Reviews

# A Systematic Review of the Potential Effects of *Nigella sativa* on Rheumatoid Arthritis

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## ABSTRACT

Considering the different untoward effects of the drugs prescribed for the treatment of rheumatoid arthritis (RA), there has been an increasing interest in adjuvant therapies devoid of such unfavorable reactions. Although the beneficial effects of *Nigella sativa* (*N. sativa*) on RA have been established, it seems that its mechanisms of action have not still been reviewed. The present review is designed to evaluate the effects of *N. sativa* on RA systematically. We searched these electronic databases until April 2019: PubMed, Scopus, ISI Web of Science, Cochrane Library, Embase, Ovid, ProQuest, and Google scholar. No restriction was conducted based on language or publication date. We selected all of the related clinical, animal, and *in vitro* studies. Review papers, abstracts in conferences, book chapters, and papers regarding the effects of *N. sativa* combined with other herbs, as well as articles regarding the effects of *N. sativa* on other diseases, were excluded. Each article was assessed critically for the possible risk of bias. Nineteen articles were reviewed. Animal and *in vitro* investigations supported the favorable effects of *N. sativa* on clinical, inflammatory, oxidative, and immunologic parameters on RA, whereas results of limited clinical studies did not illustrate any change or improvement of inflammatory and oxidative biomarkers in RA. *N. sativa* could control RA via multiple ways such as decreasing inflammation, inhibiting oxidative stress, and modulating the immune system. This paper provides persuasive clues to defend the efficacy of *N. sativa* in RA and justifies the significance of subsequent clinical trials.

## Introduction

Rheumatoid arthritis (RA) is a chronic ailment specified with an immune system disturbance and inflammation of the synovium, causing articular pain and stiffness, bone and cartilage destruction, disability, and deformity as well as systemic complications such as cardiovascular, pulmonary, and psychological problems [1]. RA is the most common inflammatory arthritis that is prevalent in 0.5–1% of world population with a 3-time greater incident in women than men [2]. This disease significantly leads to diminished quality of life and functional capacity together with increased morbidity and mortality rates and imposes remarkable costs for the health and social care systems [3]. ▶ **Fig. 1** presents the pathogenic pathways of RA.

Several pharmaceutical therapies have been suggested and marketed for RA treatment including nonsteroidal anti-inflammatory drugs (NSAIDs), nonbiologic and biologic disease-modifying antirheumatic drugs (DMARDs), immunosuppressants, and corticosteroids. However, as these medications are mostly accompanied by various side effects [4], in recent y, there has been an increasing interest in adjuvant therapies devoid of such unfavorable effects.

Medicinal herbs have gained a lot of attention recently and have been used all over the world to treat various diseases [5].

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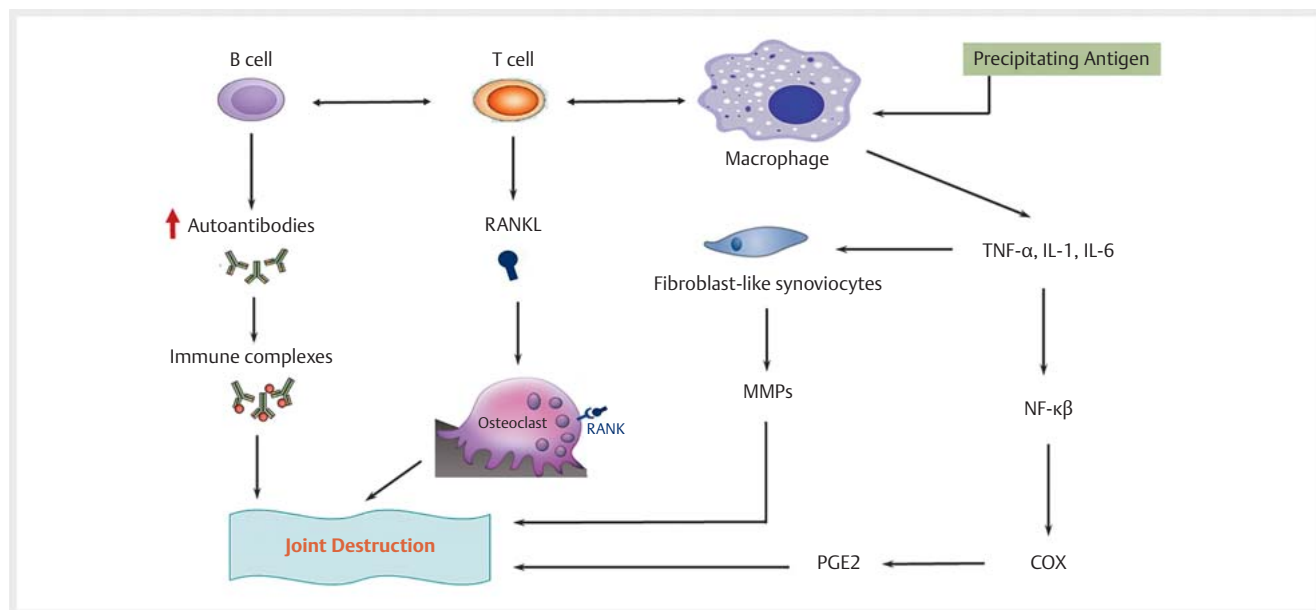
► **Fig. 1** The pathogenic pathways of rheumatoid arthritis.

There is growing evidence in the literature on how some of these medicinal herbs can be effective in the treatment of RA symptoms [6]. One such promising plant is *Nigella sativa L.* (*N. sativa*) (family Ranunculaceae), usually known as black seed [7]. *N. sativa* has been consumed customarily in the Middle and Far East and South Asian countries for the treatment of different disorders [7]. Traditional uses of *N. sativa* derive from the ancient Egyptians, Greeks, and Romans [8]. The Islamic prophet Mohammed is said to have referred to this herb as having healing capacities for every ailment except death [9]. *N. sativa* has been favored by Ibn Sina (Avicenna) as a remedy for fever, headache, toothache, and common cold [10]. It is also proposed as a sedative for skin disorders, wounds, and external irritations [10]. In folklore medicine, *N. sativa* seeds and oil have been regularly prescribed as a natural remedy for various diseases such as fever, cough, nasal congestion, bronchitis, asthma, dyspnea, hypertension, diabetes mellitus, inflammation, eczema, dizziness, gastrointestinal problems, and pain conditions [11, 12]. Furthermore, *N. sativa* has multiple biological and pharmacological functions including antioxidant [13–15], anti-inflammatory and analgesic [16, 17], anticancer [18, 19], antimicrobial [20, 21], immune enhancement [22, 23], hypoglycemic [24], hypotensive [25], hypolipidemic and cardioprotective [26–28], hepato-protective, gastro-protective [29, 30], renal-protective [31, 32], spasmolytic, bronchodilator [33–35], and increased milk production [36]. Moreover, *N. Sativa* has been beneficial for convulsion [37, 38], depression [39], men's infertility [40], and memory improvement [41]. Most of the therapeutic characteristics of *N. sativa* are attributable to the thymoquinone (TQ), a major active component and a phytochemical present in the essential oil [42]. Other components of *N. sativa* seed include carbohydrates, amino acids, proteins, both essential and fixed oil, sterols, alkaloids, saponins, organic acids, crude fiber, vitamins, and minerals [43]. The chemical structures of TQ and other chemical constituents found in *N. sativa* seed and oil are illustrated in ► **Fig. 2**. Fur-

