

Regulations of Immune Response by Astragalus in Animal Asthma Models: A Meta-analysis

Song Wang¹ , Xinhua Chen², Zuoqiao Shi³, Qianqian Shen⁴, Jing Li⁵, Wang Hua¹, Wang Shujing¹, Wanjing Wu³, Qing Xi³ and Guowen Li³

Abstract

Background: Asthma is a chronic respiratory condition by airway inflammation. Traditional Chinese Medicine, is commonly used to treat it. Astragalus is known for its immunoregulation, anti-inflammatory, and antioxidant effects.

Objectives: To systematically evaluate the efficacy of Astragalus in asthma animal models using meta-analysis methods.

Methodology: A systematic search was performed in various databases, such as China National Knowledge Infrastructure (CNKI), WanFang Data, PubMed, Web of Science, and Cochrane Library databases for animal studies on Astragalus intervention in asthma models, with the search period extending up to September 2023. Two researchers performed independent literature screening and data extraction. The quality of the included studies was assessed by the Systematic Review Center for Laboratory Animal Experimentation (SYRCLE) animal experiment bias risk assessment tool. Meta-analysis was performed with RevMan (version 3.6.1).

Results: A total of 25 experiments were included. Meta-analysis indicated that Astragalus can significantly reduce total leukocytes, neutrophils, lymphocytes, and eosinophils in animal models of asthma. It also downregulated the expression levels of interleukin-4 and interleukin-5, while upregulation of interferon- γ and interleukin-10 expression was observed; however, the impact on macrophages was found to be not statistically significant ($p > 0.05$).

Conclusion: Our systemic review suggested the potential efficiency of Astragalus in asthma treatment in China, although its mechanism is still unclear. The functions of Astragalus may be associated with attenuating airway inflammation through the regulation of immune response.

Keywords

Astragalus, animal asthma model, immune response, meta-analysis

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Introduction

About 358 million people worldwide suffer from asthma, a chronic respiratory disease (Tibrewal et al., 2023). It significantly impacts patients' quality of life and poses considerable economic loss and negative societal impacts. The pathogenesis of asthma is complex, characterized mainly by persistent airway inflammation, infiltration of eosinophils and T lymphocytes, and the release of pro-inflammatory factors (Barnig et al., 2018). Various phenotypes exhibit different degrees of inflammation, airway hyperresponsiveness, mucus secretion, and airway remodeling. The T helper 2 (Th2) cell-mediated response is considered one of the classical mechanisms of asthma pathogenesis, affecting airway eosinophil expression, mucus secretion, airway hyperresponsiveness, and immunoglobulin E (IgE) synthesis. Asthma treatment

primarily focuses on reducing airway inflammation and relieving airway obstruction, often employing inhaled corticosteroids, leukotriene modulators, long-acting β_2

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agonists for control, rapid-acting β_2 agonists, and anticholinergic drugs. However, dependency and compliance issues with these medications affect disease treatment and control (Li & Liu, 2020).

The use of animal models is essential for understanding the molecular processes involved in the development of asthma and for discovering new treatment approaches (Aun et al., 2017; Woodrow et al., 2023). Various animal models, such as mice, rats, guinea pigs, dogs, and others, are utilized in asthma research studies. However, the mouse serves as the predominant animal model utilized in research on asthma. Over the past few decades, numerous studies have utilized animal models to enhance comprehension of the pathophysiology of diseases and their immune mechanisms (Williams & Roman, 2016). Despite some inconsistencies, there has been significant evidence of cell-mediated lung inflammation and different disease mediators. The early 1980s saw a heightened recognition of the significance of inflammation in asthma, spurred by advancements in allergic immunology and observations of pronounced symptoms in human asthmatics following exposure to diverse antigens. The eosinophil was the initial inflammatory cell to be definitively associated with the pathogenesis of asthma, with the T cell following closely thereafter (Bates et al., 2009). Leukocytes and cytokines production are important outcomes assessed and diagnostic indicators in experimental models of asthma. And large number of leukocytes such as neutrophils, lymphocytes, and eosinophils accumulate in bronchoalveolar lavage fluid (BALF) (Woodrow et al., 2023). Sensitization protocols are essential components in developing an animal model of asthma, resulting in elevated levels of total IgE in response to allergens. Airways inflammation mediators were identified in BALF, with BALF inflammatory mediators being evaluated to assess lung inflammation in the animal model of asthma (Kianmehr et al., 2016).

Traditional Chinese medicine (TCM) is frequently utilized as an alternative treatment for various illnesses (Liao et al., 2022). TCM correlates the body's righteous Qi with immune function, considering recurrent asthma attacks fundamentally due to the deficiency of righteous Qi. Therefore, treating asthma should primarily involve nourishing righteous Qi and regulating immune function (Lv & Gong, 2022). Astragalus, first recorded in "Shen Nong Ben Cao Jing," is a commonly used tonic in clinical TCM, known for its effects in augmenting Qi, lifting Yang, stabilizing exterior conditions, stopping sweating, nourishing the blood, and promoting fluid production (National Pharmacopoeia Commission, 2020). Recent studies have confirmed that Astragalus enhances immune function and has anti-inflammatory, antitumor, antidiabetic, and antioxidant properties (Wang et al., 2023). Numerous experimental studies on Astragalus intervention in asthma animal models mainly discuss improvements in airway inflammation, airway hyperresponsiveness, reduction in inflammatory cell numbers, and regulation of cytokines and transcription factors. Yet, there is a shortage of systematic

reviews linking these factors. Therefore, this study aims to systematically evaluate the intervention effects of Astragalus on asthma animal models, focusing on inflammatory cells and Th cell-related inflammatory factors in BALF, thus enhancing the research's guiding value and providing a basis for experimental design and clinical practice.

Methodology

Database and Literature Search Strategies

We conducted an extensive literature search utilizing China National Knowledge Infrastructure (CNKI), WanFang Database, PubMed, Web of Science, and Cochrane Library databases. Animal research on Astragalus for asthma was analyzed. All studies were conducted up to September 2023. The following terms were selected: (a) Astragalus; (b) Asthma; (c) 1 AND 2; (d) Animals NOT human; (e) 3 AND 4. References identified in other related studies were also considered.

Eligibility Criteria

Studies meeting the inclusion criteria included a variety of asthma experiments with no limitations on species, age, strain, gender, modeling techniques, or administration methods. The treatment group had to involve Astragalus in any dosage. The control group received either inactive liquid or no treatment; primary outcome measures included leukocyte, neutrophil, lymphocyte, eosinophil, and macrophage counts, as well as levels of interleukin-4 (IL-4), interleukin-5 (IL-5), interferon- γ (IFN- γ), and interleukin-10 (IL-10) in BALF. The criteria for exclusion include (a) duplicate publication; (b) lack of control group; (c) combination with any other medicine; and (d) studies with insufficient data or inaccessible original materials.

Data Extraction

Two researchers independently searched the literature, screened for inclusion/exclusion criteria, and collected data. In cases of disagreement, a third researcher made the judgment. Information collected from the studies included the first author, publication year, study design, sample size, route of administration, animal type/source, body weight, sex, modeling method, treatment duration, and focused outcome indicators. The mean value and standard deviation of outcomes were calculated, with only the final test included for outcomes performed at different time points. The treatment group included multiple doses of Astragalus, with the highest dose being administered.

Bias Risk in Included Studies

The study used the Systematic Review Center for Laboratory Animal Experimentation (SYRCLE) for bias risk assessment

in animal experimental systematic reviews, including 10 items: sequence generation, baseline characteristics, allocation concealment, random housing of animals, implementation bias blinding, random outcome assessment, measurement bias blinding, incomplete data reporting, selective outcome reporting, and other sources of bias. Results are categorized as “yes (Y)” for low risk of bias, “no (N)” for high risk of bias, and “uncertain (U)” for unclear risk of bias.

