JOURNAL OF NATURAL REMEDIES
DOI: 10.18311/jnr/2025/49337
REVIEW ARTICLE

Current Research and Developments on Thymoquinone Nanoparticles and its Health Benefits Focusing Rheumatoid Arthritis

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Abstract

Thymoquinone (TQ) is widely recognised as safe for use in both cosmetic and therapeutic applications within the pharmaceutical industry. For its various purposes, including scientific research, sourcing pure TQ from plants is essential. There is a growing need for advanced extraction and purification methods that align with green chemistry principles, enhancing both efficiency and safety while minimising environmental impact. A number of important studies have focused on optimising the processes of extracting and purifying TQ." We have thoroughly covered the currently available TQ extraction, synthesis, and transformation techniques in this review. Moreover, TQ's numerous pharmacological properties against various ailments have been thoroughly investigated. Additionally, Thymoquinone (TQ) has faced challenges in pharmaceutical applications due to its low solubility and limited absorption in the human body. However, advancements in nanotechnology-based TQ formulations are offering promising solutions to improve their bioavailability and absorption efficiency. This review explores the different nanoformulations of TQ, particularly in the context of treating inflammatory conditions such as rheumatoid arthritis. It also outlines key areas of future research that could position TQ as a valuable therapeutic agent.

Major Findings: This review comprehensively summarises current techniques for the extraction, synthesis, transformation, and nanoformulation of Thymoquinone (TQ), highlighting its diverse pharmacological potential despite challenges related to its low solubility and limited absorption. Advances in nanotechnology-based delivery systems show promising improvements in TQ's bioavailability, positioning it as a strong candidate for future therapeutic applications, particularly in inflammatory disorders such as rheumatoid arthritis.

Keywords: Anti-arthritic, Anticancer, Antioxidant, Nanocarriers, Thymoquinone

1. Introduction

Humans have been looking for natural materials having anti-disease effects since the early days of humanity. A key aspect of alternative as well as traditional medicine has been the use of plants for medicinal purposes that seem to have medicinal properties[1]. It has been established that a group of chemical substances known as phytochemicals, such as saponins, alkaloids, and glycosides, are responsible for therapeutic. Phytochemicals are important in drug development because they may include many components that can function as pharmacophores - Naphthoquinones, anthraquinones, and benzoquinones are only a few of the well-known medicines in this class. TQ, the primary component of benzoquinones, has undergone extensive scientific investigation. The Ranunculaceae family plant, *Nigella sativa* L., has TQ in its volatile oil. Middle Easterners prefer *N. sativa* seeds, sometimes known as black cumin, for their nutritional benefits[2-4]. The oil part, which accounts for 30-48% of the seeds in *N. sativa*, is responsible for the majority of the

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Article Received on: 09.04.2025 Revised on: 14.10.2025 Accepted on: 29.10.2025

biological outcomes of the plant. Black cumin oil was given the "Generally Recognised as Safe" designation by the FDA[5]. Various qualities are effective against seizures, microbes, cancer, histamine release, diabetes, inflammation, and free radicals. TQ is a drug with pharmacological activity that can be used for both therapeutic and preventive purposes[6]. However, a number of metabolic issues hinder it from performing its pharmacological functions. TQ is a water-insoluble, thermally unstable, and photogenic molecule, all of which lower its bioavailability.

Nanotechnology is increasingly recognised as a transformative force in the field of medicine. One significant application involves the use of nanoparticles to enhance the solubility and bioavailability of lipophilic compounds such as TQ in drug delivery systems. These nanoparticles offer several advantages, including protection of the encapsulated drugs from enzymatic degradation, controlled release, and extended circulation time by interacting with blood phospholipids either within or along the bilayer interface. As a result, the use of nanoparticles in therapeutic formulations has gained substantial momentum over the past decade[7,8]. Also, liposomes can entrap any drug, regardless of its solubility, and deliver it into cells through fusion or endocytosis. Since nanoparticles can prevent drugs from degrading in the presence of enzymes, allow for their controlled release and prolonged blood circulation, alter the drugs' pharmacokinetics, reduce their toxicity, and restrict their nonspecific uptake, their use has exploded in popularity over the past decade. Numerous research have focused on the creation and *in vitro*. This review explores the potential of nano-formulated thymoquinone (nano-TQ) for targeted drug delivery, emphasising its enhanced permeability, extended circulation time, and improved biodistribution, which contribute to increased therapeutic efficacy. The paper highlights how nanotechnology can be leveraged to overcome conventional limitations of TQ by examining its key pharmacological attributes. A comprehensive literature search was conducted using databases such as ScienceDirect and Scopus, focusing on studies that investigate TQ's applications in nanotechnology-based systems, particularly for its antiarthritic, antioxidant, anticancer, and functional properties[9]. Given nanotechnology's capacity to improve the pharmacokinetic and pharmacodynamic profiles of various plant-derived compounds, it holds promising potential for advancing pharmaceutical development.

2. Thymoquinone

2.1 Sources and Chemical Structure

Nigella sativa L. is a plant species that belongs to the Ranunculaceae family. Its seeds are commonly known as black cumin, are widely recognised as the primary natural source of TQ[10]. TQ has a chemical structure as depicted in Figure 1, with a molecular formula of $C_{10}H_{12}O_2$ and a molecular weight of 164.204 g/mol[11].

