



Anti-allergic effect of bee pollen phenolic extract and myricetin in ovalbumin-sensitized mice

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ABSTRACT

Ethnopharmacological relevance: The bee pollen is used in folk medicine to alleviate allergic reactions. The bee pollen phenolic extract (BPPE) consists in phenolic compounds (flavonoids) from plants picked by *Apis mellifera* bee.

Aim of this study: Here we evaluated the anti-allergic property of the BPPE and the flavonoid myricetin (MYR) in murine model of ovalbumin (OVA)-induced allergy.

Materials and methods: The study focused on the BPPE or myricetin treatment of OVA-sensitized BALB/c mice and their effects on the IgE and IgG1 production, pulmonary cell migration, eosinophil peroxidase (EPO) activity and anaphylactic shock reaction.

Results: The BPPE treatment (200 mg/kg) showed inhibition of the paw edema, IgE and IgG1 OVA-specific production, leukocyte migration to the bronchoalveolar lavage (BAL) and EPO activity in lungs. In addition, BPPE treatment showed partial protection on the anaphylactic shock reaction induced by OVA. Treatment with myricetin (5 mg/kg) also inhibited pulmonary cell migration and IgE and IgG1 OVA-specific production.

Conclusions: These results support the hypothesis the myricetin is one of the flavonoids of BPPE responsible for the anti-allergic effect and a potential tool to treat allergies.

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1. Introduction

The number of individuals suffering with allergic illnesses is increasing in the industrialized, as well as in large cities of developing countries, such as Brazil. Allergies also have reached high prevalence and incidence in all over the world (Benicio et al., 2004; 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 (Th2) cells is the initial event responsible for the develop-

ment of allergic diseases. These cells produce interleukin (IL)-4, IL-5 and IL-13, which mediate several regulatory and effectors activities (Mayr et al., 2003). One example of this pathology is asthma that is characterized by the production of immunoglobulin E (IgE) and inflammatory mediators such as histamine, leukotrienes (LTs) and prostaglandins (PGs) (Mayr et al., 2003).

Thus, drug development has been encouraging researchers to find strategies to treat allergic diseases and the medicinal plants have been the target of these studies and an important tool to treat immediate-type allergic response (Dai et al., 2004).

The bee pollen is used in folk medicine to alleviate colds, ulcers, anemia and allergies (Lynghem and Scagnetti, 1979) and recently German Federal Board of Health has officially recognized bee pollen as a medicine (Linsens and Jorde, 1997).

The bee pollen contains male germ seeds of plants, flowers or blossoms (Linsens and Jorde, 1997) and also flavonoids, products of plants metabolism that present several phenolic structures (Linsens and Jorde, 1997).

Flavonoids are known by their immunomodulatory and anti-inflammatory activities and inhibiting pro-inflammatory cytokine

Abbreviations: BPPE, bee pollen phenolic extract; MYR, myricetin; IgE, immunoglobulin E; IgG₁, immunoglobulin G₁; OVA, ovalbumin; LTs, leukotrienes; PGs, prostaglandins; GM-CSF, granulocyte-macrophage colony-stimulating factor; DMSO, dimethyl sulfoxide; BAL, bronchoalveolar lavage; EPO, eosinophil peroxidase; OPD, ortho-phenylenediamine; OD, optical density; PCA, cutaneous anaphylaxis.

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production and their receptors (Dudov et al., 1994; Qin and Sun, 2005). Some flavonoids are also recognized by their antioxidant and radical scavenging properties (Campos et al., 2003). In addition, flavonoids have been shown to affect many enzyme systems involved in allergic and inflammatory responses such as tyrosine and serine–threonine protein kinases, phospholipase A2, phospholipase C and lipoxygenase (Middleton et al., 2000).

Flavonoids such as myricetin (MYR), kaempferol, quercetin, flavone and luteolin inhibited histamine, LTs, PGs and granulocyte-macrophage-colony stimulating factor (GM-CSF) production from cultured human mast cells, IL-4 and IL-13 from activated basophils and intracellular calcium levels and protein kinase C θ phosphorylation in human mast cells (Kimata et al., 2000; Theoharides et al., 2001; Alexandrakis et al., 2003; Hirano et al., 2004; Kempuraj et al., 2005).

