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Review

Role of *Nigella sativa* L. in the Management of Osteoarthritis: A Systematic Review

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Abstract

Because of the anti-inflammatory properties of thymoquinone (TQ), as the main bioactive substance of *Nigella sativa* L., this systematic review aimed at assessing the therapeutic effects of *N. sativa* and its main bioactive substance in the management of patients with osteoarthritis (OA) based on the *in vivo*, *in vitro*, and in clinical studies. The methodology was adjusted based on the Cochrane Handbook recommendations. All published articles focusing on *N. sativa* as a therapeutic agent for the treatment of OA or its animal model were searched up to 20 April 2022 in PubMed, Medline, Web of Sciences, and Scopus databases. The search process was carried out using the following keywords: "*Nigella sativa*", "Black seed', "Black cumin", and "Thymoquinone" in combination with "Osteoarthritis". Finally, 14 articles remained, including five intervention clinical trial, two human studies, and seven animal studies. Four of five intervention studies showed that *N. sativa* administration led to relief in pain intensity. In the other clinical trial, no difference was reported between the *N. sativa* and control groups in terms of pain relief among OA patients. Studies demonstrated the anti-inflammatory and chondroprotective effects of TQ as the main bioactive substance of *N. sativa*. The evidence confirmed the anti-inflammatory and chondroprotective effects of *N. sativa* in the management of OA patients. Considering the lack of significant adverse effects such as allergic reaction to *N. sativa* in the aforementioned studies, this substance can be recommended as a safe adjuvant treatment to relieve OA pain, compared to nonsteroidal anti-inflammatory drugs and other analgesics.

Keywords: Arthrosis; *Nigella sativa*; Osteoarthritis; Thymoquinone

Introduction

Functional and structural degeneration of the organ systems due to degenerative diseases may lead to bone and joint diseases, including osteoarthritis (OA), rheumatoid arthritis (RA), and osteoporosis [1,2]. Osteoarthritis, also known as degenerative joint disease, is the most prevalent type of arthritis and joint disease, which can result in chronic pain and severe disability. Among the large joints of the body, the knee is the most common site affected by this disease. Osteoarthritis is the most common cause of disability among adults that imposes a lot of costs on the medical system every year [3]. Some pieces of evidence show that about one-third of the population older than 45 years in the United States suffer from OA [4].

Risk factors associated with OA include aging, family history, obesity, race, joint damage, frequent or excessive use of joints, joint deformity, bone density, and female gender [5,6]. The main symptoms of OA have been reported as articular cartilage damage, inflammation, swelling, pain, movement constraints, and stiffness [7,8]. The diagnosis of OA is based on history, clinical examination, and plain radiography, and there are currently no specific tests.

Commonly, OA patients with partial cartilage damage are recommended to change their lifestyle, do

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exercise, and take analgesics and cyclooxygenase inhibitors [9]. Surgery (e.g., microfracture, mosaicplasty, and total knee replacement) is administered in patients having chronic OA with more advanced symptoms [10]. Today, nonsteroidal anti-inflammatory drugs (NSAIDs) are increasingly used to relieve pain in these patients. However, as with most medications, the long-term use of such drugs can have harmful side effects [11]. Based on some evidence, the use of NSAIDs increased the risk of cardiovascular or gastrointestinal diseases [12,13]. Therefore, it is important to find the medications that are highly effective while causing fewer side effects.

It seems that mesenchymal stem cells (MSCs) play an important role in the management of OA. The hypoimmunogenicity and anti-inflammatory characteristics of Human Wharton's jelly stem cells, as an MSC derived from the umbilical cord, have been known in recent years [14].

First of all, the inflammatory activity in OA should be controlled to integrate and contribute to cartilage repair or regeneration. Recently, the use of stem cell-based therapies and herbal supplements are suggested to decrease chronic inflammation in OA patients. *Nigella sativa* L., belonging to the Ranunculaceae family, is a plant native to Asia and the Mediterranean region and is commonly used for therapeutic purposes [15]. *N. sativa* has been reported to increase the ratio of helper T lymphocytes to suppressor T lymphocytes and enhance the activity of normal killer cells and interleukin-1 (IL-1) levels [16]. Thymoquinone (TQ) is the main active chemical component of *N. sativa*. The analgesic, anti-inflammatory, and antioxidant characteristics of TQ, as the main constituent of *N. sativa*, have been confirmed by some pieces of evidence. Moreover, it seems that TQ has anticancer properties and immunomodulatory benefits [17].

Due to the increasing trend in the population aging [18] and the higher prevalence of OA among the elderly [19], the assessment of the treatment process of the disease is highly important. Because of the anti-inflammatory properties of TQ, as the main bioactive substance of *N. sativa*, this systematic review aimed at assessing the therapeutic effects of *N. sativa* and its main bioactive substance on the management of OA patients based on *in vivo*, *in vitro*, and clinical studies.

Materials and Methods

This systematic review was conducted on the studies focusing on the use of *N. sativa* or its bioactive substance (i.e., TQ) as a therapeutic agent for the treatment of OA or its animal model. The methodology was adjusted based on the Cochrane Handbook recommendations in seven domains, including asking a question, defining the exclusion and inclusion criteria, searching, removing the irrelevant articles and entering the eligible papers, measuring the risk of bias, extracting information, and interpreting [20]. In the present review, all published articles focusing on *N. sativa* as a therapeutic agent for the treatment of OA or its animal model were searched up to 20 April 2022 in PubMed, Medline, Web of Sciences, and Scopus databases.

Inclusion and exclusion criteria

In the present review, Participants, Intervention, Comparison, Outcome, and Study design was applied to determine the eligibility criteria. In general, all clinical trial articles on human or animal subjects were entered into this study. All cross-sectional and comparative studies assessing the therapeutic effects of *N. sativa* on OA were also included. Inclusion criteria were 1) assessing the therapeutic effects of *N. sativa* on OA or its animal model, 2) providing an obvious explanation of methodological approaches, and 3) being published in English. On the other hand, exclusion criteria were 1) inaccessibility, 2) insufficient data, 3) qualitative, narrative, systematic/meta-analysis studies, and 4) consensus statements or editorial letters.

