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Phytomedicine

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Anti-allergic rhinitis effect of caffeoylxanthiazonoside isolated from fruits of *Xanthium strumarium* L. in rodent animals

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ARTICLE INFO

Article history:

Received 13 April 2013

Received in revised form 24 October 2013

Accepted 11 January 2014

Keywords:

Xanthium strumarium L.

Fructus Xanthii

Allergic rhinitis

AR

Caffeoylxanthiazonoside

ABSTRACT

The fruits of *Xanthium strumarium* L. (Asteraceae) have been used extensively in China for treatment of various diseases such as allergic rhinitis (AR), tympanitis, urticaria and arthritis or ozena. This study was designed to systemically investigate the effects of the caffeoylxanthiazonoside (CXT) isolated from fruits of *X. strumarium* on AR in rodent animals. Animals were orally administered with CXT. Anti-allergic activity of CXT was evaluated by passive cutaneous anaphylaxis test (PCA); acetic acid-induced writhing tests were used to evaluate the analgesic effects of CXT; acetic acid-induced vascular permeability tests were performed to evaluate anti-inflammatory effect of CXT. Then, the model AR in rats was established to evaluate the effects of CXT on AR with the following tests: the sneezing and nasal scratching frequencies, IgE level in serum, and histopathological examinations. Our results demonstrated that CXT had favorable anti-allergic, anti-inflammatory and analgesic effects. Additionally, we found that CXT was helpful to ameliorate the nasal symptoms and to down-regulate IgE levels in AR rats. Thus, we suggested that CXT can be treated as a candidate for treating AR.

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Introduction

Allergic rhinitis (AR) has emerged as a serious public health problem, with an increasing prevalence in recent years (Salib et al., 2003; Kemp, 2009). AR, which is a heterogeneous disorder, is defined as abnormal inflammation of membrane lining the nose and is characterized by one or more of the following main nasal symptoms: sneezing, itching, rhinorrhea, and nasal congestion (Suleimani and Walker, 2007; Mandhane et al., 2011). Currently, the most popular drugs for treating AR are antihistamines and intranasal corticosteroids. However, each of them only provides short or long term relief from one or more of the symptoms of AR instead of cure it. Additionally, the prolonged use of these drugs can induce rhinitis medicamentosa (Black and Remsen, 1980; Suleimani and Walker, 2007). Therefore, to find novel treatment strategies against RA with less side-effect became an urgent problem.

Traditional Chinese medicine (TCM) has been used for centuries and has been demonstrated to be effective in the control of allergic

diseases. What's more, plant-derived medicines could be safer than synthetic drugs (Qiu, 2007). Recent studies showed that TCMs had an immunomodulatory effect on the regulation of TH1/TH2 pathways (Ikeda et al., 2002), the suppression of IgE production (Yang et al., 2001), and the promotion of the activities of natural killing cells and macrophages (Blyth et al., 1996).

The fruits of *Xanthium strumarium* L. (Asteraceae), called Fructus Xanthii, have been used for treatment of various inflammatory diseases including allergic rhinitis, tympanitis, urticaria, arthritis and ozena (Zhu, 1998; Han et al., 2007; Pharmacopoeia PR China I, 2010). Additionally, the genus of *Xanthium* was known to have lots of saponins, flavones, caffeic acids, caffeoylquinic acids, and sesquiterpene lactones (Han et al., 2006; Qin et al., 2006; Bui et al., 2012; Yoon et al., 2008). Although the Fructus Xanthii was claimed to be clinically effective for AR, we did not find sufficient evidence about its efficacy based on well-controlled scientific research. The previous report revealed the aqueous extract of Fructus Xanthii in its role to inhibit mast cell-mediated allergic reaction in murine model (Hong et al., 2003). However, no active components for anti-allergic reaction have been reported yet.

