

Original Paper

Comparison of Histopathological Effects of Thymoquinone and Local Nasal Corticosteroids in Allergic Rhinitis in a Rabbit Model

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Key Words

Histopathology · Rhinitis · Thymoquinone · Steroid · Nasal mucosa

Abstract

Background/Aims: In this study, we aimed to evaluate the histopathological effects of thymoquinone treatment of the nasal mucosa in a rabbit model of allergic rhinitis, and we compared its effects with those of nasal mometasone furoate. **Methods:** A total of 24 male New Zealand rabbits were used. The animals were randomly assigned to one of four groups. Group 1 received no treatment, while group 2 underwent ovalbumin (OVA) sensitization only. Group 3 was the study group; after OVA sensitization, the rabbits were treated with intranasal thymoquinone. The group 4 rabbits received mometasone furoate for 7 days after OVA sensitization. Mucosal structures were stained with hematoxylin and eosin, while toluidine blue was used to stain mast cells. Apoptosis was evaluated using a TUNEL assay. **Results:** In the positive control groups, including the thymoquinone and intranasal mometasone furoate groups, intraepithelial and submucosal inflammation and goblet cell hypertrophy were significantly decreased compared to group 2 ($p < 0.001$). The ciliary structure was normal, as was the chondrocyte structure in both treatment groups. **Conclusion:** This is the first study to evaluate the histopathological effects of thymoquinone in an allergic rhinitis model. Thymoquinone reduced allergic inflammation and may be valuable for treating allergic rhinitis. However, additional studies are needed.

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Introduction

Allergic rhinitis is characterized by nasal inflammation caused by IgE-mediated immune responses to specific allergens [1, 2]. Although it is not a life-threatening condition, quality of life is usually adversely affected [3, 4]. Allergic rhinitis is characterized by nasal symptoms, including rhinorrhea, itching, sneezing, and nasal congestion [1]. Antihistamines and nasal steroids are the principal treatment options; however, complete resolution is not attained in some patients [3]. The medications used share some common side effects, including nasal irritation and epistaxis, and the use of such drugs remains controversial in certain patients such as breastfeeding or pregnant females [5, 6]. Therefore, alternative treatment options are needed.

Thymoquinone, the active ingredient in *Nigella sativa* (black cumin) seeds, has chemopreventive, antiproliferative, antioxidative, and anti-inflammatory effects [7]. A few studies have shown that thymoquinone exerts anti-inflammatory effects in patients with certain allergic diseases, including asthma, food allergies, and allergic conjunctivitis [8–12], but its effect on allergic rhinitis remains unknown.

In the present study, we evaluated the histopathological effects of thymoquinone treatment of the nasal mucosa in a rabbit model of allergic rhinitis, and we compared its effects with those of nasal mometasone furoate.

Methods

The study was conducted between September 2014 and March 2015 at the Abant İzzet Baysal University Faculty of Medicine, Bolu, Turkey. A total of 24 male New Zealand rabbits (2,000–3,000 g each; 20–24 weeks of age) were used in the present study. The animals were purchased from the Uludağ University Faculty of Medicine Animal Application and Research Center, Bursa, Turkey. The study was approved by the Committee on Animal Research of the Abant İzzet Baysal University Institute for Experimental Medicine Research, Bolu, Turkey. All rabbits were given free access to food and water and were kept in individual rubber-covered iron cages under a consistent 12-hour:12-hour light:dark protocol, at 18–23°C and 40–60% humidity. The animals were randomly assigned to one of four groups composed of six animals each. Group 1 received no treatment, while group 2 underwent ovalbumin (OVA) sensitization only. Group 3 was the study group; after OVA sensitization, the rabbits were treated with intranasal thymoquinone. Briefly, starting on day 15 after sensitization, five drops of 0.4% (w/v) thymoquinone were instilled in the nasal cavity three times per day for 7 days. This dose was shown to be as effective as topical steroids in a model of allergic conjunctivitis [13]. The group 4 rabbits received mometasone furoate 2 puffs/day (100 µg) for 7 days after OVA sensitization.

