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Study on Proinflammatory Response and Regulation of JAK2/STAT3 Signaling Pathway of Ethanol Extract of *Anacyclus pyrethrum* Root (EEAP) in Rats with Cough-Variant Asthma

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Abstract: *Objective:* To explore the modulation of the proinflammatory response and the tyrosine protein kinase 2/signal transducer and activator of transcription 3 (JAK2/STAT3) pathway in the lung of rats with cough-variant asthma (CVA) treated with ethanol extract of *Anacyclus pyrethrum* root (EEAP). *Methods:* Sixty specific pathogen-free (SPF) male Sprague-Dawley (SD) rats (with a weight range of 220–260 g, eight to ten weeks) were randomly assigned into six different groups, encompassing a control group, a model group, a positive control group (prednisone acetate, the dosage was 2.5 mg/kg, ig), the high EEAP group (640 mg/kg), medium EEAP group (320 mg/kg), and low EEAP group (160 mg/kg), with a total of 10 participants in each group. The medication was provided on a daily basis for 30 days, commencing on the 29th day. The rodents in each cohort were maintained until the 60th day. Following humane euthanasia, lung tissues and peripheral blood samples were collected. Two groups were formed by dividing rat lung epithelial type II cells: the OVA group, which received 2.5 mg/L ovalbumin stimulation and sensitization, and the treatment group, which was administered 640 mg/kg EEAP. The peripheral blood T-lymphocytes of rats in the control group and the high EEAP cohort were designated as CVA group and intervention group, respectively. The concentrations of interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α) were measured in each cohort using the enzyme-linked immunosorbent assay (ELISA). Utilizing real-time quantitative polymerase chain reaction (RT-PCR), the levels of IL-6, JAK2, and STAT3 were analyzed within each group. *Results:* In contrast to the control group, the concentrations of IL-1β, IL-6, and TNF-α in the model group exhibited a substantial rise ($P < 0.05$). Moreover, the mRNA expression levels of IL-6, JAK2, and STAT3 in the experimental group were observed to be substantially elevated ($P < 0.05$). In contrast to the control group, the positive controlled group, the elevated EEAP group, and the intermediate EEAP group demonstrated substantial decreases in the mRNA expression of IL-6, JAK2, and STAT3 ($P < 0.05$), while the low EEAP group exhibited a notable decrease in the mRNA expression of JAK2. The mRNA expression levels of IL-6 and STAT3 did not exhibit any notable variation in the low EEAP group ($P > 0.05$). The concentrations of IL-1β, IL-6, and TNF-α, along with the mRNA expression levels of IL-6, JAK2, and STAT3, demonstrated an upward trend as EEAP concentration diminished ($P < 0.05$) across the higher EEAP category, the intermediate EEAP category, and the low EEAP group. In rat type II alveolar epithelial cells, the concentrations of IL-1β, IL-6, and TNF-α, as well as the mRNA expression levels of IL-6, JAK2, and STAT3 were diminished in the intervention

cohort in comparison to the OVA cohort ($P < 0.05$). When compared to the OVA group, the treatment group demonstrated a notable decrease in IL-1β, IL-6, and TNF-α, along with mRNA expression levels of IL-6, JAK2, and STAT3 ($P < 0.05$). In contrast to the CVA group, the intervention group demonstrated a significant reduction in IL-1β, IL-6, and TNF-α levels, along with the mRNA expression levels of IL-6, JAK2, and STAT3 in T lymphocytes ($P < 0.05$). *Conclusion:* The study demonstrated that EEAP effectively suppressed the proinflammatory response mediated by IL-6 and TNF-α, along with the JAK2/STAT3 pathway, in the lungs of rats suffering from cerebral CVA.