In previous study, we have reported that the extracts of Fructus Xanthii showed significant anti-inflammatory and analgesic properties *in vivo* (Han et al., 2007). As part of our continuing investigation of this plant, we isolated and identified some new thiazinedione heterocyclic compounds (Han et al., 2006; Qin

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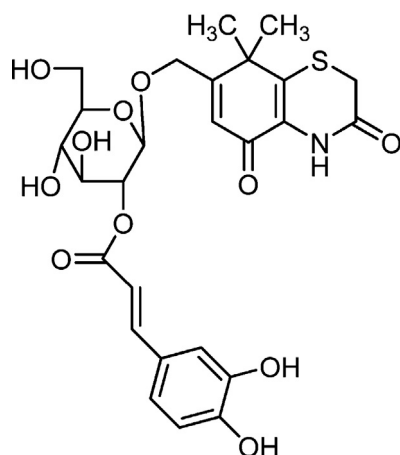


Fig. 1. Structure of the CXT.

et al., 2006) from it. Thus, we further investigated the anti-allergic, anti-inflammatory and analgesic effects of the new compound (caffeoylxanthiazonoside (CXT)) isolated from fruits of *Fructus Xanthii*, with the aim to provide a scientific basis for the clinical use of this compound.

Materials and methods

Plant material

Fructus Xanthii, the ripe fruits of *X. strumarium* were collected from a local research farm in Sunqiao town, Shanghai, in China, and authenticated by Prof. Lu-Ping Qin, Second Military Medical University, Shanghai, China. The voucher specimen of the plant was deposited at the Herbarium of the Department of Pharmacognosy, School of Pharmacy, Second Military Medical University, Shanghai, PR China (No. CE20031107).

Animals

Experimental groups consisted of ICR mice (Institute of Cancer Research) (20 ± 2 g) or SD rats (Sprague-Dawley) (220 ± 20 g). They were housed at 21 ± 1 °C under a 12 h light/dark cycle and had free access to standard pellet diet (Purina chow) and tap water. The animals were deprived of food for 15 h before the experiment, with free access to drinking water. Each animal was used only once in the experiment. The experimental protocols were approved by the Animal Care and Use Committee of our university.

Chemicals

Dimethyl benzene (AR) and CMC-Na were purchased from the Sino. Pharm. Chemical Reagent Co. (Shanghai, China); silica-gel (100–200 and 200–300 mesh) was purchased from Qingdao Haiyang Chemical Co. (Qingdao, China); sephadex LH-20 was purchased from GE Healthcare Co. (USA); Indometacin was purchased from Aladdin Reagent Co. (Shanghai, China); ketotifen fumarate, Evans Blue, and Rat Immunoglobulin E, IgE ELISA Kits were purchased from RuiQi biotechnology Co. (Shanghai, China); ovalbumin was purchased from Worthington (Lakewood, USA); DNP-human serum albumin (DNP-HSA) was purchased from Sigma Chemical Co. (St. Louis, USA); pertussis vaccines were purchased from Shanghai Institute of Biological Products Co. (Shanghai, China). All other chemicals used in this study were analytical reagent grade.

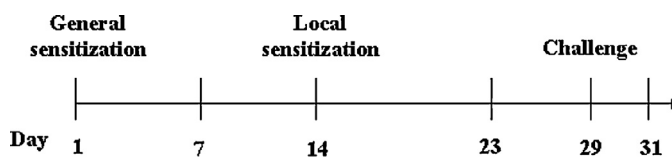


Fig. 2. Schematic diagram for preparation of AR model rats.

Extraction, isolation and preparation of pure compounds

Dried *Fructus Xanthii* was ground and extracted with 75% aqueous ethanol by reflux three times (each extraction period lasted 2 h). The solvent was evaporated under vacuum to afford crude extract. Then the extract was suspended in water and partitioned with petroleum ether, chloroform, ethyl acetate, and aqua-saturated *n*-butanol successively. The *n*-butanol fraction was subjected to repeated column chromatography over silica gel (100–200 mesh) column chromatography and eluted with ethyl acetate–methanol (20:1 to 1:2). Combination of similar fractions on the basis of TLC analysis afforded 5 fractions (I–V). By using a series of chromatographic techniques, such as silica gel column chromatography (200–300 mesh) and Sephadex LH-20 chromatography, the compound was isolated from fraction IV. In addition, the isolated compound was identified as caffeoylxanthiazonoside (Fig. 1) (Qin et al., 2006).

