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Astragalus Polysaccharides Attenuate Ovalbumin-Induced Allergic Rhinitis in Rats by Inhibiting NLRP3 Inflammasome Activation and NOD2-Mediated NF-κB Activation

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ABSTRACT Allergic rhinitis (AR) is an IgE-mediated chronic inflammatory disease of the allergic nasal mucosa. It has a significant effect on quality life; most patients with AR also suffer from sleep disorders, mood disorders, and deterioration in social relationships. As increasing numbers of medicinal plants show productive anti-inflammatory activity against inflammatory diseases, there is growing interest in natural medicinal plant ingredients. To this end, we selected *Astragalus* polysaccharides (APS) to evaluate its anti-inflammatory effect on ovalbumin-induced AR rats, and we further explored its impact on NLRP3 inflammasome activation and NOD2-mediated NF-κB activation. We found that APS can alleviate the nasal symptom of AR rats and attenuate pathological alterations. APS also reduced the inflammatory cytokine levels. APS not only inhibited the NLRP3 inflammasome activation but also inhibited NF-κB activation by decreasing NOD2 expression and blocking the phosphorylation of NF-κB (p65). In conclusion, APS can effectively improve the inflammatory symptoms of nasal mucosa in AR rats, which may be mediated by the inhibition of NLRP3 inflammasome activation and NOD2-mediated NF-κB activation. These findings indicate that APS has the potential to be used as a therapeutic agent for AR.

KEYWORDS: • ***allergic rhinitis*** • **Astragalus *polysaccharides*** • ***Caspase-1*** • ***NF-κB*** • ***NLRP3 inflammasome*** • ***Nod2***

INTRODUCTION

ALLERGIC RHINITIS (AR) is a common chronic disease in otolaryngology that is mainly caused by allergens and interacts with various immune cells and cytokines, the typical symptoms of AR include nasal sneezing, itching, rubbing, and discharge.[1] The prevalence of AR is high, affecting ~400 million people worldwide. In addition, if effective treatment is not available, 40% of AR patients will develop asthma.[2,3] The pathogenesis of AR remains unclear, but it is generally considered to be mediated by IgE in response to Th2 cell-mediated inhaled allergen response and mucosal inflammation.[3] Emerging research has shown that inflammatory mechanisms play an essential role in the development of AR.[4] In recent years, increasing numbers of studies have focused on the role of innate immune-sensing receptors in inflammatory diseases. The inflammasome is a cytoplasmic multiprotein complex that contains the Nod-like receptor protein family,[5] including NLRP3 and NOD2. The NLRP3 inflammasome is arguably the most studied inflammasome to date, the NLRP3 inflammasome components are present in the airway epithelium of normal mice and change with inflammation, NLRP3-mediated secretion of IL-18 and IL-1β through the Caspase-1 pathway, indicating that the activation of inflammation is a characteristic of allergic airway inflammation and may be related to the pathogenesis of AR.[6] NOD2 plays a vital role in the activation of NF-κB during inflammation reactions.[7] The previous research reports indicated that the NLRP3 inflammasome and NOD2/NF-κB signaling pathway have a crucial effect on the occurrence of AR. These signals may be important targets for the treatment of AR. Current therapies for AR, include antihistamines, corticosteroids, and antileukotrienes. However, the long-term use of these drugs can cause undesirable side-effects. Therefore, it is essential to develop safe and effective natural products for the treatment of AR.

Astragalus membranaceus(Fisch.)Bge. var. *mongholicus* (Bge.) is an important medicine in TCM, it has been used in China for more than 2000 years and has food and medicinal

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characteristics.[8,9] *Astragalus* polysaccharides (APS) are the primary biological active component of the herb. Astragalus membranaceus is sold as an over-the-counter dietary supplement in the U.S. health food market.[10] Accumulating research focuses on the biological activities of APS, such as anti-inflammatory, immunomodulatory, antitumor, and antidiabetic activities.[11] According to previous studies, its anti-inflammatory effects mediated via suppression of inflammation-related proteins, including PD-1, PD-L2, and ICAM-1.[12–14] Because of its abundant biological activities, in recent years, it has gradually been applied to functional foods.

So far, there have been no reports of the use of APS in the ovalbumin (OVA)-induced model of AR. The purpose of the present study was to determine whether APS showed any protective effects in the OVA-induced AR rat model. To this end, we estimated the levels of OVA-sIgE, histamine and inflammatory cytokines, inflammatory cell infiltration, goblet cell mucous secretion, and collagen deposition. In addition, we discuss the potential molecular mechanism of anti-inflammatory responses induced by inhibiting NLRP3 inflammasome activation and NOD2-mediated NF-κB activation.

MATERIALS AND METHODS

Animals

Specific pathogen-free male Sprague-Dawley rats weighing 200–220 g purchased from Laboratory of Experimental Animal, Guangzhou University of Chinese Medicine. All rats were housed under standard conditions: temperature, 23°C ± 2°C; relative humidity, 50% ± 10%; and water and food were readily available. The Experimental Animal Ethics Committee of Guangzhou University of Chinese Medicine approved all animal procedures (Ethics No. 20191008003).

Drug

The purity of APS was more than 98%, and was purchased from Kamaishu Biological Technology Co., Ltd. (Shanghai, China). Loratadine was purchased from Bayer Medicine Co., Ltd. (Shanghai, China). OVA and aluminum hydroxide were purchased from Sigma-Aldrich Chemical Co. (USA).