Keywords: Ethanol extract of *Anacyclus pyrethrum* root (EEAP); Proinflammatory response; Tyrosine protein kinase 2/signal transducer and activator of transcription 3 (JAK2/STAT3) pathway; Cough-variant asthma (CVA)

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1. Introduction

Cough-variant asthma (CVA) represents a unique form of bronchial asthma, distinguished by persistent coughing in the absence of wheezing manifestations [1]. Over the past few decades, there has been a notable rise in the occurrence of CVA cases globally, and the prevalence of pediatric CVA has progressively risen. Without prompt intervention, certain CVA cases may progress to characteristic asthma symptoms [2]. Currently, there are numerous medications available for the management of CVA [3]. Nevertheless, the adverse effects of these medications have led to a decrease in patient adherence. Therefore, it becomes crucial to identify options with superior therapeutic attributes [4].

Anacyclus pyrethrum, a time-honored Chinese herbal remedy, primarily comprises flavonoids, polysaccharides, volatile oils, tannins, amino acids, and other constituents. It has a positive therapeutic impact [5]. Recent research has demonstrated that EEAP exhibits anti-inflammatory properties and has been proven to mitigate oxidative stress-induced injury [6]. Nevertheless, the molecular mechanism of EEAP efficacy in managing CVA is still a mystery. Within this research paper, we investigated the possible role of EEAP in dampening pulmonary inflammatory reactions in CVA, both *in vivo* and *in vitro*. This paper aimed to establish a theoretical foundation for the practical implementation of EEAP in the management of CVA.

2. Materials and methods

2.1. Materials

2.1.1. Instruments

Gongyi Yuhua Instrument Co., LTD., China, produces the R-2003 rotary evaporator. Gongyi Yuhua Instrument Co., LTD., located in Gongyi, China, offers the R-2003 water bath. China]s Zhengzhou Great Wall Technology Industry and Trade Co., LTD. manufactures the SHB-III recirculating water-type multi-purpose vacuum pump, a versatile device that finds applications in various industries. Shanghai Yiheng Instrument Co., LTD., China, offers the DZF-6090 vacuum drying oven. Full wavelength microplate reader (Biotek, Epoch, USA); Leica Corporation, located in China, produces the DMI4000B fluorescence inverted microscope. RT-PCR device, KS18 biosafety cabinet (Thermo Fisher Scientific); Galaxy 170R CO_2 incubator (New Brunswick Scientific); Electrophoresis equipment (BIO-RAD); Electronic scale (Sartorius, Germany); high-speed refrigerated centrifuge available (3-18 K, Sigma).

2.1.2. Preparation of drug products

The root of *Anacyclus pyrethrum* was sourced from Xinjiang Bencao Tang Co., LTD (batch number: 20210901) and was recognized as the origin of *Anacyclus pyrethrum* (L.) DC. within the family Asteraceae by Shalameti Mati (director and pharmacist at Xinjiang Institute for Food and Drug Control). In the course of the investigation, a suitable quantity of *Anacyclus pyrethrum* (L.) was collected, ground, and the proportion of solid to liquid was determined to be 1:6 (the solution had a concentration of 6 g/mL), and it was obtained through reflux extraction using 65% ethanol at a temperature of 50℃, with the process being repeated three times for a duration of two hours. The sample underwent a series of processing steps, including filtration, integration, concentration via a rotary evaporator, freeze-drying to obtain powder form, and subsequent storage in a refrigerator maintained at 4℃ until its intended application. Acetate prednisone tablets were obtained from Tianjin Tianyao Pharmaceutical Company, Limited (5 mg, Lot: 2109027), while sodium chloride injection was sourced from Sichuan Kelun Pharmaceutical Co., LTD (0.9 g:100 ml, Lot: C22011802).

2.1.3. Reagents

Type II alveolar epithelial cell RLE-6TN can be obtained from Shanghai Binsui Biotechnology Co., LTD. The provided sentence fragment can be rewritten as follows: A quantity of 500,000 cells is present in each T25 culture bottle, with a purity level of no less than 99%, and the batch number is 2022011325. Takara was the source of the Trizol reagent, which came in a 100 ml bottle with the lot number 9909020.Sigma (48T, Lot: 1002638271) supplied ovalbumin (OVA) for this study. Capsaicin was acquired from Sigma Corporation, with a batch number of 102711388. A 10% solution of Freund's adjuvant was obtained from Sigma Corporation (Lot: 10025515070). ELISA kits for interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α) were acquired from Shanghai Biyuntian Biotechnology Co., LTD (96T, Lot: 041814002105000418). The construction of primers for IL-6, JAK2, and STAT3 was carried out by Beijing Sangon Company. The DMEM medium (with 10% FBS) was obtained from Wuhan Punosay Life Technology Co., LTD (125 ml, Lot: WHO11122121SP08), chloroform, 100% ethanol (all analytically pure), and purified water.

