



Herb pair of Ephedrae Herba-Armeniacae Semen Amarum alleviates airway injury in asthmatic rats

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ABSTRACT

Ethnopharmacological relevance: Ephedrae Herba (EH, *Ephedra sinica* Stapf.) and Armeniacae Semen Amarum (ASA, *Prunus armeniaca* L. var. *ansu* Maxim.) have been used to treat asthma, cold, fever, and cough in China for thousands of years.

Aim of the study: In this study, we aimed to investigate the optimal ratio of EH and ASA compatibility (EAC) to reduce airway injury in asthmatic rats and its possible mechanism.

Methods: Rats were sensitized with a mixture of acetylcholine chloride and histamine bisphosphate 1 h before sensitization by intragastric administration of EAC or dexamethasone or saline for 7 days. Subsequently, the ultrastructure of rat airway epithelial tissue changes, apoptosis of the airway epithelial cells, and the expression of mRNA and protein of EGFR and Bcl-2 were detected.

Results: Transmission electron microscope: EAC (groups C and E) had the most prominent effect on repairing airway epithelial cells' ultrastructural changes in asthmatic rats. TUNEL: dexamethasone and EAC (groups B, C, E and F) inhibited the apoptosis of airway epithelial cells in asthmatic rats ($P < 0.05$). In situ hybridization: EAC (group E) inhibited the overexpression of EGFR and Bcl-2 mRNA ($P < 0.05$). Western Blotting: EAC (groups A, B, C, E and F) inhibited the upregulation of airway epithelial EGFR and Bcl-2 protein expression ($P < 0.01$).

Conclusions: Our findings indicate that EAC can inhibit abnormal changes in airway epithelial structure and apoptosis of airway epithelial cells, thereby alleviating airway injury. In this study, the best combination of EH and ASA to alleviate airway epithelial injury in asthmatic rats was group E (EH: ASA = 8: 4.5).

1. Introduction

Ephedrae Herba (EH) and Armeniacae Semen Amarum (ASA) are traditional Chinese herbal medicines. According to the Chinese Pharmacopoeia (2015), EH is derived from the dry herbaceous stems of *Ephedra sinica* Stapf., and ASA is derived from the dried and peeled mature seeds of *Prunus armeniaca* L. var. *ansu* Maxim.. Both EH and ASA are commonly used to treat asthma, cold, fever, and cough (Han et al.,

2018; Song et al., 2015). Ephedrine and pseudoephedrine contained in EH (Chen et al., 2019) can stimulate heart rate, increase blood pressure, promote bronchiectasis, stimulate the central nervous system, and be used to prevent asthma, nasal congestion, hypotension, and urinary incontinence caused by spinal anesthesia (Ibragic and Sofić, 2015). The ingredients in ASA and ASA extracts have various good pharmacological effects, such as suppressing asthma, allergies, mutagenesis, cancer, and inflammation (Park et al., 2013). The development of traditional

Abbreviations: EH, Ephedrae Herba; ASA, Armeniacae Semen Amarum; EAC, the Compatibility of EH and ASA; DEX, Dexamethasone; ALC, Acetylcholine Chloride; HBP, Histamine Bisphosphate; NS, Normal Saline; ECs, Epithelial Cells; AE, Airway Epithelium; AR, Airway Remodeling; AHR, Airway Hyperresponsiveness.

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Chinese medicine compounds is limited due to its multi-component, multi-target, and unclear action mechanisms. The herb pair is the simplest form of compatibility of traditional Chinese medicine, and the study of the drug pair is the basis and entry point of the entire prescription study (Song et al., 2015; Wang et al., 2012).

Asthma is a complex and variable chronic respiratory disease, which is mainly manifested as bronchial inflammation, airway remodeling (AR), airway hyperresponsiveness (AHR), and reversible airflow obstruction (Fu et al., 2019; Xu et al., 2016). Common symptoms are wheezing, coughing, chest compression, chest tightness, and difficulty breathing. The accumulation of inflammatory cells such as IL-4, IL-5, IL-13 into the trachea will release various reactive free radicals and produce an oxidative microenvironment in the airway, thereby damaging bronchial epithelial cells (ECs) (Mabalirajan et al., 2013). Bronchial epithelial injury is one of the main pathogenic mechanisms of asthma, and the degree of injury is related to the severity of airway hyperresponsiveness (Lin et al., 2015). After the AEs come into contact with the allergen, excessive chemokines and cytokines are released, thereby inducing an acute inflammatory response (Frey et al., 2020). Although there are various treatments for asthma patients, the control of a large number of asthma patients is still very poor, and the use of traditional drugs is a new trend for effective treatment of asthma.

