

The histopathological effect of thymoquinone on experimentally induced rhinosinusitis in rats

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ABSTRACT

Background: Rhinosinusitis is a common disorder and its treatment includes a variety of topical and systemic drugs. This study was designed to determine the histopathological effect of thymoquinone on experimentally induced rhinosinusitis in rats.

Methods: Sixty rats were randomly allocated into 3 test and 2 control groups, each of which consisted of 12 animals. The rhinosinusitis model was induced using intranasal application of platelet-activating factor. In test groups, the animals were separated into groups: (1) rhinosinusitis-antibiotherapy, (2) rhinosinusitis-thymoquinone, (3) rhinosinusitis-combination therapy. The positive and negative control groups were defined: rhinosinusitis group without any treatment and the group without rhinosinusitis, respectively. The histopathological features (vascular congestion, inflammation, and epithelial injury) in nasal respiratory and olfactory mucosa of animals were examined and graded according to their severity. A quantitative and statistical analysis of histopathological features was performed.

Results: All histopathological features showed statistically significant differences between negative and positive control groups, respectively. Conversely, neither the group with rhinosinusitis-antibiotherapy nor the group with rhinosinusitis-thymoquinone had a statistically significant difference with the negative control group. Moreover, none of the histopathological features showed a statistically significant difference, when the group with rhinosinusitis-antibiotherapy and the group with rhinosinusitis-thymoquinone were compared. A statistically significant difference was not determined when the group with rhinosinusitis-combination therapy was compared with the group with rhinosinusitis-thymoquinone. The histopathological features did not show a statistically significant difference between the group with combination therapy and the negative control.

Conclusion: Thymoquinone is a promising bioactive agent for the treatment of rhinosinusitis, and its histopathological effect is as equivalent as an antibiotic.

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Rhinosinusitis is a common disorder that is characterized by the inflammation involving the mucosa of the nose and paranasal sinuses. It is clinically divided into five categories according to history, symptomatology, and duration of complaints as acute, subacute, chronic, recurrent acute, and acute exacerbation of chronic rhinosinusitis.¹ The inflammatory process is common for both acute and chronic rhinosinusitis; however, the underlying pathophysiological mechanism is somewhat different.² Acute rhinosinusitis is associated with an impaired paranasal sinus drainage and ventilation due to an infectious inflammation triggered mainly by viral or bacterial agents.^{1,3} On the other hand, chronic rhinosinusitis is a multifactorial disease that is presumably associated with infectious and/or noninfectious inflammation elucidated by extrinsic (viral and bacterial infections, allergy, superantigens, biofilms, etc.) and intrinsic factors (genetic disorders, mucociliary impairment, mucosal immune dysregulation, neoplasms, etc.); however, the exact etiology and pathophysiology still remains elusive.^{4–7} Although antibiotherapy is the mainstay for the treatment of acute and chronic rhinosinusitis, some different aspects should not be ignored. There is very little debate concerning the efficacy of antibiotics on acute bacterial rhinosinusitis.^{3,8} Despite the fact that the efficacy of antibiotics has been emphasized in evidence-based schemes, there is still limited and controversial data supporting the role of antibiotics in the treatment of chronic rhinosinusitis.^{4,5,8} Moreover, recently, topical and systemic corticoste-

roids are also suggested for the treatment of both acute and chronic rhinosinusitis because of their anti-inflammatory activities.^{8,9}

Nigella sativa, commonly known as black seed or black cumin, belongs to dicotyledon of the Ranunculaceae family. It is an annual plant that is generally found in the areas bordering the Mediterranean Sea, Pakistan, and India. Historically, the seeds of *N. sativa*, the source of active ingredients, has been used for thousands of years as a natural remedy for a number of diseases and conditions such as asthma, hypertension, diabetes, bronchitis, eczema, fever, dizziness, gastrointestinal problems, and infections.^{10,11} In the Holy Bible, it is identified as “curative black cumin” and also referred to as “universal healer” in Arabic culture. In the past, black seed had been prescribed by the Greek physician Dioscorides to treat headaches, toothaches, and nasal congestion.

