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Original Article

Anti-allergic rhinitis effects of caffeoylquinic acids from the fruits of *Xanthium strumarium* in rodent animals *via* alleviating allergic and inflammatory reactions

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ABSTRACT

The fruits of *Xanthium strumarium* L., Asteraceae, have been used for various diseases in Chinese folk medicine, including allergic rhinitis, tympanitis, arthritis, ozena *etc.* The current study was aimed to investigate the therapeutic effects of caffeoylquinic acids from fruits of *X. strumarium* on allergic rhinitis in animals. The toxicity test indicated that the caffeoylquinic acids have no obvious toxicity. By using HPLC assays combined with reference standards, ten caffeoylquinic acids were identified as the predominant constituents. Anti-allergic activities of the caffeoylquinic acids were evaluated using passive cutaneous anaphylaxis test and Schultz-Dale test; dimethylbenzene induced ear edema test was performed to evaluate its anti-inflammatory effect. Then, the allergic rhinitis model in rats was established to evaluate the therapeutic effects of the caffeoylquinic acids against allergic rhinitis with the following indexes: allergic rhinitis symptom scores, serum levels of pro-inflammatory cytokines, histopathological examination, and histamine release. Our study revealed that the caffeoylquinic acids showed obvious anti-allergic and anti-inflammatory properties, and its treatments were beneficial for ameliorating the nasal symptoms, decreasing pro-inflammatory cytokines, and inhibiting the releases of histamine. Collectively, the caffeoylquinic acids might be utilized as effective and safe disease therapeutic agents for allergic rhinitis.

Introduction

The rhinitis, defined as the nasal mucosa inflammation, affects up to 40% of the global population with as table increasing prevalence, and the allergic rhinitis (AR) is the overriding type, accounting for up to 50% of the total cases of rhinitis (Mandhane et al., 2011; Kakli and Riley, 2016). Symptoms of AR, characterized by sneezing, rhinocnesmus, rhinorrhea, and nasal congestion, could seriously interfere with daily life of AR patients, and result in headache, irritability, decreased emotional well-being, and declined sleep quality and performance of learning and work (Andrew, 2009; Kakli and Riley, 2016). In addition, it is also reported that chronic nasal airway obstruction may even induce craniofacial abnormalities and orthodontic disturbances in some children AR patients due to chronic mouth breathers (Suleimani and Walker, 2007). Currently, besides allergen avoidance, it is considered that oral antihistamines are the predominant efficacy treatment for AR (Kakli and Riley, 2016). However, the current available antihistamines, including diphenhydramine, chlorpheniramine, cetirizine, desloratadine, fexofenadine *etc.*, may result in some annoying side effects, such as headache, drowsiness, thirst, fatigue, and even cardiovascular side effects (Suleimani and Walker, 2007; Kakli and Riley, 2016). Furthermore, there are also other treatments for AR, such as intranasal corticosteroids, leukotriene receptor antagonists, and allergen immunotherapy, however, these mentioned treatments are costly or also have some unwanted side-effects (Stewart et al., 2010; Kakli and Riley, 2016). Consequently, it is necessary to discover more economical reliable drugs with few side effects for treating AR.

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In China, the Traditional Chinese Medicines (TCM) have been utilized for centuries to cure various difficult miscellaneous diseases, including allergic disorders (Patwardhan, 2005; Peng et al., 2014, 2018). Furthermore, previous investigations have revealed that some TCM have potential therapeutic effects on AR through immunomodulation of Th1/Th2, suppression of IgE production, and inhibition of pro-inflammatory cytokines *etc.* (Ikeda et al., 2002; Yang et al., 2001). The fruits of *Xanthium strumarium* L., Asteraceae, also called "*Cang-Er-Zi*" in Chinese, are one of the known and representative TCM for treating rhinitis (Editorial Committee of Chinese Pharmacopoeia, 2015). The current works have reported that extracts from the fruits of *X. strumarium* possess anti-inflammatory and analgesic properties, and could also inhibit mast cell-mediated allergic reactions (Hong et al., 2003; Han et al., 2007); we have reported that a new thiazinedione constituent, called caffeoylxanthiazonoside, extracted from the fruits of *X. strumarium* possess potential anti-allergic rhinitis effects *in vivo* (Peng et al., 2014). Furthermore, previous phytochemical researches have indicated that phenolic acids are the characteristic constituents of fruits of *X. strumarium* (Han et al., 2007). Consequently, as part of our continuing investigation of the fruits of *X. strumarium*, we aimed to investigate the anti-allergic rhinitis effects of caffeoylquinic acids from the fruits of *X. strumarium* (XSF) in rodent animals, which could be beneficial for providing a scientific basis for the clinical use of XSF to treat allergic rhinitis (AR) in the future.