OVA Sensitization

A 0.4% (w/v) OVA solution was prepared by emulsifying OVA in Tween 80 combined with Al(OH)₃, and mixing the emulsion with 100 ml of saline. In groups 2, 3, and 4, 5 ml of the suspension was administered intraperitoneally on days 0, 2, 4, and 6. Next, 10 ml of the same solution was given intranasally once a day for 7 days. Serum OVA-specific IgE levels were measured via an ELISA to confirm sensitization.

Histopathological Evaluation

After the conclusion of thymoquinone treatment, all animals were sedated via an intraperitoneal injection of ketamine (80 mg/kg) and xylazine (5 mg/kg), and the nasal septum was totally resected. For histological evaluation, paraffin-embedded tissue specimens were cut into 5-µm slices. Mucosal structures, degree of inflammation and apoptosis were looked at in the histopathological evaluation. Mucosal structures were stained with hematoxylin and eosin, while toluidine blue was used to stain mast cells. Apoptosis was evaluated using a TUNEL assay (Gen-Script apoptosis kit). A Nikon photomicroscope was employed.

The inflammation was evaluated in five different microscopic areas for each rabbit at 200 power magnification. Inflammation severity was grouped into three grades: grade 1: 1–2 lymphocytes (minimal inflammation); grade 2: sparse lymphocytes in all areas (moderate inflammation); grade 3: diffuse and intense lymphocytic infiltration (severe inflammation). At the end, there were 30 results for each group, and median inflammation grades were compared with Mann Whitney U test between groups (table 1).

Table 1. Microscopic findings of five different areas in the three groups according to severity of inflammation

	Allergic rhinitis group					Mometasone furoate group					Thymoquinone group				
Rabbit 1	3	2	3	2	2	2	1	2	2	2	1	2	2	1	1
Rabbit 2	3	3	3	2	2	2	1	1	1	2	1	1	2	1	1
Rabbit 3	3	2	3	3	2	2	2	2	1	1	1	2	1	2	2
Rabbit 4	3	3	3	3	2	2	2	1	1	1	2	1	1	2	2
Rabbit 5	3	2	2	3	3	2	1	1	2	2	2	1	2	1	1
Rabbit 6	3	3	2	3	3	2	1	1	1	1	2	1	1	1	2

1 = Minimal inflammation; 2 = moderate inflammation; 3 = severe inflammation.

Results

The structures of the nasal mucosa were normal in the control group (group 1). Group 2 had notably increased numbers of inflammatory cells, mainly neutrophils and mast cells, in the intraepithelial and submucosal layers compared to group 1. Also, significant intraepithelial goblet cell hypertrophy and hyperplasia were evident. Vascular congestion and ciliary loss were noted in some regions of the nasal epithelium (fig. 1).

In the thymoquinone group (group 3), the intraepithelial and submucosal inflammatory cell numbers were significantly lower than in group 2 ($p < 0.001$; table 1). Although hypertrophic goblet cells were evident in some areas, their intensity and number were reduced. Compared to the mometasone furoate group, the histological structure was slightly more irregular, but significant improvements in structure were evident compared to group 2. The ciliary structure was normal, as was the chondrocyte structure (fig. 2).

In the mometasone furoate group, the extent of intraepithelial and submucosal inflammation and the extent of goblet cell hypertrophy were significantly lower than in group 2 ($p < 0.001$; table 1). The distribution of cilia was also normal, and no vascular congestion was apparent (fig. 3).

When we compared the inflammation status of the mometasone furoate and thymoquinone groups, the intraepithelial and submucosal inflammatory cell numbers were similar ($p = 0.608$).

A TUNEL assay was used to measure apoptosis in epithelial and connective tissue cells. The extent of apoptosis did not differ between the mometasone furoate and thymoquinone groups, and the TUNEL-positive cell counts were similar. Thus, thymoquinone did not induce apoptosis in epithelial cells or those of the nasal septum.

