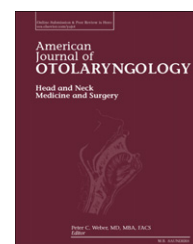


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Original Contribution

Effects of propolis in an experimental rat model of allergic rhinitis[☆]Mehmet Yasar, MD^{a,*}, Yasemin Savranlar, MD^b, Hatice Karaman, MD^c, Mustafa Sagit, MD^a, Sibel Silici, PhD^d, Ibrahim Ozcan, MD^a^a Kayseri Training and Research Hospital, Department of ENT^b Kayseri Training and Research Hospital, Department of Histology^c Kayseri Training and Research Hospital, Department of Pathology^d Erciyes University Faculty of Agriculture, Department of Agricultural Biotechnology Agriculture Research Unit

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ABSTRACT

Purpose: The aim of this study was to determine the anti-allergic activity of propolis in an ovalbumin-induced rat model of allergic rhinitis.**Materials and methods:** This prospective experimental study was conducted at Hakan Çetinsaya Clinical and Experimental Animal Research Center with 30 rats. After sensitization of all rats with 0.3 mg intraperitoneal ovalbumin plus 30 mg aluminum hydroxide for 14 days (first phase), rats were divided to five groups. In the second phase of the study 10 µL of ovalbumin was applied to each nostril for 21 days. Together with second phase, ketotifen (n:6), oral propolis (n:6), intranasal propolis (n:6) and intranasal mometasone furoate (n:6) were given to rats. A control group (n:4)(salin) and sham group (n:2) were planned. Symptoms were assessed on days 19, 22, 25, 30 and 35, resulting in 5 symptom scores: symptom scores 1–5. On day 35, nasal tissue was removed and histological examination was performed.**Results:** When rats that received systemic and intranasal propolis were compared to controls, ciliary loss, inflammation, increase in goblet cells, vascular proliferation, eosinophil count, chondrocytes and allergic rhinitis symptom score were found to be decreased ($p < 0.05$).**Conclusions:** It was found that propolis had anti-allergic effects on allergic symptom scores and nasal histology.

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1. Introduction

Allergic rhinitis is a common disease with increasing incidence that leads to serious public health problems. It presents with

rhinorrhea, sneezing, itching and symptoms of nasal congestion, which is characterized by inflammation of the nasal mucosa [1].

Many medical treatment modalities such as antihistamines, steroids, montelukast inhibitors and immunotherapy are used in

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the treatment of allergic rhinitis. In addition, many agents have been reported to have anti-allergic activity in traditional folk medicine in recent years. In contemporary medicine, these agents are being investigated using animal models of allergic rhinitis.

Propolis gains a strong, adhesive structure through the transformation of plant resin by honey bees. Botanical origin identification and chemical analysis studies were performed for Poplar propolis produced in Turkey [2]. Propolis is a pharmacologically active substance and contains flavonoids, phenolic acid and their esters and its components have anti-inflammatory and immunomodulatory effects. It was reported that propolis was used in the treatment of burns and wounds, gastric ulcer and against prostate hyperplasia [3,4]. In addition, it has been reported that propolis also has anti-microbial, anti-viral, antioxidant, anti-inflammatory and immunomodulatory effects [5–8].

In this study, we aimed to evaluate the potential anti-allergic activity of propolis on allergic rhinitis using an ovalbumin-induced rat model through assessing changes in mucosal histology and by a subjective symptom score.

2. Materials and methods

2.1. Animals

The study was conducted with 30 male Sprague-Dawley rats aged ≥ 6 weeks (weighing 250–300 g) at the Hakan Çetinsaya Clinical and Experimental Animal Research Center after approval of the Local Ethics Committee of Erciyes University on Animal Studies (approval #:2014/113). Rats were housed in cages ($n = 5$ for each cage) under standard conditions at a temperature of 21 °C, maintaining a 12-h dark-light cycle. All animals were fed with a standard commercial pellet diet and water ad libitum.

