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# Therapeutic implications of thymoquinone and its molecular and functional mechanisms against oral and lung cancer

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ABSTRACT

Thymoquinone, a bioactive component of the black seed of *Nigella sativa*, has received great attention from researchers due to its wide spectrum of pharmacological potentials. The present review highlights the molecular and functional mechanisms of thymoquinone serving as a therapeutic molecule combating oral and lung cancer by following the most recent literature. This summarized the latest nanotechnological interventions enhancing the efficacy and availability of thymoquinone in the *in-vitro* and *in-vivo* cancer models. Thymoquinone exhibits a significantly promising anticancer effect against oral and lung cancer by prompting intrinsic and extrinsic pathways of apoptosis through the activation of different caspases cascades, up and downregulation of apoptotic genes, antitumor cell proliferation, ROS regulation, *etc*. The accomplished insight into molecular and functional mechanisms of thymoquinone particularly combating oral and lung cancer will provide a better understanding and exploration of thymoquinone in an ethnopharmacological context.

## 1. Introduction

In recent years extensive research has been shifted towards finding an alternate therapeutic agent, where the bioactive compounds having low toxicity and availability at affordable cost received much attention from the researchers. From this perspective, thymoquinone a bioactive constituent of seeds of *Nigella sativa* is frequently investigated for the treatment of diverse ranges of disease (Ma and Ms, 2011). *Nigella sativa* is an emerging source of other bioactive compounds, including dithymoquinone, thymohydroquinone, saponine, and nigellicine (K. M and PM, 2010). Thymoquinone has shown promise as a chemoprotective phytochemical and anti-oxidative therapeutic molecule, protecting healthy cells from toxic side effects (Ma and Gw, 2010). Thymoquinone has been assessed for its anti-proliferative activities in *in-vitro* studies on different cell lines of the larynx, lung, liver, and colon (A. J et al., 2021). Various molecular pathways are involved in regulating the different stages of cancer including invasion, proliferation, and migration (A. J et al., 2021). However, understanding the anti-cancer activity of thymoquinone is still a challenge. Also, the recent advancements in nanobiotechnology may facilitate the development and delivery of phytoactive compounds for an effective anti-cancer effect. So, a comprehensive study highlighting the detailed anti-cancer molecular mechanisms, nanoformulations, *in-vivo*, and *in-vitro* models with special reference to oral and lung cancer needs to be reported.

*Abbreviations:* AhR, aryl hydrocarbon receptor; ALL, acute lymphoblastic leukemia; CAM, chorioallantoic membrane; CAPE, caffeic acid phenyl ester; CDDP, cis-diamminedichloridoplatinum ii; DMBA, 7,12-dimethylbenz(*a*)anthracene; EGFR, epidermal growth factor receptor; EPR, enhanced permeability retention; HNSCC, head and neck squamous cell carcinoma; HSP70, human shock protein 70; I3M, indirubin-3-monoxime; IV, intravenous; mPEG-PCL, methoxy poly(ethylene glycol)-b-polycaprolactone; NF-κβ, nuclear factor-kappa beta; NS, nanosphere; NSCLC, non-small cell lung cancer; OEC, oral epithelial cell; PBS, phosphate buffer saline; ROS, reactive oxygen species; SCLC, small cell lung cancer; SNEDDS, self nano emulsifying drug delivery system; TQ, thymoquinone; WHO, World Health Organization.

