

Anti-allergic Effects of Total Bakkenolides from *Petasites tricholobus* in Ovalbumin-sensitized Rats

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The anti-allergic effect of total bakkenolides from the rhizome of *Petasites tricholobus* (BAPT) was evaluated in an ovalbumin-induced allergic rhinitis model in male Wistar rats. The major components of the bakkenolide fraction are bakkenolide-D, bakkenolide-B, bakkenolide-IIIa and bakkenolide-IVa, which account for 60.04% of the total. The rats were treated with 40 mg/kg, 20 mg/kg, 10 mg/kg or 5 mg/kg BAPT, and 0.942 mg/kg loratadine and 0.5% gum tragacanth were used as positive and negative controls, respectively. The frequency of nose rubbing and sneezing was observed, the number of eosinophils infiltrating into the nasal tissue was counted, and serum levels of IL-4 and histamine were determined by ELISA. The results showed that BAPT had a beneficial effect on allergic rhinitis in ovalbumin-sensitized Wistar rats, which was evidenced by a significant decrease in the frequency of sneezing, the number of eosinophils infiltrating into the nasal tissue, and the serum levels of IL-4 and histamine. BAPT may therefore be a potential antiallergic drug. Copyright © 2010 John Wiley & Sons, Ltd.

Keywords: *Petasites tricholobus*; total bakkenolides; allergic rhinitis; antiallergic.

INTRODUCTION

Allergic rhinitis is defined as abnormal inflammation of the nasal mucosa, characterized by nasal congestion, rhinorrhea, sneezing and nasal itching with or without postnasal drainage (Bousquet *et al.*, 2001). The number of individuals suffering from allergic illnesses is increasing in industrialized countries and large cities of developing countries such as Brazil. The prevalence and incidence of allergic disorders are becoming high throughout the world (Benicio *et al.*, 2002; Peden, 2005). In some individuals the interaction of environmental and genetic factors can lead to the development of allergic disorders following allergen exposure. The generation of allergen-specific CD4⁺ T helper (Th-2) cells is the initial event responsible for the development of allergic diseases. These cells produce interleukin IL-4, IL-5 and IL-13, which can mediate several regulatory and effector activities. One example of this pathology is allergic rhinitis, which is characterized by the production of immunoglobulin E (IgE) and inflammatory mediators including histamine, leukotrienes (LTs) and prostaglandins (PGs) (Mayr *et al.*, 2003). Studies have been attempted to find strategies to treat allergic diseases through pharmaceutical research, including using medicinal plants as the target and as an important tool to treat immediate-type allergic response (Dai *et al.*, 2004).

Petasites species (Asteraceae) are distributed mainly in the northern Eurasian and North American continents.

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The reported characteristic chemical components of *Petasites* species include sesquiterpenes (bakkenolides) and triterpenoids (Abe *et al.*, 1993; Wu *et al.*, 1999a, 1999b; Yaoita and Kikuchi, 1995; Tori *et al.*, 1998; Neuen-schwander *et al.*, 1979). *Petasites trichinous* Franch, which is distributed widely in southwestern and northwestern China, has been utilized as a folk medicine for the treatment of palsy, hypertension, cough and snakebite. Our previous study reported the structural elucidation of three known bakkenolides and five novel bakkenolides (bakkenolide-Ia, -IIa, -IIIa, -IVa and -Va) that have sesquiterpene lactone functionality (Wang *et al.*, 2006, 2009; Zhang *et al.*, 2008). However, petasin and its isoforms, that do not have lactone functionality, were not found in *P. trichinous*. Other researchers reported that petasins, its isoforms (neopetasin and isopetasin) and the extract of *P. hybridus* growing in Europe had antiinflammatory activity in allergic rhinitis (Thomet *et al.*, 2001, 2002a, 2002b; Brattström *et al.*, 2003; Käufeler *et al.*, 2001). However, the antiallergic activity of bakkenolides from *P. trichinous* has not been studied. To determine the pharmacological efficacy, the effect of total bakkenolides from the rhizome of *P. tricholobus* (BAPT) was evaluated in a Wistar rat model of ovalbumin-induced allergy with respect to biological parameters including IgE, IL-4, serum histamine and histopathology.