2.1.4. Animals

The animal source for this study consists of specific pathogen-free (SPF) male Sprague-Dawley (SD) rats, which were obtained from the Shanghai Slaker Laboratory Animal Center (license number: SCXK: 2022-0004). These rats were aged between 6 and 8 weeks and had a weight range of 220 to 260 g. The rats were kept in the Shanghai Slake Laboratory Animal Center. The surrounding temperature ranged between 20℃ and 24℃, with relative humidity varying between 50% and 60%. The day and night cycle was maintained at a duration of 12 hours, and water was provided freely. All protocols associated with this study were in compliance with the ethical guidelines sanctioned by the hospital Ethics Committee (Approval No. 2022-05-01).

2.2. Methods

2.2.1. Experimental animals and grouping scheme

The rodents were provided unrestricted access to hydration and sustenance [7]. In the OVA model group, rats were sensitized using a 1 mg/mL freshly prepared OVA solution, which consisted of 1% normal saline solution mixed with

10% complete Freund's adjuvant solution. Ten positions were selected on the hind foot, groin, waist, back, and neck of each rat. Each point received an injection of 0.05 mL, while 0.5 mL was intraperitoneally administered simultaneously, resulting in a total volume of 1 mL [8]. Following a week's interval, the rodents were subjected to another feeding session to reinforce the sensitization process, which was then maintained for a duration of 14 days. In the study involving animals, the rodents were allocated into six distinct groups: a control group (administered normal saline), a model group, a positive control group (treated with prednisone, ig, at a dosage of 2.5 mg/kg), the high (640 mg/kg), medium (320 mg/kg), and low (160 mg/kg) EEAP groups were administered through gavage, with the dosage determined based on the alcohol extract's quality. Water was utilized as the dissolving medium, the dosage was established based on the preceding acute toxicity study conducted by EEAP and the findings of relevant research [9]. On the 15th day following the creation of the model, the rodents were housed within a custom-built transparent aerosol chamber. Daily administration of a 1% OVA solution under constant pressure of 400 mmHg (1 mmHg equivalent to 0.133 kPa) for 20 minutes, over a period of 15 days, was employed to induce asthma symptoms. For 30 consecutive days, beginning on day 30, the rats were administered OVA on a daily basis. Over a period of 60 days, the rodents in each cohort were provided with sustenance and subsequently humanely dispatched. Concurrently, lung tissue samples and peripheral blood specimens were obtained. Lung tissue was utilized to assess the presence of inflammatory factors and JAK2/STAT3 signaling molecules, while peripheral blood samples were employed to isolate T lymphocytes.

2.2.2. Culture and grouping of rat lung epithelial type II cells

The epithelial type II cells of rat lung, designated as RLE-6TN, were propagated in a DMEM growth medium supplemented with 10% fetal calf serum (FBS) and antimicrobial agents (100 U/ml penicillin and streptomycin), under an incubator condition maintained at 37°C, 5% CO_2, humid and sterile. Cells in the logarithmic growth stage were chosen and partitioned into two categories: OVA group (subject to 2.5 mg/L OVA stimulation and sensitization) and treatment group (exposed to 640 mg/kg EEAP). Following a duration of 24 hours for the treatment process, both the cells and the culture supernatant were collected. The investigation focused on the detection of JAK2/STAT3 signaling pathway components and the quantification of inflammatory mediators. The previous findings of ELISA analysis of inflammatory mediators in the plasma of CVA rats indicated that EEAP can decrease the levels of IL-6 and TNF-α, and the impact was displayed in a dose-responsive manner. Thus, the dosage plan was established upon the findings of the preceding *in vivo* study, and the higher dosage was chosen as the therapeutic group [10].