Statistical Analysis

RevMan (version 3.6.1) was utilized for the statistical analysis. The effect measure employed was the standardized mean difference (SMD), along with a 95% confidence interval (CI) provided. Statistical significance was determined at p value < 0.01 . I^2 was used to measure heterogeneity. When significant heterogeneity was observed ($I^2 > 50\%$), a random effects model was employed for analysis, while a fixed effects model was utilized in other instances.

Results

Literature Selection and Results

Based on specific criteria for inclusion and exclusion, a preliminary search in databases yielded 954 related articles. After eliminating duplicates, a total of 517 articles were reviewed based on their titles and abstracts. A full-text reading of 44 articles was conducted, and finally, 25 articles were included. Figure 1 displays the selection process and outcomes.

Basic Characteristics and Bias Risk Assessment of Included Studies

The 25 included animal studies were all Chinese journal articles (Table 1) involving guinea pigs, Sprague-Dawley (SD) rats, Wistar rats, BALB/c mice, and C57BL/6J mice. The experiments included 16 male-only studies, 7 female-only studies, and 2 studies with both sexes. All asthma animal

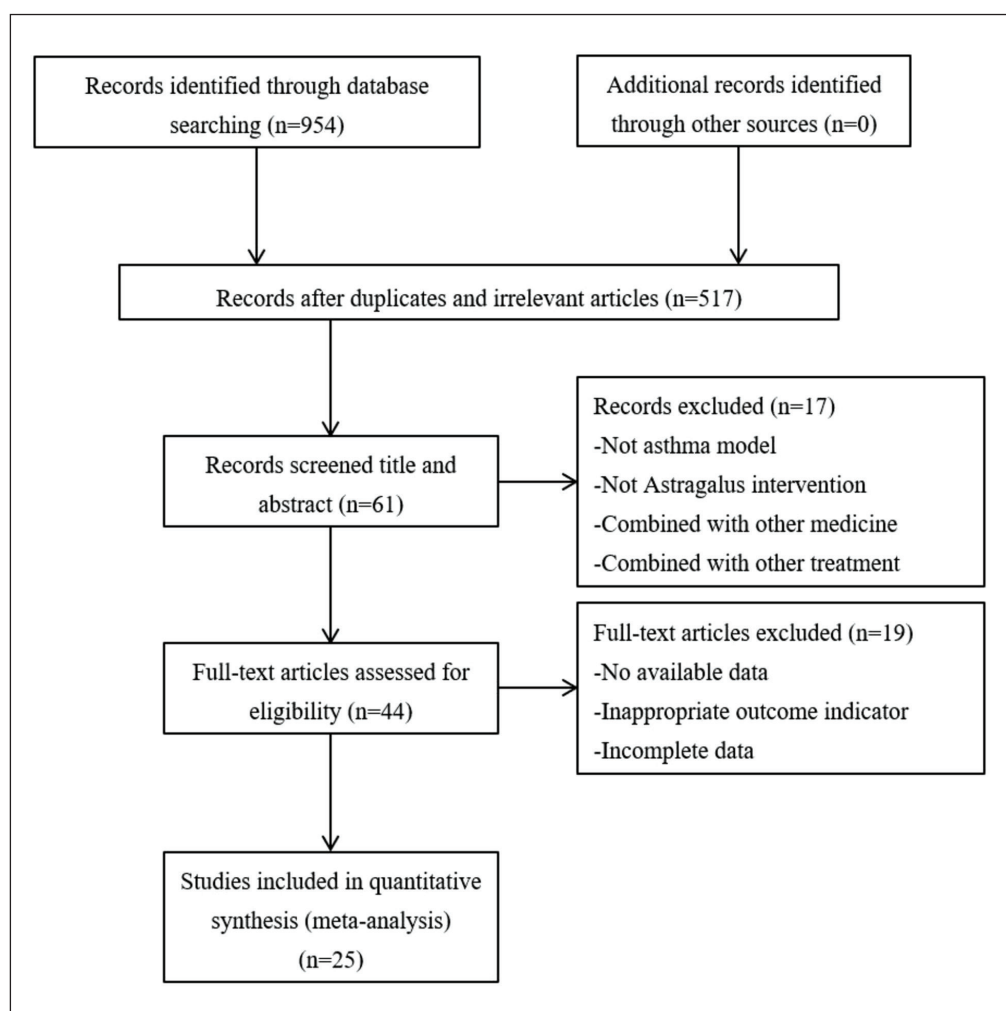


Figure 1. Summary of the Process for Identifying Candidate Studies.

Table 1. Design Features of Studies Included.

Study	Number (T/C)	Route	Strain/Species	Weight (g)	Gender	Model Method	Treatment Duration	Outcome
Mo and Wang (2001)	20 (10/10)	i.p	Guinea pigs	200–350	Male	Sensitization and stimulation by OVA	4 weeks	④
Li et al. (2006)	20 (10/10)	Gavage	SD rats	120–180	Male	Sensitization and stimulation by OVA	25 days	④
Huang and Yang (2009)	16 (8/8)	i.p	SD rats	22 ± 40	Male	Sensitization and stimulation by OVA	4 weeks	④ ⑦
Li et al. (2007)	20 (10/10)	Gavage	SD rats	120–180	Male	Sensitization and stimulation by OVA	25 days	⑥
Ye et al. (2006)	20 (10/10)	i.p	SD rats	120–180	Male	Sensitization and stimulation by OVA	Undefined	④ ⑥
Liu et al. (2011)	20 (10/10)	i.p	BALB/c mice	16–20	Female	Sensitization and stimulation by OVA	22 days	⑥ ⑧ ⑨
Zhang et al. (2019)	20 (10/10)	i.p	BALB/c mice	18–20	Female	Sensitization and stimulation by OVA	28 days	⑥
Zhang et al. (2011)	20 (10/10)	Gavage	Wistar rats	120–180	Male	Sensitization and stimulation by OVA	35 days	① ⑦
Li et al. (2013)	20 (10/10)	i.p	Wistar rats	200 ± 20	Male	Sensitization and stimulation by OVA	42 days	① ③ ④ ⑤ ⑨
Yan and Chang (2020)	40 (20/20)	i.p	Wistar rats	200 ± 25	Male	Sensitization and stimulation by OVA	44 days	⑥ ⑨
Jiang et al. (2009)	24 (12/12)	Gavage	SD rats	180–220	Male	Sensitization by inactivated pertussis vaccine and OVA, stimulation by OVA	24 days	④
Wang et al. (2019)	12 (6/6)	i.p	BALB/c mice	18–22	Male	Sensitization and stimulation by OVA	28 days	① ⑥ ⑧
Yu et al. (2011)	20 (10/10)	Gavage	BALB/c mice	18–20	Female	Sensitization and stimulation by OVA	11 weeks	① ④
Shang et al. (2021)	20 (10/10)	i.p	BALB/c mice	18–22	Female	Sensitization and stimulation by OVA	28 days	⑥ ⑦
Song et al. (2010a)	30 (15/15)	i.p	BALB/c mice	18 ± 2	Female	Sensitization and stimulation by OVA	7 weeks	① ④
Song et al. (2010b)	27 (12/15)	i.p	BALB/c mice	16–20	Half male and half female	Sensitization and stimulation by OVA	52 days	④

(Table 1 continued)

(Table 1 continued)