Materials and methods

Plant material

The ripe fruits of *Xanthium strumarium* L., Asteraceae, were collected from the *Bozhou* Chinese traditional medicinal materials market (Bozhou, China), and authenticated by Prof. Ting Han (Second Military Medical University, Shanghai, China). The voucher specimen of the fruits of *X. strumarium* was deposited at the Department of Pharmacognosy, School of Pharmacy, Second Military Medical University, Shanghai, PR China (No. CE20140703).

Animals

Institute of Cancer Research (ICR) mice (20 ± 2 g), Sprague Dawley (SD) rats (220 ± 20 g) and guinea pig (230 ± 20 g) were purchased from the Shanghai SLAC Laboratory Animal Co., Ltd (Shanghai, China; Animal Certificate No. SCXK (Hu) 2007-0003). All animal treatments were conducted strictly in accordance with international ethical guidelines and the National Institutes of Health Guide concerning the Care and Use of Laboratory Animals. The experiments were carried out with the approval of the Animal Experimentation Ethics Committee of the Animal Care and Use Committee of the Second Military Medical University.

Chemicals

The RPMI 1640 media, FBS, MEM nonessential amino acid solution and interleukin (IL)-3 were purchased from Invitrogen Co. (Carlsbad, California, CA, USA); dimethyl benzene and $A1(OH)_3$ powder were purchased from the Sino. Pharm Chemical Reagent Co. (Shanghai, China); HPD-500 macroporous resin was purchased from the Qinshi Science and Technology Ltd. (Zhengzhou, China); loratadine was purchased from Aladdin Reagent Co. (Shanghai, China); Rat Immunoglobulin E (IgE), IL-1, IL-4, IL-5, interferon (IFN)-γ and histamine ELISA kits were purchased from Abcam Biotechnology (Cambridge, MA, USA); Evans blue, Hematoxylin and Eosin (H&E), stem cell factor (SCF) and ovalbumin were purchased from Sigma Chemical Co. (St. Louis, USA); Pertussis vaccines were purchased from Shanghai Institute of Biological Products Co. (Shanghai, China). All the reference standards were purchased from National Institute for the Control of Drug and Biological Products (Beijing, China) or Sigma (St. Louis, Missouri, USA).

Preparation of the XSF samples

Dried fruits of *X. strumarium* were grounded and extracted with 75% aqueous ethanol by reflux three times (each extraction period lasted 2 h). The solvent was subjected to column chromatography over macroporous resin and eluted with 10% aqueous ethanol. Subsequently, the 10% aqueous ethanol fraction was concentrated and dried under 50 °C *in vacuum* to afford the XSF for the further chemical analysis and pharmacological investigations. Dosages of the positive drugs were determined on the basis of references and clinical uses, and the dose selection was based on the results of our preliminary experiments. For the animal experiments, all the positive drugs and XSF were dissolved in 0.5% CMC-Na. Furthermore, normal and control rats were treated with an equivalent volume of the0.5% CMC-Na which had been utilized to dissolve the testing samples.

HPLC analysis of the XSF

The HPLC analysis of the XSF was carried out followed our previous reported method using an Agilent 1100 HPLC system (Agilent 1100, USA), equipped with a quaternary pump, an autosampler, a DAD detector and a Chemstation softwar (10.2 version) (Han et al., 2009). In addition, a Zorbax SB C_{18} reversed-phase column (250 mm × 4.6 mm, 5 μm) was used with column temperature set at 25 °C. The mobile phase consisted of methanol (elute A) and 0.5% aqueous phosphoric acid (elute B), and the gradient program was as follows: 0–10 min, 10%–20%; 10–20 min, 20%–40% methanol; 20–50 min, 40%–50% methanol. Furthermore, the flow rate was set as 0.8 ml/min, and detector wavelength was set at 327 nm for acquiring chromatograms.

Toxicity tests

Total 90 ICR mice were randomly divided into nine groups ($n = 10$). Mice of groups 1–8 were orally administered 1, 5, 10, 25, 50, 100, 250 and 500 mg/kg of XSF, respectively, and mice of the 9th group were orally administered with 0.5% CMC-Na (20 ml/kg). The mortality rates and neuro-behaviors of mice within a 14 days period were observed and recorded.

Passive cutaneous anaphylaxis test

To evaluate anti-allergic effect of the XSF, the passive cutaneous anaphylaxis (PCA) test was carried out according to the previous reported method with minor modifications (Peng et al., 2014; Poulsen and Hau, 1987). Total sixty SD rats were divided randomly into six groups, and each group consisted of ten rats, including normal rats, control rats, positive drug (loratadine, 1 mg/kg) treated rats, and three doses of XSF treated rats (2.5, 5 and 10 mg/kg).