OVA sensitization/challenge AR model

According to previous research, OVA was used for sensitization and stimulation.[15] Forty-eight rats were divided randomly into four groups (Fig. 1) as follows: Control group, OVA group, Loratadine group, and APS group. OVA (0.3 mg) and 30 mg aluminum hydroxide were dissolved in saline solution. The resulting mixture was injected into the rats by the intraperitoneal route every other day for 14 days. Then, the rats were challenged through intranasal administration of 50 μL of OVA solution (5%, dissolved in saline) into each nasal cavity once daily from day 15 to 21. Loratadine and APS were dissolved in saline solution. Rats in the APS group at 400 mg/kg and loratadine group at 0.9 mg/kg were orally administered daily 30 min before intranasal OVA challenge on day 22 to 31, while the control group and OVA group were orally administered with saline.[16,17] During the period of oral administration, all groups, except for the standard group, were challenged through intranasal administration of 50 μL of OVA solution once every other day to maintain the nasal stimulation.

Evaluation of nasal symptom

On day 31 of the study, all rats were placed into a separate cage and observed for 30 min after the final OVA challenge.[18,19] The numbers of sneezing and rubbing episodes were recorded.

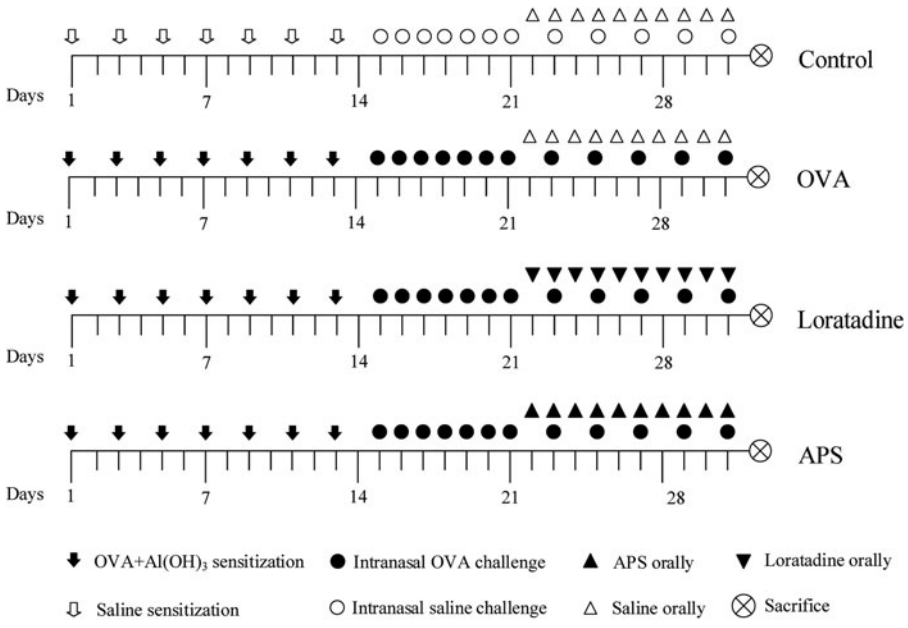

FIG. 1. Experimental design and treatment procedure. Rats were divided into four groups: Control group, OVA group, Loratadine group, and APS group. Rats were intraperitoneally sensitized with OVA and $Al(OH)_3$ dissolved in saline once every other day for 14 days and then challenged through nasal intranasal with 5% OVA once daily from day 15 to 21. Rats in loratadine and APS groups were orally administered with loratadine solution and APS solution once daily from day 22 to 31, respectively. They were challenged through intranasal administration once every other day to maintain the nasal stimulation. Rats in the control group were sensitized, challenged, and orally administered with saline. All the rats sacrificed on day 31. APS, *Astragalus* polysaccharides; OVA, ovalbumin.

Histopathological analysis

Histopathological analysis of the nasal mucosa tissue samples from each group were carried out to observe and compare the histological changes. The samples were fixed in 10% neutral buffered formalin solution for 48 h. Then, these samples were cut into 5-μm sections and used for hematoxylin and eosin (H&E) staining, AB-PAS staining, and Sirius red staining. Each section was observed manually in a random manner using a light microscope (Olympus, Japan), randomly selected nonoverlapping areas per section, quantitative analysis of eosinophils, AB-PAS-positive cells, and collagen deposition performed by Image-Pro Plus software (v6.0, USA).

Measurement of OVA-sIgE, histamine, and inflammatory cytokines in serum

The levels of OVA-sIgE and Histamine were measured by enzyme-linked immunosorbent assay (ELISA; Shanghai Enzyme-linked Biotechnology Co., Ltd.). The levels of IL-1β (Thermo Fisher Scientific, USA) and IL-18 (Neobioscience Technology Co., Ltd.) were measured by ELISA. IL-6 (BD Biosciences, USA) and TNF-α (BD Biosciences) were measured by ELISA. The levels of IL-4, IL-5, and IL-13 (Shanghai Enzyme-linked Biotechnology Co., Ltd.) in serum were also measured by ELISA. Procedures were performed according to the instructions, absorbance was read on a multifunctional enzyme labeling instrument (Bio-Tek, USA) at 450 nm.

Measurement of mRNA expression

RNA isolation performed using TRIzol and reverse transcribed to generate first-strand cDNA, according to the instructions. The quantitative polymerase chain reaction analyses were performed on an RT-PCR system by using iTaq Universal SYBR Supermix, according to instructions, and the mRNA expression data were calculated by the $2^{-\triangle\triangle Ct}$ method. The primers are listed in Table 1.