In seed oil, TQ is reported to be present at the concentration of 8 and 25 µg/mL[12]. TQ is known to possess a range of important properties, including antioxidant, neuromodulatory, antimicrobial, anti-inflammatory, and also inhibits membrane lipid peroxidation. These properties have made TQ an attractive candidate for modern pharmacology research. The thermogravimetric analysis study demonstrated that the decomposition of TQ initiated at 65°C and ended at ~213°C. TQ is a natural substance that corresponds to the monoterpenes class (Figure 2) and exhibits keto-enol tautomerism[13].

TQ is a tautomeric molecule, and it has its main pharmacological effects in keto-form[5,6]. The primary quinone ring component of TQ, which has a total of 10 carbons, has 6 carbons. The 7th methyl group carbon is located at position C2, and the 8th, 9th, and 10th propyl groups are located at position[5,13]. Dithymoquinone and thymohydroquinone are two of the closest derivatives of TQ that are most commonly synthesised (Figure 3)**4,5**. TQ has important applications to the pharmaceutical,

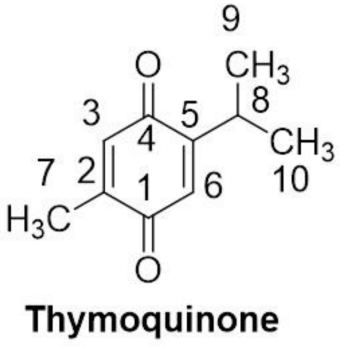
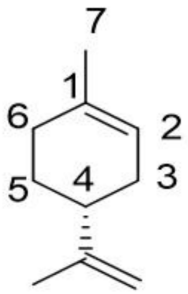


Figure 1. Chemical structure of TQ.

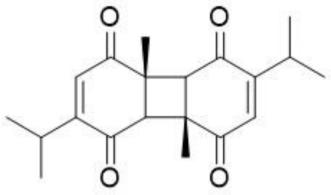
cosmetic, and food sectors because it matches closely the basic monoterpene skeletal molecule with a 10-carbon structure and the basic molecular formula C_6H_{16}[14].

In addition to Ranunculaceae, small quantities of TQ were also found in *Nigella arvensis* L. seeds[15]. The existence of this substance was previously verified in a number of genera of the Lamiaceae family, including *Agastache*, *Coridothymus*, *Monarda*, *Mosla*, *Origanum*, *Satureja*, *Thymbra*, and *Thymus*. Additionally, it has been discovered in the species Tetraclinis and Cupressus and Juniperus of the Cupressaceae family in a glycosidic form[16,17].

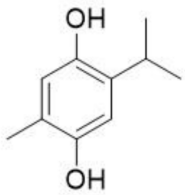


Monoterpene

Figure 2. Chemical structure of the basic ring (monoterpene) of TQ.



Dithymoquinone



Thymohydroquinone

Figure 3. Chemical structure of thymoquinone derivatives.

The compound Thymohydroquinone (THQ), which is thought to have potential antibacterial, antifungal, anti-inflammatory and antioxidative[18-21] and acetylcholinesterase inhibitory effects[22], coexists with TQ in numerous species of plants. In many Lamiaceae genera, including *Coridothymus*, *Origanum*, *Monarda*, *Mosla*, *Satureja*, and *Thymus*, THQ is spread similarly as TQ is[23]. Additionally, the genera *Tetraclinis* and *Nigella* of the Ranunculaceae and Cupressaceae families, respectively, have demonstrated their existence[24].

2.2 Isolation of TQ

Volatile oil contains TQ, also known as 2-isopropyl-5-methyl-1,4-benzoquinone[25]. TQ compound with an aromatic ketone structure that can also be generated by oxidising carvacrol or thymol[26]. *N. sativa* seeds could be processed using supercritical fluid extraction, solvent extraction, microwave-assisted extraction, and cold press extraction to produce the crude oil[27]. TQ concentration of *N. sativa* oil extracted using various techniques as shown in Table 1. The characteristics of oil basically depend on the extraction process and process conditions[28]. The supercritical CO_2 (SC-CO_2) extraction method stands out as being environmentally benign and producing products without solvent residues. The separation and synthesis of medication raw materials are thus primarily preferred[29].

2.2.1 Solvent Extraction

The chemical stability of the solvent is crucial in this technique, and it must remain stable throughout the extraction and recovery processes. Methanol, ethanol, hexane, and chloroform are examples of frequently used chemical solvents[30]. By using hexane extraction along with cold pressing, the crude oil extraction rate from *N. sativa* was determined to be 37% and 27% (wt

Table 1. Thymoquinone concentration of oil from *N. sativa* by different extraction methods

Extraction technique	Processing conditions	Thymoquinone content	References
Cold pressing	30°C, without heating	1.56 mg/mL oil	33
Cold pressing	At 25°C, without heating	14.40 µg/g oil	34
Cold pressing	Mechanical pressing without heating	8.73 mg/g oil	35
Microwave extraction	10 min, 1 atm, 50 mL distilled water	0.49 mg/g black seed	36
Soxhlet extraction	n-hexane	6.20 µg/g oil	34
Supercritical carbon dioxide extraction	350 bar, at 50°C	4.07 mg/g oil	37
Supercritical carbon dioxide extraction	600 bar, at 40°C	6.63 mg/mL oil	33

%), respectively[31]. Moroccan plants cultivated for their crude oil had a comparable content to that of other Mediterranean nations. Ethyl alcohol was employed as a cosolvent during the SC-CO_2 separation of *N. sativa* to produce a crude oil[32]. Pressure of 350 bar, at 60°C, and 120 min were found to be the ideal conditions for the greatest oil yield of 23.20%. On the contrary, under the following conditions—150 bar, 40°C, 120 min—the highest TQ concentration was found to be 4.09 mg/mL[33]. The solvent traces that remain in the raw components after extraction are the major drawback associated with this method.