To prepare the anti-serum, total 12 SD rats were received subcutaneous injection of ovalbumin (10 mg/rat) and intraperitoneal injection of pertussis vaccines (2.0×10^{10}/rat) in rats every 2 days. The rats were received total three sensitizations by injection of ovalbumin and pertussis vaccines, and the anti-serum was collected in the 12th day since the last sensitization. All the positive drug and XSF were administered orally for continuous 7 days, and the PCA test was performed after drug treatments for 5 days. Briefly, rats were received intradermal injection of 0.1 ml the diluted anti-serum (dilution at 1:5 and 1:10), and 4% Evans blue was injected *via* the tail vein after 48 h of the anti-serum injection. Then, rats

were sacrificed 30 min after the antigen-challenge under anesthetized by 10% chlora hydrate (2 ml/kg), and the dorsal skins with the pigment of rats were collected. Finally, the amount of dye was determined using colorimetry with a microplate reader at 630 nm. The inhibition was calculated as follows: inhibition (%) = $(OD_A - OD_B) \times 100\%/OD_A$, where *A* is control rats and *B* is the XSF or positive drug treated rats.

Schulty-Dale test

Furthermore, the Schulty-Dale test (SDT) was also performed to investigate the anti-allergic effect of the XSF according to the previous reported method with minor modifications (Corcostegui et al., 2005). Total fifty guinea pigs were divided randomly into five groups, and each group consisted of ten animals, including control rats, positive drug (chlortrimeton, 5 mg/kg) treated rats, and three doses of XSF treated rats (2.5, 5 and 10 mg/kg).

Guinea pigs were immunized by intraperitoneal injection of 5% ovoalbumin saline solution for 1 ml and intramuscular injection of 5% ovoalbumin saline solution for 0.4 ml. After 28 days, animals were sacrificed underanesthesia by intraperitoneal injection of sodium pentobarbital (45 mg/kg), and the ileum was removed for further experiments. Subsequently, the ileum of the guinea pig were cut into 2 cm fragments, and were kept at 37 ± 0.5 °C in 20 ml Tyrode solution at pH 7.4, constantly oxygenated with carbogen, and the baseline contractile tension was recorded. Then, ileum fragments were exposed to 50 μg/ml ovoalbumin Tyrode solution until contractions of homogeneous intensity were recorded. The inhibition was calculated as follows: inhibition (%) = $(A - B) \times 100\%/A$, where *A* is the contractile tension of control rats, and *B* is the contractile tension of XSF or positive drug treated rats.

Assessment of dimethylbenzene-induced edema in mice

To evaluate the anti-inflammatory effect of XSF, the dimethylbenzene-induced ear edema in mice was carried out as described by Wang et al. (2014). Briefly, positive drug (dexamethasone, 5 mg/kg) and XSF (2.5, 5 and 10 mg/kg) were administered orally for 5 continuous days before dimethylbenzene topical application to the right ear of mice. Then, mice were sacrificed by cervical vertebra dislocation at 1 h after dimethylbenzene treatment, and the ear edema was cut and measured by subtracting the weight of the left ear from that of the right. The inhibition was calculated as follows: inhibition (%) = $(A - B) \times 100\%/A$, where *A* is ear edema of the control rats, and B is ear edema of the XSF or positive drug treated rats.

Preparation of AR model rats

The AR model rats were prepared to evaluate the therapeutic effects of XSF against AR *in vivo* following the previous reported method (Peng et al., 2014; Zhang et al., 2016). Total sixty SD rats were divided randomly into six groups, and each group consisted of ten rats, including normal rats, control rats, positive drug (loratadine, 1 mg/kg) treated rats, and three doses of XSF treated rats (2.5, 5 and 10 mg/kg).

Rats were immunized by intraperitoneal injection of 1 ml saline solution containing ovoalbumin (2 mg) and $A1(OH)_3$ (30 mg) every 2 days (for 14 days). After sensitization for total seven times, local sensitization was performed by dripping 50 μl ovoalbumin saline solutions (1 mg/ml) into the bilateral nasal cavities once a day (for 7 days). The total AR symptom scores over 5 means the AR rats were prepared successfully (Xiang et al., 2017), and the total AR symptom scores were calculated by superposition according to the following items: (A) rhinocnesmus: few nasal-scratching (1 score), frequent nasal-scratching (2 score), severe nasal-rubbing (3 score); (B) runny nose: not over anterior nares (1 score), overanterior nares (2 score), massive and flow to face (3 score); (C) sneezing: ≤3 times during 30 min (1 score), 3–9 times during 30 min (2 score), >9 times during 30 min (3 score).

Determination of the levels of Ig E, IL-1, IL-4, IL-5 and IFN-γ in serum of AR rats

Blood samples of rats in different groups were collected from the abdominal aorta after calculation of AR symptom scores. Serum samples were prepared after incubation in ice-temperature storage and centrifugation at 2000 × *g* for 20 min. Contents of Ig E, IL-1, IL-4, IL-5 and IFN-γ in serum was determined by using commercial ELISA kits according to their corresponding manufacturer's instructions.