Literature search

Considering the predetermined goals, the search process was initiated by two trained researchers. A detailed search was carried out on four main electronic databases, including PubMed, Medline, Web of Sciences, and Scopus, from 30 May to 20 April 2020. In addition to the searching databases, manual research was performed up to 20 April. The search process was performed using various keywords, including "*Nigella sativa*", "Black seed", "Black cumin", and "Thymoquinone" in combination with "Osteoarthritis".

Study selection, data extraction, and design

All stages of the search, study selection, data extraction, design, and interpretation were performed by two trained researchers, who were in contact with each other in all steps of this review. In the first step, all the papers focusing on *N. sativa* as a therapeutic agent for the treatment of OA or its animal model were searched with the specified keywords up to 20 April 2022. The inaccessible, unavailable, and duplicate articles and those published in other languages except English were excluded from the study. Subsequently, the titles and abstracts of the remained studies were separately reviewed by each researcher. Considering the eligible criteria, the irrelevant studies were excluded. The final screening was performed by extracting the full-text version of the remained studies and reviewing them precisely. The selection process of the chosen studies is shown in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA)

flow diagram (Figure 1). In the next step, the two researchers extracted the main information of the studies and recorded them in a checklist while they were continuously in contact with each other.

Quality assessment

All articles were assessed in terms of quality based on Cochrane instructions considering the nature of the studies (i.e., clinical, *in vivo*, and *in vitro* studies) [21]. The clinical studies were investigated in terms of eight domains, namely random sequence generation, allocation concealment, blinding of participants and personal, blinding of outcome assessment, attrition bias, incomplete outcome data, selective reporting, and free of other biases [22]. The level of risk of bias was assessed by "Yes", "No", and "Unclear" responses representing low, high, and unclear risks of bias, respectively (Table 1).

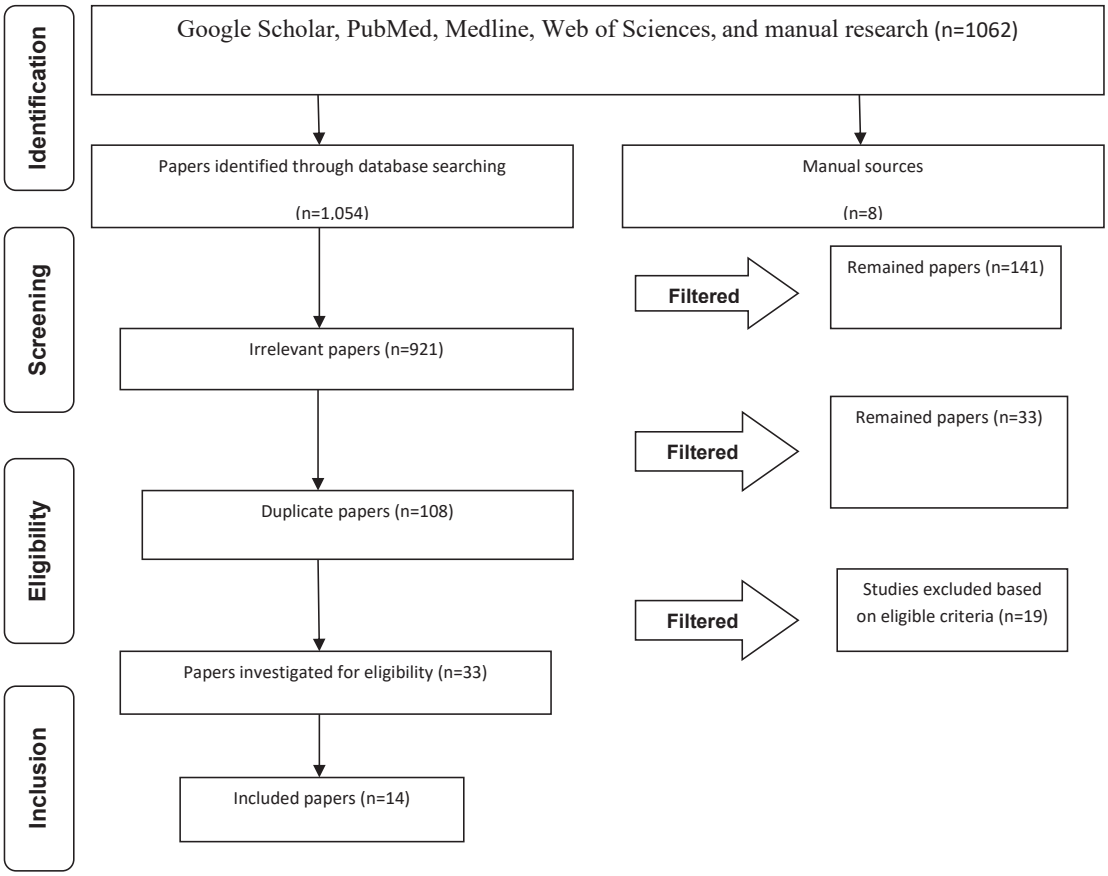

Figure 1. PRISMA flowchart representing the study selection process

Table 1. Quality assessment of reviewed papers

Author Reference	Random sequence generation	Allocation Concealment	Blinding of participant, personal	Blinding of outcome assessment	Attrition bias	Incomplete outcome data	Selective reporting	Free of other biases	Risk of bias
Kooshki et al. [23]	No	Yes	No	No	No	No	No	Unclear	Intermediate
Salimzadeh et al. [26]	Yes	Yes	Yes	Yes	No	No	No	Yes	Low
Tuna et al. [24]	No	Yes	No	No	No	No	No	Unclear	Intermediate
Azizi et al. [15]	Yes	Yes	Yes	Yes	No	No	No	Yes	Low
Amirtaheri et al. [25]	Yes	Yes	Yes	Yes	No	No	No	Yes	Low

