



Phytochemical profiles and biological activity evaluation of *Zanthoxylum bungeanum* Maxim seed against asthma in murine models



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ABSTRACT

Ethnopharmacological relevance: *Zanthoxylum bungeanum* Maxim seed (ZBMS) has been used in Traditional Chinese Medicine (TCM) as an ingredient of polyherbal formulations for the treatment of inflammation and asthma. The aim of this study was to analyze the major composition and to evaluate the anti-asthma activity of ZBMS.

Materials and methods: Some murine models including acetylcholine/histamine-induced asthma, ovalbumin-induced airway inflammation, ear edema and toe swelling measurement, citric acid-induced cough, and anti-stress abilities were investigated to fully study the anti-asthma activity of ZBMS. GC chromatography was also performed to analyze the major fatty acid composition of ZBMS.

Results: The results demonstrated that the major fatty acid composition of ZBMS includes oleic acid (20.15%), linoleic acid (26.54%), and α -linolenic acid (30.57%), which was the leading component of ZBMS, and that the total fatty acid content of ZBMS was 77.27%. The murine models demonstrated that ZBMS displays a protective effect on guinea pig sensitization, a dose-dependent inhibition of the increases in R_L and decreases in C_{dyn} , which resulted in the relief of auricle edema and toe swelling in mice and anti-stress activity.

Conclusion: Our results validate the traditional use of ZBMS for the treatment of asthma and other inflammatory joint disorders, and suggest that ZBMS has potential as a new therapeutic agent for asthma management.

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1. Introduction

Asthma is a major public health problem worldwide, and its morbidity and mortality have increased in the past several decades, particularly in Western and industrialized countries (Li et al., 2000; Zhang et al., 2010). It is estimated that as many as 300 million people of all ages, races, and ethnicities suffer from asthma, and 250,000 asthma-related deaths occur each year. In developed countries, asthma continues to be a great burden to healthcare resources (Adams and Saglani, 2013). Asthma is also the

most common chronic condition in childhood, specifically in poor communities (Page et al., 2013). According to the findings from the National Health Interview Survey from 2001 to 2010 (Oraka et al., 2013), the current asthma cases in American children (9.0%) are slightly more prevalent than adult cases (7.2%), which makes asthma the leading childhood chronic disease (Li, 2011). The reasons for the rising prevalence are often linked to increasing urbanization and environmental and genetic factors (Masoli et al., 2004; Chatzimichail et al., 2013).

The approaches for asthma management stress the importance of controlling the disease. To improve asthma management, the Global Initiative for Asthma (GINA) guidelines recommend evaluating the control of asthma through day- and night-time awakenings, activity limitation, lung function and exacerbations (Vernon et al., 2012). The current standard for asthma management is

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primarily airway inflammation suppression with inhaled corticosteroids (ICSs) and bronchoconstriction relief with bronchodilators (Li, 2011). Of these treatments, ICSs are the most potent, non-specific anti-inflammatory agents and substantially improve lung function in asthmatics (Li et al., 2000). However, prolonged ICS use, especially at higher doses, has been associated with an increased risk of both systemic and local side effects, particularly in children with osteoporosis (Lipworth, 1999; Li, 2011) and esophageal candidiasis (Fukushima et al., 2003). Consequently, novel approaches for asthma treatment are needed.

Complementary or alternative medicine (CAM) approaches are attracting increasing interest in Western countries. It is estimated that up to 30% of adults and 60% of US children are currently using CAM for asthma management (Li and Brown, 2009). Traditional Chinese Medicine (TCM), the major component of CAM therapies, is one of the oldest medical practices in the world and has a long history of use for the prevention and treatment of diseases in China and other Asian countries such as Japan and Korea. Chinese herbal medicines are a major component of TCM practice and are commonly used in Chinese hospitals as either monotherapies or in complex formulations based on the unique system theory of TCM (Li, 2007). Asthma was recognized in ancient China and a number of classical formulations have been established for use in TCM practice such as MSSM-002 (Li et al., 2000), mMMDT (Hsu et al., 2005), STA-1 (Chang et al., 2006) and ASHMI (Zhang et al., 2010). Similarly, some herbal extracts and individual components were also evaluated for their possible therapeutic effect in asthma based on murine models (Park et al., 2002; Brattström et al., 2010; Bao et al., 2011).

