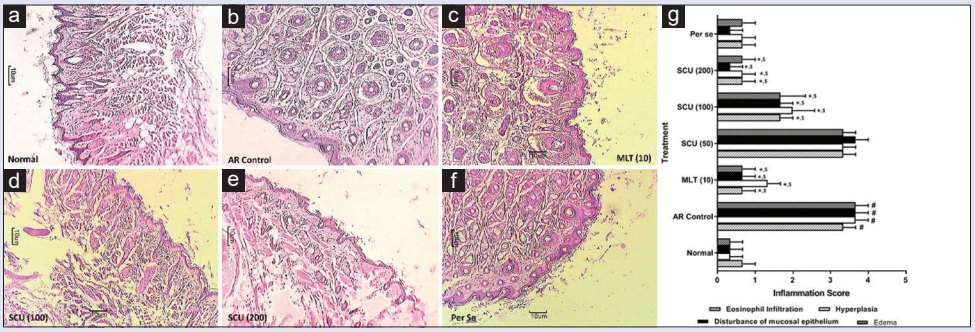

Figure 3: Effect of SCU treatment on OVA-induced alteration in nasal histopathology in AR mice**.** Photomicrograph of sections of nasal tissue from normal (a), AR control (b), MLT (10 mg/kg) (c), SCU (100 mg/kg) (d), SCU (200 mg/kg) (e), and *Per se* (f) treated mice (H and E stain). The quantitative representation of histological score (g). Data were expressed as mean ± SEM (n = 3), and one-way ANOVA followed by Kruskal–Wallis test was applied for *post hoc* analysis. $^{\#}P < 0.05$ as compared with normal group, $^{*}P < 0.05$ as compared with AR control group and $^{\$}P < 0.05$ as compared MLT with SCU. AR: allergic rhinitis; OVA: ovalbumin; MLT (10): montelukast (10 mg/kg) treated; SCU (100): scutellarin (100 mg/kg) treated; SCU (200): scutellarin (200 mg/kg) treated mice

which further decreased hyperplasia, edema in the nasal mucosa, and reduced disturbance of mucosal epithelium [Figure 3d, 3e and 3g].

DISCUSSION

AR is an immune-inflammatory system disorder affecting the daily lives of numerous people.[1] Although AR is not a life-threatening disorder, its increased prevalence is a leading cause of public health care concern.[28] Several therapies have been developed to counteract allergic prophylaxis however, most of them are either unable to regulate the allergic reaction or produce resistance. Additionally, associated side effects and high cost limit their clinical implication during AR.[1] Previously, several investigators have documented the antiallergic property of various moieties of plant origin against seasonal rhinitis clinically.[19,21,29] In the current study, the potential of SCU was determined against AR induced in experimental mice. The findings of this investigation suggested that SCU exerts its antiallergic potential via inhibition of the GATA3/p-STAT6 pathway, thus improving the Th1/Th2 imbalance during AR (Graphical Abstract).

Several researchers have reported that biphasic allergic reaction is a hallmark of allergen-induced AR.[1,2,9] During early phases, the interaction of allergen and IgE receptor cause activation of basophils and mast cells.[1,30] These immune-inflammatory cells cause a release of various inflammatory mediators, including leukotrienes, prostaglandins, histamine, and cytokines, which further contribute to the development of various nasal symptoms, including sneezing, discharge, and itching.[30] In the late-phase reaction, recruitment and accumulations of basophils, eosinophils, and mast cells in the deeper lamina propria result in an aggravated response of histamine, leukotrienes, pro-inflammatory cytokines, and chemokines which further sustain the allergic response.[1,9] In the present murine models of IgE-mediated allergic response, OVA sensitization followed by its nasal challenge potentiated the immune response, reflected by the elevated recruitment of eosinophils, neutrophils, lymphocytes, and macrophages in NLF of AR control mice. However, SCU treatment ameliorated elevated levels of differential cell count in NLF, suggesting its anti-inflammatory potential. The findings of the current study are by previous researchers where SCU treatment showed its effectiveness via down-regulation of recruitment of macrophages.[23] Additionally, histopathological evaluation of nasal tissue from SCU-treated mice showed a significant reduction in inflammatory infiltration into the nasal mucosa, providing further evidence of its therapeutic ability.