2.2.3. Isolation of rat peripheral blood T lymphocytes

In the model group and high EEAP group (640 mg/kg) of section **2.2.1.** (prior to the termination of 60-day-old rats), T-lymphocytes were isolated from freshly collected anticoagulated peripheral blood of rats. Following gradient centrifugation, isolated nucleated cells were suctioned, and T-lymphocytes were procured utilizing nylon hair columns, and T-lymphocytes derived from the model group and the high EEAP group (640 mg/kg) were designated as the CVA and the therapeutic groups respectively. The subgroups were administered the respective doses as outlined above. RPMI 1640 culture medium supplemented with 10% FBS was used to cultivate T cells for a duration of 24 hours at a temperature of 37°C and an atmosphere of 5% CO_2. Cell culture supernatant was obtained for the purpose of identifying

the presence of JAK2/STAT3 signaling pathway components, as well as assessing the concentration of inflammatory mediators.

2.2.4. Enzyme-linked immunosorbent assay

Supernatants were obtained from rat lung tissue homogenates that underwent centrifugation in each group, including the T lymphocyte CVA group and the treatment group, and from rat lung epithelial type II cell OVA group and control group. The concentrations of IL-1β, IL-6, and TNF-α were measured following the experimental protocol outlined in the ELISA kit manual. The absorption level was determined at a wavelength of 450 nm utilizing an enzyme indicator (Biotek Epoch, United States).

2.2.5. Real-time quantitative PCR Analysis (qRT-PCR)

Total RNA extraction was performed by incorporating Trizol reagent (as per the manufacturer's guidelines) into the supernatant obtained from centrifugation of rat lung tissue homogenates from each respective group. The supernatant of the T-lymphocyte CVA group and the therapy cohort, and the supernatant obtained from the rat lung epithelial type II cells in the OVA group and the control group. The RNA was converted into cDNA for quantitative PCR analysis. The parameters for PCR amplification were as follows: Before denaturation, the temperature is set to 95°C for a duration of 1 minute. This is then followed by 30 successive cycles, each consisting of denaturation at 95°C for a duration of 30 seconds, annealing at 58°C for a duration of 5 seconds, and a final extension at 72°C for a duration of 5 seconds. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) served as the standard gene for quantifying mRNA levels of IL-6, JAK2, and STAT3, and every gene in every sample was replicated thrice. The accuracy of PCR outcomes was verified through the dissolution curve method. The difference in Ct values for the target gene and the internal reference is calculated as ΔCt = Ct (target gene) – Ct (internal reference). The difference in ΔCt between the experimental group and the control group is then determined as ΔΔCt = ΔCt (experimental group) – ΔCt (control group). The analysis of target gene expression was conducted using the $2^{-\Delta\Delta Ct}$ method. The qRT-PCR primer sequence (5'-3') was composed of the following components: IL-6 forward primer: TGAGGCGAAGTCTGAATTG, and reverse primer: CTACCCGTTGCCCTATTACT; GAPDH forward: ACCGGATATGCTCTTATTCGG, reverse: CCACTCCTAGATGATTGTGAG; JAK2 forward: TGCTACGTATACGTGTCC, reverse: CACTGGCATCGAGGCGT; STAT3 forward: CTCACTTTGGCACCACA, reverse: TACGCTTCTCGACTTTGATT.

2.3. Statistical analysis

Data analysis was performed using GraphPad Prism 7.0 statistical software, and a statistical diagram was generated. The comparative expression levels of normal distribution measurement data, including IL-1β, IL-6, TNF-α, and JAK2. The expression of p-JAK2 and STAT3 was presented as mean ± standard deviation (SD). A *t*-test was employed to compare T lymphocytes between the CVA group and treatment group, as well as the OVA group and treatment group in the RLE-6TN group. To analyze the given data, a one-way analysis of variance (ANOVA) was employed for conducting multiple comparisons. Furthermore, the LSD-*t* test was utilized for evaluating pairwise comparisons. The examination threshold was set at $\alpha = 0.05$, and a *P*-value less than 0.05 was deemed statistically significant.