Study	Number (T/C)	Route	Strain/Species	Weight (g)	Gender	Model Method	Treatment Duration	Outcome
Li et al. (2018)	20 (10/10)	Gavage	C57BL/6j mice	18 ± 2	Female	Sensitization and stimulation by OVA	30 days	②③④⑦⑧
Wang et al. (2016)	16 (8/8)	i.p	Wistar rats	200 ± 20	Male	Sensitization and stimulation by OVA	6 weeks	②③④⑤
Li and Xu (2017)	20 (10/10)	Gavage	BALB/c mice	18–20	Male	Sensitization and stimulation by OVA	28 days	①④
Cai et al. (2013)	16 (8/8)	i.p	BALB/c mice	16–18	Female	Sensitization and stimulation by OVA	8 weeks	④
Wu et al. (2021)	24 (12/12)	i.p	SD rats	70–100	Both	Sensitization and stimulation by OVA	23 days	①②③④⑤
Huang et al. (2008a)	16 (8/8)	i.p	SD rats	220 ± 40	Male	Sensitization and stimulation by OVA	26 days	①③④⑥⑧
Wang and Huang (2012)	16 (8/8)	i.p	SD rats	220 ± 40	Male	Sensitization by OVA and inactivated pertussis vaccine; stimulation by OVA	4 weeks	④⑥
Huang et al. (2008b)	16 (8/8)	i.p	SD rats	220 ± 40	Male	Sensitization by OVA and inactivated pertussis vaccine; stimulation by OVA	4 weeks	②③④⑦
Kong et al. (2007)	20 (10/10)	i.p	BALB/c mice	20–25	Male	Sensitization and stimulation by OVA	22 days	⑥⑧

Notes: ① Leukocytes; ② neutrophils; ③ lymphocytes; ④ eosinophiles; ⑤ macrophage; ⑥ interleukin-4 (IL-4); ⑦ interleukin-5 (IL-5); ⑧ interferon- γ (IFN- γ); ⑨ interleukin-10 (IL-10). OVA, ovalbumin; SD, Sprague-Dawley.

models were sensitized and stimulated with ovalbumin (OVA). The models of administration included intraperitoneal injection in 18 studies and gavage in 7 studies. The treatment duration ranged from 22 days to 11 weeks, and 1 study was not specified. There were no adverse reactions documented in these studies that were included. Bias risk assessment results of the 25 studies, using SYRCLE, are shown in Table 2.

Meta-analysis Results

Total Leukocytes

A total of 8 studies (Huang et al., 2008a; Li & Xu, 2017; Li et al., 2013; Song et al., 2010a; Wang et al., 2019; Wu et al.,

2021; Yu et al., 2011; Zhang et al., 2011) involving 162 animals examined total leukocytes. A random effects model was used for meta-analysis due to substantial heterogeneity. Results indicated that Astragalus significantly decreased total leukocytes in asthma animal models compared to the control group, with a statistically significant difference (SMD = -2.52, 95% CI = -3.75 to -1.29, $p < 0.01$) (Figure 2A).

Neutrophil Count

Four studies (Huang et al., 2008b; Li et al., 2018; Wang et al., 2016; Wu et al., 2021) involving 76 animals examined neutrophil count. A random effects model was used for meta-analysis due to substantial heterogeneity. Results indicated that Astragalus significantly decreased neutrophil count in

Table 2. Quality Assessment of Included Studies.

Study	①	②	③	④	⑤	⑥	⑦	⑧	⑨	⑩
Mo and Wang (2001)	U	U	U	U	U	U	U	Y	Y	Y
Li et al. (2006)	U	U	U	U	U	U	U	Y	Y	Y
Huang and Yang (2009)	U	U	U	U	U	U	U	Y	Y	Y
Li et al. (2007)	U	U	U	U	U	U	U	Y	Y	Y
Ye et al. (2006)	U	U	U	U	U	U	U	Y	Y	Y
Liu et al. (2011)	U	U	U	Y	U	U	U	Y	Y	Y
Zhang et al. (2019)	U	U	U	U	U	U	U	Y	Y	Y
Zhang et al. (2011)	U	U	U	U	U	U	U	Y	Y	Y
Li et al. (2013)	U	U	U	U	U	U	U	Y	Y	Y
Yan and Chang (2020)	U	U	U	U	U	U	U	N	Y	Y
Jiang et al. (2009)	U	U	U	U	U	U	U	Y	Y	Y
Wang et al. (2019)	U	U	U	U	U	U	U	Y	Y	Y
Yu et al. (2011)	U	U	U	U	U	U	U	Y	Y	Y
Shang et al. (2021)	U	U	U	U	U	U	U	Y	Y	Y
Song et al. (2010a)	U	U	U	U	U	U	U	Y	Y	Y
Song et al. (2010b)	U	U	U	U	U	U	U	N	Y	Y
Li et al. (2018)	U	U	U	U	U	U	U	Y	Y	Y
Wang et al. (2016)	U	U	U	U	U	U	U	Y	Y	Y
Li and Xu (2017)	Y	U	U	Y	U	Y	U	Y	Y	Y
Cai et al. (2013)	U	U	U	U	U	U	U	N	Y	Y
Wu et al. (2021)	Y	U	U	Y	U	Y	U	Y	Y	Y
Huang et al. (2008b)	U	U	U	U	U	U	U	Y	Y	Y
Wang and Huang (2012)	U	U	U	U	U	U	U	Y	Y	Y
Huang et al. (2008a)	U	U	U	U	U	U	U	Y	Y	Y
Kong et al. (2007)	U	U	U	U	U	U	U	Y	Y	Y

Notes: ① Whether the distribution sequence is generated or applied sufficiently; ② whether the baselines of each group are the same; ③ whether the distribution hiding is sufficient; ④ whether the animals are randomly placed during the experiment; ⑤ whether the researchers are blinded; ⑥ whether the animals are randomly selected in the result evaluation; ⑦ whether the blind method is used for the evaluators of the results; ⑧ whether the incomplete data are reported; ⑨ whether the research report has nothing to do with the selective results report; ⑩ whether there is no other bias. Y, yes; N, no; U, uncertain.