Results

In general, 1,062 articles were found in the first search of the databases. After removing irrelevant studies, which did not focus on the effect of *N. sativa* in OA, 141 articles remained. In this regard, the research focusing on autoimmune diseases, including RA, systemic lupus erythematosus, multiple sclerosis, spondyloarthropathy, and ankylosing spondylitis, was also removed. Moreover, 108 papers were omitted due to being duplicated, and 33 articles remained that were screened by reviewing both titles and abstracts. In the next step, studies were excluded due to being inaccessible in full-text (n=3), published in a language other than English (n=1), a book (n=2), a qualitative and narrative review article or a systematic review (n=12), and an editorial letter (n=1). Finally, 14 articles remained to be reviewed (Figure 1).

Out of the final 14 studies, 5 (35.7%) articles were intervention clinical trials (3 double-blind randomized controlled trials and 2 controlled interventional clinical trials) conducted on human samples, 2 *in vivo* studies on human samples (14.2%), and 7 animal studies (50%). In general, 9 (64.3%) studies were performed in the Middle East (6 in Iran, 2 in Turkey, and 2 in Saudi Arabia), 4 (28.5%) studies in the Far East (2 in China and 2 in Korea), and 1 (7.1%) in Southeast Asia (Indonesia).

Human studies

In clinical trials, 274 cases were entered, 143 of whom received topical or oral *N. sativa*. The mean age of the participants was obtained at 55-67 years. Female: male ratio was 1.7: 1. The tools used to assess pain and mobility included the Visual Analogue Scale (VAS), Knee Injury and Osteoarthritis Outcome Score, Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC), and Timed Up and Go Test. *N. sativa* oil was administered topically in three studies and prescribed orally in one study. In one study, *N. sativa* was used both in the form of topical and oral. Consumption instructions were different; in topical consumption, *N. sativa* was administered at a range of 2.5 mg to 2 g, which was rubbed two or three times a day for 6-12 weeks; while, in the oral consumption, 1 mL of *N. sativa* was administered every 8 hours or twice a day for 3 weeks. The data extracted from clinical articles, including sample size, mean age, male: female ratio, weight or body mass index, applied tools, type of treatment, dosage and duration of medication, and pain score before and after treatment with *N. sativa*, are presented in table 2.

The obtained results of four out of five studies showed that *N. sativa* administration led to the relief of pain intensity [15,23-25]. In the other study, no difference was reported between *N. sativa* and control groups in terms of pain relief [26]. Based on the findings of a study by Kooshki et al., the topical application of *N. sativa* oil could relieve pain, in comparison to acetaminophen pills, among elderly patients with knee OA. The participants in the mentioned study administered 1 mL of *N. sativa* oil every 8 hours for 21 days, which was rubbed topically on the knee joint and continued for 5 min with the entire palm and in a clockwise direction [23]. In another study by Azizi et al., further pain relief was reported in the knee joint of OA patients after using *N. sativa* oil for 21 days, compared to diclofenac gel. However, no difference was observed between *N. sativa* and diclofenac gel on the 10th day, which emphasized the analgesic effect of *N. sativa* over time [15]. Moreover, Tuna et al. confirmed the pain-relieving properties of *N. sativa* oil (3 times a week for 1 month) [24].

In another study by Amirtaheri et al., patients with mild-to-moderate knee OA, who received topical or oral *N. sativa* for 6 weeks, were compared with the placebo. Accordingly, the application of *N. sativa* led to the improvement of the WOMAC total score, WOMAC limitations of physical function, WOMAC pain, and VAS scale in both oral and topical *N. sativa* groups after 6 weeks. In comparison to the placebo group, VAS and WOMAC scores showed a significant improvement in the topical *N. sativa* group, however, not in the oral *N. sativa* group. Furthermore, topical *N. sativa* application was more effective in improving the total scores of WOMAC and its physical function, compared to the oral *N. sativa* group. The scores of WOMAC stiffness and Timed Up and Go Test revealed that using the topical and oral *N. sativa* oil was not more effective than the placebo. In addition, the inflammatory factor of C-reactive protein (CRP) was significantly reduced in the oral *N. sativa* group [25].

Since the analgesic effects of *N. sativa* were reported only in the topical *N. sativa* group but not in the oral *N. sativa* group, one of the issues that should not be overlooked is the possibility of the effect of massage in reducing pain where *N. sativa* oil was administered topically. Due to the lack of control of massage as an intervening variable, it seems that more clinical trials are needed to be performed to assess the therapeutic effect of *N. sativa* in OA patients. In this regard, the results obtained from *in vitro* and *in vivo* studies could be helpful.

Animal studies

In general, seven animal studies were conducted on the animal model of OA, in two of which the intervention included the intra-articular injection of 0.3 mL of TQ (10 mmol/L) in the knee. The characteristics of animal studies considering the effect of *N. sativa* on the animal model of OA are summarized in table 3.

