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BAICALIN ATTENUATES ALLERGIC RHINITIS IN RATS VIA SUPPRESSING TLR4/AP-1 INFLAMMATORY SIGNALING PATHWAY

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ABSTRACT

Allergic rhinitis (AR) is a type of non-infectious inflammatory disease of the nasal mucosa with a complex pathogenesis. This study investigated the mechanism of Baicalin (BAI, main active component of *Radix Scutellariae* extract) on the immune-inflammatory response in AR rat model through Toll-like receptor 4 (TLR4)/activator protein-1 (AP-1) pathway. Healthy male Sprague-Dawley (SD) rats were used as Control group. The AR model (AR group) was constructed by ovalbumin sensitization, and treated with 0.9 mg/kg loratadine (LRD group) and 20, 40, 80 mg/kg of BAI (LD-BAI group, MD-BAI group and HD-BAI group) via intragastric administration for 28 days. Symptom scores of the rats were assessed, peripheral blood was collected to determine Th1/Th2 cell ratios and changes ininflammatory cytokines, and nasal mucosa tissues were harvested to analyze pathological morphological changes and the expression of proteins related to the TLR4/AP-1 signaling pathway. As against Control group, AR group suggested increased symptom scores; nasal mucosa tissue edema, congestion, and inflammatory cell infiltration; decreased $CD3^+CD4^+IFN\text{-}\gamma^+$Th1 proportion and increased $CD3^+CD4^+IL\text{-}4^+$Th2 proportion in peripheral blood; elevated levels of inflammatory cytokines IgE, IL-4, IL-17, and TNF-α, and decreased levels of IL-10 and IFN-γ; increased relative expression (RE) of TLR4, Ikkβ, NF-κB, p-JNK, and AP-1 proteins in nasal mucosa tissue ($P \leq 0.05$). As against AR group, LRD group, LD-BAI group, MD-BAI group, and HD-BAI group all suggested visible improvements in symptom scores, nasal mucosa tissue morphology, peripheral blood immune cell ratios, immune cytokine levels, and the expression of TLR4 pathway-related proteins in nasal mucosa, with HD-BAI group (80 mg/kg) demonstrating the most significant improvements in all indicators ($P \leq 0.05$ vs. AR group). These findings suggest that BAI may alleviate AR symptoms by modulating immune-inflammatory responses via TLR4/AP-1 signaling. BAI can suppress the activation of TLR4 and its downstream Ikkβ/NF-κB, JNK/AP-1 inflammatory signaling pathways in AR rat model. BAI shows the developing novel treatment modalities for AR, warranting further investigation.

Keywords: Allergic rhinitis; Baicalin; nasal mucosa; inflammatory cytokines; TLR4; Ikkβ/NF-κB; JNK/AP-1

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INTRODUCTION

Allergic rhinitis (AR) is primarily a nasal mucosa allergic disease caused by IgE-mediated mediator release, immune-active cells, and their cytokines (Siddiqui *et al*., 2022). Patients with AR mainly exhibit symptoms such as paroxysmal sneezing, nasal congestion, and itching, and are characterized by easy recurrence and long disease duration, which has seriously affected patients' daily life and work (Ponda *et al*., 2023). However, the pathogenesis of AR is not yet clear. Clinically, glucocorticoids, antihistamines are commonly used for the treatment of AR, but the long-term efficacy is not ideal, and the relapse rate is very high (Tosca *et al*., 2024).

Traditional Chinese medicine (TCM) has shown good outcomes in AR, with no toxic side effects. *Radix Scutellariae*'s root is used in medicine for detoxifying and stopping bleeding, mainly for treating upper respiratory tract infections, lung heat cough, pneumonia, and other symptoms (Wen *et al*., 2023). Baicalin (BAI), an important active ingredient in *Radix Scutellariae*, has

anti-inflammatory, antibacterial, anti-platelet aggregation, and other pharmacological outcomes (Gupta *et al*., 2022). Moreover, BAI has a broad antibacterial spectrum and can suppress bacteria such as *Pseudomonas aeruginosa*, *Staphylococcus*, *Streptococcus*, *Diplococcus pneumoniae*, and *Neisseria meningitidis* (Wang *et al*., 2023). Intraperitoneal injection of BAI can improve intestinal inflammation in mice with ulcerative colitis through the IL-22 pathway, reduce intestinal permeability, and enhance intestinal barrier function (Li *et al*., 2022).BAI is a core compound in bacterial pneumonia treatment. It synergizes with meropenem or colistin E by stably binding to bacterial targets, thereby inhibiting the expression of quorum-sensing genes LuxS and LuxR, thereby inhibiting the formation of multidrug-resistant *Klebsiella pneumoniae* biofilms (Qin *et al*., 2024). Moreover, BAI can effectively block the coagulase activity of von Willebrand factor-binding protein, reduce the virulence of *Staphylococcus aureus* after binding, and ultimately protect mice from fatal pneumonia induced by this bacterium (Zhang *et al*., 2020). Currently, BAI is also used for the treatment of upper respiratory tract infections. Respiratory syncytial virus (RSV) infection can lead to airway inflammation, and BAI has been shown to suppress the secretion of IL-6 and IL-8 cytokines induced by RSV infection. Additionally, BAI can mitigate airway inflammation caused by this viral infection by activating the IFN pathway (Feng *et al*., 2024). Kang *et al.* (2022) conducted proteomic analysis on the nasal mucosa tissue of AR patients and explored the mechanism of AR causing mild cognitive impairment, finding that BAI, an effective component of the Qi-supplementing, heat-clearing, and yin-nourishing method, can target cytochrome C protein to play a defensive role in cells resisting external stimuli (Kang *et al*., 2022). However, the potential mechanism of BAI in treating AR still needs further exploration.