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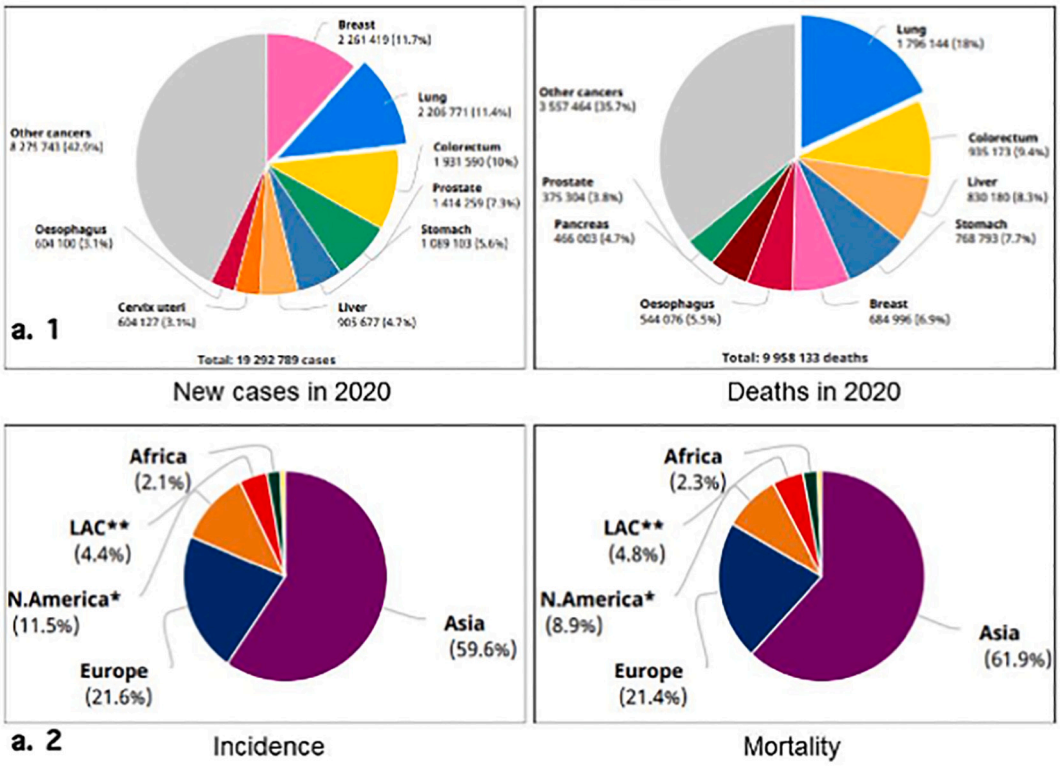


**Fig. 1.1.** Pie-chart depicting the incidence and mortality rate along with the geographical distribution of lung cancer reported in 2020 (GLOBOCAN, 2020).

## 2. Phytoactive thymoquinone: sources, chemistry, and properties

Thymoquinone (2-isopropyl-5-methyl-benzoquinone) is a quinone that is present in plant species like *Thymus vulgaris*, *Monarda didyma*, *Thymus pulegioides*, and *Nigella sativa* (Taborsky et al., 2012). Thymohydroquinone and dithymoquinone are the most frequently synthesized close derivatives of thymoquinone, where thymohydroquinone is relatively less stable, and its oxidation results formation of thymoquinone. Thymoquinone is extracted from the essential oil of the *Nigella sativa* seeds (18–25 μg/mL) using supercritical fluid extraction using liquid $CO_2$ in high-pressure cylinders, hydrodistillation using solid $CO_2$ in clevenger apparatus, and soxhalation using soxhlet apparatus (Salman et al., 2016; Alhaj et al., 2008; Salea et al., 2013). Thymoquinone is a derivative of terpene and is considered a secondary metabolite where its synthesis followed the biogenic isoprene rule and cyclization, rearrangement, and dimerization of geranyl diphosphate precursor (A. A et al., 2019). *Nigella sativa* is native to southern Europe, North Africa, Southwest Asia, India, and Pakistan (R et al., 2015). It has been extensively in use as a traditional medicinal herb that has been known for anti-bacterial, anti-fungal, anti-viral, anti-inflammatory, antioxidative anticancer, anti-diabetic, and other pharmacological activities (Salomi et al., 1992).