It seems that *N. sativa* had a remarkable effect on ar-

Table 2. Characteristics of human studies considering the effect of *Nigella sativa* L. on osteoarthritis

Author (years) Reference	Country	Type of study	Sample Size	Age mean (year)	Female/ male ratio	Weight or BMI	Tool	Treatment	Dosage	Pain score	Pain score	Outcome
Kooshki et al. (2016) [23]	Iran	Randomized, Cross-over study	40 NS[1]:20 OA[2]:20	75.66 >65	22/18	69.67	VAS[7]	Topical use of NS oil	1 cc of every 8 hours for 3 weeks	NS: 4.23 OA: 4.76 P>0.05	--	*Nigella sativa* oil leads to pain intensity relief 0.53 times more compared to oral acetaminophen.
Salimzadeh et al. (2017) [26]	Iran	Randomized, double-blind, intervention controlled study	77 NS:37 Placebo:40	NS: 55.04 Placebo: 55.85 Matched	NS: 24/13 Placebo:34/16 Unmatched	NS: 30.82 Placebo: 29.96 Matched	KOOS[8], VAS	Oral application of N	2 g of NS seed powder every day for 12-week	P>0.05	NS: 6.67 OA: 5.38 P>0.05	No difference was reported between the two groups in terms of KOOS categories.
Tuna et al. (2018) [24]	Turkey	Randomized, intervention clinical trial	60 NS:30 Control: 30	NS: 67.87 Control: 67.97 Matched	NS: 23/7 Control: 23/7 Matched	--	VAS	Topical use of NS oil	3 times a week for 30 days (30 ml)	NS: 7.5 Control: 7.33 P>0.05	NS: 6.30 Control: 7.53 P<0.05	Pain severity was decreased in the *N. sativa* group; while it did not show a difference in the control group.
Azizi et al. (2019) [15]	Iran	Randomized, double-blind, intervention study	52 NS: 26 DG[3]: 26	NS: 66.44 DG: 67 Matched	NS:18/7 DG:17/8 Matched	NS: 27.54 DG: 27.38 Matched BMI	KOOS	Topical use of NS oil	twice a day in the morning and night for 21 days	NS: 75±16.29 DG: 69.88±18.24 P>0.05	NS: 38.88 DG: 50.33 P<0.05	A better pain relief effect was reported for *N. sativa* than diclofenac gel.
Amirtaheri Afshar (2021) [25]	Iran	Randomized, double-blind, intervention controlled study	45 ONO[4]:15 TNO[5]: 15 OPO[6]:15	Matched	Matched	Matched	WOMAC[9], VAS, TUG10	Oral application of N	ONO: 2.5 ml of NS twice a day for 6 weeks TNO: 2.5 ml of three times a day for 6 weeks	--	--	An improvement was observed in VAS and WOMAC pain in the topical *N. sativa* group, in comparison to the placebo group; however, the oral *N. sativa* group showed no improvement.

1- *Nigella sativa*, 2-Oral acetaminophen, 3-Diclofenac Gel, 4-Oral *Nigella sativa* oil, 5- Topical NS oil, 6- Oral placebo oil, 7-Visual Analogue Scale, 8-Knee injury and Osteoarthritis Outcome Score, 9- Western Ontario and McMaster Universities Osteoarthritis Index, 10- Timed up & go test scores

Table 3. Characteristics of animal studies considering the effect of *Nigella sativa* on the animal model of osteoarthritis

Author (years) Reference	Country		Subjects	Sample size	Intervention	Dosage	Duration	Findings
Chen et al. (2010) [29]	China	Both *in vitro* and *in vivo*	Rabbit chondrocytes and animal mode of OA[1]	20 New Zealand rabbits used for *in vitro* assessment 10 five-week-old female China rabbits used for chondrocyte culture	Intra-articular injection of 0.3 mL of TQ[2] (10 mmol/L) in the left knee and vehicle (DMSO[3]) in the right knee	0.3 mL of TQ	Weekly injections continued for five weeks.	- TQ led to down-regulation in MMP[4]-1, MMP-3, and MMP-13 expressions and an up-regulation in the tissue inhibitors of metalloproteinase-1 expression. - TQ inhibited the NF-κB[5] p65 protein level and its translocation induced by IL-1β
Yu et al. (2013) [30]	Korea	*In vitro*	Rabbit articular chondrocytes	Two-week-old New Zealand White rabbits	20 mmol/L TQ for 24 h	20 mmol/L	24 h	TQ significantly increased apoptosis dose- and time-dependently with a focus on reactive oxygen species production. - TQ-induced ROS generation
Yu et al. (2015) [31]	Korea	*In vivo*	Rabbit articular chondrocytes	Two-week-old New Zealand white rabbits	TQ (0, 5, 10 and 20 μM) for 2 h	5, 10 and 20 μM	2 h	- TQ induced the generation of ROS in a dose-dependent manner. - TQ application led to the induction of dedifferentiation via losing type II collagen and decreasing the levels of chondroitin sulfate proteoglycan. - TQ application resulted in the induction of COX-2[6] and PGE2[7] expressions and a dose-dependent increase in p38, p-ERK[8], and PI3K[9] expressions.
Maghsoudi et al. (2018) [32]	Iran	*In vivo*	Radiocarpal joint cartilage of Mature Holstein cow	An 8-month-old Holstein cow (BFS[10] and THP-1)	Alcoholic extract of NS[11]	28.1 g from 100 g of black seeds	72 h	- Suppression of TNF-α and IL-18 expressions in activated chondrocytes was reported by alcoholic extract of *N. sativa*. - Ethanol extract of *N. sativa* could affect very high expression levels of TNF-α, PGE2, NO, iNOS, COX-2 - Ethanol extract of *N. sativa* could reduce the cartilage cells and monocytes macrophage expressions. - Low expression levels of TNF-α and IL-1β relative to LPS-activated cells were reported in human THP-1 cells after using alcoholic extract of *N. sativa*.
Maghsoudi et al. (2018) [33]	Iran	*In vivo*	Radiocarpal joint cartilage of Mature Holstein cow	An 8-month-old Holstein cow BFL[12] and THP-1	Alcoholic extract of NS	6.13 μg\ml as a media LC50	72 h	- A decrease in the expression levels of COX-2, iNOS, and TNF-α was reported by the alcoholic extract of *N. sativa*, compared to the control group. - Alcoholic extract of *N. sativa* led to a decrease in the expressions of TNF-α and IL-1β. - Anti-inflammatory effect of alcoholic extract of *N. sativa* was shown on the BFLS cells and THP-1.