Histopathological examinations

Nasal mucosa tissues of the rats were collected and fixed in 4% paraformaldehyde for 24 h. Subsequently, the tissues were received a standard processing according to the previous reported methods (Peng et al., 2015), including dehydrate with graded ethanol, vitrification by dimethylbenzene, paraffin embedding, sectioning slices (5 μm), de-paraffinization, and finally staining with hematoxylin and eosin. Then, the histopathological changes of the tissue sections were observed under an optical microscope (Olympus, Japan).

Effects of XSF on histamine releases in bone marrow-derived mast cells

The bone marrow-derived mast cells (BMMC) were isolated from the bone marrow cells and induced by IL-3 (4 ng/ml) and SCF (50 ng/ml) co-culture in RPMI 1640 media according to the reported references (Jia et al., 2015; Wang and Li, 2011). In the present study, we selected the25, 250 and 2500 μg/ml as the testing concentrations of XSF, and CCK-8 assay was subsequently used to evaluate the effects of XSF on cell viability of BMMC. For determination of the effects of XSF on histamine releases, BMMC (5×10^5/200 μl) were seeded in 96-well plates and co-cultured with XSF (finally concentrations in well: 25, 250 and 2500 μg/ml) at 37 °C for 10 min. Subsequently, 25 μl compound 40/80 was added to the well for cultivating 20 min to induce the histamine releases. Then, the cell supernatants were collected, and the histamine contents were determined using commercial ELISA kits according to the manufacturer's instructions. The inhibition was calculated as follows: inhibition (%) = $(A - B) \times 100\%/A$, where *A* is histamine contents of control rats, and *B* is histamine contents of XSF or positive drug treated rats.

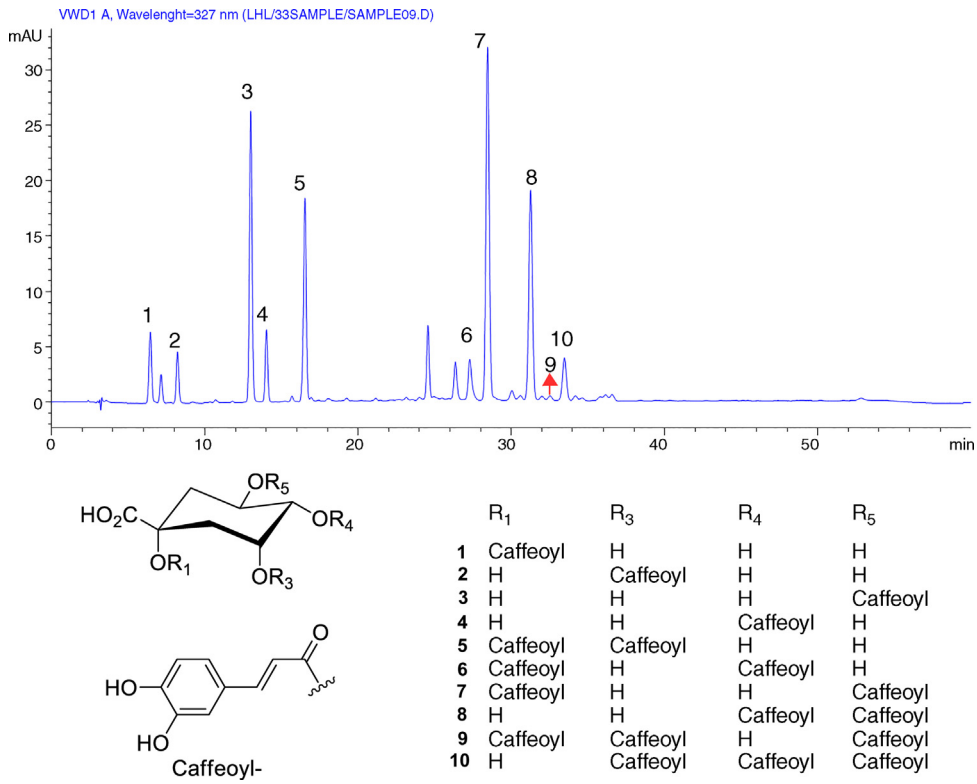
Statistical analysis

Data are represented as mean ± SD, and were evaluated with one-way ANOVA following by Dunnet *t* multiple comparisons test between different groups. The statistical significance of differences was analyzed by using SPSS software (SPSS for Windows 18.0, SPSS Inc., USA) with a significance level of $p < 0.05$.