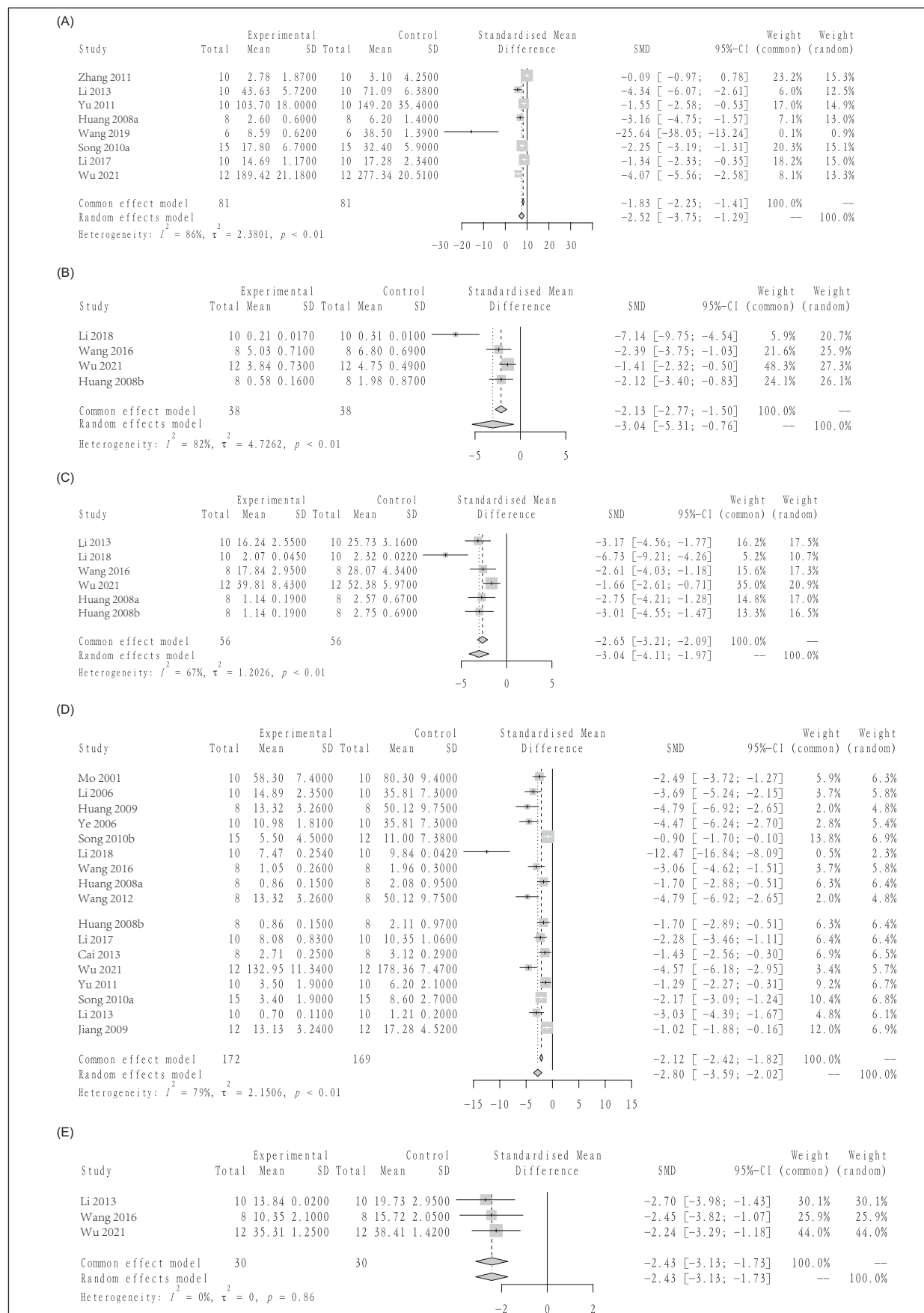


Figure 2. (A) Total Number of Leukocytes. (B) Number of Neutrophils. (C) Number of Lymphocytes. (D). Number of Eosinophils. (E) Number of Macrophages.

asthma animal models compared to the control group, with a statistically significant difference (SMD = -3.04 , 95% CI = -5.31 to -0.76 , $p < 0.01$) (Figure 2B).

Lymphocyte Count

Six studies (Huang et al., 2008a; Huang et al., 2008b; Li et al., 2013, 2018; Wang et al., 2016; Wu et al., 2021) involving 112 animals examined lymphocyte count. A random effects model was used for meta-analysis due to substantial heterogeneity. Results indicated that *Astragalus* significantly decreased lymphocyte count in asthma animal models compared to the control group, with a statistically significant difference (SMD = -3.04 , 95% CI = -4.11 to -1.97 , $p < 0.01$) (Figure 2C).

Eosinophil Count

A total of 17 studies (Cai et al., 2013; Huang & Yang, 2009; Huang et al., 2008a; Huang et al., 2008b; Jiang et al., 2009; Li & Xu, 2017; Li et al., 2006, 2013, 2018; Mo & Wang, 2001; Song et al., 2010a; Song et al., 2010b; Wang & Huang, 2012; Wang et al., 2016; Wu et al., 2021; Ye et al., 2006; Yu et al., 2011) involving 341 animals examined eosinophil count. A random effects model was utilized for meta-analysis due to substantial heterogeneity. Results indicated that *Astragalus* significantly decreased eosinophil count in asthma animal models compared to the control group, with a statistically significant difference (SMD = -2.80 , 95% CI = -3.59 to -2.02 , $p < 0.01$) (Figure 2D).

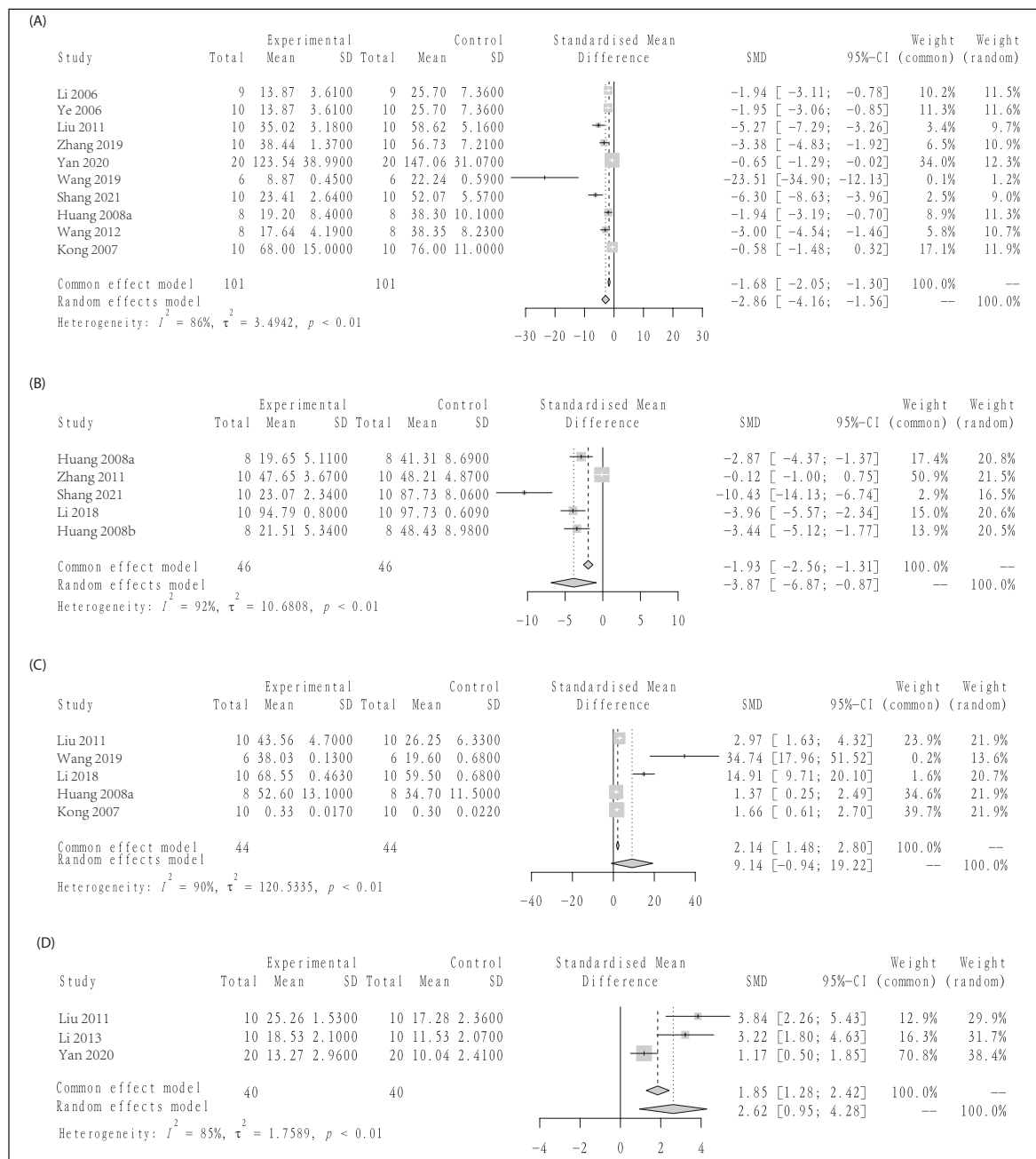


Figure 3. (A) Level of Interleukin-4 (IL-4). (B) Level of Interleukin-5 (IL-5). (C) Level of Interferon- γ (IFN- γ). (D) Level of Interleukin-10 (IL-10).

Macrophage Count

Three studies (Li et al., 2013; Wang et al., 2016; Wu et al., 2021) involving 60 animals examined macrophage count. With minor heterogeneity, a fixed effects model was employed in the meta-analysis. Results indicated that Astragalus did not significantly change macrophage count in asthma animal models compared to the control group (SMD = -2.43 , 95% CI = -3.13 to -1.73 , $p > 0.05$) (Figure 2E).