In traditional Chinese herbal medicine books, *Zanthoxylum bungeanum* Maxim (ZBM) has been described as effective for the management of inflammatory diseases and has been used traditionally for asthma relief (Wang et al., 2005). ZBM, which belongs to the *Zanthoxylum* genus of the Rutaceae family, is now widely distributed in most parts of China and some Southeast Asian countries. The reddish brown ripe seed, which is an important spice and a side product of *Zanthoxylum bungeanum*, is widely used as a prominent seasoning flavor in Chinese cuisine and in medicines (Yang et al., 2013, 2008). ZBMSs are rich in polyunsaturated fatty acids (PUFAs) and mainly contains palmitic acid, stearic acid, palmitoleic acid, oleic acid, linoleic acid, and linolenic acid. Of these, linolenic acid ($n-3$ PUFA) and linoleic acid account for 60% of total fatty acid content, and the total unsaturated fatty acid content is nearly 80% (Xiao et al., 2012). $n-3$ PUFA can reportedly compete with arachidonic acid (AA) as substrates to form pro-inflammatory mediators such as leukotrienes (LTs) and prostaglandins (PGs) and can affect the neutrophil and monocyte production of chemotactic response mediators and cytokines (Timothy, 2005). Moreover, $n-3$ PUFAs have anti-inflammatory properties that may modulate the immune response, and these fatty acids have few side effects and may be mildly beneficial for the treatment of established allergic diseases such as asthma and atopic dermatitis (Prescott and Calder, 2004).

Accordingly, the high $n-3$ PUFA content in ZBMS is regarded to be the key factor that may contribute to its anti-asthmatic effect. Numerous studies have investigated the relationships between fatty acid intake and asthma development both in animal models and children (Okamoto et al., 2000; Zhou et al., 2005; Deng et al., 2007; Miyake et al., 2008). Thus, ZBMS has the potential to be a novel therapeutic approach for asthma management.

Thus, in the present study, we describe the ZBMS fatty acid composition and performed pharmacodynamic experiments to assess its anti-asthmatic, anti-inflammatory, antitubercular, and anti-stress effects to determine its potential for use in the development of a new anti-asthmatic agent.

2. Materials and methods

2.1. Animals

Hartley guinea pigs with a body weight in the range of 350 to 500 g and Sprague-Dawley rats of either sex with weights between 180 and 240 g were provided by the Zhejiang Experimental Animal Center (Zhejiang, China) and China Medical University (Shenyang, China), respectively. The study was officially approved by the Ministry of Health of P.R. China. Outbred 6–7-week-old KunMing mice (KM mice) of either sex weighing 20 to 25 g were obtained from the Laboratory Animal Center of Zhejiang University, China. All of the animals were randomized and transferred to cages containing sawdust bedding (six mice per cage) under environmentally controlled conditions, and food and water were provided ad libitum. Before conducting any studies, the animals were acclimated for one week, and animal use was in accordance with the institutional guidelines.

2.2. Drugs and chemicals

The ZBMSs were collected from the Shanxi province of China in July 2011 and were identified by Professor Qishi Sun of Shenyang Pharmaceutical University. A voucher specimen (No. 20110721) was deposited in the Herbarium of Shenyang Pharmaceutical University (Shenyang, People's Republic of China). The following drugs and chemicals were used: salbutamol (SAL, Jiangsu Yancheng Pharmaceutical Factory), urethane (Shanghai Chemical Co.), pentobarbital sodium (Union, Belgium), ovalbumin (OVA, Reagent grades II and V, Sigma, USA), citric acid (CA, Shanghai No. 1 Chemical Co.), codeine phosphate (CP, Qinghai Pharmaceutical Factory), ephedrine (Shenyang No. 4 People's Hospital), sodium hydroxide (Zhenjiang Xiaoshan Chemical Co.) and xiaochuangning (XCN, Henan Shedian Pharmaceutical Co.). All of the other chemicals and reagents were of the highest commercial grade available.

XCN, which selected as a reference standard, is a tablet and mainly composed by eight Chinese traditional medicines including *Bile arisaema*, *Datura flower*, *Ephedra*, *Plaster stone*, *Polygala tenuifolia*, *Radix glycyrrhizae*, *Radix pseudostellariae*, and *Schisandra chinensis* with different proportions. In China, XCN has been marketed and most used for the treatment of bronchial asthma.

In traditional Chinese medical books, the doses of ZBMS used for the treatment of asthma were described as 0.15 to 0.3 g/kg for adults. Additionally, Chen et al. (1987), Pan et al. (1997) reported the effect of ZBMS oil on asthma management in clinic, and the doses used for adults were in the range of 1.8 to 3.0 g per day which resulted in a marked antiasthma action. Thus, in our paper the doses of ZBMS used for animal experiments were designed in the range of 0.25 to 3.0 g/kg.

2.3. Fatty acid preparation

Dry ZBMS (20 g) was subjected to extraction with 100 mL of *n*-hexane for 5 h using a Soxhlet apparatus (Shanghai Yarongshenhua Instrument factory, Shanghai, China). The *n*-hexane components were filtered and concentrated in vacuo. Then, 70 mg of oil was weighed in a 5-mL volumetric flask and saponified by adding 1.0 mL of 0.5 N NaOH-methanol to the mixture and heating in a water bath at 60 °C until the fat globules went into solution. The mixtures were heated for 15 min after the addition of 1.0 mL of 11% BF_3/MeOH (Sigma Co.). After cooling, saturated NaCl solution was added, and the mixtures were extracted with 3 mL of petroleum ether (PE), which was later, evaporated through incubation in a water bath at 60 °C. The fatty acid profiles were determined as fatty acid methyl esters (FAMES). The FAMES were dissolved in *n*-hexane for injection and analyzed

by GC. In addition, a 1% Tween-80 solution was used to make ZBMS oil as a suspension before it was used for animal experiments.