thermore, human [44] and animal models [45] have not shown any critical undesirable results.

The beneficial effects of *N. sativa* on RA prevention and treatment have been investigated in recent y, and some hopeful findings have been obtained from experimental investigations [46–51] and clinical trials [52–56]. It has been suggested that *N. sativa* can influence the main traits of RA via various procedures [57]. Even though some review papers have been published regarding the medicinal features of *N. sativa* [58–60], to the authors' knowledge, there has not been any review conducted in the area of *N. sativa* and RA. Therefore, in this research, we aimed to conduct a systematic study on the available literature regarding the effects of *N. sativa* on RA in clinical, animal and cellular models and possible mechanisms responsible for such effects.

## Materials and Methods

### Protocol and registration

The current systematic literature search was performed according to the preferred reporting items for systematic reviews and meta-analysis guidelines [61]. The study protocol can be observed on the international prospective register of systematic reviews (PROSPERO) database (http://www.crd.york.ac.uk/PROSPERO, registration No: CRD42019133047).

### Search Strategy and Article Selection

We searched the following electronic databases until April 2019: PubMed, Scopus, ISI Web of Science, Cochrane Library, Embase, Ovid, ProQuest, and Google scholar. The MESH and nonMESH search terms applied were ("*Nigella sativa*" OR "*sativa, Nigella*" OR "*N. sativa*" OR "Black Cumin" OR "Cumin, Black" OR "black seed" OR Kalonji OR "black caraway" OR thymoquinone) AND ("Arthri-

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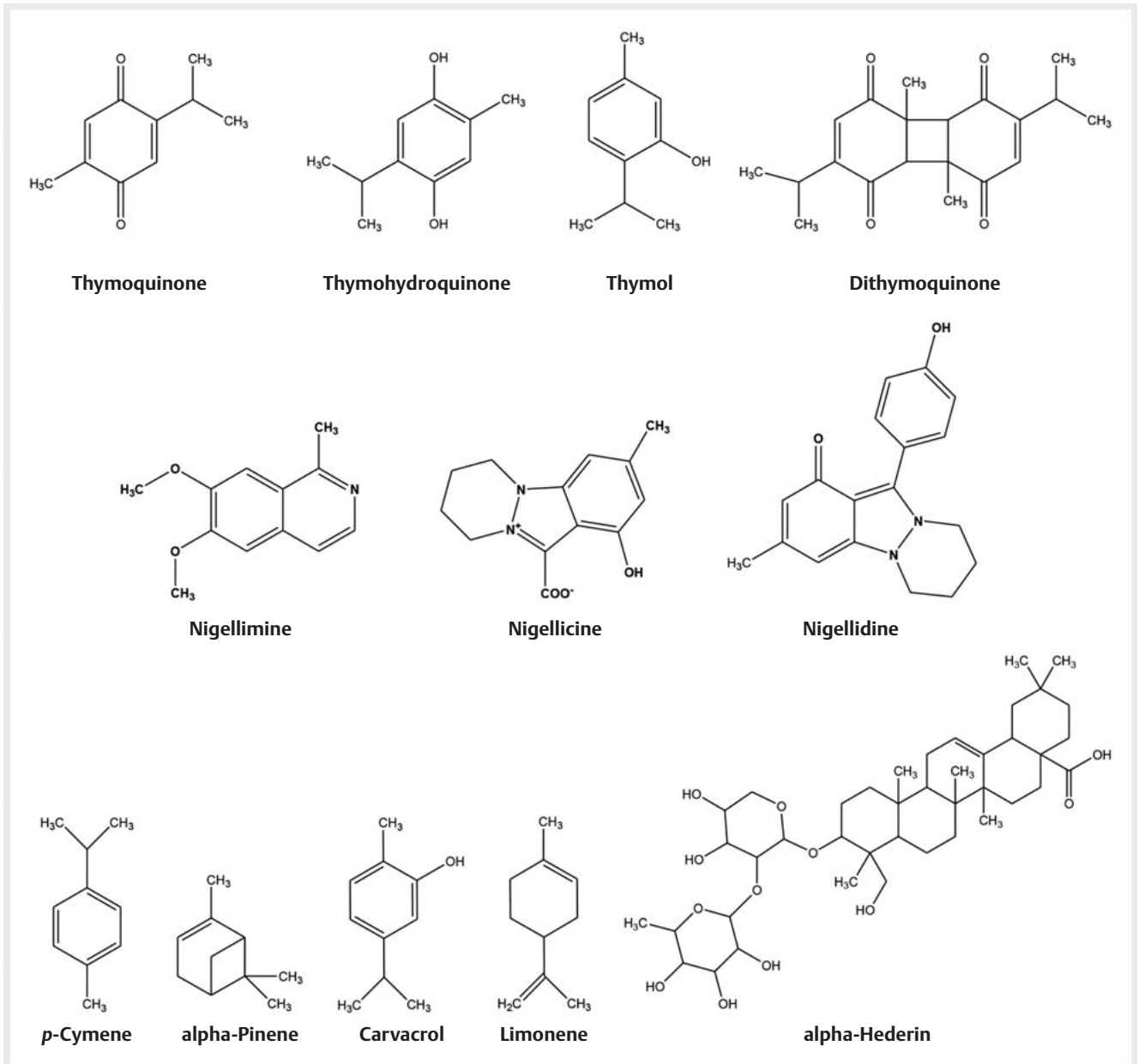
▶ **Fig. 2** The chemical structures of thymoquinone and other chemical constituents found in *N. sativa* seed and oil.
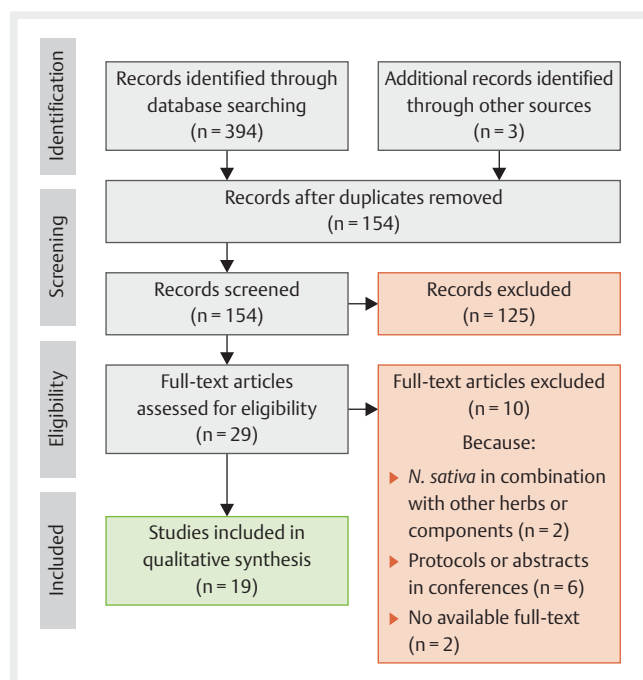
tis, Rheumatoid" OR "Rheumatoid Arthritis" OR RA OR Rheumatoid OR Arthritis). No restriction was conducted based on language or publication date. Two authors (A.KH. and A.M.M.) independently searched, screened, and extracted the data. Duplicated studies were then eliminated. In general, these 2 authors had an agreement on selecting the studies, and possible variations were removed by the third author (Z. J.).