3. Results

3.1. Effects of EEAP on inflammatory factors in lung tissue of CVA model rats

In contrast to the control group, the concentrations of IL-1β, IL-6, and TNF-α in the model group exhibited a substantial rise ($P < 0.05$). In contrast to the CVA model group, the prednisone acetate cohort exhibited a higher response in the context of medium and low-dose EEAP administration, notable decreases in IL-1β, IL-6, and TNF-α concentrations were observed ($P < 0.05$). In contrast to the prednisone acetate cohort, the high-dose EEAP group exhibited no notable variations in IL-1β, IL-6, and TNF-α ($P > 0.05$). Both the medium-dose EEAP group and the low-dose EEAP group exhibited substantial rises in IL-1β, IL-6, and TNF-α levels ($P < 0.05$). Furthermore, the concentrations of IL-1β, IL-6, and TNF-α in the high-dose EEAP cohort, the medium-dose EEAP group and the low-dose EEAP group showed an increase in their respective concentrations ($P < 0.05$) as the EEAP concentration decreased. See **Figure 1**.

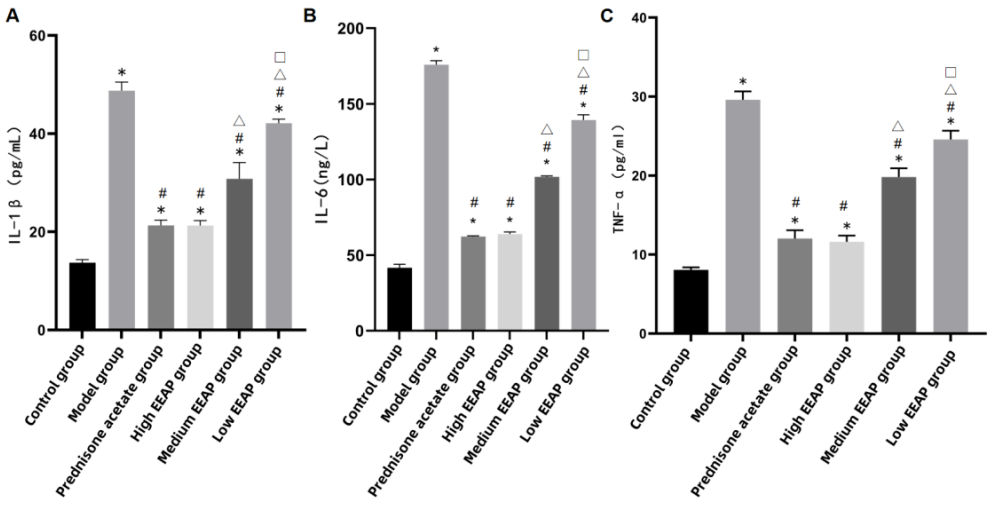

Figure 1. Analysis of the mRNA expression levels of IL-1β, IL-6, and TNF-α in lung tissues across different groups. A series of experiments were conducted using the ELISA technique to assess the expression levels of three key inflammatory markers, namely IL-1β, IL-6, and TNF-α, in lung tissues obtained from different experimental groups. In comparison to the control group, *$P < 0.05$; In contrast to the control group, #$P < 0.05$; Compared with the high EEAP group, △$P < 0.05$. Compared with the middle EEAP group, □$P < 0.05$.

3.2. Effect of EEAP on activation of JAK2/STAT3 signaling pathway in lung tissue of CVA model rats

In contrast to the control group, the model group exhibited significantly elevated concentrations of IL-1β, IL-6, and TNF-α ($P < 0.05$). In contrast to the CVA model group, the concentrations of IL-1β, IL-6, and TNF-α levels were notably reduced in the prednisone acetate cohort, as well as in the high-, medium-, and low-dose EEAP groups ($P < 0.05$). In the high-dose EEAP group, there were no statistically significant differences in the levels of 1β, IL-6, and TNF-α ($P > 0.05$). However, the levels of IL-1β, IL-6, and TNF-α in the medium-dose EEAP group and the low-dose EEAP group were notably elevated ($P < 0.05$). As the concentration of EEAP decreased, the low-dose EEAP group experienced an increase ($P < 0.05$). See **Figure 2**.