2.2. Study protocol and groups

It was planned to conduct this study with 30 rats, with 2 rats initially assigned as a sham group. Subsequently, in the first phase of the study, 28 rats, 0.3 mg of ovalbumin (Sigma-Aldrich, St. Louis, MO, USA) plus 30 mg aluminum hydroxide (in 1 ml saline) were given daily via an intraperitoneal route over 14 days in order to sensitize the rats. Drugs were applied

at 12:00–13:00 pm. In the second phase of the study, 10 μ L ovalbumin (20 mg/mL) was applied to each nostril using a micropipette during inspiration on alternate days. The rats were separated to five groups according to treatment patterns. Intranasal mometasone furoate (50 μ g; Nasonex®, Merck, İstanbul, Turkey) group ($n:6$), oral ketotifen (10 mg/kg; Zaditen®, Novartis, İstanbul, Turkey) group ($n:6$), intranasal propolis (200 mg/kg) group ($n:6$), oral propolis (200 mg/kg) group ($n:6$) and control group (0.5 ml intranasal saline) ($n:4$). All medications were given for 21 days after the first phase of the study. Both nasal passages of the rats were included in the study and 60 nostrils (4 sham group) were assessed in total (Fig. 1).

2.3. Drug administration

The propolis sample was collected from Kayseri (Central Anatolia) in Turkey. Hand collected propolis was kept desiccated, in the dark, until processing. Thirty grams of propolis powder was dissolved in 100 ml of 70% ethanol solution for a week at room temperature. After a week, the ethanol extract was filtered and then evaporated using a vacuum evaporator [9]. The chemical content of propolis used in this study had previously been identified by gas chromatography/mass spectrophotometry [10].

Active substances were given in the second phase of the study. Ketotifen (10 mg/kg) was given via gavage until day 35 of the study. In the oral propolis group, 200 mg/kg propolis was given via gavage while propolis (diluted in saline) was applied to each nostril of rats during inspiration using a PPD injector in order to ensure that active substance was delivered to the nasal passage. In the positive control group of topical administration, mometasone furoate (50 μ g) was applied to each nostril of the rats during inspiration using a PPD injector. All active substances were given 0.5 h after ovalbumin in order to avoid interaction of substances within the nasal passage. In the control group, saline was applied via an intranasal route. In the sham group, no substance was applied to the nasal passage.

2.4. Symptom assessment

Rats were subjectively monitored on day 16 to rate symptoms. Symptoms were assessed on days 19, 22, 25, 30 and 35, resulting in 5 symptom scores: symptom scores 1–5. An

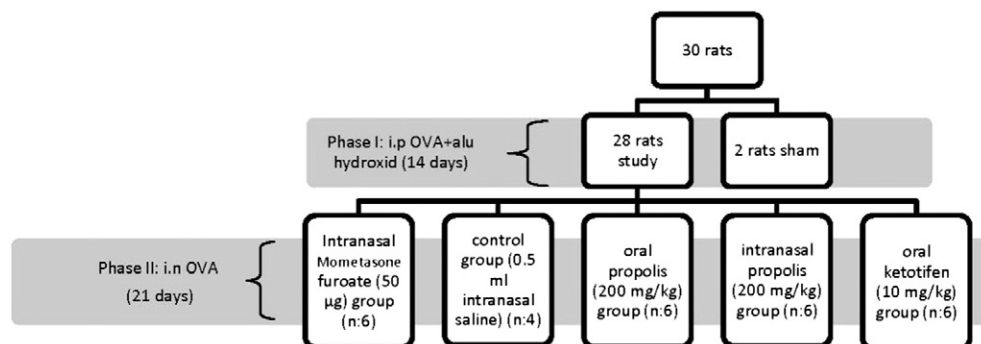


Fig. 1 – Experimental design. Abbreviations: i.p; intraperitoneal, i.n; intranasal, OVA; ovalbumin, alu; aluminum hydroxide.

Table 1 – Subjective symptom scoring of allergic rhinitis.

Variable	0	1	2	3
Nasal itching motion (time/min)	None	2	4–6	>6
Sneezes (time/min)	None	2	4–6	>6
Amount of nasal flow	None	In one nostril	In both nostrils	Out-flowing

investigator blinded to experimental groups rated symptoms (nasal irritation, sneezing and nasal secretion) by placing each rat in a transparent cage for 10 min. Symptom scoring was performed based on nasal irritation, sneezing and amount of nasal secretion. Each symptom was rated on a 0–3 point scale (Table 1.) [11].