2.4. Analysis conditions

A GC-8A Shimadzu instrument (Shimadzu, Japan) was used for the fatty acid measurements. The fused-silica gas chromatographic capillary column was a Chromosorb W (AW-DMCS column, $\phi 3\text{ mm} \times 2\text{ m}$). The injector temperature was 162°C , and the column temperature was increased from 162°C to 190°C at a rate of $3^\circ\text{C}/\text{min}$. The split ratio was 10:1; the detector temperature was 250°C ; the injection volume was $1.0\text{ }\mu\text{L}$.

2.5. In vivo experiments

2.5.1. Murine model of acetylcholine/histamine-induced asthma

For drug-induced asthma evaluation, guinea pigs were pre-screened for their latent period of acetylcholine and histamine-induced asthma. Conscious guinea pigs were placed in a 4-L inhalation chamber equipped with a jet nebulizer (BARI Co. Ltd; Germany). The nebulizer was connected to the inhalation chamber air entry, and 2% acetyl choline and 0.1% histamine solution was delivered for a period of 20 s. The asthma-inducing period was counted by a trained observer, and animals that recorded time ranges greater than 120 s were screened through the next test.

The experimental guinea pigs ($n=60$) were divided into six groups of ten animals each. The reference drugs (6 mg/kg ephedrine and 1.0 g/kg XCN), three different ZBMS doses (0.25, 0.5, and 1.0 g/kg) and the vehicle (1% Tween-80 solution) were administered intragastrically to the tested animals. After 1 h, the tested animals were treated using the same methods described above.

2.5.2. Protective effects on asthma symptoms in the guinea pig allergen sensitization model

The six-week-old Hartley guinea pigs were randomly divided into five groups (10 per group), and asthma was induced in four of the groups. To sensitize the mice, $100\text{ }\mu\text{g}$ of ovalbumin (Grade V, Sigma Chemical Co., St. Louis, MO, USA) was adsorbed on a 2-mg aluminum hydroxide gel as an adjuvant and subcutaneously injected on days 0 and 14. On day 14, a $50\text{-}\mu\text{g}$ ovalbumin and 2 mg of aluminum hydroxide booster was intraperitoneally injected 1 h after the sensitized mice were treated with a low, medium, and high dose of ZBMS (0.5, 1.5, and 3.0 g/kg, respectively). The control mice were injected with an aluminum hydroxide gel following the same protocol.

2.5.3. R_L and C_{dyn} measurements in sensitized guinea pigs

The guinea pigs were sensitized through a single intraperitoneal ovalbumin injection (1.0 mL of 10 mg OVA mixed with 100 mg of aluminum hydroxide in saline). The sensitized guinea pigs were anesthetized with pentobarbital sodium (mg/kg) and placed in a whole body plethysmograph to measure the R_L and C_{dyn} . The sensitized mice were administered the reference drug salbutamol (4 mg/kg), ZBMS (0.25, 0.5, and 1.0 g/kg) and vehicle (1% Tween-80 solution, 4 mL/kg) 1 h before being exposed to ovalbumin aerosol for 1 min. After antigen challenge, the R_L and C_{dyn} values were monitored for a period of 30 min and the maximal changes from baseline for each parameter were continuously recorded using a Medlab (Nanjing Biotech instruments; Nanjing, China).

2.5.4. In vivo cough measurements

For the cough induction test, the guinea pigs were pre-screened to determine their cough response to citric acid. Conscious guinea

pigs were placed in a 4-L inhalation chamber equipped with an YC-Y800 ultrasonic nebulizer (Beijing Yadu Science and Technology Co. Ltd. Beijing, China). The nebulizer was connected to the inhalation chamber air entry, and 17.5% citric acid solution was delivered at a rate of 3 mL/min for 30 s; the chamber ventilator was turned off during this period. The cough incubation and cough number were counted by a trained observer during the next 5 min. The animals that coughed more than 10 times were selected for further assessment the following day.

The screened guinea pigs ($n=60$) were divided into six groups of ten animals each. The reference drugs (7 mg/kg CP and 1.0 g/kg XCN), ZBMS at three different doses: 0.25, 0.5, and 1.0 g/kg and the vehicle (1% Tween-80) were injected intraperitoneally. One hour after treatment, the animals were administered an additional dose according to the methods described above.