Numerous researchers have documented that elevated IgE production in response to environmental allergens is a characteristic feature of AR.[7,9,15] IgE antibody is the vital mediator for allergic reactions, formatted by stimulated allergen-specific B cells.[1,12,31] The IgE level increased due to the presence of allergens in the serum, which is susceptible to stimulation.[7] IgE antibodies bind to IgE-specific receptors known as FcεRI, present on eosinophils, mast cells, and basophils; thus, these cells are susceptible to stimulation by an environmental allergen.[7] Furthermore, dominant modulators such as Th2 cells and B cell proliferation produce eosinophils, IgE, and IgG1, and pull enormous unite of inflammatory cell infiltrations in the nasal tissue.[14,32] Thus, elevated Th2 cytokines are closely connected with IgE-mediated immune reactions.[7,30,33] Patients with AR showed elevated IgE and IgG levels, which are further linked with specific symptoms of AR, including nasal congestion and discharge, redness, sneezing, itching, and rhinorrhea.[9,11,34] OVA-sensitized and challenged mice also exhibit elevated levels of OVA-specific IgE levels in sera, which further induces characteristic pathological symptoms of AR.[7] In agreement with a previous study, the present study also reported that AR mice showed elevated IgE and IgG levels in serum, which in turn showed allergen-specific nasal symptoms. However, the administration of SCU significantly reduced the allergen-induced IgE and IgG levels, which might play a vital role in attenuating nasal rubbing, sneezing, and discharge post-OVA-challenge.

The important role of Th2 cytokines in developing allergic reactions has been well supported by researchers.[14,16,35] IL-4 has been demonstrated as a mast cell chemoattractant and is responsible for initiating allergic reactions via inhibition of Th1 differentiation, whereas IL-5 has recently been documented for amplifying allergen-induced late-phase allergic response during the pathogenesis of AR.[12,35,36] Furthermore, the severity of allergic response was directly correlated with the elevated expressions of IL-17 in blood and nasal mucosa samples in patients with AR.[37] Gu *et al.*[38] (2017) showed inhibition of elevated IL-17 response by administration of anti-IL-17 antibody led to prominent suppression of Th2 response, and thus allergic symptoms in AR mice. On the other hand, boosting the Th1 immune response through IFN-γ activation is a promising strategy for inhibiting allergic response.[35,39,40] IFN-γ, a Th1 cytokine secreted by natural killer cells, plays an important role in inhibiting the switching of B cells to IgE production.[12] In the present study, the Th2 cytokines levels were found to be elevated in NLF of AR control mice. However, the administration of SCU activated the production of IFN-γ, which further suppressed increased levels of ILs, suggesting the role of SCU in the regulation of IL-4/IFN-γ ratio during allergic responses.

Induction of allergic response via a disproportion in response of Th1 and Th2 was further explained based on the elevated expression of GATA3.[41,42] GATA3 is a member of the GATA family of transcription factors that are effectively expressed in Th2 cells and induce Th2 differentiation via activation of STAT6.[43-45] Aggravated expression of IL-4 causes phosphorylation of STAT6 via activation of Janus Kinase 1, resulting in STAT6 homodimer formation, followed by its nuclear translocation.[46] Furthermore, STAT6 has been identified as an important molecule in developing Th2-cell and class switching between Ig and IgE in B cells.[39,47,48] Researchers have documented that STAT6 knockout mice failed to develop IgE-induced allergic response, and expression of IL-4 was normal.[39] Furthermore, Akei *et al.*[5] (2006) reported that STAT6 knockout mice showed fewer frequencies of sneezing induced by the intranasal challenge of fumigatus. The results of our study also showed that OVA-induced allergic responses were linked with up-regulated splenic GATA3 and p-STAT6 expression. Notably, these expressions were significantly down-regulated following treatment with SCU, suggesting its important role in regulating Th1/Th2 balance. Recent molecular network interaction also supports the finding of the present investigation where SCU showed prominent inhibitory potential against GATA3 protein.[18]

MLT, cysteinyl leukotrienes-1 receptor antagonists, is currently approved as the first-line treatment for the management of AR. Numerous investigators have well documented its antiallergic potential during AR.[49] However, post-marketing clinical surveillance analysis suggested its possible association with several psychiatric side effects, including aggression, hallucination, anxiousness, insomnia, depression, restlessness, and irritability.[7] Indeed, flavone from plant origin has proven its antiallergic efficacy in patients with AR.[19,21] Additionally, a plant containing SCU as a major phytoconstituents has already shown beneficial effects in various clinical settings.[26] Thus, SCU can serve as an important therapeutic moiety for further clinical development in the treatment of AR.