MATERIALS AND METHODS

Chemicals. The chemicals used in the present study included ovalbumin (OVA, Worthington; P7K9885), aluminum hydroxide hydrate gel (Sinopharm Chemical Reagent Co., Ltd, China; F20070513), lithium carbonate

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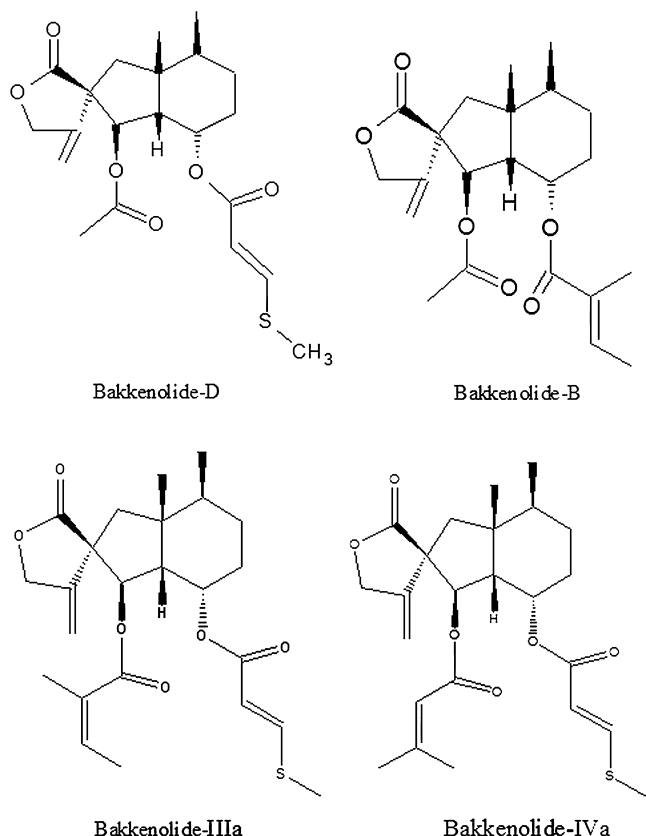


Figure 1. Molecular structures of bakkenolide-D, bakkenolide-B, bakkenolide-IIIa and bakkenolide-IVa.

(Sinopharm Chemical Reagent Co., Ltd, China; F20070408), gum tragacanth powder (Sinopharm Chemical Reagent Co., Ltd, China; WG20071126), hematoxylin (Sinopharm Chemical Reagent Co., Ltd, China; WG20071126), and fuchsin acid (Sinopharm Chemical Reagent Co., Ltd, China; F20070416), Oil Red O (Sigma; O0625-25G) and HPLC-grade acetonitrile (Merck KGaA, Darmstadt, Germany). Water was deionized distilled water.

Preparation and determination of BAPT. BAPT was extracted from the rhizome of *P. tricholobus* Franch, the main components of which were bakkenolide-D, bakkenolide-B, bakkenolide-IIIa and bakkenolide-IVa (Fig. 1) accounting for 1.93%, 4.27%, 19.69% and 34.47%, respectively, by HPLC analysis. The content of BAPT was 60.4% as measured by HPLC.

Experimental conditions: Agilent-ODS (5 μ m, 4.6 $\times$ 250 mm); mobile phase: tetrahydrofuran–acetonitrile–water (28:12:60), constant flow-rate elution, flow-rate: 1.0 mL/min; detection: 265 nm; column temperature: 55°C; time: 32 min; injection volume: 20 μ L.

Animals and experimental protocol. Eighty-four male Wistar rats weighing 100–120 g (SLACOM experimental animal company, Shanghai, China) were housed in an air-conditioned room with 12 h/12 h light-dark illumination cycles at a constant temperature $24 \pm 0.5^\circ\text{C}$ and humidity (45–50%) and acclimated to conditions for 1 week before the experiment with free access to food and drinking water.