2.6. Anti-inflammatory assessment

2.6.1. Ear edema measurement

The anti-inflammatory properties of ZBMS were investigated using the murine dimethylbenzene-induced auricle edema model. KM mice were divided into six groups of ten animals each. The reference drugs (0.1 g/kg aspirin and 2.0 g/kg XCN), ZBMS at three different doses: 0.5, 1.0 and 2.0 g/kg and vehicle (1% Tween-80) were administered intragastrically daily for a period of three days. Dimethylbenzene (0.05 mL) was spread on both sides of the right ear of each mouse 0.5 h after the final drug administration. The mice were executed 15 min later. Both mouse earlaps were amputated and weighed. The weight difference between the two earlaps was calculated.

2.6.2. Carrageenan-induced rat model of toe swelling

Sprague-Dawley rats ($n=60$) were divided into six groups of ten animals each and were fasted but allowed free access to water for 12 h. The reference drugs (0.2 g/kg aspirin and 1.2 g/kg XCN), ZBMS at three different doses: 0.5, 1.0 and 2.0 g/kg and vehicle (1% Tween-80) were administered intragastrically. Thirty minutes after treatment, 0.1 mL of 1% carrageenan was spread on both sides of the right toes through hypodermic injection to induce inflammation. The toe volume was calculated through the glass container method to measure the tumorous toe size.

2.7. Mouse model of the anti-stress effect of ZBMS

2.7.1. Mouse model of the anti-fatigue effect

The effect of ZBMS on mouse fatigue was measured through a weight-loaded swimming test. This procedure has been described according to Zhang et al., (2006). Briefly, 1 h after the last intragastric administration, the mice were placed in a swimming pool filled with fresh water at $20 \pm 1^\circ\text{C}$ and approximately 14 cm in depth to ensure that the mice could not touch the bottom with their feet. A lead block (2.0 g) was loaded onto the tail root of the mice. Exhaustion was determined by observing the swim period, which was the time that the mouse spent floating in the water struggling and moving until exhausting its strength and drowning.

2.7.2. Mouse model of anoxia

The seven-week-old animals were randomly assigned to five experimental groups of ten animals each. The reference drug (2.0 g/kg XCN), ZBMS at three different doses: 0.5, 1.0 and 2.0 g/kg and vehicle (1% Tween-80) were administered intragastrically daily during a period of seven days. One hour after the last administration, each mouse was placed inside a sealed plexiglass chamber with 15 g of lime-soda. The survival duration was assessed to determine the anti-anoxia ability of ZBMS.

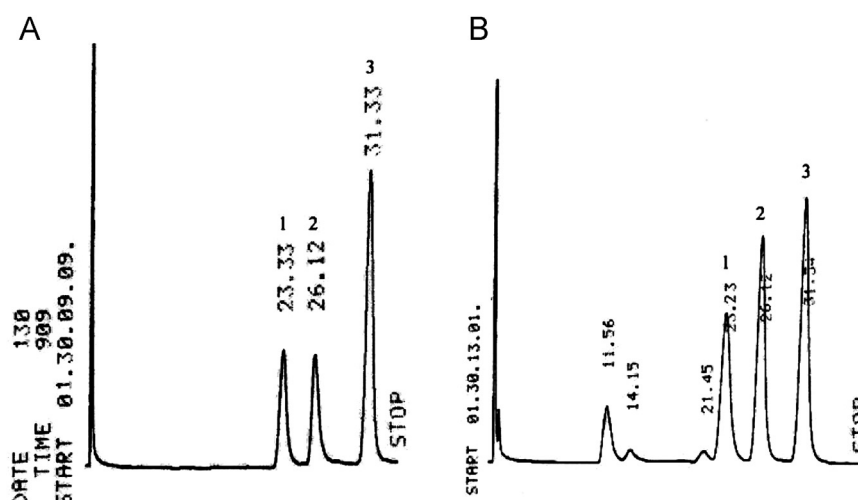


Fig. 1. GA chromatography of mixed standards (A) and fatty acids in ZBMS (B): (1) oleic acid; (2) linoleic acid; (3) α-linolenic acid.

Table 1

Major fatty acids in *Zanthoxylum bungeanum* Maxim seeds.

Fatty acids	Content (%)	Linear curves
Oleic acid	20.15 ± 1.22	Y=53.00546X+0.00367; r=0.9997
Linoleic acid	26.54 ± 1.61	Y=51.57077X+0.00153; r=0.9999
α-Linolenic acid	30.57 ± 1.90	Y=53.37407X+0.00336; r=0.9997
Total fatty acids	77.2 ± 4.71	

Data were presented as mean ± S.D. (n=3).

2.8. Statistical analysis

Data are expressed as mean ± S.D. or mean ± S.E.M. The Statistical evaluations were performed using the Sigma Stat software. ANOVA and Student's *t*-tests were used for calculating differences between each group, and a *P* < 0.05 was considered to be statistically significant.

3. Results and discussion

3.1. Fatty acids in *Zanthoxylum bungeanum* Maxim seeds

Table 1 demonstrates the most abundant acids in ZBMS, and the relative amounts are expressed as the percentage area in relation to the total acid area. The quantification of these compounds was performed by comparing the retention times with those of the standards.