Although previous studies reported the anti-inflammatory effects of BAI in allergic diseases, its role in regulating the TLR4/AP-1 pathway in AR remains unexplored. This pathway is implicated in NF-κB and JNK activation, which drive inflammatory cytokine production, yet direct evidence linking BAI to TLR4/AP-1 suppression in AR is lacking. Thus, ovalbumin (OVA) was selected as the allergen for constructing the AR model due to its ability to induce Th2-mediated allergic inflammation, thereby mimicking the pathophysiology of human AR (Piao *et al.,* 2023; Tabaru *et al.,* 2024). Our study sought to address this gap by systematically evaluating the dose-dependent effects of BAI on TLR4/AP-1 signaling and immune balance in an OVA-induced AR model.

This study used ovalbumin sensitization to construct the AR rat model, analyzed the outcomes of different doses of BAI on the morphology and immune response of the model rats' nasal mucosa tissue through TLR4/AP-1 pathway. The aim of this study was to understand the potential pathogenesis of AR and provide reference for the selection of new therapeutic targets and development of therapeutic drugs.

MATERIALS AND METHODS

Experimental animals: Sixty clean-grade healthy male SD rats (5 to 6 weeks, 150 to 220 g; Beijing Charles River Laboratory Animal Co., Ltd., China) were selected. They were conventionally housed in a room temperature environment of 18 to 22°C with a relative humidity of 45 to 55%, under a 12 h/12 h light/dark cycle, and allowed free access to food and water. All procedures were approved by the Ethical Committee of Affiliated Hospital of North Sichuan Medical College (Nanchong, Sichuan, China) and responsible authorities of research organization(s) following all guidelines, regulations, legal, and ethical standards as required.

Construction of animal model: Fifty rats were randomly selected for the construction of the AR model. A total of 0.3 mg ovalbumin and 300 mg aluminum hydroxide powder (Sigma-Aldrich, USA) were subjected to dissolving in 1 mL of physiological saline (Sichuan Kelun Pharmaceutical Co., Ltd., China) to form a suspension. The suspension was administered intraperitoneally at a concentration of 2 g/L to the rats once a day for 20 consecutive days to establish a basic sensitization reaction. Subsequently, a 10 μm ovalbumin solution was dripped into the nasal cavity of the rats once a day for 7 consecutive days. After the final sensitization, the effectiveness of the model was evaluated using a quantified scoring method (lightly touching the nose and sneezing more than 3 times was scored as 1 point; touching the nose and sneezing less than 9 times was scored as 2 points; frequently touching the nose and sneezing more than 9 times was scored as 5 points), with a score over 5 points considered a successful model. In this experiment, all AR rat models were successfully constructed.

Intervention: Ten non-modeled rats were designated as Control group, and ten successfully modeled rats were randomly selected as AR group. The remaining 40 successfully modeled rats were randomly divided into four groups in a 1:1:1:1 ratio: LRD group, LD-BAI group, MD-BAI group, HD-BAI group. The doses of BAI (20, 40, and 80 mg/kg) were selected based on preliminary experiments and previous studies demonstrating its anti-inflammatory efficacy in allergic models (Wang *et al.,* 2021). LRD group was given gavage with 0.9 mg/kg LRD (Xi'an Janssen Pharmaceutical Co., Ltd., China), while LD-BAI group, MD-BAI group, and HD-BAI group were given gavage with 20, 40, 80 mg/kg BAI (Shanghai Rui Chu Biotechnology Co., Ltd., China), respectively. The

Control group and AR group were administered physiological saline by gavage at the same volume daily for 28 days.

Observational indicators

Behavioral evaluation: The body weight changes of the rats were recorded, and a 1-h continuous observation was conducted for symptom scoring (Tabaru *et al*., 2024).

(1) Nasal itching score: the scoring is based on the observation of the rats' nasal scratching behavior during the observation period. A single brief nose touch (using the forepaw to lightly touch the nose tip 1–2 times, lasting ≤2 seconds) is scored as 1 point. Intermittent scratching (3–5 scratching actions, each lasting 2–5 seconds with an interval >10 seconds) is scored as 2 points. Continuous, intense scratching (≥6 consecutive scratches, or a single scratch lasting >5 seconds, accompanied by noticeable agitation) is scored as 3 points. The frequency and intensity of spontaneous nasal scratching within 1 hour are recorded and scored blindly by two independent observers using video playback, with consistency required to achieve a Kappa value ≥0.8. (2) Sneezing score: the scoring is based on the number of sneezes within 1 hour. 1 point is given for 1–3 sneezes (intermittent, without continuous episodes). 2 points are given for 4–10 sneezes (frequent episodes, with intervals ≤1 minute). 3 points are given for ≥11 sneezes (continuous, dense episodes, with intervals ≤30 seconds). During a fixed observation period (*e.g.,* 09:00–10:00), the sneezes are counted by sound recording and visual observation, with environmental disturbances (such as dust) excluded. (3) Rhinorrhea score: the scoring is based on the extent and quantity of nasal secretions. Secretions confined to the anterior nostrils (with a small amount of clear mucus attached to the edge of the nostrils) are scored as 1 point. Secretions extending beyond the anterior nostrils to the nasal wings (with mucus covering the outer side of the nasal wings, requiring wiping) are scored as 2 points. Secretions covering the face (with mucus spreading to the nose bridge, cheeks, or chin, requiring frequent wiping) are scored as 3 points. A non-irritating cotton swab is used to lightly touch the nose to assess the viscosity and extent of the secretions, and quantitative analysis is performed using video recordings. All symptom scores are recorded during a fixed period (on day 7 after model induction), with the environmental temperature maintained at (22±2)°C to avoid external disturbances. Prior to scoring, rats undergo a 10-minute acclimation period to reduce stress interference.