Protocols

Anti-allergic activity of CXT was evaluated by passive cutaneous anaphylaxis test (PCA); acetic acid-induced writhing test was used to evaluate the analgesic effect of CXT; acetic acid-induced vascular permeability test was performed to evaluate the anti-inflammatory effect of CXT. Then, the AR model in rats was established to evaluate the effects of CXT on AR with the following tests: the sneezing and nasal scratching frequencies, IgE level in serum, and histopathological examinations. Dosage of the positive control was determined on the basis of the principle of pharmacokinetics and clinical use. CXT was administered orally, and the dose selection of 10 mg/kg/day was based on the results of preliminary experiments. Normal and control groups were treated with an equivalent volume of the vehicle (0.5% CMC-Na) that had been used to dilute this compound.

Grouping of animals

For testing the anti-allergic, anti-inflammatory, and analgesic effects of CXT isolated from *Fructus Xanthii*, there were 5 groups were consisted, including control, positive control, CXT (5, 10, and 20 mg/kg) groups ($n = 10$). In addition, total 60 AR rats were prepared to evaluate the effects of CXT on AR, and equally divided into the following 5 groups ($n = 10$): normal, control, positive control, CXT (5, 10, and 20 mg/kg) groups.

Preparation of AR model rats

AR model of rats was prepared as the method described previously (Tasaka et al., 1995) with minor modifications (Fig. 2). Briefly, rats were generally sensitized by intraperitoneal injection of 1 ml of physiological saline containing egg albumin (3 mg) and alum (10 mg) on the first day. Five days later (day 7), they were boosted in the same manner. Then, local sensitization was performed from day 14 to day 23 by dripping the egg albumin in physiological saline (1 mg/ml, 10 μ l) into the bilateral nasal cavities. After 5 days, the animals were challenged intranasally with 1% ovalbumin saline solution, 100–150 μ l per nostril once every day for 3 consecutive days (from day 29 to day 31). Testing drugs were administered 1 h

before nasal antigen challenge from day 14 to day 31 after general sensitization.

Passive cutaneous anaphylaxis test

PCA was performed to evaluate anti-allergic activity of CXT, and the detail procedure was described in previous report (Poulsen and Hau, 1987; Shin et al., 2004). Briefly, mice were injected intradermally with 100 ng of anti-DNP IgE into each of three dorsal skin sites, and the sites were marked. Each mouse was injected of 200 μ l of the mixture of 1 mg/ml DNP–HAS in PBS (1:1) and 4% Evans blue via the tail vein after 1 day, and 1 h before the injection, testing drugs were administered orally. Then, mice were sacrificed 40 min after the antigen-challenge under anesthetized, and the dorsal skin with the pigment of the mouse was collected. Finally, the amount of dye was determined by colorimetry at 610 nm.

Assay of acetic acid-induced vascular permeability in mice

The vascular permeability test was determined by colorimetrically following the method of Li et al. (2007) and Li et al., 2013 with a minor modification. Briefly, 0.75% (v/v) acetic acid in normal saline (10 ml/kg) was injected into the abdominal cavity at 1 h after the final administration of the testing compound and positive agents. Simultaneously, 1% (w/v) Evans blue in normal saline (10 ml/kg) was injected into vena caudalis. After 20 min, the mice were sacrificed by decapitation and then the pigment that had leaked into the abdominal cavity of each mouse was rinsed with 5 ml of normal saline solution. The wash solution was recovered and centrifuged at $780 \times g$ for 15 min; the absorbance of the supernatant at a wavelength of 590 nm was measured. The vascular permeability effects were expressed in terms of the absorbance of dye that leaked into the peritoneal cavity.

Abdominal constriction induced by acetic acid in mice

The writhing test was used to evaluate the analgesic activities, and the test was carried out using the method described in previous, with minor modification (Chen et al., 2009). The testing samples were administered orally, and 30 minutes after administration, the animals received 10 ml/kg body wt. acetic acid by peritoneal injection (0.7%). The number of abdominal contractions and stretching with a jerk of the hind limb were counted for 15 min after administering acetic acid. The percentage of protection was calculated using the following ratio: (control mean-treated mean) $\times$ 100/control mean.