In a particular study, it is reported that even lower concentration of 5 mg/kg and 20 mg/kg can be used as therapeutic doses to cancer treatment through intravenous and oral routes, respectively (KM et al., 2015). Also, the estimated clearance for intravenous and oral dosing was found to be 7.19 mL/kg/min and 12.30 mL/kg/min respectively (KM et al., 2015). Thymoquinone is reported more stable at lower pH and its solubility is the same in water, phosphate buffer saline (PBS), and 0.1 N HCl. It is also extremely light-sensitive, with more than 70% of thymoquinone being depleted within the first 10 h.

## 3. Lung cancer and oral cancer: a concern

As per the World Health Organization, cancer accounts for nearly

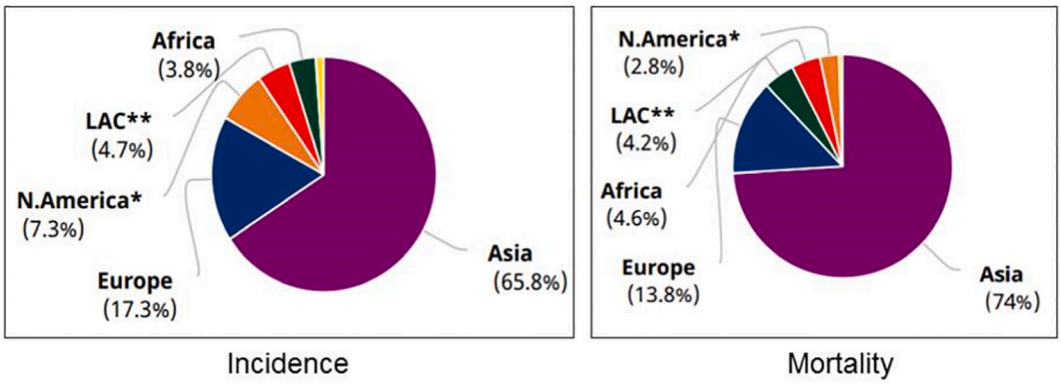


**Fig. 1.2.** Pie-chart depicting the incidence and mortality rate along with the geographical distribution of oral cancer reported in 2020 (GLOBOCAN, 2020).