2.2.2 Cold Press Extraction

Cold pressing is a method for separating liquids that are embedded in rigid matrixes[34]. This method doesn't involve the use of heat or chemicals, and especially consumers asked for natural, solvent-free products that have been extracted. Consuming natural goods and cold-pressed oils improves human health and helps to avoid some diseases[35]. It was discovered that the TQ content of oil extracted using the cold press technique and supercritical fluid was 6.63 mg/mL and 1.56 mg/mL, respectively[36]. Additionally, the percentage of TQ was found to be 14.40 µg/g, 5.65 µg/g, and 6.20 µg/g, respectively, in oil cold press, microwave-assisted extraction, and solvent extraction[37]. The quantity of oil extracted from *N. sativa* in the cold press depends on the process's temperature. The viscosity of the oil is the primary cause of this variance[38]. The amount of *N. sativa* oil produced by cold pressing can vary significantly depending on how viscosity changes with temperature. As the lowest viscosity was achieved at 60°C during the cold press, therefore temperature shouldn't be raised above that[38,39].

2.2.3 Supercritical Fluid Extraction

Supercritical fluid is one phase that displays both liquid and gaseous characteristics when both gas and liquid states are above the critical temperature and critical pressure. Over these crucial points, the coexistence of liquid and gas phases displays a unique property from that of liquids or gases under normal circumstances[40]. Regarding the quantity and quality of the intended substance, the ideal conditions influencing supercritical fluid extraction are crucial[41]. Characteristics of the finished product are influenced by variables including the size of particles and uniformity of sample pre-drying, various solvents and cosolvents, temperature, pressure, flow rate, and outlet valve temperature. Supercritical fluid extracts are highly pure and leave no trace of the liquid. According to reports, increasing the extraction pressure from 10,000 to 14,000 kPa increases the solvent's potency, and increasing the temperature from 40 to 80°C increases the supercritical fluid's ability to remove black poplar[42]. The extraction yield for ethyl acetate and ethanol, respectively, was found to be 14.54% and 16.36% in another study[38].

2.3 Characterisation of Isolated Components

2.3.1 Chromatographic Techniques

Chromatography is a method of separation that is frequently employed in the measurement of liquid components. The HPLC technique can be used for quantifying high molecular weight and high boiling point components. Moreover, the kinetics of the TQ's aqueous solubility and degradation have recently been investigated with UV spectroscopy. The peak at 254-257 nm is referred to as the quinone group's signature peak[43].

In addition to HPLC, Gas Chromatography (GC) is a popular method for figuring out number of components and their concentration in the extract. In other words, accurate identification requires a spectroscopic detection device. Furthermore, to determine a molecule's molecular weight, elemental makeup, functional groups, spatial isomerism, and geometry, Mass Spectrometric (MS) detectors are frequently used. The GC-MS device uses inert gases with various retention times in the column to transport the material[43,44].

Using SC-CO2 extraction, the unprocessed oil was recovered from *N. sativa* seeds. Utilising GC-MS, the hydrocarbon extraction yield was calculated[45]. TQ was discovered to have a peak area and retention time of 16.80 and 11.32, respectively[28]. By using GC-MS, the amount of TQ in the oil made from the grains of *N. sativa* was quantified. Characterisation of the oils extracted from six distinct samples of *N. sativa* seeds demonstrated the presence of TQ, which ranges between 27.8 and 57.0% in all samples. According to GC-MS research, TQ is primarily found in the volatile oil of *N. sativa*[44].

2.3.2 FTIR Analysis

The capability to track the vibrations of functional groups that define molecular structures and regulate the progress of chemical reactions is provided by Fourier-Transform Infrared Spectroscopy (FTIR). Stretching bands at wavenumbers 1650 cm^{-1} and 2967 cm^{-1} in the TQ, respectively, correspond to the carbonyl group of a cyclohexadiene and the C-H stretching vibration of aliphatic groups, according to the findings of FTIR analysis of the TQ. The C=C stretching band cannot be distinctly distinguished because the strong carboxylic stretching band of TQ covers within the frequency range of 1640 to 1675 cm [28].

2.4 Synthesis and Purification of Thymoquinone

Thymoquinone is currently made using a one-step chemical reaction technique that involves catalysing the oxidation of thymol with a complex Co salt, according to a report in the literature[46]. The amount of Dimethyl Formamide (DMF) is very large, making the method only appropriate for research in laboratories. Thymol is used as a starting material in the Chinese patent application with publication number CN103288618A, along with chlorosulfonic acid as a sulfonating agent, chromic acid solution as an oxidant, dichloromethane as a solvent, and chloroform as a solvent. A sulfonation-oxidation formation route is utilised, which includes alkali washing, water washing, filtering, crystallisation, distillation, cooling and solidification to generate TQ, However, since chlorosulfonic acid, $Na_2Cr_2O_7$-$2H_20$, chloroform, and other chemical reactants that cause major contamination are used in the reaction, factory workers suffer physical harm, and the three wastes produced also pose a significant environmental pollution problem[47].

The advantages of the chemical synthesis technique over the extraction and separation from plants include ample raw material sources, stable quality, a straightforward process, and high yield[48]. Though numerous chemical synthesis methods for thymoquinone are reported in the related documents, the majority of industrial pollution is severe, and the process can also lead to physical harm to operators, making it unsuitable for industrial production[48].