As described in Fig. 1 and Table 1, the most abundant fatty acids in ZBMS in decreasing order of concentration were α-linolenic acid, linoleic acid and oleic acid, which is in agreement with the data presented in the scientific literature (Xiao et al., 2012). For repeatability and reproducibility, three samples were selected for quantitative analysis. The results demonstrated that the total fatty acids in ZBMS accounted for 77%. Of these, α-linolenic acid (*n*-3 PUFA) was the leading component with a percent content of 30.57%. Accordingly, the high PUFA content in ZBMS is closely associated with the traditional use of ZBMS for asthma treatment.

Research on the anti-inflammatory properties of *n*-3 fatty acids has gained momentum in recent years. *n*-3 PUFAs suppress *n*-6 leukotriene (LT) synthesis, which is the major chemical mediator in asthma, by competing for and inhibiting the arachidonic acid (AA) metabolism. AAs are released from membrane phospholipids during cell activation through the 5-lipoxygenase

Table 2

ZBMS-mediated protection against acetylcholine/histamine-induced asthma symptoms.

Drugs	n	Dose (g/kg)	Latent period of induced-asthma (s)	
			Before	After
Vehicle	10	4.0 mL/kg	73.2 ± 12.1	75.1 ± 12.7
Ephedrine	10	6.0 mg/kg	72.8 ± 16.7	258.7 ± 97.1 ^{*,##}
XCN	10	1.0	71.4 ± 14.5	166.7 ± 82.4 ^{*,##}
ZBMS	10	1.0	74.5 ± 13.5	180.2 ± 72.6 ^{*,##}
ZBMS	10	0.5	74.2 ± 11.8	162.6 ± 68.6 ^{*,##}
ZBMS	10	0.25	72.3 ± 11.7	119.9 ± 52.8 ^{*,#}

Data were shown as mean ± S.D.

* *P* < 0.05, statistically significant compared with animals before drug treatment.

** *P* < 0.01, statistically significant compared with animals before drug treatment.

P < 0.05, statistically significant compared with the model.

P < 0.01, statistically significant compared with the model.

pathway (Okamoto et al., 2000). *n*-3 fatty acids reportedly have anti-inflammatory properties by modulating prostaglandin metabolism and suppressing LTB₄, which is generated from linoleic acid through AA, as well as suppressing the capacity of monocytes to synthesize interleukin-1 (Simopoulos, 2002). The immunomodulatory benefits of *n*-3 PUFAs may be greater during the critical stages of early immune development before allergic responses are established (Prescott and Calder, 2004). These previous studies suggest that *n*-3 PUFAs are beneficially associated with lung function, and asthma prevalence, hence ZBMS, which is mainly composed of *n*-3 fatty acids, was further investigated to determine its anti-asthma activities in the following assays.

3.2. ZBMS-induced protection against acetylcholine/histamine-induced asthma symptoms

In the acetylcholine/histamine-induced asthma model, the latency period of the induced-asthma (LPIA) in the model animals was significantly increased compared with animals that were treated with vehicle only (1% Tween-80, Table 2). ZBMS treatment also dose-dependently prolonged the LPIA, and the guinea pigs treated with 0.5 g/kg ZBMS exhibited LPIA counts similar to those of the XCN-treated controls and lower counts than the EP-treated controls.

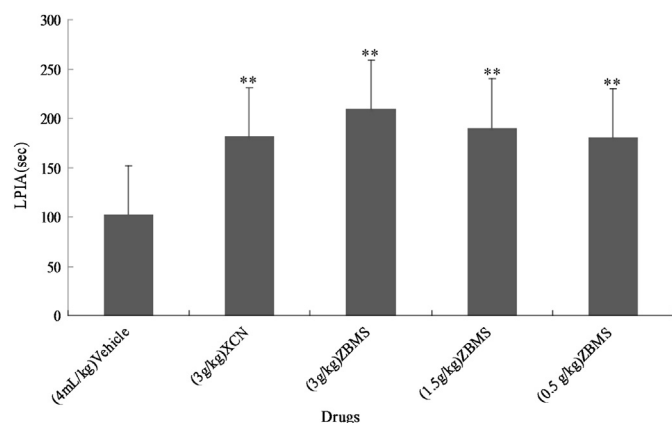


Fig. 2. ZBMS-mediated effects on the latency period of induced asthma. Data were expressed as mean $\pm$ S.D. Statistically significant differences at $^{***}P < 0.01$, as compared with vehicle group.

Table 3

Anti-inflammatory activity of ZBMS treatment on auricle edema in mice.