Previously, researchers used the mixed spray-induced asthma model of Acetylcholine Chloride (ALC)-Histamine Bisphosphate (HBP) to evaluate the anti-asthma effects of *Alstonia scholars* (Shang et al., 2010) and *Citrus reticulata* 'Chachi' (Fu et al., 2019) in vivo. Histamine is a major mediator released from mast cells during allergic reactions. It causes airway blockage through smooth muscle contraction, bronchial secretion, and airway mucosal edema (Hirota and Martin, 2013; Yamauchi and Ogasawara, 2019). Histamine's ability to contract the strength of bronchial smooth muscle is comparable to muscarinic M1 receptor agonists. This study intends to use the uniform design table U_6 (6^4) to design the protection and repair effect of different combinations of EH and ASA on AE injury in rats and explore the more potential combination ratio of EH and ASA in the prevention and treatment of asthma in EAC and its potential mechanism.

2. Materials and methods

2.1. Collection and preparation of plant materials

Herb pair (Ephedrae Herba (Mahuang), the dried herbaceous stems of *Ephedra sinica* Stapf.; Armeniacae Semen Amarum (Kuxingren), the dried mature seeds of *Prunus armeniaca* L. var. *ansu* Maxim.) was purchased from Jiangxi Jiangzhong Traditional Chinese Medicine Pieces Co., Ltd. (Nanchang, Jiangxi, China), and was verified by Associate Professor Xiaomei Fu, a pharmaceutical expert at Jiangxi University of Traditional Chinese Medicine. The specimens of medicinal materials are kept in the specimen hall of Jiangxi University of Traditional Chinese Medicine (Certificate No.: 20190624 and 20190628). Different compatibility groups were designed according to uniform design table U_6 (6^4) (Table 1); the dosage of the two plant materials in this table meets the dosage range specified in the Chinese Pharmacopoeia (2015) (Commission, 2015). According to the traditional Chinese medicine decoction method (Yang et al., 2017), EH was soaked in 8 times double-distilled water for 30 min, then boiled at high temperature for 30 min, then added ASA to continue to boil 40 min, the medicinal decoction was filtered with three layers of gauze, and the filtrate was concentrated to $1 \text{ g} \cdot \text{mL}^{-1}$ under standard atmospheric pressure (Fig. 1).

Table 1

Composition of EAC (A ~ F).

EAC groups	A	B	C	D	E	F
<i>Ephedra sinica</i> Stapf.	2	3.5	5	6.5	8	9.5
<i>Prunus armeniaca</i> L. var. <i>Ansu</i> Maxim.	6.3	9	5.4	8.1	4.5	7.2

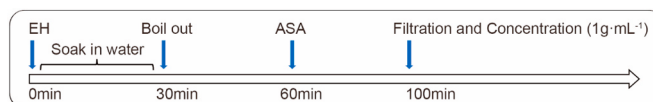


Fig. 1. Preparation of EAC extract.

2.2. Chemicals and reagents

Acetylcholine Chloride (ALC) was purchased from Solarbio Science & Technology (Beijing, China). Histamine Bisphosphate (HBP) was obtained from Sigma-Aldrich (St. Louis, MO, USA). Glutaraldehyde was purchased from Beijing Chemical Works (Beijing, China). Pentobarbital sodium was purchased from Merck Millipore (Billerica, MA, USA). All other chemicals used in this study were reagent grade.

2.3. Animals experiment

Male Sprague-Dawley rats ($200 \pm 20 \text{ g}$ body weight) were purchased from Hunan SJA Laboratory Animal Co., Ltd. (Changsha, Hunan, China). At least three days before the experiment, all rats were individually housed in cages under standard conditions and controlled environment ($22\text{--}24^\circ\text{C}$, relative humidity $50 \pm 10\%$, light/dark cycle 12 h). During the study period, the animals were allowed ad libitum access to food and water. Our research is based on internationally recognized principles for the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources, National Research Council, and published by the National Academy Press [revised 1996]. This research protocol was approved by the Experimental Animal Ethics Committee of Jiangxi University of Traditional Chinese Medicine. All animals have received humane care.

2.4. ALC-HBP induced rats asthma model and treatment

Seventy-two male Sprague-Dawley rats were fed adaptively for 3 days and then were randomly divided into the following 9 groups ($n = 8$): normal control (CTRL), model control (ALC-HBP), dexamethasone (DEX), and EAC (A ~ F) treatment groups. The EAC treatment group's dose was based on the clinically used dose converted according to the rat surface area. The sensitization and treatment of different groups in this study were following Table 2. Asthma model rats were continuously sensitized with a nebulized 2% ALC and 0.4% HBP mixed solution in a self-made nebulizer, CTRL group with normal saline (NS) instead for 7 days, once a day. One hour before the rats were sensitized, the CTRL and ALC-HBP groups were treated with an equal volume of NS (i.g.), and the DEX and A ~ F groups were treated with DEX and EAC (i.g.). One hour after the end of the last nebulization, the rats were anesthetized with pentobarbital sodium (60 mg kg^{-1} , i.p.), then the tracheas were

Table 2

Rats asthma model and treatment.