The bee pollen phenol extract (BPPE) used in this study consists basically in phenolic compounds from several plants picked by *Apis mellifera* bee and combined with plant nectar and bee saliva (Campos et al., 1997).

Since flavonoids are phenolic compounds and some of them have shown immunomodulatory activity, we evaluated the effect of the BPPE and the flavonoid myricetin, one of the compounds presents into the extract, in the murine model of ovalbumin-induced allergy. The study focuses on their effects, such as anaphylactic immunoglobulin (IgE and IgG₁) production, pulmonary cell migration, lung eosinophil peroxidase (EPO) activity and anaphylactic shock reaction.

2. Methodology

2.1. Animals

Male or female BALB/c mice (25–30 g) and Wistar rats (250–300 g) were used throughout the study. Animals were maintained with free access to water. They were obtained from the animal facility of the Laboratório de Tecnologia Farmacêutica (Federal University of Paraíba) and from the Oswaldo Cruz Foundation breeding unit. In each experiment were used groups of 5–10 animals. The experimental protocols for the use of animal subject were approved by the Institutional Review Committee of the Federal University of Paraíba, Brazil (CEPA No. 0612/05).

2.2. Bee pollen phenolic extract preparation

The bee pollen (palynotheca – PUEF-528, -529, -530, -531 – *Eucalyptus*, *Cecropia*, *Eupatorium*, *Mimosa*) was identified by palynotheca's slides comparison of the Plant Micromorphology Laboratory (Universidade Estadual de Feira de Santana, Brazil) by Dr. Francisco de Assis Ribeiro dos Santos. The freshly collected pollen was dried at 40 °C with light protection and grounded into powder. The material (162 g) was extracted with 70% ethanol three times under reflux during 2 h. After dissolved in MeOH and water (1:1), the extract was partitioned with *n*-hexane and ethyl acetate. The combined solution was concentrated under reduced pressure in rotavapour at 45 °C to evaporate the solvent, yielding about 9 g of bee pollen phenolic extract.

The BPPE was redissolved in methanol high-performance liquid chromatography (HPLC) grade (10 mg/mL). Aliquot sample was filtered through a 0.45 μ m filter unit (SUPELCO, USA) and then analyzed by HPLC equipment. The HPLC-DAD analysis was carried out on a product from Shimadzu Instruments Co., Ltd., with a pump model SCL-10Avp and a detector model SPD-M10Avp (photo diode array detector). A supelcosil LC-18 (125 cm $\times$ 10 mm $\times$ 5 μ m, Supelco, Bellefonte, USA) analytical HPLC column was employed for analysis. The mobile phase consisted of solvents A (0.1% formic acid in distilled water) and B (MeOH). The solvent flow rate was 1 mL/min, and the wavelength of the PDA was 360 nm. The injection volume was 10 μ L of the sample, the linear gradient of HPLC solvent was as follows: 0–3 min 40%B, 5–15 min 45%B, 17–25 min 50%B and 27–43 min 55%B, 45 min 40%B. The next sample was injected after 15 min. The genuine standards of flavonoids were acquired as follows: myricetin (Acros Organics), kampferol (Sigma–Aldrich) and taxifolin, isoquercetin, tricetin, naringenin, quercetin, luteolin, selagin and isoramnetin were previously isolated from pollen loads studied in our laboratory (Lins et al., 2003; Silva et al., 2006). The chromatogram of phenolic extract (Fig. 1) showed the presence of five flavonoids, four of them identified as myricetin, tricetin, quercetin and luteolin. One of these compounds (myricetin) was purchased from Sigma–Aldrich (St. Louis, MO) for tests.

After analysis by HPLC-DAD 400.0 mg of BPPE were submitted to column chromatography using Sephadex LH-20 and MeOH as eluent furnished the flavonoids myricetin (7 mg, ~2%) and tricetin (12 mg, 3%). The NMR spectrum was used to identify the structures of natural products isolated from the pollen.

