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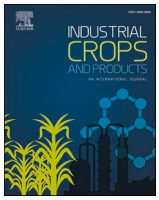

Review Article

Nanodelivery systems of thymoquinone for improving its bioavailability and efficiency in the food and biomedical applications

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ABSTRACT

Thymoquinone (TQ), a hydrophobic bioactive constituent of *Nigella sativa* seeds, has garnered attention for its potential in treating various ailments due to its antioxidative and anti-inflammatory properties. However, TQ's hydrophobicity, instability in varying pH environments, photosensitivity, rapid hepatic metabolism, and low bioavailability present major challenges for its application in pharmaceutical and nutraceutical formulations. Nanotechnology offers innovative nanocarriers that can overcome these limitations. Notable among these are lipid-based nanocarriers (*e.g.*, nano-liposomes, nano-emulsions, niosomes, solid lipid nanoparticles, and nano-structured lipid carriers), biopolymeric systems (*e.g.*, nano-hydrogels, nanofibers, nanotubes, and cyclodextrin inclusion complexes), and inorganic nanocarriers. These delivery systems are designed to enhance TQ's solubility, protect it from degradation, and improve its bioavailability and therapeutic performance. Despite numerous advances, the clinical and industrial translation of these nano-delivery systems remains limited, primarily due to scalability issues, regulatory constraints, and a lack of standardized evaluation protocols for food and biomedical use. This review provides a comprehensive analysis of these nanocarriers, emphasizing their mechanisms for TQ encapsulation, controlled release, and bioaccessibility enhancement. It also highlights current limitations and outlines future directions for their development. Unlike previous reviews, this work offers a comparative evaluation of nanocarrier systems for both food and biomedical applications, addressing their effectiveness, limitations, and readiness for real-world translation. The key takeaway is that among the various approaches, lipid-based and biopolymeric nanocarriers have demonstrated the greatest potential for enhancing TQ delivery, particularly in oral and functional food formulations, as well as targeted cancer therapy, due to their biocompatibility, scalability, and effective release profiles.

1. Introduction

Harnessing the advantageous properties of nanotechnology in the domains of food and medicine has spurred the creation of cutting-edge bioactive-loaded nanocarriers aimed at mitigating a myriad of health conditions, including cardiovascular diseases (Lobatto et al., 2011), infectious diseases (Zazo et al., 2016), cancer (Shamsi et al., 2019), and diabetes (Veiseh et al., 2015). Nanocarriers have primarily surfaced to address the critical pharmacokinetic challenges that impede the delivery of conventional free bioactives/therapeutics to their intended sites of action. Such a potent vehicle must navigate a multitude of biophysical barriers within the biological milieu to guarantee efficient delivery of bioactives/medications to their targeted locations and ensure the prolonged dispensation of bioactives. Nanocarriers are specifically designed to improve the bioavailability, pharmacokinetics, and distribution of bioactives, safeguard them against enzymatic degradation, and control

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their release by acting as a cellular and tissue reservoir for the bioactives.

Thymoquinone (TQ), first isolated and identified in the oil of *Nigella sativa* seeds in the early 1960s, is the key hydrophobic active component responsible for the plant's therapeutic potential. Over the past six decades, research interest in TQ has expanded exponentially, focusing on its potent bioactivities, including antioxidative, anti-inflammatory, anti-apoptotic, and hepatoprotective effects (Gupta et al., 2016; Harwansh et al., 2019; Imran et al., 2018; Negi et al., 2017). Notably, TQ mitigates the activation of hepatic stellate cells and offers liver protection by neutralizing free radicals directly or by enhancing the activity of endogenous antioxidant enzymes and molecules (Zhang et al., 2017). Additionally, TQ reduces inflammation in liver cells by blocking the action of enzymes such as cyclooxygenase (which produces pro-inflammatory prostaglandins) and 5-lipoxygenase (which generates leukotrienes), thereby decreasing the production of eicosanoids—signaling molecules that mediate inflammation—in white blood cells (AbuKhader and Khan, 2017). The use of TQ is on the rise in the development of functional foods, medicinal products, and beauty items (Pottoo et al., 2022). Notably, it's becoming a common ingredient in anti-aging products, hydrating face creams, and eye treatments, celebrated for its exceptional protective qualities against UV damage and its ability to prolong the effects of tanning (Cobourne-Duval et al., 2016). Further, TQ is rich in nutrients and can be added to foods to enhance their nutritional profile (Bashir et al., 2021).

Despite its promising pharmacological properties, TQ faces significant barriers to clinical application due to its poor water solubility (~ 0.015 mg/mL at room temperature) (Salmani et al., 2014), instability under various pH conditions, light sensitivity, and rapid hepatic metabolism. These physicochemical and pharmacokinetic drawbacks—including low oral bioavailability (typically < 60 %) and a short plasma half-life of 1.5–2 h (El-Far et al., 2018; Negi et al., 2017; Rathore et al., 2020; Salmani et al., 2014)—limit its systemic availability and therapeutic potential as a nutraceutical or pharmaceutical agent. Moreover, its lipophilic nature, degradation under gastrointestinal conditions, and limited stability further complicate its formulation and delivery, posing major challenges for effective therapeutic application. To overcome these obstacles, an array of nanocarriers has been developed to ensure the safe transport of TQ through the gastrointestinal tract (GIT) and its subsequent controlled and sustained release at the desired site, by encapsulating it within an appropriate nanocarrier (Negi et al., 2019; Rathore et al., 2023). Nanocarriers have shown exceptional potential for TQ delivery and can be broadly categorized as nanoparticles (NPs) crafted from lipid-based nano-formulations (*e.g.*, nano-liposomes, nano-emulsions (NEs), niosomes, solid lipid NPs (SLNs), and nano-structured lipid carriers (NLCs)), biopolymers (*e.g.*, nano-hydrogels, nanofibers, nanotubes, and cyclodextrin (CD)-based inclusion complexes), and inorganic nanocarriers. These nanocarriers are instrumental in formulating TQ-based products that exhibit reduced degradation during circulation, improved solubility in both drug and food formulations, and enhanced targeted delivery. Moreover, they contribute to increased bioavailability and stability of TQ, ensuring that its therapeutic potential is fully realized (Rathore et al., 2023, 2020; Salmani et al., 2014).

From a regulatory standpoint, TQ is generally recognized as safe (GRAS) for use in certain food and supplement applications; however, its approval as a pharmaceutical ingredient requires more rigorous safety and efficacy evaluations by agencies such as the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA) (Burdock, 2022). This regulatory gap underlines the need for nano-delivery systems that can meet strict quality, safety, and efficacy standards.

Several review articles have explored various aspects of TQ encapsulation. For instance, reviews by El-Far et al. (2018) and Pottoo et al. (2022) focused on the therapeutic effects of nano-formulated TQ, primarily within biomedical frameworks. Other reviews have addressed specific carrier systems, such as lipid-based (Negi et al., 2018) or polymeric nanoparticles (Rathore et al., 2020), or evaluated nano-formulations from a pharmacological standpoint (Rathore et al., 2020). However, these studies often lacked a comprehensive and integrative focus on several key aspects—namely, the comparative performance of diverse nanocarriers in both food and biomedical applications, detailed mechanisms of bioavailability enhancement and controlled release, and thorough evaluation of *in vivo*, *in vitro*, and cytotoxicity outcomes related to TQ encapsulation. In particular, there remains a gap in knowledge concerning the comparative efficacy of different nanocarrier types for TQ across diverse applications, mechanisms underlying bioavailability improvements, and the translation of these findings to practical food and biomedical products. This highlights the necessity of an integrative review addressing these multidimensional aspects. The novelty of this review lies in its broad and comparative perspective; this work provides an integrated analysis of nanodelivery systems for TQ that spans both food and biomedical contexts, bridging disciplinary gaps and offering insights into the multifunctionality of TQ nanocarriers.

Specifically, this review presents: (i) a concise overview of TQ, detailing its structural characteristics, sources, applications, methods of extraction and purification, health benefits, factors influencing its stability, and the necessity for encapsulation; (ii) the current advancements in diverse nanocarriers that incorporate TQ, as well as their distinctive features; (iii) a thoughtfully selected assortment of studies centered on the encapsulation of TQ within various nanocarriers; (iv) clarifying the elements that affect the bioavailability and release, as well as the *in vivo*, *in vitro*, and cytotoxicity outcomes of TQ when encapsulated in these nanocarriers; and finally, (v) exploring the pharmaceutical and food potential applications of TQ-loaded nanocarriers.

2. An overview of thymoquinone

2.1. Chemical structure, sources and applications

TQ (Fig. 1) is a valuable substance that plays a significant role in the field of medicine and treatment. It is found in plants belonging to the *Asteraceae*, *Cupressaceae*, *Lamiaceae*, *Ranunculaceae* family. Although the amount of TQ in the aerial parts of plants named *Eupatorium cannabinum* L. (TB-017), *Juniperus communis* L. (TB-002, TB-056), *Satureja hortensis* L. (TB-010, TB-044, TB-077), *Satureja montana* L. (TB-011), *Thymus pulegioides* L. (TB-014), *Thymus serpyllum* L. (TB-075), *Thymus vulgaris* L. (TB-015, TB-048) are insignificant to note, it is found in substantial amounts in the aerial parts of *Monarda didyma* L., chemotype 1 (TB-005), *M. didyma* L., chemotype 2 (TB-035), *M. didyma* L. 'Pink Lace' (TB-037), *Monarda media* Willd. (TB-072) *Monarda menthifolia* Graham (TB 073), and particularly in the seeds of *N. sativa* L. (TB-007, TB-067, TB-068). The seeds of *N. sativa* are noted for their higher yield of TQ, making them a primary source for the extraction of this substance (Taborsky et al., 2012).

TQ is a compound that is available as a white to tan white crystalline

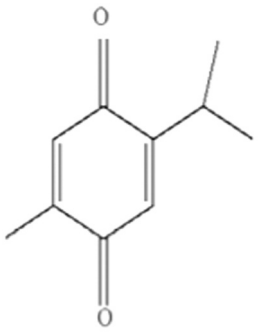


Fig. 1. Thymoquinone or 2-methyl-5-(propane-2-yl)cyclohexa-2,5-diene-1,4-dione (Mathur et al., 2024).

solid, has a melting point of 49–50 °C, and a molecular weight of 164.204 g/mol. It has an aroma very similar to that of pepper. TQ exhibits poor water solubility but is more readily soluble in organic solvents such as ethanol, DMSO, and methanol, as well as in lipid-based media (Ahmad et al., 2018). The most important bioactives in *N. sativa* are TQ and *p*-cymene. Additionally, proteins and peptides are present as other bioactives within this seed (Alu'datt et al., 2024). Wild bergamot (*Monarda fistulosa* L.) is another medicinal plant whose essential oils (EOs) contains 20–32 % TQ (Igor et al., 2020). *N. sativa* L. is an annual herbaceous plant that was originally cultivated in southwest Asia and is native to that region, and has been cultivated and domesticated in Europe and North Africa. *N. sativa* L. seeds are commonly known as black cumin and are used as a spice and seasoning. In traditional medicine, *N. sativa* L. has been used in various forms to treat many diseases, including diabetes, asthma, bronchitis, high blood pressure, inflammation, fever, cough, headache, eczema, dizziness, and influenza (Khader and Eckl, 2014; Mathur et al., 2024). This plant even before the advent of modern medicine, this miracle herb was being used in folkloric traditional medicine across the globe for treatments of all kinds of ailments and diseases. This plant has been mentioned in the written traditional medicinal texts of major civilizations like the Ayurveda, Siddha, Unani, Greek-Roman, Malay, Tibb-e-Nabawi, and Jewish texts (Mathur et al., 2024).

TQ has many applications; *e.g.*, TQ was used in combination with electrospun polycaprolactone (PCL)-gelatin nanofibrous matrix as a periosteum substitute to promote bone healing. This compound had osteoconductive and osteoinductive properties and increased bone mineralization, making it a suitable alternative to periosteum (Jeyakumar and Sivagnanam, 2024). Another application of TQ is its use in the combined system of hydrogel and nanotechnology in skin treatment in such a way that it causes rapid wound closure in the rat wound model, which was associated with a decrease in the size of the wound and an increase in collagen deposition (Algahtani et al., 2021). Another way to use TQ in the form of NLCs is to deliver lipophilic chemotherapy drugs, namely TQ orally for the treatment of breast cancer (Gupta et al., 2023). In addition to the medicinal uses of TQ, this material is also used in the preservation of perishable food. Recently, in a study, zein nanofibers with polyvinyl alcohol (PVA) containing 4 % TQ were made through electrospinning. This formulation was able to increase the shelf life of fish by at least three days (Ahmadi et al., 2024).

2.2. Extraction

To extract TQ, different methods have been described. For example, Dimitrijević et al. (2024) reported that in order to obtain EOs with a high content of TQ, 30 g of *N. sativa* seeds were extracted by *n*-hexane in a Soxhlet apparatus. After 2 h extracting and changing the solvent 20 times, it was placed in a rotary evaporator to evaporate the solvent to the maximum extent, and the remainder was subjected to distillation with water for another 2 h in the Cloninger machine. Finally, EOs extracted with the help of diethyl ether solvent, which is known as extract 1 in Fig. 2. For the second sample, the same amount of seed was placed in the Soxhlet apparatus with petroleum ether solvent and the operation was repeated and the extraction process was performed by changing the solvent 24 times, and then the residue was distilled with water for 2 h in the Clevenger apparatus. EOs were separated by diethyl extraction and this sample was identified as extract 2. The third extract was obtained by supercritical fluid extraction (SFE). In this method, seeds were subjected to pressure 500 unit (Eurotechnica GmbH). Extraction was performed with supercritical fluid at 10 MPa and 40 °C. The powder was placed in a 280 mL stainless steel extractor. Liquid CO_2 is cooled in a cryostat to prevent evaporation. This mixture is supplied with a siphon tube and pumped into the system by a liquid metering pump to achieve the required pressure. The continuous flow starts when the necessary conditions for the extraction become operational. The back pressure regulator is responsible for providing the operational pressure of the extraction. The consumption of CO_2 was 125 g/g plant material, and the extraction duration was 5 h (Dimitrijević et al., 2024).

TQ extraction from *N. sativa* L. is achieved by Supercritical CO_2 extraction, solvent extraction, Cold pressing and Microwave extraction methods. According to previous works, Supercritical CO_2 extraction has the highest purity and highest extraction efficiency compared to other methods. Its extraction efficiency at 60 °C and 150 bar pressure has been reported to be 20.80 mg/mL oil. The other methods do not differ much

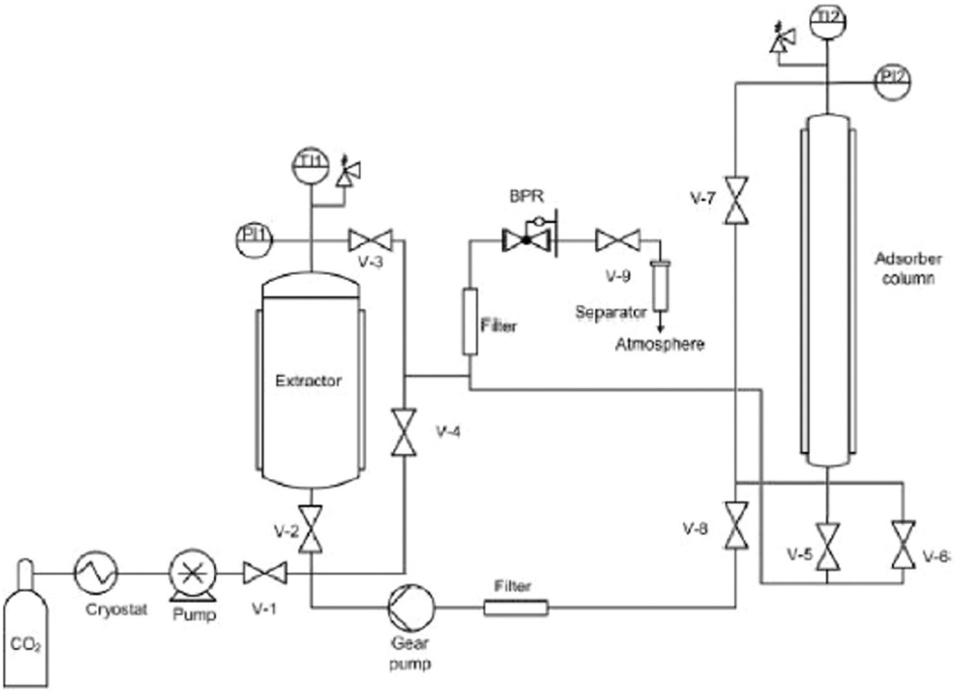


Fig. 2. Schematic view of HPEA 500 unit.

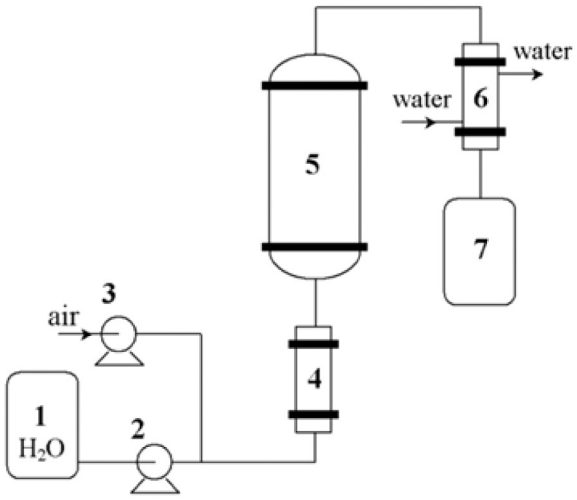
in terms of extraction efficiency, and their values have been reported to be between 1 and 9 mg/mL oil, depending on the extraction conditions. Supercritical CO_2 extraction (SC-CO_2), though requiring a higher initial investment, can be more cost-effective in the long term compared to cold-press extraction. While cold pressing is simpler and may be more economical for small-scale operations, SC-CO_2 offers greater efficiency and yield, making it advantageous for larger-scale production. In the case of Microwave extraction and Cold pressing, although they are economically viable, high peroxide levels have been reported in the extracted samples, respectively.in the case of solvent extraction although the solvent extraction is inexpensive, there are some issues which hinder its use such as toxicity of solvent and solvent losses in recovery step. In this method, the chemical stability of solvent is very important, and it is expected to be stable during the extraction and recovery steps. The use of CO_2 as a supercritical fluid has several advantages, such as solvent recovery, easy separation from the extracted material, not non-flammable, abundant, low cost, and requiring low energy in the region close to the critical point. Supercritical CO_2 extraction is largely the preferred technique in a wide range of industrial separations because there is no solvent residue in the product and it is an environmentally friendly method; However, solvent extraction is not environmentally friendly. Microwave-assisted extraction (MAE) is also a greener and more efficient alternative to solvent extraction, but in terms of other advantages, Supercritical CO_2 extraction is better than all methods overall (Yalçin and Gönen, 2019).