2.5. Histological assessment

At the end of the experiment, rats were sacrificed using 100 mg/kg ketamine hydrochloride (Ketalar, Eczacıbaşı, Turkey) plus 7.5 mg/kg xylazine (Rompun, Bayer, Germany) via an intraperitoneal route. Then, en bloc maxilla resection including the nose was performed via surgical dissection, which passed below the orbit in a horizontal plane in order to spare surrounding tissues. Nasal tissues were stored in 10% formaldehyde. For histopathological evaluation, nasal tissue samples were embedded in paraffin blocks. Specimens then underwent decalcification using formic acid and sodium citrate. Tissue sections (5 µm in thickness) were cut from paraffin blocks, which were then deparaffinized using xylene. Subsequently, tissue sections were rinsed with ethanol and stained with hematoxylin–eosin for evaluation under a light microscope. A pathologist blinded to the groups assessed nasal septum and mucosa. Tissue samples were subjectively rated as –, +, ++ or +++ regarding inflammation, vascular proliferation, eosinophils, increase in goblet cells and chondrocytes.

2.6. Statistical analysis

Data were analyzed using SPSS (Statistical Package for Social Sciences) for Windows version 16.0. Independent sample t test and one-way ANOVA were used to compare histological parameters and symptom scores. Data were expressed as mean ± standard deviation. A p value <0.05 was considered to be statistically significant.

3. Results

When control and sham groups were compared, it was found that there were significant differences in ciliary loss, inflammation, increase in goblet cells, chondrocytes, vascular congestion, eosinophils and symptom scores 1–4, which were found to be significantly higher in the control group than in the sham group ($p < 0.05$) (Fig. 2). This indicated that allergic morphology was induced in the mucosa. There was no significant difference in symptom score 5 ($p > 0.05$) (Table 2).

In rats that received mometasone furoate, it was found that ciliary loss, inflammation, vascular congestion, vascular proliferation, eosinophils and symptom scores 1, 2, 3, and 4 were significantly decreased when compared to the control group ($p < 0.05$). No significant difference was found regarding increase in goblet cells, chondrocytes and symptom score 5 ($p > 0.05$) (Table 2).

In the rats that received ketotifen (positive controls), ciliary loss, inflammation, increase in goblet cells, chondrocytes, vascular proliferation, eosinophils and symptom scores 1, 3, and 4 were significantly decreased when compared to the control group ($p < 0.05$). No significant difference was found in vascular congestion and symptom scores 2 and 5 ($p > 0.05$) (Table 2).

In the rats that received propolis via an intranasal route, chondrocytes, vascular congestion, eosinophils and symptom scores 1, 3, and 4 were significantly decreased when compared to the control group ($p < 0.05$) (Fig. 3). No significant difference was found in ciliary loss, inflammation, increase in goblet cells, vascular proliferation and symptom scores 2 and 5 ($p > 0.05$) (Table 2).

In the rats that received propolis via an oral route, ciliary loss, inflammation, increase in goblet cells, vascular proliferation, eosinophils and symptom scores 1, 2, 3, and 4 were significantly decreased when compared to the control group ($p < 0.05$) (Fig. 4). No significant difference was found in chondrocytes, vascular congestion and symptom score 5 ($p > 0.05$) (Table 2).

When groups receiving mometasone furoate and systemic propolis were compared, inflammation, goblet cells and symptom scores 2, 3, and 4 were significantly decreased in the systemic propolis group ($p < 0.05$). When groups receiving ketotifen and systemic propolis were compared, inflammation, goblet cells, chondrocytes and symptom scores 2 and 3

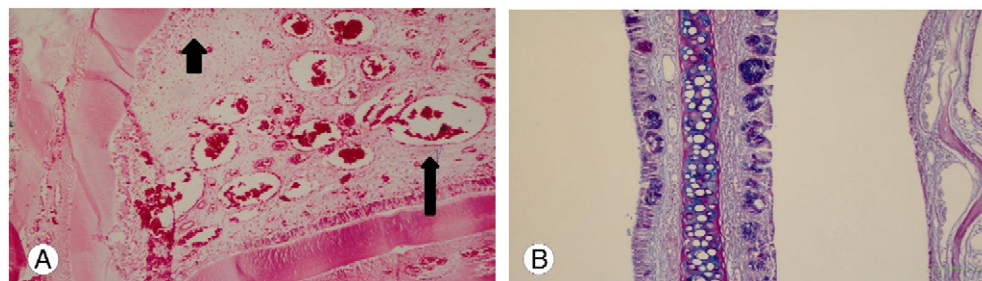


Fig. 2 – Comprehensive of (A) represents control group while the picture (B) represents sham group. Vascularity, inflammation, eosinophil and chondrocyte were increased in control group when compared to sham group. Short arrow indicates increased vascularity and long arrow indicates increased congestion in control group (H&E, ×100).