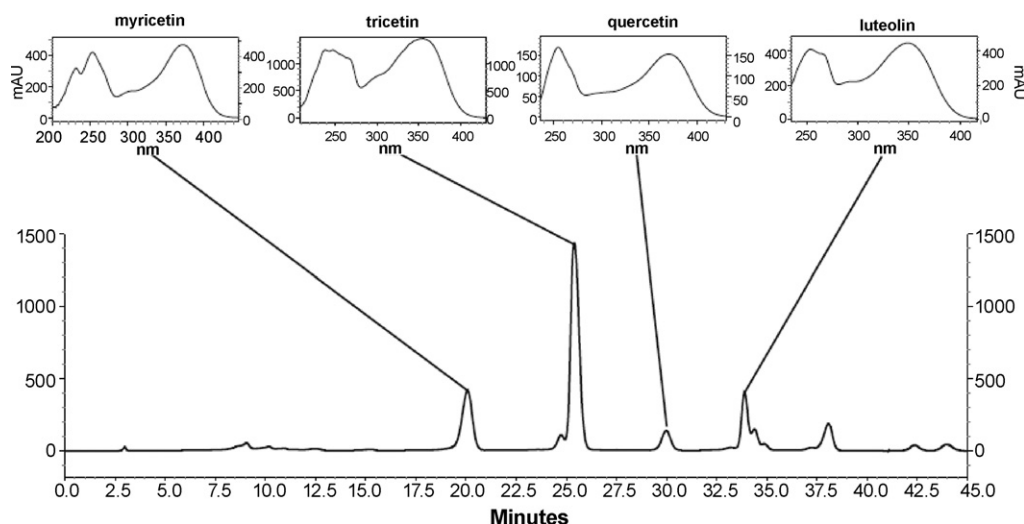


Fig. 1. The high-performance liquid chromatogram (HPLC-DAD) of bee pollen phenolic extract showing the presence of flavonoids (myricetin, tricetin, quercetin and luteolin).

2.3. Bee pollen phenolic extract treatment

One hour before each ovalbumin sensitization and challenge, the animals were treated with BPPE (50, 100 or 200 mg/kg) dissolved in 3% cremor and 1% DMSO in saline. The groups of animals were defined as: saline: non-sensitized and non-treated mice; BPPE 200: treated with 200 mg/kg of the BPPE and non-sensitized mice; OVA: non-treated and OVA-sensitized mice; BPPE/OVA: treated with BPPE and OVA-sensitized mice.

2.4. Sensitization and ovalbumin-induced paw edema

On days 0 and 14 BALB/c mice ($n = 7–10$) were actively sensitized with OVA (10 μ g; grade V Sigma Chemical Co., St. Louis, MO, USA) adsorbed to 2.25 mg of $\text{Al}(\text{OH})_3$ using a modified protocol (Oshiba et al., 1996). Three weeks after the first OVA injection, animals were exposed to aerosol of OVA (1%, w/v) during 30 min. Saline replaced OVA for the non-sensitized group (Randolph et al., 1999). Seventy-two hours after the challenge, the treated, non-treated and non-sensitized groups were challenged with 20 μ L OVA solution (400 μ g) in the left footpad and with 20 μ L of saline in the right footpad. The edema's measurement was performed with the aid of a gauge caliper (Mitutoyo, Japan) before and after (30 min) the OVA challenge (De Siqueira et al., 1997).

2.5. Determination of IgE OVA-specific titer by the PCA reaction

The determination of the IgE titers were done using passive cutaneous anaphylaxis (PCA) reaction. Serum dilutions were inoculated intradermally on the shave backs of Wistar rats. After 48 h the rats received intravenous injection of OVA (10 mg/kg) in Evans blue solution (1%). Thirty minutes after the rats were euthanized and the dorsal skins were removed. The IgE OVA-specific titer was determined by measurement of pigment area. The highest serum dilution giving a 5 mm diameter flare or bluing reaction was taken as the PCA titer (Holt et al., 1981).