In addition to extraction and purification methods, one of the methods of TQ production is synthesis. In previous research, it has been reported that using manganese complex, CH_3CN, TBHP and monoterpene, TQ can be obtained by oxidation of thymol and carvacrol in acetonitrile at 80 °C (Dimitrijević et al., 2024). For the detection of EOs using GC-MS, analytical conditions were established with GC-FID and GC-MS. The GC-FID setup included a slotless injector and an automatic liquid sampler connected to an HP-5 column. The carrier gas (H2) flow rate was set at 1 mL/min, and the detector temperature was noted to be 300 °C. In the GC-MS analysis, the same HP-5MS column was utilized, with helium as the carrier gas. Additionally, the transmission line was heated to 260 °C (Dimitrijević et al., 2024).

In general, thymol and carvacrol are converted into TQ due to the drying of the wild bergamot plant and also due to the effect of oxidative enzymes. In the essence distillation method from plant materials, the amount of TQ is reduced and the amount of thymohydroquinone is added due to the presence of reducing agents. Various techniques have been used to increase the efficiency of TQ extraction from this plant (Igor et al., 2020), including: (i) Due to the reactivation of enzymes, plant materials were fermented to a greater extent before distillation, and dried plant materials were moistened with water and then exposed to air fermentation. In this process, fermentation was done with continuous air flow, and the moistened plant material was loaded into the distillation extractor. The main advantage of this method is the use of one device in all stages without reloading the wild bergamot plant and all parameters such as water, air, humidity, temperature and flow were optimized. (ii) EOs distillation using a combination of air and steam. According to this method, oxygen prevents the reduction of TQ during distillation. Air is pumped together with water into the mixer (Fig. 3). The volume of the extractor was adjusted to 2.8 L, because by increasing the volume, the time will also increase, which causes the conversion of TQ to thymohydroquinone.

2.3. Stability challenges and the need for encapsulation

Pharmaceutical studies indicate that if TQ is designed as a phospholipid nanostructure, it has 3 times more absorption capacity compared to unmodified TQ. This is due to the fat matrix serving as a protective cover, which prevents the decomposition of the active substance containing TQ as it passes through the intestine. Furthermore, the aforementioned structure functions as an effective solvent (Abdullah,



1 – feeding vial with water, 2 – water pump,
3 – air pump, 4 – electric flow evaporator,
5 – extractor with plant material, 6 – flow cooler
7 – recipient for condensate.

Fig. 3. Scheme of lab installation for isolation of essential oils.

2024). Given that TQ is a significant natural compound with therapeutic properties (Badary et al., 2021), TQ, a naturally occurring compound with anticancer properties, faces significant physical and chemical stability challenges, particularly in aqueous solutions. Its instability is exacerbated by alkaline pH, light exposure, and temperature. In case of Non-Targeted Drug Distribution, TQ's hydrophobic nature can lead to non-specific distribution within the body issues need to be addressed for effective formulation and delivery of TQ. Previous findings demonstrate that TQ underwent minimal degradation at acidic pH. Its degradation kinetics follow first-order reactions at extreme pH values and second-order reactions at pH 5–7.4. Light sensitivity of TQ indicate that less than 20 % of the drug remained intact after 24 h and this decreased further to less than 10 % after 48 h and sensitivity to light, high temperature and alkaline environments and most importantly, non-targeted distribution of this drug due to its hydrophobic nature has led to the need to encapsulate this very important drug compound for better and more targeted protection and distribution (Salmani et al., 2014) and it is essential to enhance its stability and the efficacy of its effects during usage. Employing NEs and NPs is one method to increase the stability of TQ (Karaman, 2020; Mishchenko et al., 2023; Tubesha et al., 2013a).

It has been reported that due to the poor solubility of TQ in water, its use in the body is problematic and leads to low absorption in the body (Tubesha et al., 2013a). Therefore, lipid-based nanocarriers have been used for better stability and better use of this substance. For example, in 2023, NEs and SLNs were designed to create a better delivery system for TQ and doxorubicin. NEs with an average droplet diameter of 50 nm and SLNs with an average size of 30 nm are stable against aggregation for $>$ 90 days. Also, the effectiveness of NEs and SLNs against cancer cells was much higher than when the structure of NEs and SLNs was not used for medicinal effects. It should be noted that the size of the particles also had a great effect on the efficacy of TQ and doxorubicin, and in this research it was mentioned that the size of the particles should be $<$ 100 nm for better efficacy. This occurs as a result of the inhibition of DNA replication, followed by the subsequent induction of cell apoptosis (Mishchenko et al., 2023).

In another study in 2020, the stability and preservation of TQ concentration in black cumin oil seed was investigated by encapsulating in plasmolized and non-plasmolized yeast cells. The results showed that the degradation rate of TQ in plasmolyzed yeast cells was about 53 %

and the control sample of black seed oil phytochemical compounds was about 97 %. The stability of TQ in the plasmolysis yeast cell was better than the non-plasmolysis one and the control sample of oil seed phytochemical compounds. Plasmolysis as a pretreatment can make TQ more stable due to increased oil absorption (Karaman, 2020). In another study, TQ was made into NEs by high-pressure homogenization (HPH). The researchers reported that TQ NEs could provide better stability of this compound over a period of 6 months. In another method, a multi-step self-heating MN patch by micromolding method consists of poly(lactic-co-glycolic acid) (PLGA) tips loaded with TQ for sustained release of TQ for skin cancer treatment. The researchers highlighted the successful outcomes of developing this device, which include: (i) Long-term inhibition of tumor growth; (ii) Minimal side effects; (iii) Outstanding heat-responsive drug release capabilities; (iv) Potential for sustained delivery; (v) Safe, prolonged, and controllable mild self-heating reaction for MN; (vi) Cost-effective MN patch with convenient storage, easy transportation, and no need for external equipment; (vii) Safe and efficient treatment process; (viii) Potential for an excellent clinical experience (Shao et al., 2024).

2.4. Health benefits

TQ has many health properties including antioxidant, anti-cancer, anti-inflammatory hepatoprotective, reduced blood pressure and immune system modulating properties (Adinew et al., 2022; Mahmoud and Abdelrazek, 2019; Pal et al., 2021). The neuroprotective, antioxidant and anti-inflammatory properties of *N. sativa* plant have made it a potential drug for the treatment of multiple sclerosis in human and animal models (Raimi et al., 2024).

2.4.1. Anti-cancer activity

Colorectal cancer (CRC) is one of the most common cancers that kill one million people every year. TQ prevents tumor progression with its proapoptotic effect and inhibiting the proliferation of cancer cells by affecting the regulation of signaling pathways and it controls this type of cancer (Kurowska et al., 2023). Recently, the use of TQ for the treatment of bladder cancer has made significant progress and has significantly reduced the metastasis of tumor cells by inhibiting Wnt/β-catenin/EMT axis (Xue et al., 2024). TQ ameliorates experimental autoimmune encephalomyelitis and this improvement occurs through the regulation of pro-inflammatory and anti-inflammatory cytokines (Kazemi et al., 2024). In addition to these, this substance has antioxidant capacity and LoVo has shown high anticancer activities in colon cancer cells (Bozali et al., 2024).

Existing investigations on the action of TQ on human breast cancer (MCF7) cell lines revealed that 10, 25, and 50 μM of TQ inhibited tumor cell growth within 24 h (Shabani et al., 2023) also TQ (orally and daily 375 mg/kg body weight (B.W.) for 12 weeks) decreased the number of large polyps in the small intestine and reduced polyp growth through selective induction of apoptosis (Darakhshan et al., 2015). Overexpression of the chemokine receptor CXCR4 has been linked to heightened tumour growth, enhanced metastatic behavior, and poorer clinical outcomes in breast cancer patients. As such, therapeutic agents capable of downregulating CXCR4 may offer promising anti-metastatic strategies. One study investigated the regulatory effects of TQ, a bioactive constituent from *N. sativa* seeds, on CXCR4 expression in breast cancer cells. Results showed that TQ significantly reduced CXCR4 levels in MDA-MB-231 triple-negative breast cancer (TNBC) cells in both a dose- and time-dependent fashion. This reduction appeared to involve transcriptional mechanisms, as TQ treatment suppressed NF-κB activation and reduced its binding activity at the CXCR4 promoter site. Importantly, the inhibitory effect of TQ on CXCR4 expression was not reversed by blocking proteasomal degradation or lysosomal pathways. Furthermore, the decrease in CXCR4 expression corresponded with a reduction in CXCL12-induced cell migration and invasion in the same breast cancer cell line. *In vivo*, TQ also led to a dose-dependent reduction in tumor growth and vascular development, as demonstrated using the chick chorioallantoic membrane (CAM) model.

In a mouse model of metastatic breast cancer, administration of TQ at doses of 2 and 4 mg/kg over a 10-day period led to a marked, dose-dependent reduction in metastases to the lungs, brain, and bones. This inhibitory effect is believed to be associated with disruption of the CXCR4/SDF-1 signaling axis. Histological analysis using hematoxylin and eosin (H&E) staining, along with immunohistochemistry of bone samples from TQ-treated animals, revealed fewer osteolytic lesions and diminished levels of metastasis-related markers. These findings support the conclusion that TQ's anti-metastatic properties may stem largely from the suppression of NF-κB-driven CXCR4 expression, positioning it as a promising agent in the treatment of breast cancer (Shanmugam et al., 2018; Zhu et al., 2019).

In another investigation, the individual anticancer effects of alpha-hederin and TQ—the main active compounds found in *N. sativa*—were evaluated against four human cancer cell lines: A549 (lung carcinoma), HEp-2 (laryngeal carcinoma), HT-29 (colorectal adenocarcinoma), and MIA PaCa-2 (pancreatic carcinoma). Alpha-hederin was also assessed for its potential as a prodrug. Both compounds demonstrated cytotoxicity and induced cell death (apoptotic and necrotic) in a concentration- and time-dependent manner. Among the tested lines, HEp-2 cells exhibited the highest sensitivity, especially to TQ, with pronounced apoptotic response. However, pre-treatment with α-hederin followed by TQ or cisplatin did not result in any synergistic increase in cytotoxic or apoptotic effects. These results suggest that despite the membrane-disrupting capacity typically attributed to saponins like α-hederin, no enhancement of anticancer activity was observed within the 24-hour exposure period (Rooney and Ryan, 2005).

2.4.2. Effects of TQ on Dox-induced cardiotoxicity

Another investigation assessed the antibacterial properties of *N. sativa* seed extract against several pathogenic bacteria, including *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Proteus vulgaris*. The methanol extract, when applied at 100 mg/mL, demonstrated strong inhibitory effects on all bacterial strains tested. However, *Klebsiella pneumoniae* and *Proteus vulgaris* exhibited resistance to the aqueous extract at a concentration of 20 mg/mL. At 50 mg/mL, the methanol extract showed notable antibacterial activity, particularly against *Streptococcus pyogenes*, producing an inhibition zone measuring 19 mm. Meanwhile, the inhibition zones for *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Proteus vulgaris* measured approximately 15 mm. Statistical analysis *via* the Kruskal-Wallis test revealed that both the aqueous and methanol extracts of black seed had a stronger inhibitory effect on Gram-positive bacteria (*Streptococcus pyogenes*) than on Gram-negative species (*Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Proteus vulgaris*). The study further suggested that factors such as bacterial species, strain variability, and the concentration of *N. sativa* extract significantly affect bacterial susceptibility. Additionally, a meaningful correlation was found between the size of the inhibition zone and the extract concentration (Al-Ameedy and Omran, 2019).

TQ with curcumin is effective in preventing the progression of breast cancer cells (Sharma et al., 2024). TQ increases apoptosis of damaged nerve cells and improves cell survival and neurogenesis in healthy hippocampus (Beker et al., 2018). Oxidative stress, development of membrane permeability, blockage of efflux pumps, and the use of potent biocidal effects are various TQ-related mechanisms that may lead to cell death (Ahmad et al., 2020; Wahab and Alsayari, 2023).

2.4.3. Hepatoprotective

TQ is a promising agent in maintenance of normal hepatic function during treatment with anti-tuberculosis drugs and had therapeutic effect against anti-tuberculosis drugs-induced liver damage. TQ administration for 8 weeks (3 days/week) attenuated the increases in the levels of hepatic AST, ALT and ALP enzymes, and caused a consequent recovery toward normalization indicating stabilization of plasma membrane as

well as repair of hepatic tissues (Kazemi et al., 2024). In a controlled study, fifty Wistar rats were divided into five groups: control, sham-operated, hepatic ischemia-reperfusion (I/R) for 45 min plus 45 min reperfusion, TQ treatment (50 mg/kg), and TQ pretreatment for ten days before I/R. The I/R model involved temporarily blocking the hepatic artery and portal vein. After the experiment, liver and blood samples were analyzed histologically and biochemically, and TRPM gene expression in liver tissue was measured. TQ-treated groups, with or without I/R, showed significantly less liver damage—*e.g.*, reduced swelling, vascular congestion, neutrophil buildup, and cell death—compared to the I/R-only group. TQ also suppressed calcium entry by downregulating TRPM2, TRPM6, TRPM7, and TRPM8 gene expression. These findings indicate that TQ, from *N. sativa*, may protect against ischemia-reperfusion liver injury (Kazemi et al., 2024).

2.4.4. Anti-inflammatory activity

TQ ameliorates experimental autoimmune encephalomyelitis and this improvement occurs through the regulation of pro-inflammatory and anti-inflammatory cytokines (Kazemi et al., 2024). It has been reported that treatment with TQ (6.25 μg/mL) reduced the inflammatory response by suppressing the levels of IL-6 and IL-8 proteins in human vascular endothelial cells (HVECs) exposed to lipopolysaccharides (LPS, 100 ng/mL) for 24 h. Regarding the effect of TQ in neuroinflammatory, TQ has potential neuroprotective effects in acute ischemic stroke by influencing microglia, the brain's immune cells, which shift their activation states (polarization) to regulate inflammation after stroke. While microglia polarization plays a key role in controlling neuroinflammation and brain damage after ischemia, TQ's impact on this process remains unclear. Using a mouse model of middle cerebral artery ischemia-reperfusion (I/R) that simulates stroke symptoms, studies showed TQ treatment reduced brain infarct size, swelling, and tissue damage, while enhancing neuron survival and improving motor function as measured by various behavioral tests. Molecular analyses revealed that TQ shifted microglia from the pro-inflammatory M1 state toward the anti-inflammatory M2 state. Additionally, TQ inhibited the TLR4/NF-κB signaling pathway through activation of Hif-1α, which may decrease microglial activation after stroke. These findings outline a potential mechanism for TQ's protective effects in cerebral ischemia-reperfusion over 7–14 days (Abo Mansour et al., 2023).

In relation to Alzheimer's prevention, in a study, 30 mice were divided into normal, SCOP and TQ groups. Y-maze and pole climbing tests were performed to measure memory and motor function. Subsequently, histopathological and immunohistochemical examinations were performed. In addition, proteins and genes related to the peroxisome proliferator-activated receptor gamma (PPAR-γ) signaling pathway were identified, with emphasis on the role of miR-9. The results showed that TQ has the potential to improve cognitive deficits observed in the SCOP-induced Alzheimer's-like model, as evidenced by improved behavioral outcomes, histopathological changes, modulation of the expression pattern of downstream targets of PPAR-γ with a significant reduction in amyloid beta (Aβ) deposition and TQ provided significant multi-level neuroprotection through its anti-inflammatory and PPAR-γ agonistic activities. As a result, TQ may have a potentially beneficial role against the development of Alzheimer's in 14 day (Abo Mansour et al., 2023) (Table 1).

2.4.5. Immune system modulating properties

These strong anti-inflammatory and immune-modulating properties have made this substance considered as an antiviral against Sars and Covid. For example, TQ may inhibit and interfere with the binding of SARS-CoV-2 to ACE2 receptors, which can prevent the virus from entering and replicating inside the host cell (Masood et al., 2024).

Table 1
Properties of thymoquinone (TQ): usage, mechanisms, and dosage.

Form of usage	Biological properties	Mechanism of act	Dose/treatment duration	Ref.
p-benzoquinone-TQ ≥ 98 % purity	Antibacterial in *in vivo*	Decrease of *Mycobacterium tuberculosis*	256 μg/mL/24 h	(Jankowski et al., 2024)
Natural: (Scopolamine + TQ)	Alzheimer's Disease-like and anti-inflammatory in mice	Decrease cytokines such as TNF-α and IL-6	50 mg/kg/day-/14 days	(Abo Mansour et al., 2023)
Black cumin-enriched acha-based cookies were formulated with varying percentages of black cumin	Antioxidant activity of diabetic mice	Decrease in neuronal antioxidant enzymes and GSH levels	2.5 % and 5 % of black cumin/1–14 days	(Nwanna et al., 2024)
Thymoquinone purity	Treatment of liver diseases by transient receptor potential biochemical parameters, melastatin (TRPM) channels in rats with liver ischemia	Creatinine and urea levels were more decreased in rats that received TQ than in other rat	50 mg/kg/10 days	(Caglar et al., 2024)
TQ purity	Regulates microglial M1/M2 polarization in the Polarization after cerebral ischemia-reperfusion injury	Regulation of microglial M1/M2 brain was regulated by the TLR4 signaling pathway	5 mg/kg-/7–14 days	(Zhao et al., 2024)
TQ from black cumin-loaded chitosan nanoparticles	Antidiabetic activity in pediatrics	Streptozotocin-induced inhibited diabetes was completely	45 mg/1–10 days	(Xue and Lin, 2024)
TQ purity	Anticancer in MDA-MB-23 cell breast	Decrease of NF-κB and CXCR4	50 μM/24 h	(Shanmugam et al., 2018; Sharma et al., 2024; Zhu et al., 2019)
	Anticancer in H9C2 cells in rat cardiomyocytes	Increase of AMPK/mTOR pathway	5 μM/24 h	(Liu and Zhao, 2022; Yarmohammadi et al., 2024)
	Cytotoxicity and antiproliferative activity	Decrease of Larynx. Pidermoid cancer-Hp-2	22.9–34.6 μM/24 h	(Mathur et al., 2024; Rooney and Ryan, 2005)
TQ - spirulina and TQ	Cytotoxicity and antiproliferative activity in rats	Protection against MTX-induced hepatic toxicity	10 mg/kg/day	(Behairy et al., 2024)
TQ purity	Anti-apoptotic activity in rat	Protect kidney damage by cisplatin-induced	1–20 mg/kg/24 h	(Li and Zhao, 2024)
	Antidiabetic, anti-obesity	Reduction in plasma leptin and resistin levels	20 and 40 mg/day/ 12–18 week	(Ramineedu et al., 2024)
Ethanolic extract from *Nigella sativa* seed (rich in TQ)	Antidiabetic in rat	Protection of streptozotocin-induced diabetes	100 mg/kg body weight/28 days	(Khan and Zaidi, 2024)
TQ – Melanin	Antibacterial effect	Reduce of *Staphylococcus aureus*	Extract of seed (50 g)	(Al-Ameedy and Omran, 2019)

TQ with curcumin is effective in preventing the progression of breast cancer cells (Sharma et al., 2024). TQ increases apoptosis of damaged nerve cells and improves cell survival and neurogenesis in healthy hippocampus (Beker et al., 2018). Oxidative stress, development of membrane permeability, blockage of efflux pumps, and the use of potent biocidal effects are various TQ-related mechanisms that may lead to cell death (Ahmad et al., 2020; Wahab and Alsayari, 2023). *N. sativa*, whose active component is TQ, is used in traditional Indian medicine to treat cough, chest congestion, inflammation, bronchitis, and asthma, and one of the important reasons for this is that TQ inhibits the metabolism of arachidonic acid. Inhibition occurs through the COX and LOX-5 pathways (Usmani et al., 2023). A number of studies administered oral TQ dose in the range of 10–100 mg/kg B.W. without any reported toxic or lethal effects reduced blood pressure (Elghareeb et al., 2024). Clinical studies have proven that taking 300 mg of black seed extract twice a day in less than a month has reduced blood pressure during 28 day in people with high blood pressure (Rizka et al., 2017).