Table 2 – Comprehensive histopathological parameters and symptom scores in rat groups.

Parameters	Mometasone furoate	Ketotifen	Intranazal propolis	Systemic propolis	Sham	Control	P1 value	P2 value	P3 value	P4 value	P5 value	P6 value	P7 value	P8 value
Ciliary loss	1.17 ± 0.93	1.67 ± 0.49	2.00 ± 0.60	2.50 ± 0.52	0.25 ± 0.50	2.00 ± 0.00	0.01	0.039	1.00	0.007*	0.002*	<0.001*	0.001*	0.041*
Inflammation	1.50 ± 0.52	1.67 ± 0.77	2.67 ± 0.49	1.00 ± 0.00	1.00 ± 0.00	2.67 ± 0.51	0.001*	0.006*	1.00	0.001*	0.001*	0.007*	0.013*	<0.001*
Goblet cell	1.83 ± 0.71	2.33 ± 0.49	1.50 ± 0.52	0.67 ± 0.98	0.00 ± 0.00	1.67 ± 0.51	0.58	0.026	0.53	0.012*	0.001*	0.003*	<0.001*	0.019*
Chondrocyte	1.00 ± 0.00	2.00 ± 0.60	2.00 ± 0.60	1.17 ± 0.38	0.00 ± 0.00	1.33 ± 0.51	0.17	0.032*	0.032*	0.50	0.001*	0.166	0.001*	<0.001*
Vascular congestion	2.17 ± 0.38	2.33 ± 0.49	2.33 ± 0.49	2.92 ± 0.00	1.25 ± 0.50	3.00 ± 0.00	<0.001*	0.001*	0.001*	0.33	0.002*	<0.001*	0.002*	0.147
Vascular proliferation	1.33 ± 0.49	2.17 ± 0.38	2.17 ± 0.38	2.00 ± 0.00	0.00 ± 0.00	2.67 ± 0.51	<.001*	0.07	0.07	0.025*	<0.001*	0.001*	0.166	0.001*
Eosinophil	0.5 ± 0.79	0.83 ± 0.71	0.83 ± 0.71	1.67 ± 0.49	0.00 ± 0.00	2.33 ± 0.51	<0.001*	<0.001*	<0.001*	0.026*	<0.001*	<0.001*	0.004*	0.007*
Score on day 1	1.92 ± 0.28	1.92 ± 0.28	1.92 ± 0.28	1.92 ± 0.28	0.25 ± 0.50	1.17 ± 0.40	0.004*	0.004*	0.004*	0.004*	0.014*	1.00	1.00	0.004*
Score on day 2	4.50 ± 0.52	3.08 ± 0.28	3.08 ± 0.28	1.50 ± 0.52	0.25 ± 0.50	3.00 ± 0.00	<0.001*	0.339	0.339	<0.001*	<0.001*	<0.001*	<0.001*	0.055
Score on day 3	0.50 ± 0.79	0.83 ± 0.93	0.83 ± 0.93	0.00 ± 0.00	0.00 ± 0.00	3.00 ± 1.54	0.009*	0.016*	0.016*	0.005*	0.005*	0.053	0.01*	0.002*
Score on day 4	1.00 ± 1.20	0.00 ± 0.00	0.00 ± 0.00	0.17 ± 0.38	0.00 ± 0.00	2.33 ± 1.03	0.032	0.003*	0.003*	0.003*	0.003*	0.04*	0.166	0.212
Score on day 5	0.33 ± 0.49	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.33 ± 0.51	1.00	0.175	0.175	0.175	0.175	0.039*	0.66	0.166

p1 value: Mometasone furoate-control.

p2 value: ketotifen-control.

p3 value: Intranazal propolis - control.

p4 value: Systemic propolis - control.

p5 value: sham - control.

p6 value: Mometasone furoate - Systemic propolis.

p7 value: Ketotifen - Systemic propolis.

p8 value: Intranazal propolis - Systemic propolis.