In modern medicine, most of the curative effects of black seed are attributed to thymoquinone, a bioactive constituent of the volatile oil of *N. sativa*, which was initially extracted by El-Dakhkhany in 1963.¹² Thymoquinone has analgesic, antidiabetic, antiulcerous, anticoagulant, antineoplastic, antihepatotoxic, and antinephrotoxic activities; however, the exact molecular pathways of thymoquinone action have still remained obscure.¹³ In addition, antimicrobial, antioxidant, anti-inflammatory, and immunomodulatory activities of thymoquinone have been reported in the literature.^{13–16} Because of all of these biological activities, it seems worthwhile to evaluate the potential role of thymoquinone on the treatment of rhinosinusitis. Therefore, the current study was designed to investigate the histopathological effect of thymoquinone in a rat model of platelet-activated factor-induced rhinosinusitis.

MATERIALS AND METHODS

The study protocol was approved by the Animal Ethics Committee of Osmangazi University, Turkey (25.08.2010 and no. 172).

Animal Preparation

The study included 60 Sprague-Dawley rats, weighing between 250 and 350 g (mean, 300 g). Only the animals without any evidence of

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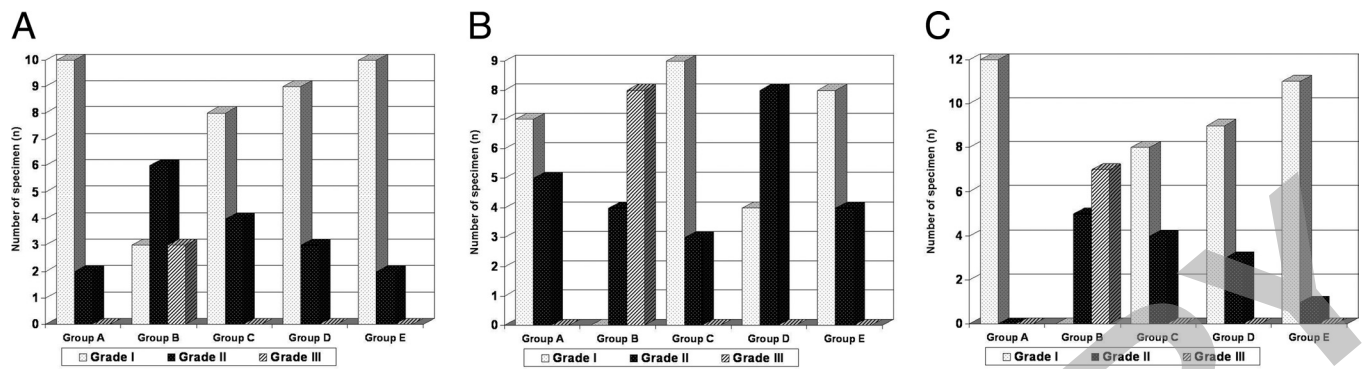


Figure 1. The distribution of all groups according to the grade of (A) vascular congestion, (B) inflammatory cell infiltration, and (C) epithelial injury.

upper airway infection were used and they were equally randomized between five groups. Every rat was anesthetized with administering i.p. ketamine hydrochloride (75 mg/kg) and xylazine hydrochloride (10 mg/kg). The induction of rhinosinusitis was performed by the technique that was offered by Jeon *et al.*¹⁷ Initially, platelet-activated factor was dissolved in ethanol to form a standard solution (1 mg/mL) and final dose was derived by diluting this solution in normal saline with 0.25% bovine serum albumin. Thereafter, 50 mL of platelet-activated factor (16 µg/mL; Sigma Chemical Co., St. Louis, MO) was applied to both nasal cavities of all the rats, except the group without rhinosinusitis to which normal saline solution (50 µL) with 0.25% bovine serum albumin and ethanol was applied (negative control, group A). The rats, to which rhinosinusitis were induced (experimental groups) and were separated into four groups as follow:

Rhinosinusitis without any treatment (positive control, group B): physiological saline (50 mg/kg per day) was applied i.p. for 5 days.

Rhinosinusitis-antibiotherapy (group C): Cefazolin sodium (50 mg/kg per day) was applied i.p. for 5 days.

Rhinosinusitis-thymoquinone (group D): Thymoquinone (80 mg/kg per day) was applied i.p. for 5 days.

Rhinosinusitis-combination therapy (group E): Both cefazolin sodium (50 mg/kg per day) and thymoquinone (80 mg/kg per day) was applied for 5 days.