Western blot analysis

Western blot was carried out as described in a previous study.[20] Primary antibodies and their dilutions were as follows: NLRP3 (1:1000), Caspase-1 (1:1000), NF-κB p65 (1:1000), p-NF-κB p65 (1:1000)—Cell Signaling Technology (USA); NOD2 (1:1000)—Santa Cruz Biotechnology (USA); β-actin (1:5000)—Gene Tex International Corporation (USA). The density of each protein band was analyzed with ImageJ software (v1.51; NIH, USA).

Statistical analyses

All results are expressed as means ± standard errors of the mean of at least three independent experiments. GraphPad 8.0 (CA, USA) used for statistical analyses. ANOVA (analysis of variance) and LSD (least significant difference) *t*-test, respectively, were utilized for comparisons between multiple groups and two groups. The differences were considered statistically significant at $P < .05$.

RESULTS

Effects of APS on OVA-induced nasal symptom OVA-sIgE and histamine of AR rats

We analyzed the number of rubbing and sneezing of nasal symptom. We observed that OVA-induced increased the number of rubbing and sneezing episodes ($^{***}P < .001$) in rats. Contrarily, APS treatment significantly alleviated in the number of rubbing and sneezing episodes ($^{\#\#\#}P < .001$; Fig. 2a). The data show that APS could relieve nasal symptoms of AR.

To further assess the efficacy of APS, we estimated the levels of OVA-sIgE and histamine in AR rats by ELISA. We observed that OVA-induced rats increased OVA-sIgE and histamine significantly ($^{***}P < .001$) and the levels of OVA-sIgE were significantly alleviated by APS administration ($^{\#\#\#}P < .001$, $^{\#}P < .05$; Fig. 2b, c), indicating that APS could reduce serum OVA-sIgE and histamine levels and relieve the inflammatory response of AR.

Effect of APS on OVA-induced pathological damage

To evaluate the protective effect of APS on nasal mucosa tissue, we performed the pathological analysis of the nasal mucosa of rats by H&E, AB-PAS, and Sirius Red staining methods. OVA-induced pathological alterations were improved, and the number of eosinophils was reduced

Table 1. Primer Sequence of Genes Used for Quantitative Real-Time Polymerase Chain Reaction

Gene	*Forward*	*Reverse*
IL-1β	ACAGCAATGGTCGGGACATAG	CTGCCCATTCTCGACAAGG
IL-4	TCTGTAGAGGTGTCAGCGGTCTG	TATTTCCCTCGTAGGATGCTTTT
IL-5	TCACCGAGCTCTGTTGACAA	CCACACTTCTCTTTTTGGCG
IL-6	GTTGCCTTCTTGGGACTGATGT	GGTCTGTTGTGGGTGGTATCCT
IL-13	AGACCAGACTCCCCTGTGCA	TGGGTCCTGTAGATGGCATTG
IL-18	CAGCCAACGAATCCCAGACC	AGATAGGGTCACAGCCAGTCC
TNF-α	TGATCGGTCCCAACAAGGA	TGCTTGGTGGTTTGCTACGA
Nod2	GGGAGCACTTCCATTCCATC	CACCCTGCAAAACGTCAACTT
NLRP3	GAGGACCTGGAAGATGTGGA	CCAAGTGATCTGCCTTCTCC
Caspase-1	TGCCTGGTCTTGTGACTTGGA	CCTATCAGCAGTGGGCATCTGTA
β-Actin	CCCATCTATGAGGGTTACGC	TTTAATGTCACGCACGATTTC

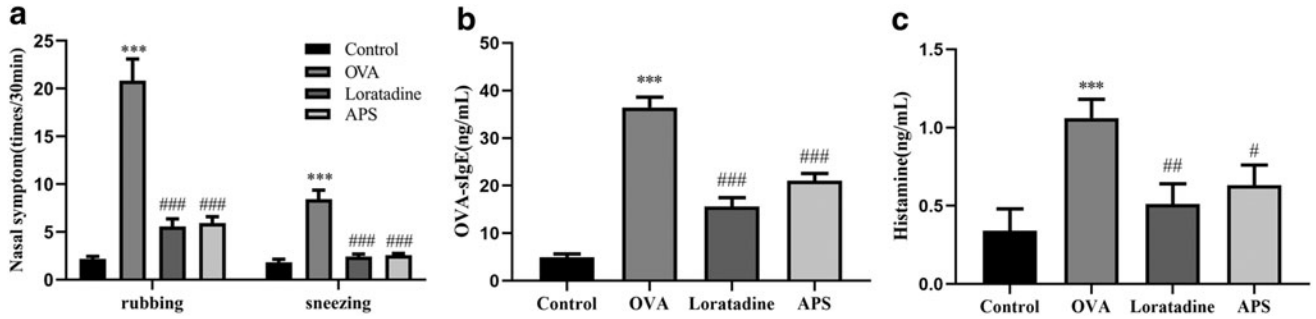

FIG. 2. Effects of APS on nasal symptom (rubbing and sneezing), OVA-sIgE and histamine in AR rats. **(a)** Nasal symptom (rubbing and sneezing), **(b)** OVA-sIgE, **(c)** histamine. Values are presented as means ± SEM ($n = 12$). Significantly different from Control, $^{***}P < .001$; significantly from OVA, $^{\#\#\#}P < .001$; $^{\#\#}P < .01$; $^{\#}P < .05$. AR, allergic rhinitis; SEM, standard error of the mean.