Group	Treatment ($\text{g} \cdot \text{kg}^{-1}$)			Asthma model	
	NS	DEX	EAC	NS	ALC-HBP
CTRL	+	–	–	+	–
ALC-HBP	+	–	–	–	+
DEX	–	0.0005	–	–	+
EAC A	–	–	10	–	+
B	–	–	10	–	+
C	–	–	10	–	+
D	–	–	10	–	+
E	–	–	10	–	+
F	–	–	10	–	+

Note: "+" means to perform this operation, and "-" means not to do so. NS: Normal Saline; DEX: Dexamethasone; EAC: the compatibility of EH and ASA; CTRL: Normal Control; ALC-HBP: Acetylcholine Chloride-Histamine Bisphosphate.

collected, and finally, the rats were euthanized.

2.5. Transmission electron microscope (TEM)

The tracheas removed from the rat's neck were fixed with 2% glutaraldehyde and placed at 4 °C for storage. After cleaning the tracheas with phosphate buffer saline, fixed them with hungry acid, and then performed dehydration treatment with gradient alcohol. Epon812 was treated with propylene oxide before embedding. The embedded tracheal tissues were cut into ultra-thin slices with an ultra-thin microtome (ULTRA CUT UC6, Leica, Germany) and stained with 3% uranyl acetate-lead citrate. The effect of EAC on the ultrastructure of airway ECs in asthmatic rats was observed under the TECNAI SPIRIT TEM (FEI, USA). For each sample, the visual field 2550× and 8200× were randomly selected to take pictures, and the two professionals were analyzed independently, and the final results were obtained after synthesis.

2.6. TUNEL

The tracheal tissues were cut into small pieces (1.5 cm * 1.5 cm * 0.3 cm) and fixed in 10% formalin for 48 h. The fixed tissues were washed with running water and then subjected to alcohol gradient dehydration, followed by xylene dehydration, and then a series of embedding, sectioning, and staining (DAB). Finally, according to the operating instructions, the terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) system (Roche, Basel, Switzerland) was used to detect and quantify the apoptosis cells. A CX41 microscope (Olympus, Tokyo, Japan) was used for observation and photographing, and a sample image was collected with a Lecia Application Suite imaging system. The apoptosis index is the average of the total number of positive nuclei in the order of 200× from three random observation fields.

2.7. In situ hybridization

In situ hybridization was used to detect the expression of EGFR mRNA and Bcl-2 mRNA. Simply put, the tracheal tissues were fixed in 4% paraformaldehyde for 48 h for cleaning, embedding in paraffin, dehydration, and then cut into 3 μm slices and then dewaxed. The slices were denatured by treatment with protease and then hybridized with StatSpin ThermoBrite™ in situ hybridization instrument (Thermo Fisher Scientific, Waltham, MA, USA), and finally, nuclear staining (DAB). MD100 microscope (Leica, Wetzlar, Germany) was used for imaging. Each image was randomly selected from 5 different visual fields of the mucosa layer and its surrounding parts, and its integrated option density (IOD) was determined using Image-pro-plus image analysis software.

2.8. Western blotting

To determine whether EAC can affect EGFR and Bcl-2 proteins' expression in asthmatic rats' tracheal tissue, we analyzed the changes of these two proteins by western blotting. The total protein in the tracheal tissues was obtained from RIPA lysis buffer, and the protein concentration was determined using a BCA kit (Cowin Bio, Jiangsu, China). The proteins were separated by 7% SDS-PAGE gel electrophoresis, and then electro-transfer to PVDF membrane. TBST buffer containing 5% skimmed milk powder was used for non-specific binding to the membrane. The membranes with anti-EGFR, anti-Bcl-2 antibodies and mouse anti-β-actin monoclonal antibodies were incubated overnight at 4 °C. Next, wash the membrane and incubate with an HRP conjugated secondary antibody. Finally, the gel imaging software (Bio-Rad, Hercules, CA, USA) was used to detect and analyze according to the ECL method (Cowin Bio, Jiangsu, China).

2.9. Statistical analysis

All data were expressed as Means ± SEM. One-way analysis of variance and Dunnett's multiple comparisons test were used to analyze the statistical differences between groups. Data analysis and graphics were performed with GraphPad Prism 8.0 (GraphPad Software Inc., San Diego, CA, USA). $P < 0.05$ was considered statistically significant.