Turhan, et al. (2019) [27]	Turkey	*In vivo*	Rabbit osteoarthritis model (anterior cruciate ligament)	20 New Zealand rabbits Case group:14 Control:6	Intraarticular injections in their right knees weekly after the anterior cruciate ligament transection surgery	0.3 ml of NS oil	5 weeks	- Better total results and better total OARSI[13] scores were reported in the macroscopic grading of rabbits receiving *N. sativa*, compared to the control group.
Dwita et al. (2019) [28]	Indonesia	*In vitro*	Carrageenan-induced paw edema and granuloma pouch	Rat (Acute and subacute inflammatory models)	Topical NS balm contains 10% NS			- Acute and sub-acute inflammations were improved by topical *N. sativa* balm. - Significant reduced TNF-α levels were reported in 7.5% and 10 % *N. sativa* balm groups, compared to the control group.

1- Osteoarthritis, 2- Thymoquinone, 3-Dimethylsulfoxide, 4-Matrix metalloproteinase, 5- Nuclear factor kappa B, 6- cyclooxygenase-2, 7-Prostaglandin E2, 8- Phosphorylated extracellular signal-regulated kinase, 9- Phosphoinositide 3-kinase, 10-Bovine Fibroblast synoviocytes, 11-*Nigella sativa*, 12- Bovine Fibroblast-like 13- Osteoarthritis Research Society International

ticular cartilage. The results of a study conducted by Turhan et al. confirmed the chondroprotective effect of the intra-articular injections of 0.3 mL of *N. sativa* oil for 5 weeks in the animal models of knee OA. Based on the findings of the aforementioned study, in which *N. sativa* oil was used as a whole rather than the isolated TQ component, *N. sativa* had a potential effect of protecting cartilage from degeneration in the early stages of OA [27]. In another study, Dwita et al. assessed the anti-inflammatory activity of balm sticks containing 10% *N. sativa* applied topically in rats and reported that this production could improve the acute and sub-acute inflammation by high edema inhibition (about 60%) [28]. Chen et al. investigated the effect of TQ on the matrix metalloproteinase (MMP) expression was examined in the animal model of OA. Accordingly, downregulation of MMP-1, MMP-3, and MMP-13 expressions and an up-regulation of tissue inhibitors of metalloproteinase-1 expression were reported due to the use of TQ in both chondrocytes and cartilage in the animal model. They showed that TQ could inhibit the NF-κB p65 protein level [29]. The effects of TQ on the apoptosis of chondrocytes were assessed in another study by Yu et al., the results of which showed that TQ significantly increased apoptosis dose- and time-dependently with a focus on reactive oxygen species (ROS) production [30].

To the best of our knowledge, dedifferentiation and inflammation are considered the main characteristics of cartilage degeneration in OA pathogenesis. The findings of a study conducted by Yu et al. on rabbit articular chondrocytes showed that TQ induced the generation of ROS in a dose-dependent manner. This substance played an important role in the induction of cyclooxygenase-2 (COX-2) and prostaglandin E2 (PGE2) expressions. In general, they indicated that TQ-induced production of ROS led to dedifferentiation via the extracellular signal-regulated kinase (ERK) pathway. Moreover, it caused inflammation via phosphoinositide 3-kinase (PI3K) and p38 pathways [31].

In one study by Maghsoudi et al., the effects of alcoholic extract of *N. sativa* were evaluated on the anti-inflammatory activity in bovine fibroblast-like synoviocytes (BFLSs). In the above-mentioned study, cells were activated with 100 ng/mL lipopolysaccharide (LPS) for 24 h. Increased levels of tumor necrosis factor alpha (TNF-α) and IL-18 expressions were observed in synoviocytes activated for 1 h. In comparison to activated control, about 60% of TNF-α and IL-18 expressions in activated chondrocytes were suppressed using the alcoholic extract of *N. sativa*. It was also revealed that the alcoholic extract of *N. sativa* decreased TNF-α and IL-1β expressions in LPS-activated THP-1 cells. It was shown in the afore-

said study that the anti-inflammatory activity of the alcoholic extract of *N. sativa* was not only limited to synoviocytes but also could affect monocyte macrophage-like cells [32]. Similarly, the findings of another study by Maghsoudi et al. reported the effect of alcoholic extract of *N. sativa* in decreasing TNF-α and IL-1β expressions in LPS-activated THP-1 cells [33].

In vivo and in vitro studies on human samples

The immunomodulatory, anti-inflammatory, and antioxidant properties of TQ, as the main bioactive substance of *N. sativa*, have been confirmed by some evidence. Wang et al. investigated the anti-inflammatory effect of TQ on IL-1β-stimulated human osteoarthritis chondrocytes. Based on the obtained results of the mentioned study, IL-1β-induced inflammatory response could be remarkably attenuated by TQ. This substance inhibited the protection of IL-1β-induced COX-2, inducible nitric oxide synthase (iNOS), nitric oxide (NO), and PGE2. Moreover, the production of IL-1β-induced MMP-1, MMP3, and MMP13 was suppressed by TQ. They elucidated that TQ suppressed the inhibition of IL-1β-induced NF-κB activation and IκBα degradation in a dose-dependent manner [34].

In another *in vivo* study by Kalamegam et al., conducted on human cells, the effects of TQ on bone marrow mesenchymal stem cells (BM-MSCs) derived from OA were examined, and the stemness properties of BM-MSCs were identified. Additionally, the interrelated pathways of TQ in inflammation and OA were evaluated using ingenuity pathway analysis in the aforementioned study. Based on the obtained results, morphological changes, such as cell shrinkage, membrane damage, and the loss of characteristic fibroblastic shape, were reported after the treatment of BM-MSCs with TQ for 2 days in various concentrations (100 nM to 5 mM). Cell death and cell number reduction were reported in high concentrations of TQ; while in low concentrations, a mild-to-moderate increase was observed in cell numbers. In various doses of TQ, a significant concentration-dependent reduction (range: 27.80-73.67%) and a concentration-dependent decrease in cell viability (range: 20.04-69.76%), especially on days 2 and 3, have been reported [17]. Therefore, it is highly important to investigate the effectiveness of TQ on the stem cells and normal tissue-specific cells to specify its optimal concentration (Table 4).