Expression Level of IL-4

Ten studies (Huang et al., 2008a; Kong et al., 2007; Li et al., 2007; Liu et al., 2011; Shang et al., 2021; Wang & Huang, 2012; Wang et al., 2019; Yan & Chang, 2020; Ye et al., 2006;

Zhang et al., 2019) involving 202 animals examined IL-4 expression level. A random effects model was used for meta-analysis due to substantial heterogeneity. Results indicated that Astragalus significantly decreased IL-4 expression level in asthma animal models compared to the control group, with a statistically significant difference (SMD = -2.86 , 95% CI = -4.16 to -1.56 , $p < 0.01$) (Figure 3A).

Expression Level of IL-5

Five studies (Huang & Yang, 2009; Huang et al., 2008b; Li et al., 2018; Shang et al., 2021; Zhang et al., 2011) involving 92 animals examined IL-5 expression levels. A random effects model was used for meta-analysis due to substantial heterogeneity. Results indicated that Astragalus significantly

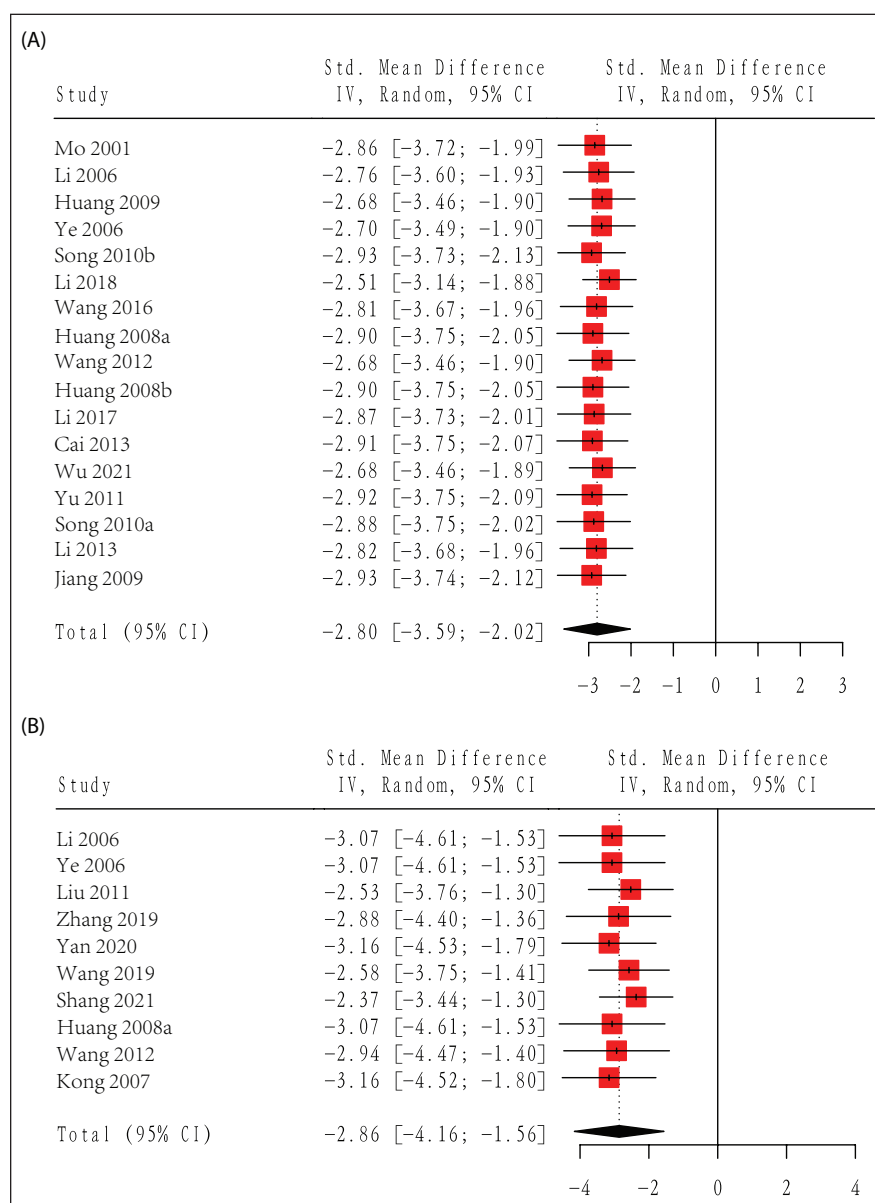


Figure 4. (A) Sensitivity Analysis of Eosinophil. (B) Sensitivity Analysis of Interleukin-4 (IL-4).

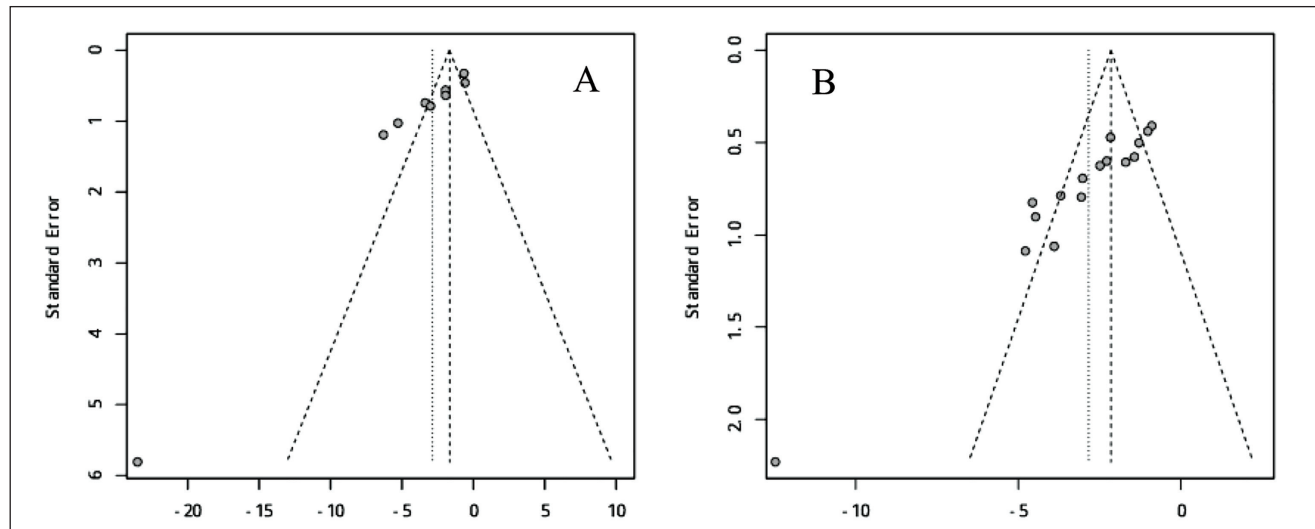


Figure 5. Funnel Plot of the Size of Eosinophil (A) and Interleukin-4 (IL-4) (B).

decreased IL-5 expression level in asthma animal models compared to the control group, with a statistically significant difference (SMD = -3.87 , 95% CI = -6.87 to -0.87 , $p < 0.01$) (Figure 3B).

Expression Level of IFN- γ

Five studies (Huang et al., 2008a; Kong et al., 2007; Li et al., 2018; Liu et al., 2011; Wang et al., 2019) involving 88 animals examined IFN- γ expression levels. A random effects model was used for meta-analysis due to substantial heterogeneity. Results indicated that Astragalus significantly increased IFN- γ expression level in asthma animal models compared to the control group, with a statistically significant difference (SMD = 9.14 , 95% CI = -0.94 to 19.22 , $p < 0.01$) (Figure 3C).

Expression Level of IL-10

Three studies (Li et al., 2013; Liu et al., 2011; Yan & Chang, 2020) involving 80 animals examined IL-10 expression levels. A random effects model was used for meta-analysis due to substantial heterogeneity. Results indicated that Astragalus significantly increased IL-10 expression level in asthma animal models compared to the control group, with a statistically significant difference (SMD = 2.62 , 95% CI = 0.95 – 4.28 , $p < 0.01$) (Figure 3D).

