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Impact of volatile oils from processed products of *Schisandra chinensis* fruits on a mouse model of allergic asthma

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Abstract: We established a mouse model of allergic asthma by sensitizing with chicken ovalbumin. The volatile oils and decoctions from raw, wine- and vinegar-steamed *Schisandra chinensis* fruits were intragastrically administrated to the mice. Atomization, serum IgE, IL-2, IL-4 and IFN-γ in the lung homogenates and pathological sections were evaluated to compare the effect of these volatile oils and decoctions on allergic asthma in mice. The results showed that all Schisandra volatile oils could significantly suppress allergic asthma in mice. Raw Schisandra volatile oil was most effective followed by volatile oils extracted from wine-steamed and vinegar-steamed Schisandra. The decoctions had no significant impact. Our findings demonstrated that volatile oil was the active ingredient in Schisandra, and raw Schisandra could be used to prevent cough and asthma.

Keywords: *Schisandra chinensis* fruits, processed, volatile oil, allergic asthma.

INTRODUCTION

Schisandra chinensis is the dried ripe fruit of *Schisandra chinensis* (Turcz.) Baill a widely used traditional Chinese herbal medicine. It is officially listed in the Chinese Pharmacopoeia and is used as a tonic and sedative (Tang *et al.*, 1992). According to traditional Chinese medicine, it tastes sweet and sour, is warm in nature, enters into the lung, heart and kidney meridians, plays a role in astringency, promotes the production of body fluids and calms the mind. It is mainly used to treat cough, asthma, nocturnal enuresis, frequent urination, diarrhea, night sweat, thirst, internal heat, diabetes, heart palpitations and insomnia (Chinese Pharmacopoeia Commission, 2015).

Unlike western herbs, many Chinese herbs are subjected to specific treatments before they are used as materia medica, such as boiling, steaming, frying, etc, that be called "processing" (Zhao *et al.*, 2010).The common processed products that are clinically used include wine-steamed and vinegar-steamed Schisandra. According to traditional Chinese medicine theory, vinegar-steamed Schisandra has a stronger healing effect in diarrhea, while wine-steamed Schisandra helps in strengthening the kidney to prevent spermatorrhea (Jia *et al.*, 2010). According to the Compendium of Materia Medica《本草纲目》, Schisandra should be processed in tonic, and used raw in cough medicine. Raw and processed Schisandra have different medical effects. However, currently Schisandra is incorrectly used in some clinical applications. Therefore, we compared the effects of raw and processed Schisandra in order to provide guidance for the correct usage of Schisandra in clinical applications.

Bioactive components in *Schisandra chinensis* contain lignans, volatile oil, polysaccharides and triterpene acid, etc. Lignans are the most important components.Volatile oil from *Schisandrae chinensis* can protect the pancreatic β-cells in diabetic rats (An *et al.*, 2015). Our preliminary study on the active ingredient of Schisandra in cough prevention was performed in an ammonia-induced cough model (Ge, 2007), which indicated that the volatile oil extracted from Schisandra might be the major component in cough prevention. Here we established a mouse model of allergic asthma using chicken ovalbumin and compared the impact of volatile oils and decoctions from various processed Schisandra in order to clarify whether volatile oil was the key component that could prevent cough and asthma. We also compared the efficacies of volatile oils from various processed Schisandra to provide experimental evidence for the usage of raw Schisandra in cough medicine.

In patients with asthma, the expression of Th_1 cytokines remains unchanged or decreases, while the expression of Th_2 cytokines significantly increases. The main cytokines secreted by Th_1 include IL-2 and IFN-γ, while IL-4 is the major cytokine produced by Th_2 (Lin *et al.*, 2001). IgE, as the mediator of allergy, is the result of immune dysfunction as well as the reason for asthma. IgE levels are higher in asthma patients than in healthy controls. Therefore, we selected IgE, IL-2, IL-4 and IFN-γ levels as well as lung tissue pathological sections as the indices to evaluate the effects of various processed products of Schisandra chinensis fruits.