2.6. Measurement of IgE and IgG₁ OVA-specific by enzyme-linked immuno sorbent assay (ELISA)

Immediately after sera sampling collected, the IgE and IgG₁ OVA-specific levels, were measured by ELISA. Briefly, a 96-well microtiter plates (Corning, NY, EUA) were coated with 4 μ g/mL of anti-IgE (Pharmingen, USA) or 20 μ g/mL of OVA, respectively in 50 μ L coating buffer and kept overnight at 4°C. The plates were washed three times with PBST (PBS containing 0.05% Tween 20) and blocked with 50 μ L of blocking buffer (1% bovine serum albumin—Sigma in PBST). It was kept at 37°C for 2 h. After washing three times with PBST, 100 μ L of serum it was added dilutions to the wells of plates and it was kept overnight under a temperature 4°C. Once again, the plates were washed three times with PBST. For the IgE analysis the plates were incubated with biotinylated OVA in PBST and for the IgG₁ analysis they were incubated with biotinylated anti-IgG₁ (1:1000 dilution, Pharmingen, USA) in PBST. After washing, the plates were incubated with 50 μ L of horseradish peroxidase-labeled avidin (Pharmingen, USA) under room temperature for 30 min and then washed three times with PBST. A solution of substrate (o-phenylenediamine and H_2O_2) was added within each well and incubated under room temperature. To stop the reaction it was used 2.4 M H_2SO_4 . The plates were read at 450 nm in the ELISA plate reader (BioRad, USA). The results were given in optical density (OD) values.

2.7. OVA-induced anaphylactic shock reaction

In a separated experiment, BPPE-treated OVA-sensitized mice were injected intravenously (i.v.) with 200 μ L Evans blue solution (0.5%) containing OVA (500 μ g) as described (Grunewald et al., 1998), 23 days after initiation of OVA sensitization. The mortality was monitored for 1 h after induction of anaphylactic reaction.

2.8. Bronchoalveolar lavage (BAL) fluid

BALB/c mice ($n = 5$) were sensitized with two subcutaneous injections (days 0 and 7) of OVA (100 μ g; grade V Sigma Chemical, St. Louis, MO, USA) adsorbed to 4 mg/mL of $\text{Al}(\text{OH})_3$ in saline. One week after the second injection, the animals were challenged intranasally (i.n.) with OVA (10 μ g) in 50 μ L of saline (Gaspar Elsas et al., 1997). Twenty-four hours later, the groups were sacrificed using pentobarbital (37 mg/(kg body weight), i.p.). The BAL was collected after serum collection (Waxman et al., 1998). Shortly, the trachea was cannulated and lungs were washed four times with 0.5 mL of HBSS. About 1.5 mL of BAL was recovered per mouse. The count of total leukocytes was immediately performed in a hemocytometer after BAL collection. Differential cell counts were obtained by using May–Grunwald–Giemsa-stained cytopsin preparations. A differential count of at least 100 cells was made blinded fashion and according to standard morphologic criteria.

2.9. Eosinophil peroxidase (EPO) activity

The eosinophil peroxidase activity in the cell-free supernatant of lung from BPPE treated BALB/c measured according to the method (Strath et al., 1985). The lung cell suspension was freeze and thawed three times in liquid nitrogen. After centrifugation under 4° during 10 min at 1000 g, the lung extract were placed in 96-well plates (75 μ L/well), followed by the addition of 1.5 mmol/L o-phenylenediamine [OPD] and 6.6 mmol/L H_2O_2 in 0.05 mol/L Tris–HCl, pH 8.0. After 30 min under room temperature, the reaction was stopped with 75 μ L of 0.2 mol/L citric acid, and absorbance of the sample determined at 492 nm in the ELISA plate reader (BioRad, USA).

2.10. Myricetin treatment

To study the effect of the flavonoid myricetin treatment on established airway inflammation, BALB/c mice were sensitized and challenge as described above (Section 2.4) and 1 h before each ovalbumin sensitization and challenge, the animals were treated with myricetin (Sigma–Aldrich, St. Louis, MO) at 5 mg/kg. Animal groups defined as: MYR/OVA: treated with MYR and OVA-sensitized mice; OVA: non-treated and OVA-sensitized mice; saline: non-sensitized and non-treated mice. The total leukocyte and eosinophil numbers on the BAL as well as the IgE and IgG₁ serum quantifications were determined as described above.