Sensitivity Analysis

To assess how studies affect meta-analysis results, sensitivity analysis was conducted for two outcome indicators (eosinophil and IL-4) that included 10 or more articles. Excluding each study one by one in a random effects model, the results remained unchanged, indicating the overall reliability of the meta-analysis results (Figure 4A and B).

Publication Bias Analysis

The risk of publication bias was evaluated through the utilization of funnel plots for two outcome measures (eosinophil and IL-4) that included 10 or more articles. The funnel plots exhibited a degree of symmetry; however, the presence of points outside the funnel plot indicates a possible inclination towards publication bias (Figure 5). Other indicators were not analyzed for publication bias due to smaller sample sizes.

Discussion

With advanced research into the mechanisms of asthma onset and development, asthma is recognized as a multifaceted condition primarily distinguished by inflammation of the airway. Factors such as age, sex, environment, and race can influence physiological and biological characteristics. The pathogenesis of asthma is closely related to innate and adaptive immunity involving inflammatory cells like lymphocyte subgroups, eosinophils, macrophages, mast cells, and cytokines such as interleukins (Lambrecht, 2019). The cells play a crucial role in asthma development, with T helper 1 (Th1) cells and their secreted cytokines like IFN- γ mediating cytotoxic and local inflammatory immune responses (Lin et al., 2022). Enhancement of Th2 specificity is one of the immunological mechanisms in asthma development. Studies have found that the development of IgE and Th2 cytokines, such as IL-4 and IL-5, was responsible for most childhood and adult asthma cases (León & Ballesteros-Tato, 2021). Therefore, research on asthma-related inflammatory cells, pro-inflammatory cytokines, and other proteins plays a crucial role in prevention, diagnosis, and treatment, providing a basis for the development of targeted drugs.

Astragalus, a key herb in TCM for strengthening Qi, is often used in combination with other herbs, especially in classic formulas like Yu Ping Feng powder and Bu Zhong Yi Qi decoction. It is listed among the initial 100 “Ancient Classic Formulae” released by the National Administration of TCM (Jiang et al., 2023). As basic research advances, Astragalus is widely used in respiratory, digestive, endocrine, and hematological systems, exhibiting immunomodulatory, anti-inflammatory, and antioxidant effects (Jiang et al., 2023). Recent studies on Astragalus in treating asthma have been popular. Astragalus acts on the spleen and lung meridians in TCM, corresponding to the etiology and clinical symptoms of asthma in TCM. Several studies have found that Astragalus reduces inflammation, improves airway remodeling, regulates immune balance, and alleviates drug dependence after steroid use (Zhu et al., 2021). Our group has previously studied the anti-inflammatory and immunoregulatory effects of Yu Ping Feng powder, which contains Astragalus, and Astragaloside IV, an active component of Astragalus, in asthma animal models (Huang et al., 2020; Wang et al., 2017). Our research systematically evaluated the impact of Astragalus intervention in asthma animal models through meta-analysis, to provide evidence-based support for further research.

When developing models of human respiratory disease in non-human animals, it is imperative to take into account a multitude of factors, including lifestyle, lifespan, anatomy, and genetics. Nevertheless, despite these disparities, utilizing animal models has greatly enhanced our understanding of how respiratory illnesses develop. Various inflammatory biomarkers, including BALF, bronchoscope biopsy, and blood cell counts, are used for asthma diagnosis and characterization (Rupani et al., 2021). Many animal model studies on asthma use inflammatory cell counts in BALF. Therefore, this study analyzed inflammatory cells and certain Th-related cytokines in pulmonary alveolar lavage fluid. Leukocytes, including neutrophils, eosinophils, and macrophages, are important indices for evaluating asthma models. In experimental models of asthma, particularly in the airways, a pronounced influx of eosinophils can be observed in pulmonary inflammation (Kurup et al., 2007). Elevated levels of eosinophils are observed in bronchoalveolar lavage fluid, along with increased numbers of lymphocytes, macrophages, and neutrophils (Aun et al., 2017). The prevailing asthma model is characterized by Th2 predominance, resulting in an immune imbalance between Th1 and Th2. The assessment of cytokine production serves as a crucial outcome measure in experimental models of asthma (Akdis et al., 2016). Meta-analysis showed that Astragalus treatment decreased leukocytes, neutrophils, lymphocytes, eosinophils, IL-4, and IL-5, and increased IFN- γ and IL-10. The results indicated that Astragalus can alleviate asthma symptoms by inhibiting inflammatory cells and pro-inflammatory cytokines, exerting anti-inflammatory and immunoregulatory effects. Asthma, a respiratory inflammatory disease with complex mechanisms involving

numerous inflammatory mediators, requires anti-inflammatory and bronchodilator treatments, with reduction in inflammation markers being one of the key efficacy assessments (Papi et al., 2018). The exacerbation of asthma is intricately linked to the secretion of cytokines by Th2 cells, particularly those associated with the Th2 subset, leading to eosinophils and neutrophils. IL-4, IL-5, and other Th2 cytokines impact airway eosinophil expression, mucus secretion, airway hyperresponsiveness, and IgE synthesis. IL-4 is vital for sensitization and IgE synthesis, while IL-5 plays a central role in eosinophil production (Lambrecht et al., 2019). IFN- γ , a Th1 cytokine secreted by immune cells following acute inflammatory stimulation, promotes type I immune responses and inhibits type II immune responses (Guo et al., 2023). Increased IFN- γ expression helps alleviate symptoms and restore Th1/Th2 immune balance in asthma animal models. Anti-inflammatory factor IL-10 inhibits inflammatory responses and is an important expression gene in asthma, with lower expression observed in asthma patients, deactivating neutrophils, eosinophils, and mast cells (Kawano et al., 2016). The balance of IL-10/IL-5 also plays a role in alleviating asthma (Tomiita et al., 2015). Asthma models help improve understanding of asthma’s different aspects, with a focus on type 2 (Th2 high) phenotype. There is a scarcity of data available on the T-helper activation profile in relation to cytokine measurement among various species. The Th2 cytokines have been extensively studied in rodent experiments (Aun et al., 2017).

The quality exhibited in these studies pertaining to Astragalus intervention in asthma models is suboptimal. The results may be biased due to not fully complying with SYRCLE’s requirements in design and implementation. In animal experiments, the application of blinding in intervention implementation and outcome measurement differs from randomized controlled trials (RCT) studies. Although blinding is not required for animals, most researchers are also animal caretakers, and if caretakers are not blinded, this may lead to subjective bias in expected results (Chen et al., 2014). Different strains also have different immune responses in animal asthma models. The most common mouse strains utilized are typically BALB/c or C57BL/6. BALB/c demonstrate a heightened IgE response and are classified as Th2 immune responders, while C57BL/6 typically exhibit a preference for a Th1 response and display reduced levels of IgE production (Woodrow et al., 2023).

This research has certain limitations: (a) All included literature has a small sample size, and some studies were included due to inaccessible data, possibly causing publication bias. (b) High heterogeneity in the nine included indicators may be related to animal species and modeling methods, especially medication duration. (c) Due to limited study numbers, subgroup and dosage analyses were not conducted.

In conclusion, this meta-analysis demonstrates that Astragalus effectively decreases the pro-inflammatory cytokines IL-4 and IL-5 in asthma model animals while

increasing anti-inflammatory cytokines IFN- γ and IL-10. It has certain anti-inflammatory and immunoregulatory effects, providing evidence-based support for Astragalus intervention in asthma. However, the quality of the studies objectively reduces the scientific nature of animal experiments. Engaging in systematic study and research of the SYRCLE framework for assessing bias risk in animal experiments has the potential to enhance the credibility of findings derived from such experiments. Future inclusion of high-quality experimental studies in systematic reviews, reducing bias risk, can provide more reliable support for animal experiment design, implementation, and guiding clinical medication research. Enhancing the understanding of asthma pathophysiology in humans through the use of animal models is intended to enhance diagnostic and therapeutic approaches. The selection of the most suitable animal model for addressing research inquiries is ultimately at the discretion of the researcher, with each model presenting distinct advantages and disadvantages.