MATERIALS AND METHODS

Devices

Microplate reader (Thermo Fisher, Shanghai); TGL-16C table-top centrifuge (Anting Scientific Instrument Factory, Shanghai); SHA-C thermostatic oscillator (Guohua

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Electronic Appliance Co. Ltd, Changzhou); AE240 analytical scale (1/100,000 accuracy) (METTLER, Switzerland); paraffin slicing machine (Leica); and Olympus BX51 microscope were used in this study.

Drugs and reagents

Schisandra was purchased from the GAP Schisandra base in Dalishu, Dandong, and its quality was evaluated by Professor Bing Wang in the School of Pharmacy, Liaoning University of Traditional Chinese Medicine. The wine-steamed Schisandra was processed as follows: raw Schisandra was mixed well with wine (use 20 litres wine per 100 kilogram Schisandra) for 1 hour, steamed for 4 hours followed by a drying process at 50^{o}C until a constant weight was reached. The vinegar-steamed Schisandra was processed as follows: raw Schisandra was mixed well with vinegar (use 20 liters vinegar for 100 kilogram Schisandra) for 1.5 hours, steamed for 5 hours followed by a drying process at 50^{o}C until a constant weight was reached. Ovalbumin (OVA) was purchased from Sigma, USA; Ketotifen fumarate tablets from Hengshang Pharmaceutical Co. Ltd, Shanghai (Lot No. H31021309); and ELISA kits for mouse IgE, IL-2, IL-4 and IFN-γ from Kexing Trade Co. Ltd, Shanghai (Lot No. 201405).

Animals

Specific pathogen free grade male KM mice with body weight ranging from 18 to 22g were provided by Changsheng Biotech Co. Ltd, Liaoning (SCXK (Liao) 2010-0001). The protocol was approved by the Ethics Committee of Liaoning University of Traditional Chinese Medicine.

Methods

Material preparation

Volatile oil was extracted from raw, wine- and vinegar-steamed Schisandra by distillation according to Method A of determination of volatile oil in the appendix XD of Chinese Pharmacopoeia 2010 version for use. The extraction rates were 1.31%, 1.19% and 1.27%, respectively. The decoction was filtered, dried and resuspended in 0.5% sodium carboxymethyl cellulose solution. The final concentration of each solution was 240 mg/mL. Ketotifen fumarate tablets were dissolved in 0.5% sodium carboxymethyl cellulose solution, and the final concentration of the active ingredient was 0.02 mg/mL.

Grouping of animals

The mice were randomly assigned to nine groups, with eight mice per group as follows: The blank control group, allergic asthma model group, model group treated with positive control Ketotifen fumarate, model groups treated with volatile oils from raw, wine- and vinegar-steamed Schisandra and model groups treated with decoctions from raw, wine- and vinegar-steamed Schisandra.

Establishment of allergic asthma model and drug administration

The mice were given allergen solution (0.2 mL each) intraperitoneally on day 0 (Wang *et al.*, 2013) and day 14. From day 18 to 26, mice were activated by atomization of 1% OVA solution for 20 minutes daily. The blank control group received similar treatment with the same volume of saline. Drugs were intragastrically administered from day 0 of model establishment for 26 days. The blank control group and the allergic asthma model group were given corresponding 0.5% sodium carboxymethyl cellulose solution, and all drugs were given before allergen injection and atomization (Hamelmann *et al*., 1997; Daoui *et al*., 2000).

Examination of serum IgE

Retro-orbital blood samples were collected and centrifuged at 8000 r/min for 5 min. The supernatants were subjected to ELISA according to the manufacturer's manual.

Examination of IL-2, IL-4 and IFN-γ in lung homogenates

The right lung of each mouse, weighing about 80 mg, was homogenized in ice-cold saline for 10% homogenate. The supernatants after centrifugation were subjected to ELISA for IL-2, IL-4 and IFN-γ according to the manufacturer's manuals.

