Review Article

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TANAFFOS

Thymoquinone Targeting T Helper 2 Cytokines in Animal Models of Asthma: A Systematic Review

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Background: Thymoquinone (TQ) is one of the active components of *Nigella sativa* L. It has therapeutic properties in allergic diseases, such as the antihistamine effect on the airways of patients with asthma and inhibition of inflammatory changes. This systematic review was conducted to investigate the effect of TQ on T helper 2 (Th2) cytokines, including IL-4, IL-5, and IL-13, in the treatment of animal models of asthma.

Materials and Methods: A comprehensive article search was conducted using Web of Science, Scopus, and PubMed to find articles published until 2022 regarding the efficacy of TQ in treating animal models of asthma. We found 399 articles in Scopus, 927 in Web of Science, and 790 in PubMed, from a total number of 2116 articles. After deleting duplicate articles, we read the remaining 1126 titles and abstracts. Finally, 37 articles were selected for full reading. After excluding papers without full text, duplicates, letters, case studies, and those whose topic did not meet the criteria of this study, 8 articles remained. In the manual search, we did not find any deviating articles from the systematic search.

Results: Our results showed that TQ had a significant effect on the reduction of Th2 cytokines, including IL-4, IL-5, and IL-13, in animal models of asthma.

Conclusion: Current evidence shows the anti-inflammatory effects of TQ on Th2 cytokines, but its association with the reduction of Th2 cytokines in animal models of asthma needs further studies.

Keywords: Asthma; Thymoquinone, *Nigella sativa* L.; Inflammation; T helper 2

INTRODUCTION

Asthma is a chronic inflammatory disease which is known by airway hyperresponsiveness and reversible airway obstruction (1). Different studies have shown that the cause of bronchoconstriction is the release of inflammatory mediators (2). The hallmark of allergic asthma is allergic inflammation related to airway hyperreactivity. The inflammatory response is characterized by the activation of T cells (3), an increase in the numbers of mast cells and eosinophils (4), and mucus hypersecretion (3). Many studies showed that the important cause of the initiation and maintenance of allergic airway inflammation and asthma is the overproduction of T-helper 2 (Th2) type cytokines, including IL-4, IL-5, and IL-13 (5). These mediators are produced in bronchial tissue by epithelial cells, mast cells, and alveolar macrophages, which have an important role in the airway inflammation pathogenesis (6). Th2 cytokines mediate the activation of several events, including maturation of B cells, IgE isotype switching, activation and regulation of eosinophils, mast cells, and neutrophil function, regulation of adhesion molecules and chemokines, and mucus production in the inflammatory cascade, which ultimately leads to allergic asthma (7).

A lot of people nowadays have asthma, which is a common and long-lasting illness. Many people with asthma use alternative medicine to help them feel better (8). Researchers are trying to find new efficient therapeutic agents with few side effects. They are investigating novel treatment methods from folk or traditional medicines, besides conventional medicine (2, 9). Lots of individuals with asthma use herbal remedies to lessen symptoms and enhance asthma management. When herbal medicines are combined with routine medications, they enhance treatment outcomes more effectively than medications alone. A systematic review by Shergis et al. on 29 studies involving 3,001 participants who were taking some medicinal herbs showed improved lung function and reduced salbutamol use, and acute asthma exacerbations over one year (10). In fact, the approach of integrative medicine is to provide evidence-based practice of complementary medicines through conducting evidence-based research (11).

Nigella sativa L. and its oils are traditionally used to treat different inflammatory conditions, including asthma (12, 13). Thymoquinone (TQ) (Figure 1) is the major component of *Nigella sativa* L. which previous in vitro and in vivo studies have revealed its therapeutic properties (14) such as anti-inflammatory, antioxidant, and antimicrobial effects (15). Therapeutic effects of TQ in patients with bronchial asthma, allergic rhinitis, atopic eczema, and COVID-19 have also been demonstrated (16, 17). It has a potent antihistaminic effect on the airways of patients with asthma (18). TQ reduces airway inflammation by inhibiting Th2 cytokines and eosinophil infiltration into the airways. As a potent inhibitor of inflammatory changes in asthma, it demonstrates its potential anti-inflammatory role during the allergic response in the lungs (19).

This study aimed to explore the impact of TQ from *Nigella sativa* L. on Th2 cytokines, including IL-4, IL-5, and IL-13, in animal models of asthma.