Researchers have utilized lipid nanocarriers made of poly(D-glucosamine) to inhibit the progression of glioblastoma multiforme (GBM), a grade IV malignant brain tumor associated with high mortality rates. These nanocarriers enhance the absorption of TQ in the brain, offering a potential therapeutic strategy. Administering black seed oil containing 13 % TQ improved the structure of thyroid, ovary and uterine tissues in mice. It also regulated antioxidant enzymes, anti-apoptotic protein, bone morphogenetic protein and thyroid stimulating hormone. Another beneficial effect was that it reduced apoptotic proteins, inflammatory cytokines, and estrogen receptors (Elghareeb et al., 2024).

2.4.6. Anti-diabetic activities

Another very important use of TQ in health is its anti-diabetic properties. TQ exhibits notable anti-diabetic properties in addition to its broad pharmacological effects. Streptozotocin (STZ)-induced diabetes is a widely accepted animal model for studying human diabetes. In this model, TQ significantly lowered blood glucose levels, including in cases of gestational diabetes. A 30-day oral regimen of TQ (50 mg/kg) in diabetic hamsters led to marked reductions in fasting glucose and HbA1c, as well as suppression of excessive gluconeogenesis. Furthermore, six weeks of TQ treatment improved insulin secretion and significantly decreased plasma glucose concentrations (Elghareeb et al., 2024). The accumulation of advanced glycation end products resulting from non-enzymatic glycosylation is one of the factors that underlie long-term pathogenesis in diabetes. The use of TQ in the amount of 10 mM can prevent non-enzymatic glycosylation (Liu et al., 2023).

Diabetic ketoacidosis (DKA) is a common complication in children and adolescents with both types of diabetes. Prompt diagnosis and close monitoring are essential for effective treatment. TQ combined with chitosan nanoparticles (ChNPs) demonstrated rapid anti-diabetic activity within 24 h. Laboratory tests showed TQ inhibits key enzymes, α-amylase and α-glucosidase. Protecting insulin from degradation is critical for its delivery. In diabetic cell studies, TQ-ChNPs reduced apoptosis by modulating the NF-κB signaling pathway. This formulation enhances therapeutic benefits and shows promise for diabetes management (Liu et al., 2023).

To investigate the potential protective effect of ethanolic extract of black cumin seeds and its active ingredient, TQ, on streptozotocin-induced diabetic rats, the following procedure was used: To induce diabetes, the male Wistar rats were administered an intraperitoneal injection of STZ at a dosage of 90 mg/kg B.W in 0.9 % normal saline after being fasted for 16 h and made diabetic Group 1; 7 rats non-diabetic control (saline-treated), Group 2; 7 untreated diabetic rats, Group 3; 7 diabetic rats treated orally with *N. sativa* extract at a dose of 100 mg/kg B.W., Group 4; 7 diabetic rats treated orally with TQ at a dose of 10 mg/kg B.W and Group 5; 7 diabetic rats treated orally with Metformin at a dose of 5 mg/kg B.W. After the treatment of 28 days, all groups were examined for B.W. and biochemical alterations. The results showed a significant decrease in blood glucose, urea, creatinine, uric acid, total protein, total cholesterol, low-density lipoprotein, and very low-density lipoprotein, while high-density lipoprotein was increased. Hepatic enzymes—alanine transaminase, aspartate aminotransferase, and alkaline phosphatase—were also normalized. Additionally, body weight significantly increased. These preliminary findings demonstrate that the ethanol extract of *N. sativa* seeds and its active ingredient, TQ, have a protective effect against streptozotocin-induced diabetes in rats (Liu et al., 2023).

2.4.7. Antioxidant activity

Antioxidant enzymes such as GSH, SOD, catalase (CAT), glutathione-S-transferase (GST), and glutathione peroxidase (GSHPx) constitute the main components of the antioxidant system in most cells. It is well known that these enzymes are responsible for neutralizing free radical–induced oxidative damage. TQ induces the expression and/or activity of GST, GSH-Px, SOD, and glutathione reductase. Additionally, TQ improves plasma and liver antioxidant capacity as well as the expression of liver antioxidant genes.

In models with induced hypercholesterolemia, it can up-regulate the genes coding for GST, GSH-Px and CAT leading to elevation of hepatic levels of these enzyme to overcome oxidative stress induced during diethylnitrosamine metabolism. Treatment with TQ showed a protective effect, particularly at a dose of 5 mg/kg/day, both prophylactically and curatively (Elghareeb et al., 2024). In another study Black cumin-enriched acha-based cookies have also been shown to exhibit high antioxidant properties. After receiving STZ (50 mg/kg B.W.) intraperitoneally to induce diabetes and high blood glucose levels of more than 200 mg/dL, mice were infected with *Plasmodium berghei* (NK65 strain). Then, as a treatment, black cumin-enriched acha-based cookies were given to the experimental mice. The effect of the cookies on the activities of neuronal cholinesterases (AChE and BChE), reactive oxygen species (ROS), thiobarbituric acid reactive species (TBARS), and reduced glutathione (GSH) levels as well as neuronal antioxidants [superoxide dismutase (SOD), catalase, glutathione-s-transferase (GST), and glutathione peroxidase (GPx)] was evaluated. The result showed an increase ($P < 0.05$) in cholinesterases and MAO, ROS and TBARS activities with a concomitant decrease in neuro-antioxidant enzymes and GSH levels in diabetic mice infected with *P. bergei* for 2–3 weeks (Nwanna et al., 2024).

2.4.8. Antibacterial activity

p-benzoquinone is effective against various bacteria, including multidrug-resistant *Mycobacterium tuberculosis*. To better understand the antimycobacterial activity of TQ, metabolomic and transcriptomic analyses of bacteria exposed to the compound were performed. Expression of genes encoding stress-responsive sigma factors showed that TQ rapidly induced the production of sigE transcripts. At the same time, long-term exposure resulted in the overexpression of all sigma factor genes and a significant increase in sigF. Metabolomic analysis confirmed that the antimycobacterial activity of TQ was related to the depletion of NAD and ATP stores and the downregulation of plasma membrane lipids. This was observed after 24 h and continued the next day (Jankowski et al., 2024).

In another study, antibacterial activity of *N. sativa* seed extract against some pathogenic bacterial strains (*Streptococcus pyogenes*, *Pseudomonas aeruginos*a, *Klebseilla pneumoniae* and *Proteus vulgaris*) was evaluated. Methanol extract at the concentration of 100 mg/mL had a remarkable sensitivity towards all tested bacteria in this study. *K. pneumonia* and *P. vulgaris* showed resistance against aqueous extract at 20 mg/mL. Methanol extract of *N. sativa* exhibited significant antibacterial activity at the concentration of 50 mg/mL against *S. pyogenes* with a greater inhibition zone of 19 mm, while a 15 mm zone of inhibition was observed in *P. aeruginosa*, *K. pneumonia* and *Proteus vulgaris*. Kruskal Wallis analysis showed that both aqueous and methanol extract of black seed exhibited a greater inhibition on Gram positive bacteria

(*S. pyogenes*) compared with Gram negative bacteria (*P. aeruginosa*, *K. pneumonia* and *P. vulgaris*). Their study also showed that species, strains and concentrations of *N. sativa* extract are some of the factors that may influence the sensitivity of the tested bacteria. A significant correlation was observed between zone of inhibition and concentration of extract (Jankowski et al., 2024).

2.4.9. Reduced the toxicity and apoptosis induced by methotrexate

Methotrexate is widely used in chemotherapy but is known to induce liver toxicity. This study evaluated whether *Arthrospira platensis* (Spirulina) and TQ could protect against methotrexate-induced liver damage. Seven groups of rats ($n = 7$ per group) received different treatments: saline (control), A. platensis (500 mg/kg orally), TQ (10 mg/kg orally), methotrexate (20 mg/kg intraperitoneally), and combinations of methotrexate with either or both protective agents. Methotrexate administration significantly raised liver enzymes, lipids (cholesterol, triglycerides, LDL), and oxidative stress markers, while reducing HDL levels and key antioxidants such as glutathione, superoxide dismutase, and catalase. Liver histology also revealed tissue damage and elevated TNF-α and caspase-3 expression. However, co-treatment with A. platensis and/or TQ notably alleviated oxidative stress, biochemical changes, and apoptosis caused by methotrexate (Jankowski et al., 2024).

2.4.10. Effect of thymoquinone on reduce renal toxicity

In a study in rat model of cisplatin-induced renal damage was established, with TQ treatment groups (receiving 1, 3, 5, 10, or 20 mg/kg of TQ) and determined serum creatinine (Cr) and blood urea nitrogen (BUN), measured the expression of the anti-apoptotic protein Bcl-2, the pro-apoptotic protein Bax, caspase-3, kidney injury molecule-1 (KIM-1) and neutrophil gelatinase-associated lipocalin (NGAL) in renal tissue. Additionally, they observed pathological changes in renal tissue and performed paller score for renal tubule injury. Relative to the control, the cisplatin group showed significantly increased expression of Bax, caspase-3, NGAL, and KIM-1, elevated serum creatinine (Cr) and blood urea nitrogen (BUN) levels, and significantly decreased Bcl-2 expression ($P < 0.05$). Histopathological examination of the cisplatin-treated group revealed vacuolar degeneration, tubular epithelial cell swelling, and loss of brush borders in renal tubules. The Paller score was significantly elevated in the cisplatin group compared to the normal control group. TQ dose-dependently ameliorated these effects after 24 h treatment (Jankowski et al., 2024).

2.4.11. Anti-obesity of thymoquinone

A study investigated the anti-obesity properties of TQ, a compound derived from *N. sativa*, using male Wistar rats subjected to either a standard or high-fat diet over 18 weeks. Beginning at week 12, select high-fat diet groups received TQ at doses of 20 mg or 40 mg, or a reference drug, orlistat (5 mg). Treatment with TQ notably curtailed body weight gain, lowered blood glucose and insulin levels, and improved glucose metabolism and insulin responsiveness. Biochemical tests revealed reduced levels of liver enzymes, pro-inflammatory adipokines like leptin and resistin, and enzymes involved in fat synthesis, while beneficial markers such as adiponectin and lipoprotein lipase increased. Genetic analysis showed decreased activity of fat-producing genes and elevated insulin receptor signaling. Additionally, TQ stimulated activation of AMPK, a key regulator of energy balance. Microscopic examination confirmed that TQ alleviated fat accumulation and tissue damage in the liver and adipose tissue. Overall, these results highlight TQ's promise as a natural therapeutic agent against diet-induced obesity and metabolic dysfunction (Jankowski et al., 2024).

3. Nanocarriers for the delivery of thymoquinone

3.1. Lipid-based nanocarriers

Lipids serve as a versatile and widely-used medium for crafting nanocarriers for bioactives, offering numerous benefits. These benefits include scalable production, the biodegradable and biocompatible nature of lipids, minimal toxicity, and the ability to control the release of the encapsulated bioactives under various conditions, thereby improving the solubility of bioactives (Puri et al., 2009). Hydrophobic agents like TQ, which possess limited solubility in water, may not dissolve completely in GIT fluids, affecting their bioaccessibility. Incorporating these hydrophobic agents into consumable lipids can significantly enhance their bioaccessibility (McClements et al., 2015). The digestion of triglycerides into free fatty acids (FFAs) and monoglycerides (MGs) through the action of gastric and pancreatic lipases in the stomach and small intestine leads to the formation of minuscule colloid particles. These particles, upon interacting with bile salts and phospholipids in the intestinal fluids, improve the solubility and absorption of hydrophobic substances. As outlined in Table 2, a variety of lipid-based nanocarriers, including nano-liposomes; NEs/double emulsions/Pickering emulsions (PEs); microemulsions/cubosomes/hexosomes; niosomes; SLNs; NLCs; self-nano-emulsifying delivery system (SNEDS) (Fig. 4), have been employed to deliver TQ, enhancing its functional attributes, which will be further explored in subsequent sections.

Despite their many advantages, lipid-based nanocarriers also present several limitations that must be considered when designing delivery systems for TQ. One major challenge is their physical and chemical instability, as nano-liposomes and emulsions may be prone to fusion, aggregation, or leakage of the encapsulated drug during storage (Mozafari, 2005). The oxidation of lipid components, especially in unsaturated phospholipids, can compromise the integrity and shelf-life of the formulation (Torchilin, 2005). Another limitation involves the complexity of scale-up and reproducibility, as achieving consistent particle size, drug loading, and colloidal stability across batches can be technically demanding (Danaei et al., 2018). Lipid-based systems such as nanoemulsions and SNEDDS often require high surfactant concentrations, which may lead to GI irritation or toxicity at higher doses (Pouton, 2006). Moreover, drug expulsion during digestion or systemic circulation, particularly in lipophilic environments, can reduce bioavailability and therapeutic efficacy (Porter et al., 2007). In topical or parenteral applications, these systems may also face limited penetration depth or rapid clearance, unless modified with surface stabilizers or targeting ligands (Bozzuto and Molinari, 2015). While lipid-based carriers are generally biocompatible, the need for additional excipients—*e.g.*, cholesterol, co-solvents, or polymers—to improve performance can introduce regulatory and formulation complexities (Sercombe et al., 2015).

From a regulatory perspective, the clinical translation of lipid-based nanocarriers remains limited. Approval by authorities such as the U.S. Food and Drug Administration (FDA) or the European Medicines Agency (EMA) demands extensive safety evaluations, strict quality control, and robust manufacturing reproducibility (Csóka et al., 2021). The inclusion of novel lipid components or nanostructures often necessitates additional preclinical toxicology studies (Csóka et al., 2021). Challenges such as large-scale production, long-term stability, and biological interactions must be considered early in development. Integration of quality-by-design (QbD) principles and alignment with regulatory frameworks (*e.g.*, ICH Q8–Q10) are essential to facilitate the transition of these systems from research to pharmaceutical and functional food markets (Buya et al., 2024; Cunha et al., 2020). Therefore, although lipid-based nanocarriers are promising for TQ delivery, their practical and regulatory limitations in formulation, stability, scalability, and clinical translation must be carefully addressed during development.

3.1.1. Nano-liposomes

Nanoliposomes are adept at encapsulating both hydrophilic and hydrophobic compounds, making them an ideal carrier for TQ, a hydrophobic phytochemical. These nanocarriers consist of a phospholipid bilayer, with hydrophobic tails facing inward and hydrophilic heads facing outward (Shaddel and Rajabi-Moghaddam, 2024). Typically, the

Table 2
Lipid-based nanocarriers for thymoquinone (TQ).