HE staining of the lung tissues

The mice lung tissues were fixed, dehydrated, permeated, waxed, embedded, dewaxed, stained and observed under the microscope.

STATISTICAL ANALYSIS

All data were represented as mean ± standard deviation ($\bar{x} \pm SD$). One-way ANOVA was performed using SPSS 17.0. $P < 0.05$ was considered to be statistically significant.

RESULTS

Observations during atomization

During atomization, all groups except the blank control showed shortness of breath, scratching, muzzle bruising, irritability, incontinence and other symptoms, with the model group exhibiting the most severe symptoms.

Serum IgE levels in mice

Serum IgE level in OVA-induced allergic asthma model group was significantly higher than in the blank control group ($P<0.05$). Serum IgE levels in the model groups treated with positive control and volatile oils from various processed Schisandra were significantly lower than in the model group. Volatile oil from raw Schisandra was most effective followed by the positive control and volatile oils from wine- and vinegar-steamed Schisandra. Serum IgE

Table 1: Effect of various Schisandra on serum IgE levels in different groups

Group	Number	IgE/ (μg/mL)
Blank control	8	162.73 ± 13.14*
Model	8	190.57 ± 19.99
Model + positive control	8	165.85 ± 15.44*
Model + volatile oil from raw Schisandra	8	159.03 ± 14.70*
Model + volatile oil from wine Schisandra	8	168.41 ± 21.11*
Model + volatile oil from vinegar Schisandra	8	169.55 ± 12.86*
Model + decoction from raw Schisandra	8	180.62 ± 27.22
Model + decoction from wine Schisandra	8	186.59 ± 20.51
Model + decoction from vinegar Schisandra	8	184.60 ± 16.45

Table 2: Effect of various Schisandra on IL-2, IFN-γ and IL-4 levels in mouse lung homogenates

Group	No.	IL-2/(ng/L)	IFN-γ/(ng/L)	IL-4/(pg/mL)
Blank control	8	2191.31 ± 127.20*	880.71 ± 141.04*	357.63 ± 26.55*
Model	8	1996.88 ± 140.43	711.23 ± 94.11	391.47 ± 15.30
Model + positive control	8	2181.25 ± 239.51*	857.99 ± 125.46*	358.49 ± 27.21*
Model + volatile oil from raw Schisandra	8	2190.63 ± 196.14*	861.88 ± 81.68*	352.03 ± 13.38*
Model + volatile oil from wine Schisandra	8	2164.06 ± 233.56*	856.04 ± 54.28*	361.72 ± 16.12*
Model + volatile oil from vinegar Schisandra	8	2178.13 ± 89.33*	859.29 ± 80.82*	364.09 ± 21.26*
Model + decoction from raw Schisandra	8	2050.00 ± 182.25	765.78 ± 139.46	380.47 ± 32.78
Model + decoction from wine Schisandra	8	2001.56 ± 115.52	793.70 ± 157.67	375.52 ± 33.15
Model + decoction from vinegar Schisandra	8	1984.38 ± 95.14	810.58 ± 119.19	382.84 ± 29.08

Compared to model group, *P <0.05.

levels in the model groups treated with decoctions from various processed Schisandra showed a decreasing albeit insignificant trend as compared to the model group (table 1).

IL-2, IFN-γ and IL-4 levels in mouse lung homogenates

IL-2 and IFN-γ levels in lung homogenates were significantly reduced (P<0.05) in OVA-induced allergic asthma model mice as compared to the blank control, while IL-4 level was significantly increased (P<0.05). Treatment with positive control and volatile oils from various processed Schisandra significantly increased the IL-2 and IFN-γ levels and reduced the IL-4 levels in lungs. Treatment with decoctions had no significant effect on the IL-2, IFN-γ and IL-4 levels (table 2).