Discussion

This is the first study to compare the histopathological effects of intranasal thymoquinone with those of a nasal steroid; we found that intranasal thymoquinone-exerted histopathological effects on the nasal mucosa were similar to those caused by mometasone furoate in an allergic rhinitis rabbit model. Intranasal thymoquinone decreased inflammation and goblet cell hyperplasia and hypertrophy, without inducing apoptosis.

Allergic rhinitis is an IgE-mediated chronic inflammatory disease of the nasal mucosa [14]. Although the condition is not life-threatening, it usually has a negative impact on a patient's quality of life [3, 4]. The prevalence of allergic rhinitis has increased substantially in recent decades; it varies between 4.6 and 31.8% in different populations [15]. Treatments

Fig. 1. Histopathological findings of the untreated allergic rhinitis group (group 1). There were increased inflammatory cells and congestion.

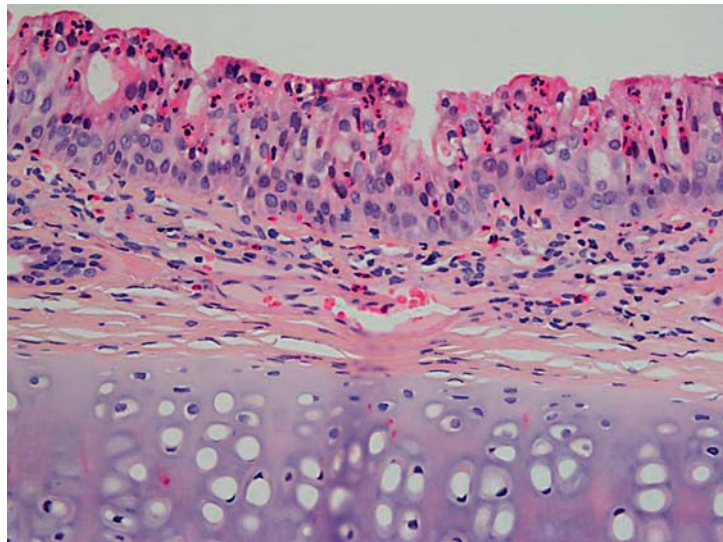


Fig. 2. Histopathological findings of animals that were treated with thymoquinone after OVA sensitization. It was shown that inflammation was significantly decreased compared to the untreated group.

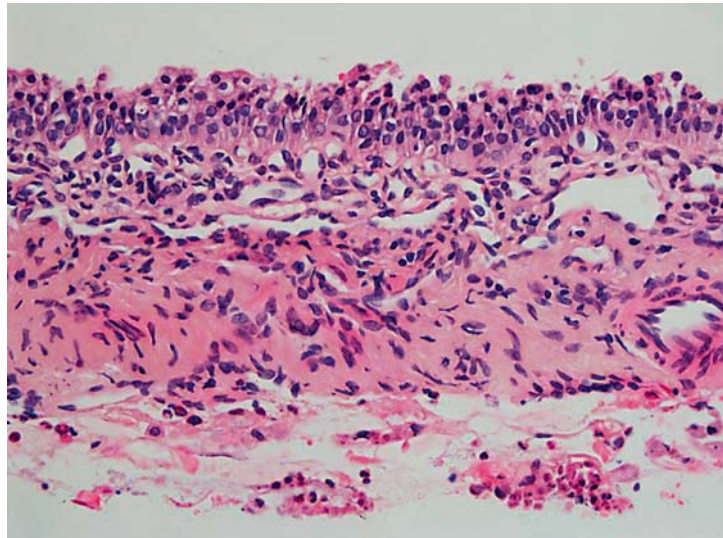
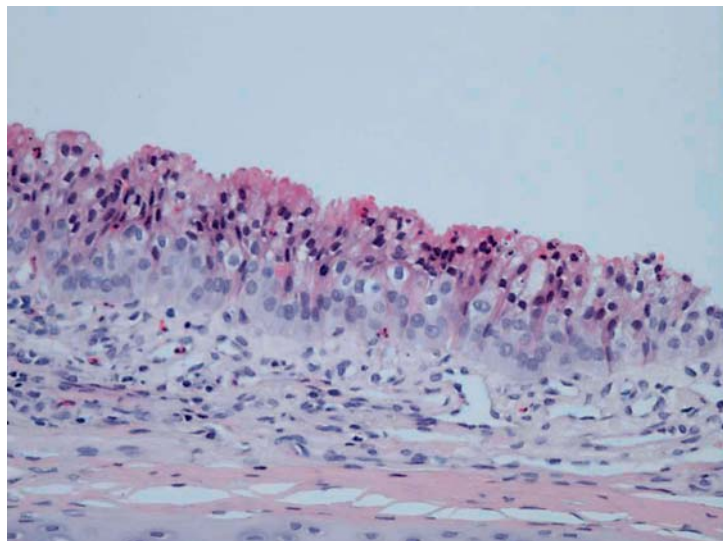