CONCLUSION

The observations of the current investigation suggested that SCU inhibits OVA-induced allergic reactions developed in experimental mice. Furthermore, scutellarin exerted its antiallergic potential by suppressing the activation of the GATA3/p-STAT6 pathway, thus improving the Th1/Th2 imbalance during AR.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Eifan AO, Durham SR. Pathogenesis of rhinitis. Clin Exp Allergy 2016;46:1139-51.
2. Mims JW. Epidemiology of allergic rhinitis. Int Forum Allergy Rhinol 2014;4(Suppl 2):S18-20.
3. National Bureau of Statistics of China. Available from: http://www.stats.gov.cn/. [Last accessed on 2021 Aug 24].
4. Chen J, Xiang J, Wang Y, Shi Q, Tan H, Kong W. [Health economics analysis of specific immunotherapy in allergic rhinitis accompanied with asthma]. Lin Chung Er Bi Yan Hou Tou Jing Wai Ke Za Zhi 2013;27:925-8.
5. Akei HS, Brandt EB, Mishra A, Strait RT, Finkelman FD, Warrier MR, *et al.* Epicutaneous aeroallergen exposure induces systemic TH2 immunity that predisposes to allergic nasal responses. J Allergy Clin Immunol 2006;118:62-9.
6. Fröhlich M, Pinart M, Keller T, Reich A, Cabieses B, Hohmann C, *et al.* Is there a sex-shift in prevalence of allergic rhinitis and comorbid asthma from childhood to adulthood? A meta-analysis. Clin Transl Allergy 2017;7:44.
7. Mandhane SN, Shah JH, Thennati R. Allergic rhinitis: An update on disease, present treatments and future prospects. Int Immunopharmacol 2011;11:1646-62.
8. Mukherjee AA, Kandhare AD, Rojatkar SR, Bodhankar SL. Ameliorative effects of Artemisia pallens in a murine model of ovalbumin-induced allergic asthma via modulation of biochemical perturbations. Biomed Pharmacother 2017;94:880-9.
9. Hoyte FCL, Nelson HS. Recent advances in allergic rhinitis. F1000Res 2018;7. doi: 10.12688/f1000research. 15367.1
10. Kim TH, Kim K, Park SJ, Lee SH, Hwang JW, Park SH, *et al.* Expression of SOCS1 and SOCS3 is altered in the nasal mucosa of patients with mild and moderate/severe persistent allergic rhinitis. Int Arch Allergy Immunol 2012;158:387-96.
11. Passali D, Cingi C, Staffa P, Passali F, Muluk NB, Bellussi ML. The International study of the allergic rhinitis survey: Outcomes from 4 geographical regions. Asia Pac Allergy 2018;8:e7.
12. Chen B, Qu S, Li M, Ye L, Zhang S, Qin T, *et al.* Effects of 1,25-dihydroxyvitamin D3 in an ovalbumin-induced allergic rhinitis model. Int Immunopharmacol 2017;47:182-9.
13. Mohod SM, Kandhare AD, Bodhankar SL. Gastroprotective potential of pentahydroxy flavone isolated from Madhuca indica J.F. Gmel. leaves against acetic acid-induced ulcer in rats: The role of oxido-inflammatory and prostaglandins markers. J Ethnopharmacol 2016;182:150-9.
14. Kandhare AD, Liu Z, Mukherjee AA, Bodhankar SL. Therapeutic potential of Morin in ovalbumin-induced allergic asthma via modulation of SUMF2/IL-13 and BLT2/NF-kB signaling pathway. Curr Mol Pharmacol 2019;12:122-38.
15. Aswar UM, Kandhare AD, Mohan V, Thakurdesai PA. Anti-allergic effect of intranasal administration of type-A procyanidin polyphenols based standardized extract of cinnamon bark in ovalbumin sensitized BALB/c mice. Phytother Res 2015;29:423-33.