2.11. Statistical analysis

Data were statistically analyzed by one-way ANOVA followed by Bonferroni's test using the software GraphPad Prism Version 3.02. Data were expressed as a mean $\pm$ S.E.M. Results with $p < 0.05$ were considered statistically significant. For the OVA-induced anaphylactic shock reaction the statistical analysis were realized using the Fisher's test.

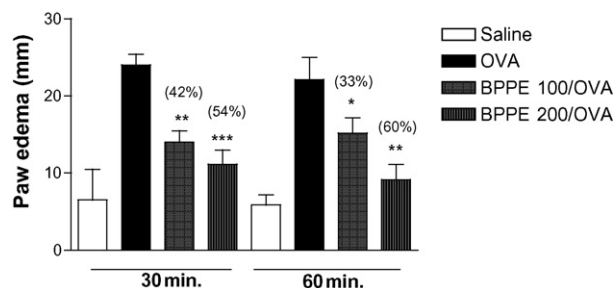


Fig. 2. Effect of the BPPE treatment in the OVA-induced paw edema. The paw edema was measured in mm, at 30 and 60 min after OVA challenge. The results are expressed as means $\pm$ S.E.M. ($n = 5-10$). * $p < 0.05$, ** $p < 0.001$ and *** $p < 0.0001$ as compared with OVA group and analyzed by one-way ANOVA followed by Bonferroni's test.

3. Results

3.1. Effect of BPPE in the ovalbumin-induced paw edema

BALB/c mice treated with BPPE (100 or 200 mg/kg) 1 h before each OVA sensitization and challenge showed an inhibition of the paw edema formation induced by OVA at 42% and 54%, respectively, as compared with OVA group. This phenomenon was observed at 30 and 60 min after challenge. The non-sensitized group (saline) did not present footpad edema after OVA challenge (Fig. 2).

3.2. Effect of BPPE in the production of IgE and IgG₁ OVA-specific

We investigated the ability of BPPE to inhibit the production of anaphylactic IgE and IgG₁. BPPE treatment (200 mg/kg) inhibited both IgE and IgG₁ OVA-specific at 29% and 27%, respectively, when compared with the OVA group (Table 1). In the passive cutaneous anaphylaxis assay, the OVA sensitization induced an IgE OVA-specific titer at 1:128 whereas administration of BPPE at dose of 200 mg/kg reduced the IgE titer by 1:16 corroborating with the results observed above (Fig. 3).

3.3. Effect of BPPE on systemic anaphylactic shock induced by OVA

To demonstrate the possible protective effect of BPPE treatment in the systemic anaphylactic shock reaction it was used the OVA-sensitized and challenge model. The OVA challenge induced a fatal shock in non-treated and OVA-sensitized mice (100% of death). However, BPPE treatment inhibited the mortality at rate by 20% (50 mg/kg), 30% (100 mg/kg) and 50% (200 mg/kg) showing a partial protection of the systemic anaphylactic shock. This inhibition was statistically significant ($p < 0.05$) at the dose of 200 mg/kg of BPPE in relation to the OVA group.

Table 1
IgE and IgG₁–OVA specific levels in BPPE treated and OVA-sensitized BALB/c mice^a

Groups	OVA-specific IgE (OD)	OVA-specific IgG ₁ (OD)
Saline	0.100 $\pm$ 0.0162	0.068 $\pm$ 0.029
OVA	0.253 $\pm$ 0.052	0.426 $\pm$ 0.072
BPPE 200	0.095 $\pm$ 0.011	0.054 $\pm$ 0.096
BPPE 50/OVA	0.221 $\pm$ 0.056	0.327 $\pm$ 0.073
BPPE 100/OVA	0.207 $\pm$ 0.041	0.369 $\pm$ 0.057
BPPE 200/OVA	0.179 $\pm$ 0.024*	0.294 $\pm$ 0.055*

Statistically significant: means $\pm$ S.E.M. of the OD ($n = 5-10$). * $p < 0.05$ as compared with OVA group and analyzed by one-way ANOVA followed by Bonferroni's test.