significantly after APS administration ($^{\#\#}P < .01$; Fig. 3a, c). To further assess the effect of APS on AR rats, goblet cells and collagen fiber were stained. The data showed that the number of AB-PAS-positive cells in nasal mucosa of AR rats was significantly increased compared with normal rats ($^{***}P < .001$). However, the intranasal administration of APS significantly reduced AB-PAS-positive cells ($^{\#\#}P < .01$; Fig. 3b, e). Besides, the significant reduction in collagen deposition was observed after APS administration ($^{\#\#\#}P < .001$; Fig. 3c, f). The data indicate that APS could relieve the pathological damage in the nasal mucosa tissue of AR rats.

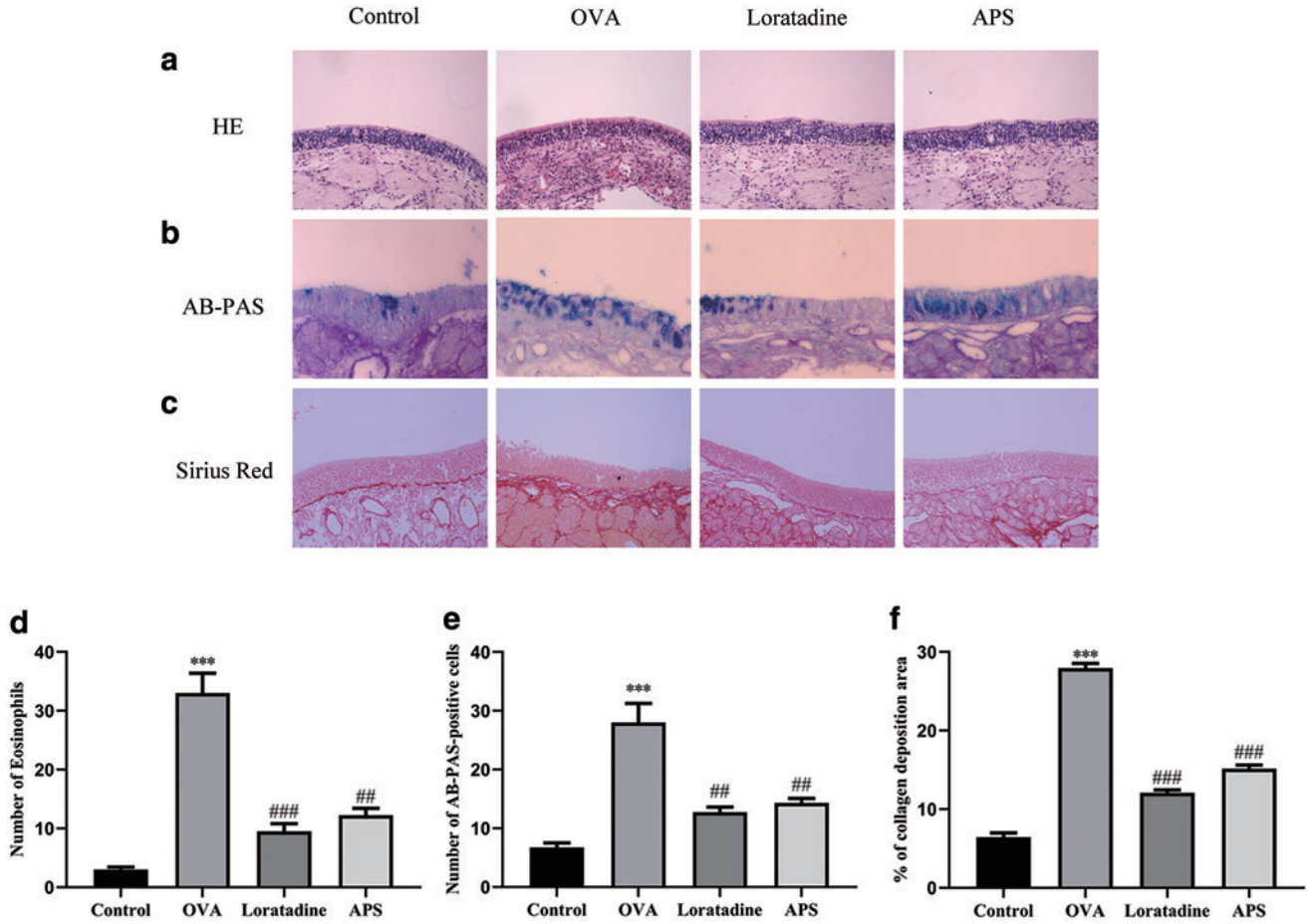

FIG. 3. Effects of APS on OVA-induced pathological changes of the nasal mucosa tissue (200×). **(a)** HE staining, **(b)** AB-PAS staining, **(c)** Sirius red staining, **(d)** the number of eosinophils, **(e)** the number of AB-PAS-positive cells, and **(f)** the area of collagen deposition. Values are presented as means ± SEM ($n = 4$). Significantly different from Control, $^{***}P < .001$; significantly from OVA, $^{\#\#}P < .01$; $^{\#\#\#}P < .001$. Color images are available online.