Hematoxylin-eosin staining observation of nasal mucosa tissue: Rats were subjected to anesthesia with 10% pentobarbital sodium (Sigma-Aldrich, USA) at 30 mg/kg, and following complete anesthesia, they were sacrificed. The maxillary bone tissue was removed, and the nasal septum and bilateral nasal cavities were exposed by cutting along the midline of the nasal back. The nasal septum and its mucosal tissue were sequentially stripped, and bilateral nasal mucosa tissues were collected. Some tissues were fixed in 4% polyformaldehyde solution (Sigma-Aldrich, USA) for 24 h, dehydrated through a gradient alcohol series, and then routinely embedded in paraffin. Sections (4 μm thickness) were sliced, dewaxed and rehydrated, and then stained with hematoxylin-eosin according to the instructions of the hematoxylin-eosin staining kit (Beijing Solarbio Technology Co., Ltd., China). After being dehydrated again through a gradient alcohol series, the sections were mounted with neutral resin (Sigma-Aldrich, USA) and observed for tissue pathological morphological changes under a BX53M optical microscope (Olympus, Japan).

Flow cytometry detection of peripheral blood $CD3^+CD4^+IFN\text{-}\gamma^+$Th1 and $CD3^+CD4^+IL\text{-}4^+$Th2 levels: Before anesthesia and euthanasia, 3 mL of blood was collected from the abdominal aorta, in duplicate. One portion of the peripheral blood sample was used for the separation of peripheral blood mononuclear cells using a peripheral blood lymphocyte separation kit (Beyotime Biotechnology, China), placing in RPMI-1640 medium (Thermo Fisher Scientific, USA). The cells were subjected to culture in an Incubator 311 at 37°C with 5% carbon dioxide (Thermo Fisher Scientific, USA), and stimulated with phorbol 12-myristate 13-acetate at 81 ng/mL, ionomycin at 1.34 μg/mL, and brefeldin A (Sigma-Aldrich, USA) at 3.0 μg/mL for 6 h. At 25°C, protected from light, the samples were incubated with fluorescein isothiocyanate (FITC)-labeled anti-rat CD3 antibody and phycoerythrin (PE)-labeled anti-rat CD4 antibody (Abcam, UK) for 30 minutes, followed by cell fixation. The samples were rinsed and then incubated with allophycocyanin (APC)-labeled anti-rat IFN-γ, IL-4, and IgG antibodies (Abcam, UK) for 15 minutes at 25°C, avoiding light. After washing with phosphate-buffered saline, the levels of the targeted objects were detected using a CytoFLEX nano flow cytometer (Beckman Coulter, Inc., USA).

ELISA for the detection of cellular immune factors in peripheral blood: The other portion of the peripheral blood sample was centrifuged at 3000 rpm for 15 min to separate the serum. Serum samples were analyzed for IgE, IL-4, and other cytokines using an ELISA kit (Beyotime Biotechnology, China), following the manufacturer's instructions. The detection wavelength was set to 450 nm, with the standard curve range of 0.1–100 ng/mL. According to the guidance of the ELISA kit, incubation was carried out adopting antigen-coated microplates at 37°C for 1 h, and rinsing was performed to remove unbound antigens before the addition of 100 μL of the serum sample to be tested, followed by sealing the plate and incubating at 37°C for another hour. Unbound samples were washed off, and 100μL of diluted

biotinylated IgE, IL-4, IL-10, IL-17, TNF-α, and IFN-γ antibody working solution (Abcam, UK) was added, followed by sealing of the plate and incubation at 37°C for 1 h. The plate was washed and the liquid was discarded, then 300 μL of washing solution was added for soaking for 1 min, the liquid was flicked off. 100 μL of diluted enzyme-conjugated working solution was added, followed by sealing the plate and incubating at 37°C for 10 min. After washing, 100 μL of TMB substrate solution was added, followed by sealing of the plate and incubation at 37°C in the dark for 15 min. When a clear color gradient change occurred in the standard wells, 100 μL of 2 mmol/L sulfuric acid was added to the reaction wells, and the absorbance (OD value) at 450 nm of each well was detected within 10 min using a SpectraMax iD5 multifunctional microplate reader (Shanghai Molecular Devices, China).

Western Blotting: Western blotting was used to detect the activation status of TLR4 and its downstream key inflammatory pathways, Ikkβ/NF-κB, and JNK/AP-1. A suitable amount of nasal mucosa tissue was homogenized and lysed to extract total protein using radio-immunoprecipitation assay reagent (Sigma-Aldrich, USA), and the density was measured by the bicinchoninic acid assay (Beyotime Biotechnology, China). Proteins were denatured by heating, separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, transferred to a membrane, and blocked with a blocking solution containing 5% skim milk at 25℃ for 1 h. Incubation was implemented with diluted primary antibodies against TLR4, Ikkβ, NF-κB p65, JNK, p-JNK, and AP-1 at a ratio of 1:1000 and β-actin at a ratio of 1:2000 (Abcam, UK) at 4°C overnight. After washing, incubation was conducted again with horseradish peroxidase-labeled IgG secondary antibodies at a ratio of 1:5000 (Abcam, UK) at 25℃ for 1 h. After washing, the membrane was exposed and developed, and the grayscale value of the protein bands was measured using *ImageJ*. β-actin was used as the internal reference gene and the relative expression of each protein was calculated.

Statistical methods: All data were represented as the mean ± standard deviation ($\bar{x} \pm s$) and were statistically analyzed using *SPSS 23.0*. One-way ANOVA was used for multiple groups, and independent sample *t*-tests were used for comparisons between two groups. A difference was considered statistically significant when $P \leq 0.05$.