3. Results

3.1. EAC alleviates ultrastructural changes of the trachea

To determine EAC's effect on ultrastructural changes in the tracheal epithelium, we performed TEM examination of tracheal slices of each group of animals. As shown in Fig. 2, CTRL shows continuous pseudostratified columnar ciliated ECs with normal structure, long and dense cilia, narrow cell spaces, and dense cellular connections. Compared with CTRL, ALC-HBP showed apparent structural abnormalities, short and sparse cilia, wide cell gap, and no cellular connections. Some nuclear

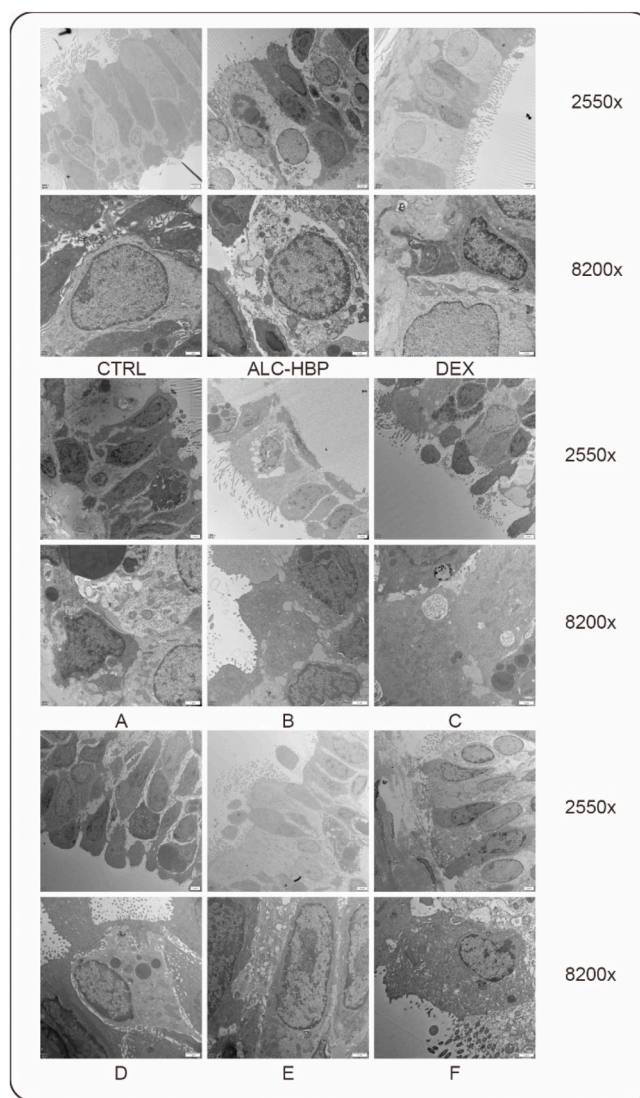


Fig. 2. Transmission electron microscope was used to observe the ultrastructure changes of airway epithelium. EAC can alleviate airway epithelial damage and restore the ultrastructural changes of ECs in asthmatic rats. Representative images of airway epithelial structure status in each group on TEM 2550× and 8200×. CTRL: Normal Control; ALC-HBP: Acetylcholine Chloride-Histamine Bisphosphate; DEX: Dexamethasone; ECs, Epithelial Cells.

membranes shrunk, chromatin increased, darkened, edged, and most of the nucleoli disappeared. The organelles disappeared, vacuoles and apoptosis bodies, and autophagic vesicles were visible in the cytoplasm. DEX and EAC alleviated these changes to a certain extent, and group C and E in the EAC treatment group could significantly alleviate the ultrastructural changes of airway epithelium in asthmatic rats.

3.2. EAC inhibits apoptosis of airway ECs

Those with brown-yellow staining in the nucleus are positive cells, while the normal nucleus is not stained. Compared with CTRL, ALC-HBP significantly increased the apoptosis rate of tissue cells ($P < 0.01$), which is consistent with the increase in the distribution of ALC-HBP apoptosis cells presented in Fig. 3a. It is worth noting that compared with ALC-HBP, EAC and DEX reduced the apoptosis rate to different degrees (Fig. 3b), in which B, C, D, E, and F reduced ($P < 0.05$) apoptosis rate. The inhibitory effect of group B and F is more pronounced ($P < 0.01$).