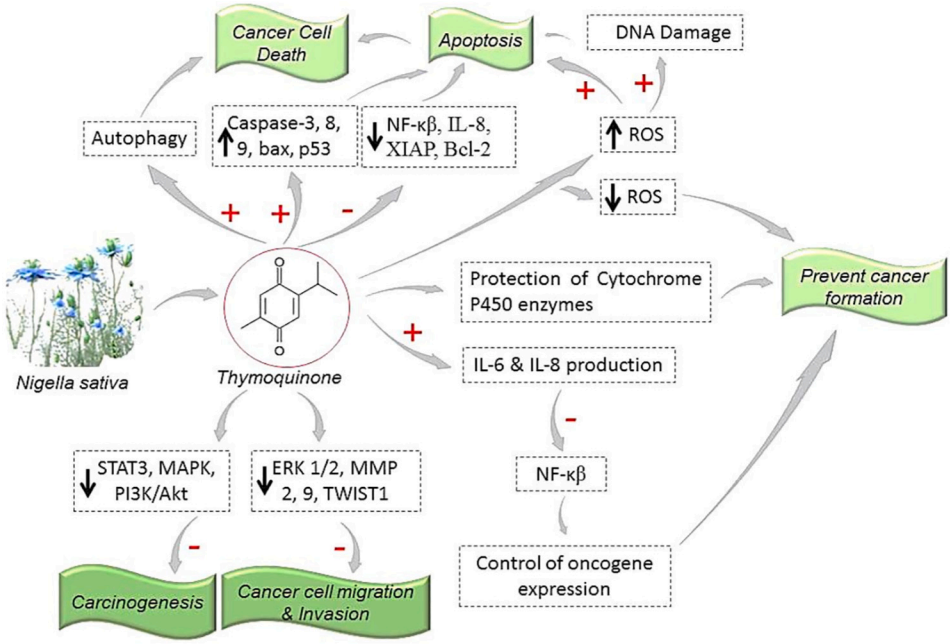


**Fig. 2.** Schematic representation of molecular pathways involved in the thymoquinone anticancer activity.

19.3 million deaths in 2020 (Today, n.d.). Lung cancer results in 1.80 million deaths among the total 2.21 million cases of lung cancer around the world (Cancer, n.d.). Asia accounts for more than 50% of all the incidence and mortality cases among other continents (GLOBOCAN, 2020) (Fig. 1.1). Lung cancer is categorized into small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC), where NSCLC accounts for 80–85% of all lung cancers (squamous cell carcinoma, adenocarcinoma, and large cell carcinoma) and SCLC accounts for 10–15% (Types of lung cancer | Cancer Research UK, n.d.). An incessant upsurge in tobacco consumption is the prime cause of the high prevalence of lung cancer. Smokers, due to their high exposure to benzopyrene hydrocarbons, are 20-fold more susceptible in comparison to non-smokers (PE et al., 2014). Systemic chemotherapy, especially with cisplatin, along with paclitaxel, and docetaxel, are common therapeutics for both NSCLC and SCLC (JP et al., 2008). Other approved therapeutic agents are erlotinib and bevacizumab (Sandler et al., 2009; FA et al., 2005).

*Oral cancer* is the collective term for lip, mouth, and oropharynx cancer. The global incidence varies from 4 to 20 cases per 100,000 people (Bray et al., 2018). Oral cancer causes 377,713 cases and kills 177,757 people worldwide, with Asian countries accounting for more than 65% of cases and deaths (Fig. 1.2) (GLOBOCAN, 2020).

Consumption of alcohol, tobacco, and areca nuts are the leading causes of oral cancer in Asian-Pacific countries, whereas papillomavirus infection is the main aetiology in America and European countries (M. H et al., 2017). Other associated risk factors which may increase the development of oral cancer are poor nutrition, exposure to ultraviolet light, genetic syndromes like Fanconi anaemia, and Lichen planus. Lung cancer is dominated in men and older age people relative to women. Men are more susceptible than women to developing oral cancer, likely as twice develop oral cancer. Only squamous cell carcinoma accounts for more than 90% of oral cavity cancer, whereas only 5% constitutes verrucous carcinoma. The treatment options available for oral cancer are chemo- and immunotherapy, surgery, radiations, and therapies with drugs targeting epidermal growth factor receptors (EGFR) (Bray et al., 2018; CTCA, n.d.).

### 3.1. Molecular targets and anticancer mechanism of Thymoquinone

Thymoquinone has been known for its significant role in manipulating cancer-mediated molecular pathways. The anti-oxidative potential is the widely accepted mechanism of thymoquinone for its anticancer potential owing to its oxidative stress-reducing property. Additionally, inducing apoptosis in cancer cells by exerting oxidative damage is a contradiction; therefore, thymoquinone has both antioxidant and pro-oxidant properties in a dose-dependent manner (Khan et al., 2017). Thymoquinone promotes intrinsic pathways of apoptosis through the activation of caspases cascades (Ma and Ms, 2011). Studies have shown that by reducing the expression of catalase and glutathione peroxidase, thymoquinone limits hepatic carcinoma in rats, thereby supporting the antioxidant perspective, whereas its pro-oxidant property induces apoptosis as reported in hepatic ischemia-reperfusion injury (Z. H et al., 2013; WY et al., 2013).

In a clinical trial, reactive oxygen species (ROS) based modulation of breast cancer has been reported with the use of thymoquinone. ROS is presumed to induce apoptosis through the acceleration of mitochondrial oxidative stress (Khan et al., 2017). Other potential targets like heat-shock protein 70 (HSP70) down-regulation, Bcl-2 upregulation accompanied with ROS production in acute lymphoblastic leukemia (ALL) revealed other mechanisms routed by thymoquinone (LZ et al., 2013). The protective and therapeutic potential of thymoquinone adopted various molecular pathways at the transcriptional and translational stages in cancer ranging from breast, bone, cervical, hepatic, colon, pancreatic, blood, brain, lung, prostate, skin, ovarian, kidney, oral, and lung (Fig. 2).