RESULTS

Influence on behavioral symptoms in AR rats: In Fig. 1A, the change in body weight of rats was not visible over time ($P > 0.05$). In Fig. 1B, the symptom scores of AR group, LRD group, LD-BAI group, MD-BAI group, and HD-BAI group decreased over time. As against Control group, the scores of the other five groups of rats were visibly increased at all time points; those of AR group were visibly greater than those of LRD group, LD-BAI group, MD-BAI group, and HD-BAI group; as the dose of BAI increased, the scores of the rats decreased, with HD-BAI group showing a visible decrease ($P \leq 0.05$).

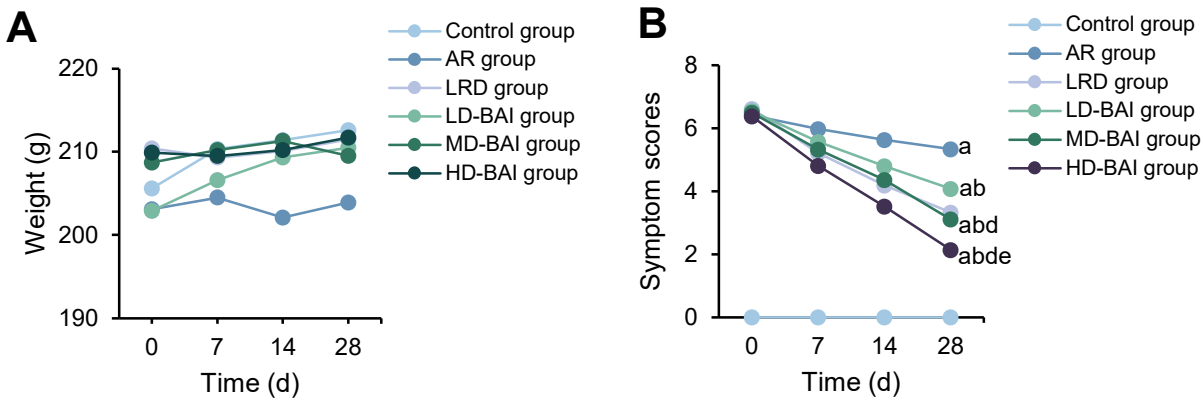

Fig.1. Changes in behavioral characteristics of rats. A: body weight-time change curve; B: symptom score-time change curve. [a] As against Control group, $P \leq 0.05$; [b] as against AR group, $P \leq 0.05$; [c] as against LRD group, $P \leq 0.05$; [d] as against LD-BAI group, $P \leq 0.05$; [e] as against MD-BAI group, $P \leq 0.05$.

Influence on the pathological morphology of nasal mucosa tissue: In Fig. 2, rats in the Control group exhibited normal nasal mucosa tissue structure, without inflammatory reactions. The nasal mucosa of rats in the AR group exhibited obvious edema and congestion, accompanied by inflammatory cell infiltration and necrosis of mucosal epithelial cells. In contrast, the nasal mucosa tissue of rats in the LRD, LD-BAI, MD-BAI, and HD-BAI groups showed partial repair, with a reduction in cell gaps following epithelial cell proliferation, and only

minimal inflammatory cell infiltration was observed. Quantitative analysis revealed that HD-BAI reduced inflammatory cell infiltration by 68% (*vs.* AR group, $P \leq 0.01$) and restored epithelial thickness to 92% of Control group levels ($P \leq 0.05$).

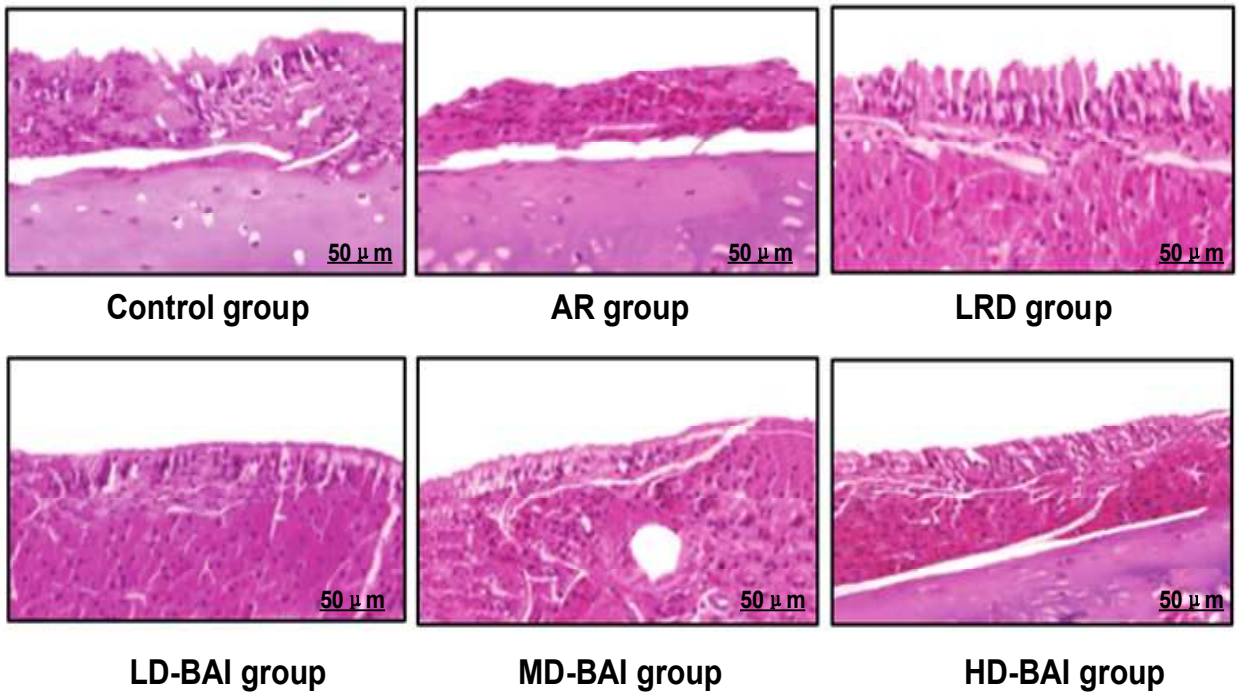

Fig. 2. Hematoxylin-eosin staining of rat nasal mucosa tissue (×200).