Fig. 3. Histopathological findings of animals that were treated with intranasal corticosteroids (mometasone furoate) after OVA sensitization. It was shown that inflammation was significantly decreased compared to the untreated group.



include allergen elimination, pharmacotherapies, and immunotherapies [16, 17]. Nasal corticosteroids are the preferred first-line treatment for moderate-to-severe disease [18, 19]. Although the main goal of treatment is symptom control, current therapies (including nasal steroids) do not provide adequate control in many patients [3, 16, 20]. Also, nasal corticosteroids may have side effects, including nasal irritation, epistaxis, and (rarely) nasal septum perforation and *Candida* overgrowth [5]. Therefore, alternative treatments are needed.

Thymoquinone is the active ingredient in *N. sativa* (black cumin), which possesses anti-oxidative, anti-inflammatory, chemopreventive, and antiproliferative activities. No earlier work evaluated the effect of thymoquinone in allergic rhinitis, but a few studies on its effects on other allergic diseases have been published [8–12]. Besides, thymoquinone has a very low toxicity, and a few cases of contact dermatitis have been reported after its use; otherwise, no adverse effects are listed [21]. Negative effects on kidney and liver do not occur. Keyhanmanesh et al. [9] showed that thymoquinone pretreatment significantly decreased tracheal responsiveness to methacholine and OVA and reduced the bronchoalveolar lavage eosinophil count in an animal model. Kalemci et al. [10] reported that thymoquinone alleviated the pathological changes of chronic asthma. Duncker et al. [12] focused on food allergies and showed that an *N. sativa* seed extract significantly improved the symptoms and immune parameters (including mast cell numbers) in a murine model of OVA-induced allergic diarrhea. Using an animal model of allergic conjunctivitis, Hayat et al. [11] showed that thymoquinone significantly reduced the associated ocular symptoms by attenuating eosinophil recruitment and reducing the levels of IgE, histamine, and cytokines. Allergic rhinitis shares several common pathogenic features with the allergic diseases mentioned above. Therefore, we investigated the histopathological effects of thymoquinone in an allergic rhinitis model and found that allergic inflammation was significantly reduced.

Intranasal steroids are the cornerstone of allergic rhinitis treatment. In the present study, we compared the effects of a nasal corticosteroid (mometasone furoate) and thymoquinone. Both agents reduced allergic inflammation significantly. Neither agent increased apoptosis, nor did they disturb the ciliary structure. Thus, thymoquinone may be an alternative safe option for the treatment of allergic rhinitis.

In conclusion, this is the first study to evaluate the histopathological effects of thymoquinone in an allergic rhinitis model. Thymoquinone reduced allergic inflammation and may be valuable for treating allergic rhinitis. However, additional studies are needed.

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Erratum

In the article by Wang J, Fang R, Peterson A and Jiang JJ, entitled ‘The protective role of autophagy in human vocal fold fibroblasts under cigarette smoke extract exposure: a new insight into the study of Reinke’s edema’ [ORL 2016;78:26–35, DOI: 10.1159/000442022], the corresponding author Jack J. Jiang is affiliated with both institutes:

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