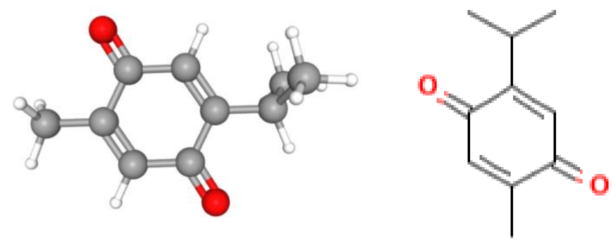

Figure 1. Chemical structure of thymoquinone with the molecular formula of $C_{10}H_{12}O_2$ (adapted from https://pubchem.ncbi.nlm.nih.gov).

MATERIALS AND METHODS

Eligibility criteria

We used the following items to define the eligibility criteria, including population (asthma in animal models), intervention and controls (use of TQ to treat Th2 cytokines, including IL-4, IL-5, and IL-13), outcomes (reduction of IL-4, IL-5, and IL-13 in the treatment of asthma models), and study type (experimental studies).

All electronic search titles, selected abstracts, and full-text articles were independently reviewed. Disagreements as to whether texts met the inclusion/exclusion criteria were resolved by consensus.

Inclusion criteria

The inclusion criteria were: in vivo studies, the use of TQ, treatment of asthma, and evaluation of IL-4, IL-5, and IL-13.

Exclusion criteria

The exclusion criteria were: studies that did not meet the abovementioned characteristics, reviews, meta-analyses, abstracts, conference proceedings, editorials/letters, and case reports, in vitro and randomized clinical trials (RCTs) (Table 1).

Search strategy

Three databases were used to search for articles that met the inclusion criteria: PubMed, Web of Science, and

Scopus with the various combinations of the following keywords:

(thymoquinone[Title/Abstract] OR 2-isopropyl-5-methlbenzo-1,4-quinone[Title/Abstract] OR TQ[Title/Abstract] OR *Nigella sativa*[Title/Abstract] OR black cumin[Title/Abstract] OR black caraway[Title/Abstract] OR black seed[Title/Abstract]) AND (Inflammation[Title/Abstract] OR inflammatory[Title/Abstract] OR tumor necrosis factor[Title/Abstract] OR TNF-α[Title/Abstract] OR TNF[Title/Abstract] OR C-reactive protein[Title/Abstract] OR c reactive protein[Title/Abstract] OR high sensitivity CRP[Title/Abstract] OR hs-CRP[Title/Abstract] OR CRP[Title/Abstract] OR interleukin-4[Title/Abstract] OR IL-4[Title/Abstract] OR interleukin-5[Title/Abstract] OR IL-5[Title/Abstract] OR interleukin-13[Title/Abstract] OR IL-13[Title/Abstract] OR Th2[Title/Abstract] OR T helper 2[Title/Abstract] OR cytokine[Title/Abstract]).

The databases were searched for studies conducted in the period up to September 2022. The search strategy was designed to identify experimental studies related to the effect of TQ on IL-4 and/or IL-5 and/or IL-13 levels in animal models of asthma.

RESULTS

Selection of the studies

The study flowchart is shown in Figure 2. We found 399 articles in Scopus, 927 in Web of Science, and 790 in PubMed (a total number of 2116 articles). After deleting duplicate articles, we read the remaining 1126 titles and abstracts. Then, 37 articles were selected according to the inclusion criteria. After excluding 29 papers, 8 articles remained for final assessment.

Characteristics of included studies

The 8 selected studies were conducted in three countries: Iran (four), China (one), and the USA (three). They were published between 2006 and 2016 (Table 1). The studied lung tissues were from animal models of asthma.

TQ was administered intraperitoneally in six studies and orally in one study. Doses included one injection daily for 5 days before the first ovalbumin (OVA) challenge with 3 mg/kg TQ (20-23), 3 mg/kg TQ on day 10 of the induction protocol (24, 25), TQ from day 15 to 56 one hour before each nebulization (1% OVA) (26), and drinking water with 20 and 40 µM TQ (27).