Drugs	Dose (g/kg)	Weight difference	Inhibition (%)
Vehicle	10 mL/kg	7.56 $\pm$ 1.10	
Aspirin	0.1	1.85 $\pm$ 0.54	76.53 ^{***}
XCN	2.0	2.53 $\pm$ 0.69	66.54 ^{**}
ZBMS	2.0	2.13 $\pm$ 0.59	71.83 ^{***}
ZBMS	1.0	2.24 $\pm$ 0.61	70.43 ^{**}
ZBMS	0.5	2.38 $\pm$ 0.60	68.52 ^{**}

Data were shown as Mean $\pm$ S.D. ($n = 10$).

^{**} $P < 0.01$, statistically significant compared with the model.

^{***} $P < 0.001$, statistically significant compared with the model.

different ZBMS doses (0.25, 0.5, and 1.0 g/kg) markedly prolonged the LPIA of the guinea pigs. In particular, the LPIA time found for the high dose ZBMS-treated guinea pigs was longer than that obtained for the guinea pigs treated with XCN, which is a drug that is marketed and used for asthma management in China. Therefore, we concluded that ZBMS exerts a protective effect on acetylcholine/histamine-induced asthma.

3.3. Ovalbumin-induced airway inflammation in sensitized guinea pigs

Antigen attack often induces an inflammatory reaction that eventually results in asthma. Consequently, we investigated the protective effect of ZBMS on sensitized guinea pigs in an ovalbumin-induced airway inflammation model. The LPIA of the tested animals was observed after treatment with 0.5, 1.5, and 3.0 g/kg ZBMS and the reference drug XCN. As demonstrated in Fig. 2, the LPIA of the sensitized animals was significantly improved compared with that of the vehicle treated animals (vehicle group was given 1% Tween-80). Moreover, ZBMS treatment dose-dependently prolonged the LPIA. The guinea pigs that were treated with 0.5 and 1.5 g/kg ZBMS had LPIAs similar to those of the XCN-treated controls, and the animals that were treated with a high dose of ZBMS (3.0 g/kg) exhibited a longer LPIA than those that were treated with the reference drug. Thus, ZBMS protects against ovalbumin-induced airway inflammation in sensitized guinea pigs.

3.4. Ovalbumin-induced changes in R_L and C_{dyn} in sensitized guinea pigs

Aerosolized ovalbumin causes immediate bronchoconstriction that peaks within 10 min in sensitized guinea pigs (Fig. 3). Compared with the baseline before aerosol antigen challenge value, the bronchial OVA challenge-sensitized guinea pigs induced an increase of 18.8 cm H₂O/L/h in the R_L and a reduction of 1.5 mL/cm H₂O in C_{dyn} in the vehicle group ($n = 8$), and the maximal response (35.2 and 1.0 mL/cmH₂O for R_L and C_{dyn} , respectively) was obtained at 5 min. The mean R_L and C_{dyn} values from 1 to 30 min after antigen challenge were increased to 28.8 cm H₂O/L/h and 1.3 mL/cm H₂O in the saline group, respectively. The i.g. administration of 0.25, 0.5, and 1.0 g/kg ZBMS dose-dependently inhibited the aerosolized OVA-induced bronchoconstrictive responses. The maximal R_L increases at 5 min were 24.0 $\pm$ 3.80, 25.7 $\pm$ 8.99, and 31.6 $\pm$ 16.3 cmH₂O/L/h, respectively, and the maximal C_{dyn} reductions were 1.60 $\pm$ 0.57, 1.24 $\pm$ 0.54, and 1.15 $\pm$ 0.51 mL/cmH₂O, respectively. The positive control salbutamol (4.0 mg/kg) also inhibited antigen-induced bronchoconstriction, and its maximal R_L increase and C_{dyn} decrease at 5 min were 20.2 $\pm$ 2.71 cmH₂O/L/h and 1.87 $\pm$ 0.50 mL/cmH₂O, respectively. Therefore, in this assay, ZBMS dose-dependently inhibited the R_L increases and the C_{dyn} decreases.