Histopathological Examination

All of the animals were killed at day 21 of the experiment by i.p. injection of high-dose ketamine (250 mg/kg). Each head was decapitated, to preserve the nasal respiratory and olfactory mucosa, and immersed in the fixative (10% formaldehyde) overnight. Thereafter, they were decalcified and coronal sections from the middle segment of the sinonasal cavity (maxillary sinus and olfactory region) were chosen as the principal area of the histopathological examination. Paraffin blocks were formed and anatomically similar sections were collected. Finally, 5-µm-thick sections were stained with hematoxylin and eosin (H&E).

All the histopathological examinations were performed under light microscopy (Olympus BH-2; Olympus Optical Co., Ltd.; Tokyo, Japan) by a senior pathologist (DB) who was blinded to the specimens. Every slide was precisely assessed for histopathological features using a semiquantitative grading system:

- Degree of vascular congestion: Grade I, absence of vascular congestion or very mild vascular congestion; grade II, mild-to-moderate vascular congestion; grade III, severe vascular congestion with erythrocyte extravasation.
- Intensity of inflammatory cell infiltration: Grade I, absence of inflammation; grade II, presence of few inflammatory cell clusters

in mucosa and/or submucosa; grade II, severe inflammatory cell infiltration in mucosa and/or submucosa with/without disruption of mucosa.

- Degree of epithelial injury: Grade I, absence of epithelial injury; grade II, partial degeneration between epithelial cells; grade III, loss of epithelial polarity and/or loss of cilia.

The grade for every rat was determined according to the examination of two sections (nasal respiratory and olfactory mucosa). In addition, a scoring system that was designed by rating every grade (grade I, 1; grade II, 2; and grade III, 3) was also used. In this system, a total score was obtained for every group by adding the scores of every animal for each histopathological feature. Finally, the total score was divided by the number of animals in each group and a mean value was detected.

Statistical Analysis

The results were evaluated using Windows for SPSS 15.0 (SPSS, Inc., Chicago, IL). The statistical significance was analyzed with Pearson and Fisher's exact chi-square test. The values of $p < 0.05$ were accepted as statistically significant.

RESULTS

The degree of vascular congestion and intensity of inflammatory cell infiltration were very low in group A (negative control) and no sign of epithelial injury was detected (Figs. 1, A–C, and 2, A and B; Table 1). On the other hand, high degree of vascular congestion and extensive infiltration of inflammatory cells with significant epithelial injury were determined in group B (positive control; Table 1; Fig. 3, A and B). In addition, the statistical analysis between these groups showed significant differences for vascular congestion ($p = 0.010$), inflammatory cell infiltration ($p < 0.001$), and epithelial injury ($p < 0.001$).

In contrast to group B, groups C, D, and E had a remarkably low degree of vascular congestion and epithelial injury and mild inflammatory cell infiltration (Figs. 1, A–C, 4, A and B, 5, A and B, and 6, A and B; Table 1). Moreover, statistically significant differences between groups B and C were detected when they were compared according to inflammatory cell infiltration ($p < 0.001$) and epithelial injury ($p < 0.001$); however, no statistically significant difference was determined for vascular congestion ($p = 0.071$). Similarly, the comparison of vascular congestion, inflammatory cell infiltration, and epithelial injury between groups B and D showed statistically significant differences, respectively ($p = 0.025$, $p < 0.001$, and $p < 0.001$). In addition, the grades of vascular congestion, inflammatory cell infiltration, and epithelial injury between groups A and D had slight differences (Fig. 1, A–C; Table 1), although they were not statistically significant ($p = 1.000$, $p = 0.414$, and $p = 1.000$).

When groups C and D were compared according to vascular congestion, inflammatory cell infiltration, and epithelial injury, none of