Effect of APS on Th2-related cytokines of IL-4, IL-5, and IL-13 in serum and nasal mucosa tissue of AR rats

We observed that the levels of IL-4, IL-5, and IL-13 were significantly increased in AR rats (***$P < .001$). Contrarily, APS administration significantly reduced levels of the cytokines ($^{###}P < .001$, $^{##}P < .01$; Fig. 4a–c). Besides, the mRNA expression of IL-4, IL-5, and IL-13 also increased (***$P < .001$), which were significantly reduced by APS treatment ($^{###}P < .001$, $^{##}P < .01$, $^{#}P < .05$; Fig. 4d–f). These data indicate that APS treatment effectively relieved OVA-induced cytokines secreted by Th2 cells.

Effect of APS on NLRP3 inflammasome activation in rats with AR

We observed that the levels of IL-1β and IL-18 significantly increased in AR rats (***$P < .001$). Contrarily, APS treatment significantly reduced and decreased the levels of these cytokines ($^{###}P < .001$; Fig. 5a, b). Besides, the mRNA expression of IL-1β and IL-18 also increased (***$P < .001$), which were significantly reduced by APS treatment ($^{###}P < .001$; Fig. 5c, d). We also observed that OVA-induced rats significantly increased protein and gene expression of NLRP3 and Caspase-1 in nasal mucosa compared with normal rats (***$P < .001$). APS treatment reduced protein and gene expression compared with AR rats ($^{###}P < .001$, $^{##}P < .01$; Fig. 5e–h). The data indicate that APS treatment effectively inhibited OVA-induced NLRP3 inflammasome activation.

Effect of APS on NOD2-mediated NF-κB Activation of AR rats

We observed that the levels of IL-6 and TNF-α significantly increased in AR rats (***$P < .001$), but APS treatment significantly reduced levels of these cytokines ($^{###}P < .001$; Fig. 6a, b). Besides, the gene expression of IL-6 and TNF-α also increased in AR rats (***$P < .001$), which were significantly reduced by APS treatment ($^{###}P < .001$; Fig. 6c, d). We also observed that OVA induced significant increases in gene mRNA and protein expression of Nod2 (***$P < .001$). APS treatment significantly reduced protein and gene expression compared with AR rats ($^{###}P < .001$, $^{##}P < .01$; Fig. 5e, f). We also observed that APS treatment significantly reduced protein expression and phosphorylation of NF-κB p65/NF-κB p65 compared with AR rats ($^{##}P < .01$; Fig. 5f, h). These data indicate that APS treatment effectively inhibited NOD2-mediated NF-κB activation.

DISCUSSION

While AR is not life-threatening, most AR patients always have complications, such as asthma and chronic rhinosinusitis.[21] The clinical symptoms lead to inconvenience in daily life, resulting in increased socioeconomical costs and lower quality of life.[22] Preventing contact between the allergen and nasal mucosa is the primary prevention method, but it is not enough in most cases, drug treatment for symptom control is also required. Currently, the commonly used treatment agents are nasal steroids, antihistamines, and