^a The BPPE (50, 100 or 200 mg/kg) was given 1 h before each sensitized and challenge with OVA.

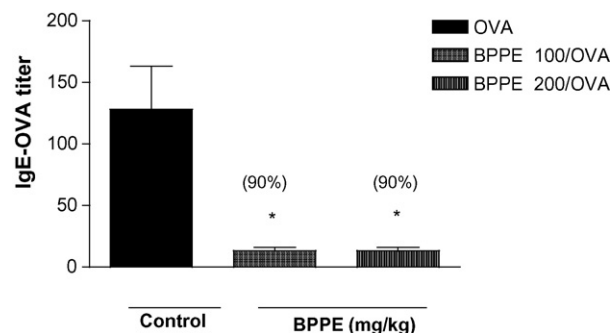


Fig. 3. Effect of the BPPE treatment on the IgE–OVA-specific titer. The PCA reaction was used to measure the IgE–OVA specific. The titers were expressed as the reciprocal of the highest dilution that gave a lesion of >5 mm in diameter. The results are expressed as means $\pm$ S.E.M. ($n = 5-10$). * $p < 0.05$ as compared with OVA group and analyzed by one-way ANOVA followed by Bonferroni's test.

3.4. Effects of BPPE in the pulmonary cell migration

To demonstrate whether BPPE treatment inhibited the lung leukocyte migration it was used the BAL analyses of treated and OVA-sensitized mice. It was observed that BPPE treatment (100 and 200 mg/kg) significantly decreased the total leukocyte numbers in bronchoalveolar fluid by 30% and 52%, respectively, when compared with OVA group. The total cell numbers of the BPPE control group (animals treated with 200 mg/kg of the BPPE and non-sensitized) were similar as the saline group (Fig. 4).

3.5. Lung eosinophil peroxidase activity

The eosinophil infiltration was evaluated by EPO levels (pH 8.0) in the lung tissue. The BPPE treatment inhibited EPO activity at 25% (50 mg/kg), 33% (100 mg/kg) and 62% (200 mg/kg) as compared with OVA group. The OVA group showed an increased EPO activity in comparison with saline and BPPE 200 groups indicating the presence of eosinophil infiltration in the OVA group (Fig. 5).

3.6. Anti-allergic property of the myricetin treatment

The flavonoid myricetin was able to reproduce the inhibition of the pulmonary cell migration and the IgE and IgG₁ productions in the actively sensitized mice. The myricetin treatment was able to reduce both the total leukocyte numbers (43%) and eosinophil (73%) into the lungs of these animals (Fig. 6) as compared with the OVA group. The myricetin treatment also decreased statistically ($p < 0.001$) the production of IgE and IgG₁ OVA-specific (0.29 ± 0.02

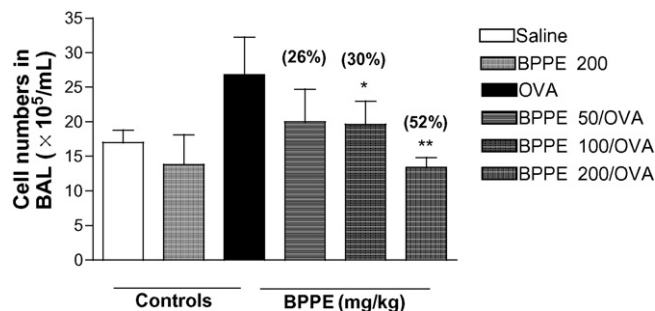


Fig. 4. Effect of BPPE treatment on the airway cell migration. The airway cell migration was measured in the BAL of OVA-sensitized and challenges BALB/c mice. The BPPE treatment was made 1 h before the OVA challenge. The results are expressed as means $\pm$ S.E.M. ($n = 5-10$). * $p < 0.05$, ** $p < 0.001$ as compared with OVA group and analyzed by one-way ANOVA followed by Bonferroni's test.