Y. J et al. (2015) investigated the anti-metastatic, anti-proliferative, and anti-invasive effects of thymoquinone in A549 cells. Thymoquinone was found to inhibit A549 proliferation and migration at a 40 μmol/L concentration in a dose and time-dependent manner. Wherein, mRNA and protein expression levels of proliferating cell nuclear antigen (PCNA), matrix metalloproteinase-2 (MMP2), MMP9, and cyclin D1 are observed to be inhibited with the increased expression of P16 has been reported. The tissue inhibitors of metalloproteinase-1 (TIMP1) and TIMP2 expression, on the other hand, remain unchanged. They explored the extracellular signal-regulated kinase 1/2 (ERK1/2) pathway, where reduced phosphorylation of ERK1/2 mounts to inhibitory effects of proliferation and invasion on A549 cells, whereas P38 and c-Jun N-terminal kinase 1/2 (JNK1/2) protein levels remain unaltered. Akt/mTOR signaling pathway proteins p-53 and caspase-3 activation during

apoptotic induction, when treated with a combination of thymoquinone and indirubin-3-monoxime (I3M), suggested the involvement of this pathway in the xenograft model of lung cancer (AA et al., 2020). Further, combined thymoquinone+I3M induces apoptosis in both *in-vitro* and *in-vivo* lung cancer models by down-regulating Akt, mTOR, and NF-κβ. MicroRNAs (miRNAs) are 18–22 long nucleotide RNAs known for the regulation of gene expression at post-transcriptional levels, and therefore act as attractive targets for therapeutic intervention. The promising effect of thymoquinone for controlled gene expression of miRNA and limited migration of NSCLC has also been evidenced (A. J et al., 2021). miRNA circulatory regulation which is controlled through p53 gene up-regulation and feedback loop to induce A549 cells apoptotic pathways is being reported by PEGylated PLGA thymoquinone (TQ) nanoparticle (Np) with transferrin (TF) (TF-TQ-Np) (U. P, 2019).

### 3.2. In-vitro and in-vivo lung cell line model

SH et al. (2010) evaluated the anti-cancer effect of thymoquinone alone and also in combination with cisplatin in NSCLC (NCI-H460) and SCLC (NCI-H146) lung cancer cell lines using both *in-vitro* and *in-vivo* techniques. Thymoquinone inhibits the proliferation of NCI-H460 and NCI-H146 lung cancer cell lines and induces apoptosis. The Matrigel assay demonstrated inhibition of NCI-H460 cells, suggesting thymoquinone influences the extracellular environment and the immune system.

In a study by BR et al. (2014) microtubule-targeting activity of thymoquinone was appreciated for its anticancer effect. Microtubules networks are involved in maintaining cellular physiology and regulating mitosis and therefore have emerged as crucial targets for cancer therapeutics (de, 2012). Direct interaction of thymoquinone with tubulin at the colchicine binding site inhibits tubulin polymerization and induced depolymerization of microtubule in an *in-vitro* human A549 cell line. Such an alteration in microtubule dynamics results in cell arrest in the $G_2$/M phase and further proceeds to the intrinsic pathway of mitochondrial apoptosis. Even in a cell-free system, thymoquinone restricts tubulin polymerization with an $IC_{50}$ of 27 μM (BR et al., 2014).