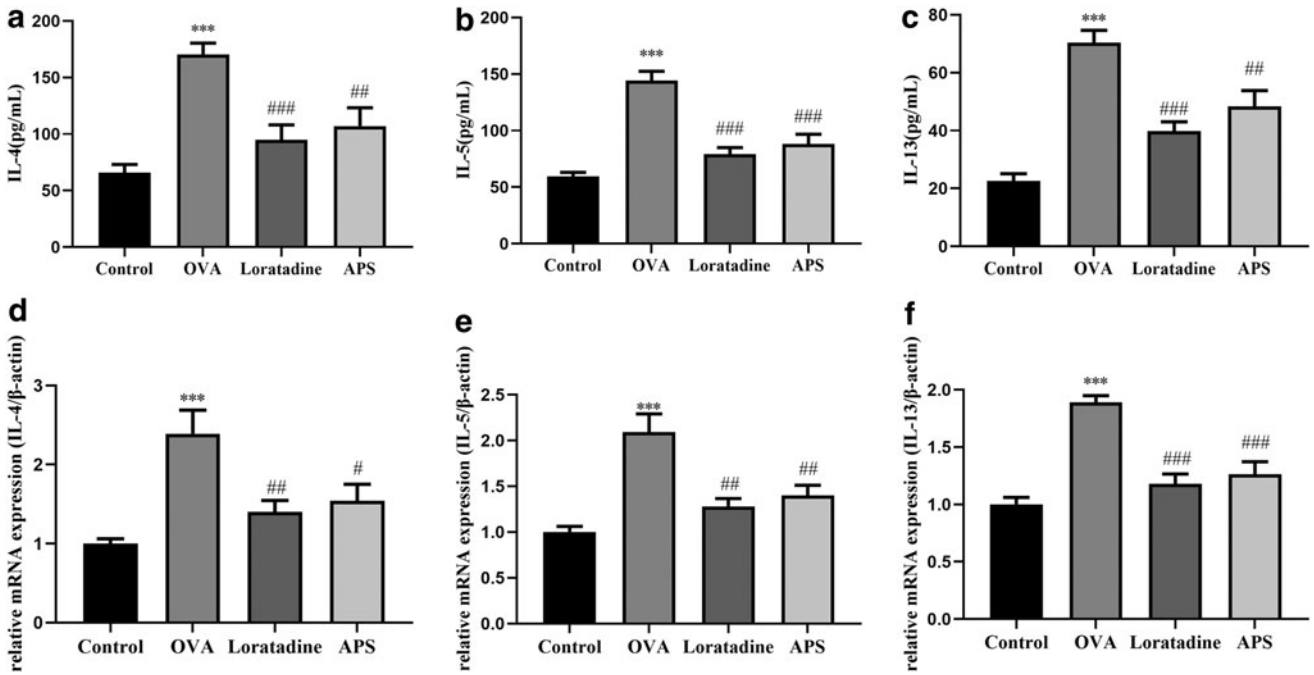

FIG. 4. Effects of APS on cytokines in serum and nasal mucosa tissue. **(a–c)** The levels of IL-4, IL-5 and IL-13 in serum were measured using ELISA ($n = 12$). **(d–f)** The mRNA expression of IL-4, IL-5 and IL-13 in nasal mucosa tissue was measured using qRT-PCR ($n = 8$). Values are presented as means ± SEM. Significantly different from Control, ***$P < .001$; significantly from OVA, $^{#}P < .05$; $^{##}P < .01$, $^{###}P < .001$. ELISA, enzyme-linked immunosorbent assay; qRT-PCR, quantitative real-time polymerase chain reaction.

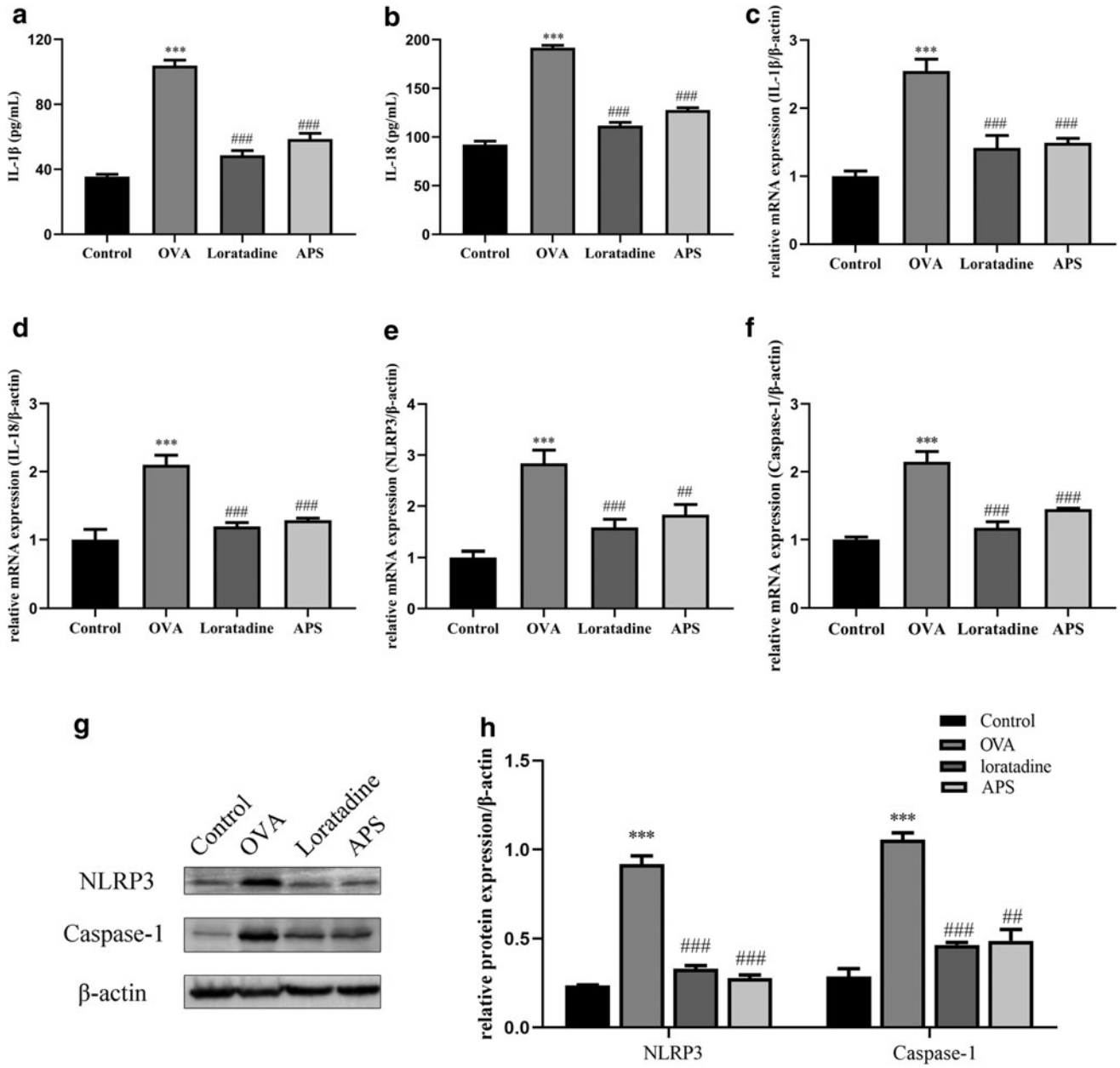

FIG. 5. Effects of APS on NLRP3 inflammasome activation. **(a, b)** The levels of IL-1β, IL-18 in serum were measured using ELISA ($n=12$). **(c, d)** The mRNA expression of IL-1β, IL-18 in nasal mucosa tissue was measured using qRT-PCR ($n=8$). **(e, f)** The mRNA expression of NLRP3, Caspase-1 were measured using qRT-PCR ($n=8$). **(g, h)** Quantification of NLRP3, Caspase-1 ($n=3$) protein expressions were normalized to β-actin. Values are presented as means ± SEM. Significantly different from Control, $^{***}P<.001$; significantly from OVA, $^{\#\#}P<.01$, $^{\#\#\#}P<.001$.

leukotriene receptor antagonists. However, side-effects due to long-term use force patients to stop taking the agents. For this reason, it is imperative to investigate new alternative agents that can treat AR. We estimated the protective effect of APS treatment by using an OVA-induced AR rat model, focusing on inflammatory responses and underlying mechanisms.