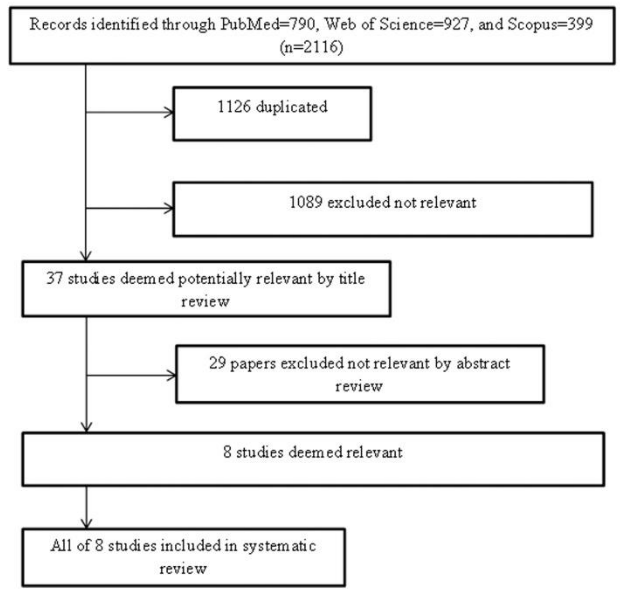

Figure 2. Flowchart for article research and screening

The levels of Th2 inflammatory cytokines, including IL-4 (20-24, 26, 27), IL-5 (20-22, 26), and IL-13 (20-22, 25) were analyzed in this systematic review. The results of one study demonstrated a significant effect of TQ on the inhibition of IL-4 when compared to the OVA challenge group. Specifically, the data revealed a remarkable reduction in IL-4 levels in the group treated with TQ. This finding suggests that TQ has a strong inhibitory effect on IL-4 production in response to the OVA challenge. In addition, the treatment of mice with TQ for five days before airway challenge with OVA antigen normalized the increased levels of IL-5 and IL-13 (20). The study by Su X et al. showed that TQ attenuates the inflammatory response by antagonizing IL-4, and administration of TQ inhibits the increase in IL-5 level. Also, the effect of TQ was compared to that of Dexamethasone, showing no significant differences between the two groups (26). In another study, the results showed a non-significant decrease in IL-4 blood levels in the sensitized (S) +TQ-treated group compared to that of the saline group, which is higher than in the control group (24).

Table 1. Summary of the included studies in the systematic review

Ref.	First author	Year	Country	Study design	Model	Sample size	Methodological characteristics of TQ	Assessed markers	Main results
(18)	El Gazzar	2006	USA	Animal study	Mice	3 groups in each of them were 6 mice	TQ i.p. injection once daily for 5 days preceding the first OVA-challenge, with 3 mg/kg TQ in 10% DMSO	IL-4, IL-5, and IL-13	Inhibition of IL-4, IL-5, and IL-13
(24)	Su	2016	China	Animal study	Mice	5 groups in each of them were 6 mice	Treatment with 3 mg/kg TQ i.p. 1 h before every nebulization	IL-4, and IL-5	Inhibition of IL-4, and IL-5
(22)	Keyhanmanesh	2014	Iran	Animal study	Pig	3 groups in each of them were 10 pigs	TQ with a 3 mg/kg dose was injected i.p. on day 10 of the induction protocol	IL-4	Decreased of IL-4
(23)	Fallahi	2016	Iran	Animal study	Rat	4 groups in each of them were 10 rats	TQ 3 mg/kg injected i.p. on the 10th day of sensitization.	IL-13	Decreased mRNA expression of IL-13
(19)	El Gazzar	2006	USA	Animal study	Mice	3 groups in each of them were 6 mice	TQ 3 mg/kg i.p. injected once daily for the 5 days preceding the first OVA challenge in 10% DMSO	IL-4, IL-5, and IL-13	Decreased of IL-4, IL-5, and IL-13
(20)	El Mezayen	2006	USA	Animal study	Mice	3 groups in each of them were 6 mice	TQ 3 mg/kg in sensitized mice was injected i.p. once daily for the 5 days preceding the first OVA challenge in 10% DMSO	IL-4, IL-5, and IL-13	Decreased of IL-4, IL-5, and IL-13
(21)	Keyhanmanesh	2015	Iran	Animal study	Pig	6 groups in each of them were 8 pigs	Sensitized groups pretreated with TQ 3 mg/kg	IL-4	Decreased of IL-4
(25)	Keyhanmanesh,	2010	Iran	Animal study	Pig	4 groups in each of them were 8 pigs	1) Drinking water containing 20 μM (0.0033 w/v) TQ (Aldrich Chemical Co., Germany) (group S+LTQ). 2) Drinking water containing 40 μM (0.0066 g %) TQ (group S+HTQ).	IL-4	Decreased of IL-4

TQ: Thymoquinone, I.P.: Intraperitoneal, IL-4: Interleukin 4, IL-5: Interleukin 5, IL-13: Interleukin 13, OVA: Ovalbumin, DMSO: Dimethyl Sulfoxide, LTQ: Low Dose of Thymoquinone, HTQ: High Dose Of Thymoquinone, S: Sensitized.