A combination of different anticancer agents is necessary to achieve improved efficacy by targeting different overlapped signaling pathways. In this concern, AA et al. (2020) have evaluated the synergistic therapeutic impact of thymoquinone combining it with I3M in A549 cells of xenografted lung cancer model of a mouse. The low and high dose combinations of 5 mg/kg body weight thymoquinone plus 3 mg/kg body weight I3M and 10 mg/kg body weight thymoquinone plus 6 mg/kg body weight I3M, respectively, were reported to be safe and effective in reducing tumor size in xenografted lung cancer model of a mouse. The anticancer activity of TF-TQ nanoparticles was reconfirmed in the chick chorioallantoic membrane (CAM) xenograft model and xenograft immunosuppressed Balb/c mice model owing to its p53/miR-34a/miR-16 axis circuitry (U. P, 2019). SS et al. (2013) *in-vitro* study investigating the effect of thymoquinone with resveratrol and caffeic acid phenyl ester (CAPE) showed decreased Bcl2 and cyclin D expression and increased Bax expression and p21 protein in cells owing to an increase in their regulatory effect on p53 levels. The increased concentration of TQ additive to lung cancer cells (LNM 35) has been reported to be cell viability reduction by following Akt phosphorylation which leads to DNA damages and singling of mitochondrial proapoptotic pathways. In athymic mice inoculated with LNM35 lung cells, 10 mg/kg/i.p. administration of thymoquinone for 18 days inhibited tumor growth by 39% due to caspase-3 activation (Attoub et al., 2013). Thymoquinone increases apoptosis and inhibits the pulmonary arterial remodeling in an *in-vivo* study by Z. N (2016) in monocrotaline-treated rats *via* the p38MAPK/NF-κβ signaling pathway.

### 3.3. In-vitro and in-vivo oral/neck-head cell line model

The anti-cancerous effect of thymoquinone on head and neck squamous cell carcinoma (HNSSC) is being investigated, where Sc et al. (2014) reported the caspase-9 dependent apoptotic cell death in human oral SASVO3 cancer cells by increasing Bax and caspase-9 expression. The $IC_{50}$ for the SASVO3 cell was reported as 45.02 μM, in comparison to 56.02 μM and 59.13 μM for SCC-4 and OC2 respectively. Thymoquinone increased the levels of autophagosomes, autophagic vacuoles, and LC3-II proteins in the thymoquinone exposed cells in a dose-dependent manner. They further validated the results using BALB/c nude

**Table 1**
Anticancer activities of thymoquinone and combinatorial drugs in lung and oral cancer *in-vitro* and *in-vivo* models (A. J et al., 2021).