We selected all of the related clinical, animal, and *in vitro* studies. We also checked the references of selected papers to identify possible novel investigations. In addition, we used search alert services in order to identify any relevant article published after the primary search. Review papers, abstracts in conferences, book chapters, and papers regarding the effects of *N. sativa* combined with other herbs on RA were not included. Also, papers regarding the

effects of *N. sativa* in other diseases were not included. Considering the specified search terms and the inclusion criteria, 18 papers were assessed. Furthermore, 1 article published after the initial search was identified *via* search alert services and reviewed. ▶ **Fig. 3** presents the flowchart of screening and choosing papers.

## Data extraction

Two independent reviewers (A.KH. and A.M.M.) extracted data from the eligible papers. The following information was obtained from the papers: first author's name, publication y, location, subject features, type and dosage of *N. sativa* supplement, treatment duration, and main results of studies. Any probable controversies were removed with the third reviewer (Z. J.). A summary of the included studies is presented in ▶ **Tables 1**–**3**.

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## Assessment of risk of bias

Each of the qualified papers was evaluated for the potential risk of bias using Cochrane Collaboration's tool [62] for clinical studies, Checklist for Reporting *In vitro* Studies (CRIS) Guideline [63], for *in vitro* studies and SYRCLE's risk of bias tool [64] for animal studies. The SYRCLE's risk of bias tool is based on the Cochrane Rob tool and has been adapted for aspects of bias that have a particular function in animal intervention. Both of the tools have 6 domains (▸ **Fig. 4**), and each domain was evaluated as having a low, unclear, or high risk of bias.

## Results

### Study characteristics

We identified 397 related papers. After removing duplicates, 154 papers remained that were screened by reviewing both titles and abstracts. Of those, 125 papers were excluded after the revision. Eventually, out of 29 potentially relevant publications, 10 studies were removed for the following reasons: *N. sativa* had been combined with other herbs or components (n = 2), they were protocols or abstracts in conferences (n = 6), and there was no available full-text (n = 2). Finally, 19 papers were entered in the systematic review (▸ **Fig. 3**). The entered studies were classified into clinical (n = 5), animal (n = 13) and *in vitro* (n = 3) studies.



▸ **Fig. 3** The flowchart of screening and choosing eligible articles.

▸ **Table 1** Characteristics of human studies regarding the effect of *Nigella sativa* on rheumatoid arthritis.

| Author (date) | Country | Subjects | Intervention | Dosage | Duration | Results |
|---|---|---|---|---|---|---|
| Hadi et al. (2016) [55] | Iran | Female RA patients (n = 25 per group) | *Nigella sativa* oil capsules | 1 g/day | 8 wk | 1. Significant decrease in DAS28 score compared with the placebo group. 2. No significant differences in serum IL-10, TNF-$\alpha$, MDA, SOD, catalase, TAC, and NO compared with the placebo group. |
| Kheirouri et al. (2016) [52] | Iran | Female RA patients (n = 25 per group) | *Nigella sativa* oil capsules | 1 g/day | 8 wk | 1. Significant reduction in DAS28 and CD8$^+$ percentage compared to the placebo. 2. Significant increase in CD4$^+$/CD8$^+$ ratio and CD4$^+$CD25$^+$ regulatory T cell percentage compared to the placebo. 3. No significant changes in percentage of CD4$^+$ T cells compared to the placebo. |
| Gheita et al. (2011) [54] | Egypt | Female RA patients (n = 40) | *Nigella sativa* oil capsules | 1 g/day | 4 wk | Significant decrease in DAS28, number of swollen joints, duration of morning stiffness, VAS for pain, and WBC count compared to the placebo. |
| Mahdy et al. (2009) [56] | Egypt | Female R A patients (n = 36) | *Nigella sativa* oil capsules | 1 g/day | 2 wk | Significant decrease in DAS28, RAI, morning stiffness, and WBC count compared to the placebo. |
| Al-Okbi et al. (2000) [53] | Egypt | Male RA patients (n = 28 per group) | *Nigella sativa* oil capsules | 2 g/day | 8 wk | 1. No significant changes in ESR, CRP, RF, PGE2, SOD, vitamin C, vitamin E, and uric acid compared to the other treatment groups. 2. Significant decrease in plasma Cu, creatinine, AST, and ALT compared to the other treatment groups. 3. Significant increase in plasma Zn compared to the other treatment groups. |

RA: rheumatoid arthritis; DAS: disease activity score; IL-10: interleukin-10; TNF: tumor necrosis factor; MDA: malondialdehyde; SOD: superoxide dismutase; TAC: total antioxidant capacity; NO: nitric oxide; RAI: ritchie articular index; WBC: white blood cell; VAS: visual analog scale; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; RF: rheumatoid factor; PGE2: prostaglandin E2; AST: aspartate aminotransferase; ALT: alanine aminotransferase.