Sneezing and nasal scratching frequencies test

Before the test, the AR rat was placed into the observation cage (32 cm $\times$ 22 cm $\times$ 10 cm) for about 10 min for acclimatization. After

nasal instillation of 10 μ l of egg albumin dissolved in physiological saline solution (1 mg/ml) into the bilateral nasal cavities, the rats were placed into the observation cage (1 rat/cage). The numbers of sneezes and nasal scratching movements induced by the antigen were counted for 30 min (Narita et al. 1996).

Assay of IgE level in serum by enzyme-linked immunosorbent assay (ELISA)

Blood samples were collected from the abdominal aorta after the behavior experiments mentioned in above. Serum samples were prepared after incubation in ice-temperature storage and centrifugation at $1800 \times g$ for 15 min, and were stored at -80°C until analysis. IgE level in serum of the testing rats was analyzed with commercial kits according to the manufacturer's instructions.

Histopathological examinations

Nasal mucosa tissues were harvested and fixed in 10% formaldehyde, then processed routinely, embedded in paraffin, sectioned to 5 μ m thickness, de-paraffinized, rehydrated using standard techniques, stained with hematoxylin and eosin (H&E) (Wang et al., 2011). The histopathological changes were evaluated in tissues sections with microscope (Olympus, Japan).

Statistical analysis

Data are presented as $\bar{x} \pm s$, and were evaluated with one-way ANOVA following by Dunnett 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 the passive cutaneous anaphylaxis test

To evaluate the anti-allergic activity of CXT *in vivo*, the ability of it to inhibit the PCA reaction in mice were tested. The results of the PCA test of our present study were showed in Table 1. As can be seen from the results, CXT (5, 10 and 20 mg/kg) can significantly decrease the OD value compared with the control group, with the average inhibitions of 34.5%, 41.50% and 55.35%, respectively. In addition, the anti-allergic activity of CXT showed a good dose-dependent manner, which indicated that CXT is a potential anti-allergic agent.

Results of the acetic acid-induced vascular permeability test in mice

CXT isolated from Fructus Xanthii had significant inhibitory effect on increased vascular permeability induced by acetic acid in

Table 1
Effect of CXT on PCA in mice ($n = 10$).

	Dose (mg/kg)	OD value		Average inhibition
		1/5 antiserum	1/10 antiserum	
Control		0.054 $\pm$ 0.015	0.056 $\pm$ 0.022	–
Ketotifen	0.4	0.016 $\pm$ 0.007***	0.034 $\pm$ 0.005*	54.85%
	5	0.037 $\pm$ 0.022	0.035 $\pm$ 0.016*	34.50%
CXT	10	0.032 $\pm$ 0.016*	0.028 $\pm$ 0.012*	41.50%
	20	0.026 $\pm$ 0.006**	0.023 $\pm$ 0.007**	55.35%

The vehicle (control, 10 ml/kg), ketotifen, and CXT were administered orally. Asterisks indicated significant difference from control.

* $p < 0.05$.

** $p < 0.01$.

*** $p < 0.001$.

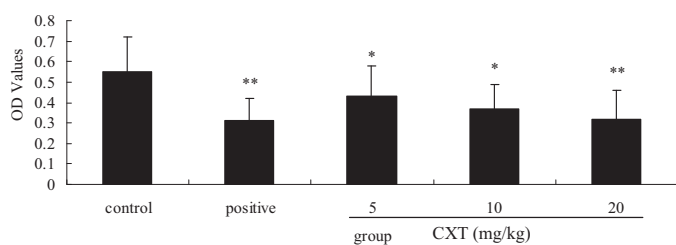


Fig. 3. Effect of CXT on leakage of dye into the peritoneal cavity of mice. Indomethacin was treated as positive control. The vehicle (control, 10 ml/kg), indomethacin, and CXT were administered orally. Each column represented the $\bar{x} \pm s$ ($n=10$). Asterisks indicated significant difference from control. * $p<0.05$ and ** $p<0.01$.

Table 2
Effect of CXT on acetic acid-induced writhing responses in mice ($n=10$).

	Dose (mg/kg)	Number of writhings	Inhibition
Control		38.39 ± 8.62	
Indomethacin	10	$14.22 \pm 1.78^{**}$	62.96%
	5	34.40 ± 5.61	10.39%
CXT	10	$24.14 \pm 3.86^{*}$	37.12%
	20	$18.45 \pm 2.32^{**}$	51.94%

The vehicle (control, 10 ml/kg), indomethacin, and CXT were administered orally. Asterisks indicated significant difference from control.