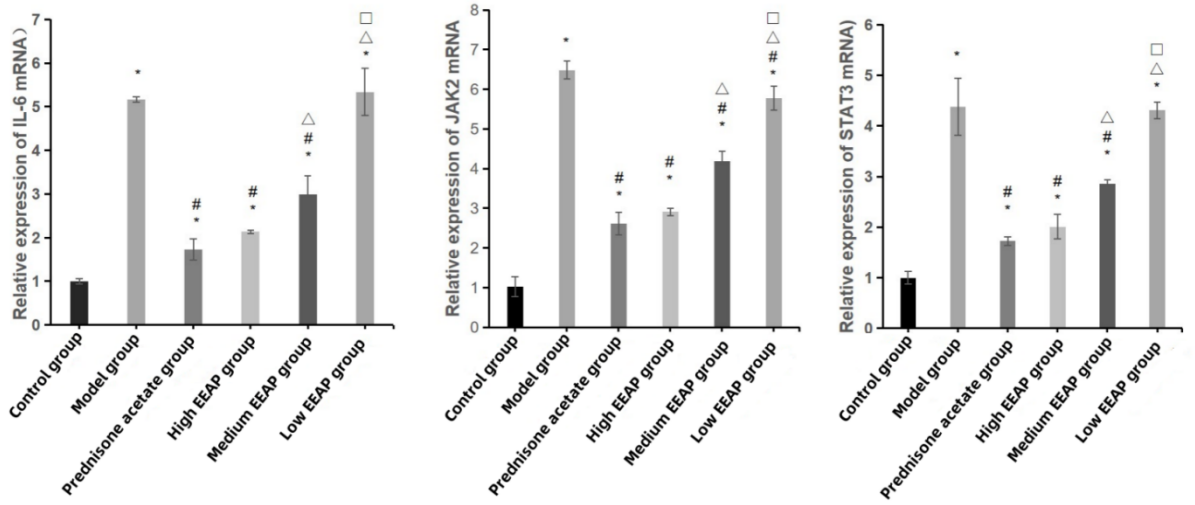

Figure 2. Comparison of mRNA expression levels of IL-6, JAK2, and STAT3 in lung tissues from various experimental groups. (A) ~ (C) Quantitative real-time polymerase chain reaction (qRT-PCR) technique was employed to measure the mRNA expression levels of IL-6, JAK2, and STAT3 in lung tissues derived from various experimental groups. In comparison to the control group, $*P < 0.05$; In contrast to the control group, $\#P < 0.05$; Compared with the high EEAP group, $\triangle P < 0.05$. Compared with the middle EEAP group, $\square P < 0.05$.

3.3. Effect of EEAP on inflammatory factors in OVA-stimulated lung epithelial cells and T lymphocytes of CVA rats

In contrast to the OVA group, the treatment group exhibited substantially reduced concentrations of IL-1β, IL-6, and TNF-α in lung epithelial cells ($P < 0.05$). In contrast to the CVA group, the concentrations of IL-1β, IL-6, and TNF-α in T lymphocytes within the treatment group exhibited a substantial reduction ($P < 0.05$). See **Figure 3**.