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▶ **Table 2** Characteristics of animal studies regarding the effect of *Nigella sativa* on rheumatoid arthritis.

| Author (date) | Country | Subjects | Intervention | Dosage | Duration | Results |
|---|---|---|---|---|---|---|
| Nasuti et al. (2019) [65] | Italy | FCA-induced arthritic rats | *Nigella sativa* oil | 1596 and 798 mg/kg BW | 25 days | 1. Significant reduction in paw volume compared to the CFA control group.<br>2. No significant reduction in paw volume and the arthritis score compared to the CFA control group.<br>3. No significant change in % of inhibition of arthritis, spontaneous locomotor activity, plasma levels of IL-6, CRP, and albumin compared to the CFA control group.<br>4. Significant anti-nociceptive activity in contralateral hind paw compared to the CFA control group.<br>5. No significant anti-nociceptive activity in the inoculated hind paw compared to the CFA control group. |
| Arjumand et al. (2019) [48] | Pakistan | FCA-induced arthritic rats | Thymoquinone | 10 mg/kg BW | 20 days | 1. Significant decrease in the arthritic score and CRP levels compared to the arthritic control group.<br>2. Significant normalization of TLC, lymphocytes, neutrophils, and monocytes compared to the arthritic control group.<br>3. Significant attenuation of inflammation, pannus formation, and bone erosion compared to the arthritic control group.<br>4. Significant decrease in mRNA expression levels of TLR2, TLR4, IL-1, NF-κB, and TNF-α compared to the arthritic control group.<br>5. No significant change in RF compared to the arthritic control group |
| Faisal et al. (2018) [66] | Pakistan | Pristine-induced arthritic rats | Thymoquinone | 2 mg/kg BW | 15 days | Significant reduction in paw weight and score of histopathological parameters (*e.g.*, inflammatory cells, synovial hyperplasia, villous hyperplasia, and pannus formation) compared to the arthritic control group. |
| Faisal et al. (2015) [68] | Pakistan | Pristine-induced arthritic rats | Thymoquinone | 2 mg/kg BW | 15 days | Significant reduction in TLC and clinical score of inflammation, and improvement in blood urea and serum creatinine compared to the arthritic control group. |
| Faisal et al. (2015) [49] | Pakistan | Pristine-induced arthritic rats | Thymoquinone | 2 mg/kg BW | 15 days | Significant reduction in TLC and number of inflammatory cells compared to the arthritic control group. |
| Faisal et al. (2015) [67] | Pakistan | Pristine-induced arthritic rats | Thymoquinone | 2 mg/kg BW | 15 days | Significant decrease in clinical score of inflammation and TLC and normalization of DLC. |
| Umar et al. (2012) [51] | India | Collagen-induced arthritic rats | Thymoquinone | 5 ml/kg BW | 21 days | 1. Suppression of severity and progression of collagen-induced arthritis in a dose-dependent manner.<br>2. Significant decrease in articular elastase and myeloperoxidase levels, neutrophil activation, and infiltration in the synovial tissues of the joints compared with the untreated disease group.<br>3. Significant inhibition of lipid peroxidation and decrease in TBARS level in the cartilage tissue compared with the untreated disease group.<br>4. Significant inhibition of down regulation of GSH and SOD compared with the untreated disease group.<br>5. Significant replenishment of catalase activity compared with the untreated disease group.<br>6. Significant decrease in nitrite concentration compared with the untreated disease group.<br>7. Significant suppression of the increase in the level of IL-1β, IL-6, TNF-α, IFN-γ, and PGE2, and significant increase in IL-10 compared with the untreated disease group.<br>8. Amelioration of the changes at histological level and restoring the degenerative changes. |
| Vaillancourt et al. (2011) [46] | Canada | Adjuvant-induced arthritic rats | Thymoquinone | 5 mg/kg BW | 28 days | 1. Significant reduction in serum HNE, IL-1β, TNF-α, and PGE2 compared to the arthritic control group.<br>2. Significant reduction in bone turnover markers, such as alkaline phosphatase and tartrate-resistant acid phosphatase compared to the arthritic control group.<br>3. Significant inhibition of the increase in arthritis score and paw swelling compared to the arthritic control group.<br>4. Significant suppression of bone resorption compared to the arthritic control group. |

*continued*

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▶ **Table 2** *Continued*

| Author (date) | Country | Subjects | Intervention | Dosage | Duration | Results |
|---|---|---|---|---|---|---|
| Hou et al. (2011) [69] | China | Pristine-induced arthritic rats | Black seed oil | 2.5 and 5 ml/kg BW | 5 days | 1. No significant changes in clinical arthritis severity, ankle joint pathological scores, and plasma NO level compared with the disease group. 2. Declining trend in IL-17A mRNA expression compared with the disease group. 3. No significant change in mRNA expressions of IL-4, TGF-β, and TNF-α. |
| Sajad et al. (2010) [70] | India | Collagen-induced arthritic rats | *Nigella sativa* aqueous methanolic extract | 400 and 500 mg/kg BW | 7 days | 1. Significant decrease in myeloperoxidase activity and elastase activity in the synovial tissue of the joints in a dose-dependent manner compared with the untreated group. 2. Significant inhibition of lipid peroxidation and decrease in MDA in the cartilage tissue in a dose-dependent manner compared with the untreated group. 3. Significant inhibition of the decrease in GSH levels compared with the untreated group. 4. Significant increase in SOD and catalase activities compared with the untreated group. 5. Significant decrease in articular nitrite content compared with the untreated group. 6. Amelioration of the changes at histological level. |
| Mahdy et al. (2009) [56] | Egypt | Adjuvant-induced arthritic rats | *Nigella sativa* seed oil | 400 mg/kg BW | 2 wk | No significant change in paw edema, ALT, and AST levels compared to the arthritic control group. |
| Tekeoglu et al. (2007) [50] | Turkey | FCA-induced arthritic rats | Thymoquinone | 2.5 & 5 mg/kg BW | 2 wk (on days 1, 4, 7, 10, 14) | Significant reduction in clinical arthritis, radiological scores, and serum TNF-α and IL-1β levels compared with the control group. |
| Budancamanak et al. (2006) [71] | Turkey | Collagen-induced arthritic rats | Thymoquinone | 10 mg/kg BW once a week | 3 wk | 1. Significant decrease in the incidence and severity of arthritis compared with the control group. 2. Significant reduction in serum NO, urea, and creatinine levels compared with the control group. 3. Significant inhibition of kidney dysfunction and histopathologic alterations compared with the control group. |

FCA: Freund's complete adjuvant; CRP: C-reactive protein; TLC: total leukocyte count; TLR: toll-like receptor; IL-1: interleukin-1; TNF: tumor necrosis factor; NF-κB: nuclear factor kappa-light-chain-enhancer of activated B cells; RF: rheumatoid factor; AST: aspartate aminotransferase; ALT: alanine aminotransferase; DLC: differential leukocyte count; TBARS: thiobarbituric acid reactive substances; GSH: glutathione; SOD: superoxide dismutase; IFN: interferon; PGE2: prostaglandin E2; HNE: $H_2O_2$-induced 4-hydroxynonenal; NO: nitric oxide; TGF: transforming growth factor; MDA: malondialdehyde.