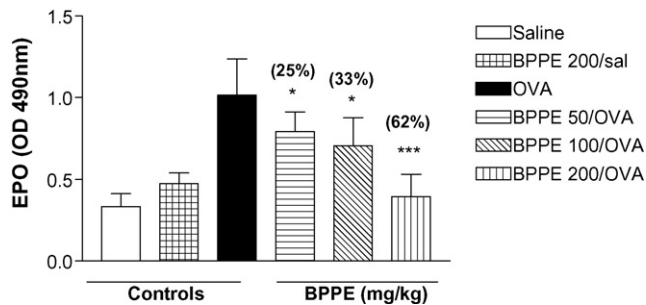


Fig. 5. Effect of the BPPE treatment in the lung eosinophil peroxidase (EPO) activity. The EPO activity was measure in the cell-free supernatant of lung from BPPE treated or non-treated BALB/c mice ($n=5-10$). The results are expressed as means $\pm$ S.E.M. ($n=5-10$). * $p < 0.05$, *** $p < 0.0001$ as compared with OVA group and analyzed by one-way ANOVA followed by Bonferroni's test.

and 0.54 ± 0.06 OD, respectively) as compared with the OVA group (0.50 ± 0.02 and 0.80 ± 0.04 OD, respectively). These results demonstrated that myricetin is one of the flavonoids into the bee pollen extract responsible for the anti-allergic property observed in the BPPE treatment.

4. Discussion and conclusions

We report here for the first time that BPPE effectively reduced immunological parameters in murine model of OVA-induced allergy. The parameters analyzed were the paw edema, anaphylactic IgE and IgG₁ OVA-specific production, the cell migration to the lungs, EPO production and the mortality caused by anaphylactic shock reaction. These activities are correlated to the immunomodulatory and anti-inflammatory properties of the flavonoid myricetin present into the BPPE.

The BPPE-treatment inhibited paw edema induced by OVA, an immediate-type allergy reaction. Several studies have showed that 5 min after antigen challenge the mast cells release histamine, proteoglycans (heparin, chondroitin sulfate E) and proteases (chymase, tryptase carboxypeptidase A). Lipid-derived mediators are produced in about 5–30 min, including the arachidonic acid metabolites: prostaglandins, thromboxanes and leucotrienes. All of these mediators contribute to the edema formation in an established acute inflammatory process (Mayr et al., 2003). In this way, BPPE treatment could be inhibiting the production of the inflammatory mediators responsible for the edema formation or interfering with the mast cell degranulation.

Our results also showed that BPPE inhibited both IgG₁ and IgE OVA-specific production. Elevated levels of IgE and IgG₁ antibodies are associated with allergies such as asthma. IgG₁ and IgE bind to FcγRIII and FcεRI, respectively, on the surface of murine mast cells and activate these cells to release the inflammatory mediators including pro-inflammatory cytokines (Miyajima et al., 1997). The results present here suggest that BPPE treatment has anti-allergic effect through the decrease of IgE and IgG₁ production and concomitantly mast cell inhibition.

The BPPE treatment reduced the total leukocyte number into the BAL and the EPO levels in the airway. The eosinophil infiltration and the synthesis of inflammatory mediators such as cytokines in the airway are responsible for the epithelial damage in allergic reactions (Rothenberg, 1998; Sabroe et al., 2001; Lampinen et al., 2004). Eosinophil peroxidase is one of the most abundant proteins in eosinophils granules, and it is used as a cell specific marker (Metso et al., 2002). It has been shown that patients with allergic rhinitis show an increase in the levels of EPO-stained mucosal eosinophils and free EPO-stained granules in nasal biopsies (Erjefalt et al., 1999). It was also demonstrated the presence of eosinophils in

the blood, bronchoalveolar lavage fluid and thoracic lymphnodes after allergen sensitization and challenge in mice. Our results suggest that the BPPE inhibited the cell migration to the lung as well as the eosinophil activation.

In addition, the BPPE treatment partially protected the mice to the anaphylactic shock reaction. This result corroborated with others showing that plant extract rich in flavonoids can block the anaphylactic shock responses associated with IgE level (Kaneko et al., 2000; Kim et al., 2001; Dai et al., 2004, 2005). The partial inhibition of the anaphylactic shock reaction by the BPPE treatment may be associated with the decrease of the IgE and IgG₁ OVA-specific levels.