Results

Results of HPLC analysis on XSF

Using the established separation conditions, HPLC chromatograms of XSF were recorded (Fig. 1). By comparing individual peak retention times with those of the authentic reference standards, ten caffeoylquinic acids, including 1-*O*-caffeoylquinic acid (**1**), 3-*O*-caffeoylquinic acid (**2**), chlorogenic acid (**3**), 4-*O*-caffeoylquinic acid (**4**), 1,3-*O*-dicaffeoylquinic acid



	R_1	R_3	R_4	R_5
1	Caffeoyl	H	H	H
2	H	Caffeoyl	H	H
3	H	H	H	Caffeoyl
4	H	H	Caffeoyl	H
5	Caffeoyl	Caffeoyl	H	H
6	Caffeoyl	H	Caffeoyl	H
7	Caffeoyl	H	H	Caffeoyl
8	H	H	Caffeoyl	Caffeoyl
9	Caffeoyl	Caffeoyl	H	Caffeoyl
10	H	Caffeoyl	Caffeoyl	Caffeoyl

Fig. 1. HPLCchromatogram of the caffeoylquinic acids extracted from the fruits of *Xanthium strumarium*. Peaks of 1–9 represented the 1-*O*-caffeoylquinic acid (**1**), 3-*O*-caffeoylquinic acid (**2**), chlorogenic acid (**3**), 4-*O*-caffeoylquinic acid (**4**), 1,3-*O*-dicaffeoylquinic acid (**5**), 1,4-*O*-dicaffeoylquinic acid (**6**), 1,5-*O*-dicaffeoylquinic acid (**7**), 4,5-*O*-dicaffeoylquinic acid (**8**), 1,3,5-*O*-tricaffeoylquinic acid (**9**), 3,4,5-*O*-tricaffeoylquinic acid (**10**), respectively.

(**5**), 1,4-*O*-dicaffeoylquinic acid (**6**), 1,5-*O*-dicaffeoylquinic acid (**7**), 4,5-*O*-dicaffeoylquinic acid (**8**), 1,3,5-*O*-tricaffeoylquinic acid (**9**), 3,4,5-*O*-tricaffeoylquinic acid (**10**), were identified from the XSF, and the chemical structures of these nine compounds are shown in Fig. 1.

Toxicity study results

In the present toxicity investigation, neither death nor any abnormal neuro-behaviors of mice were observed during the observation period. Consequently, the 50% lethal dose (LD_{50}) of the XSF was not calculated due to lacking of observable toxicity at any of the tested dosages.

Results of PCA and SDT in vivo

In order to investigate the anti-allergic activities of XSF *in vivo*, the PCA and SDT tests were carried out. The results of our present research were shown in Tables 1 and 2. As can be seen from our results of PCA, after sensitization by anti-serum (1:5 and 1:10), the OD value was statistically significantly increased ($p < 0.01$), compared to the normal rats. Interestingly, similar to the positive drugs, XSF (2.5, 5.0 and 10.0 mg/kg) can statistically significantly decrease the OD value compared with the control rats ($p < 0.01$), with the average inhibitions of 28.40, 38.95 and 45.60%, respectively. Furthermore, the results of the SDT also indicated that compared to the control animals, the positive drug ($p < 0.01$) and XSF (2.5, 5 and 10 mg/kg, $p < 0.01$) treatments statistically significantly inhibited the ileum contractile tension of guinea pig induced by ovoalbumin with the inhibition of 83.88, 40.41, 55.17 and 80.69%, respectively. These results above suggested that XSF possesses potential anti-allergic activities.

Results of the dimethylbenzene-induced edema test

As shown in Table 3, theanti-inflammatory effects ofXSF were evaluated by calculating the inhibitory rate of XSF on dimethylbenzene-induced ear edema in mice. Our present results revealed that compared to the control mice, the positive drug (dexamethasone, 5 mg/kg) showed statistically significant inhibitory activity on ear edema induced by dimethylbenzene with the inhibitory rate of 69.14%. Interestingly, similar to the dexamethasone, XSF also showed statistically significant inhibitory effects on ear edema by dimethylbenzene at 2.5, 5 and 10 mg/kg ($p < 0.01$) with inhibitory rates of 46.82, 62.61 and 66.34%, respectively. Importantly, at the10 mg/kg dose, the anti-inflammatory activity of XSF was comparable to the positive drug of dexamethasone at the dose of 5 mg/kg (69.14% *vs.* 66.34%).

Effects of XSF on AR symptom scores

As can be seen from our present results shown in Table 4, after the last ovoalbumin sensitization in bilateral nasal cavities of rats, the AR symptom scores were statistically significantly increased ($p < 0.01$), compared to the normal rats, indicating that the AR rats were prepared successfully. Similar to the positive drugs (loratadine, 1 mg/kg), XSF at the doses of 2.5, 5 and 10 mg/kg could statistically significantly decrease the AR symptom scores ($p < 0.01$), compared to the control rats.

Effects of XSF on serum levels of Ig E, IL-1, IL-4, IL-5 and IFN-γof AR rats

As shown in Fig. 2, in the AR control group, the levels of IgE, IL-1, IL-4 and IL-5 in serum statistically significantly increased compared to the normal rats ($p < 0.05$), whereas the IFN-γ level in serum

Table 1
Effect of XSF on PCA in rats.