▶ **Table 3** Characteristics of *in vitro* studies regarding the effect of *Nigella sativa* on rheumatoid arthritis.

| Author (date) | Country | Subjects | Treatment | Dosage | Duration | Results |
|---|---|---|---|---|---|---|
| Umar et al. (2015) [47] | USA | Human RA synovial fibroblasts | Thymo-quinone (TQ) | 1–5 µM | 2 hr | 1. Induction of apoptosis by blocking Mcl-1 expression.<br>2. Inhibition of TNF-$\alpha$-induced IL-6 and IL-8 production in a dose-dependent manner.<br>3. Decrease in TNF-$\alpha$-induced ICAM-1, VCAM-1, and Cad-11 expression.<br>4. Inhibition of TNF-$\alpha$-induced phosphorylation of p38 and JNK in a dose-dependent manner.<br>5. Inhibition of TNF-$\alpha$-induced ASK1 activation and suppression of p38/JNK-mediated phospho-c-Jun expression.<br>6. Inhibition of ASK1 phosphorylation and subsequent activation to decelerate TNF-$\alpha$ signaling. |
| Vaillancourt et al. (2011) [46] | Canada | Human RA fibro-blast-like synoviocytes | Thymo-quinone (TQ) | 0–10 µM | 1 hr | 1. Moderate antiproliferative (inhibition of cell growth by 20%) and strong antioxidative effects (prevention of HNE generation up to 70%).<br>2. Inhibition of the LPS-induced mRNA expression of IL-1$\beta$ and TNF-$\alpha$.<br>3. Inhibition of the LPS-induced PGE$_2$ release and COX-2 expression in a dose-dependent manner.<br>4. Prevention of LPS-induced MMP-13 expression at the protein and mRNA levels in a dose-dependent manner.<br>5. Inhibition of LPS-induced p38 MAPK, ERK½, and NF-$\kappa$B-p65 phosphorylation and their signaling pathways. |
| May et al. (2007) [72] | USA | HTB-93 Female RA Cell Line | Thymo-quinone (TQ) | 10, 50, 100 µM | 24, 48, 72 hr | 1. Significant reduction in cell proliferation and cell number compared to the controls.<br>2. No significant change in MDA levels compared to the controls.<br>3. Significant changes in total cellular protein levels.<br>4. Significant increase in glutathione levels only at high-dose treatment (100 µM) at 72 h compared to the control.<br>5. No significant change in glutathione levels at doses of 10–50 µM treatment over 24, 48, and 72 h compared to the control.<br>6. Significant decrease in NO levels over 24 h treatment compared to the control.<br>7. No significant change in NO production over 48 and 72 h compared to the control. |

RA: rheumatoid arthritis; MCL: myeloid cell leukemia; ICAM: intercellular adhesion molecule; VCAM: vascular cell adhesion molecule; CAD: cadherin; ASK: apoptosis-regulated signaling kinase; JNK: c-Jun N-terminal kinase; LPS: lipopolysaccharides; HNE: H$_2$O$_2$-induced 4-hydroxynonenal; PGE2: prostaglandin E2; COX: cyclooxygenase; MMP: matrix metalloproteinase; MAPK: mitogen-activated protein kinase; ERK: extracellular-signal-regulated kinase; NF-$\kappa$B: nuclear factor kappa-light-chain-enhancer of activated B cells; MDA: malondialdehyde; NO: nitric oxide.

One study included both human and animal models [56], and 1 study included both animal and *in vitro* models [46]. Characteristics of the investigations are demonstrated in ▶ **Tables 1–3**.

## Human studies

The effect of *N. sativa* consumption on RA cases was assessed in 5 investigations only (▶ Table 1). In a research by Hadi et al. [55] on 50 female RA patients, *N. sativa* oil supplementation (1 g/day) for 8 wk caused a significant decrease in disease activity score 28 (DAS28) compared with the placebo group, while there were no considerable differences in serum interleukin-10 (IL-10), tumor necrosis factor-$\alpha$ (TNF-$\alpha$), malondialdehyde (MDA), superoxide dismutase (SOD), catalase, total antioxidant capacity (TAC), and nitric oxide (NO) between the groups. Furthermore, in a study by

Kheirouri et al. [52] on 50 female RA patients, *N. sativa* oil supplementation (1 g/day) for 8 wk led to a significant reduction in DAS28 and CD8$^+$ percentage and a significant increase in CD4$^+$/CD8$^+$ ratio and CD4$^+$CD25$^+$ regulatory T cell percentage compared to the placebo, while there were no considerable differences in percentage of CD4$^+$ T cells. In a study by Gheita et al. [54] on 40 female patients with RA, *N. sativa* oil supplementation (1 g/day) for 4 wk caused a significant decrease in DAS28, number of swollen joints, morning stiffness duration, visual analog scale (VAS) for pain, and white blood cell (WBC) count compared to the placebo. Another study by Mahdy et al. [56] on 36 female RA patients indicated that *N. sativa* oil supplementation (1 g/day) for 2 wk caused a significant decrease in DAS28, Ritchie articular index (RAI), morning stiffness, and white blood cell cin comparison
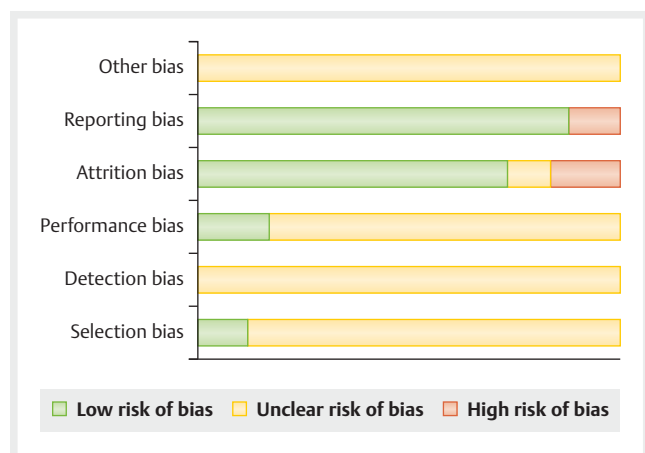
▶ **Fig. 4** The risk of bias assessment across the studies (n = 21).

with the placebo. In a research by Al-Okbi et al. [53] on 56 male RA patients, *N. sativa* oil supplementation (2 g/day) for 8 wk led to a remarkable reduction in plasma Cu, creatinine, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) and a significant increase in plasma Zn, compared to the other treatment groups, whereas there were no significant changes in erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), rheumatoid factor (RF), prostaglandin E2 (PGE2), SOD, vitamin C, vitamin E, and uric acid compared to the other treatment groups.