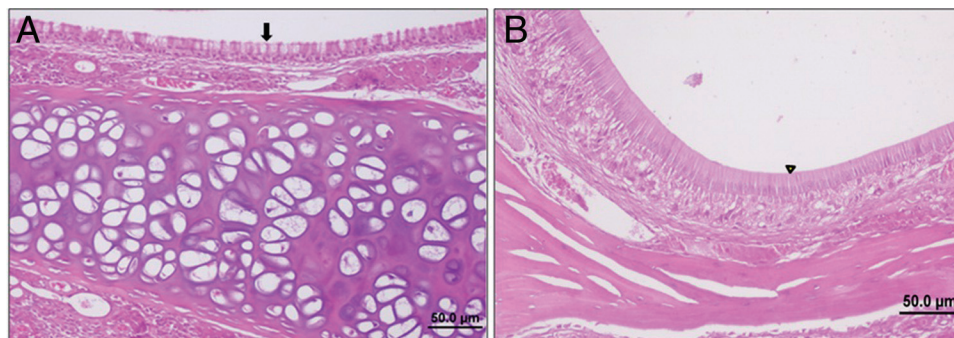


Figure 2. Light micrographs of the rats in group A (group without rhinosinusitis, negative control). (A) Nasal respiratory mucosa was normal with cilia (black arrow; hematoxylin and eosin [H&E], original, $\times 20$). (B) No evidence of vascular congestion, inflammation, or epithelial injury was detected in olfactory mucosa (arrowhead; H&E, original, $\times 20$).

Table 1 The distribution of histopathological features in test and control groups

	Vascular Congestion (mean value and range)	Inflammatory Cell Infiltration (mean value and range)	Epithelial Injury (mean value and range)
Group A (group without rhinosinusitis)	1.17 (1–2)	1.41 (1–2)	1.00 (1)
Group B (rhinosinusitis without any treatment)	2.00 (1–3)	2.67 (2–3)	2.58 (2–3)
Group C (rhinosinusitis-antibiotherapy)	1.33 (1–2)	1.25 (1–2)	1.33 (1–2)
Group D (rhinosinusitis-thymoquinone)	1.25 (1–2)	1.67 (1–2)	1.25 (1–2)
Group E (rhinosinusitis-combination therapy)	1.17 (1–2)	1.33 (1–2)	1.08 (1–2)

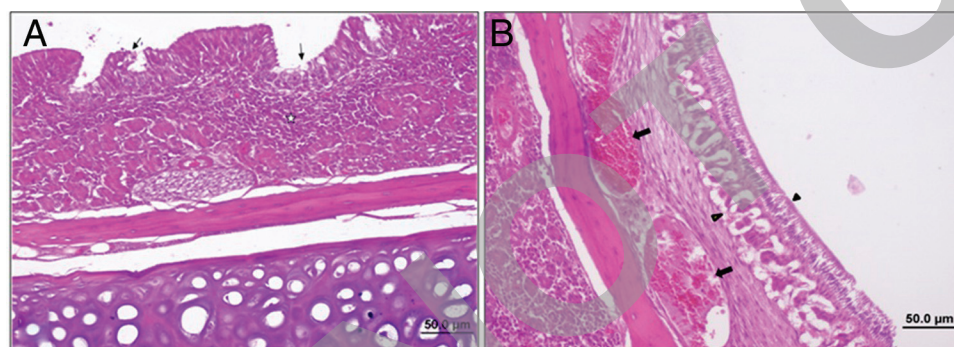


Figure 3. Light micrographs of the rats in group B (rhinosinusitis without any treatment, positive control). (A) Loss of epithelial polarity (black arrows) in nasal respiratory epithelium and severe inflammatory cell infiltration (hollow star) in lamina propria of nasal respiratory mucosa were revealed (hematoxylin and eosin [H&E], original, $\times 20$). (B) Significant injury in olfactory epithelium (arrowhead) and extensive vascular congestion (black arrow) in lamina propria of olfactory mucosa were detected (H&E, original, $\times 20$).