	OD value		Inhibition (%)		
	1/5 antiserum	1/10 antiserum	1/5 antiserum	1/10 antiserum	Average
Normal	0.26 ± 0.03	0.23 ± 0.02	–	–	–
Control	0.78 ± 0.05[a]	0.74 ± 0.03[a]	–	–	–
Positive	0.36 ± 0.04[b]	0.35 ± 0.04[b]	53.5	52.2	52.85
2.5 mg/kg	0.57 ± 0.03[b]	0.52 ± 0.05[b]	27.1	29.7	28.40
5.0 mg/kg	0.47 ± 0.04[b]	0.46 ± 0.04[b]	39.4	38.5	38.95
10.0 mg/kg	0.42 ± 0.02[b]	0.41 ± 0.03[b]	46.3	44.9	45.60

Loratadine (1.0 mg/kg) was used as the positive drug, and the XSF and positive drug were administered orally *(p.o)*. Data were expressed as mean ± SD ($n = 10$).
[a] $p < 0.01$, compared to normal.
[b] $p < 0.01$, compared to Model.

Table 2
Effect of XSF on Schultz-Dale tests in guinea pig.

	Dose (mg/kg)	Contractile tension (g/100 mg wet weight)	Inhibition (%)
Control		2.66 ± 0.11	–
Positive	5.0	0.43 ± 0.11[a]	83.88
XSF	2.5	1.59 ± 0.15[a]	40.41
	5.0	1.19 ± 0.15[a]	55.17
	10.0	0.51 ± 0.14[a]	80.69

Chlortrimeton was used as the positive drug, and the XSF and positive drug were administered orally (p.o). Data were expressed as mean ± SD ($n = 10$).
[a] $p < 0.01$, compared to control.

Table 3
Effects of XSF on ear edema induced by dimethylbenzene in mice.

	Dose (mg/kg)	Edema weight (mg)	Inhibition (%)
Control		16.29 ± 3.90	
Positive	5.0	5.03 ± 4.37[a]	69.14
XSF	2.5	8.65 ± 4.77[a]	46.82
	5.0	6.09 ± 4.15[a]	62.61
	10.0	5.48 ± 3.56[a]	66.34

Dexamethasone was used as the positive drug, and the XSF and positive drug were administered orally (p.o). Data were expressed as mean ± SD ($n = 10$).
[a] $p < 0.01$, compared to control.

Table 4
Effects of XSF on scores of nasal symptoms in AR rats.

	Dosage (mg/kg)	Prior-treatment	Post-treatment
Normal		0.5 ± 0.71	0.6 ± 0.84
Control		6.6 ± 0.70[a]	6.6 ± 0.52[a]
Positive	1.0 mg/kg	6.7 ± 0.48	2.4 ± 0.70[b]
XSF	2.5 mg/kg	6.6 ± 0.84	5.3 ± 0.79[b]
	5.0 mg/kg	6.7 ± 0.94	4.5 ± 0.75[b]
	10.0 mg/kg	6.7 ± 0.67	3.1 ± 0.74[b]

Loratadine was used as the positive drug, and the XSF and positive drug were administered orally (p.o). Data were expressed as mean ± SD ($n = 10$).
[a] $p < 0.01$, compared to normal.
[b] $p < 0.01$, compared to control.

statistically significantly decreased ($p < 0.01$). However, compared to the control rats, the positive drug treatment (loratadine, 1 mg/kg) could statistically significantly decrease the IgE, IL-1, IL-4 and IL-5 in serum of AR rats, while increase the IFN-γ ($p < 0.01$). Interestingly, similar to the positive drug, after treatment with XSF at the doses of 2.5, 5 and 10 mg/kg, the levels IgE, IL-1, IL-4 and IL-5 in serum were statistically significantly decreased compared to the control group ($p < 0.01$); furthermore, the IFN-γ level in serum statistically significantly increased compared to the control rats ($p < 0.01$).

Results of the histopathological examination

Histopathological examinations were carried out to evaluate the effects of XSF on pathological changes of the nasal mucosa tissues of AR rats, and the related results were shown in Fig. 3. Our present results revealed that no obvious visible injury could be found in the nasal mucosa sections of normal rats (Fig. 3A). In contrary, for the AR control rats, obvious hyperplasia of epithelial cells, hemangiectasis, edema and severe inflammatory cell infiltration could be observed in the nasal mucosa tissues (Fig. 3A). Similar to the positive treated rats, these pathological changes mentioned above could be significantly alleviated. Interestingly and importantly, for the rats treated with the high dose of XSF (10 mg/kg), it is showed that mucosa structure seems to be intact with mild inflammatory cell infiltration, compared to the AR control rats.

Effects of XSF on the releases of histamine in BMMC

As shown in Table 5, effects of XSF on the releases of histamine in BMMC were described. Besides the positive drug, our results indicated that the histamine releases could be also statistically significantly suppressed by treatment with XSF at the concentrations of 25, 250 and 2500 mg/ml ($p < 0.01$), compared to the AR control rats with the inhibitions of 22.76, 59.44 and 80.89%, respectively.