Influence on the proportions of peripheral blood $CD3^+CD4^+IFN\text{-}\gamma^+Th1$ and $CD3^+CD4^+IL\text{-}4^+Th2$: In Fig. 3A-C, the proportion of peripheral blood $CD3^+CD4^+IFN\text{-}\gamma^+Th1$ and $CD3^+CD4^+IFN\text{-}\gamma^+Th1/CD3^+CD4^+IL\text{-}4^+Th2$ were visibly lower, while the proportion of $CD3^+CD4^+IL\text{-}4^+Th2$ was visibly higher in AR group; as against the LRD group, the proportion of peripheral blood $CD3^+CD4^+IFN\text{-}\gamma^+Th1$ and the ratio were visibly reduced, and the proportion of $CD3^+CD4^+IL\text{-}4^+Th2$ was visibly increased in the LD-BAI group ($P \leq 0.05$). As against LRD group, LD-BAI group, and MD-BAI group, the proportion of peripheral blood $CD3^+CD4^+IFN\text{-}\gamma^+Th1$ and the ratio were visibly increased, and the proportion of $CD3^+CD4^+IL\text{-}4^+Th2$ had a visibly decrease in HD-BAI group ($P \leq 0.05$).

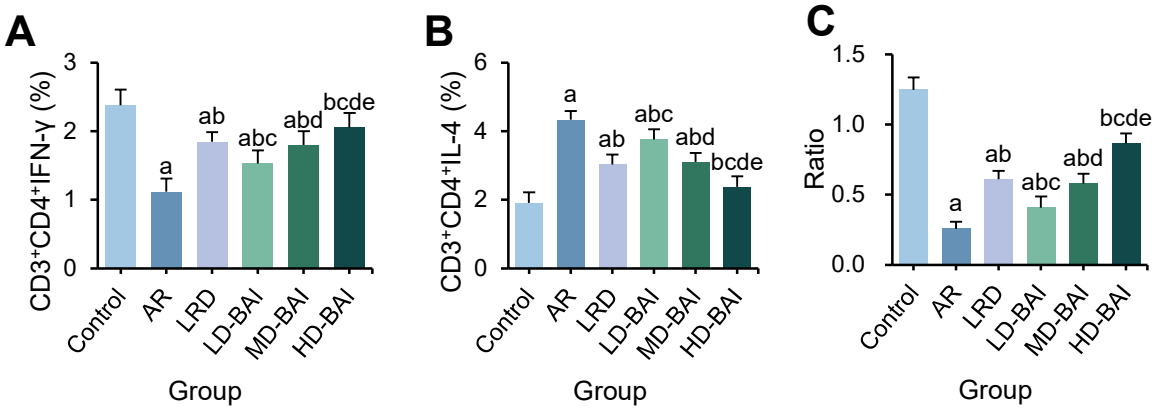

Fig. 3. Comparison of $CD3^+CD4^+IFN\text{-}\gamma^+Th1$ and $CD3^+CD4^+IL\text{-}4^+Th2$ proportion in rat peripheral blood. A: $CD3^+CD4^+IFN\text{-}\gamma^+Th1$; B: $CD3^+CD4^+IL\text{-}4^+Th2$; C: ratio. [a] As against Control group, $P \leq 0.05$; [b] as against AR group, $P \leq 0.05$; [c] as against LRD group, $P \leq 0.05$; [d] as against LD-BAI group, $P \leq 0.05$; [e] as against MD-BAI group, $P \leq 0.05$.

Influence on cellular immune factors in peripheral blood: In Fig. 4, IgE, IL-4, IL-17, and TNF-α were visibly higher, while IL-10 and IFN-γ were visibly lower in the peripheral blood of AR group ($P \leq 0.05$). No visible distinctions were noted in any of the peripheral blood cellular immune factors between LRD group and MD-BAI group ($P > 0.05$). With the increase of BAI dosage, IgE, IL-4, IL-17, and TNF-α in the peripheral blood of rats decreased, while IL-10 and IFN-γ increased, with HD-BAI group showing the most visible changes in the levels of all inflammatory cytokines($P \leq 0.05$).