Nanocarrier	Components	Method	Encapsulation/ Entrapment Efficiency (% EE)	Target application	Key findings	Refs.
Nano-liposomes	Cholesterol/Phospholipid extracted from egg yolk	Ethanol-injection	73.40–87.10	Breast and cervical anticancer & therapeutic purposes	Improved physicochemical stability and performance in cancer cell line studies, indicating its potential for cancer treatment A controlled and targeted delivery of TQ, enhancing its therapeutic efficacy	(Shariare et al., 2022)
	Cholestrol/1,2-dipalmitoyl-Sn-glycero-3-phosphocholine (DPPC)	Thin-film hydration	94.30	Therapeutic efficacy of TQ against both drug-sensitive and drug-resistant *A. baumannii*	Effectively reduction of the bacterial load in the lung tissues of the mice infected with the drug-sensitive and drug-resistant *A. baumannii* Effective therapeutic formulation in the treatment of the drug-sensitive or drug-resistant *A. baumannii* infection, achieving up to a 70 % survival rate in drug-resistant infections	(Allemailem et al., 2021)
	Cholesterol/Phospholipon 90H® (PL 90H®)		93.50	Anti-inflammatory effect for topical application	Clear and suitable skin permeation with acceptable rheological properties and superior anti-inflammatory activity	(Mostafa et al., 2018)
	1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC)/Triton X-100		80–83	Breast anticancer purposes	Highly stable, safe with anti-proliferative activity on cancer cells *in vitro* and improved bioavailability	(Odeh et al., 2012)
	Dipalmitoylphosphatidylcholine (DPPC)/Dicetylphosphate (DCP)/ Cholesterol (CHOL)		70	Therapeutic purposes	Improving the delivery and efficacy of TQ in various therapeutic applications	(Mohammadabadi and Mozafari, 2019)
	1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC)/1-palmitoyl-2-oleoylsn-glycero-3-phospho-(10 -rac-glycerol) sodium salt (POPG) (phospholipids)		71.10	Anti-melanoma therapies	An enhanced cytotoxic impact on the examined cell lines, surpassing the effects of TQ in its free state.	(Kłos et al., 2022)
Nanoemulsions (NEs)	Triolein/TQ/Tween-80/double distilled water	High-pressure homogenization (HPH)	~ 89	Improving the solubility and bioavailability	Good stability of NEs over 6 months, with a slow degradation of TQ, indicating the chemical stability of TQ in NEs	(Tubesha et al., 2013a)
	Curcumin + TQ + resveratrol Oleic acid as the oil phase/Tween 20 as the surfactant/PEG 200 as the co-surfactant	Spontaneous emulsification method	~ 81.20	Improving the anti-psoriatic activity for therapeutic purposes	Improved anti-psoriatic conditions approved by *in vitro* and *in vivo* studies in Balb/c mice model	(Khatoon et al., 2021)
Double emulsions	Topotecan +TQ Poly vinyl alcohol (PVA)/Poly lactic glycolic acid (PLGA)	Solvent evaporation	62.60	Co-delivery of opotecan and TQ for therapeutic purposes	A sustained release pattern of both the drugs with a minimal burst release	(Verma et al., 2017)
Pickering emulsions	Alginate/Chitosan/Red palm olein/ Cellulose nanocrystals-soy protein isolate (CNC/SPI) complex	Ionic gelation method	78	Developing the colloids-based nutraceutical delivery system	The highest stability against structural deformation during the drying process; Good stability during gastric digestion; High swelling degree with a superior water uptake capacity during intestinal digestion in simulated intestinal fluid (SIF)	(Wong et al., 2021)
Microemulsions	Formulation 1: Cremaphor EL (Surfactant)/Akoline –MCM (Cosurfactant)/Capryol 90 (Oil) Formulation 2: Solutol (Surfactant)/	Dissolution and homogenization	92	Anticancer applications	Improved stability and a significant increase in the anticancer activity against various cell lines	(Velho-Pereira et al., 2017)

(*continued on next page*)

Table 2 (*continued*)

Nanocarrier	Components	Method	Encapsulation/ Entrapment Efficiency (% EE)	Target application	Key findings	Refs.
	Ethanol (Cosurfactant)/Ethyl oleate (Oil)					
Cubosomes	Glyceryl monooleate (GMO)/ Pluronic F127 (PLX)	Emulsification method	88.60	Human breast anticancer purposes	Enhanced antitumor activity with high entrapment efficiency and favorable physicochemical properties	(Mehanna et al., 2020)
Hexosomes	Glycerol monooleate (GMO)/Vit E/ D—α—tocopheryl poly (ethylene glycol)$_{2000}$ succinate (TPGS-PEG2000)	Dissolution, homogenization, and ultrasonication	~ 90	Development of TQ nanomedicines	TQ in a concentration-dependent manner dramatically alters the internal nanostructure as well as the morphological features of TPGS-PEG2000-stabilized GMO/Vit E nanoparticles	(Yaghmur et al., 2019)
Niosomal vehicles	Cholesterol/Tween 60	Thin film hydration	80.10	Therapeutic effect in treating cerebral I/R injury	Improving TQ bioavailability and efficacy in treating cerebral I/R injury	(Nemati et al., 2023)
Solid lipid nanoparticles (SLNs)	Stearic acid/Lecithin/Taurocholate	Thermo-mechanical nano-precipitation	78	Neuroprotective applications	Counteracted motor impairments and neuroinflammation in a rat model of Huntington's disease	(Ramachandran and Thangarajan, 2018)
	Glyceryl mono stearate/Aqueous P-188/Stabilizer TPGS	Combined melt emulsification ultrasonication method	83.20	Oral bioavailability for therapeutic purposes	Improved oral bioavailability	(Rahat et al., 2021b)
Nano-structured lipid carriers (NLCs)	Hydrogenated palm oil (HPO)/Lipoid S100 (soy lecithin)/Olive oil/ Sorbitol/Thimerosal/Polysorbate 80	HPH	86.90	Gastroprotective purposes	Improved gastroprotection against ethanol-induced ulcers by approximately threefold compared to free TQ; Non-toxic to normal human liver cells, suggesting that NLCs can be a safe vehicle for the oral delivery of TQ	(Abdelwahab et al., 2013)
Self-nano-emulsifying delivery system (SNEDS)	Olive oil, Tween 80, Span 85, PEG 300	Hydrophilic-Lipophilic Balance - Response Surface Methodology	~ 95	*In vitro* test of SNEDS and *in vivo* evaluations of TQ-loaded SNEDS against rat liver I/R injury for therapeutics purposes	TQ encapsulated in SNEDS significantly protects rat liver from I/R injury	(Bahloul et al., 2024)
	Oil/water/surfactant/co-surfactant	Micro-emulsification	99.80	To enhance its oral bioavailability	Significant liver-protective effects in an animal model induced with PCM by using TQ-SNEDS; SNEDS mediated a fourfold increase in the bioavailability of TQ-SNEDS over pure TQ	(Rathore et al., 2023)

size of a nanoliposome is < 200 nm, though definitions can extend up to 1000 nm in diameter (Danaei et al., 2018). Various production methods for liposomes include reversed-phase evaporation, solvent-injection, and detergent dialysis. To achieve smaller liposome sizes, high-energy techniques such as sonication, high-pressure extrusion, and micro-fluidization are employed (Huang et al., 2014; Zahedi et al., 2024).

Recent advancements in nano-liposomal formulations have focused on optimizing the encapsulation efficiency and release kinetics of TQ. For example, Shariare et al. (2022) developed stable liposomal TQ using phospholipid extracted from egg yolk. The optimized liposomal preparation exhibited improved physicochemical stability and performance in cancer cell line studies, indicating its potential for cancer treatment (Shariare et al., 2022). Another research effort characterized nano-liposomal TQ in terms of particle size distribution, zeta potential, and entrapment efficiency. The findings suggested that these nano-liposomes could provide a controlled and targeted delivery of TQ, enhancing its therapeutic efficacy (Shariare et al., 2022). Mostafa et al. (2018) focused on the optimization and characterization of TQ-loaded liposomes for enhanced topical anti-inflammatory activity. Utilizing a thin film hydration method, they developed various liposomal systems by adjusting cholesterol molar concentrations, total lipid molar concentrations, and drug-to-lipid ratios. The optimized liposomal formulation, known as F12, demonstrated improved skin permeation and was integrated into a chitosan gel for topical application. This formulation not only showed clear and suitable skin permeation but also exhibited acceptable rheological properties. *In vivo* studies using

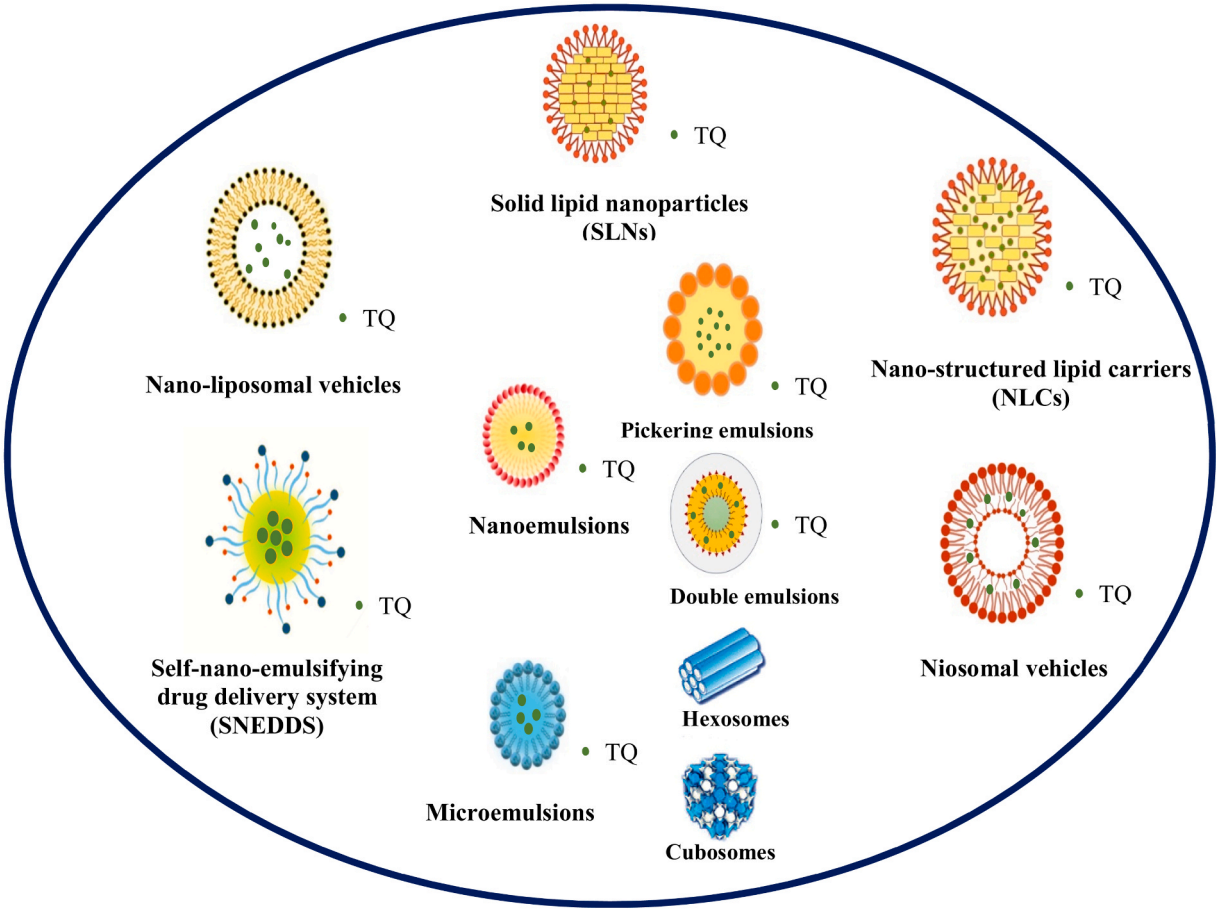


Fig. 4. Lipid-based nanocarriers including nano-liposomes; nanoemulsions/double emulsions/Pickering emulsions (PEs); microemulsions/cubosomes/hexosomes; niosomes; SLNs; NLCs and self-nano-emulsifying delivery system (SNEDS). The green circles (●) represent thymoquinone (TQ) molecules. The outermost oval shape denotes the general encapsulating structure typical of lipid-based carriers.

carrageenan-induced paw edema in rats indicated that the F12 chitosan gel had significantly superior anti-inflammatory activity compared to the TQ gel and was comparable to the marketed indomethacin gel (Mostafa et al., 2018).

Similarly, Odeh et al. (2012) encapsulated TQ within liposomes, focusing on the loading efficiency and the compound's biological activity against breast cancer. The research successfully formulated TQ-loaded liposomes using a method that ensured high entrapment efficiency, with $>$ 90 % for TQ-loaded liposomes and 49.6 % for liposomes modified with Triton X-100. These liposomes, approximately 100 nm in diameter, were effective in inhibiting the proliferation of breast cancer cell lines MCF-7 and T47D while demonstrating minimal toxicity towards normal fibroblast cells. This encapsulation technique not only preserved the stability and bioavailability of TQ but also maintained its anticancer activity, marking a significant advancement in the use of liposomes for cancer therapy (Odeh et al., 2012). In addition, Mohammadabadi and Mozafari (Mohammadabadi and Mozafari, 2019) developed nanoliposomal TQ, focusing on the evaluation of loading efficiency and particle characterization. Their research aimed to enhance the bioavailability and therapeutic potential of TQ by encapsulating it in nanoliposomes composed of dipalmitoylphosphatidylcholine (DPPC), dicetylphosphate (DCP), and cholesterol (CHOL) in a 7:2:1 molar ratio. Atomic force microscopy and light scattering techniques confirmed the successful formation of spherical vesicles with sizes around 267.8 nm for empty vesicles and 276.0 nm for TQ-loaded vesicles. The study achieved a high entrapment efficiency of 75.43 % for TQ, and the zeta potential values indicated long-term stability of the formulations. This meticulous approach to nanoliposome development holds promise for improving the delivery and efficacy of TQ in various therapeutic applications (Mohammadabadi and Mozafari, 2019).

Kłos et al. (2022) examined the effects of TQ and TQ-loaded liposomes on skin-derived and metastatic human malignant melanoma cell lines. Their research aimed to compare the anticancer activity of free TQ and TQ encapsulated in liposomes on two melanoma cell lines, A375 and WM9, representing different stages of the disease. The study assessed the proapoptotic effects and mitotoxicity of TQ, as well as the cytotoxicity of both free TQ and liposomal TQ. The results indicated a higher antiproliferative effect of TQ in the WM9 cells, while the A375 cell line exhibited a greater proapoptotic response. This suggests that nano-liposomes could potentially enhance the delivery and efficacy of TQ, offering a targeted approach to treating various stages of melanoma (Kłos et al., 2022).

These studies underscore the potential of nano-liposomes in improving the delivery and therapeutic outcomes of TQ. By leveraging the unique properties of liposomes, *e.g.*, their biocompatibility and ability to merge with cellular membranes, TQ's pharmacological profile can be significantly enhanced. Overall, the literature from the past five years indicates that nano-liposomes are a promising approach for the delivery of TQ. Ongoing research continues to explore the potential of these nanocarriers in various biomedical applications, with the aim of developing more effective treatments for a range of conditions.

The general challenges associated with lipid-based delivery systems, including liposomes, such as physical and chemical instability, scale-up difficulties, and formulation complexity, have been discussed in Section 3.1. Specifically for nano-liposomes, issues like drug leakage, vesicle fusion, oxidation of phospholipids, and sterilization challenges can

hinder their long-term stability and clinical translation (Mozafari, 2005; Torchilin, 2005). These limitations should be carefully considered alongside their therapeutic potential when designing TQ-loaded liposomal formulations.

3.1.2. Emulsion-based nanocarriers

NEs represent innovative approaches to enhance the bioavailability and controlled release of TQ. These systems can encapsulate TQ, protecting it from degradation and enabling targeted delivery to specific tissues. NEs are colloidal dispersions of two immiscible liquids where one is dispersed in the other in the form of droplets, typically ranging from 20 to 200 nm. Due to their small size, NEs can improve the solubility and stability of TQ, facilitating its absorption and distribution in the body. The use of biocompatible surfactants and co-surfactants in the formulation of NEs can further enhance the delivery of TQ, allowing for a more efficient penetration through biological membranes.

The enhancement of TQ bioavailability by emulsion-based nanocarriers is primarily attributed to their ability to improve solubility, protect TQ from degradation, and facilitate absorption across biological membranes. The nanoscale droplet size (typically 20–200 nm) increases the surface area, promoting greater dissolution and interaction with the gastrointestinal mucosa, which helps overcome the poor water solubility of TQ (Khatoon et al., 2021; Tubesha et al., 2013a). The incorporation of biocompatible surfactants and co-surfactants enhances the permeability of these nanocarriers by transiently disrupting epithelial tight junctions and facilitating transcellular and paracellular transport (Khatoon et al., 2021; Tubesha et al., 2013a). Double emulsions provide an additional barrier to premature drug release, enabling sustained delivery and protection from enzymatic degradation in the gastrointestinal tract, thus improving systemic absorption (Verma et al., 2017). Pickering emulsions stabilized by solid nanoparticles create a rigid interfacial layer that improves the physical stability of the formulation during gastrointestinal transit, while the swelling behavior of alginate-chitosan beads promotes controlled release and targeted delivery in the intestine, further enhancing TQ absorption (Wong et al., 2021). Overall, these mechanisms allow emulsion-based nanocarriers to effectively overcome solubility and permeability barriers, resulting in improved bioavailability and therapeutic efficacy of TQ.

As an example, Tubesha et al. (2013a) prepared NEs of TQ by HPH, using 5 % Triolein with 4.45 % TQ and an aqueous phase consisting of 2 % Tween-80 and 93 % double distilled water. The study aimed to improve the solubility and bioavailability of TQ due to its poor water solubility. NEs showed good stability over 6 months, with a slow degradation of TQ, indicating the chemical stability of TQ in NEs (Tubesha et al., 2013a). In another study, Khatoon et al. (2021) developed a nanoemulsion gel containing a combination of curcumin, TQ, and resveratrol. The formulation used oleic acid as the oil phase, Tween 20 as the surfactant, and PEG 200 as the co-surfactant. This study focused on improving the anti-psoriatic activity of these compounds, demonstrating that the nanoemulsion gel effectively managed psoriasis in *in vitro* and *in vivo* studies (Khatoon et al., 2021).

Double emulsions, also known as W/O/W or O/W/O systems, consist of droplets containing an inner water phase dispersed within an oil phase, which is in turn dispersed in an outer water phase. This configuration can be particularly useful for the encapsulation of both hydrophilic and lipophilic drugs like TQ. The double-layered structure provides an additional barrier, potentially reducing the rate of drug release and allowing for a more sustained delivery over time. Verma et al. (2017) presented a novel dual-drug delivery system using a modified double emulsion solvent evaporation method to co-deliver topotecan (hydrophilic) and TQ (lipophilic). In this system, topotecan was dissolved in the inner aqueous phase, and TQ was incorporated into the organic phase. The resulting nanocarriers had a well-characterized particle size of 240.7 ± 8.3 nm, confirming the formation of nanoscale droplets. Using central composite design (CCD), the authors optimized formulation variables to ensure desirable characteristics, including drug entrapment efficiency, particle stability, and sustained release. The nanoparticles exhibited a spherical shape, negative zeta potential (−1.16 mV), and good reconstitution properties, and they demonstrated minimal burst release with over 90 % cumulative release of both drugs within 96 h. These results confirm the potential of this nanoscale double emulsion system for effective co-delivery of chemotherapeutic agents (Verma et al., 2017).

PEs are stabilized by solid particles rather than traditional surfactants. These particles adsorb at the oil-water interface, creating a rigid layer that can enhance the stability of the emulsion. For TQ delivery, NPs such as silica or biodegradable polymers can be employed to form PEs. This method not only improves the stability of TQ but also offers the possibility of a controlled release profile, as the particle size and surface properties can be manipulated to adjust the release rate. In a study, Wong et al. (2021) investigated the *in vitro* digestion and swelling kinetics of TQ-loaded PEs incorporated in alginate-chitosan hydrogel beads. The study aimed to explore the release behavior of TQ, using a simulated GIT model. PEs were formulated with 20 % red palm olein and 0.5 % (w/v) cellulose nanocrystals-soy protein isolate (CNC/SPI) complex, followed by encapsulation within beads (Fig. 5). The results showed that the alginate-chitosan beads had excellent stability in simulated gastric fluid with a low water uptake. During intestinal digestion, the beads demonstrated a high swelling degree and a superior water uptake capacity. The release profile of TQ reached up to 83 % in intestinal digestion, following a diffusion-dominated release *via* the bead swelling process (Wong et al., 2021). In summary, these advanced emulsion systems provide versatile platforms for the effective delivery of TQ. By optimizing the composition and preparation methods, it is possible to achieve desired therapeutic outcomes with improved safety and efficacy.