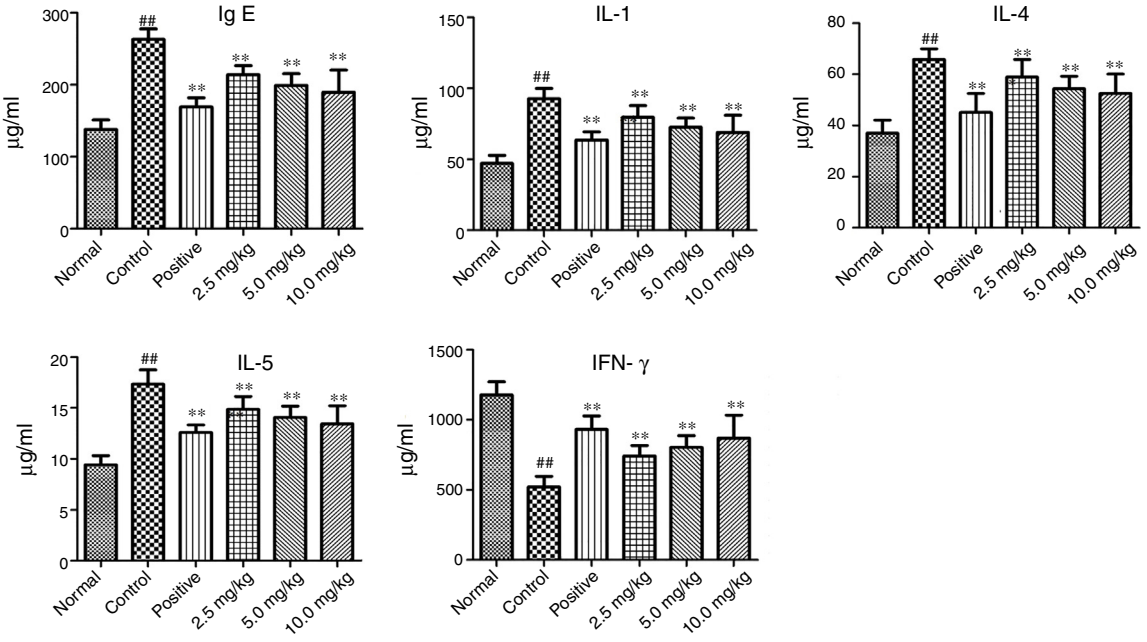


Fig. 2. Effects of fruits of *Xanthium strumarium* on the serum levels of Ig E, IL-1, IL-4, IL-5 and IFN-γ of AR rats. Loratadine was used as the positive drug, and the fruits of *Xanthium strumarium* and positive drug were administered orally (*p.o.*). Data were expressed as mean ± SD ($n = 10$); $^{\#\#}p < 0.01$, compared to normal, $^{**}p < 0.01$, compared to Control.

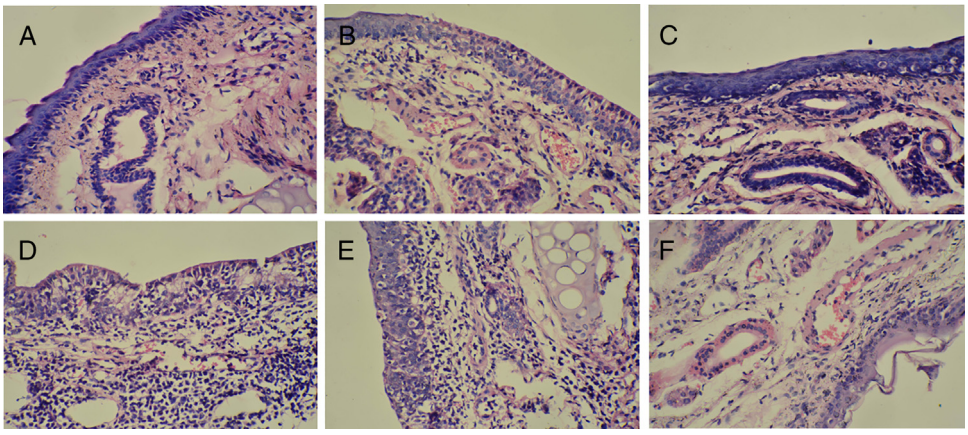


Fig. 3. Results of the histopathological examinations of nasal mucosa tissues of AR rats. A–F represented the histopathological changes of normal, AR control, positive control, fruits of *Xanthium strumarium* (2.5, 5.0, 10.0 mg/kg), respectively. Loratadine was used as the positive drug, and the fruits of *Xanthium strumarium* and positive drug were administered orally (*p.o.*). Tissue sections were stained with H&E (magnification, 200×).

Table 5
Effects of XSF on the releasesof histamine in BMMCs.