Twelve rats were treated with 0.5% west gum tragacanth at a dose of 5 mL/kg as the normal control group. The remaining 72 rats were sensitized by 1 mL physiological saline injection containing egg albumin (1 mg), alum (2 mg) and 10^{10} B pertussis into the four footpads on the first day. Five days later, they were boosted by subcutaneous injection of 1 mL physiological saline containing egg albumin (0.5 mg) in 10 sites on the back. Then, local sensitization was performed every day. From day 14 to day 27, the egg albumin in physiological saline (1 mg/mL, 10 μ L) was dropped into the bilateral nasal cavities with a micropipette (Sakairi *et al.*, 2005; McLeod *et al.*, 2002). On day 20, blood was drawn from all groups via the orbit for the assessment of total immunoglobulin E (IgE). The 72 rats were randomized into six groups according to the frequency of rubbing the nose, one of which was the negative group. The other five groups were treated with BAPT 40 mg/kg, 20 mg/kg, 10 mg/kg, 5 mg/kg and loratadine tablet 0.942 mg/kg in 0.5% west gum tragacanth by intragastric administration at a dose of 5 mL/kg (the positive group) for 7 consecutive days respectively. On day 28, blood was drawn from all groups via the abdominal artery for the assessment of IL-4 and histamine. Nasal mucosa samples were collected and fixed in 10% formalin for histopathological examination and eosinophil counting. This experiment was approved by the Bioethics Committee of the Second Military Medical University (Shanghai, China), and the procedures of the experiment were strictly according to the generally accepted international rules and regulations.

Morphological observations. Between day 21 and 27, the rats were treated with OVA 1 h after administration, and the frequency of rubbing the nose in 30 min after sensitization was observed.

Determination of biological parameters in serum. Each blood sample was centrifuged at 4500 rpm for 10 min; the supernatant serum was used for the determination of IgE and IL-4. Serum IgE, IL-4 and histamine levels were measured by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions.

Histopathological examinations. The nasal mucosa was dissected and fixed in 10% formalin, processed routinely, paraffin embedded, sliced to 4 μ m sections, paraffinized, dehydrated by standard technique and stained with Luna (Nakamaru *et al.*, 2005).

Statistical analysis. Data were expressed as mean $\pm$ SD. Differences between different groups were analysed using one-way analysis of variance (ANOVA) with Student's *t*-test. Values of $p < 0.05$ were considered statistically significant.

RESULTS

Evaluation of the allergic rhinitis model

Serum IgE increased significantly in the treatment groups after OVA administration compared with the control group (Table 1), indicating that the allergic rhinitis model was established successfully.

Morphological observation

From day 21 to 27, the frequency of rubbing the nose was counted between 30 min and 2 h after drug administration. Compared with the normal control group, the frequency of rubbing the nose decreased markedly and progressively with prolonged duration of drug administration in the loratadine group and the four BAPT groups (Fig. 2), but remained almost unchanged in the 0.5% west gum tragacanth powder group.

Biological parameters in serum

As shown in Table 2, the serum IL-4 level of rats in the positive group and four BAPT groups was significantly lower than that in the negative group, and significantly higher in all five treatment groups than that in the control group, indicating that administration of BAPT and loratadine tablet significantly reduced the serum IL-4 level. In addition, high-dose BAPT was as effective as the loratadine tablet. It was also found that BAPT and loratadine tablet significantly reduced the serum histamine level compared with the negative group (Table 3).

Histopathological change

The number of infiltrating eosinophils in the nasal mucosa decreased significantly in the treatment groups

Table 1. Effect of OVA on serum IgE level

Group	IgE level in serum ($\mu\text{g/mL}$)
Treatment group	16.32 ± 12.67^a
Non-treatment group	6.77 ± 5.67

The values are expressed as mean $\pm$ SD.

^a $p < 0.05$, compared with non-treatment group.

compared with the control group (Fig. 3A). Large numbers of infiltrating eosinophils were seen overlapping in the negative group (Fig. 3B). However, the number of infiltrating eosinophils was decreased in different degrees in the positive group and BAPT groups.