16. Bozkurt MK, Tülek B, Bozkurt B, Akyürek N, Öz Mehmet M, Kiyici A. Comparison of the efficacy of prednisolone, montelukast, and omalizumab in an experimental allergic rhinitis model. Turk J Med Sci 2014;44:439-47.
17. Kandhare AD, Raygude KS, Ghosh P, Gosavi TP, Bodhankar SL. Patentability of animal models: India and the globe. Int J Pharm Biol 2011;2:1024-32.
18. Du L, Ye X, Li M, Wang H, Zhang B, Zheng R, *et al.* Mechanisms of traditional Chinese medicines in the treatment of allergic rhinitis using a network biology approach. J Tradit Chin Med Sci 2021;8:82-9.
19. Guo R, Pittler MH, Ernst E. Herbal medicines for the treatment of allergic rhinitis: A systematic review. Ann Allergy Asthma Immunol 2007;99:483-95.
20. Shao YY, Zhou YM, Hu M, Li JZ, Chen CJ, Wang YJ, *et al.* The anti-allergic rhinitis effect of traditional Chinese medicine of Shenqi by regulating mast cell degranulation and Th1/Th2 cytokine balance. Molecules 2017;22:504.
21. Wang S, Tang Q, Qian W, Fan Y. Meta-analysis of clinical trials on traditional Chinese herbal medicine for treatment of persistent allergic rhinitis. Allergy 2012;67:583-92.
22. Li X, Wang L, Li Y, Bai L, Xue M. Acute and subacute toxicological evaluation of scutellarin in rodents. Regul Toxicol Pharmacol 2011;60:106-11.
23. Liu Y, Jing YY, Zeng CY, Li CG, Xu LH, Yan L, *et al.* Scutellarin suppresses NLRP3 inflammasome activation in macrophages and protects mice against bacterial sepsis. Front Pharmacol 2017;8:975.
24. Liu Y, Wang J, Zhang X, Wang L, Hao T, Cheng Y, *et al.* Scutellarin exerts hypoglycemic and renal protective effects in db/db mice via the Nrf2/HO-1 signaling pathway. Oxid Med Cell Longev 2019;2019:1354345.
25. Tan ZH, Yu LH, Wei HL, Liu GT. Scutellarin protects against lipopolysaccharide-induced acute lung injury via inhibition of NF-kappaB activation in mice. J Asian Nat Prod Res 2010;12:175-84.
26. Wang L, Ma Q. Clinical benefits and pharmacology of scutellarin: A comprehensive review. Pharmacol Ther 2018;190:105-27.
27. Wang S, Zhang H, Xi Z, Huang J, Nie J, Zhou B, *et al.* Establishment of a mouse model of lipopolysaccharide-induced neutrophilic nasal polyps. Exp Ther Med 2017;14:5275-82.
28. Brożek JL, Bousquet J, Baena-Cagnani CE, Bonini S, Canonica GW, Casale TB, *et al.* Allergic Rhinitis and its Impact on Asthma (ARIA) guidelines: 2010 revision. J Allergy Clin Immunol 2010;126:466-76.
29. Walanj S, Walanj A, Mohan V, Thakurdesai PA. Efficacy and safety of the topical use of intranasal cinnamon bark extract in seasonal allergic rhinitis patients: A double-blind placebo-controlled pilot study. J Herb Med 2014;4:37-47.
30. Tanaka T, Iuchi A, Harada H, Hashimoto S. Potential beneficial effects of wine flavonoids on allergic diseases. Diseases 2019;7:8.
31. Kandhare AD, Bodhankar SL, Singh V, Mohan V, Thakurdesai PA. Anti-asthmatic effects of type-A procyanidine polyphenols from cinnamon bark in ovalbumin-induced airway

hyperresponsiveness in laboratory animals. Biomed Aging Pathol 2013;3:23-30.