Conclusion

In this research, we have conducted a systematic review of Astragalus in asthma animal models, with the results suggesting that its mechanisms of action may involve the modulation of immune response to reduce airway inflammation.

Abbreviations

BALF: Bronchoalveolar lavage fluid; CI: Confidence interval; CNKI: China National Knowledge Infrastructure; IFN- γ : Interferon γ ; IgE: Immunoglobulin E; IL-4: Interleukin 4; IL-5: Interleukin 5; IL-10: Interleukin 10; OVA: Ovalbumin; TCM: Traditional Chinese medicine; Th: T helper; SMD: Standardized mean difference; SYRCLE: Systematic Review Center for Laboratory Animal Experimentation.

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Ethical Approval and Informed Consent

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References

- Akdis, M., Aab, A., Altunbulakli, C., Azkur, K., Costa, R. A., Crameri, R., Duan, S., Eiwegger, T., Eljaszewicz, A., Ferstl, R., Frei, R., Garbani, M., Globinska, A., Hess, L., Huitema, C., Kubo, T., Komlosi, Z., Konieczna, P., Kovacs, N., ... Akdis, C. A. (2016). Interleukins (from IL-1 to IL-38), interferons, transforming growth factor β , and TNF- α : Receptors, functions, and roles in diseases. *The Journal of Allergy and Clinical Immunology*, 138(4), 984–1010. <https://doi.org/10.1016/j.jaci.2016.06.033>
- Aun, M. V., Bonamichi-Santos, R., Arantes-Costa, F. M., Kalil, J., & Giavina-Bianchi, P. (2017). Animal models of asthma: Utility and limitations. *Journal of Asthma and Allergy*, 10, 293–301. <https://doi.org/10.2147/JAA.S121092>
- Barnig, C., Frossard, N., & Levy, B. D. (2018). Towards targeting resolution pathways of airway inflammation in asthma. *Pharmacology and Therapeutics*, 186, 98–113. <https://doi.org/10.1016/j.pharmthera.2018.01.004>
- Bates, J. H. T., Rincon, M., & Irvin, C. G. (2009). Animal models of asthma. *American Journal of Physiology. Lung Cellular and Molecular Physiology*, 297(3), L401–L410. <https://doi.org/10.1152/ajplung.00027.2009>
- Cai, C., Dong, J. C., Du, W. J., Gong, W. Y., Liu, B. J., Wu, J. F., & Cao, Y. X. (2013). Study of Astragaloside on infiltration of eosinophils in mouse model of allergic asthma. *China Journal of Traditional Chinese Medicine and Pharmacy*, 28(08), 2278–2283.
- Chen, K. Y., Ma, B., Wang, Y. N., Chen, C. H., Zhao, Y. Q., Zheng, J. X., Huang, C. B., Zheng, D. X., Wang, X. Y., Zhu, X. G., & Wang, Y. P. (2014). STRCLE's risk of bias tool for animal studies. *Chinese Journal of Evidence-Based Medicine*, 14(10), 1281–1285.
- Guo, C. Y., Su, M., Guo, Y., Wang, L. L., Yang, Z., Zhang, X., & Bai, Y. (2023). Effect of IFN- γ on inflammatory response of asthmatic mice and its possible mechanisms. *Advances in Anatomical Sciences*, 29(02), 116–119.
- Huang, C. P., & Yang, H. P. (2009). Effects of astragalus injection on the expression of p38 mitogen-activated protein kinase in asthmatic rats. *Journal of Xianning University (Medical Sciences)*, 23(01), 1–3.
- Huang, S. J., Wang, S., Yu, L., Xi, Q., Li, G. W., & Shen, C. B. (2020). Inhibitory effect of Astragaloside IV on th2 transcription factor expression in asthmatic mice. *Modern Immunology*, 40(1), 28–33.
- Huang, C. P., Yang, H. P., Yang, Y. Q., Zhang, Z. X., & Xu, Y. J. (2008a). Protective effects and mechanism of astragalus injection on asthmatic rats. *Chinese Journal of Pathophysiology*, 24(12), 2386–2390.
- Huang, C. P., Yang, H. P., & Yang, Y. Q. (2008b). Protective effects and mechanism of astragalus injection on asthmatic rats. *Chinese Journal of Clinical Pharmacology and Therapeutics*, 13(08), 900–904.
- Jiang, Y. L., Feng, N., Shao, X. Y., Huang, J., & Huang, F. H. (2023). Progress of modern pharmacological function of Astragalus. *Journal of Southwest Medical University*, 46(5), 456–460.
- Jiang, Y., Li, D. M., & Chen, M. (2009). Effects of astragalus polysaccharides on airway inflammation and interleukin-3