Table 2. IL-4 level in serum

Group	Number	IL-4 level in serum (pg/mL)
Control group	12	135.86 ± 61.61^b
Negative group	12	621.58 ± 87.03
Small dose group	12	575.33 ± 87.90
Low dose group	12	544.92 ± 96.59
Middle dose group	12	518.25 ± 124.85^a
High dose group	12	443.44 ± 93.10^b
Positive group	12	364.95 ± 126.94^b

The values are expressed as mean $\pm$ SD of the OD.

^a $p < 0.05$.

^b $p < 0.0001$ compared with the negative group.

Table 3. Histamine level in serum

Group	Number	Histamine level in serum (ng/mL)
Control group	12	380.87 ± 123.37^a
Negative group	12	556.61 ± 82.15
Small dose group	12	517.38 ± 71.09
Low dose group	12	477.05 ± 67.67
Middle dose group	12	485.96 ± 94.50
High dose group	12	434.58 ± 77.93^a
Positive group	12	423.81 ± 104.66^a

The values are expressed as mean $\pm$ SD of the OD.

^a $p < 0.05$, compared with the negative group.

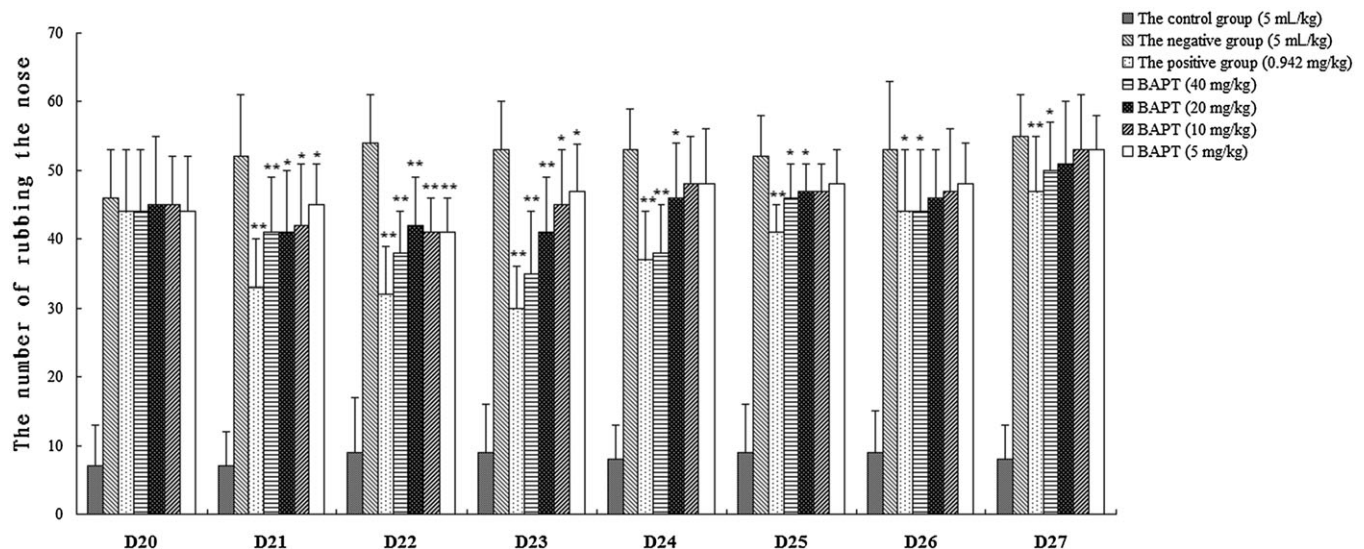


Figure 2. The frequency of rubbing the nose during 30 min after sensitization. The values are expressed as mean $\pm$ SD. * $p < 0.05$, ** $p < 0.01$ compared with the negative group.