2.5 Molecular Targets of Thymoquinone

The primary active ingredient obtained from the therapeutic *N. sativa* is TQ. Through poorly understood mechanisms, a few studies have demonstrated that TQ displays anti-inflammatory activities in experimental models of Rheumatoid Arthritis (RA)[49]. TQ substantially reduced the LPS-induced levels of Interleukin-1beta (IL-1b), Tumour Necrosis Factor-alpha (TNF-a), metalloproteinase-13, cyclooxygenase-2, and prostaglandin E2[49]. TQ also prevented the time-dependent phosphorylation of nuclear factor-kappaB-p65, extracellular regulated kinases 1 and 2, and p38 mitogen-activated protein kinase that was triggered by LPS[50]. The oral ingestion of TQ 5 mg/kg/day substantially decreased serum levels of HNE, IL-1b, and TNF-a, as well as bone turnover indicators like alkaline phosphatase and tartrate-resistant acid phosphatase in an animal Rheumatoid Arthritis (RA) model[51]. TQ's protection against RA was also demonstrated by a decline in arthritic severity scores and bone resorption[52].

Additionally, TQ has been found to have antioxidant effects through a variety of pathways in a number of recent studies. Both 5-lipoxygenase and 5-hydroxyeicosatetraenoic acid, which are necessary for the survival of colorectal cancer cells, were inhibited by TQ. Additionally, TQ was able to significantly lower liver catalase, glutathione peroxidase, and superoxide dismutase levels. TQ was also found to reduce oxidative stress by increasing glutathione in female Lewis's rat models of experimental allergy encephalomyelitis. TQ has also been noted as a strong regulator of leukotriene synthesis in human blood cells. This inhibiting impact affected the activity of 5-lipoxygenase and leukotrien C4 synthase, and it was dose- and time-dependent[52].

3. Therapeutic Potential of Thymoquinone

Thymoquinone's numerous pharmacological properties have been thoroughly investigated in the following section.

3.1 Anticancer Activity

One of the most common medical conditions in the modern world is cancer. Chemotherapy, radiotherapy, and surgery are the most common cancer remedies today; however, these treatments have a number of

drawbacks, such as adverse effects, toxic effects, and the potential for secondary tumours. TQ has been known for years for its antitumor benefits as well as its capacity to protect healthy cells from toxins[53].

3.2 Breast Cancer

The most aggressive form of cancer that affects women is breast cancer. Age, high levels of hormones, race, socioeconomic status, infections caused by viruses, deficiency of iodine in the diet, mutations in the genome, and epigenetic modifications are some of the various risk factors for breast cancer[54]. Breast cancer is primarily brought on by oestrogens. These substances accelerate the growth of cancer cells, cytochrome P450 (CYP 450) metabolism, and the start of aneuploidy[55]. The CYP 450 family 1 subfamily A member 1 (CYP1A1) and CYP1B1 genes, which have roles in the chemical breakdown of estradiol to produce 2-hydroxyestradiol, have been shown through research to be less expressed when TQ is administered[56]. Additionally linked with elevated cell division and spreading in breast cancer, overexpression of the Chemokine Receptor type 4 (CXCR4) is blocked by TQ in the MDAMB-231 triple-negative breast cancer cell line in a dose- and time-dependent way[57].

3.3 Liver Cancer

Some of the main reasons for liver cancer include the hepatitis virus, aflatoxin, pollution from the environment, hazardous industrial chemicals, and heavy alcohol consumption[58]. Additionally, diethylnitrosamine is a primary hepatocarcinogen as a result of tobacco smoke, also identified in cooked and preserved foods, and some pharmaceuticals. It raises oxidative stress and can cause liver cancer. TQ has been demonstrated to cure liver cancer by reducing this oxidative stress and boosting the activity and mRNA expression of antioxidant enzymes[59]. TQ has also been observed to promote apoptosis in thioacetamide-induced hepatocellular carcinoma by raising caspase-3 and upregulating the Tumour Necrosis Factor (TNF) apoptosis-inducing ligand genes (TRAIL/TRAIL2), while downregulating the B-Cell Lymphoma 2 gene. (Bcl-2)[60].

3.4 Colon Cancer

The most prevalent cancer, particularly in Western nations, is colon cancer. Interestingly, TQ was found to inhibit nuclear factor kappa B (NF-jb) via induction of pyrolidine dithiocarbamate, to chemosensitise colorectal cancer cells[61]. In a murine model where colon tumorigenesis was promoted by oxidative stress brought on by 1-2 dimethylhydrazine, TQ also inhibited invasion of cells and tumour development. TQ also aids in the treatment of LoVo colorectal cancer cells by enhancing autophagic cell death via the stimulation of MAPK and c-Jun N-terminal Kinase (JNK)[62].

3.5 Neuroprotective Activity

Numerous neurological diseases have been linked to TQ's neuroprotective effects[63]. Neuro-inflammation is a result of activation of the innate immune system to protect the CNS from different types of infection[64]. The inflammatory cytokines are released because of the activation of microglia[65]. It has been demonstrated that TQ inhibits the generation of inflammatory mediators like prostaglandin 2, TNF-a, and interleukin-b in BV2 microglial cells[66]. Additionally, TQ therapy can boost the expression of neuroprotective and antioxidant proteins like Glutaredoxin-3 (GLRX3), Biliverdin Reductase-A (BVR-A), and mitochondrial ion protease. The NAD(P)H:Quinone Oxidoreductase 1 (NQO1) gene is upregulated, demonstrating the antioxidant impact and preventing cellular damage[67]. In Alzheimer's Disease (AD), TQ also has a neuroprotective impact by directly inhibiting beta amyloid peptide accumulation[68].