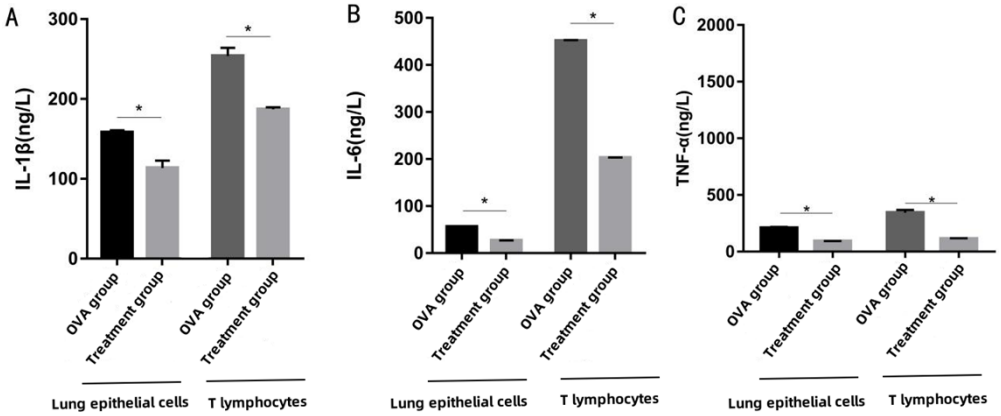

Figure 3. Comparison of the expression levels of IL-1β, IL-6, and TNF-α in the culture medium of cellular samples from various groups. (A) ~ (C) Enzyme-linked immunosorbent assay (ELISA) was employed to measure the expression levels of IL-1β, IL-6, and TNF-α in the various cell groups. $*P < 0.05$.

3.4. Effect of EEAP on JAK2/STAT3 signaling pathway in lung epithelial cells and T lymphocytes of rats with cough-variant asthma

In contrast to the OVA group, mRNA levels of IL-6 in the treatment group were significantly reduced. In the lung epithelial cells of the OVA treatment group, JAK2 and STAT3 levels were notably reduced ($P < 0.05$). In contrast to the CVA group, the mRNA expression of IL-6 in the treatment group was significantly reduced. In the T lymphocytes of the treatment group, both JAK2 and STAT3 exhibited substantial reduction ($P < 0.05$). See **Figure 4**.

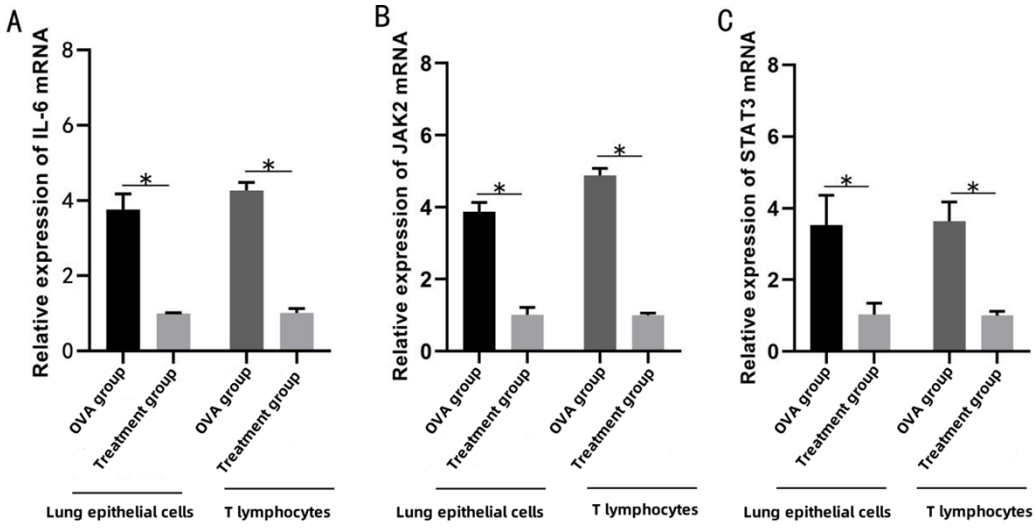

Figure 4. Analysis of mRNA expression levels for IL-6, JAK2, and STAT3 in various cellular groups. Note: (A) ~ (C) Quantitative real-time polymerase chain reaction (qRT-PCR) technique was employed to measure the mRNA expression levels of IL-6, JAK2, and STAT3 in various cell groups. *$P < 0.05$.