| Experimental model and intervention | Outcome |
|---|---|
| *Lung cancer (in-vitro) models* | |
| A549 cells treated with 25–100 μM thymoquinone for 72 h | ↑ Apoptosis (S. S, 2019; R. S et al., 2013) |
| NCI-H460 and NCI-H146 cells treated with 20–100 μM/L thymoquinone for 24 h | |
| A549 cells treated with 5–160 μmol/L thymoquinone for 24, 48, or 72 h | ↓ Invasion, migration *via* ERK 1/2 pathway<br>↓ PCNA, Cyclin D, MMP2, MMP9 mRNA, and P16 expression (Y. J et al., 2015) |
| A549 cells treated with 10 and 25 μM thymoquinone for 24 h | Depolymerization of the microtubule network<br>↑ Apoptosis (BR et al., 2014) |
| LNM35 cells treated with 10 and 50 μM thymoquinone and 10 μM Cisplatin for 24 h | ↓ Cellular viability, proliferation (SH et al., 2010; Attoub et al., 2013) |
| NCI-H460 and NCI-H146 cells were treated with 80–100 μM thymoquinone and 1.25–5 μM cisplatin for 24, 48, and 72 h | |
| *Lung cancer (in-vivo) models* | |
| HS766T cells treated with 25–75 μM thymoquinone for 3, 6, 24 h | ↑ p21 activity<br>↓ Histone deacetylase activity<br>↓ MCP-1, TNF-α, IL-1β and Cox-2 *via* NF-κβ modulation (C. N et al., 2009) |
| MCT treated male Sprague-Dawley rats treated with 8–16 mg/kg/day thymoquinone for 14 days | ↓ Pulmonary arterial remodeling *via* p38MAPK/NF-κβ signaling pathway<br>↑ Apoptosis (Z. N, 2016) |
| MUC4 expressed FG/COLO357 and CD18/HPAF cells treated with 10–100 μmol/L thymoquinone for 24 h | ↑ Apoptosis<br>↓ MUC4 expression (MP et al., 2010) |
| MiaPaCa-2, BxPC-3, AsPC-1, and HPAC cells treated with 10 μM thymoquinone for 72 h | ↓ Cell growth<br>↑ Apoptosis and G2/M phase cell-cycle arrest<br>Modulation of NF-κβ transcription ( Banerjee et al., 2010) |
| SCID mice treated with 5 and 20 mg/kg/2 days (thymoquinone) and 2.5 (cisplatin) mg/kg/week for 21 days | ↓ Tumor volume and weight (SH et al., 2010) |
| *Oral cancer (in-vitro) models* | |
| SCC-4, SAS, SASVO3, OC2 cells treated with 20–60 μM thymoquinone for 24 h | ↑ Autophagic cell death<br>↑ Caspase-9-dependent apoptosis (Sc et al., 2014) |
| T28 and N28 cells treated with 5–100 μM thymoquinone for 24 h | ↑ Apoptosis |
| UMSCC-14C cells treated with 0.01–100 μM thymoquinone and cisplatin for 24, 48, and 72 h | ↓ Cell proliferation, viability (Alaufi et al., 2017; A. E et al., 2013; D. S et al., 2012) |
| Human SCC A431, and Hep2 cells treated with 10 μM thymoquinone and 20 μM diosgenin for 48 h | |
| *Oral cancer (in-vivo) models* | |
| BALB/c AnN.CgFoxn/CrlNarl mice treated with 10 and 25 mg/kg body wt thymoquinone for 20 days | ↓ Tumor weight and volume (Sc et al., 2014) |
| DMBA induced hamster rats treated with 30 mg/kg body wt thymoquinone for 14 weeks | Potent chemopreventive efficacy (R. G and MA, 2010) |