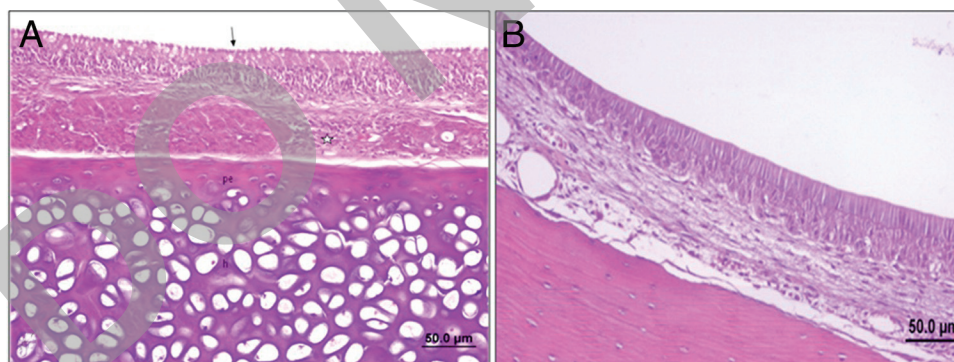


Figure 4. Light micrographs of the rats in group C (rhinosinusitis-antibiotherapy). (A) Nasal respiratory epithelium was almost normal histologically (black arrow); however, mild inflammatory cell infiltration (hollow star) in lamina propria of nasal respiratory mucosa was observed (hematoxylin and eosin [H&E], original, $\times 20$). (B) Partial degeneration between cells in olfactory epithelium without inflammatory cell infiltration in olfactory mucosa was revealed (H&E, original, $\times 20$). pe, perichondrium; h, hyaline cartilage.

the histopathological features was statistically significant ($p = 1.000$, $p = 0.100$, and $p = 0.317$). Finally, the histopathological features of group E were slightly better than group D (Fig. 1, A–C; Table 1); however, the statistical analysis did not show a significant difference in vascular congestion ($p = 1.000$), inflammatory cell infiltration ($p = 0.220$), and epithelial injury ($p = 1.000$).

DISCUSSION

Recently, there is a substantial body of scientific interest in medicinal plants and natural products, and $>25\%$ of drugs that have been manufactured in the last 20 years are directly derived from plants.¹⁸ Among these, thymoquinone is a bioactive compound that exhibits a

potent action against Gram-positive and Gram-negative bacteria even those that are resistant to antibiotics.¹⁶ Thymoquinone can also function as a scavenger of various reactive oxygen species including superoxide radical anion and hydroxyl radicals and inhibit cyclooxygenase and 5-lipoxygenase pathways of arachidonate metabolism.^{19–22}

Recently, significant relationship between rhinosinusitis and bacterial colonization within the nose and/or paranasal sinuses, which lead to exacerbation of inflammatory processes, has been established.^{6,23,24} Thus, both acute and chronic states of inflammation generally trigger the generation of reactive oxygen species, mainly produced by certain cells of the immune system including macrophages

Figure 5. Light micrographs of the rats in group D (rhinosinusitis-thymoquinone). (A) Nasal respiratory epithelium was almost normal histologically (black arrow); however, mild inflammatory cell infiltration (black star) in lamina propria of nasal respiratory mucosa was revealed (hematoxylin and eosin [H&E], original, $\times 20$). (B) Histologically normal appearing olfactory epithelium; however, a few inflammatory cell clusters were observed in lamina propria of olfactory mucosa (H&E, original, $\times 20$).

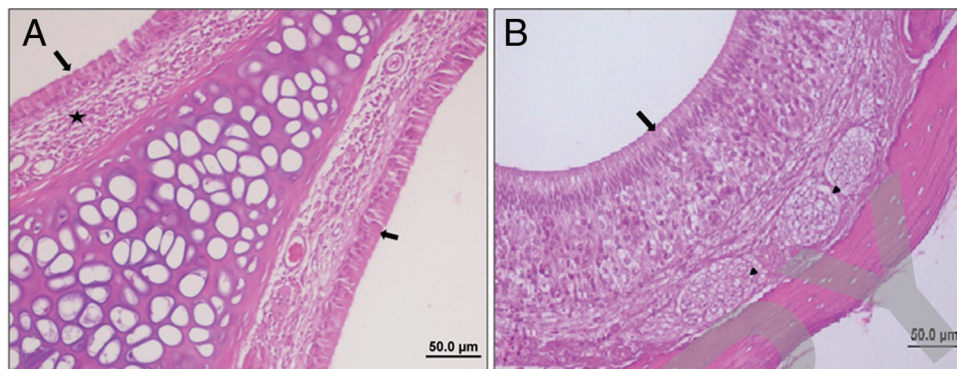
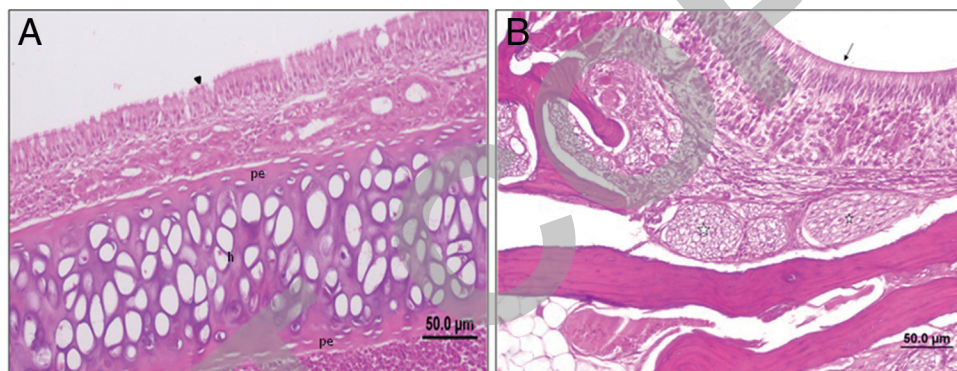