3.1.3. Microemulsions/cubosomes/hexosomes

Microemulsions, cubosomes, and hexosomes are at the forefront of nanotechnology, offering novel pathways to enhance the bioavailability and controlled release of TQ. These nanocarriers are adept at encapsulating TQ, safeguarding it against degradation, and facilitating its targeted delivery. Microemulsions are clear, thermodynamically stable systems composed of oil, water, and surfactant, often in combination with a co-surfactant. Their droplet size typically falls below 100 nm. The ultra-small size of microemulsions allows for increased solubility and stability of TQ, promoting its absorption and distribution. The surfactants used in microemulsions can be tailored to enhance the delivery of TQ, optimizing its penetration through biological barriers for effective therapeutic action. Velho-Pereira et al. (2017) investigated the biodistribution of microemulsion formulations of technetium-99m-radiolabeled TQ. The research focused on enhancing the bioavailability of TQ for anticancer applications. The microemulsions demonstrated improved stability and a significant increase in the anticancer activity against various cell lines compared to plain TQ (Velho-Pereira et al., 2017).

Cubosomes are NPs with a unique internal structure resembling a three-dimensional cubic phase. They are formed from certain lipids that self-assemble into a bi-continuous cubic liquid crystalline phase in water. Cubosomes offer a high surface area and unique morphology, which can be leveraged for the encapsulation of both hydrophilic and lipophilic drugs like TQ. Their structure provides a sustained release mechanism, potentially improving the bioavailability of TQ. Mehanna et al. (2020) explored the anticancer activity of TQ cubic phase NPs against human breast cancer. The study aimed to overcome the poor bioavailability of TQ by using cubosomes. The TQ-loaded cubosomes showed enhanced antitumor activity and were characterized by a high entrapment efficiency and favorable physicochemical properties (Mehanna et al., 2020).

Hexosomes are similar to cubosomes but are characterized by a hexagonal phase. They are composed of a lipid bilayer forming a continuous honeycomb-like structure. Hexosomes can encapsulate a

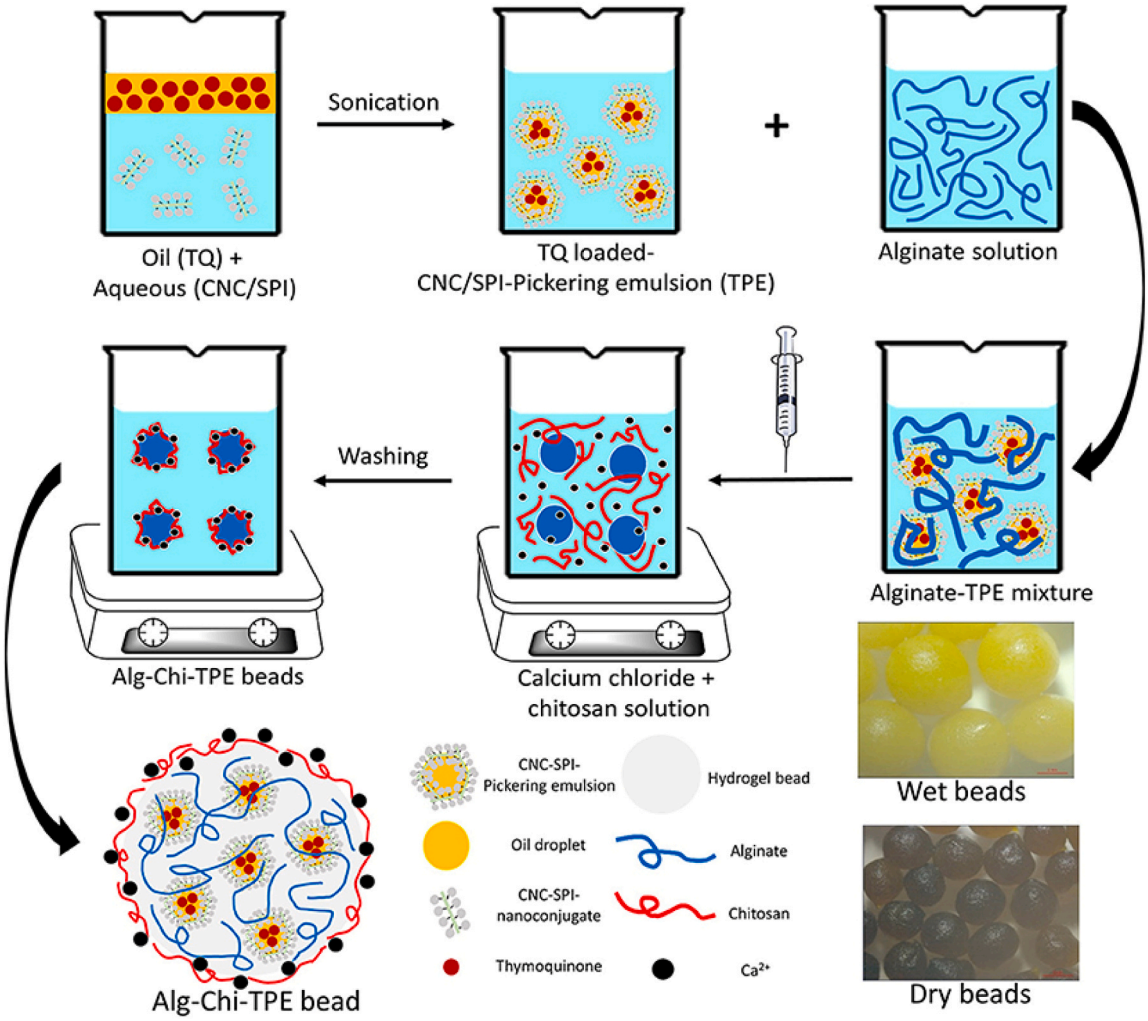


Fig. 5. The schematic illustration of the preparation of thymoquinone loaded alginate-chitosan beads by immobilized CNC/SPI-stabilized Pickering emulsion. Reproduced with permission from Wong et al. (2021).

large amount of drug and release it in a controlled manner. The incorporation of TQ into hexosomes could lead to novel formulations with enhanced stability and a tailored release profile. An investigation conducted by Yaghmur et al. (2019) reported on non-lamellar liquid crystalline nanocarriers for TQ encapsulation. The study highlighted the potential of hexosomes to accommodate TQ, with findings suggesting that TQ loading led to structural transitions within nanocarriers, which could be beneficial for the development of TQ nanomedicines (Yaghmur et al., 2019).

3.1.4. Niosomal carriers

Niosomes are emerging as a significant nanocarrier in the realm of drug delivery, particularly for bioactives like TQ. These vesicular structures are composed of non-ionic surfactants and cholesterol, offering a biocompatible and stable means of encapsulating both hydrophilic and lipophilic drugs. Recent studies have highlighted the potential of niosomes for enhancing the bioavailability and therapeutic efficacy of TQ. For instance, a study by Kurowska et al. (2023) discussed the therapeutic potential of TQ in the treatment of CRC, emphasizing the role of niosomal encapsulation in enhancing the bioactivity and delivery of TQ. The niosomal formulation was found to improve the proapoptotic effect of TQ and inhibit proliferation by regulating crucial signaling pathways involved in tumor progression (Kurowska et al., 2023). Another research by Pal et al. (2021) reviewed the advancements in the delivery of TQ for cancer and rheumatoid arthritis (RA) treatment using nanocarriers, including niosomes. The study underscored the importance of nanocarriers in overcoming the pharmacokinetic limitations of TQ, such as its short half-life and low bioavailability, thereby maximizing its therapeutic potential (Pal et al., 2021).

Nemati et al. (2023) developed a niosomal formulation loaded with TQ to enhance its therapeutic effects for cerebral ischemia-reperfusion (I/R) injury. The niosomes, averaging 77.8 nm in size, were prepared using cholesterol and Tween 60 and assessed for morphology and stability. *In vivo* studies on male Wistar rats subjected to middle cerebral artery occlusion (MCAO) showed that intravenous administration of TQ niosomes (TQN) significantly reduced brain damage and inflammation compared to free TQ and control groups. The TQN group exhibited increased antioxidant enzyme activities and decreased oxidative stress markers. The study concluded that TQN could be a promising nanocarrier for TQ, improving its bioavailability and efficacy in treating cerebral I/R injury (Nemati et al., 2023).

3.1.5. Solid lipid nanoparticles

SLNs have emerged as versatile and efficient nanocarriers for TQ, offering advantages such as controlled drug release, increased drug stability, and improved bioavailability. SLNs are submicron colloidal carriers that are composed of physiological lipid materials, which solidify at room and body temperatures. Encapsulation of TQ in SLNs can protect it from degradation due to environmental factors such as light and oxygen. Moreover, the solid matrix of SLNs provides a sustained

release of TQ, which is beneficial for achieving prolonged therapeutic effects. The lipid matrix of SLNs can be tailored to modify the release profile of TQ, allowing for a customizable delivery according to therapeutic needs.

Studies have shown that TQ-loaded SLNs can enhance the solubility of TQ and facilitate its transport across biological barriers. For instance, a study by Ramachandran and Thangarajan (2018) demonstrated that TQ-loaded SLNs counteracted motor impairments and neuroinflammation in a rat model of Huntington's disease, suggesting a potential for SLNs in neuroprotective applications. Furthermore, research on chitosan-modified SLNs aimed to improve the oral bioavailability of TQ, addressing the challenges of high lipophilicity and low bioavailability. These SLNs were optimized using a quality by design (QbD) approach, showcasing the precision and adaptability of SLNs for TQ delivery (Rahat et al., 2021b). In summary, SLNs represent a promising nanocarrier for TQ, providing protection, enhanced bioavailability, and controlled release. The adaptability of SLNs allows for their application in various therapeutic areas, making them a valuable tool in the advancement of TQ-based treatments.

3.1.6. Nano-structured lipid carriers

NLCs are advanced nanocarriers extensively studied for their potential to enhance the bioavailability and controlled release of bioactives like TQ. NLCs are composed of a blend of solid and liquid lipids, which form a matrix capable of loading both lipophilic and hydrophilic drugs. The unique structure of NLCs allows for the stabilization of TQ, protecting it from degradation and enhancing its solubility. Due to their solid lipid content, NLCs offer a controlled release profile, which is crucial for maintaining therapeutic levels of TQ over an extended period. This can be particularly beneficial for chronic treatments where long-term drug release is desired. Studies have demonstrated that TQ-loaded NLCs can significantly improve the gastroprotective activity of TQ, as well as its pharmacokinetic properties (Abdelwahab et al., 2013). For example, TQ encapsulated in NLCs has shown improved gastroprotection against ethanol-induced ulcers, and pharmacokinetic studies have indicated that TQ behaves linearly, with improved Tmax, Cmax, and elimination half-life, making it suitable for extravascular administration (Abdelwahab et al., 2013). Moreover, the safety profile of TQ-NLCs has been tested and found to be non-toxic to normal human liver cells, suggesting that NLCs can be a safe vehicle for the oral delivery of TQ (Abdelwahab et al., 2013). The versatility of NLCs also allows for the modulation of particle size and surface charge, which can be optimized for targeted delivery to specific tissues or cells.

3.1.7. Self-nano-emulsifying delivery systems

SNEDS are innovative colloidal carriers that have shown promise in enhancing the oral bioavailability and stability of hydrophobic compounds like TQ. SNEDS are isotropic mixtures of natural or synthetic oils, surfactants, solvents, and co-solvents/surfactants that spontaneously form fine oil-in-water NEs when introduced to aqueous phases under gentle agitation. Encapsulation of TQ in SNEDS can significantly protect it from degradation and improve its dissolution rate, leading to enhanced bioavailability. For instance, a study demonstrated that TQ-loaded SNEDS exhibited good *in vitro* characteristics, including stable NPs structure and size, with a high drug release rate (Bahloul et al., 2024). In another study, Rathore et al. (2023) used SNEDS for the encapsulation of TQ to enhance its oral bioavailability. The size of the droplets was within the nanometer scale, measuring < 90 nm. The zeta potential, recorded at -11.35 mV, indicated a high level of stability for these oil droplets. Studies conducted in living organisms revealed that the bioavailability of TQ when delivered through SNEDS was quadrupled compared to its pure form. Additionally, significant liver-protective effects were observed in an animal model induced with PCM when using TQ-SNEDS, surpassing the results of pure TQ and the standard silymarin. This was further corroborated by a decrease in liver enzymes typically used as biomarkers and by histological analysis of liver tissue. The findings of this research suggest that SNEDS is a superior method for orally delivering TQ, as it not only reduces hepatotoxicity but also significantly improves bioavailability (Rathore et al., 2023).

Moreover, SNEDS can be engineered to target specific absorption sites in GIT, which can further optimize the therapeutic efficacy of TQ. The surface properties of SNEDS can also be modified to achieve mucosal adhesion, providing an extended residence time at the absorption site.

The stability and performance of SNEDS formulations are critically influenced by factors such as droplet size, zeta potential, surfactant concentration, and the nature of oils and co-surfactants used. Smaller droplet sizes (typically below 100 nm) provide a larger surface area for dissolution and absorption, which is essential for improving TQ bioavailability, contributing to the reported fourfold increase in the bioavailability of TQ-SNEDS over pure TQ. A sufficiently high absolute zeta potential (positive or negative) ensures physical stability by preventing droplet aggregation through electrostatic repulsion, thus maintaining consistent drug release profiles. Additionally, the choice and ratio of surfactants and co-surfactants determine the robustness of the nanoemulsion, impacting its self-emulsification efficiency and resistance to precipitation or phase separation during storage and gastrointestinal transit (Zhao et al., 2010)

Furthermore, SNEDS can be tailored for targeted delivery within the gastrointestinal tract by modifying their surface properties to promote mucosal adhesion, thereby extending residence time at absorption sites and optimizing drug uptake (Batool et al., 2020). These critical quality attributes—droplet size, surface charge, surfactant composition, and mucoadhesive potential—collectively determine the formulation's stability and therapeutic performance, making SNEDS a promising platform for efficient oral delivery of TQ.

3.2. Biopolymeric nanocarriers

Although TQ has an approved anti-oxidant, anti-inflammatory and anti-cancer activity, it's poor solubility and strong hydrophobicity can limit these activities in application (Almajali et al., 2021). Biopolymeric nanocarriers are promising to overcome this. Nanocarriers of TQ provides protection and enhances bioavailability of it, thus improves therapeutic potential for human health. Biopolymeric NPs, nanogels (NGs), nanotubes (NTs), nanofibers (NFs), nanocomplexes and dendrimers can provide unique physicochemical properties such as high surface area and reactivity, allowing high loading capacity (Fig. 6). Thus, they can be occupied for TQ delivery by improving efficient transport through biological membranes in controlled manner and exhibiting the remarkable pharmacokinetic properties. Due to their natural content, biocompatibility and biodegradability, mainly chitosan, gelatin, zein, dextrin and alginate can be used to encapsulate, transport and delivery of TQ. This approach holds promise for enhancing therapeutic efficacy and minimizing side effects through targeted delivery. Besides, it is essential to optimize formulation parameters and properly evaluate the biopolymeric nanocarriers through *in vitro* an *in vivo* studies in terms of cytotoxicity, stability and cellular uptake to ensure their safety and efficacy for target purposes.

Selection of biopolymers to encase, carry and deliver TQ differs due to their particular characteristics and capabilities tailored to the proposed applications. For instance, chitosan and its derivatives are the most commonly occupied biopolymers for wound dressing and tissue scaffolding mainly due to their excellent film-forming ability, antimicrobial activity and non-toxicity (Shukla et al., 2013). Besides, biocompatibility and biodegradability of chitosan, since purified from natural biological sources, strongly guide its employment in nanogel and nanoparticle forms to cargo TQ for therapeutic purposes. When chitosan is blended with another natural biopolymer, cellulose exhibiting hydrophilic structure, high elasticity, biocompatibility and perfect mechanical properties, formed nanogels are considered as coatings and wound dressing matrices, promising for the controlled release of TQ

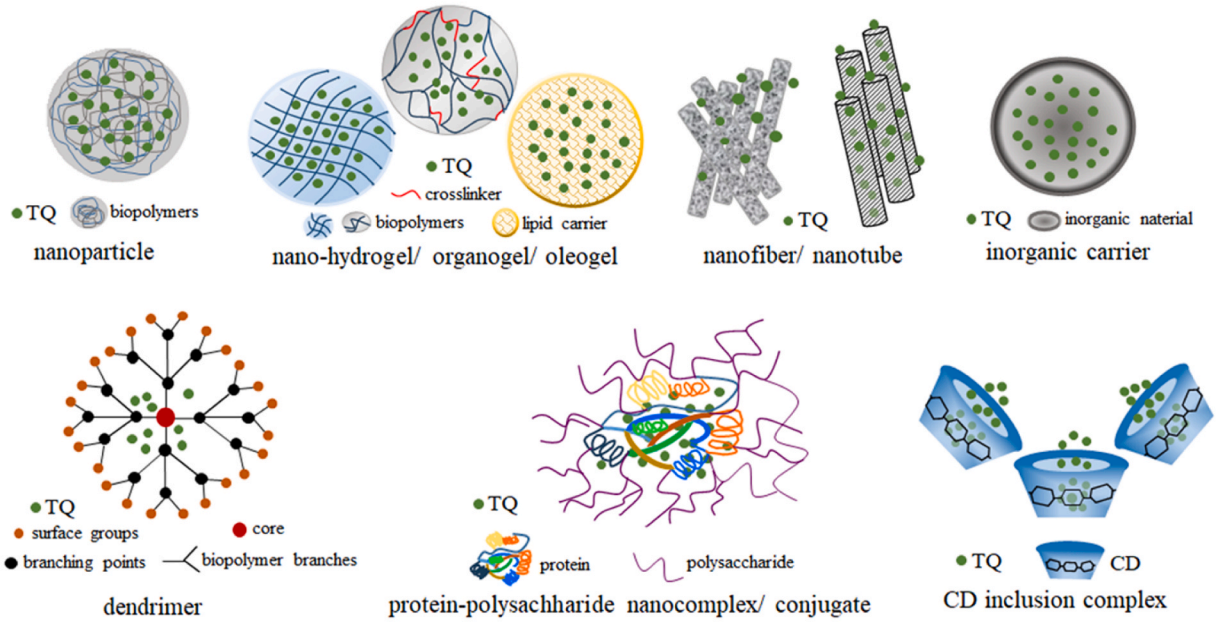


Fig. 6. Biopolymeric nanocarriers including biopolymer-based nanoparticles, nanogels, nanotubes, nanofibers, nanocomplexes and dendrimers.

(Bayraktar and Tunç, 2024). Cost-effectiveness, performance for target application and stability are the other factors guiding the selection of biopolymers for generating TQ delivery matrices.