	Concentration (μg/ml)	Histamine concentration (μg/ml)	Inhibition (%)	Cell viability (%)
Control	–	2.88 ± 0.25		100
Positive	25	0.57 ± 0.12[a]	80.26	94.28
XSF	25	2.22 ± 0.23[a]	22.76	96.48
	250	1.17 ± 0.24[a]	59.44	95.67
	2500	0.55 ± 0.17[a]	80.89	90.37

The azelastine was used as the positive drug. Data were expressed as mean ± SD ($n = 4$).
[a] $p < 0.01$, compared to control.

Interestingly, at the 2500 mg/ml concentration, the inhibitory effect of XSF on histamine releases was comparable to the positive drug of azelastine at the concentration of 25 mg/ml (80.89% *vs.* 80.26%). Besides, our results also revealed that the testing concentrations of XSF (25, 250 and 2500 mg/ml) have no obvious effect on cell viability of BMMC.

Discussion

Nowadays, allergic rhinitis (AR) is commonly recognized as a chronic disease that markedly affects life quality of patient and may result in substantial medical care expenditures (Corren, 2000; Kakli and Riley, 2016). From time immemorial, plants are the primary

sources of food and drugs, moreover, the plants have continued to provide human beings with new therapeutic remedies and candidate drugs to against various diseases (Wu et al., 2014; Pu et al., 2016). It is known that plant-derived drugs may be safer than the synthetic drugs (Newman and Cragg, 2016), and therefore, searching therapeutic agents for AR from plants is an apparently feasible approach. The fruit of *X. strumarium* is a TCM used to treat rhinitis in the form of compound Chinese medicine or simple recipes (Han et al., 2009). To the best of our knowledge, this report is the first work regarding the anti-allergic rhinitis effects of caffeoylquinic acids from the fruits of *X. strumarium* (XSF) and its possible mechanisms.

The passive cutaneous anaphylaxis test (PCA) and Schulty-Dale test (SDT) are two of the most frequently used and simple models for searching for potential anti-allergic candidate drugs (Corcostegui et al., 2005; Bryce et al., 2016). In our present results, similar to the used positive drugs, XSF at all the testing doses or concentrations exhibited significant inhibitory effects against allergic reactions induced by PCA and SDT, which indicated that XSF is a potential anti-allergic agent for AR treatment. It is reported that the main symptoms of AR in human are sneezing, pruritus and mucosal edema *etc*., and ovoalbumin induced AR rat model is one of the most used tools for investigating the therapeutic effects of the candidate drugs due to its similar nasal allergic symptoms with AR patients (Wang et al., 2009). The immune response related to AR could be divided into the early-phase responses and the late-phase responses. The typical symptoms of early phase response include sneezing, secretions and nasal blockage, which are induced by the histamine released from the IgE-activated nasal mucosal mast cells or other inflammatory cells (Suleimani and Walker, 2007). Interestingly, our present results revealed that XSF treatment could obviously decrease the AR symptom scores of the AR rats, and inhibit the releases of histamine in bone marrow-derived mast cells (BMMC), which indicated that XSF is an effective agent that can alleviate the nasal allergic symptoms. AR is an inflammation of the nasal mucosa and is closely related to the IgE mediated immune response corresponding to specific allergens, such as pollens, dust mites, spores of fungi, animal hair *etc*. (Kawase et al., 2006; Skoner, 2001). Thus, IgE plays a crucial role in the pathogenesis of AR, and drugs targeted IgE with fewer side effects are ideal agents for treating AR (Ciprandi et al., 2015). Furthermore, some over-released pro-inflammatory cytokines are close to the exacerbation of inflammatory and allergic reactions of AR, including IL-1, IL-4, as well as IL-5, whereas the IFN-γ is a helpful cytokine for control inflammatory and allergic reactions of AR (Kakli and Riley, 2016; Suleimani and Walker, 2007). The results of our present study revealed that XSF significantly decreased the serum levels of IgE, IL-1, IL-4 and IL-5 of AR rats, whereas increased the IFN-γ level in serum. In addition, histopathological results also suggested that XSF can alleviate the inflammatory reactions in nasal mucosa of AR rats, and the dimethylbenzene-induced edema test in mice also revealed the XSF has potential anti-inflammatory activity.

In conclusion, our results demonstrated that caffeoylquinic acids from the fruits of *X. strumarium* (XSF) possess favorable anti-allergic rhinitis effects *via* alleviating allergic and inflammatory reactions of AR rats *in vivo*, and can be utilized as effective and safe disease preventive or therapeutic agent for AR treatment. However, more investigations are also necessary to fully elucidate the further mechanism of action of XSF in the future.

Ethical disclosures

Protection of human and animal subjects. The authors declare that the procedures followed were in accordance with the regulations of the relevant clinical research ethics committee and with those of the Code of Ethics of the World Medical Association (Declaration of Helsinki).

Confidentiality of data. The authors declare that no patient data appear in this article.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

Authors' contributions

TH and HLX conceived the paper, PH, LYY and BZY completed the experiments, PH and WP analyzed the data, WP, TH, YC and LPQ wrote the paper.

Conflicts of interest

The authors declare no conflicts of interest.

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