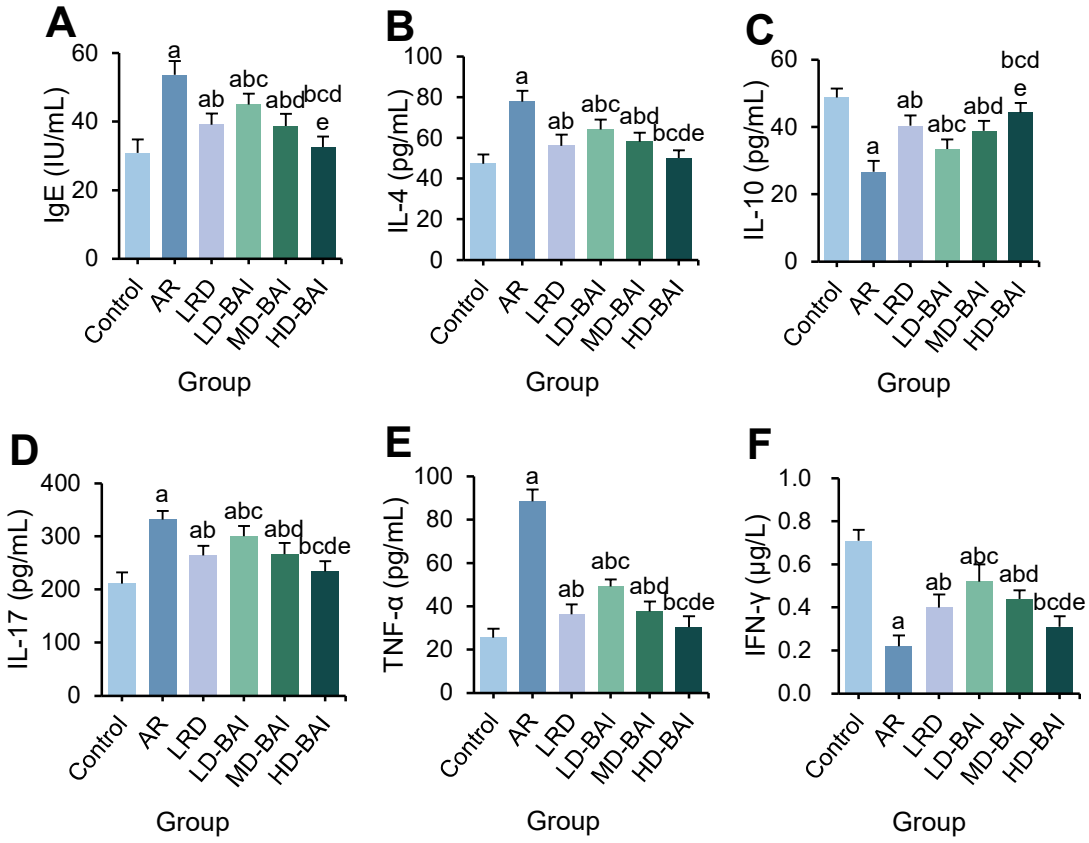

Fig. 4. Comparison of cellular immune factors levels in rat peripheral blood. A: IgE; B: IL-4; C: IL-10; D: IL-17; E: TNF-α; F: IFN-γ. [a] As against Control group, $P \leq 0.05$; [b] as against AR group, $P \leq 0.05$; [c] as against LRD group, $P \leq 0.05$; [d] as against LD-BAI group, $P \leq 0.05$; [e] as against MD-BAI group, $P \leq 0.05$.

Influence on the TLR4/AP-1 pathway: In Fig. 5A-B, the relative expression of TLR4 protein in the nasal mucosa of AR group was significantly higher than that of Control group (1.85 ± 0.21 *vs.* 0.42 ± 0.08, $P=0.003$). No statistically significant difference was observed between LRD group (0.91 ± 0.15) and MD-BAI group (0.88 ± 0.12) ($P=0.712$). However, as the BAI dosage increased, TLR4 expression progressively decreased, with HD-BAI group showing the lowest expression (0.51 ± 0.09, $P \leq 0.001$ *vs.* AR group) (Fig. 5B).

In Fig. 5C-E, the relative expression of Ikkβ (2.13 ± 0.32 *vs.* 0.55 ± 0.11, $P=0.001$) and NF-κB p65 (1.97 ± 0.28 *vs.* 0.48 ± 0.09, $P=0.002$) in AR group were markedly elevated compared to Control group. No significant differences were found between LRD group (Ikkβ: 1.02 ± 0.18; NF-κB: 0.95 ± 0.16) and MD-BAI group (Ikkβ: 0.98 ± 0.14; NF-κB: 0.92 ± 0.12) ($P > 0.05$). The HD-BAI group exhibited the most pronounced reduction in both proteins (Ikkβ: 0.62 ± 0.10, $P \leq 0.001$; NF-κB: 0.58 ± 0.08, $P \leq 0.001$) (Fig. 5D-E).

In Fig. 5F-H, the expression of total JNK protein showed no significant differences among groups (AR: 1.05 ± 0.12 *vs.* Control: 1.02 ± 0.10, $P=0.892$). However, the phosphorylation levels of JNK (p-JNK: 2.08 ± 0.25 *vs.* 0.50 ± 0.07, $P \leq 0.001$, Cohen's d=2.4) and AP-1 (1.94 ± 0.22 *vs.* 0.45 ± 0.06, $P \leq 0.001$) were significantly elevated in the AR group. The LRD group (p-JNK: 1.02 ± 0.15; AP-1: 0.98 ± 0.14) and MD-BAI group (p-JNK: 0.95 ± 0.12; AP-1: 0.92 ± 0.10) showed no significant differences ($P > 0.05$), while HD-BAI group demonstrated the lowest expression levels (p-JNK: *0.55 ± 0.08, $P \leq 0.001$; AP-1: 0.52 ± 0.07, $P \leq 0.001$)* (Fig. 5G-H)..