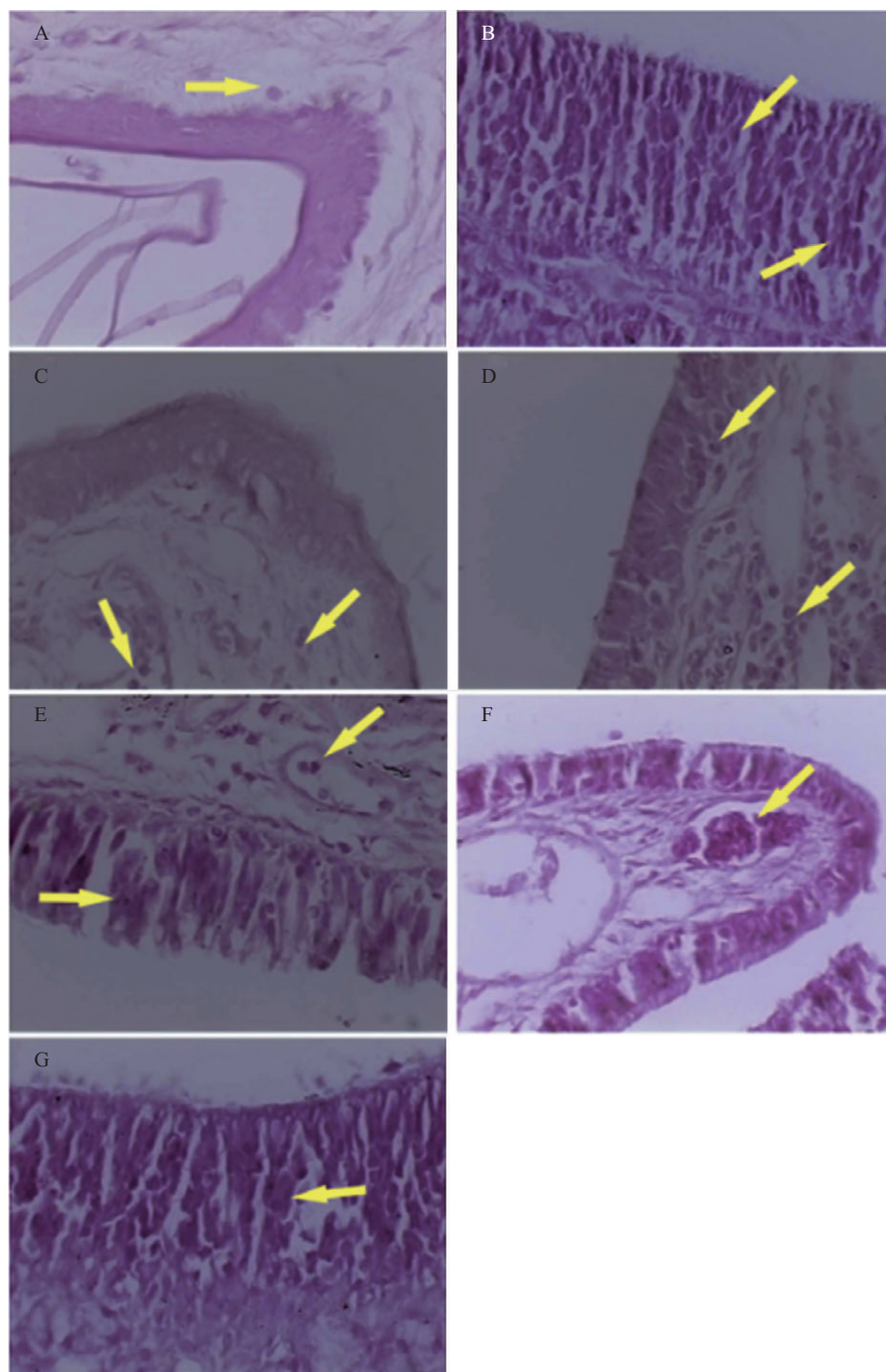


Figure 3. Histopathology of the nasal mucosa (×400), all yellow arrows in figures refer to one infiltrating eosinophil, or many. (A) The normal control group (treated with 0.5% west gum tragacanth powder). (B) The negative group (treated with 0.5% west Gum tragacanth powder). (C) Treated with the loratadine tablet (0.942 mg/kg). (D) Treated with BAPT (40 mg/kg). (E) Treated with BAPT (20 mg/kg). (F) Treated with BAPT (10 mg/kg). (G) Treated with BAPT (5 mg/kg).