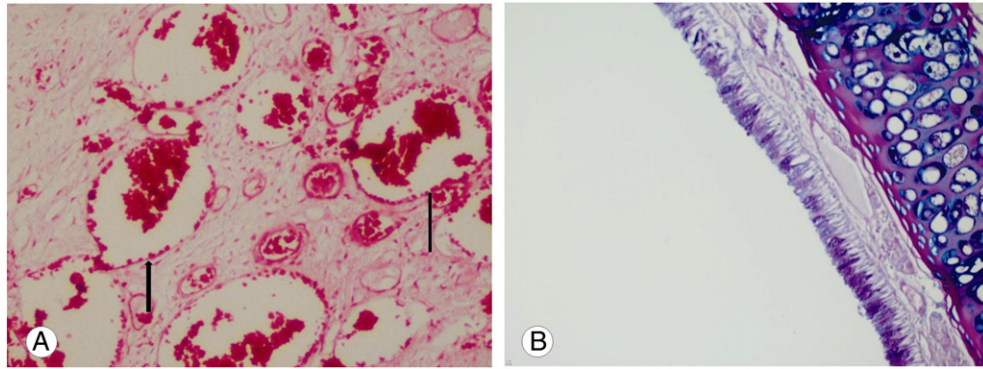


Fig. 3 – The picture (A) represents control group while the picture (B) represents systemic propolis group. Marked decrease in histological findings of allergic rhinitis in rats received systemic propolis when compared to controls. Arrows indicate increased vascularity in control group (H&E, $\times 100$).

were significantly decreased in the systemic propolis group. When groups receiving intranasal or systemic propolis were compared, inflammation, increase in goblet cells, chondrocytes, vascular proliferation and symptom scores 1, 2 and 3 were significantly decreased in the systemic propolis group ($p < 0.05$).

4. Discussion

Allergic rhinitis is a disease of the upper respiratory tract characterized by symptoms such as rhinorrhea, sneezing, itching in the nose and eyes, tearing and anosmia with an incidence of 20%, which is gradually increasing worldwide [12]. In the treatment, many modalities are used, including steroids, antihistamines, montelukast inhibitors, mast cell stabilizers, immunotherapy etc. However, it should be noted that these treatment modalities have only partial effectiveness on symptoms. Thus, several novel agents are being tested in experimental animal models of allergic rhinitis.

The effectiveness of agents used in allergic rhinitis, considered as a systemic disease, is assessed by the extent of decrease in symptoms such as nasal congestion, nasal itching and sneezing. When testing novel agents in an animal model of disease, symptoms must be induced in the subjects. In the literature, it has been reported that ovalbumin induced symptoms of allergic rhinitis by both intranasal and systemic administration [11]. In in vivo models, it was reported that ovalbumin induced an increase in inflammatory cell infiltration in nasal lavage by increasing histamine and IL-4 release [13]. In previous studies, the effectiveness of many novel agents was assessed in rat models of allergic rhinitis, including *Gleditsia sinensis*, doxycycline, bamboo salt, ostericum coreanum extract [14–17].

It was reported that ketotifen, a H1 receptor antagonist, prevented an increase in vascular congestion and decreased release of chemical mediators from mast cells and basophils in a rat model induced by allergen [18]. Ketotifen is used in the treatment of allergic disorders in contemporary medicine. As is known, mometasone furoate used in our study as a topical steroid has anti-inflammatory activity at the cellular level.

Corticosteroids exert their effects through prevention of antigen presentation, reduction of cytokines (IL-3, IL-4, IL-5 and IL-13) and chemokine release, and decrease in cellular infiltration (T cells, eosinophils, mast cells and basophils) and mediator release from these cells [19]. In a rat study, it was reported that mometasone exerts its anti-allergic and anti-inflammatory effects via a mechanism involving increased specific IgA release from the basal membrane of nasal mucosa [20]. Similarly, it was found that ciliary loss, inflammation, vascular congestion, vascular proliferation, eosinophils and symptom scores 1–4 were significantly decreased in rats receiving mometasone furoate when compared to controls in our study ($p < 0.05$). Histopathological examination can objectively establish the anti-inflammatory activity of the drugs used. Some cellular and structural changes in nasal mucosa can be detected by light microscopy, including loss of cilia in the mucosa of the upper respiratory tract, chondrocyte hypertrophy, increased eosinophil count, increased goblet cells, enhanced inflammation, vascular congestion and vascular proliferation. These changes are mediated by substances released from inflammatory cells due to allergen-organism interaction [21].