3.6 Hepatoprotective Activity

Hepatocellular carcinoma, a primary liver cancer, is the third most common cause of death globally and is more prevalent in both men and women than any other type of cancer[69]. As both TQ and N-acetylcysteine have the ability to suppress a number of inflammatory pathways, including cyclooxygenase-2 (COX-2), NF-jb, and STAT-3, it has been discovered that TQ therapy can avoid hepatic injury brought on by sodium fluoride in combination therapy[70]. According to studies, TQ has similar hepatoprotective properties to the well-known medication silymarin and has an advantageous effect on total oxidant status. TQ functions as an antioxidant and also inhibits liver fibrosis, which is a significant contributor to persistent liver injury[71].

3.7 Cardioprotective Activity

TQ has promise as a treatment for myocardiopathy. TQ reduces blood levels of serum oxidative stress

indicators that are increased during doxorubicin-induced cardiocytotoxicity[72]. Cardiotoxicity is known to be at danger from high cholesterol levels. Low-Density Lipoprotein (LDL) and total cholesterol levels are higher in ApoE mice, whereas they are reduced in ApoE mice that have received TQ treatment[73]. TQ has also been shown to lessen lipid buildup, LOX-1m gene expression in mouse hearts[74]. Adult albino rats receiving TQ orally showed Positive ionotropic effects after two months, and consisted of elevated baseline peak tension without any accelerated force decrease or increases in myocardial flow rate[75].

3.8 Anti-Inflammatory Activity

A physiological or immune reaction called inflammation serves as a defence system toward different pathogenic and non-infectious factors[76]. TQ is known for its ability to reduce inflammation, which is regulated by a number of proinflammatory transcription factors, inflammatory cytokines, and inflammatory pathways, highlighting the significance of TQ in conditions like heart disease, diabetes, asthma, encephalomyelitis, and arthritis[77]. Treatment with TQ for rheumatoid arthritis decreases TNF-a-induced inflammatory processes, which in turn results in less IL-6 and IL-8 being produced[78]. Additionally, it prevents cell adhesion to endothelial cells that are found at areas of inflammation or in damaged tissue by reducing the expression of Intercellular adhesion molecule 1 (ICAM-1), Vascular cell adhesion molecule-1 (VCAM-1), and Cad-11[79]. TQ suppresses tissue-factor-mediated clotting and disables platelets via the NF-jb pathway in cancer-associated thrombosis, further demonstrating its beneficial effects in this condition[80]. In corrosive esophagitis, TQ therapy is also helpful in reducing inflammatory cytokine accumulation. TQ controls a variety of inflammatory reactions by inhibiting the Interleukin-1 Receptor-Associated Kinase 1 (IRAK1)[81].

3.9 Antioxidant Activity

The reduced form of TQ (thymohydroquinone) is recognised for its high antioxidant activity[82]. Additionally helpful in addressing liver and kidney damage brought on by prolonged cyclosporine use. Additionally, it boosts constitutive nitric oxide synthase activity and nitrate concentrations while lowering iNOS activity, which is helpful for addressing endothelial dysfunction[83]. TQ also lessens oxidative stress in microglial cells by reducing lipid hydroperoxide levels[84]. Raising glutathione levels as an antioxidant, and boosting oxidative enzyme levels like SOD and CAT[85].

4. Challenges Associated with Thymoquinone as an Anti-Arthritic Agent

TQ is a pharmacologically active substance used both therapeutically and preventively. Yet it has a number of metabolic problems that prevent it from performing its pharmacological functions. TQ has poor solubility in water, is thermally unstable, and is photosensitive, all of which reduce its bioavailability. Additionally, the method of administration has a significant impact on the bioavailability of TQ[86]. Following oral (20 mg/kg PO) and intravenous (5 mg/kg) administration of TQ to rabbits, the absolute Bioavailability (BA) of TQ showed a *58% lag time of 23 min with slower uptake and faster elimination rates[87]. It is an acidic molecule that extensively degrades in the presence of water, especially at higher pH levels. The main obstacles to maximising its medicinal potential are its hydrophobicity, light-sensitive nature, and low bioavailability[66]. The nanocarriers have a particular targeting ability to the inflamed cells and subsequently, effectively decrease the pro-inflammatory sequence of events and may reduce RA indications and subsequent bone damage[88]. Additionally, NPs may lower doses and off-target toxicities, improving the possibility for treating arthritis with drug delivery[89]. A variety of nanoformulations, such as an oral phospholipidic nanomatrix, have been developed to increase the stability and bioavailability of TQ[88]. Moreover, when tested in a carrageenan-induced paw inflammation model, topical ethosomes and liposomal gel improved the therapeutic effectiveness of TQ[90].

5. Thymoquinone Nanoformulations for the Effective Management of Rheumatoid Arthritis

According to the biopharmaceutical classification system, TQ has poor aqueous solubility. The approaches like nanosuspension, nanoemulsion, microemulsion,

nanocrystal, *etc*., preferred surfactant compositions and optimised cosurfactant ratios are commonly employed to improve solubility and dissolution profiles. Novel surfactant blends or chemically altered surfactants are often specifically engineered to enhance solubility, offering distinct physicochemical properties tailored for this purpose.