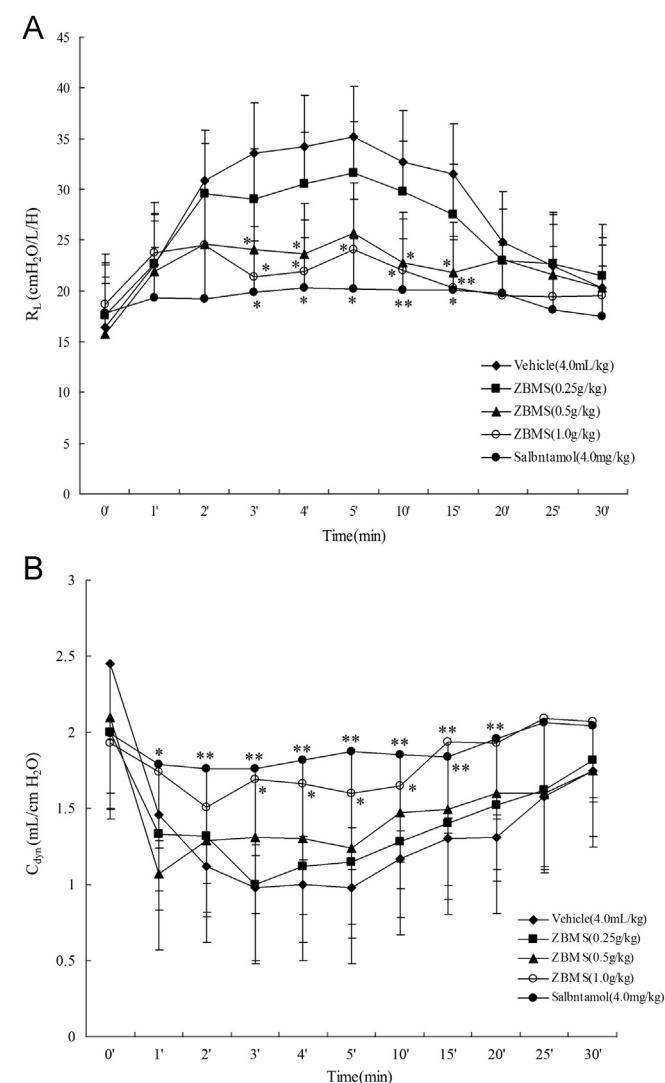


Fig. 3. Time-course curves for the antigen-induced lung resistance (R_L) increase and dynamic lung compliance (C_{dyn}) reduction. Vehicle group was given 1% Tween-80 and the data was demonstrated as the means $\pm$ S.D. Statistically significant differences at $^{*}P < 0.05$, $^{**}P < 0.01$, as compared with the model.

In this model, the induced asthma latency period is the time it takes the guinea pigs to develop asthma symptoms, including hyperspasmia movements until falling down after acetylcholine/histamine inhalation. Our results demonstrated that the three

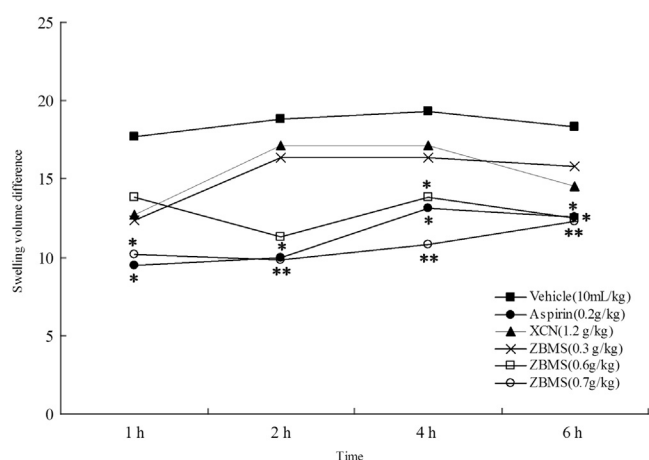


Fig. 4. Toe swelling volume difference at different times after treatment with aspirin (0.2 mL/kg), XCN (1.2 g/kg), and ZBMS (0.3, 0.6, and 1.2 g/kg). The data was illustrated as the means ± S.D. ($n=10$). * $P < 0.05$, ** $P < 0.01$, statistically significant compared with the model.

Table 4

ZMBS-mediated inhibition of citric acid-induced cough.

Drugs	<i>n</i>	Dose (g/kg)	LPIA (s)	Number of coughs (within 5 min)	Inhibition rate (%)
Vehicle	10	10 mL/kg	65.2 ± 35.8	17.3 ± 4.1	
CP	10	7.0 mg/kg	162.5 ± 69.4**	4.5 ± 2.2**	75.99
XCN	10	1.0	92.8 ± 42.4	12.7 ± 6.8*	26.59
ZBMS	10	1.0	96.6 ± 49.7	10.8 ± 5.1**	37.57
ZBMS	10	0.5	93.8 ± 45.2	11.5 ± 5.3*	33.53
ZBMS	10	0.25	83.4 ± 50.3	11.8 ± 5.0*	31.79

Data were demonstrated as means ± S.D.

* $P < 0.05$, statistically significant compared with the model.

** $P < 0.01$, statistically significant compared with the model.

Table 5

Effect of ZBMS on weight-loaded swimming time of mice.

Groups	Dose (g/kg)	<i>n</i>	Swimming time (s)
Vehicle	10 mL/kg	10	187.5 ± 81.2
XCN	2.0	10	371.8 ± 116.9**
ZBMS	2.0	10	353.2 ± 96.1**
ZBMS	1.0	10	315.3 ± 90.0**
ZBMS	0.5	10	289.4 ± 79.9*

Data were demonstrated as means ± S.D.

* $P < 0.05$, statistically significant compared with the vehicle.