- expression in asthmatic rats. *China Journal of Modern Medicine*, 19, 1779–1781.
- Kawano, H., Kayama, H., Nakama, T., Hashimoto, T., Umemoto, E., & Takeda, K. (2016). IL-10-producing lung interstitial macrophages prevent neutrophilic asthma. *International Immunology*, 28(10), 489–501. <https://doi.org/10.1093/intimm/dxw012>
- Kianmehr, M., Ghorani, V., & Boskabady, M. H. (2016). Animal model of asthma, various methods and measured parameters: A methodological review. *Iranian Journal of Allergy, Asthma, and Immunology*, 15(6), 445–465.
- Kong, C. Y., Xie, X. Z., & Mou, C. Z. (2007). Effects of astragalus injection on th1/th2 immunity in asthmatic mice. *Clinical Medicine*, 27(10), 82–83.
- Kurup, V. P., Barrios, C. S., Raju, R., Johnson, B. D., Levy, M. B., & Fink, J. N. (2007). Immune response modulation by curcumin in a latex allergy model. *Clinical and Molecular Allergy: CMA*, 5, 1. <https://doi.org/10.1186/1476-7961-5-1>
- Lambrecht, B. N., Hammad, H., & Fahy, J. V. (2019). The cytokines of asthma. *Immunity*, 50(4), 975–991. <https://doi.org/10.1016/j.immuni.2019.03.018>
- León, B., & Ballesteros-Tato, A. (2021). Modulating Th2 cell immunity for the treatment of asthma. *Frontiers in Immunology*, 12, 637948. <https://doi.org/10.3389/fimmu.2021.637948>
- Li, F. X., & Liu, J. (2020). Research progress in drug treatment of refractory bronchial asthma. *Medical Recapitulate*, 26, 1573–1577.
- Li, S., & Xu, L. (2017). Effects of Astragaloside on respiratory inflammation and oxidative stress in asthma model mice. *International Journal of Traditional Chinese Medicine*, 39, 1007–1010.
- Li, Y., Yang, L. L., Bai, Y. L., & Ming, H. X. (2018). Effect of astragalus polysaccharides on airway inflammation and aquaporin 5 expression in mice with bronchial asthma. *Journal of Gansu University of Chinese Medicine*, 35, 16–20.
- Li, C.-C., Ye, L.-P., Su, M.-S., Li, M.-R., Zhang, H.-L., Cai, X.-H., Dong, L., & Luo, Y.-C. (2007). Effect of radix astragali on signal transducer and activator of transcription activator-4 and its expression in a rat model of asthma. *Zhong Hua Er Ke Za Zhi*, 45(10), 727–731.
- Li, C. C., Ye, L. P., Yu, X. C., Li, M. R., & Luo, Y. C. (2006). Effect of radix astragali on signal transducer and activator of transcription 6 and its mRNA expression in a rat asthma model. *Journal of Chinese Pharmaceutical Ences*, 41, 104–107.
- Li, C. D., Zhou, W. B., Sun, Y., Fang, L., Yu, J., & Mao, S. M. (2013). Effects of Astragalus polysaccharide on Th17/Treg associated cytokines and airway inflammation in lung. *Chinese Pharmacological Bulletin*, 29, 1275–1278.
- Liao, P. F., Wang, Y. T., Wang, Y. H., Chiou, J. Y., & Wei, J. C. C. (2022). Traditional Chinese medicine use may reduce medical utility in patients with asthma: A nationwide population-based retrospective cohort study. *QJM: Monthly Journal of the Association of Physicians*, 114(12), 857–864. <https://doi.org/10.1093/qjmed/hcaa252>
- Lin, L., Xu, S., Peng, F., Jin, H., & Xiao, F. (2022). MDSCs aggravate the asthmatic progression in children and OVA-allergic mice by regulating the Th1/Th2/Th17 responses. *Evidence-Based Complementary and Alternative Medicine: eCAM*, 2022, 6157385. <https://doi.org/10.1155/2022/6157385>
- Liu, R. W., Li, C. X., Li, Z. H., & Wang, J. P. (2011). Effect of astragalus on CD4⁺ CD25⁺ T cells and cytokines in the mice model of asthma. *Journal of Jining Medical University*, 34, 232–235.
- Lv, Z. Y., & Gong, J. (2022). Research progress on the effect of Chinese medicine treatment on the immune function of asthma. *Journal of Medicine of the People's Liberation Army*, 34(10), 111–113.
- Mo, B. W., & Wang, C. M. (2001). Study on the effect of Astragalus on the expression of nuclear factor kb in guinea pig asthma model. *Chinese Journal of Tuberculosis and Respiratory*, 24, 43+68.
- National Pharmacopoeia Commission. (2020). *Pharmacopoeia of the People's Republic of China part I*. China Pharmaceutical Science and Technology Press
- Papi, A., Brightling, C., Pedersen, S. E., & Reddel, H. K. (2018). Asthma. *The Lancet*, 391(10122), 783–800. [https://doi.org/10.1016/S0140-6736\(17\)33311-1](https://doi.org/10.1016/S0140-6736(17)33311-1)
- Rupani, H., Fong, W. C. G., Kyaly, A., & Kurukulaaratchy, R. J. (2021). Recent insights into the management of inflammation in asthma. *Journal of Inflammation Research*, 14, 4371–4397. <https://doi.org/10.2147/JIR.S295038>
- Shang, Y. L., Guo, C. X., Shi, H. M., & Ye, L. Q. (2021). Effect of astragalus polysaccharide on the expression of TSP and DCs in airway inflammation of asthmatic mice. *Chinese Journal of Antibiotics*, 46, 711–716.
- Song, Z. Q., Zhu, Y. F., Yao, W. M., & Wang, H. (2010a). Interventional effects of astragalus polysaccharide on airway remodeling in asthmatic mice. *Chinese Clinical Pharmacology and Therapeutics*, 15, 385–390.
- Song, Z. Q., Lin, L., & Zhu, Y. F. (2010b). The enhancement of astragalus polysaccharide on specific immunotherapy in asthmatic mouse. *Chinese Journal of Immunology*, 26, 132–135.
- Tibrewal, C., Modi, N. S., Bajoria, P. S., Dave, P. A., Rohit, R. K., Patel, P., Gandhi, S. K., Gutlapalli, S. D., Gottlieb, P., & Nfonoyim, J. (2023). Therapeutic potential of vitamin D in management of asthma: A literature review. *Cureus*, 15(7), e41956. <https://doi.org/10.7759/cureus.41956>
- Tomiita, M., Campos-Alberto, E., Shima, M., Namiki, M., Sugimoto, K., Kojima, H., Watanabe, H., Sekine, K., Nishimuta, T., Kohno, Y., & Shimojo, N. (2015). Interleukin-10 and interleukin-5 balance in patients with active asthma, those in remission, and healthy controls. *Asia Pacific Allergy*, 5(4), 210–215. <https://doi.org/10.5415/apallergy.2015.5.4.210>
- Wang, X. H., & Huang, C. P. (2012). Effects of Astragalus injection on the expression of p38 protein kinase and interleukin-4 in asthmatic rats. *Lishizhen Medicine and Material Medica Research*, 23(02), 310–311.
- Wang, J. L., Li, C. D., Sun, H. W., Mao, S. M., Zhang, X. J., & Qu, M. H. (2016). Astragalus polysaccharide regulates NF-κB signaling pathway and attenuates airway inflammation in ova-induced asthmatic rats. *Chinese Pharmacological Bulletin*, 32, 489–493.
- Wang, H., Wang, S., Ding, X. Y., Lu, L., Cai, H., Wang, Q. E., & Shen, C. B. (2017). Influence of Yupingfeng powder on the expression of thymic stromal lymphopoietin (TSLP) in airway epithelial cells of asthma model mice. *Shanghai Journal of Traditional Chinese Medicine*, 51(2), 69–72.
- Wang, P., Wang, Z., Zhang, Z., Cao, H., Kong, L., Ma, W., & Ren, W. (2023). A review of the botany, phytochemistry, traditional uses, pharmacology, toxicology, and quality control of the *Astragalus memranaceus*. *Frontiers in Pharmacology*, 14, 1242318. <https://doi.org/10.3389/fphar.2023.1242318>
- Wang, L. H., Zhang, Y., Lan, K., Zhang, H. Y., Cao, X. F., & Li, S. Y. (2019). Inhibitory effect of astragalus polysaccharides on

- pulmonary inflammation in asthma model mice and its mechanism. *Journal of Jilin University (Medicine Edition)*, 45, 313–318+472.
- Williams, K., & Roman, J. (2016). Studying human respiratory disease in animals—Role of induced and naturally occurring models. *The Journal of Pathology*, 238(2), 220–232. <https://doi.org/10.1002/path.4658>
- Woodrow, J. S., Sheats, M. K., Cooper, B., & Bayless, R. (2023). Asthma: The use of animal models and their translational utility. *Cells*, 12(7), 1091. <https://doi.org/10.3390/cells12071091>
- Wu, S., Li, W., Li, D., Xin, D., Chen, X. F., & Li, X. (2021). Effects of Astragaloside II on the IL-21/STAT3 pathway and airway inflammation in young asthmatic rats. *Chinese Journal of Comparative Medicine*, 31(12), 53–59.
- Yan, W. H., & Chang, J. X. (2020). Effect of astragalus polysaccharides on airway inflammation and lung tissue vascular endothelial growth factor expression of asthmatic rats. *The Chinese Journal of Clinical Pharmacology*, 36, 953–955.
- Ye, L. P., Li, C. C., Guan, S. J., Luo, Y. C., Huang, D. S., & Xu, Y. Q. (2006). Effect of astragalus membranaceus on expressions of interleukin-4 and interleukin-12 in bronchoalveolar lavage fluid in asthma rats' model. *Journal of Applied Clinical Pediatrics*, 21, 549–550+552.
- Yu, J. F., Gu, S. P., & Yang, X. Y. (2011). Effect of astragalus polysaccharides on airway hyperresponsiveness and airway remodeling in a murine model of chronic asthma. *Traditional Chinese Drug Research and Clinical Pharmacology*, 22, 418–421.
- Zhang, Y., Wei, Q., & Yi, P. F. (2011). Effect of Mongolian milk vetch root on NO, IL-5, inflammatory cells in balf and serum of bronchial asthmatic rats. *Journal of Jilin University (Medicine Edition)*, 37, 1033–1036.
- Zhang, Y., Yang, L., Jiang, R. Y., Li, R. R., Li, P. P., & Chen, S. Y. (2019). The effect of Astragalus on the changes of il 2, IL 4 and their behavior in BALF of asthmatic mice. *Journal of Taishan Medical College*, 40, 914–916.
- Zhu, X. Y., Wei, Y., & Dong, J. C. (2021). Review of pharmacological effects of Astragalus in the treatment of bronchial asthma. *Journal of Basic Chinese Medicine*, 27(1), 182–185.