Quality assessment of the entered articles

Based on the obtained results, among five entered clinical trials, three studies had a low risk of bias; while, the other two studies had an intermediate risk of bias. Table 1 and figure 2 present the quality assessment of the entered articles.

Discussion

The present systematic review was the first study focusing on the effects of *N. sativa* and its bioactive substance (i.e., TQ) on OA in clinical trials, *in vivo*, and *in vitro* studies. The obtained results of *in vitro* studies confirmed the desirable effects of TQ, as the bioactive substance of *N. sativa*, in improving the inflammatory activity, chondroprotective condition, and oxidative status of OA (35). In the same way, the findings of animal studies were indicative of the beneficial effects of *N. sativa* and its active component (TQ) on the inflammatory condition and oxidative parameters of the animal model of OA. Furthermore, the results of most human studies showed the favorable effects of *N. sativa* on reducing pain in OA patients, and the improvement of inflammatory parameters was reported in only one study, in which CRP was significantly reduced in the oral *N. sativa* group; however, not in the topical *N. sativa* group [25]. The reason for the high prevalence of studies conducted in this regard in Asia and the Mediterranean is that *N. sativa* is a plant native to these parts of the world and is commonly used for therapeutic purposes in these regions.

Analgesic effects of Nigella sativa

Analgesic properties of oleic acid, as an unsaturated fatty acid, in *N. sativa* were confirmed. Oleic acid inhibited the activity of the cyclooxygenase enzyme [36]. Based on some evidence, the use of *N. sativa* prevented the eicosanoid formation in leukocytes and lipid peroxidation [37]. Moreover, COX and 5-lipoxygenase pathways from arachidonic metabolism were inhibited by TQ, as the main bioactive substance of *N. sativa*. TQ helped the conversion of arachidonic acid to prostaglandin H2 by cyclooxygenase enzyme, and consequently, led to pain relief [38]. Moreover, there is some evidence confirming the low toxicity and high safety of the therapeutic approach with TQ [39]. Evidence on the toxicity effect of oral or intraperitoneal *N. sativa* administration showed no cardiac, hepatic, or kidney damage [40,41]. Any hepatotoxic or nephrotoxic effects of TQ have been reported in liver function, creatinine, and urea tests in another study by Arjumand et al. [42].

Anti-inflammatory effects of Thymoquinone as a bioactive substance of Nigella sativa

There is insufficient information on the mechanism of OA. In OA patients, chronic inflammation leads to the damage of articular cartilage tissue. It seems that the cause of OA is the imbalance of chondrocyte homeostasis to maintain the extracellular matrix component (e.g., reducing proteoglycan) [43]. Chronic inflammation has been introduced as the most common underlying cause of OA and other age-related degenerative diseases. The activation of the cascade of pro-inflam-

Table 4. The experimental studies on human samples considering the effect of *Nigella sativa* on osteoarthritis

Author (years) Reference	Country	Sample Size	Samples	Substances	Tools	Outcome
Wang et al. (2015) [34]	China	16	Articular cartilage samples of patients with Total knee replacement	3-(4,5-dimethylthiazol-2-y1)-2,5-diphenyltetrazolium bromide (MTT) and thymoquinone	MTT Assay and ELISA Assay, NO Measurement, and Western Blot Analysis	- TQ had no effect on cell viability at a concentration of ≤15 μM. - IL-1β significantly increased the production of NO and PGE2 in the TQ group, compared to the control group. - TQ significantly inhibited IL-1β-induced iNOS and COX- 2 expressions, in comparison to the LPS-treated group. - IL-1β significantly upregulated the production of MMP-1, MMP-3, and MMP-13 in the TQ group than in the control group.
Kalamegam et al. (2020) [17]	Saudi Arabia	10	Total knee replacement	100 nM, 300 nM, 1 mM, 3 mM, and 5 mM for 24, 48, and 72 h	MTT and CellTiter-Blue R assays	- Morphological changes were observed after the treatment of BM-MSCs with TQ for 2 days in various concentrations (100 nM to 5 mM). - Cell death and cell number reduction were reported in high concentrations of TQ; while in low concentrations, a mild to moderate increase was observed in cell numbers.

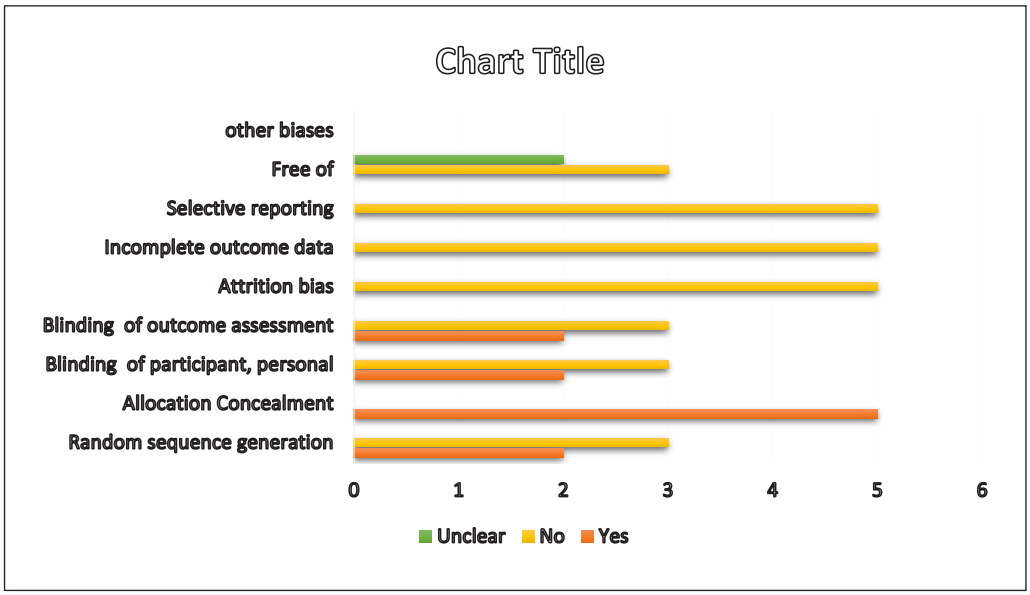

Figure 2. Quality assessment of reviewed papers in review process

matory cytokines results in cartilage degradation and joint structure deformity [44].