The study by El Gazzar et al. revealed that intraperitoneal administration of TQ before the OVA challenge normalized the elevated levels of cytokines affecting IL-4, with a great effect on IL-5 and IL-13 (21). El Mezayen et al. reported that treatment with TQ for 5 days before the OVA challenge normalized elevated IL-5 and IL-13 levels to those observed in Na¨ıve mice, and had significant inhibition of IL-4 proteins secreted by TQ into the OVA-sensitized airways (22). The study by Keyhanmanesh et al. showed that blood IL-4 levels in the high-dose hederin groups had a significant decrease compared to the sensitized group (p<0.05), which included the S+TQ and inhaled fluticasone propionate (S+FP) groups (23). Another study showed that blood IL-4 levels in animals treated with the lower TQ concentration were not significantly higher than those in the higher concentration group (27). Fallahi et al. showed in their study that there were significant decreases in IL-13 in the TQ and alpha-hederin pretreated groups compared to the synthesized group (25).

DISCUSSION

Medicinal herbs have been attracting attention for the prevention and treatment of different diseases for many years. Interest in the use of medicinal herbs is increasing due to their relatively lower price and availability compared to chemical medicines. In this regard, *Nigella sativa* L. is known as a medicinal herb with anti-inflammatory, antioxidant, and antimicrobial properties (28, 29). As mentioned earlier, TQ is a major component of *Nigella sativa* L., and there are many reports of *Nigella sativa* L.'s anti-inflammatory activities related to its main components, TQ in particular (29).

One of the anti-inflammatory effects of TQ is the inhibition of 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced cyclooxygenase-2 (COX-2) expression and the nuclear factor kappa light chain enhancer of activated B cells (NF-κB) (30). By targeting the 5-lipoxygenase and cyclooxygenase pathways of arachidonic acid metabolism, TQ inhibits the production of inflammatory mediators, leukotriene B_4, and thromboxane B_2 (31). It induces relaxation in the isolated guinea pig trachea by inhibiting the effects of serotonin and histamine on smooth muscles (32). TQ also has high potential in inhibiting inflammatory cell aggregation in lung tissue and bronchoalveolar lavage (BAL) fluid, transforming growth factor-1 (TGF-1), and mRNA expression of inducible nitric oxide synthase (iNOS) (33).

One study showed that tracheal spirals to acetylcholine and histamine were reduced in OVA-sensitized animals by pretreatment with 3 mg/kg intraperitoneally TQ for 5 days. Levels of glutathione depletion (GSH), lipid peroxidation (LP), TNF, IL-1, and infiltration of inflammatory cells in both lung tissue homogenates and BALF in animals exposed to endotoxin lipopolysaccharide (LPS) were improved by 8 mg/kg intraperitoneal TQ. It also reduced histamine release from rat peritoneal mast cells (RPMCs) (34). Moreover, the study performed by Kalemci et al. showed that the administration of 3 mg/kg TQ intraperitoneally leads to the reduction of the lung histopathological changes in OVA-sensitized mice (35). According to our findings, TQ is an effective phytochemical component to decrease the inflammation in the lungs of the animal asthmatic models by reducing IL-4, 5, and 13 levels.

Limitations

The limitation of this study was the small number of studies with methodological heterogeneity conducted on this subject. In addition, in a limited number of related articles, the simultaneous effect of TQ on all 3 interleukins 4, 5, and 13 had been measured. If the number of similar articles had been greater, it would have helped the authors to more rigorously confirm the effect of TQ on these cytokines.

CONCLUSION

This study examined how a natural compound, TQ, found in *Nigella sativa* L., may benefit asthma sufferers. Specifically, it searched whether TQ could reduce IL-4, IL-5, and IL-13 in animal models of asthma. Current evidence shows the anti-inflammatory effects of TQ on Th2 cytokines. However, additional studies are necessary to confirm these benefits and evaluate its safety and effectiveness for treating human asthma.

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