32. Hirano T, Higa S, Arimitsu J, Naka T, Shima Y, Ohshima S, *et al.* Flavonoids such as luteolin, fisetin and apigenin are inhibitors of interleukin-4 and interleukin-13 production by activated human basophils. Int Arch Allergy Immunol 2004;134:135-40.
33. Mlcek J, Jurikova T, Skrovankova S, Sochor J. Quercetin and its anti-allergic immune response. Molecules 2016;21:623.
34. Nikakhlagh S, Rahim F, Aryani FH, Syahpoush A, Brougerdnya MG, Saki N. Herbal treatment of allergic rhinitis: The use of Nigella sativa. Am J Otolaryngol 2011;32:402-7.
35. Das J, Chen CH, Yang L, Cohn L, Ray P, Ray A. A critical role for NF-kappa B in GATA3 expression and TH2 differentiation in allergic airway inflammation. Nat Immunol 2001;2:45-50.
36. Wee JH, Zhang YL, Rhee CS, Kim DY. Inhibition of allergic response by intranasal selective NF-κB decoy oligodeoxynucleotides in a murine model of allergic rhinitis. Allergy Asthma Immunol Res 2017;9:61-9.
37. Carr VM, Robinson AM, Kern RC. Tissue-specific effects of allergic rhinitis in mouse nasal epithelia. Chem Senses 2012;37:655-68.
38. Gu ZW, Wang YX, Cao ZW. Neutralization of interleukin-17 suppresses allergic rhinitis symptoms by downregulating Th2 and Th17 responses and upregulating the Treg response. Oncotarget 2017;8:22361-9.
39. Kim SH, Gunst KV, Sarvetnick N. STAT4/6-dependent differential regulation of chemokine receptors. Clin Immunol 2006;118:250-7.
40. Yano S, Umeda D, Yamashita S, Yamada K, Tachibana H. Dietary apigenin attenuates the development of atopic dermatitis-like skin lesions in NC/Nga mice. J Nutr Biochem 2009;20:876-81.
41. Kanhere A, Hertweck A, Bhatia U, Gökmen MR, Perucha E, Jackson I, *et al.* T-bet and GATA3 orchestrate Th1 and Th2 differentiation through lineage-specific targeting of distal regulatory elements. Nat Commun 2012;3:1268.
42. Li B, Jin X, Meng H, Hu B, Zhang T, Yu J, *et al.* Morin promotes prostate cancer cells chemosensitivity to paclitaxel through miR-155/GATA3 axis. Oncotarget 2017;8:47849-60.
43. Liang K, Kandhare AD, Mukherjee-Kandhare AA, Bodhankar SL, Xu D. Morin ameliorates ovalbumin-induced allergic rhinitis via inhibition of STAT6/SOCS1 and GATA3/T-bet signaling pathway in BALB/c mice. J Funct Foods 2019;55:391-401.
44. Wang J, Kandhare A, Mukherjee-Kandhare A, Bodhankar S. Chrysin ameliorates ovalbumin-induced allergic response in allergic rhinitis: Potential role of GATA-3, T-box protein expressed in T cells, nuclear factor-kappa B, and nuclear factor erythroid 2-related factor 2. Phcog Mag 2020;16:335-44.
45. Zhao L, Kandhare AD, Mukherjee AA, Bodhankar SL. Antiallergic potential of fisetin in a murine model of OVA-induced allergic rhinitis via inhibition of GATA-3 and Th2 cytokines. Biomedica 2018;34:88-101.
46. Howell MD, Kuo FI, Smith PA. Targeting the Janus kinase family in autoimmune skin diseases. Front Immunol 2019;10:2342.
47. Bloodworth MH, Newcomb DC, Dulek DE, Stier MT, Cephus JY, Zhang J, *et al.* STAT6 Signaling Attenuates interleukin-17-Producing γδ T cells during Acute Klebsiella pneumoniae Infection. Infect Immun 2016;84:1548-55.
48. Hosoya K, Satoh T, Yamamoto Y, Saeki K, Igawa K, Okano M, *et al.* Gene silencing of STAT6 with siRNA ameliorates contact hypersensitivity and allergic rhinitis. Allergy 2011;66:124-31.
49. Grainger J, Drake-Lee A. Montelukast in allergic rhinitis: A systematic review and meta-analysis. Clin Otolaryngol 2006;31:360-7.