3.3. EAC inhibits EGFR and Bcl-2 mRNA overexpression

As shown in Fig. 4a, EGFR mRNA appears brownish-yellow. CTRL rat airway epithelial EGFR mRNA expression is minimal. Compared with CTRL, ALC-HBP significantly increased the EGFR mRNA positive rate ($P < 0.01$). The DEX, E, and F groups significantly reduced the EGFR mRNA positive rate ($P < 0.01$, Fig. 4c). As shown in Fig. 4b, Bcl-2 mRNA is brown-yellow, CTRL airway ECs are almost not expressed, and individual smooth muscle cells are expressed. Compared with CTRL, ALC-HBP significantly increased the Bcl-2 mRNA positive rate ($P < 0.01$). The DEX, A, C, and E groups reduced the Bcl-2 mRNA positive rate ($P < 0.05$, Fig. 4d).

3.4. EAC inhibits the upregulation of EGFR and Bcl-2 in an asthmatic rat

The immunoblotting analysis results showed that EAC could inhibit the upregulation of EGFR and Bcl-2 protein expression in the tracheal tissue of asthmatic rats induced by HBP (Fig. 5a, b, c). Compared with CTRL, EGFR and Bcl-2 protein expression in asthmatic rats' trachea in the ALC-HBP group increased significantly ($P < 0.01$). All EAC (A~F) groups could significantly inhibit the expression of EGFR protein in the trachea of asthmatic rats ($P < 0.01$ Fig. 5b). However, for the expression of Bcl-2, except for group D, other groups of DEX, A, B, C, E, and F groups all had inhibitory effects on the expression of Bcl-2 protein. These results further confirmed the effect of EAC on the downregulation of EGFR and Bcl-2 protein expression. (Fig. 5c).

4. Discussion

Respiratory diseases such as asthma bring a heavy health burden and economic burden to asthma patients and society. The long-term use of existing drugs to manage asthma patients produces many adverse reactions, forcing us to explore more economical, effective, and safe therapeutic drugs. Beta receptor agonists and corticosteroids can achieve the purpose of controlling or relieving asthma symptoms, but long-term use of such drugs can cause side effects (Da Silva et al., 2016; Shi et al., 2019) or increase drug resistance (Banuelos et al., 2016; Song et al., 2018). Various plant extracts can be used to treat allergic diseases and their activity has been proven (Fu et al., 2019; Lee et al., 2016; Shang et al., 2010). In this study, we successfully adopted the ALC-HBP induced rat asthma model to evaluate the anti-asthmatic efficacy of EAC systematically.

AE damage can increase mucosal intrinsic and immune cells' susceptibility to antigen, resulting in severe airway hyperresponsiveness (AHR) (Wang et al., 2018). AHR has long been considered a significant feature of asthma and seems to be associated with airway inflammation and AR. AHR is considered regulated by genetics, airway inflammation, airway smooth muscle, cytokines, and other factors (Tian et al., 2019). Eosinophils, lymphocytes, macrophages, neutrophils, and inflammatory mediators, including cytokines, chemokines, histamine, and leukotrienes, are involved in the asthma process (Zhang et al., 2016a). Asthma is related to the Th2 immune response. Th2 cells coordinate the aggregation and activation of mast cells and eosinophils through the release of Th2 cytokines (IL-4, IL-5, IL-13, the primary effector cells of allergic reactions) (Lee et al., 2016). In the pathophysiological process of asthma, the activation of pro-inflammatory cytokines and cell damage keep the epithelium in a state of continuous injury and repair, which may eventually lead to a vicious circle and AR (Wang et al., 2018). The relationship between the ultrastructural changes of AE and AR in asthma includes the difference of eosinophil activity, platelet retention, mucus secretion of ciliated cells, enlargement of adjacent bronchial epithelial membrane space, an increase of airway smooth muscle mitochondria, and ultrastructural changes of bronchial epithelial mitochondria between acute asthma and chronic asthma (Leishangthem et al., 2013). As a barrier, bronchial epithelium plays a vital role in maintaining the homeostasis of the microenvironment. AR is an important part of asthma, which is characterized by epithelial goblet cell proliferation/metaplasia, leading to the increase of bronchial smooth muscle and new blood vessels, as well as interstitial collagen deposition (Davies, 2009). Mitochondrial dysfunction and epithelial damage of airway ECs are closely related to asthma (Mabalarajan et al., 2013). Our research shows that EAC can repair these ultrastructure changes in