Figure 6. Light micrographs of the rats in group E (rhinosinusitis-combination therapy). (A and B) Both nasal respiratory and olfactory mucosa were histologically normal without any evidence of vascular congestion (hollow arrows), inflammation, and epithelial injury (arrowhead and black arrow; hematoxylin and eosin [H&E], original, $\times 20$, and H&E, original, $\times 20$). pe, perichondrium; h, hyaline cartilage.



and neutrophils, that cause damage to cell structure and function.^{2,25–27} Therefore, it is likely that thymoquinone, which has antimicrobial, antioxidant, anti-inflammatory, and immunomodulatory activities, may play a role in the treatment of rhinosinusitis. In this study, remarkable histopathological changes with statistically significant differences were determined between the positive control group and the group with rhinosinusitis-thymoquinone, when they were compared according to vascular congestion ($p = 0.025$), inflammatory cell infiltration ($p < 0.001$), and epithelial injury ($p < 0.001$). In addition, the degree of vascular congestion, intensity of inflammatory cell infiltration, and degree of epithelial injury in the group with rhinosinusitis-thymoquinone were almost similar to the negative control group without having statistically significant differences (Fig. 1, A–C; Table 1; $p = 1.000$, $p = 0.414$, and $p = 1.000$). Both of these data obviously show that thymoquinone is considerably effective as a therapeutic regimen in rhinosinusitis and may accelerate the healing process in nasal respiratory and olfactory mucosa.

In the second part of this study, the histopathological changes between the group with rhinosinusitis-antibiotherapy and the group with rhinosinusitis-thymoquinone were evaluated to determine the degree of thymoquinone-related improvement on experimentally induced rhinosinusitis in rats. Indeed, antibiotherapy is accepted as the main treatment modality for rhinosinusitis.^{3,28} Sütbeyaz *et al.* reported a remarkable decrease of neutrophil infiltration in sinus mucosa of experimentally induced rhinosinusitis in rabbits after cefazolin administration.²⁹ In our study, none of the histopathological features showed a statistically significant difference between the group with rhinosinusitis-antibiotherapy and the group with rhinosinusitis-thymoquinone ($p = 1.000$, $p = 0.100$, and $p = 0.317$). Hence, there is certain evidence to suggest that thymoquinone may have an antibiotic-like effect in rhinosinusitis and facilitate the resolution of rhinosinusitis-induced histopathological changes. Finally, the histopathological features of the group with rhinosinusitis-thymoquinone were compared with the histopathological features of the group with rhinosinusitis-combination therapy to examine the presence of adjunct effect. Although histopathological features in the group with rhino-

sinusitis-combination therapy had slightly better results, none of them were statistically significant (Fig. 1, A–C; Table 1; $p = 1.000$, $p = 1.000$, and $p = 0.220$). Therefore, it can be speculated that a combination of antibiotic and thymoquinone does not provide an additional improvement in the histopathological features of nasal respiratory and olfactory mucosa in rhinosinusitis.

Of note, this study has several limitations. In this study, a semi-quantitative grading system was used to assess the qualitative changes in nasal respiratory and olfactory mucosa; however, the pharmacologic effects of thymoquinone such as antioxidant and anti-inflammatory activities should also be examined in rhinosinusitis. In addition, these data may not be extrapolated directly to the rhinosinusitis in human, although the histopathological effect of thymoquinone on experimentally induced rhinosinusitis was obvious in rats.

CONCLUSION

This study showed that thymoquinone can provide beneficial effects for the resolution of rhinosinusitis-induced histopathological changes and may be a therapeutic choice for the treatment of rhinosinusitis, although further studies are required to clarify its mechanism of action.

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