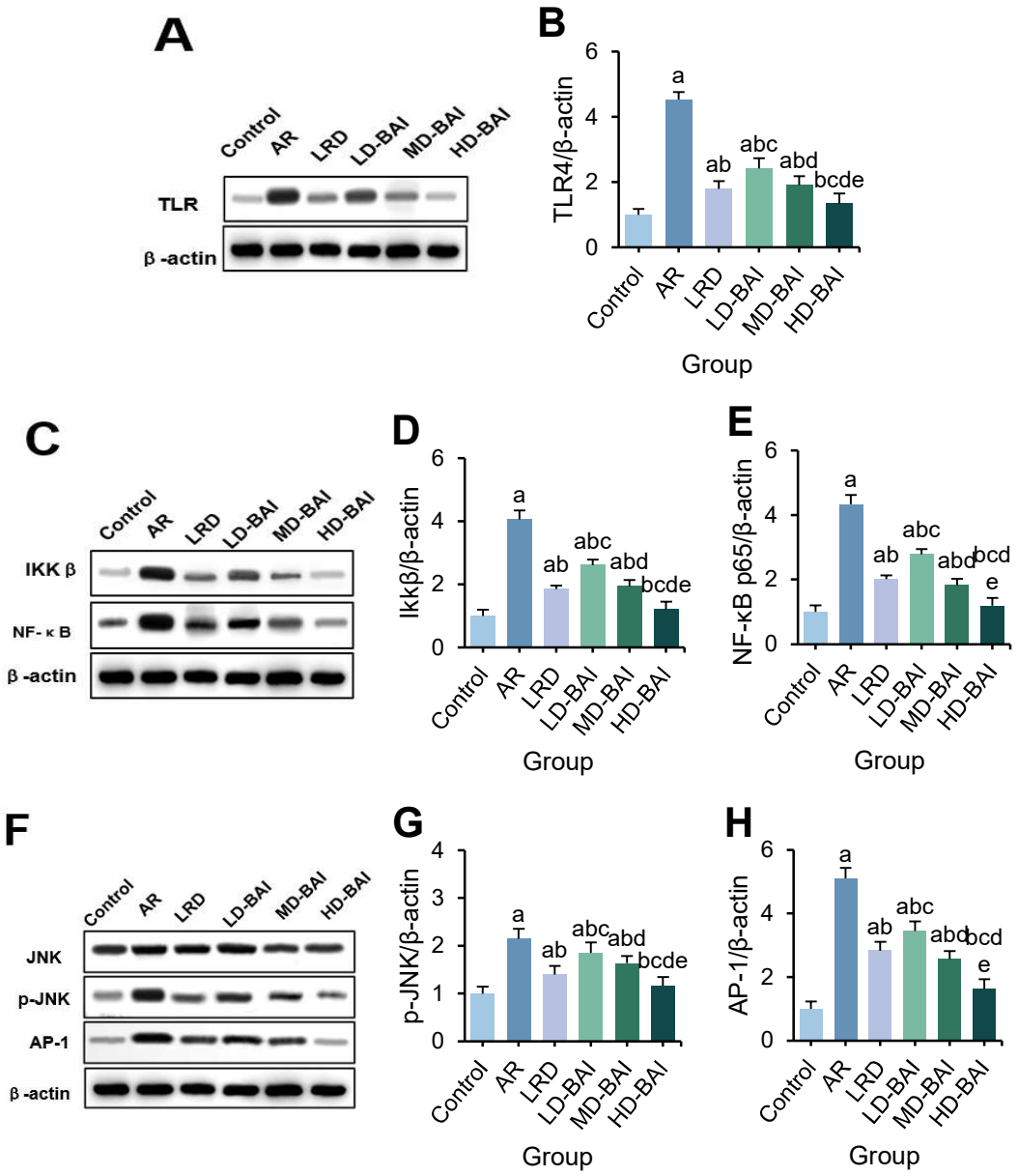

Fig. 5. Comparison of protein expression in the TLR4/AP-1 pathway of rat nasal mucosa. A: TLR4 protein blot; B: relative expression of TLR4; C: blot of Ikkβ and NF-κB p65 proteins; D: relative expression of Ikkβ; E: relative expression of NF-κB p65; F: blot of JNK, p-JNK, and AP-1 proteins; G: relative expression of p-JNK; H: relative expression of AP-1. [a] As against Control group, $P \leq 0.05$; [b] as against AR group, $P \leq 0.05$; [c] as against LRD group, $P \leq 0.05$; [d] as against LD-BAI group, $P \leq 0.05$; [e] as against MD-BAI group, $P \leq 0.05$.

DISCUSSION

In this study, the AR rat model was constructed using ovalbumin sensitization, and it was observed that the modeled rats exhibited symptoms included visible edema and congestion in the nasal mucosa tissue, accompanied by inflammatory cell infiltration. The pathogenesis of AR, a typical type I hypersensitivity reaction obvious correlated with immune imbalance in the body, is influenced by various factors (Nappi *et al.*, 2022). The main pathological characteristics of AR include structural changes in the nasal mucosa tissue, such as the accumulation of extracellular matrix, goblet cell differentiation, necrosis of mucosal ciliated epithelial cells, and proliferation of submucosal glands (Li *et al.*, 2022a). Damage to the nasal mucosa tissue can result in physiological dysfunction of the nasal cavity, ultimately leading to diseases such as AR. Therefore, inhibiting damage to the nasal mucosa and promoting its repair are

crucial for the treatment of AR. AR can cause many inflammatory cells to infiltrate the nasal mucosa tissue, subsequently destroying tissue structure, intensifying inflammatory reactions, activating immune responses in the body, and causing symptoms such as rhinorrhea (Ding *et al*., 2023; Testera-Montes *et al*., 2022). BAI is the main pharmacological substance basis of *Radix Scutellariae*. BAI has a visible anti-inflammatory effect, which can suppress the infiltration of macrophages and lymphocytes and suppress the release of pro-inflammatory cytokines to exert its anti-inflammatory effect (Sulistyowati *et al*., 2023). This study found that the treatment of AR rats with different doses of BAI caused lower scores of rhinitis, sneezing, and rhinorrhea symptoms. Liu *et al*. (2020) found that BAI used in ovalbumin-sensitized AR rats can repair abnormal tissues, inhibit the production of inflammatory cytokines in peripheral blood and nasal lavage fluid, and alleviate symptoms by inhibiting the expression of STAT3 phosphorylation (Liu *et al*., 2020).