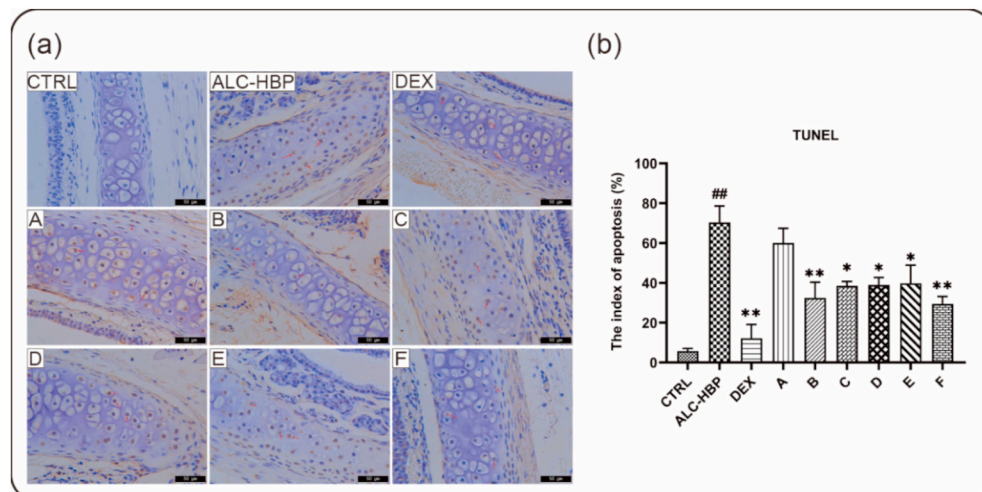


Fig. 3. TUNEL technology detects apoptosis of airway ECs. EAC can inhibit ALC-HBP-induced apoptosis of airway ECs. (a): TUNEL apoptosis measures apoptosis cells in tracheal tissue. (b): The apoptosis index is determined by the number of airway epithelial positive cells. CTRL: Normal Control; ALC-HBP: Acetylcholine Chloride-Histamine Bisphosphate; DEX: Dexamethasone; ECs: Epithelial Cells. The index data of apoptosis expressed as means $\pm$ SEM. ## $P < 0.01$ vs with CTRL, * $P < 0.05$, ** $P < 0.01$ vs with ALC-HBP.