N. sativa seeds contain 27.8-57.0% TQ [45]. IL-1β and TNF-α are two inflammatory cytokines playing an important role in the pathophysiology of OA [46]. TQ can increase the anti-inflammatory cytokines (IL-10) and suppress the proinflammatory cytokines and inflammatory factors form of TNF-α [47]. Previous evidence has shown that BFLs and chondrocytes can produce some cytokines and chemokines detected in osteoarthritis synovial fluid, including COX, TNF-α, IL-1β, and IL-18 [48]. Apoptosis induced by inflamma-

tory cytokines, including IL-1β, TNF-α, PGE2, NO, iNOS, and COX-2, has been reported in OA. In this regard, decreasing the synthesis of the cytokine is the best approach to prevent symptoms. The effect of ethanol extract of *N. sativa* has already been demonstrated in decreasing the amount of IL-1β and the very high expression levels of TNF-α, PGE2, NO, iNOS, and COX-2. It has been reported that the expressions of cartilage cells and monocytes/macrophages decrease with the ethanol extract of *N. sativa*, suggesting that the extract can be used for reducing the expressions of inflammatory cytokines and inflammation in OA by affecting the expression of these cytokines [32,33]. The expression of nucleus NF-κB p65 subunits and binding of p50 subunits are inhibited by TQ. Proinflammatory cytokines, such as TNF-α and IL-6, as activators, leads to the maintenance of proinflammatory conditions. However, TQ reduces the synthesis of monocyte chemoattractant-1 proteins, TNF-α, and IL-1β. It can also inhibit the histone COX -2 deacetylases [16]. It has been reported that TQ inhibits oxidative stress by inducing glutathione. The anti-inflammatory properties of *N. sativa* act by decreasing the NO production and inhibiting cytokines IL-1 and IL-6 [24].

The role of thymoquinone in the treatment of cartilage degradation in osteoarthritis

The induction of catabolic enzymes (i.e., MMP-1, MMP-3, and MMP-13) by IL-1β can lead to the stimulation of human osteoarthritis chondrocytes and cartilage matrix degradation [49,50]. Matrix metalloproteinases play an important role in cartilage degradation in OA, which is attributed to oxidative stress, and consequently, ROS production [51]. Among the MMPs family, MMP-1 and MMP-13 have been introduced as the two main factors related to cartilage degradation. Some evidence has shown that the agents inhibiting MMPs exert beneficial effects in the treatment of OA [52,53].

Inflammatory mediators, including NO and PGE2, may be induced by the stimulation of chondrocytes by IL-1β [54]. Based on some evidence, the reduction of IL-1β level has clinical values in the treatment of OA [55]. Wang et al. have indicated that IL-1β-induced inflammatory response can be remarkably decreased by TQ. This substance inhibits the protection of IL-1β-induced COX-2, iNOS, NO, and PGE2. Moreover, the productions of IL-1β-induced MMP-1, MMP3, and MMP13 are suppressed by TQ [34]. Similarly, Chen et al. assessed the chondroprotective properties of TQ on the inhibition of MMPs in rabbit chondrocytes. Since the expressions of MMP-1, MMP-3, and MMP-13, as the facilitating factors in cartilage degradation, were significantly inhibited by TQ in response to IL-1β, it seems that TQ could be employed for the treatment of cartilage degradation. Considering that TQ can increase TIMP-1 expression, which plays an important role in MMPs activities, this assumption is reinforced that TQ can help OA treatment [29]. However, the results of another study showed that TQ did not affect MMP production [30]. It seems future studies should be performed in this regard.

The other main finding was the suppression of the NF-kB activation pathway by TQ [29]. It was reported that NF-κB regulated the production of inflammatory mediators [56,57]. Furthermore, the mitogen-activated protein kinases (MAPKs) signaling pathway is involved in cytokine production [58]. The findings of a study by Wang et al. revealed that TQ suppressed the inhibition of IL-1β-induced NF-κB activation and IκBα degradation in a dose-dependent manner. They confirmed the effects of TQ on IL-1β-induced MAPKs activation [34]. Seemingly, the production of IL-1β-induced mediator in osteoarthritis chondrocytes could be controlled via NF-κB and MAPKs signaling pathways by TQ. These findings confirmed the anti-inflammatory effects of TQ on OA.