TQ-loaded biopolymeric nanocarriers can be characterized through morphological, physicochemical, structural and thermal analysis methods. High resolution microscopy techniques like scanning and transmission electron microscopy (SEM and TEM), and atomic force microscopy (AFM) provide information about size and morphological features of the nanocarrier particles. Light scattering measurements define particle size and size distribution of the nanoparticles, revealing TQ loading capacity of the nanocarrier system through its particular surface area. Zeta-potential measurements facilitate assessment of the surface charge, chemical interactions and colloidal stability of the system. Quantification and encapsulation efficiencies can be determined through chromatographic analyses and UV–visible spectroscopy. Structural features like chemical content and interactions, linkages and crystallinity of the TQ-loaded nanocarriers can be characterized by spectroscopic tools like Fourier transform infrared (FTIR), circular dichroism (CD) and X-ray diffraction (XRD) spectroscopies, as well. Mechanical strength and stability of the TQ-nanocarrier systems can be evaluated through rheological measurement tools. Thermal stability of these systems are mainly assessed by thermogravimetric (TGA) and differential scanning calorimetry (DSC) analyses, which determines weight loss, decomposition, phase transition, and degradation profiles of the referred nanostructures.

All the research studies reporting biopolymeric nanocarriers for TQ delivery are assessed through at least a few of these analytical methods to facilitate proper design of nanocarrier system for particular delivery purposes. Recent research works regarding common biopolymer nanocarriers for TQ delivery are mentioned in Table 3.

Despite their many advantages, biopolymeric nanocarriers face several limitations relevant to both food and biomedical applications. Batch-to-batch variability due to natural polymer sources can affect reproducibility in particle size, loading efficiency, and release behavior, impacting product consistency (del Castillo-Santaella et al., 2019). Some biopolymers may lack mechanical strength or stability under food processing or physiological conditions, leading to premature degradation or leakage of TQ (Kučuk et al., 2023). The complexity and cost of polymer modification and synthesis may hinder large-scale production for commercial food or pharmaceutical use (Milewska et al., 2021).

Interactions between the biopolymer matrix and TQ can alter loading efficiency and release profiles, potentially reducing efficacy in functional foods or therapeutic formulations (Zhang et al., 2024). Additionally, residual solvents or impurities in polymer carriers may cause immunogenic or inflammatory reactions, a concern for biomedical applications and regulatory approval (Bancos et al., 2013). Achieving precise, controlled, and targeted release often requires chemical crosslinking or surface modifications, which can introduce safety and regulatory challenges in both sectors (Wang et al., 2023). Moreover, variability in biopolymer degradation rates can lead to inconsistent release and clearance, affecting bioavailability and functional outcomes in food digestion or human metabolism (Wu et al., 2024).

Thus, while biopolymeric nanocarriers hold great promise for enhancing TQ's bioavailability and efficacy in both food and biomedical fields, addressing issues of reproducibility, stability, safety, and scalable production remains critical to their successful application and commercialization.

3.2.1. Nanoparticles

Biopolymer NPs used for TQ delivery aims to improve stability, bioavailability and targeted release. Especially, cationic biopolymer NPs are highly talented to carry and deliver lipophillic materials like TQ at target locations by minimizing off-target effects. A well-recognized cationic polysaccharide chitosan offers desirable properties including nature-based origin, non-toxicity and non-antigenicity, enabling it favorable for nanocarriers (Narmani and Jafari, 2021). Chitosan is widely applied biopolymer for NP fabrication through ionotropic gelation of positively charged water-soluble chitosan with negatively charged sodium tripolyphosphate to carry TQ for various therapeutic purposes (Al-Qubaisi et al., 2022; Sharifi et al., 2020; Xue and Lin, 2024).

Surface modification of chitosan NPs with certain polymeric ligands such as polyethylene glycol (PEG) can facilitate specific delivery of TQ to the desired site of action such as cancer cells and inflamed tissues as well (Hemananthan, 2019). PCL, polysorbate (PS), and PLGA are some synthetic polymers used in combination with chitosan to formulate TQ-encasing NPs (Alshehri et al., 2020; Fahmy et al., 2020; Rahat et al., 2021b). Those successfully increased entrapment efficiency and sustained delivery of TQ, revealing enhanced anti-cancer activity. Besides chitosan, gums, collagen and Citrem are employed to fabricate

Table 3
Biopolymeric and inorganic nanocarriers for thymoquinone (TQ).

Nanocarrier	Biopolymer/ inorganic matrix	Method	Target application	Dose	Key findings	Refs.
Nanoparticles (NPs)	Chitosan	Anionic gelation and cooling spinning	Pediatric diabet treatment	20–100 μg/mL	TQ-loaded chitosan NPs were advised for curation of diabet in children safely	(Xue and Lin, 2024)
		High pressure homogenization and spray drying	Anti-inflammatory purpose	10 % (w/w)	NPs exhibited rapid TQ release in acidic gastric medium while showing sustained release at pH 6.8 over 100 h.	(Al-Qubaisi et al., 2022)
		Ionic gelation and emulsification	Preservative in cosmetics	12,5–50 % (w/w)	NPs had sustained TQ release with max 62 % at 28 days, and improved antimicrobial rate of TQ against cosmetic microflora	(Mondéjar-López et al., 2022)
	Citrem and soy phosphatidylcholine	Microfluidic synthesis and low energy-emulsification	Therapeutic purpose	1, 2.5 and 5 mg/mL	TQ-loaded NPs with 124 nm produced through microfluidic synthesis, introduced as an efficient method for nanodelivery of therapeutics.	(Ilhan-Ayisigi et al., 2021)
	Chitosan-polycaprolactone	Single emulsion solvent evaporation	Bioavailability assessment	20 mg/mL (in organic phase)	Initial burst release of TQ followed by prolonged release from NPs which also improved oral bioavailability of TQ.	(Rahat et al., 2021a)
	Chitosan	Ionic gelation and crosslinking	Reducing infection	100 mg/mL	Cercaricidal and microbicidal activity of TQ is improved when encapsulated within NPs	(El-Menyawy et al., 2021)
	Chitosan-polysorbate 80		Depression treatment	20 mg/mL	Water solubility and bioavailability of TQ were improved in NP formulation indicating antidepression effect. Initial rapid TQ release was followed by delayed release for 72 h.	(Fahmy et al., 2020)
	Folic acid-chitosan		Treatment of ovarian cancer cells	22.5 mg/mL	TQ-loaded NPs exhibited significant effect on cancer cell lines.	(Ince et al., 2020)
	Chitosan		Anticancer purpose	200–12.5 μg/mL	Initial burst release of TQ was followed by sustained release from NPs which successfully inhibited metastasis.	(Sharifi et al., 2020)
	Palmitoyl-Chitosan	Precipitation, ionic gelation and crosslinking	Multi-drug therapy purposes	150 μM	Successful encapsulation (64.9 %) and release (97.5 %) efficiency of TQ was achieved.	(Othman et al., 2020)
	Chitosan-polyethylene glycol	Ionic gelation and crosslinking	Therapeutic effect	-	It was achieved high bioavailability of TQ encapsulated with NPs which also exhibited high toxicity against cancer cell lines.	(Hemananthan, 2019)
	Gum-rosin	Nanoprecipitation	Management of diabet	20 mg/mL (in organic phase)	Combined TQ and glycyrrhizin exhibited significant decrease in blood glucose and improved biochemical parameters in rats	(Rani et al., 2019)
	Chitosan	Ionic gelation and crosslinking	Medical and pharmaceutical purposes	100, 150, and 200 μM	Good encapsulation efficiency of TQ with 36 %	(Othman et al., 2018)
Nanogels (NGs)	Chitosan-sodium alginate/psyllium husk derived	Homogenization and film casting	Antibacterial and anticancer purposes	-	Significant antimicrobial activities against *P. aeruginosa* and *E. faecalis*, and strong anticancer activities against prostate cancer cells and adenocarcinomic epithelial cells were observed.	(Das et al., 2022)
	Black seed oil nanoemulgel	High-energy ultrasonication	Wound healing	5 % (w/w)	Significantly increased skin penetration of TQ *via* nanoemulgel, quicker and earlier wound healing than conventional hydrogel.	(Algahtani et al., 2021)
	Chitosan-lecithin	Solvent evaporation-self assembly		10 mg/mL	TQ-polymeric micelle hydrogel exhibited superior wound healing efficacy to the native TQ	(Negi et al., 2020)
	Chitosan-carboxymethyl cellulose	Solvent evaporation emulsification			Nontoxic and antibacterial nano-hydrogel was obtained with acceptable drug loading, degradation and stability under physiological conditions, and high potential for wound healing.	(Bagheri et al., 2021)
Nanogels (NGs)	Myristic acid-chitosan	Ultrasonication- self assembly	Human Breast Adenocarcinoma	0, 5, 50, 100, 200 and 300 μg/mL	High drug-targeting potential and efficiency demonstrated the significant role of the anticancer properties of TQ-loaded NPs.	(Dehghani et al., 2015)

(*continued on next page*)

Table 3 (*continued*)

Nanocarrier	Biopolymer/ inorganic matrix	Method	Target application	Dose	Key findings	Refs.
Nanofibers (NFs)	Zein/PVA	Electrospinning	Food packaging	4 % (w/w)	TQ-loaded NF matrix extended shelf life of fish fillets at least for 3 days.	(Ahmadi et al., 2024)
	Gelatin/PCL		Bone healing	1 % (w/v)	TQ-loaded nanofibrous matrix possessed the osteo-conductivity and osteo-inductivity, promoting the bone healing process.	(Jeyakumar and Sivagnanam, 2024)
	Zein/PVA		Active food packaging	%2 and %4 (w/w)	TQ-incorporated NFs with electrosprayed resveratrol improved antimicrobial and antioxidant activity of packages.	(Aminzare et al., 2024)
	Zein		Drug delivery purposes	5, 10 and 15 % (w/v)	NF diameter increased with the increasing *N. sativa* extract oil amount, and the highest TQ release was obtained at 15 % oil.	(Teilaghi et al., 2020)
Cyclodextrin (CD)-based inclusion complexes	TQ/Sulfobutylether-β-CD	Lyophilization	Leukemia treatment	50–0.39 μg/mL	Cytotoxic effect of TQ was enhanced *via* the given complex promising high treatment potential.	(Eid et al., 2023b)
			Anti-cancer purpose	10 mg/mL	Effectiveness of TQ for inhibiting cancer cell was improved *via* complexation.	(Eid et al., 2023a)
	TQ/hydroxypropyl-β-CD complex	Molecular modelling with quantum mechanical calculation	Chemical, structural and reactivity analysis providing complex stability	-	Conformational change resulted from modified geometric parameters of TQ through complexation provide complex stability.	(Rayene et al., 2022)
	TQ/hydroxypropyl-β-CD complex/bacterial cellulose	Solvent evaporation	Wound healing	15 mg/mL	TQ solubility was greatly enhanced through the given complex showed good level of adhesion, thus highly promising for clinical wound healing applications.	(Swingler et al., 2022)
	TQ/Hydroxypropyl-β-CD	Ultrasonication and freeze-drying	Anti-allergenic purpose	1 mg/mL	High encapsulation efficiency (99.93 %) of TQ in the complex which showed better antiallergenic effects.	(Al-Qubaisi et al., 2022)
Inorganic NPs	Gold acetate and silver acetate	Chemical precipitation	Anti-tumor treatments in breast cancer	4.5 mg/mL	Significantly enhanced anti-tumor activity and improved therapeutic efficacy with synergistic effect of TQ	(Gomaa et al., 2025)
	Polypropylene Glycol-Functionalized Silver	Chemical reduction	Therapeutic efficiency	0.164 mg/mL	Boosted antimicrobial activity through nanodelivery of TQ promised for it's enhanced therapeutic efficiency.	(Raja et al., 2024)
	Gold	Solid state synthesis	Anti-virulent activity	20 mg	TQ NP was found as an effective inhibitor against virulent *M. tuberculosis* and shrimp white spot syndrome.	(Supriya et al., 2023)
	Zinc oxide	Chemical precipitation	Activity against breast cancer cells	-	pH-dependent TQ release and anti-proliferating effect on cells were observed.	(Banupriya et al., 2022)
	PVPylated iron oxide	Nanoprecipitation	Anti-cancer purpose	-	Rapid delivery of TQ in acidic medium indicating efficient tumoricidal action, besides, higher cytotoxicity and significant anticancer activity was achieved.	(Kumar et al., 2020)
	Silica	Chemical precipitation	Drug delivery	1.4 mg/mL	TQ in silica nanomatrix was enhanced to delivery to the different sites in brain.	(Fahmy et al., 2019)
		Probe sonication and rotary evaporation	Anti-invasion of cancer cells	-	TQ loaded in silica NPs had higher anti-invasion properties and better cytotoxicity inducing apoptotic death in cancer cells.	(Goel and Mishra, 2019)

TQ-loaded biopolymeric NPs with desirable size, shape and encapsulation efficiency commonly through solvent evaporation, ionic gelation, precipitation and sonication. TQ is accommodated within the core of NPs during their formation, through self-assembly induced by electrostatic and hydrophobic interactions (Shaarani et al., 2017; Xue and Lin, 2024). Thus, the biopolymer shell protects TQ from degradation, helps enhancing its solubility and promotes sustained release at target site by improving its anti-microbial, anti-inflammatory, anti-cancer, anti-diabetic and anti-hyperlipidemic (Abu-Dahab et al., 2013; Fakhria et al., 2019; Ilhan-Ayisigi et al., 2021; Ince et al., 2020; Mondéjar-López et al., 2022; Rani et al., 2019; Rasheeda et al., 2019; Sweety et al., 2020).

Additionally, PLGA, PEG and PCL are biodegradable and biocompatible polymers FDA-approved for using nanodelivery purposes of bioactives and drugs. TQ-incorporated PLGA NPs are reported as effective anti-cancer agents (Noor et al., 2021; Soni et al., 2015). PLGA-encapsulated TQ NPs also showed an effective brain-targeted delivery system for epilepsy treatment with high benefit of nanoscale enabling better therapeutic effect (Ahmad et al., 2020). PCL and PEG

nanoencapsulation successfully improved oral bioavailability of TQ by indicating versatility of polymeric NPs with desirable physicochemical and sustained release properties (Sunoqrot et al., 2020).

In beside of controlled delivery, nanocarriers are essentially formulated to improve stability of TQ under various conditions. Thermal stability of the TQ-loaded chitosan nanoparticles were reported as improved due to interactions between amide functional group of TQ and the amine group of chitosan (Al-Qubaisi et al., 2022). PEGylated chitosan nanoparticles suggested a good stability of TQ at room and refrigerator storage for a month (Vignesh Kumar et al., 2017). Some evidences also exhibited maintained stability of TQ-loaded nanoparticles up to three months through monitoring particle size and zeta potential (Ibrahim et al., 2020).

3.2.2. Nano-hydrogels/organogels/oleogels

Nano-hydrogels, organogels and oleogels are promising therapeutic vehicles for natural therapeutics like TQ. Self-assembly, crosslinking, chemical modification and ionizing radiation induced polymerization results in gelation of biopolymers. Relevant therapeutics can be incorporated into NG matrix during the gelation process. Hydrogen bonding, hydrophobic and electrostatic interactions mainly play role in physical crosslinking (Sasaki and Akiyoshi, 2010). Intramolecular crosslinkers like gluteraldehyde, emulsion polymerization, click chemistry and photo-induced assembly reveal chemically-crosslinked NG formation (Ashfaq et al., 2021; Mauri et al., 2021). Biopolymer NGs can provide high surface area and biocompatibility to enhance solubility of lipophilic bioactives like TQ, thus improving their biological activity (Dehghani et al., 2015). Morphology, physicochemical, structural and muco-adhesive properties of biopolymer NGs can be modified by the preparation method, content and stimulants.

Nano-hydrogels are composed of hydrophilic polymer networks swelling in water to form gel structure. TQ can be encapsulated within the hydrogel matrix keeping it dispersed in highly aqueous content. Chitosan-based micellar hydrogels formed *via* homogenization and solvent evaporation are capable of encasing TQ, giving rise to strong anticancer activity and high wound healing efficacy (Das et al., 2022; Negi et al., 2020). Besides helping wound healing *via* TQ content, *N. sativa* oil can also help to skin penetration of other healing agents loaded on chitosan nano-hydrogels (Bagheri et al., 2021). NEs-based hydrogel systems can improve therapeutic efficacy of TQ with deeper penetration helping to formation of extensive and organized cell structure in readily healing skin as well (Algahtani et al., 2021). TQ-administrated polymeric films and hydrogels are also successful in the skin target site delivery to manage bacterial infection and wound healing (Haq et al., 2020).

Free TQ has low stability at heating and light exposure, thus carrier systems are expected to improve their stability. Degradation ratio of TQ in yeast cell-based encapsulation systems have been decreased during storage at 65 °C for 8 days (Karaman, 2020). Thermal stability of TQ-loaded nanogels showed differences due to the composition of the gel matrix. Chitosan/alginate gels encasing TQ resulted in higher degradation than chitosan/psysllium husk gels containing TQ at elevated temperatures from 50 to 400 °C (Das et.al., 2022).

Organogels are composed of organic solvents or oils administrated in a three dimensional network of self-assembled gelating agents (Sahoo et al., 2011). Lipophilic therapeutics like TQ can be solubilized in the organic phase or oil phase which then mixed with the gelators to form therapeutic-loaded organogels. Lipophilic environment of organogels can provide enhanced solubility and stability to lipophilic agent (Martin et al., 2017). Polymer-based, TQ-loaded organogel/hydrogel bigels can be successfully produced through high speed homogenization and microwave heating with preserved antioxidant activity and without any degradation of TQ (Algin Yapar et al., 2020). Oleogels are structured systems consisting liquid oil phase immobilized within the gel network induced by oleogelators or solid lipids, through capillary forces, van der Waals and hydrogen interactions (Mao et al., 2020). The structural features and stability of oleogels are strongly related with the type and content of the oleogelators, oil composition and processing conditions (Yang et al., 2022). Oleogels are introduced as successful delivery systems capable of increasing bioaccessibility and delayed release of poorly water-soluble ingredients and therapeutics (Chloe et al., 2017; Macoon et al., 2020). Besides, hydrogel/oleogel biphasic systems are promising for loading and delivery of both hydrophilic and lipophilic bioactives simultaneously (Zheng et al., 2020). Thus, these gel systems can serve good opportunities for TQ encasing and dispatching. TQ can be dispersed in the oil phase of oleogel matrix which can act as an excellent nest and provide sustained release of TQ over time. In fact, more research works are needed to certify the competencies of these NGs for TQ delivery.