* $p<0.05$.

** $p<0.01$.

mice at the dose of 5, 10 and 20 mg/kg ($p<0.05$, $p<0.05$ and $p<0.01$, respectively) (Fig. 3). The positive control drug, indomethacin (10 mg/kg), also reduced the dye leakage considerably as compound at the dose of 20 mg/kg.

Results of the acetic acid-induced writhing test in mice

The acid-induced writhing test in mice was performed to evaluate the anti-nociceptive activities of CXT isolated from *Fructus Xanthii*, and the results were presented in Table 2. From our study, CXT was able to significantly reduce the writhes at the doses of 10 and 20 mg/kg ($p<0.05$ and $p<0.01$) compared with the control group, with the inhibitions of 37.12% and 51.94%, respectively.

Effects of the CXT on nasal symptoms on the experimental model of allergic rhinitis in rats

As can be seen from Fig. 4, in the AR control group, the numbers of sneezing and nasal scratching were significantly increased compared with the normal group ($p<0.001$). In contrary, the ketotifen significantly inhibited antigen-induced sneezings and nasal scratchings ($p<0.01$). CXT significantly induced the decrease of

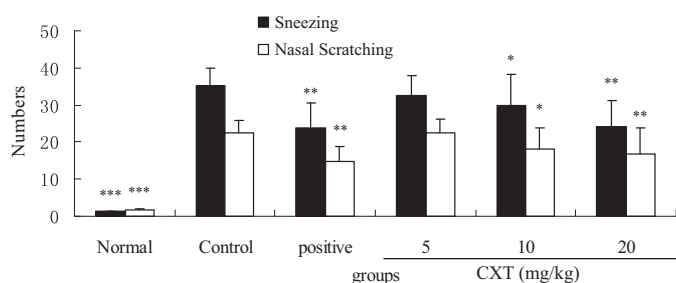


Fig. 4. Effect of CXT on antigen-induced sneezings and nasal scratchings in AR rats. Ketotifen was treated as positive control. The vehicle (control, 10 ml/kg), ketotifen (0.4 mg/kg), and CXT were administered orally. Each column represented the $\bar{x} \pm s$ ($n=10$). Asterisks indicated significant difference from control. * $p<0.05$ and ** $p<0.01$.

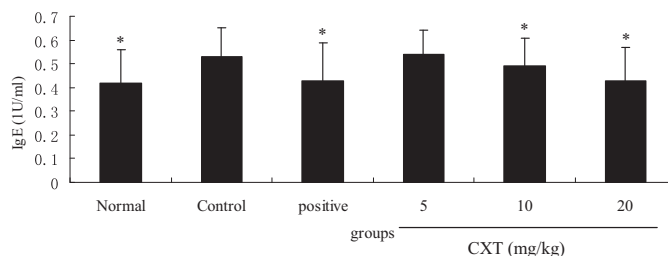


Fig. 5. Effect of CXT on IgE level in serum of AR rats. Ketotifen was treated as positive control. The vehicle (control, 10 ml/kg), ketotifen (0.4 mg/kg), and CXT were administered orally. Each column represented the $\bar{x} \pm s$ ($n=10$). Asterisks indicated significant difference from control. * $p<0.05$ and ** $p<0.01$.

sneezings at the doses of 10 and 20 mg/kg ($p<0.05$ and $p<0.01$); in addition, the nasal scratching frequencies can be also decreased by treating with CXT at the doses of 10 and 20 mg/kg ($p<0.05$ and $p<0.01$).

Effects of the compound on IgE level in serum of AR rats

As shown in Fig. 5, in the AR control group, the IgE level in serum was increased significantly compared with normal rats ($p<0.05$). However, after administration of CXT (10 and 20 mg/kg), the IgE level in serum was significantly decreased compared with the control group ($p<0.05$ and $p<0.05$).