## Animal studies

Several animal investigations supported the effect of *N. sativa* on RA (▶ **Table 2**). According to the inclusion criteria, 13 animal investigations were entered in the current systematic review. Arjumand et al. [48] reported that 10 mg/kg BW TQ caused a considerable reduction in the arthritic score and CRP levels, significant normalization of total leukocyte count (TLC), lymphocytes, neutrophils, and monocytes; significant alleviation of inflammation, pannus formation, and bone erosion; and a significant decrease in mRNA expression levels of toll-like receptor (TLR)2, TLR4, IL-1, nuclear factor kappa-light-chain-enhancer of activated B cells (NF-$\kappa$B), and TNF-$\alpha$ in rats with Freund's Complete Adjuvant-induced arthritic compared to the arthritic control group, while there was no significant change in RF compared to the arthritic control group. Nasuti et al. [65] noted that *N. sativa* oil administration (1596 and 798 mg/kg BW for 25 days) contributed to significant alleviation in paw volume and had significant antinociceptive activity in contralateral hind paw in Freund's Complete Adjuvant-induced arthritic rats compared to the arthritic control group; however, reduction in paw volume and the arthritis score was not significant compared to the arthritic control group. Moreover, no considerable change occurred in % of inhibition of arthritis, spontaneous locomotor activity, plasma levels of IL-6, CRP, and albumin compared to the arthritic control group. Additionally, no significant antinociceptive activity was reported in the inoculated hind paw compared to the arthritic control group [65]. Another study in Freund's Complete Adjuvant-induced arthritic rats also indicated that TQ (2.5 and 5 mg/kg BW) led to a significant reduction in clinical arthritis, radiological scores, and serum

TNF-$\alpha$ and IL-1$\beta$ levels in comparison with the control group [50]. Faisal et al. [66] reported that administering TQ (2 mg/kg BW) for 15 days to pristine-induced arthritic rats significantly decreased paw weight and score of histopathological parameters (*e.g.*, inflammatory cells, synovial hyperplasia, villous hyperplasia, and pannus formation) compared to the arthritic control group. Furthermore, they showed a considerable decrease in TLC, clinical score of inflammation, and number of inflammatory cells as well as improvement in blood urea and serum creatinine and normalization of differential leukocyte count (DLC) compared to the arthritic control group [49, 67, 68]. Another study in pristine-induced arthritic rats [69] indicated that 2.5 and 5 ml/kg BW black seed oil did not cause significant changes in clinical arthritis severity, ankle joint pathological scores, and plasma NO level compared with the disease group. There was no significant change in mRNA expressions of IL-4, transforming growth factor (TGF)-$\beta$, and TNF-$\alpha$; however, a declining trend in IL-17A mRNA expression was observed compared with the disease group. In a study in collagen-induced arthritic rats, Umar et al. [51] found that 5 ml/kg BW TQ for 21 days lowered the severity and the development of collagen-induced arthritis in a dose-dependent manner and resulted in a significant decrease in articular elastase and myeloperoxidase concentrations, neutrophil activation, and infiltration in the synovial tissues of the joints, as well as a significant decrease in thiobarbituric acid reactive substances (TBARS) level in the cartilage tissue compared with the untreated disease group. In addition, TQ considerably prevented down-regulation of glutathione (GSH) and SOD and replenished catalase activity compared with the untreated disease group [51]. Also, TQ significantly decreased nitrite concentration, suppressed the increase in IL-1$\beta$, IL-6, TNF-$\alpha$, interferon (IFN)-$\gamma$, and PGE2 levels, and enhanced IL-10 levels compared with the untreated diseased group. Furthermore, it improved the changes at the histological level and restored the degenerative changes [51]. Another study in collagen-induced arthritic rats showed that the administration of 400 and 500 mg/kg BW *N. sativa* aqueous methanolic extract led to a significant decrease in myeloperoxidase and elastase activities in the joints as well as a significant suppression of lipid peroxidation and a decrease in MDA in the cartilage tissue in a dose-dependent manner compared with the untreated group [70]. Moreover, *N. sativa* aqueous methanolic extract significantly restored GSH levels, increased SOD and catalase activities, and decreased articular nitrite content compared with the untreated group. It also improved the changes at histological level [70]. Moreover, Budancamanak et al. [71] stated that giving 10 mg/kg BW TQ by gavage for 3 wk to collagen-induced arthritic rats can cause a significant reduction in the incidence and severity of arthritis, serum NO, urea, and creatinine levels. It can also inhibit kidney dysfunction and histopathologic alterations significantly compared to the control group. In a study in adjuvant-induced arthritic rats, 400 mg/kg BW *N. sativa* seed oil for 2 wk did not cause a significant change in paw edema, ALT, and AST levels compared to the arthritic control group [56]. TQ administration (5 mg/kg BW) by oral gavage has also been shown to cause a significant reduction in serum $H_2O_2$-induced 4-hydroxynonenal (HNE), IL-1$\beta$, TNF-$\alpha$, PGE2, and bone turnover markers such as alkaline phosphatase and tartrate-resistant acid phosphatase [46]. TQ was also able to

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inhibit significantly the arthritis score elevation and paw swelling compared to the arthritic control group [46].