Lung pathology in mice

The morphology of lungs in the blank control group was normal with a few inflammatory cells scattered around the terminal bronchioles; very little secretions were observed in the lung bronchioles; the thickness of alveolar wall was even and the size of alveolar sacs was appropriate; no abnormal changes were seen (fig. 1A). In the model group, alveolar wall was thickened and infiltration with large number of inflammatory cells was observed. Little secretions were observed in the lung bronchioles and the center of the lung was heavy (fig. 1B). In the model groups treated with positive control and volatile oil from raw Schisandra, the lung morphology was close to normal with a few inflammatory cells near bronchioles. No secretions were observed (fig. 1C, D). In the model groups treated with volatile oils from wine- and vinegar-steamed Schisandra, the lung morphology was close to normal with some inflammatory cells. A small amount of secretions were observed in bronchioles. Alveolar wall thickening and congestion were seen in some areas (fig. 1E, F). In the model group treated with decoction from raw Schisandra, infiltration with large amount of inflammatory cells was observed. The periphery of lung tissue was heavy. Abundant secretions were seen in the bronchioles and alveolar wall was thickened. Congestion was found in some alveolar sacs (fig. 1G). In the model groups treated with decoctions from wine- and vinegar-steamed Schisandra, severe congestion was seen and the alveolar wall was thickened. Severe infiltration with large number of inflammatory cells was observed in bronchioles with abundant secretions (fig. 1H, I).

DISCUSSION

Schisandra plays a role in healing and cough prevention. In this study, we established an OVA-induced allergic asthma mouse model to compare the inhibitory effects of volatile oils and decoctions from various processed Schisandra on allergic asthma. The results showed that volatile oils from all processed Schisandra were beneficial for allergic asthma in mice, and significantly increased IL-2 and IFN-γ levels while lowering IL-4 and serum IgE levels. The decoctions from all processed Schisandra showed no significant effect, suggesting that volatile oil