Histopathological changes

Histopathological examinations were performed to evaluate the effects of CXT on histopathological changes, and the results of histopathological changes were shown in Fig. 6. No obvious visible injury can be observed in the tissue sections of normal rats. However, the local necrosis, degeneration, edema were occurred in mucous membrane, and obvious dilatation and congestion were observed in submucosal vascular; in addition, large number of eosinophils infiltrating were found in subepithelial cells and lamina propria. The gland-body hyperplasia in the lamina propria was also observed in tissue sections of the AR model rats (control group). In contrary, the inflammatory reactions of the rats treated with positive agents and the CXT (10 mg/kg) were significantly lessened compared with the AR model group.

Discussion

Fructus Xanthii was a TCM used to treat AR in forms of compound recipes or simple recipe (Pharmacopoeia PR China I, 2010). However, there have been no reports on the bioactive compounds of Fructus Xanthii, which have therapeutical effects on AR. Thus, this study is the first report on the anti-AR effects of CXT, which is one of the bio-active constituents isolated from fruits of *X. strumarium*.

Approximately half of AR cases are caused by allergy, and AR is an inflammation of the nasal mucosa caused by gamma globulin E (IgE) mediated immune response to specific allergens such as pollens, molds, animal dander, and dust mites (Kawase et al., 2006; Skoner, 2001). IgE is considered to be the key mediator in pathogenesis of allergic diseases. IgE is the least abundant antibody class found in human serum, and the presence of elevated levels of serum IgE had been demonstrated to be associated with allergic diseases (Owen, 2007). Thus, drugs targeted IgE with fewer side effects are desirable for treating AR (Chang, 2000). PCA in mice is one of the most frequently used and simple model for searching for potential anti-allergic drugs (Kabu et al., 2006). In our results, CXT could significantly inhibit PCA reaction, which indicated that