The myricetin, one of the flavonoids that represent about 2% into the BPPE, was analyzed in this allergic experimental model. It was observed that this flavonoid also inhibited the allergic parameters. Animals treated with myricetin showed decrease of IgE and IgG₁ OVA-specific and inhibited cell migration to the pulmonary cavity. We also evaluated the effect of BPPE main flavonoid, tricetin, although no effect on allergic parameters could be seen (data not shown).

Previous studies demonstrated that bee pollen phenolic extract significantly reduced the thymus and spleen weights and depressed the immune response in rats (Qin and Sun, 2005). It was also showed that the extract inhibited the neutrophil function and NO and PGE₂ productions (Park et al., 1999). Therefore several other studies described that plant extracts rich in flavonoids such as *Chrysanthemum indicum* Linne (Cheng et al., 2005), *Egletes viscosa* Less (Souza et al., 1992) and *Tephrosia purpurea* (Damre et al., 2003) have anti-inflammatory and immunomodulatory effects.

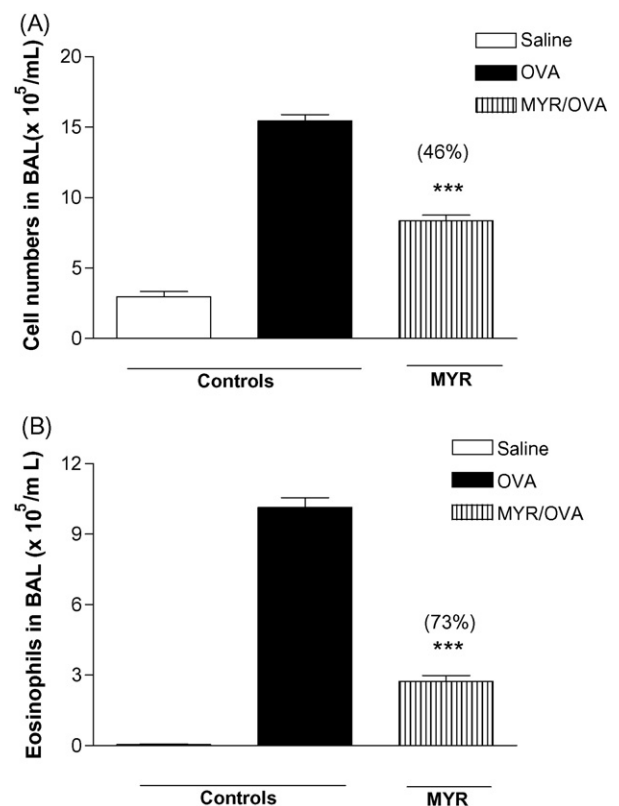


Fig. 6. Effect of myricetin treatment on the airway cell migration. The total numbers of cell (A) and eosinophils (B) was measured in the BAL of OVA-sensitized and challenges BALB/c mice. The myricetin treatment was made concomitantly each OVA immunization. The results are expressed as means $\pm$ S.E.M. ($n=5-10$). *** $p < 0.0001$ as compared with OVA group and analyzed by one-way ANOVA followed by Bonferroni's test.

Such studies powerfulness the hypothesis that myricetin may be responsible for the BPPE activities. Alexandrakis et al. (2003) and Kempuraj et al. (2005) showed that several flavonoids including myricetin were able to inhibit the proliferative response of human leukemia mast cell, the production of tryptase, IL-6, IL-8, TNF- α , histamine, to suppress the intracellular calcium and also to inhibit the phosphorylation of the calcium-insensitive protein kinase C θ in human mast cells.

The results presented here showed that the intraperitoneal BPPE and myricetin treatments are effective in ameliorate the allergic reaction in the experimental model of allergy. Hence these data support the hypothesis that myricetin is a one of flavonoids of the BPPE responsible for the anti-allergic effect observed and a potential molecule to treat allergies.

Acknowledgments

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