DISCUSSION

The present study showed that BAPT had bioactivity on allergic rhinitis in ovalbumin-sensitized Wistar rats; the parameters analysed were IgE, IL-4 and histamine OVA-specific production in serum, the number of infiltrating eosinophils in the nasal mucosa and the frequency of rubbing the nose. Elevated levels of IgE antibody are associated with allergy such as allergic rhinitis. In sensitized individuals, the nasal mucosa is full of IgE-binding mast cells (Enerback *et al.*, 1986), during the period of sensitization, increasing numbers of IgE

coated mast cells traverse the epithelium and once challenged, these recognize the mucosally deposited allergen and degranulate (Naclerio, 1991). Products of degranulation include investigated mediators such as histamine, tryptase and other enzymes. In addition mast cells also create some inflammatory mediators including prostaglandin D₂ and the sulfidopeptidyl leukotrienes LTC₄, LTD₄. These mediators cause blood vessel leakage, produce mucosal edema, and the watery rhinorrhea characteristic of allergic rhinitis, it also stimulates sensory nerves to cause nasal itch and congestion as well as recruiting systemic reflexes such as sneezing (Mygind and Naclerio, 1993).

The other major feature of allergic rhinitis is the high expression of Th-2-type cytokines; T lymphocytes of the T-helper 2 subpopulation can generate IL-3, IL-4, IL-5 and other cytokines that promote IgE production, eosinophil chemoattraction and their own survival in tissues, as well as mast cell recruitment (Mosmann and Coffman, 1989; Durham *et al.*, 1992). The Th2 phenotype is thought to influence subsequent T cell activation and IgE production by B cells in addition to promoting the attraction, activation, growth and differentiation of specific leukocytes such as eosinophils. In this way, activated T cells can initiate and propagate allergic inflammation and participate directly in the events responsible for allergic rhinitis.

Histamine plays a pivotal role in allergic inflammation; it is mostly released from the mast cells after the crossing-linking of the surface IgE by allergen, or through mechanisms of IgE (Enerback *et al.*, 1986; Kaliner, 1994; Howarth, 1995), nasal challenge with histamine cause sneezing, pain, pruritus, rhinorrhea and nasal blockade (Doyle *et al.*, 1990). Molecular biology studies have found that four histamine receptor subtypes (H1, H2, H3 and H4), occur in normal nasal mucosa studies with a higher expression of H1 and H2 in atopic individuals (Nakaya *et al.*, 2004; Hirata *et al.*, 1999). Most of the effects of histamine in allergic rhinitis are mediated through H1 receptors (Schmelz *et al.*, 1997; Schneider *et al.*, 2002; Akdis and Blaser, 2003).

Mast cells are constitutive cells within the normal nasal mucosa; connective tissue mast cells constitute 85% of the IL-4 positive mast cells population in the nasal lamina propria (Bradding *et al.*, 1995). The development and maturation of eosinophils also occurs *in situ*

at peripheral sites of inflammation where there are increased numbers of tissue eosinophils (Adamko *et al.*, 2005). After they have infiltrated from the blood into the tissue, there are a lot of eosinophils within the sub-mucosa and epithelium in those with symptomatic rhinitis by the immunohistochemistry of the nasal mucosa. Cytokines secreted by Th-2 cells help to recruit and activate eosinophils in the nose, IL-4 is considered to be pivotal since it up-regulates adhesion molecules that are selective for eosinophil recruitment (Ciprandi *et al.*, 2004).

In conclusion, the results of this study showed that elevated levels of IgE antibody in allergic rats confirmed our model successfully; the results also showed that BAPT which has sesquiterpene lactone functionality from *P. trichinosis* inhibited IL-4 and histamine OVA-specific production, so it is concluded that BAPT mainly reduces the level of histamine by reducing the IL-4 level in serum in allergic rats, thus alleviating the symptoms of allergic rhinitis finally. Also BAPT may have the same mechanism of action as oral H1 antihistamines. As a result, BAPT may be a potential antiallergic drug.

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Conflict of Interest

The authors have declared that there is no conflict of interest.

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