The balance of Th1/Th2 cells has a major role in AR (Kaczynska *et al*., 2022). Th1 cells can suppress Th2 cell activity and promote macrophage activation, contributing to nasal mucosa inflammation and damage (Piao *et al*., 2023). Th2 cells can promote the release of cytokines such as IL-4, which activates vascular endothelial cell adhesion molecules, promoting the migration, adhesion, and localization of various lymphocytes in the local nasal mucosa (You *et al*., 2021). During the occurrence of AR, the proportion of Th2 cells markedly increases, promoting the synthesis and secretion of IL-4, which in turn accelerates the production of IgE and inhibits the release of IFN-γ by Th1 cells (Ke *et al*., 2023; Jafarinia *et al*., 2020). Wang *et al.* found that BAI can improve the imbalance of Th1/Th2 in asthma and alleviate inflammatory reactions to control asthma symptoms (Wang *et al*., 2021). This suggests that BAI can alleviate the symptoms of AR by regulating the balance of Th1 and Th2 cells, inhibiting the activity of Th2 cells, or enhancing the activity of Th1 cells. IgE is an important monitoring indicator in AR. When patients are exposed to allergens, Th cells in the body are activated to initiate an immune response, which subsequently activates B cells to produce allergen-specific IgE (Bernstein *et al.,* 2024). At this stage, IgE binds to its receptor, triggering the release of inflammatory mediators such as histamine and bradykinin, which promote the abnormal expression of pro-inflammatory cytokines and the activation of eosinophils. IL-4 is a Th2-type cytokine that can induce the differentiation of B cells into plasma cells, promoting the production of IgE (Bayar Muluk and Cingi, 2023). IL-10 can suppress the synthesis of inflammatory factors, suppress the activation of Th2 cells and the production of IgE, and inhibit Ikkβ to reverse the inflammatory reactions induced by TNF-α (Numata *et al*., 2024). IL-17 is a pro-inflammatory cytokine with the function of recruiting neutrophils. It can promote the release of inflammatory cytokines and increase airway hyperresponsiveness by promoting the secretion of mucus by airway mucous glands, thereby playing a role in airway remodeling in airway allergic reactions (Wen *et al*., 2020). TNF-α has multiple biological activities and exacerbates nasal mucosa inflammation through the promotion of the infiltration of inflammatory cells and the release of mediators (Sujata *et al*., 2023). IFN-γ is a Th1-type cytokine that can suppress the activation of Th2 cells (Ai *et al*., 2021). In this study, IgE, IL-4, IL-17, and TNF-α in the peripheral blood of AR rats were increased, while IL-10 and IFN-γ were decreased. Subsequently, this study found that the levels changed negatively after BAI treatment in AR rats. Peng *et al.* found that BAI can inhibit the release of peripheral blood cytokines (Peng *et al*., 2023). Bui *et al.* (2017) found that BAI can inhibit the generation of IL-1β, etc., in ovalbumin-induced asthma mouse models (Bui *et al*., 2017). This suggests that BAI treats AR by modulating inflammatory cytokine levels, including IgE.

TLRs are widely expressed on dendritic cells and mucosal epithelial cells in the nasal respiratory mucosa and function as innate immune receptors (Chen *et al*., 2022). The TLR4/Ikkβ/NF-κB pathway is obviously correlated with anti-inflammatory and immune mechanisms. Activated TLR4 can activate Ikkβ, and excessive activation of Ikkβ leads to the nuclear translocation and activation of NF-κB (Liu *et al*., 2022). Zhang *et al*. (2020) found that the TLR4/NF-κB pathway is abnormally activated in AR mice. This is consistent with the results of this study, which showed that the increased expression levels of TLR4, Ikkβ, and NF-κB p65 proteins in the nasal mucosa tissue of allergic rats (Zhang and Jin, 2020). High expression level of TLR4 can cause an overreaction of immune cells, leading to AR symptoms (Ebrahim Soltani *et al*., 2022). NF-κB is considered a hub in inflammation and immune responses; it can also be involved in IgE production (Qiao and Chen, 2021). Ran *et al*. (2021) confirmed that BAI inhibits TLR4/NF-κB pathway activation and promotes microglial polarization toward an anti-inflammatory phenotype, thereby mitigating post-stroke neuronal damage (Ran *et al*., 2021). The activation of the JNK/AP-1 pathway after lipopolysaccharide stimulation of macrophage activation is an important pathway for the generation of inflammatory factors (Yu *et al*., 2023). JNK/AP-1 is a key inflammatory pathway downstream of TLR4. This study found that p-JNK and AP-1 were abnormally increased in the nasal mucosa tissue of AR rats. In an *in vitro* AR model constructed with IL-13-induced JME/CF15 human nasal epithelial cells, the phosphorylation levels of c-Fos and c-Jun in the AP-1 pathway were markedly elevated, and the addition of an AP-1 inhibitor can inhibit AP-1 pathway activity and improve IL-13-induced inflammation and mucus formation (Gao *et al*., 2021). Therefore, it is believed that

the activation of the AP-1 pathway is involved in the regulation of the occurrence of AR. The TLR4/AP-1 pathway is a key regulator of inflammatory responses in AR. TLR4 activation promotes NF-κB and AP-1 nuclear translocation, driving IgE and IL-4 production (Zhang and Jin, 2020; Qiao and Chen, 2021). Our data align with these findings, as OVA sensitization significantly upregulated TLR4, p-JNK, and AP-1 ($P \leq 0.05$), while BAI treatment dose-dependently suppressed these markers, providing novel evidence for BAI's mechanism in AR. This study confirmed that BAI improves the inflammatory response in AR rats by inhibiting the TLR4/AP-1 pathway, which is consistent with the recent findings by Xu *et al.* (2024) regarding the mechanism of TLR4 regulation of Th2 differentiation. However, the therapeutic effects of BAI still need to be further validated through clinical trials. Although BAI shows promising anti-inflammatory effects in this preclinical model, its translation to human AR requires rigorous clinical trials to confirm safety and efficacy.

Conclusion: BAI suppresses the activation of TLR4 and its downstream inflammatory pathways, IKKβ/NF-κB and JNK/AP-1, in AR rats. Moreover, high-dose BAI demonstrates significantly enhanced therapeutic efficacy. This study contributes to a better understanding on the mechanism of BAI, which is critical in developing novel treatment modalities for AR.

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