4. Discussion and conclusion

CVA is noted to occur independently and is usually defined by a chronic, enduring cough, throat irritation, and the absence of wheezing [11]. Moreover, it has been noted that CVA occurs in individuals with asthma as well. Despite its non-life-threatening nature, CVA exerts a substantial influence on the patient's daily living experience [12]. It is crucial to acknowledge that no particular drugs are currently prescribed for CVA. Therefore, further investigation into the management of CVA is essential. Studies have shown that persistent airway inflammation is a characteristic feature of CVA. The severity of this condition can be measured by evaluating peripheral blood eosinophil counts and conducting anti-hyaluronidase reaction tests. Recent investigations have shown that upon translocation to inflamed tissue and activation, T lymphocytes emit a range of cytokines and inflammatory factors, such as IL-6 and IL-1β. IL-12 is a substance that has the ability to cause inflammation in the airways [13]. Within this research paper, it was verified that EEAP suppressed inflammation in rat lungs and decreased the expression levels of IL-1β and IL-6. TNF-α is found in lung tissue, lung epithelial cells, and T lymphocytes.

In this study, a rat model was employed to explore the mechanism of action of EEAP in cough-variant asthma. *In vivo* studies demonstrated that EEAP showed a tendency towards inflammatory response and immunomodulation in the CVA rat model. IL-6 and TNF-α have been demonstrated to be crucial in the pathogenesis of asthma [14]. IL-6 has been discovered to play a role in controlling inflammatory and immune reactions, Numerous studies have provided evidence indicating that a certain substance impacts cellular functionality by activating the JAK2/STAT3 signaling pathway [15]. Moreover, IL-1β and TNF-α have been recognized as crucial pro-inflammatory mediators capable of eliciting airway inflammation and bronchospasm [16]. Moreover, the investigation demonstrated that EEAP reduced the expression levels of IL-1β, IL-6, and TNF-α in OVA-stimulated lung epithelial cells and T-lymphocytes in the CVA group. This discovery highlights the crucial role of EEAP in the treatment of lung inflammation associated with CVA.

Moreover, our research revealed that EEAP can exert its anti-inflammatory impact on stroke model rats by reducing TNF-α production and limiting inflammatory cell invasion [17]. STAT3 transcription factors, belonging to the STAT family, play a crucial role in numerous biological processes. Numerous studies indicate that STAT3 is crucial in the development of asthma [18]. STAT3 is activated in peripheral blood mononuclear cells and airway smooth muscle tissues of individuals with asthma, and this activation is linked to an increase in cytokines [19]. Blocking STAT3 signaling reduces airway inflammation [20], thereby reversing the adverse impacts of CVA. The JAK/STAT signaling pathway plays a crucial role in the development of asthma. The intracellular signaling pathway known as JAK/STAT facilitates the transmission of chemical messages from the cell membrane to gene promoters localized within the nucleus [21]. JAK/STAT frequently interacts with signaling pathways such as PI3K/AKT, NF-κB, HIF1α, and others, leading to the generation of diverse intricate biological responses [22]. In this study, we demonstrated that EEAP disrupted JAK2 expression and suppressed upstream IL-6 signaling following cell incubation *in vitro*. The evaluation also demonstrated that the control group exhibited markedly increased mRNA expression of IL-6, JAK2, and STAT3 were observed in the EEAP treatment group *in vivo*, where there was a substantial decrease in levels of IL-6, JAK2, and STAT3 mRNA at medium and high doses. This investigation indicated that EEAP may exhibit its anti-inflammatory properties by suppressing the JAK2/STAT3 signaling pathway. In the future, our research team will aim to delve into the impact of lung inflammation on cognitive function and focus on the JAK2/STAT3 signaling pathway specifically.

There are certain limitations in this research. Initially, the investigation was conducted solely using a rat model. Further animal experimentation and clinical trials are required to corroborate the findings of this research. In investigating the induction of JAK2/STAT3 signaling pathway by EEAP in lung tissues of CVA model rats, the expression levels of IL-6 and STAT3 mRNA in the low EEAP group did not exhibit a statistically significant difference when compared to the model group. The cause of this requires further exploration. Secondly, we have not conducted an in-depth analysis of the standalone impacts of EEAP and only examined the impact on inflammatory mediators and signaling pathways in the CVA model. Hence, further investigation and enhancement of the dosage regimen are necessary.

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Disclosure statement

The authors declare no conflict of interest.

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