OVA is widely used to induce AR models; OVA-induced symptoms are similar to human AR.[23] The primary clinical manifestations of AR include sneezing, rubbing, blockage.[19] High OVA-sIgE and histamine levels are a vital sign of the presence of allergic diseases. When the OVA-induced mouse model is established, the levels of OVA-sIgE and histamine significantly increased.[24] In the present study, Obvious clinical symptoms in OVA-induced rats: nasal symptom increased considerably, and the levels of OVA-sIgE and histamine were higher. In contrast, APS treatment relieved the frequency of rubbing and sneezing, and downregulated the levels of OVA-sIgE and histamine. These findings indicate that a model of AR was successfully established, and APS improved some nasal allergy symptoms of AR.

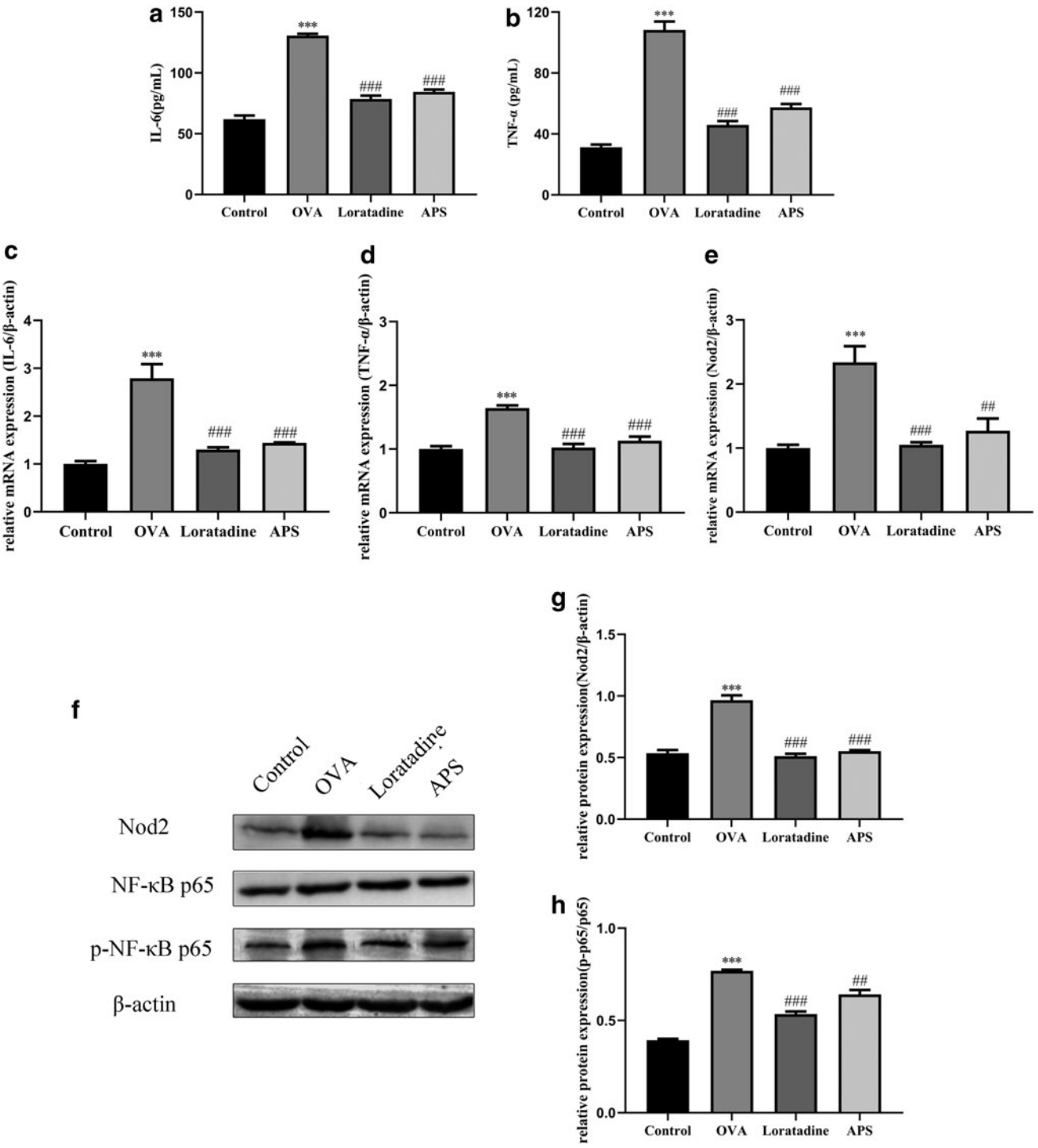

FIG. 6. Effects of APS on NOD2-mediated NF-κB Activation. **(a, b)** The levels of IL-6 and TNF-α in serum were measured using ELISA ($n = 12$). **(c–e)** The mRNA expressions of IL-6, TNF-α and Nod2 were measured using qRT-PCR ($n = 8$). **(f–h)** Quantification of Nod2 and NF-κB p65/p-NF-κB p65 ($n = 3$); protein expressions were normalized to β-actin. Values are presented as means ± SEM. Significantly different from Control, $^{***}P < .001$; significantly from OVA, $^{\#\#}P < .01$, $^{\#\#\#}P < .001$.