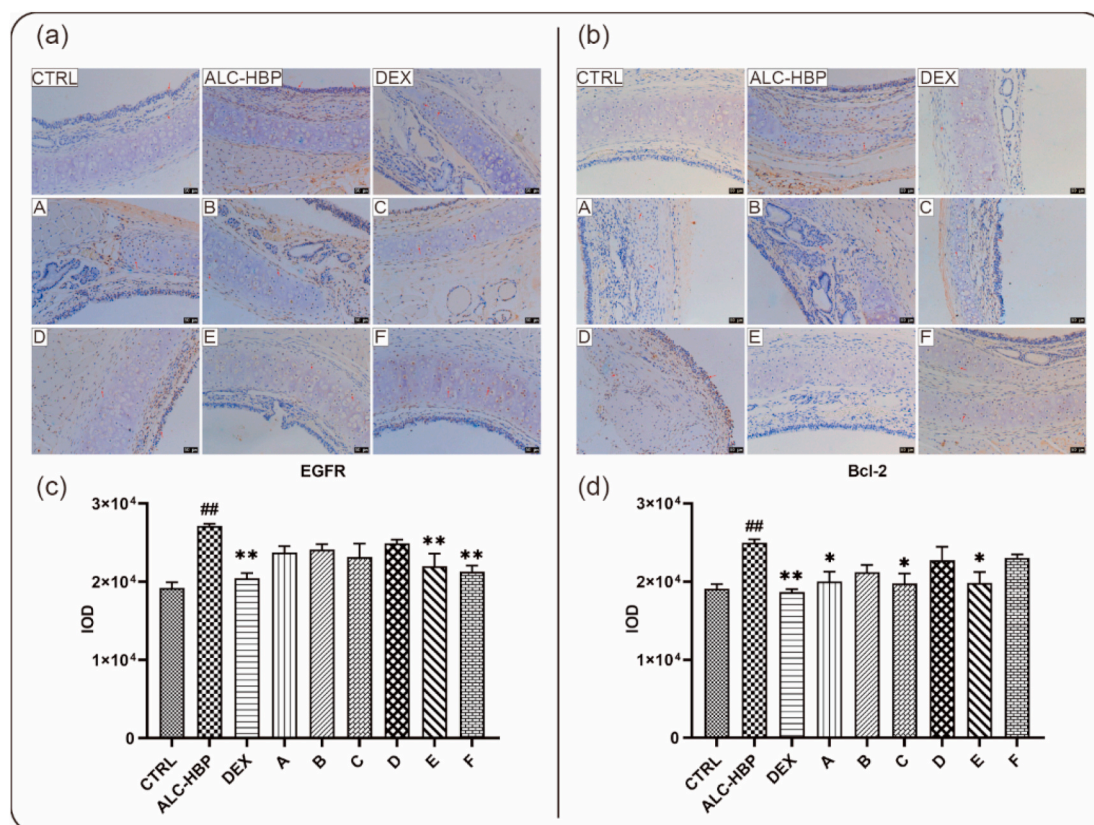


Fig. 4. The expression of EGFR and Bcl-2 mRNA in asthmatic rats' airway epithelium was detected by in situ hybridization. (a) and (c): EGFR mRNA expression and relative expression of EGFR mRNA. (b) and (d): Bcl-2 mRNA expression and relative expression of Bcl-2 mRNA. CTRL: Normal Control; ALC-HBP: Acetylcholine Chloride-Histamine Bisphosphate; DEX: Dexamethasone. Data were expressed as means $\pm$ SEM. ## $P < 0.01$ vs with CTRL, * $P < 0.05$, ** $P < 0.01$ vs with ALC-HBP.

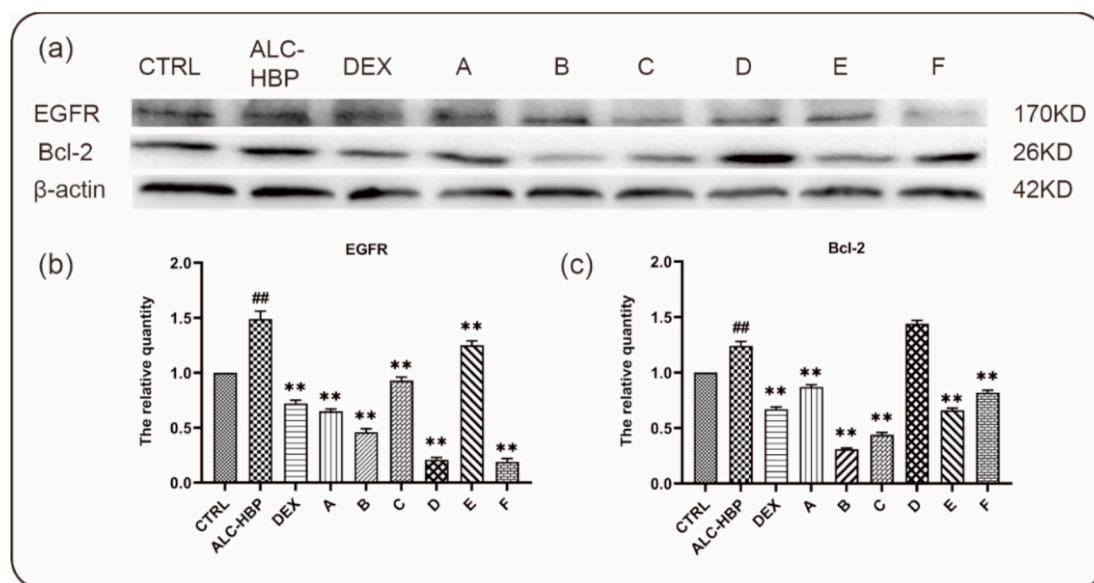


Fig. 5. EAC inhibited EGFR and Bcl-2 protein expression in the tracheal tissue of asthmatic rats treated with ALC-HBP. (a): The expression of EGFR and Bcl-2 protein in the airway epithelium of asthmatic rats were detected by Western Blotting. (b): The relative expression of EGFR protein. (c): Relative expression of Bcl-2 protein. CTRL: Normal Control; ALC-HBP: Acetylcholine Chloride-Histamine Bisphosphate; DEX: Dexamethasone. Data were expressed as means $\pm$ SEM. ## $P < 0.01$ vs with CTRL, * $P < 0.05$, ** $P < 0.01$ vs with ALC-HBP.

airway ECs.

Apoptosis is controlled by the balance of pro-apoptotic and anti-apoptotic proteins. Once this balance is broken, it will cause severe consequences. Transitional apoptosis of airway ECs can cause columnar

ciliated cells in patients with COPD and asthma to shed, ECs aggregation in sputum, increased permeability to allergens, and disorder of connecting molecules in epithelial dissection sites, leading to AR (Lin et al., 2015). ECs play an essential role in coordinating an inflammatory

response; they are damaged after interacting with various environmental factors and cannot be repaired entirely to produce chronic wounds involving tissue damage and abnormal repair, accompanied by the secretion of a series of secondary growth factors that drive structural changes associated with AR (Holgate, 2007). AR is an important part of asthma and is related to the severity of asthma. Our results show that EAC can inhibit the apoptosis of airway ECs, thus maintaining the dynamic balance of the mechanism of promoting apoptosis and anti-apoptosis.