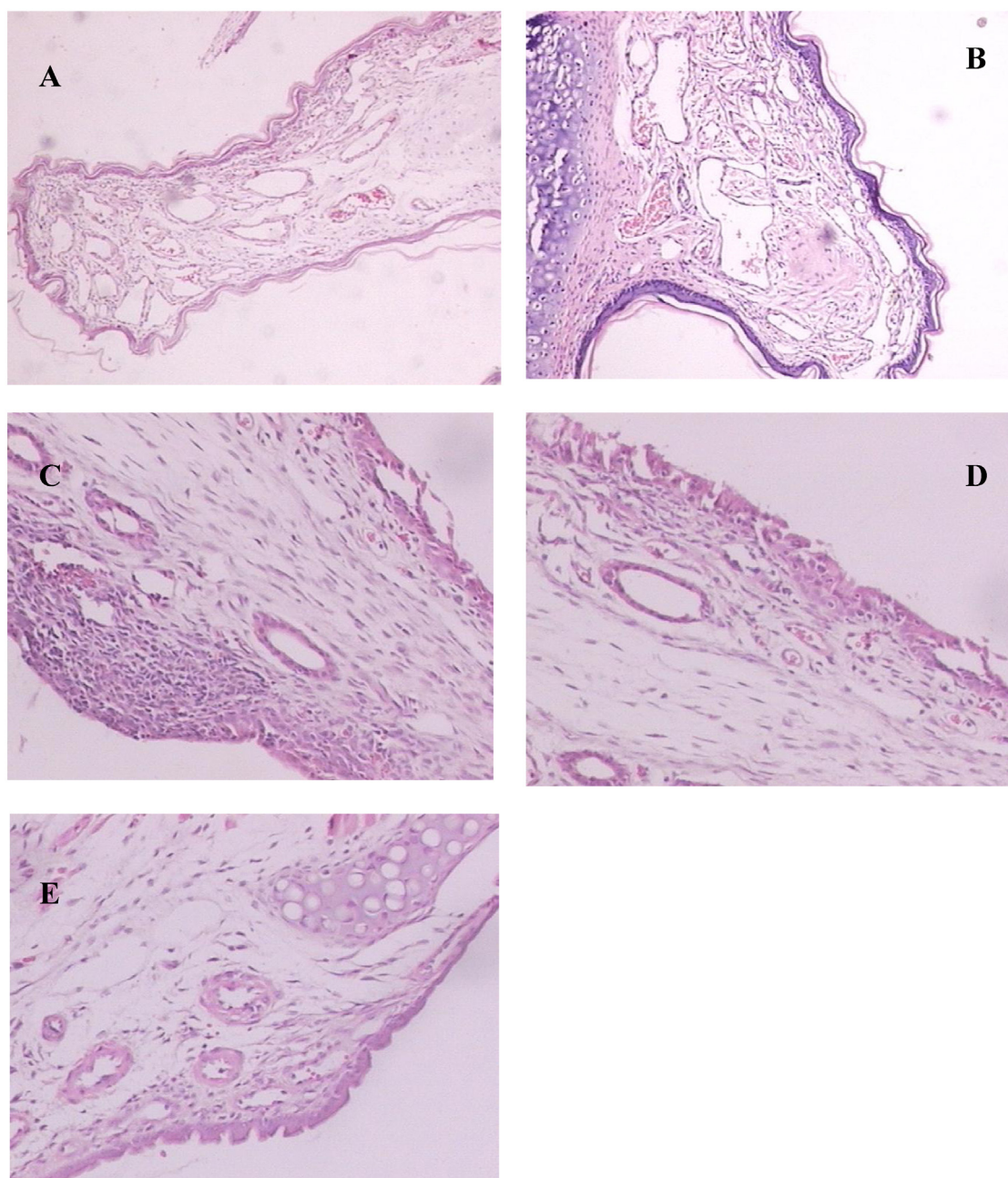


Fig. 6. Results of the histopathological examination. A–E represented the histopathological changes of normal, AR control, positive control, CXT (5, 10, 20 mg/kg), respectively. The vehicle (control, 10 ml/kg), ketotifen (0.4 mg/kg), and CXT were administered orally. Tissue sections were stained with H&E (magnification, 20 $\times$).

CXT is a potential anti-allergic agent for treating AR. Furthermore, the anti-allergic ability of CXT was demonstrated in AR mice model by determining the IgE level in serum.

The main symptoms of AR in humans are sneezing, pruritus, mucosal edema, etc. Therefore, it is necessary to establish an animal model to show the similar nasal allergic symptoms for evaluation therapeutical effects of the test drugs. In our study, CXT can decrease numbers of sneezings and nasal scratchings in AR model rats. Additionally, mucosal edema of AR rats can also be lightened by treating with CXT, which indicated that CXT was an effective agent that can alleviate the nasal allergic symptoms.

The immune response associated with AR can be divided into the early-phase response and the late-phase responses. The characteristic symptoms during the early phase response such as sneezing, secretions and nasal blockage are mediated by histamine

which was released from nasal mucosal mast cells or other inflammatory cells after activation of IgE. As the consequence of the early inflammatory responses involved in AR, enhanced nasal vascular permeability plays a key role in the development of early and late phase symptoms of AR (Kawase et al., 2006). Therefore, decreasing the enhanced vascular permeability of nasal mucosa is one of the feasible approaches for alleviation of AR symptoms. In our study, we demonstrated that CXT can significantly inhibit the increased vascular permeability induced by acetic acid. Additionally, results of histopathological changes also indicated that CXT can alleviate the inflammatory reactions in AR rats. What is more, headache could be appeared in the AR symptoms of some patients (Mandhane et al., 2011). The anti-nociceptive activity of CXT was also revealed by acetic acid-induced writhing which is the most useful model in studying anti-nociceptive drugs (Chen et al., 2009).

In conclusion, the results obtained in this work are noteworthy, and our results demonstrated that CXT have the favorable anti-allergic, anti-inflammatory and analgesic effects. What's more, we also found that CXT was helpful to ameliorate the nasal symptoms and to down-regulate IgE levels in AR rat model. Thus, we suggested that CXT can be treated as a candidate for treatment of AR. However, because of the low amount of CXT, it can be suggested that these observed activities may be associated with the presence of phenolics of known analgesic activity, as well as a probable synergistic effect of the heterocyclic components and other secondary metabolites (Han et al., 2007). However, more investigations are necessary to fully elucidate the mechanism of action of the compound in the future.

Acknowledgement

This work was supported in part by the TCM Modernization Foundation of Science and Technology Commission of Shanghai (Nos. 10DZ1972000 and 13401900102).

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