## *In vitro* studies

Findings from *in vitro* studies supported the effect of *N. sativa* on RA via showing moderate antiproliferative and strong anti-inflammatory and antioxidant features of TQ (▶ **Table 3**). These results indicate that the treatment of human RA synovial fibroblasts with 1–5 µM TQ can induce apoptosis by blocking myeloid cell leukemia (Mcl)-1 expression and inhibit TNF-α-induced IL-6 and IL-8 generation in a dose-dependent style. TQ treatment can additionally decrease levels of TNF-α-induced intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1, reduce the expression of cadherin (Cad)-11, and inhibit TNF-α-induced phosphorylation of p38 and c-Jun N-terminal kinase (JNK) in a dose-dependent style. TQ treatment has also been illustrated to prevent the phosphorylation and the subsequent activation of TNF-α-induced apoptosis-regulated signaling kinase (ASK)-1 to decelerate TNF-α signaling and to suppress the expression of p38/JNK-mediated phospho-c-Jun [47]. Vaillancourt et al. [46] suggested that treatment with 0–10 µM TQ in human RA fibroblast-like synoviocytes can inhibit the lipopolysaccharides (LPS)-induced mRNA expression of IL-1β and TNF-α, PGE2 release, and cyclooxygenase (COX)-2 expression and prevent LPS-induced matrix metalloproteinase (MMP)-13 expression at the protein and mRNA levels in a dose-dependent style. The authors further reported the inhibition of LPS-induced p38 mitogen-activated protein kinase (MAPK), extracellular-signal-regulated kinase (ERK½) and NF-κB-p65 phosphorylation and their signaling pathways [46]. In a study by May et al. [72] in HTB-93 female RA Cell Line, TQ treatment (10, 50, and 100 µM) caused a significant reduction in cell proliferation, cell number, and NO levels compared to the controls. A significant decrease and/or increase in total cellular protein levels, and a significant increase in GSH levels compared to the controls were also reported; while no significant change in MDA levels and NO production were shown [72].

## Methodological quality and risk of bias

The risk of bias was evaluated for all of the above-mentioned types of investigations. An unclear risk of selection bias (owing to lack of information regarding the randomization procedure, n = 19 and concealment, n = 21), performance bias (owing to lack of information regarding blinding of participants and personnel, n = 18) and detection bias (blinding of outcome evaluation, n = 21) was noticed. The risk of bias for the studies was low for reporting bias and attrition bias (▶ **Fig. 4**).

## Discussion

This is the first systematic review assessing the available literature on the effects of *N. sativa* on RA in *in vitro*, animal, and clinical studies. *In vitro* studies have noted the desirable effects of *N. sativa* in improving the inflammatory and oxidative status of RA [46, 47, 72]. Furthermore, almost all animal studies [46, 48–51, 65–68, 70, 71] have shown that *N. sativa* and its active component TQ can have beneficial effects on clinical, inflammatory, oxidative, and immunologic parameters in RA. However, 2 studies in

animal models [56, 69] did not report any significant effect of *N. sativa* in RA. In addition, human studies [52–56] have suggested favorable effects of *N. sativa* on clinical and immunologic parameters of RA; however, no change or improvement of inflammatory and oxidative biomarkers was declared in the clinical trials [53, 55]. The controversy in findings between clinical and experimental investigations may be attributed to the differences in the measures of inflammatory, oxidative, and anti-oxidative markers *in vivo* or *in vitro*, as well as intensity and the type of stimulators of inflammation and oxidative stress. Furthermore, different preparations used in various studies may also affect the results. In clinical trials studied in this review, *N. sativa* oil was administered in capsules, whereas in a majority of experimental studies, the active component TQ was used. It seems that concentration of the principal compounds within *N. sativa*, namely TQ, varies greatly depending on the storage and preparation of *N. sativa* products, which can cause significant differences in bioactive compounds between studies. A primary limitation of the reviewed studies is the lack of information regarding quantification or standardization of bioactive compounds in the *N. sativa* preparations used. Standardization of herbal formulations is necessary in order to evaluate quality of drugs, based on the concentration of their active principles, physical, chemical, phytochemical, *in vitro*, and *in vivo* parameters [73]. Standardization of herbal medicines is the process of prescribing a set of standards or inherent features, constant parameters, and qualitative and quantitative values that carry an assurance of quality, efficacy, safety, and reproducibility [74]. However, among clinical trials and animal investigations reviewed here, only 1 study [70] discussed standardization of *N. sativa* preparation used. As mentioned above, TQ is the most bioactive component of *N. sativa* seeds and the oil that exists in tautomeric forms including the enol, keto and mixtures. Comprising the major fraction (~ 90%), the keto form is responsible for the pharmacological features of TQ [75]. TQ is a hydrophobic molecule, so its solubility can affect its bioavailability and cause limitations in drug formulation. In addition, its solubility depends on time as it ranges from 549–669 µg/ml in 24 h elevating to 665–740 µg/ml [76] in 72 h. TQ can be consumed via various methods including oral, intravenous, and intraperitoneal. Oral administration of TQ can cause biotransformation due to the metabolizing liver enzymes that catalyzes TQ into a hydroquinone [75]. Not many reports are detectable regarding its oral pharmacokinetics, which can be due to its poor solubility and low oral bioavailability [77]. The clearance of TQ following intravenous administration is 7.19 ml/kg/min, and the estimated volume of distribution at steady state (VSS) is 700.90 ml/kg. After oral administration, the apparent clearance value is 12.30 ml/min/kg, and VSS is 5109.46 ml/kg. The absorption half-life (T1/2) of TQ is calculated about 217 min, and it is rapidly removed from plasma [78]. The lack of bioavailability and pharmacokinetic parameters, as well as formulation problems, delayed the usage of TQ in the clinical phase. Thus, more investigations are required for a better understanding of TQ pharmacological properties meant for future clinical development.

Several potential mechanisms can be proposed for the observed ameliorating influences of *N. sativa* on RA. ▶ **Fig. 5** summarizes the potential mechanisms and pharmacological properties of
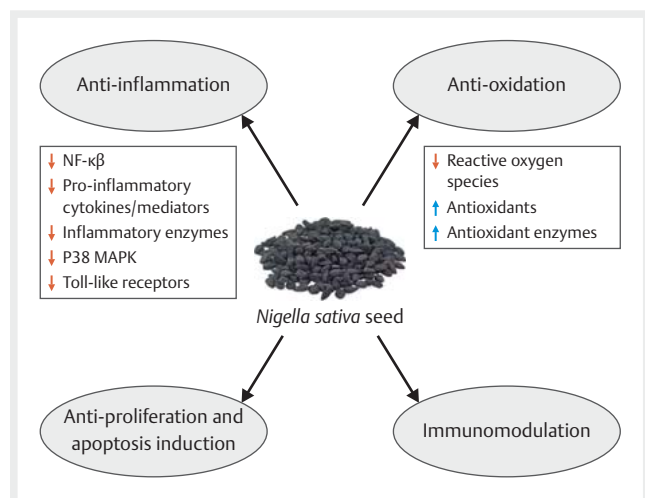
▶ **Fig. 5** The potential mechanisms and pharmacological properties of *N. sativa* in rheumatoid arthritis.