3.2.3. Nanofibers and nanotubes

NFs are elongated structures in nanoscale diameters, having active surfaces to accommodate bioactives. Both natural and synthetic polymers including cellulose, zein, gelatin, PEO, PLGA, PEG, PVA are used in different formulations to fabricate NFs mainly through electrospinning and the resultant NF mats can be employed for shipping of bioactives and therapeutics (Jain et al., 2020). Recent findings demonstrated that biopolymer NF mats are appropriate matrices for TQ which can be incorporated during electrospinning process (Ahmadi et al., 2024). High surface area-to volume ratio offered by the NF matrix enhances TQ loading efficiency and subsequently controlling release profile. NF mats or patches incorporated with TQ offer successful use for localized delivery to the specific tissues and organs for healing and regenerative medical applications (Almukainzi et al., 2022; Jeyakumar and Sivagnanam, 2024). Besides, in comparison to its direct use, therapeutic effect of TQ is improved when incorporated in NFs (Zidan et al., 2018). TQ incorporated in zein/PVA NFs maintained its stability within the NF mat under thermal exposure while revealing enhanced bioactivity (Aminzare et al., 2024).

NTs are hollow cylindrical structures with their nanoscale diameters and/or wall thicknesses. Organic polymers and inorganics are used to fabricate NTs through template synthesis and self-assembly for delivery of bioactives and drugs (Manoukian et al., 2021; Mashatooki and Ghalami-Choobar, 2021; Tarhan and Harsa, 2014). Among those, protein NTs have a great potential in delivery of bioactives to be encased in the cavity of the NTs or adsorbed onto their surfaces (Liu et al., 2024). High aspect ratio, desirable mechanical properties and tunable surface chemistry of biopolymer NTs provide advanced protection and target controlled release of quinone-based drugs with enhanced therapeutic potential (Ilbasmis-Tamer et al., 2016). In addition, boron-nitrite NTs incorporated with a quinone-based drug successfully delivered for anticancer purpose (Mashatooki and Ghalami-Choobar, 2021). Although they have toxicity potential, single and multi-walled carbon NTs (SWCNTs and MWCNTs) are used to develop efficient nanocarriers to deliver drugs and quinones for cancer therapy (Dineshkumar et al., 2015; Grushevskaya and Krylova, 2018). Functionalization of CNTs with biopolymeric agents improves their efficiency. For instance, gelatin-functionalized CNTs can be used in cancer therapy (Li et al., 2023). Hyaluronic acid-conjugated CNTs have also facilitated release of quinone-based anticancer drugs (Datir et al., 2012). NF and NT envelopes show promise in providing physical and chemical stability to TQ. However, further research is needed to evaluate the stability of TQ in these nanocarrier systems under various storage conditions.

3.2.4. Protein-polysaccharide nanocomplexes/conjugates

Proteins and carbohydrates are versatile biopolymers occupied in industry individually or in combination due to their functional properties (Shaddel et al., 2024). Merged formulations enable benefiting from the advantages of each biopolymer. Nanocomplexes are formed through self-assembly of proteins and polysaccharides due to hydrophobic interactions, electrostatic attractions and hydrogen bonding. Certain chemical modifications of proteins and polysaccharides give rise to

formation of reactive functional groups which then conjugate, thus, protein-polysaccharide conjugates are obtained. Both formulations can be considered as carrier matrices for plant-based bioactives including EOs, vitamins and phytochemicals like terpenes (Paliya et al., 2023; Zhang et al., 2021). These nanocarriers can be desirable matrices for the terpene, TQ by providing improved solubility, stability and targeted delivery. For instance, chitosan and hydroxymethyl cellulose (HMC) are capable of forming nanoconjugates to deliver TQ for colon cancer treatment (Sweety et al., 2020). Additionally, surface modifications of protein-polysaccharide nanocomplexes/conjugates with certain ligands can facilitate TQ delivery specifically to the diseased tissues or cells (Pal et al., 2021). This approach helps to improve target therapeutic activity of TQ. New evidences are expected to bring deep insight in protein-polysaccharide nanocarriers for TQ delivery and therapeutic efficacy.

3.2.5. *Cyclodextrin-based inclusion complexes*

CDs are cyclic oligosaccharides capable of forming inclusion complexes with different molecules under certain conditions. Hydrophobic cavity of CDs can successfully host hydrophobic compounds such as TQ by providing high stability, solubility and bioavailability (Del Valle, 2004). Inclusion complexes of TQ with CD can be formed through hydrophobic interactions, van der Waals and hydrogen bonding, enhancing its delivery and therapeutic efficiency (Eid et al., 2023b; Rayene et al., 2022). As evident with the recent findings, especially TQ/β-CD inclusion complexes successfully improved anticancer effect of TQ *via* enhancing its solubility and cellular uptake, thus considered as highly promising nanocarriers of TQ-based drugs (Eid et al., 2023a). In beside of anti-proliferative activity of TQ/β-CD complexes, potential side effects like little cytotoxicity to normal tissue cells should be fully understood prior to further use (Abu-Dahab et al., 2013; Eid et al., 2023a).

3.3. *Inorganic nanocarriers*

Inorganic NPs derived from silicon dioxide, metals like gold, silver and iron, and metal oxides such as zinc oxide are promising carriers for bioactives including TQ (Goel and Mishra, 2019; Kumar et al., 2020; Raja et al., 2024; Supriya et al., 2023). Multifunctional use potential, high stability and tunable properties are some important advantages of inorganic nanocarriers for drug delivery. Mesoporous silica NPs have gained interest in recent years for delivery of bioactives and drugs due to their highly porous structure, adjustable size and shape, desirable mechanical stability and high loading efficiency. Mesopores of silica NPs can accommodate TQ through physical adsorption and conjugation which retards cell invasion and encourage cytotoxicity due to reactive oxygen species (ROS)-mediated apoptosis (Goel and Mishra, 2019). Surface modification of mesoporous silica can enhance loading efficiency and controlled release (Guo et al., 2015). Solid-state synthesis, nanoprecipitation, ultrasonication and solvent evaporation are common methods to produce TQ-loaded inorganic NPs. Antimicrobial, anticancer and other therapeutic activities of TQ are significantly improved through NP formulations (Fahmy et al., 2019; Kumar et al., 2020; Raja et al., 2024). Incorporation of TQ in iron oxide NPs exhibited that TQ enhanced stability of the NPs without changing magnetic features of iron (Fathy, 2020). In addition, encasing TQ in iron oxide NPs improved cellular uptake and bioavailability of TQ, suggesting increased effectiveness in cancer therapy. Gold NPs are biocompatible and can show plasmonic properties which can aid photothermal therapy. TQ-adsorbed gold and silver NPs exhibited targeted delivery and better cytotoxicity for cancer cells than TQ alone and thus promising for combined chemo-photothermal therapy of cancer (Gomaa et al., 2025).

Dendrimers with numerous binding sites branched from central core have the unique 3D structure offering advantages for nutraceutical and drug delivery (Chauhan et al., 2004). Central core is composed of organic polymers or inorganic molecules while branches have multiple functional groups able to bind plant-derived bioactives (Zhang et al., 2024). Besides, surface groups can be modified with some chemicals or ligands to direct the physicochemical properties and interactions with bioactives and drugs. The quinone-based drug-loaded polymeric micelles consisting dendrimers give rise to enhanced anti-tumor activity (Zhang et al., 2023). High loading capacity, adjustable surface chemistry, increased stability, sustained release and target delivery are major advantages of the polymeric dendrimers to be purposed for TQ delivery (Mistry, 2016).

Metal organic frameworks (MOFs) are porous structures serving good opportunities for loading and delivery of bioactives for treatments through their high surface area, cavities, tunable shape and stability. A recent research work revealed that *N. sativa* oil (containing TQ) loaded copper-benzene tricarboxylic acid MOF indicated the best curative effect on chronic murine toxoplasmosis, as demonstrated by *in vivo* studies (Mohammad et al., 2023). More future works on MOFs as nanocarriers of TQ are expected to reveal supportive findings.

As with all nanodelivery systems, scaling up the production of TQ-loaded nanocarriers from laboratory to industrial scale presents significant challenges, including technological, economic, and regulatory barriers. The large-scale manufacturing of these carriers for food and biomedical applications demands an integrated approach that considers high raw material costs (*e.g.*, biopolymers, surfactants), complex production setups, and optimization of yields. Additional operational burdens may arise due to the need for specialized equipment, waste management, and the variability introduced at intermediate or pilot scales. Moreover, commercialization efforts may be hindered by delayed time-to-market and the need to establish reliable supply chains (Paliwal et al., 2014).

In particular, inorganic nanocarriers, despite their promising functionality for TQ delivery, raise specific concerns. Their potential cytotoxicity, long-term biocompatibility, and tissue accumulation necessitate thorough safety evaluations, especially for chronic or repeated use (Nel et al., 2006). Ensuring batch-to-batch consistency and achieving precise surface functionalization for targeted delivery can further complicate large-scale production (Muthu and Wilson, 2012). Environmental impacts and regulatory scrutiny regarding metal-containing nanomaterials must also be addressed (Kumah et al., 2023). Therefore, although inorganic nanocarriers offer significant advantages, overcoming these manufacturing, safety, and regulatory hurdles is critical to their successful application in real-world settings (El-Kalliny et al., 2023).

4. Bioavailability, release, and safety of nanoencapsulated thymoquinone

4.1. *In vivo bioavailability*

Research indicates that nanoencapsulation markedly enhances the bioavailability of TQ (TQ). For example, a study conducted on rats by Zakarial Ansar et al. (2020) examined the bioavailability of TQ-loaded NLCs (TQ-NLC) administered both orally and intravenously. In this study, the TQ-NLC was radiolabeled with technetium-99m, facilitating the evaluation of biodistribution and pharmacokinetic parameters at various time points. Similarly, Rahat et al. (2021a) found that chitosan-modified NPs entrapping TQ improved its oral bioavailability. These NPs demonstrated a burst release within the first 2 h, followed by a sustained release for up to 24 h in simulated intestinal fluids. Techniques such as the use of NLCs and chitosan-modified NPs have shown significant promise in enhancing TQ's bioavailability, thereby boosting its therapeutic potential. Nonetheless, while nanoencapsulation offers a promising method to increase the bioavailability of bioactives like TQ, further research is essential to address the associated challenges and fully leverage its potential in various applications (Rahat et al., 2021a).

Nihei et al. (2016) explored a nano-formulation of TQ using a cold wet-milling (CWM) system to enhance its dissolution behavior and

pharmacokinetic properties. This formulation, termed TQ/CWM, resulted in nano-sized TQ particles (143 nm). To assess the biopharmaceutical properties of this formulation, a pharmacokinetic study was conducted comparing crystalline TQ (10 mg/kg) and TQ/CWM (2 mg/kg) in rats. Plasma concentrations of TQ were measured at different intervals following oral administration using HPLC analysis (Fig. 7). Key pharmacokinetic parameters, including maximum concentration (C_{max}), time to reach maximum concentration (T_{max}), area under the curve from 0 to 24 h (AUC0–24), and absolute bioavailability, are detailed in Table 4. The absolute bioavailability of crystalline TQ was determined to be 9.6 %, based on the AUC0–24 value (4.1 h·mg/mL) for intravenously administered TQ (1 mg/kg). In contrast, TQ/CWM exhibited improved pharmacokinetic behavior, reducing T_{max} by 1.4 h and increasing oral bioavailability six-fold, thanks to the nano-pulverization achieved with CWM technology. Rapid systemic exposure to TQ was observed in the TQ/CWM group (Nihei et al., 2016).

Various nanocarrier systems have been explored to enhance the bioavailability of TQ, each offering distinct advantages. For instance, nanostructured lipid carriers (NLCs) provide a lipid matrix that improves solubility and protects TQ from degradation, while chitosan-modified nanoparticles enhance mucoadhesion and facilitate paracellular transport across the intestinal epithelium. Cold wet-milled (CWM) formulations reduce particle size significantly, increasing the surface area and dissolution rate, which leads to faster and more efficient absorption. These systems operate through mechanisms such as improved aqueous solubility, protection from enzymatic and pH-induced degradation, and enhanced permeability and retention (EPR) effects. A comparative evaluation suggests that while NLCs and chitosan NPs are more suited for sustained oral delivery, CWM formulations may be preferable for rapid systemic exposure.

Fig. 8 presents a decision tree designed to guide the selection of appropriate nanocarrier systems for TQ delivery based on specific application needs. Starting with the application type, oral or transdermal, the flowchart incorporates critical factors such as desired release profile (immediate or sustained), stability requirements (high or low), and target tissue (gut or skin). Depending on the pathway, the tree leads to recommended delivery systems, including nanostructured lipid carriers (NLCs), chitosan nanoparticles (NPs), cellulose-based wound matrices (CWM), and transdermal patches. This structured approach facilitates rational design and optimization of TQ formulations for targeted therapeutic outcomes.

4.2. Release of nanoencapsulated thymoquinone

The release profile of TQ from nanocarriers is vital for its therapeutic effectiveness. Dahmash et al. (2024) demonstrated that TQ-loaded

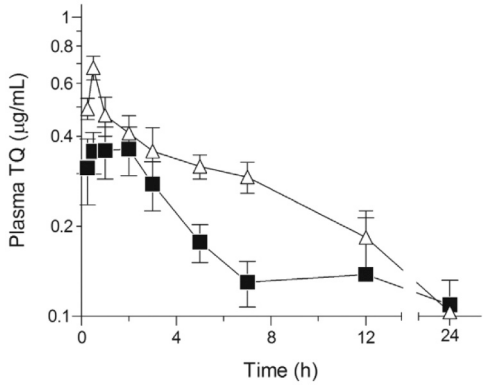


Fig. 7. Plasma concentration-time profiles of TQ after oral administration of TQ samples in rats. Square: Crystalline TQ (10 mg/kg); Triangle: TQ/CWM (2 mg-TQ/kg). Data represent mean ± S.E. of 5–6 experiments (Nihei et al., 2016).

Table 4
An example of pharmacokinetic parameters of orally-dosed TQ samples (Nihei et al., 2016).

	C_{max}	T_{max}	AUC_{0-24}	BA
TQ (10 mg/kg)	0.40 ± 0.066	1.9 ± 0.51	3.9 ± 0.70	9.6
TQ/CWM (2 mg-TQ/kg)	0.65 ± 0.041	0.54 ± 0.092	4.8 ± 0.74	59

C_{max}: maximum concentration; T_{max}: time to maximum concentration; AUC0–24: area under the curve of blood concentration *vs.* time from t = 0 to t = 24; and BA: oral bioavailability. Data represent mean±S.E. of 5–6 experiments.

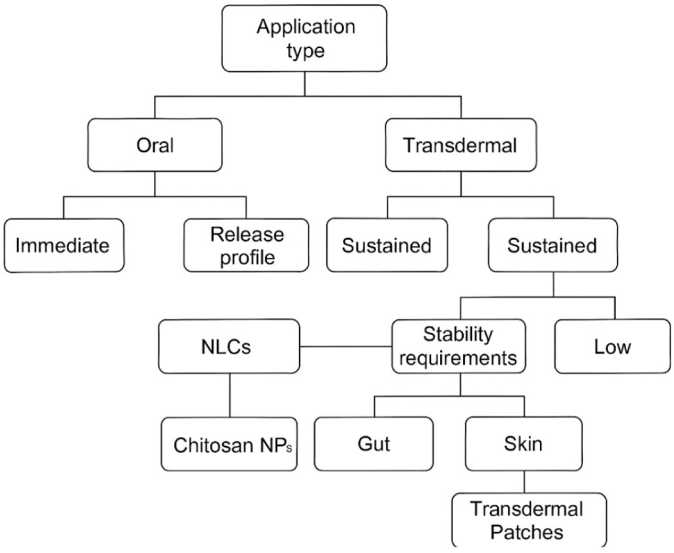


Fig. 8. Decision tree for selecting appropriate nanocarrier systems for thymoquinone based on application type, release profile, stability requirements, and target tissue.

L-arginine-based polyamide nanocarriers incorporated into transdermal patches provided an extended-release of TQ over 24 h. Likewise, in another study (El-Far et al., 2018) it was reported that TQ and its nanoformulations exhibited a sustained release profile compared to their free forms. Furthermore, Hemananthan (2019) investigated the *in vitro* digestion of encapsulated TQ, showing that the micellization rate of TQ from the developed nanocarriers was significantly higher than that of mixed micelles. Using the everted gut sac method, the study confirmed a higher absorption rate of the nanoencapsulated product compared to mixed micelles, indicating enhanced bioavailability of TQ through these nanocarriers (Hemananthan, 2019). Hemananthan (2019) also found that nanocarriers were toxic to MCF-7 cell lines but compatible with HEK 293 cell lines, suggesting target specificity. Based on these results, further *in vivo* testing and potential pharmaceutical development of the carrier are recommended.

4.3. Safety aspects of nanoencapsulated thymoquinone

Safety is a critical consideration for the clinical application of nanoencapsulated compounds (Akbari-Alavijeh et al., 2025). Evaluating the dosage level and toxicity of a new drug is a crucial step before human testing, especially when bioactives are intended for food delivery. Various *in vitro* and *in vivo* studies have been conducted on TQ to assess its toxicological properties and appropriate dosages. TQ is highly unstable under conditions of temperature, light, and low pH, necessitating the use of nanocarriers for its delivery (Navadeepthy and Ponpandian, 2022). Studies have indicated that TQ NPs, with their anti-inflammatory, antioxidant, anti-asthma, and antitumor activities, can be safely used to treat lung disorders (Al-Gabri et al., 2021). Given

TQ's water insolubility, its lethal dose (LD_{50}) varies depending on the administration route and carrier used. For instance, when TQ was orally administered to mice, LD_{50} was 870.9 mg/kg, whereas for intraperitoneal (i.p.) injection, it was 104.7 mg/kg. In rats, LD_{50} was 794.3 mg/kg for oral administration and 57.5 mg/kg for i.p. injection (Tubesha et al., 2013b). Post-oral administration side effects, such as respiratory difficulties and peritonitis, were observed in both rats and mice, and long-term administration of TQ could lead to liver toxicity. However, tolerable dosages of TQ did not adversely affect human organs (AbuKhader, 2013), suggesting that careful dosage management could mitigate potential risks.

Stability and shelf-life are critical factors, particularly for food applications where long-term storage under variable conditions is common. Nanoencapsulation can significantly improve the stability of TQ by protecting it from light, heat, and acidic environments (Hemananthan, 2019). For example, lipid-based carriers and polymeric nanoparticles have demonstrated the ability to maintain TQ integrity over extended periods under refrigerated and ambient conditions. However, food-grade formulations must also meet additional criteria such as non-toxicity of excipients, regulatory approval, and compatibility with food matrices. Compared to pharmaceutical applications, food systems often require more robust encapsulation to withstand processing and storage stresses. Therefore, further research is needed to optimize nanoformulations for food applications, including accelerated stability testing and real-time shelf-life studies (Al-Gabri et al., 2021).