5.1 Polymeric Nanoparticles

Nanoparticles (NPs) have a diameter of up to 100 nm with a higher surface-to-volume ratio [15]. Nanoparticles, formulated with biodegradable polymers like Poly-Lactic-Co-Glycolic Acid (PLGA), Polylactic Acid (PLA), are known as Polymeric Nanoparticles (PNPs) that are Nanocarriers are considered effective drug delivery systems due to their large surface area, ability to retain drugs at the target site, and improved cellular uptake resulting from their nanoscale dimensions. Various studies have reported that TQ, when encapsulated within PNPs, exhibits significantly enhanced anti-inflammatory effects[91]. Recently, formulated TQ-loaded chitosan Nanocomposites (TQ-NCs) were formulated using the high-pressure homogeniser method. The lateral dimensions of the TQ-NC NPs have a mean diameter of 12 nm, according to TEM images, but the SEM photos show that the blank chitosan exhibited a broad size and shape (Figure 4). The NPs exhibited an immediate release of TQ in the stomach's acidic environment. TQ-NC demonstrated prolonged release of TQ over a 100-hour duration in a pH 6.8 medium. Additionally, TQ-NC successfully inhibit the LPS-stimulated RAW 264.7 cells› synthesis of inflammatory cytokines, demonstrating that they possess anti-inflammatory characteristics. TQ-NC NPs suppressed these cells' generation of NO, PGE2, IL6, TNF-a, and IL-1b in a dose-dependent way (Figure 4). Secondary chemokines, cytokines, and non-cytokine messengers that have separate roles in inflammation may be released as a result of TNF-a and IL-1b induction. TQ-loaded poly (Lactic-co-Glycolic Acid) (PLGA) NPs dramatically reduced the blood levels of IL-10 and TGF-1 in LPS-induced rats, according to another study[91]. Thus, prevented inflammation and tissue

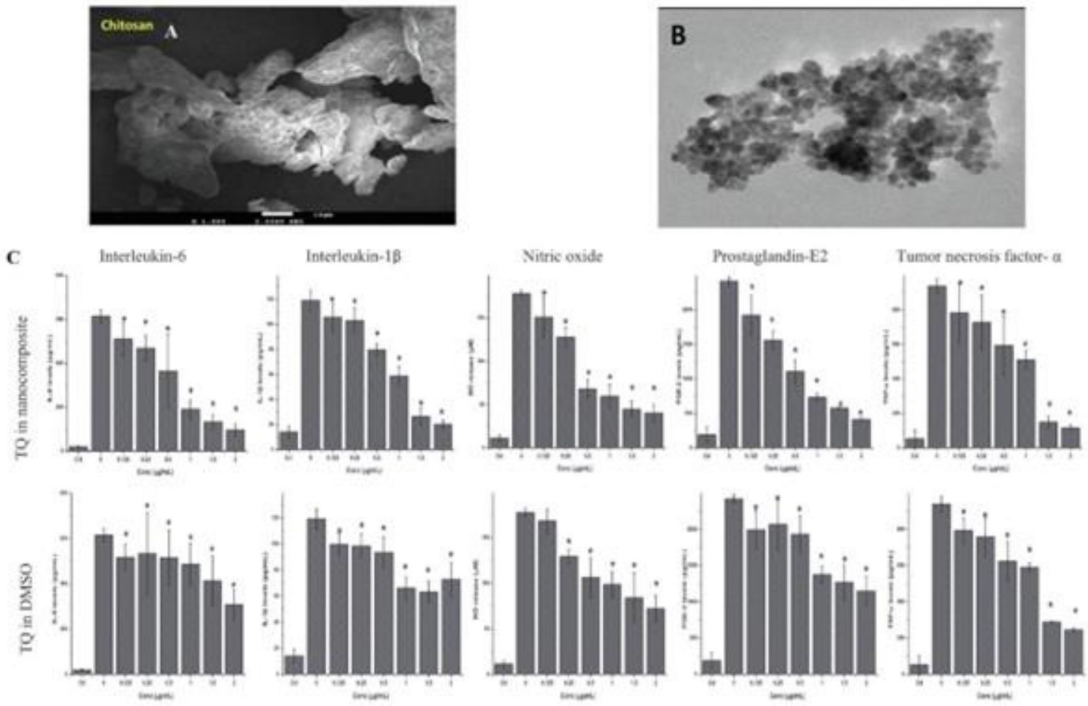


Figure 4. **(A)** SEM image of chitosan, **(B)** TEM image of TQ-NC, **(C)** Levels of Interleukins, Nitric oxide, Prostaglandin and TNF-a produced by Lipopolysaccharide (LPS)-stimulated RAW 264.7 cells treated with TQ dissolved in DMSO or encapsulated within chitosan NPs. Reproduced from[90]. Reproduced with kind permission of the copyright holder, Elsevier. [This is an open-access article distributed under the terms of the Creative Commons CC-BY license, which permits unrestricted use, distribution, and reproduction in any medium].

injury in the rat model. In conclusion, polymeic NPs could be the promising carriers for the delivery of TQ in the treatment of inflammatory diseases like arthritis.

5.2 Self-Nanoemulsifying Drug Delivery System

Emulsification has emerged as an effective strategy for delivering hydrophobic drugs by incorporating them within oil globules. By employing surfactants—and often cosurfactants—the size of these globules can be minimised to the nanometer range, resulting in the formation of nanoemulsions. Depending on the drug's nature and phase behaviour, these systems are typically classified as either oil-in-water (o/w) or water-in-oil (w/o) nanoemulsions. Unlike conventional emulsions, nanoemulsions are transparent and offer thermodynamic stability. However, they are considered non-equilibrium systems, as external energy input is required to generate nanoscale droplets.