EGFR is a tyrosine kinase receptor widely distributed in the human cell membrane, enhancing the immunoreactivity of bronchial smooth muscle in patients with asthma. EGFR is thought to regulate the progression of asthma through the PI3K pathway's function and its effects on cell differentiation, T, or B cells (Dong et al., 2018). EGFR inhibitors can reduce collagen deposition and mucus accumulation after antigen treatment, suggesting that EGFR may be involved in AR (Zhang et al., 2016b). Goblet cell metaplasia is related to EGFR activation, and ROS-induced goblet cell metaplasia in normal human ECs is related to hyaluronic acid depolymerization and the treatment of EGF by tissue kallikrein after EGFR signal transduction (Casalino-Matsuda et al., 2006). Goblet cell differentiation/proliferation is related to the increase of MUC5AC. Inhibiting the activation of the EGFR pathway can reduce the expression of MUC5AC, reduce airway mucus, and relieve airway damage (Yang et al., 2012). Activation of EGFR in the AE of asthmatic patients can cause AR, mucinous metaplasia, and neutrophil inflammation (Habibovic et al., 2016). Gefitinib, a selective EGFR inhibitor, significantly reduced airway smooth muscle proliferation/remodeling, eosinophil aggregation, AHR, ECs, and goblet cell proliferation. (El-Hashim et al., 2019). IL-33 is an essential mediator of allergic asthma, and its expression is increased in nasal ECs of asthmatic patients (Hristova et al., 2016). IL-33 secretion is mediated by the oxidative modification of cysteine residues inside EGFR (Danyal et al., 2016), and our study found that EAC can inhibit HBP-induced upregulation of EGFR.

The Bcl-2 gene is a proto-oncogene that suppresses apoptosis and plays an essential role in the molecular regulation of apoptosis and has a close relationship with asthma occurrence and development. High expression of the Bcl-2 gene will inhibit the apoptosis of eosinophils, causing many eosinophils to infiltrate into peripheral blood, lung tissue, and BALF (Yan et al., 2018). The eosinophils release inflammatory mediators, causing damage to bronchial ECs and inducing asthma (Wang et al., 2018). Overexpression of Bcl-2 can also significantly increase neutrophils in BALF (Geng et al., 2018). The high secretion of mucus in inflammatory and obstructive airway diseases is related to the metaplasia of airway goblet cells, and the continuous increase in the number of goblet cells is related to the upregulation of Bcl-2 in airway ECs (Casalino-Matsuda et al., 2006). Beauveria plays a role in preventing asthma by inhibiting the upregulation of Bcl-2 (Zhang et al., 2016a). We checked the expression of Bcl-2 by Western Blotting analysis and found that the level of Bcl-2 protein in ALC-HBP was significantly higher than that in the CTRL group and EAC may inhibit the Bcl-2 gene and protein in the tracheal tissue of asthma rats Expression, promote eosinophil apoptosis and reduce airway inflammation.

EAC inhibits apoptosis of airway ECs, possibly because it directly targets EGFR and/or Bcl-2, affecting its downstream signaling pathways. Although the amygdalin contained in ASA is toxic, the hydrocyanic acid produced by its hydrolysis can have a specific inhibitory effect on the respiratory center, making the respiratory movement quieter and achieving the antitussive and anti-asthmatic effect (Xu et al., 2017). Based on traditional Chinese medicine theory, the combined application of EH and ASA has a synergistic effect and reduces toxic and side effects (Wang et al., 2012). It may be because EH can detoxify ASA by promoting its stereoselective metabolism (Song et al., 2016), which has special significance for the modernization of traditional Chinese medicine.

5. Conclusion

To sum up, the treatment of ALC-HBP induced asthma by EAC can reduce the trachea's ultrastructural changes and inhibit the apoptosis of airway ECs, thus relieving or repairing AR. EAC may down-regulate EGFR and Bcl-2 to reduce the inflammatory reaction, alleviate AE damage, inhibit AR, and repair airway physiological structure to prevent and treat asthma effectively. Our study found that group E (EH: ASA = 8:4.5) had a better effect on alleviating/inhibiting these changes. Our study can provide a basis for the clinical application of EH and ASA. Due to the limited conditions, we still need more perfect experiments to clarify the central components that play a significant role in the mechanism of the combination of EH and ASA in preventing and treating asthma.

Author's contributions

Jia-xin Ma and Kai-fang Zhou carried out and analyzed the experiment. Jia-xin Ma wrote a manuscript. Xiong Xiao assisted in designing the experiment and provided technical advice on the experiment with Wen-jun Guo, Gang Huang, and Li Yang. Bo Ao, Ying Zhang, Ting Lei, and Xue-cheng Fan are responsible for preparing experimental materials and collecting experimental data. Wen-hong Li designed and supervised the study and reviewed and proofread the manuscript. All authors approved the final manuscript.

Declaration of competing interest

All authors declare no conflicts of interest.

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