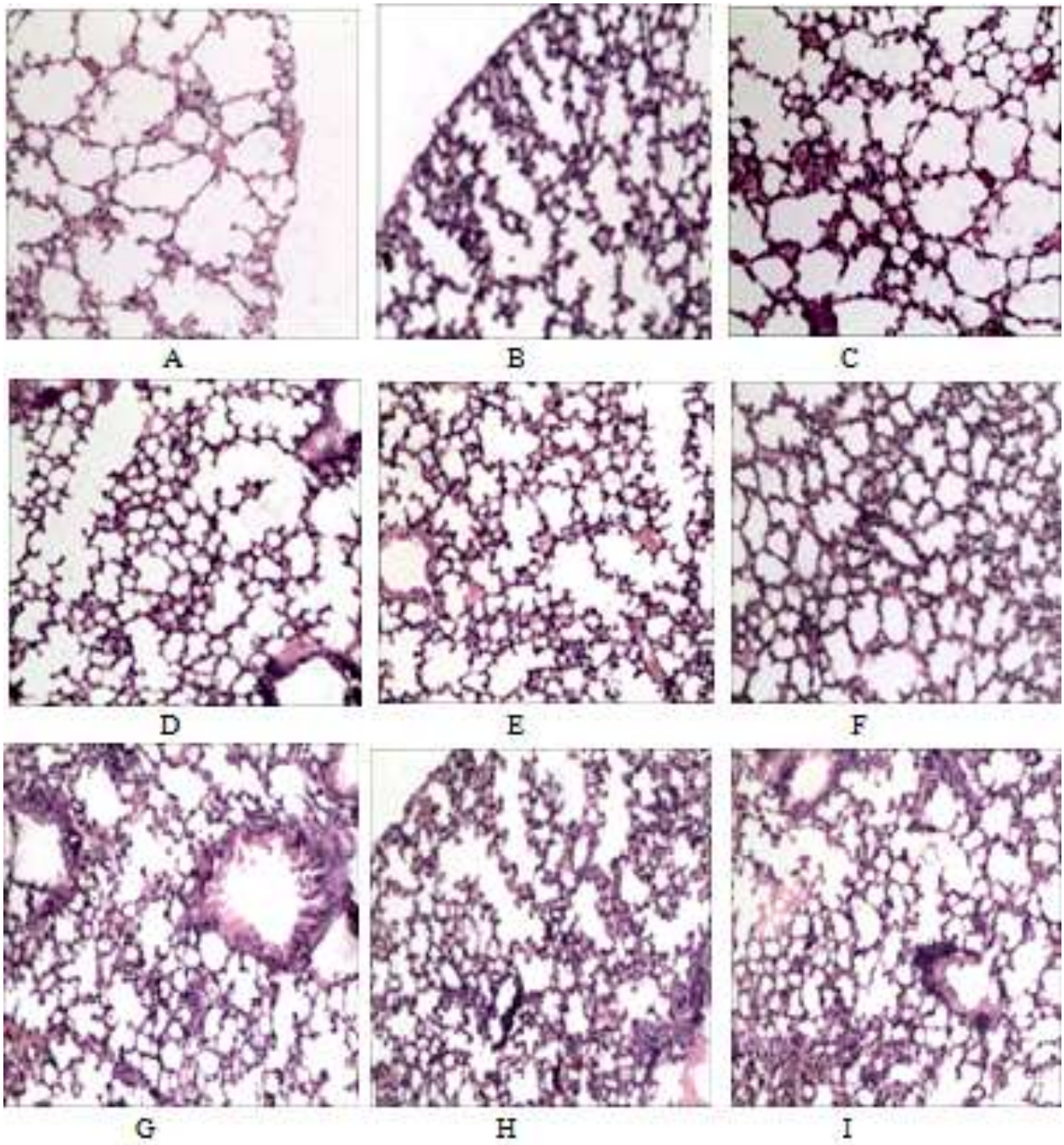

Fig. 1: Lung pathology in mice (10×). (A) Blank control, (B) OVA-induced allergic asthma model, (C) Model treated with positive control, (D) Model treated with volatile oil from raw Schisandra; (E) Model treated with volatile oil from wine-steamed Schisandra; (F) Model treated with volatile oil from vinegar-steamed Schisandra, (G) Model treated with decoction from raw Schisandra, (H) Model treated with decoction from wine-steamed Schisandra; (I) Model treated with decoction from vinegar-steamed Schisandra.

was the active ingredient in preventing cough. The volatile oil from raw Schisandra was most effective, which corroborated with the traditional medicine theory that "raw Schisandra should be used to prevent cough". This is because the extraction rate was higher in raw Schisandra than other processed products and some of the ingredients in the volatile oil could change during the process (Han HX *et al.*, 2011).Through the experiment that the volatile oil can increase IL-2 and IFN-γ levels while lower IL-4 and serum IgE levels, is beneficial to correct the Th_1/Th_2 imbalance.

Lai KF showed Schisandra chinensis reduces cough frequency and pulmonary inflammation in the cigarette

smoke-induced cough hypersensitivity guinea pigs, and lignans may be the active components (Zhong *et al.*, 2015). Lignans contents changed little before and after wine-steamed or vinegar-steamed (Tong, 2014), so that may be not the reason for the difference for preventing cough.

Thirty-three volatile compounds have been separated and identified from the fruits of *Schisandra chinensis* by Gas Chromatography-Mass Spectrometry (Deng *et al.*, 2003). We plan to examine the differences in volatile oil compositions before and after processing, and the effect of processing on volatile oil in order to better understand why raw Schisandra should be used in cough prevention. The ingredients in volatile oil and decoction from Curcuma were reported to be completely different (Zhu *et al.*, 2013). Future studies should investigate whether this is true for Schisandra as well.

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