Anti-apoptosis effects of thymoquinone in osteoarthritis

ROS are the main factor in modulating cellular responses, including immune-regulatory responses, the production of high levels of which leads to oxidative stress [59]. The accumulation of ROS and its excessive generation can lead to apoptotic cell death via facilitating mitochondrial permeability transitioning pore opening and activating caspase-3 and caspase-9 [60]. TQ acts via the inhibition of proliferation, induction of apoptosis, and remarkable upregulation of ROS expression in the articular chondrocytes, which showed that TQ-induced ROS might regulate apoptosis [30]. Moreover, the role of N-acetyl-L-cysteine has been demonstrated in neutralizing the cytotoxic effects of quinines because it can decrease the toxicity of quinines [61]. Pretreatment of chondrocytes with N-acetyl-L-cysteine can neutralize the induction of ROS and protect these cells against TQ-induced apoptosis, which confirms the anti-apoptosis role of TQ in chondrocytes by the ROS production [30]. The apoptosis-induced effect may be involved as a stimulant of joint diseases, such as OA. Therefore, TQ may act in the etiology of cartilage disease by inducing chondrocyte apoptosis [30]. TQ should be considered an effective inducer of ROS generation in chondrocytes, which involves cartilage destruction via ROS-mediated pathways [31]. Due to the therapeutic effects of TQ in low concentrations (<5 μM), its effect on ROS accumulation and COX-2 expression can be rejected, nevertheless, the results of a study indicated that the treatment of chondrocytes with TQ (5-20 μM) led to apoptosis, which confirmed the role of TQ in explaining the mechanisms responsible for apoptosis, and

consequently, dedifferentiation and inflammation in chondrocytes [30].

Bone marrow mesenchymal stem cells derived from OA patients have the determined minimal criteria of MSCs. To the best of our knowledge, TQ led to morphological changes and cell death at higher concentrations (5 mM). It has been reported that TQ has a neuroprotection effect on the reduction of the pro-inflammatory cytokines in interferon-gamma-activated microglial cells [62]. In a study by Kalamegam et al., an upregulation of the anti-inflammatory genes IL-4 and IL-10 was observed 2 days after the treatment of BM-MSCs with TQ in doses of 1 and 3 mM [17]. It appears that TQ not only upregulates the anti-inflammatory cytokines, but also leads to the downregulation of pro-inflammatory cytokines. Additionally, the anticancer and antioxidant effects of TQ should not be ignored. TQ leads to an increase in the expression of pro-apoptotic BAX in the ovarian carcinoma (SKOV3) cell line [63]. Kalamegam et al. reported a down-regulation of pro-apoptotic BAX and up-regulation of survivin in both 1- and 3-mM concentrations of TQ. In addition, a higher decrease in BAX gene expression was reported, compared to B-cell lymphoma 2 (3 mM concentration of TQ). Therefore, unlike the abnormal cancer cells, TQ may have a protective effect on normal cells. The results of the mentioned study showed that the pro-inflammatory genes (interferon gamma, TNF-α, COX-2, IL-6, IL-8, IL-16, and IL-12A) were upregulated by TQ; however, they were decreased at a 3-mM concentration of TQ, in comparison to lower concentrations. Finally, they introduced TQ as an effective anti-inflammatory therapy against inflammation in OA, which could be used in combination with other conventional therapies [17].

Challenges of thymoquinone use in the treatment of osteoarthritis

Some discrepancies have been observed between the results of clinical and experimental studies, which could be attributed to the differences in the measures of inflammatory, oxidative, and antioxidant markers *in vivo* or *in vitro*, as well as the intensity and type of stimulators of inflammation and oxidative stress. Another factor that might have affected this discrepancy in the results was the different preparations used in various studies. Regarding this, it should be considered that *N. sativa* oil was administered in capsules or prescribed as an ointment in clinical trials; whereas the active component TQ was used in experimental studies. The reason for inconsistent findings might also be due to the storage and preparation of concentrations of bioactive compounds of TQ in *N. sativa* products.

To determine the quality of agents, the herbal formulations should be standardized regarding the concentration of active constituents and chemical, phytochemical, and physical properties [64]. The major limitation of the entered studies was the lack of enough information regarding the standardization of bioactive compounds in *N. sativa* preparations. Therefore, the standardization of herbal medicines, including quality, efficacy, safety, and reproducibility, should be considered an integral part of experimental studies [65].

Since TQ is a hydrophobic molecule, its bioavailability may be affected by its solubility. On the other hand, the solubility of TQ depends on time [66]. It should be considered that TQ can be used differently, including topical, oral, intravenous, and intraperitoneal administration. Liver enzymes can catalyze TQ into hydroquinone; therefore, biotransformation may occur in the oral administration of TQ [67]. Moreover, the absorption half-life of TQ is short (about 217 min), which is rapidly eliminated from plasma. The use of TQ in the clinical phase has been delayed due to the lack of formulation problems and bioavailability. In this regard, future research is needed to be performed to assess the pharmacological properties of TQ.

Study limitations and risk of bias in outcomes

We investigated all clinical trials in terms of quality assessment in eight domains based on the Cochrane guidelines. The quality assessment was high in the majority of domains, except in random sequence generation, blinding of participants, and blinding of outcome assessment, in which 40% (n=2) of clinical trials had a high risk of bias. The low number of randomized clinical trials was the main limitation of the present study. The obtained results from various studies might have been affected by interfering factors, including demographic characteristics and unknown interventional variables. Being a single-center study and the lack of follow-up were the other limitations of the entered clinical trials. Moreover, the age group of the subjects was limited to elderly people [15,23]. These limitations could affect the generalizability of the findings. The other limitation of included studies was the failure to review important indicators, such as the effect of *N. sativa* on inflammation and treatment factors in clinical trials. Polypharmacy was unavoidable because preventing the elderly from taking their medications was unethical. In addition, in none of the studies, the severity of OA was specified; therefore, it was not clear whether the *N. sativa* oil had any effect on the patients with severe OA. Finally, we found no homogeneous studies to convert this study to a meta-analysis.

Conclusion

In summary, the evidence confirmed the anti-inflammatory and chondroprotective effects of *N. sativa* in

the management of OA patients. It was also found that *N. sativa* had no toxicity effects, compared to NSAIDs and steroid agents that may stimulate gastrointestinal and metabolic disorders. Due to the lack of allergic reaction to *N. sativa* in the aforementioned studies, this substance can be used as a safe adjuvant treatment to relieve OA pain, in comparison to NSAIDs and other analgesics.

Conflict of Interests
None.

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None.

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