** $P < 0.01$, statistically significant compared with the vehicle.

3.5. Anti-inflammatory activity of ZBMS in mouse models

We assessed the anti-inflammatory activity of ZBMS in a murine model of auricle edema (see Table 3) and toe swelling (Fig. 4). Both vehicle control groups were given 1% Tween-80. Dimethylbenzene application to the anti-inflammatory model animals resulted in a marked increase in ear thickness with clear evidence of edema. The results shown in Table 3 illustrate the effect of ZBMS on the inhibition of dimethylbenzene-induced auricle edema in mice with an inhibition rate similar to that of XCN. Moreover, when a high ZBMS dose (2.0 g/kg) was administered, the edema inhibition rate was similar to that obtained with aspirin which was used as a positive control.

Table 6

Effect of ZBMS on survival time of mice under anoxia.

Groups	Dose (g/kg)	<i>n</i>	Survival time (min)
Vehicle	10 mL/kg	10	26.81 ± 3.52
XCN	2.0	10	37.25 ± 4.98**
ZBMS	2.0	10	44.64 ± 6.70**
ZBMS	1.0	10	41.33 ± 4.81**
ZBMS	0.5	10	40.04 ± 4.77**

Data were demonstrated as means ± S.D.

** $P < 0.01$, statistically significant compared with the vehicle.

Toe or paw swelling, as signs of arthritis, were assessed by the level of swelling of each toe, including the tarsus or carpal joints. The following scoring system was used: 0.5=swelling of the toes only or very slight ankle/wrist swelling; 1=slight paw swelling; 2=moderate paw swelling; 3=marked paw swelling; and 4=substantial paw swelling (Patten et al., 2004). As shown in Fig. 4, all ZBMS doses markedly inhibited toe swelling in a dose-dependent manner. Treatment with ZBMS at a dose of 1.2 g/kg dose resulted in an inhibition similar to that obtained with aspirin, which was used as the positive control.

3.6. Effect on citric acid-induced cough in guinea pigs

Table 4 demonstrates the mean cough number for the vehicle-, XCN-, codeine phosphate (CP)-, and ZBMS-treated guinea pig groups after treatment with 17.5% citric acid. The vehicle treatment (1% Tween-80) did not significantly change the number of citric acid-induced coughs. The treatment of three different ZBMS doses significantly reduced the citric acid-induced cough number compared with those of vehicle treatment (17.3 ± 4.1). The positive control CP (4.5 ± 2.2) also significantly decreased the number of coughs compared with vehicle treatment. The latency period of induced asthma (LPIA) was obviously increased in a dose-dependent manner with ZBMS treatment compared with those of vehicle treatment. Although CP treatment resulted in a longer LPIA (162.5 ± 69.4) and a higher inhibition rate (75.99%) than ZBMS, ZBMS treatment induced a similar LPIA and inhibition rate to those of XCN, which has been marketed in China.

3.7. Antistress activity of ZBMS

To evaluate the anti-stress ability of ZBMS, its anti-anoxia and anti-fatigue effect were investigated in mouse models. Swimming is an experimental exercise model because other methods of forced exercise can cause animal injury and may not be routinely acceptable (Zhang et al., 2006). Therefore, in this study the anti-fatigue activity of ZBMS was measured as the mouse swimming endurance capacity using an adjustable current swimming pool. A weight-loaded swimming apparatus measured the maximal swimming time. As demonstrated in Table 5, the swimming time of the mice in the three ZBMS treatment groups (0.5, 1.0, and 2.0 g/kg) increased significantly ($P < 0.05$) compared with that obtained the vehicle group. Of them, the high dose of ZBMS-treated mice exhibited a swimming time similar to that of XCN-treated mice.

To ascertain the anti-anoxia ability of ZBMS treatment, the mice were divided into three group: the vehicle-treated group (1% Tween-80), the XCN (2.0 g/kg)-treated group, and the ZBMS (0.5, 1.0, 2.0 g/kg)-treated group. As illustrated in Table 6, the ZBMS-treated mouse survival time was significantly increased ($P < 0.01$) compared with the vehicle-treated group. ZBMS treatment also resulted in a similar survival time to that obtained with the reference drug XCN. Accordingly, ZBMS had a positive anti-stress effect.

4. Conclusion

In the present study, we describe the main fatty acid composition and the pharmacodynamics, including the assessments of the anti-asthmatic, anti-inflammatory, antitubercular, and antistress effects of ZBMS, which has been traditionally used for asthma management in Chinese medicine. The *n*–3 polyunsaturated fatty acids as substrate fatty acids are an essential determinant of eicosanoid production by reducing AA and interfering with the production of 6-series LT, which results in the suppression of inflammation (Deng et al., 2007). ZBMS is rich in PUFAs and mainly contains α -linolenic acid (18:3, *n*–3), linoleic acid (18:6, *n*–6), and oleic acid (total content > 70%), which has a protective effect on chronic inflammatory lung diseases, such as asthma. All of these results suggest that ZBMS treatment is an effective therapeutic approach for the improvement of asthma, as determined using some murine models. These results may be useful for the development of research agents and for assisting clinicians with the treatment or prevention of asthma.

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