*N. sativa* in RA. The major bioactive component of *N. sativa* is the phytochemical TQ, which has been illustrated to convey anti-inflammatory, antioxidant, and immunomodulatory properties [48]. As noted previously, inflammation, oxidative damage, and immune system activation are major players in the development and progression of RA. Increased generation and activity of pro-inflammatory cytokines, specifically TNF-$\alpha$, IL-1, and IL-6, leads to the uncontrolled inflammation, which destructs the bone and cartilage and causes RA manifestations [79]. It has been suggested that the TQ in *N. sativa* can be involved in anti-inflammatory processes by preventing the production of eicosanoids such as thromboxane B2 and leukotriene B4 via suppressing COX and 5-lipoxygenase [80]. Furthermore, TQ can significantly inhibit the leukotriene C4 synthase activity [81]. *In vitro* treatment of stimulated neutrophils with TQ has shown to inhibit the generation of 5-lipoxygenase products and 5-hydroxy eicosatetraenoic acid [82]. TQ treatment in human blood cells can also inhibit the transformation of arachidonic acid to 5-hydroxy eicosatetraenoic acid [81].

In addition to the inhibitory effects of TQ on eicosanoid production, TQ has been proposed to retain its anti-inflammatory effects via inhibiting various pro-inflammatory transcription factors such as NF-$\kappa$B/STAT3, by inducing several stimuli including cytokines, free radicals, etc. [83]. NF-$\kappa$B activation triggers multiple cascades that cause inflammatory and immune responses [79]. According to animal studies of inflammatory arthritis, NF-$\kappa$B had a predominant function in expansion of arthritis [84]. Furthermore, NF-$\kappa$B activation has been seen in RA synovial tissue in early as well as later phases [79]. Therefore, NF-$\kappa$B inhibitors are supposed to have therapeutic effects and are appropriate for RA treatment [85]. TQ can decrease pro-inflammatory responses predominantly by modulating NF-$\kappa$B activity and suppressing IL-1$\beta$, IL-6, TNF-$\alpha$, and IFN-$\gamma$ production [86–88].

Anti-inflammatory properties of *N. sativa* in RA are further supported by studies showing the preventive effects of TQ on the generation of NO [51]. NO is a pro-inflammatory mediator pro-

duced from activated macrophages as part of the inflammatory response [89]. Inflammatory cytokines in chondrocytes can up-regulate the activity of inducible nitric oxide synthase (iNOS) with the subsequent production of NO. The iNOS activity and plasma NO levels have been declared to be higher in RA cases compared with the normal controls [90, 91]. Excessive NO production has been proposed in the induction of apoptosis in chondrocytes [92]. Thus, agents that prevent extra NO production may have helpful therapeutic influences on arthritis by blocking cartilage destruction [93].

Another possible mechanism for the observed positive effects of *N. sativa* in RA is its free radicals scavenging properties. TQ is a potent antioxidant scavenging reactive oxygen species (ROS) such as superoxide anion, hydroxyl radical, and singlet molecular oxygen [28, 94]. The antioxidant activity of TQ may be attributed to the redox characteristics of its quinone structure and the capability of TQ to pass physiological barriers and easily reach the subcellular sections, all of which assist the radical cleaning function [95, 96]. Moreover, it can increase the activity of several antioxidant enzymes such as catalase, SOD, glutathione transferase, glutathione peroxidase, glutathione reductase, and GSH [97]. TQ may enhance the activation of Nrf2, and thus increase the heme oxygenase-1 (HO-1) expression [98].

The immunomodulatory activity of *N. sativa* is another possible mechanism through which it can influence RA. It has been indicated that *N. sativa* can enhance the immune response, particularly T lymphocytes [99, 100], increase the proportion of helper T cells to suppressor T cells, and increase the activity of natural killer cells [101]. *N. sativa* has also been shown to modulate Th1/Th2 pattern, and partially inhibit the Th2 [102]. Furthermore, a reduction in B cell-mediated immunity has been reported *in vitro* [103]. The immunomodulatory features of *N. sativa* and TQ have also been observed in mixed lymphocyte cultures, where it reduced IL-1$\beta$ and IL-8 secretion [104]. Additionally, it has been announced that *N. sativa* can stimulate interleukin-3 secretion by T cells [105].

The first limitation of our study is the limited number of relevant clinical trials while the number of experimental studies is great and the majority of them have similar procedures. Due to lack of clinical trials and incomparable experimental information, we were not able to carry out meta-analysis on clinical, animal, and cellular studies. Furthermore, according to the risk of bias assessment, all experimental studies showed unclear risk of bias regarding randomization, allocation concealment, and masking ways because of the lack of reporting. Since the clinical investigations are often justified based on the findings from animal studies, this systematic review illustrated the need for randomization, allocation concealment, and masking outcome assessment of animal studies to reduce the risk of bias [106]. The strength of the present study was to systematically review all of the related human, animal, and *in vitro* studies. Furthermore, there was no limitation for time and language in our systematic review.

In this systematic review article, we tried to give persuasive clues on the efficacy of *N. sativa* in RA management and its mechanisms of function. Generally, there is an agreement regarding the impressive effects of *N. sativa* on clinical, inflammatory, oxidative, and immunologic parameters in animal models of RA, while

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the findings of the existing clinical investigations did not declare any change or improvement of inflammatory and oxidative biomarkers in RA subjects. Nevertheless, it should be noted that the majority of the animal research used *N. sativa* extract or its bioactive compound in high doses compared to *N. sativa* powder in clinical studies. In general, given the absence of sufficient clinical information, further randomized controlled clinical trials are warranted to confirm the promising effects of *N. sativa* on RA.

## Contributors' Statement

Conception and design of the work: A. Khabbazi, Z. Javadivala, N. Seyedsadjadi, A. Malek Mahdavi; data collection: A. Khabbazi, Z. Javadivala, N. Seyedsadjadi, A. Malek Mahdavi; analysis and interpretation of the data: A. Khabbazi, Z. Javadivala, N. Seyedsadjadi, A. Malek Mahdavi; drafting the manuscript: A. Khabbazi, Z. Javadivala, N. Seyedsadjadi, A. Malek Mahdavi; critical revision of the manuscript: A. Khabbazi, Z. Javadivala, N. Seyedsadjadi, A. Malek Mahdavi.

### Conflict of Interest

The authors declare that they have no conflict of interest.

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