In the literature, there are a few studies on the anti-allergic activity of propolis [22–23]. It was reported that propolis significantly reduced itching behavior, which was achieved by inhibiting vascular permeability via decreased histamine release [22]. In an experimental study by Shinmei et al., propolis was given to rats for itchy nose and sneezing. The authors reported that symptoms were improved through decreased histamine release and that long-term effects were favorable [22]. In our study, it was found that there was a significant decrease in ciliary loss, inflammation, increase in goblet cells, vascular proliferation, eosinophil count and symptom scores 1–4 in rats receiving systemic propolis, similar to those receiving mometasone furoate and ketotifen ($p < 0.05$). In rats that received intranasal propolis, a significant decrease was detected in chondrocytes, vascular congestion, eosinophil count, and symptom scores 1, 3 and 4 ($p < 0.05$). No significant difference was found in ciliary loss, inflammation, increase in goblet cells, vascular proliferation and symptom scores 2 and 5 ($p > 0.05$). We found that topical use of propolis was less effective than systemic use for

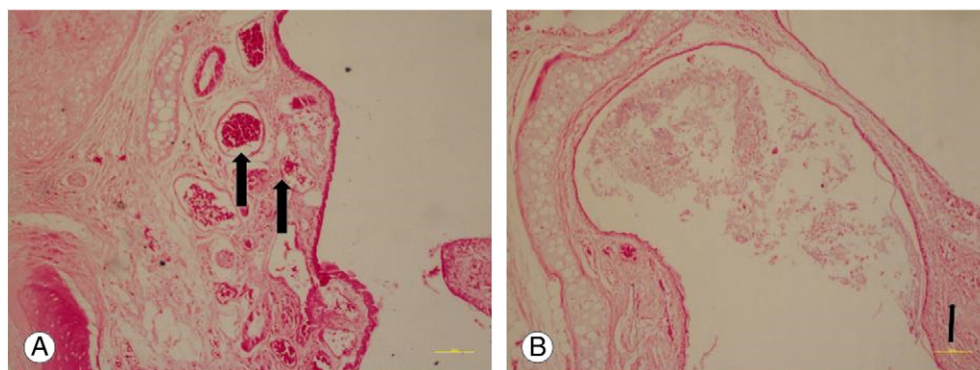


Fig. 4 – The picture (A) represents control group while the picture (B) represents intranasal propolis group. There is a decrease in vascular congestion, eosinophil and chondrocyte with ongoing acute inflammation in rats received intranasal propolis when compared to controls (H&E, $\times 100$). Thick arrows indicate increased vascularity and congestion in the control group. Thin arrow indicates ongoing inflammation in intranasal propolis group.

allergy. The sensitization rate to propolis in patients suffering from dermatitis has been reported to vary between 1.2% and 6.6% [24]. Machackova (1988) identified 4.2% of 650 patients as being sensitized to propolis [24], whereas in a study in Germany performed on 3177 subjects, 1.2% of the tested population was positive to propolis [25]. The allergic effect of propolis may cause the difference effect of oral and intranasal use of propolis on allergic rhinitis. In our study, it was seen that symptom score 5 was lower in the systemic propolis group when compared to mometasone furoate and ketotifen groups. In other words, reduction in symptoms on day 35 was greater in the systemic propolis group. These results support the anti-allergic activity of propolis. In our study, the major limitation was lack of OVA-specific IgE measurements before or after treatment.

5. Conclusion

In the present study, we found that systemic propolis use has favorable effects on allergic symptoms and nasal histology when compared to ketotifen and mometasone furoate. In particular, it was shown that it has greater anti-inflammatory activity than mometasone furoate and ketotifen. In addition, a decrease in symptom scores obtained at a late period emphasizes that it is more effective than mometasone furoate and ketotifen in the long-term. Propolis has better systemic bioavailability than intranasal use. Further studies are however needed to introduce propolis into clinical practice.

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