Pathological changes in the nasal mucosa tissue due to AR are as follows: infiltration of inflammatory cells such as eosinophils, goblet cell hyperplasia, over deposition of collagen, and vascular congestion.[25] Th2 cytokine-mediated tissue eosinophilia is a typical characteristic of AR. The release of toxic products from eosinophils prolongs allergic symptoms.[26] Nasal mucosa of AR also has characteristics similar to asthma tissue remodeling, including massive shedding of epithelial cells, collagen deposition, and goblet cell hyperplasia.[27] In the present study, APS inhibited eosinophil

infiltration, goblet cell proliferation, and excessive collagen deposition. The data show that APS effectively alleviated histopathological response of AR.

Th2 cells have an important regulatory function of allergic inflammatory responses by secreting relevant cytokines and promoting IgE-producing B cells. The IgE released by B cells bind to the mast cell surface, further leading to histamine release.[28,29] Th2-related cytokines (IL-4, IL-13, and IL-5) have been shown to attract basophils, eosinophils, and mast cells to inflammatory sites. When the allergen challenge aggregates again, it will bind to the mast cell surface IgE, and cause allergic reactions.[30,31] In this study, the APS treatment significantly inhibited the secretion of type II cytokines.

Generally, allergic inflammation is mainly controlled by Th2-mediated immune response. we found that APS inhibited Th2-related cytokine secretion, improved nasal allergy symptoms of AR, and alleviated the histopathological response of AR. APS showed a good antiallergic effect on AR and further alleviated the aggravation of inflammation in rhinitis. In this study, we chose loratadine as the positive control. Loratadine (Claritin, the second-generation H1 antihistamine) has been shown to be a selective antagonist of histamine response, and the mechanism of action of loratadine as an H1 receptor blocker is well known. Interestingly, we found that APS has a significant inhibitory effect on the release of histamine in the serum of rats with AR, showing an antiallergic effect similar to that of antihistamines. The evidence suggests that the mechanism of APS inhibition of AR may be similar to that of loratadine, but whether APS can be used as an antihistamine to treat AR is unknown and requires more experimental data to support the assertion.

Previous studies have shown that the inflammatory response in AR is related to the NLRP3 inflammasomes activation.[32] Inflammatory corpuscles are multiprotein complexes composed of nucleotide-binding oligomerization domain, leucine-rich repeat, and pyrin domain-containing family members, apoptosis-related speckle proteins, and caspase-1, whose function is to initiate the inflammatory process.[33] NLRP3 inflammatory corpuscles play a key role in the normal function of the innate immune system. Leucine-rich repeat domain of NLRP3 enters a self-inhibitory state by binding ubiquitin ligase-associated proteins and molecular chaperone Hsp90 in the absence of exogenous or endogenous stimuli.[33] However, once activated, the NLRP3 inflammasome induces Caspase-1 activation, thereby generating mature IL-18 and IL-1β from their respective precursors, pro-IL-18 and IL-1β.[32] The results showed that the NLRP3 and its downstream cytokine Caspase-1 were upregulated in AR rats and then IL-18 and IL-1β were also upregulated. By contrast, APS treatment could downregulate NLRP3 and Caspase-1, then further inhibit NLRP3 inflammasome activation which leads to the downregulation of IL-18 and IL-1β compared with AR rats. The findings indicate that APS attenuation of allergic inflammation may be mediated by inhibition of NLRP3 inflammasome activation.

In addition to NRLP3, other NLRs (NOD2) are expressed in human tissues of the upper respiratory tract, such as normal nasal mucosa.[34] NOD2 is a critical molecule involved in host defense. Innate immunity provides the first line of defense against pathogenic organisms while maintaining immunologic homeostasis.[35] NOD2 in host cells sense peptidoglycan components of gram-positive and harmful bacteria that signal to receptor-interacting protein 2, and then triggers NF-κB-mediated proinflammatory and antimicrobial responses.[7] The inhibition of NF-κB activity results in the decreased expression of inflammatory cytokines such as IL-6, TNF-α, and reduced inflammatory injury. In this study, the data show that NOD2 expression was significantly upregulated in AR rats; furthermore, the activation of NF-κB was enhanced considerably following OVA-induction, and IL-6 and TNF-α also increased. In contrast, APS decreased NOD2 expression and inhibited the activation of NF-κB in nasal mucosa from OVA-induced rats. Moreover, APS also reduced IL-6 and TNF-α secretion. These findings indicate that the mechanism of action may be through the inhibition of the NOD2/NF-κB signaling pathway.

In conclusion, we found that the administration of APS alleviated the nasal symptoms and histopathological changes of eosinophil infiltration, goblet cell metaplasia, and collagen deposition in AR rats and inhibited OVA-sIgE, histamine, and Th2-related cytokine secretion. APS exhibited potent antiallergic effects on AR and further alleviated the aggravation of inflammation in rhinitis. Further mechanistic research found that APS may attenuate allergic symptoms in AR rats by inhibiting NLRP3 inflammasome activation and NOD2-mediated NF-κB activation. APS may be a potential therapeutic drug for AR.

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AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist.

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