Nanoemulsions can be prepared using two main techniques: low-energy and high-energy methods. Low-energy techniques operate with minimal energy input and are largely dependent on the selection of stabilisers to modulate interfacial tension between the oil and aqueous phases. In contrast, high-energy approaches utilise intense mechanical forces—such as high-shear mixing, ultrasonication, or high-pressure homogenisation—to break down the dispersed phase into uniformly distributed nanosised droplets within the continuous phase. A team of researchers produced a black seed oil-loaded nanoemulsion employing Tween 80 and Polyethene Glycol (PEG 400) as cosurfactants and surfactants, respectively, with varying concentrations of the oil and surfactant mixture[15]. This was done in light of the huge potential of SNEDDS. The optimum formulation was made using Tween 80 and PEG 400 in a 2:1 ratio using oil and a 1:8 mix of surfactant and cosurfactant. The average globule size was determined to be 65.4 nm, with excellent physical properties and stability[92]. Reported delivery of TQ and curcumin using a self-emulsifying drug delivery system in a different study. The therapeutic effects were increased, and the toxicity related to their separate use was reduced by using the two substances together. The optimised batch had reduced droplet size, combined with an improvement in dissolution and solubility. The image in Figure 5[93]. demonstrates that, at the highest

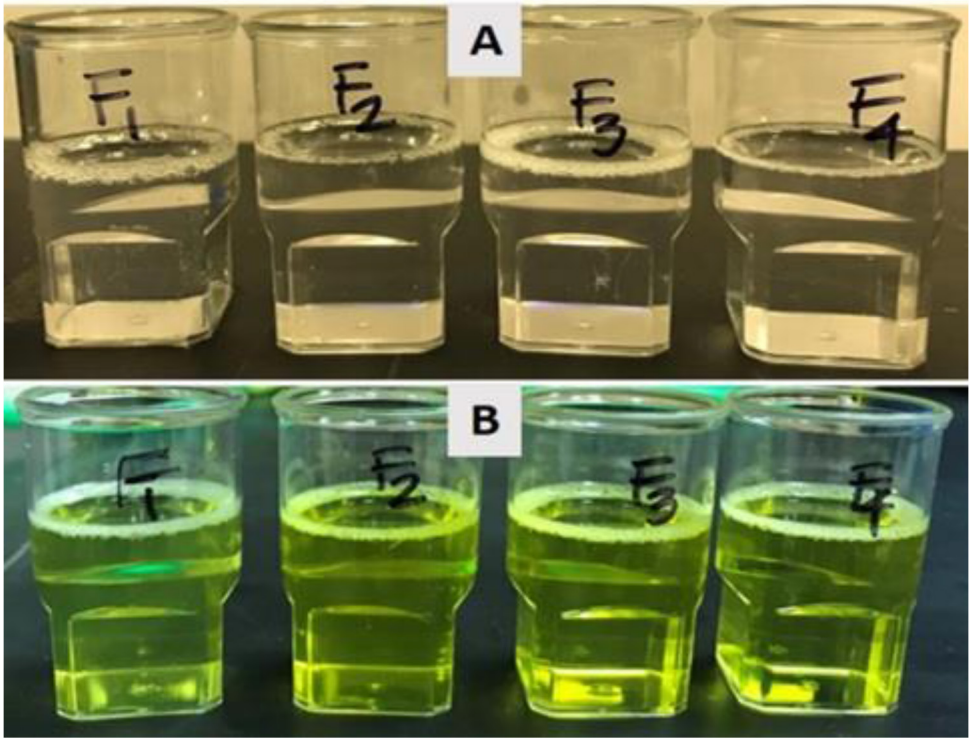


Figure 5. Image of the blank **(A)** SNEDDS and **(B)** CUR containing SNEDDS after aqueous dispersion with water. Reproduced with kind permission of the copyright holder, Elsevier.

dilution level of 1 in 1000, all formulas produced transparent dispersion. The final SNEDDS formulation had been solidified and administered in capsules made of hard gelatin[92]. When compared to the pure drug, the optimised batch F4 with liquid TQ and solid curcumin given via the SNEDDS formulation showed a greater percentage of cumulative drug release with no significant modifications to the physical characteristics.

Similar to this, TQ-loaded SNEDDS were developed[72] for improved anti-inflammatory efficacy. Various phospholipid levels were used in the microemulsification process to create TQ-loaded NPs. Using a rat paw oedema model, the NPs' oral bioavailability and anti-inflammatory efficacy were evaluated. The optimal NP composition (F1) had a surface with a negative charge, a zeta potential of -0.57 mV, a spherical shape, a small size distribution, increased drug entrapment effectiveness (>70%), and a controlled drug release pattern. F1 formulation demonstrated approximately 226.6% relative bioavailability following the ingestion of a single dosage compared to plain TQ suspension. While in the inflammatory model, a noticeably larger reduction in the % inhibition in paw oedema was found when compared to the pure drug suspension and diclofenac sodium. According to the current research, PLN holds promise as a viable carrier system to increase TQ oral bioavailability as well as an effective nanodevice for the treatment of inflammatory illnesses.

5.3 Solid Lipid Nanoparticles

SLNs are second-generation lipid nanocarriers that range in diameter from 10 to 1000 nm. Interfacial surfactants can be used primarily for stabilising SLN. For solid lipid, core compositions range from 0.1% to 30% by weight. Phospholipids, bile salts, sterols, and other substances make up the surfactant. The formulation uses a variety of important lipids, such as glycerol monostearate, tristearin, and stearic acid[94]. To improve the dissolution and bioavailability of traditional thymoquinone suspension, a new formulation of SLN containing thymoquinone was recently fabricated. Another study found that treatment with TQ suspension (20 mg/kg p.o.) and TQ solid lipid nanoparticles (20 mg/kg p.o.) decreased the raised hippocampal IL-6 and TNF as determined by the proportions of hippocampal KYN/TRP and 5HT/TRP in stressed rats[94]. The current study's findings support the anti-inflammatory and neuroprotective properties of TQ, which may be related to the 5-HT pathway[95,96].