4.4. *In vitro and cytotoxicity results*

In vitro studies have shown that TQ and its nanoformulations have equal or enhanced anticancer activity compared to TQ in various cancer cells, with no significant cytotoxicity from the blank NPs (Fakhoury et al., 2016; Kalamegam et al., 2020). TQ NPs were effectively taken up by cells in a time-dependent manner for up to 24 h (Kalamegam et al., 2020). Cytotoxicity studies revealed a significant concentration-dependent decrease in cell viability with higher doses of TQ NPs (Fakhoury et al., 2016; Kalamegam et al., 2020). However, the stability issues of the active ingredient highlighted in these studies need further investigation (Ibrahim et al., 2020). Generally, rapid oral absorption may not be desirable for safety reasons, especially for compounds with a narrow safety margin. However, in the study by Nihei et al. (2016), orally administered TQ did not show toxicity at doses of at least 2600 mg/day in a phase I clinical trial, suggesting a low toxicity risk with rapid systemic exposure. These findings support that CWM technology offers a promising approach to enhancing the biopharmaceutical properties of TQ. In summary, nanoencapsulation of TQ improves its bioavailability, controls its release, and ensures its safety, thereby enhancing its therapeutic efficacy. Nonetheless, more research is necessary to fully realize the potential of nanoencapsulated TQ in clinical applications.

5. Potential applications of thymoquinone-loaded nanocarriers

5.1. *Cancer treatment*

One of the most promising applications of TQ-loaded nanocarriers is in cancer treatment. TQ has demonstrated potent anticancer effects against various cancer cell lines, including breast and ovarian adenocarcinoma, lung carcinoma, human pancreatic adenocarcinoma, and CRC (Selmi et al., 2023). Nanoformulations of TQ have shown enhanced anticancer efficacy by maximizing drug internalization and achieving target specificity (Pal et al., 2021). TQ induces apoptosis, causes cell cycle arrest, inhibits angiogenesis and metastasis, generates ROS, and stimulates immune system responses (Homayoonfal et al., 2022). These properties position TQ as a strong candidate for anticancer therapy. Additionally, TQ has been shown to enhance the survival and activity of antigen-specific CD8-positive T cells, which are essential for adoptive T-cell therapy against cancer (Khan et al., 2017). It can also upregulate apoptotic pathways, cause DNA double-strand breaks, and potentiate the effects of certain chemotherapeutic drugs (El-Far et al., 2020). The cellular and molecular activities of TQ, including its regulatory effects on various processes (Fig. 9), underscore its potential as an anticancer agent (Fakhoury et al., 2016). However, further research is necessary to fully understand the mechanisms of TQ, both in its free form and encapsulated, and to translate these findings from preclinical studies to clinical trials (Farooqi et al., 2022). However, translation from preclinical to clinical application remains limited due to variability in nanocarrier stability, immune response, and regulatory challenges, which are common obstacles in the development of phytochemical-based therapies (Aljabali et al., 2025; Sa et al., 2023).

5.2. *Treatment of rheumatoid arthritis*

RA is characterized by immunological dysregulation with multifactorial pathogenesis, including genetic and environmental factors. Both RA and tumor tissues share common features, such as enhanced permeability and retention (EPR) and hypoxia. Consequently, the nanocarriers used for tumors could also apply to RA. The altered and fenestrated synovial membrane in RA, akin to tumor EPR, presents a potential target for nanocarriers (Xiao et al., 2019). The leaky vasculature of the synovial membrane facilitates the penetration and retention of NPs (Pal et al., 2021). Nanocarriers containing bioactives like TQ can specifically target inflammatory cells, downregulate pro-inflammatory pathways, and potentially alleviate RA symptoms and subsequent bone damage. For instance, the increased presence of macrophages at arthritic inflammatory sites can facilitate passive targeting of NPs (Pal et al., 2021; Pham, 2011).

TQ nanoformulations have shown promise in enhancing the anti-inflammatory efficacy of TQ (Pal et al., 2021). Moreover, NPs can reduce dosage requirements and off-target toxicities, improving the therapeutic potential for RA treatment (Pal et al., 2021). A phospholipidic nanomatrix composed of a lipidic core and surfactant mixture enhances TQ's aqueous solubility and intestinal absorption compared to TQ suspension. Lipidic NPs are directly absorbed by intestinal lymph and deliver drugs into the bloodstream, bypassing first-pass metabolism. This results in enhanced anti-inflammatory effects of TQ, as seen in the carrageenan-induced paw edema rat model (Rathore et al., 2019). Furthermore, alternative nanocarrier systems such as nanoemulsions and lipid-polymer hybrids may offer improved loading efficiency and controlled release profiles for TQ in RA treatment. Future research avenues hold significant promises for enhancing TQ's therapeutic and functional benefits through synergistic combinations with other bioactive compounds (Moghaddam et al., 2022). For instance, in the context of rheumatoid arthritis, exploring co-formulations of TQ with other anti-inflammatory compounds such as gallic acid or ferulic acid could lead to enhanced efficacy. Similarly, for metabolic disorders like diabetes, developing nanoformulations that co-deliver TQ with other known antihyperglycemic phytochemicals has the potential to yield novel synergistic therapeutic strategies (Alam et al., 2025). In food applications, investigating the integration of TQ nanocarriers with other functional ingredients is recommended to deliver synergistic health benefits in functional foods. Such co-loaded nanocarrier systems, potentially incorporating compounds like curcumin or quercetin (as you mentioned), could maximize therapeutic outcomes or functional properties beyond what individual compounds or TQ alone can achieve (Wang and Luo, 2021).

5.3. *Neurological diseases*

TQ and its nanoformulations have shown potential as therapeutic agents for neurological diseases due to their anti-inflammatory, antioxidant, and neuroprotective properties. A comprehensive recent review conducted by Saadat et al. (2023) highlighted these attributes,

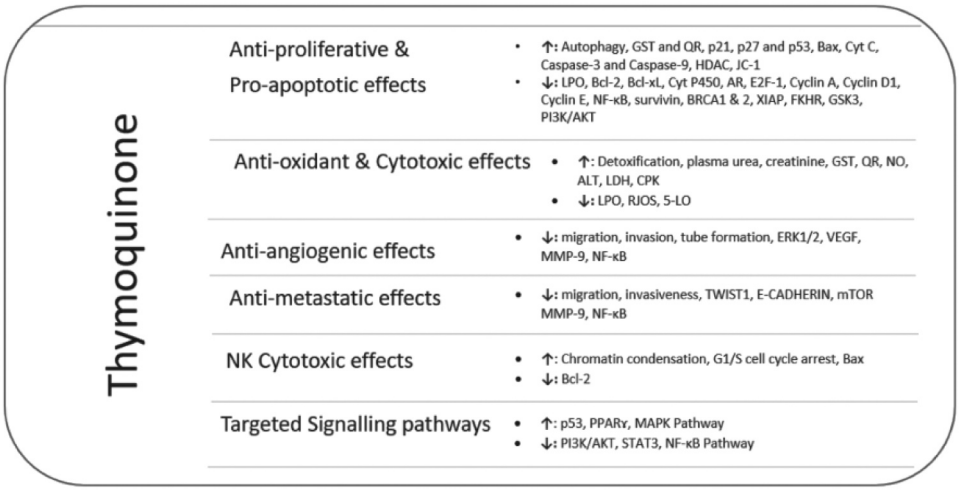


Fig. 9. A concise overview of the cellular and molecular activity in TQ, where, ↑ signifies upregulation and ↓ indicates downregulation. Withdrawn from Fakhoury et al. (2016).

concluding that TQ's antioxidant and anti-inflammatory properties enhance neuron survival in affected brain regions, improving behavioral and motor functions. Additionally, TQ and its nanoformulations can restore antioxidant enzyme levels and reduce oxidative stress. The primary mechanism for TQ's antioxidant effects is believed to involve the regulation of the nuclear factor erythroid 2–related factor 2/heme oxygenase-1 (Nrf2/HO-1) signaling pathway. Furthermore, TQ modulates key components of inflammatory signaling pathways, including toll-like receptors (TLRs), nuclear factor-κB (NF-κB), interleukin 6 (IL-6), IL-1β, and tumor necrosis factor-alpha (TNFα), exerting significant anti-inflammatory effects (Saadat et al., 2023).

5.4. Hypertension, diabetes, allergies, and immunogenic disorders

TQ has been incorporated into various nanocarriers to explore its pharmacological activities against numerous human illnesses, including hypertension, diabetes, allergies, eczema, and immunogenic disorders (Khan et al., 2021). Particularly, the antidiabetic potential of TQ-loaded nanocarriers has garnered significant interest. In a study by Rani et al. (2018), the antidiabetic potential of TQ-loaded nanocarriers was investigated in rats with streptozotocin-nicotinamide-induced type-2 diabetes, comparing it to metformin, a standard diabetes treatment. Both TQ- and metformin-loaded nanocarriers exhibited sustained release, significantly reducing blood glucose and glycated haemoglobin levels in a dose-dependent manner and improving the serum lipid profile (Rani et al., 2018). This study suggested that nanocarriers significantly enhances TQ's antidiabetic activity. Nonetheless, understanding the interaction of TQ nanocarriers with other antidiabetic agents could reveal synergistic effects that warrant further study (Tachour et al., 2024). Developing nanoformulations that co-deliver TQ with other known antihyperglycemic phytochemicals could yield new synergistic therapeutic strategies. TQ NPs also show potential in treating other conditions, such as dermal disorders, anxiety, stress-related disorders, and female urinary tract infections (Pal et al., 2021). However, further research is necessary to understand the molecular mechanisms underlying these anti-diabetic properties. Investigating how TQ-loaded nanocarriers affect pancreatic cell regeneration, insulin secretion, and sensitivity could offer new therapeutic insights for diabetes management. This underscores the potential of TQ-loaded nanocarriers as a promising avenue for diabetes treatment (Khan et al., 2021).

5.5. Food applications of thymoquinone-loaded nanocarriers

Beyond the extensively studied applications in cancer treatment, rheumatoid arthritis, neurological diseases, and metabolic disorders, TQ nanocarriers hold potential for several emerging or underexplored applications. These include areas such as wound healing, where TQ's anti-inflammatory and antioxidant properties could promote tissue repair; photoprotection in cosmetics, leveraging TQ's ability to reduce oxidative stress and potentially protect against UV-induced damage; and various veterinary uses, where TQ could offer anti-inflammatory or antimicrobial benefits for animal health.

While specific studies on these particular applications were not detailed in the provided sources, the broad therapeutic potential of TQ and the enhanced delivery capabilities of nanocarriers provide a strong rationale for future investigations in these novel areas. Nanocarriers have the potential to enhance the stability, targeting ability, bioavailability, and therapeutic efficacy of phytochemicals, which is a primary focus in Food Science (Singh and Kumar, 2023). TQ, as a potent phytochemical with several health-promoting properties, has been incorporated into various nanocarriers to evaluate its pharmacological activities against numerous human illnesses (Khan et al., 2021). However, its application in the food industry has been limited due to issues such as fast metabolism, poor absorption and stability, hydrophobic nature, and lower target specificity. Although research on encapsulated TQ in food products is still emerging, specific food categories such as functional beverages, sauces, baked goods, and dairy products are promising candidates for TQ inclusion due to their compatibility with nanoemulsions and microcapsules (Nejatian et al., 2022; Rashidinejad et al., 2016). *N. sativa* oil (NSO), rich in TQ, is widely used in food products around the world. *N. sativa* EOs (NSEO), obtained through distillation, offers even more functional benefits and is seen as a promising source of food preservatives and antioxidants (Liao et al., 2021). Despite the potential challenges like lipid oxidation, low water solubility, and volatility, advances in nanocarriers provide significant opportunities to develop innovative carriers for NSEO delivery and controlled release.

Nonetheless, consumer acceptance may also be influenced by public perceptions of nanotechnology in food, highlighting the need for transparent communication strategies. This emphasizes that beyond scientific safety, public trust and understanding are vital for the successful commercialization and adoption of these products (Gupta et al., 2015; Meijer et al., 2021). Transparent communication is identified as a key strategy to address potential scepticisms or concerns. Furthermore, regulatory concerns necessitate strict adherence to national and international food safety standards and labeling requirements for nanotechnology in food. Key aspects to emphasize include the biocompatibility and potential for immune responses to the nanocarrier materials

themselves, the GRAS status of the carrier components, and careful dose considerations to ensure that the concentration of TQ remains within safe consumption limits for specific food matrices. Transparent communication strategies are also vital to address consumer perceptions of nanotechnology in food and foster acceptance (Butt and Sultan, 2013; Coles and Frewer, 2013; Ghosh and Kumar, 2024).

A recent study highlighted the potential of nanocarriers to enhance the stability and bioavailability of TQ, making it more suitable for food applications (Singh and Kumar, 2023). Researchers explored the microencapsulation of oil from *N. sativa* seeds, which contain high concentrations of TQ, using a spray-drying technique with modified starch (MS) and maltodextrin (MD) as wall materials. The aim was to produce a functional yogurt rich in TQ. The results showed that microcapsules stored at a controlled temperature (4°C) retained the highest TQ content after four weeks. Furthermore, chemical and sensory analyses indicated that these microcapsules could be used to produce functional yogurt, owing to their high stability and satisfactory chemical and sensory properties (Liao et al., 2021; Singh and Kumar, 2023).

Challenges such as stability under food processing conditions, oxidative degradation, and cost-effectiveness remain significant barriers to commercialization (Rashidinejad et al., 2014; Salehi and Rashidinejad, 2024). Emerging encapsulation technologies such as multilayered emulsions or protein-based delivery systems are being investigated to address these limitations. The economic feasibility of scaling up TQ nanocarrier production for commercial applications is a critical consideration. Advances in established encapsulation techniques, such as spray-drying and nanoemulsion preparation, which are already utilized for other bioactives, suggest that the scale-up of nanocarriers for compounds like TQ appears feasible. However, despite this apparent feasibility, challenges related to overall cost-effectiveness remain significant barriers to broad commercialization. A comprehensive economic assessment would need to account for various cost factors, including the procurement and processing of raw materials, the energy and infrastructure required for large-scale encapsulation technologies, and the costs associated with storage, stability maintenance, and distribution (Petrovic and Barbinta-Patrascu, 2023). Future research should investigate the integration of TQ nanocarriers with other functional ingredients to deliver synergistic health benefits in functional foods.

6. Conclusions and future outlook

The exploration of nanocarriers in enhancing TQ delivery has unveiled promising strategies to maximize its therapeutic benefits. Nanocarriers, including lipid-based formulations, biopolymeric structures, and inorganic nanocarriers, have shown significant potential in improving TQ's release profiles, stability under various conditions, and targeted delivery to specific tissues or cells. This review has identified lipid-based nanocarriers (such as nano-liposomes and solid lipid nanoparticles) as particularly effective in enhancing bioavailability, while biopolymeric nanocarriers (such as chitosan-based nanoparticles and cyclodextrin complexes) offer advantages in controlled release and biocompatibility. Inorganic nanocarriers, though less extensively studied, present unique opportunities for multifunctional delivery platforms. Despite these advances, challenges remain in scaling up production from laboratory to industrial scale. Issues such as reproducibility, cost-efficiency, and quality control need to be addressed to facilitate the commercial viability of TQ nano-delivery systems. The development of scalable manufacturing processes that maintain the physicochemical and biological properties of nanocarriers is essential for their translation into real-world applications. The transition from experimental research to practical application further necessitates rigorous clinical trials to establish safety, efficacy, and optimal dosing regimens, particularly in healthcare settings. From a regulatory perspective, although TQ has GRAS status for certain food and supplement uses, the incorporation of nanocarriers introduces additional complexity. Ensuring the safety, stability, and bioavailability of TQ-loaded nanocarriers in food systems necessitates alignment with evolving guidelines by agencies such as the FDA and EFSA. Moreover, emerging approaches like stimuli-responsive nanocarriers, which release TQ in response to pH or enzyme levels, represent innovative strategies with great promise for future development, potentially enhancing regulatory acceptance by offering controlled, targeted release profiles. Economically and societally, successful implementation of TQ nano-delivery systems could transform therapeutic strategies for chronic diseases, particularly cancer, and offer innovative solutions in food preservation by extending shelf life and maintaining nutritional quality. This could lead to reduced healthcare costs, improved patient outcomes, and increased consumer acceptance of functional foods enriched with bioactive compounds like TQ.

The future of TQ nanodelivery is optimistic, with interdisciplinary research and development continuing to drive novel formulations and applications. This review underscores the necessity of integrative approaches that bridge food science, pharmacology, and nanotechnology to fully harness the multifunctional potential of TQ nanocarriers.

List of abbreviations

CHOL	Cholesterol
CDs	Cyclodextrins
CNC/SPI	Cellulose nanocrystals-soy protein isolate
CRC	Colorectal cancer
CWM	Cold wet-milling
DCP	Dicetylphosphate
DPPC	Dipalmitoylphosphatidylcholine
EPR	Enhanced permeability and retention
FDA	Food and Drug Administration
FFAs	Free fatty acids
GBM	Glioblastoma multiforme
GIT	Gastrointestinal tract
HMC	Hydroxy methyl cellulose
HPEA	High pressure extraction apparatus
HPH	High-pressure homogenization
IL-6	Interleukin 6
IL-1β	Interleukin 1β
i.p.	Intraperitoneal
I/R	Ischemia-reperfusion
LC_{50}	Lethal dose
MCAO	Middle cerebral artery occlusion
MD	Maltodextrin
MGs	Monoglycerides
MS	Modified starch
MWCNTs	Multiple-welled carbon nanotubes
NEs	Nanoemulsions
NF-κB	Nuclear factor-κB
NFs	Nanofibers
NGs	Nanogels
NLCs	Nano-structured lipid carriers
NPs	Biopolymer-based nanoparticles
NSEO	*N. sativa* essential oils
NSO	*N. sativa* oil
NTs	Nanotubes
PCL	Polycaprolactone
PEG	Polyethylene glycol
PEs	Pickering emulsions
PLGA	Poly(lactic-co-glycolic acid)
PS	Polysorbate
PVA	Polyvinyl alcohol
QbD	Quality by design
RA	Rheumatoid arthritis
ROS	Reactive oxygen species
SFE	Supercritical fluid extraction
SLNs	Solid lipid nanoparticles
SNEDDS	Self-nano-emulsifying drug delivery system
SWCNTs	Single-welled carbon nanotubes
TNFα	Tumor necrosis factor-alpha
TLRs	Toll-like receptors
TQ	Thymoquinone
TQN	Thymoquinone-loaded niosome

CRediT authorship contribution statement

Rezvan Shaddel: Data curation, Investigation, Methodology, Writing – original draft. **Ali Rashidinejad:** Data curation, Investigation, Methodology, Writing – original draft. **Mohammad Mahdi Karimkhani:** Data curation, Investigation, Methodology, Writing – original draft. **Ozgur Tarhan:** Data curation, Investigation, Methodology, Writing – original draft. **Seid Mahdi Jafari:** Writing – review & editing, Validation, Supervision, Project administration, Formal analysis, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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