5.4 Nanostructured Lipid Carriers (NLC)

The NLCs were developed as a result of limitations of SLN, such as reduced drug-loading efficiency and the leakage of drugs from the lipid core while storage, because of the recrystallisation of solid lipid. NLC dispersions have a larger drug payload and little drug ejection throughout storage since they are made up of a blend of both solid lipid and liquid lipid. Additionally, NLCs improve oral drug absorption by selectively absorbing drugs through the lymphatic system or through the payer's patches. To improve oral absorption and bioavailability of TQ in this situation, TQ-loaded NLCs were created[96]. The resulting NLCs demonstrated higher AUC0-t values in animals given NLC treatment, and their relative bioavailability was 3.97 times greater than that of TQ suspension, demonstrating that the formulation of the NLCs had increased bioavailability.

5.5 Liposomes

Bilayer self-assembled colloidal structures range in size from the nanoscale to the microscale. These systems typically consist of an internal aqueous core surrounded by a lipid bilayer that can encapsulate hydrophobic substances. The bilayer, composed of either natural or synthetic phospholipids, is capable of interacting with drugs that are hydrophobic, sensitive to light, or photodegradable. Due to these characteristics, liposomes present a promising delivery platform for transporting hydrophobic compounds such as TQ. Recently, TQ-loaded liposomes for the enhancement of the anti-inflammatory activity were prepared by Mostafa *et al.*[90] using *N. sativa* oil. The various cholesterol concentrations were selected for the preparation of a liposomal system by using the film hydration method. The optimisation of the formulation was done, and better permeation through the skin was considered as the best criterion for the selection of the optimised batch during the experiments, which was found to be F12. The selected optimised batch was incorporated into the chitosan gel for the topical application. The prepared gel formulation has shown greater skin permeation and excellent rheological properties. The anti-inflammatory activity of the optimised batch

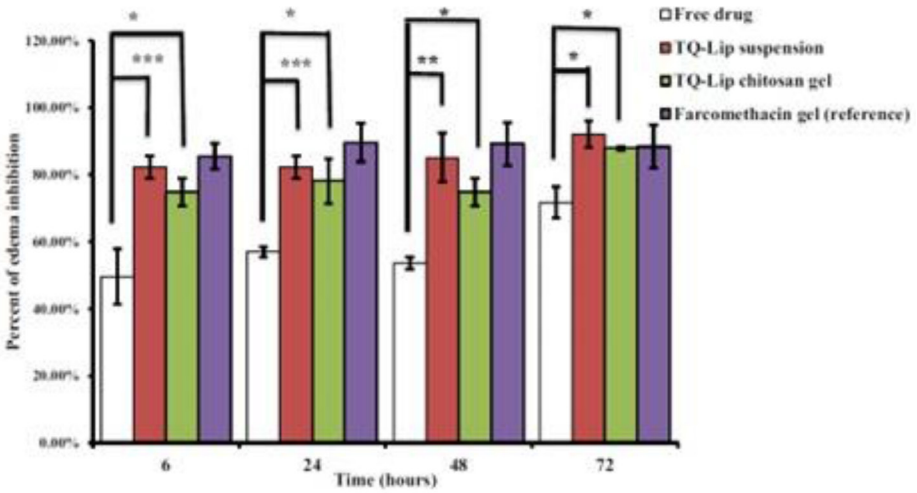


Figure 6. Anti-inflammatory (treatment effect) of free drug gel, TQ-Lip, TQ-Lip chitosan gel, and Farcomethacin gel; data are expressed as mean (n = 4). Reproduced with kind permission of copyright holder, Springer[90].

was evaluated by using the carrageenan-induced paw oedema in the rats. The newly created chitosan gel (F12) significantly outperformed the chitosan gel of the TQ in *in vivo* anti-inflammatory activity ($p = 0.05$), and its effects were comparable to those of the commercial indomethacin gel (Figure 6).

6. Conclusion and Future Perspectives

The development of nanoassembled structures requires a thorough understanding of each component involved, as their precise design is essential for improving the performance of poorly soluble bioactives such as TQ. TQ is a naturally occurring compound with a wide range of therapeutic benefits, including anti-inflammatory, antiarthritic, and anticancer properties, making it highly valuable in promoting human health. Its broad pharmacological profile underscores its significance as a multifunctional therapeutic agent.

The chemical modification or derivatisation of TQ holds great promise for enhancing its therapeutic efficacy and improving recovery outcomes. Additionally, TQ has demonstrated synergistic effects when used in combination with conventional chemotherapeutic drugs, a property that continues to attract research interest. It can induce cytotoxic effects by disrupting the structural integrity of malignant cells or their genetic material.

The integration of TQ into nano-assembled systems is primarily aimed at combining its physicochemical attributes with enhanced biological activity, particularly in the treatment of inflammatory diseases and cancer. Looking ahead, there is growing emphasis on multimodal and multitherapeutic strategies to further explore the therapeutic potential of phytopharmaceuticals like TQ and to maximise their clinical benefits.

7. Author Contribution

Ravi Manne: Conceptualized the research, collected relevant literature, and wrote the initial draft of the article. Madhavi Patel: Provided valuable insights and guidance during the literature review and article writing process and reviewed and helped refine the content, ensuring its clarity and accuracy. Mohan Maruga Raja: contributed to the overall structure and organization of the article, providing critical feedback. Also, helped with the final revisions and corrections to enhance the quality of the manuscript. All authors have read and agreed to the published version of the manuscript.

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