**Table 2**
Anticancer activity of thymoquinone in different cancer *in-vitro* and *in-vivo* models (A. J et al., 2021).

| Experimental model and Intervention | Outcome |
|---|---|
| *Breast cancer models* | |
| MCF-7 and MDA-MB-231 cells treated with 40 μM thymoquinone for 12 h | ↑ Apoptosis; ↓ Cell proliferation (CC et al., 2013; Khurshid et al., 2020) |
| MCF-7 cells treated with 5–60 μg/mL thymoquinone for 48 h | |
| Mouse breast cancer cell line 4T1 treated with 5 μM/mL thymoquinone for 6 h | ↓ N-cadherin and ↑ E-cadherin regulation (MA et al., 2015) |
| NCr-Foxn1nu treated with 2 mg/kg thymoquinone bodyweight for 4 weeks | ↓ Bone metastasis (MK et al., 2018) |
| *Hepatic cancer models* | |
| HepG2 cells treated with 6–50 μM thymoquinone for 6, 12, 18 h | Stimulation of pro-apoptotic Bcl-xS ↑ TRAIL-induced cell death (AE et al., 2014) |
| HepG2 cells treated with 20, 40, 60, 80, and 100 μM thymoquinone for 24, 48, and 72 h | ↓ VEGF (E. A et al., 2015) |
| NDEA induce male Wistar strain albino rats treated with 20 mg/kg body weight of thymoquinone | Regulation of G1/S phase; ↓ Hepatic nodule formation (R. S et al., 2013) |
| DENA induced hepatocarcinogenesis male Wistar rats treated with 250 mg/kg/day thymoquinone for 5 days | ↓ TNF-α, IL-6 levels and iNOS activity (F. M, 2018) |
| *Cervical cancer models* | |
| SiHa and C33A cells treated with 10–100 μM thymoquinone for 22 h | ↑ Apoptosis (SJ et al., 2014) |
| HeLa cells treated with 0.03 to 2 μL/mL and 6.25–100 μM thymoquinone for 48 h | ↑ Apoptosis ↓ Cell growth, migration, and proliferation (S. C, 2013; Li et al., 2017) |
| SiHa and CaSki cells treated with 1–40 μM thymoquinone for 12–48 h | |
| *Ovarian cancer models* | |
| Murine ID8-NGL cells treated with 25 μM thymoquinone for 24 h | ↑ Apoptosis and p65 ↓ NF-κβ, TNF-α, and IL-1β (AJ et al., 2015) |
| ID8-NGL treated C57BL/6 mice treated with 20 mg/kg thymoquinone thrice weekly for 10 days and 30 days | ↑ DNA damage (AJ et al., 2013) |
| *Bone cancer models* | |
| SaOS-2 cells treated with 20–80 μmol/l thymoquinone for 24 h | ↓ Chemo-resistance and angiogenesis (P. L et al., 2013) |
| Male athymic BALB/c nu/nu mice treated with 6 mg/kg/day thymoquinone for 15 days | ↓ Tumor angiogenesis (P. L et al., 2013) |
| *Brain cancer model* | |
| U87 cells treated with 100 mM thymoquinone for 24 h | α/β tubulin degradation (A. M et al., 2012) |
| *Prostate cancer models* | |
| DU-145 and PC3 cells treated with 0.1–10 μM thymoquinone for 24 h | Reverse of EMT ↓ Metastatic phenotype (K. B et al., 2017) |
| LnCaP cells treated with 1–50 μM thymoquinone for 24 h | ↑ Apoptosis by activating caspase-9 (K. G et al., 2018) |

(luciferase-expressing SASVO3 bearing) mouse xenograft model, where thymoquinone administration by oral gavage reduced the growth of the tumor *via* induced autophagy and apoptosis. A 3.04-fold reduction in tumor weight after 20 days with 25 mg/kg thymoquinone treatment showed anti-tumor effects *in vivo*.

A study on the UMSCC-14C oral cell line by Alaufi et al. (2017) using thymoquinone alone or in combination with cisplatin showed promising results. Thymoquinone when given alone, exerted considerable cytotoxicity and increased apoptotic cells (96.7 ± 1.6%) in UMSCC-14C when time extended beyond 6 h. UMSCC-14C cells apoptosis increased by up to 99.3 ± 1.2% when thymoquinone is given in combination with cisplatin. The potential mechanism of apoptosis has been shown by a significant upregulation of p53, caspase-9, and a decrease in anti-apoptotic Bcl-2 protein expression. In a study, R. G and MA (2010) reported that an oral administration of thymoquinone (30 mg/kg body weight) has the ability to reduce tumor cell propagation in the neck pouches *in-vivo* DMBA painted hamsters. Thymoquinone also down-regulates p38β MAPK and induction of chemo-preventive effects in oral cancer cells (T28) (A. E et al., 2013).

The potential of therapeutic application of *Nigella sativa*'s seeds for the inhibition of cancerous cells in the oral mucosa was proved by Dagtas and Griffin (2021). Thymoquinone alone demonstrated 30% SCC VII oral cancer cells death at 3 h. Seed extract (5% v/v) of *Nigella sativa* showed early cell detachment and cell death devoid of apoptosis. It was found that both the seed coat and the inner seed of *Nigella sativa* have active constituents like thymoquinone and thymohydroquinone. Thymoquinone being a lipophilic compound, may need lipid-surfactant-based self-emulsifying formulations for enhanced oral bioavailability. Self-nano emulsifying drug delivery systems (SNEDDS) are a mixture of drugs, lipids, surfactants, and water-soluble co-solvents which can be converted into solid dosage form using the adsorbent method (AA et al., 2018). Alwadei et al. (2019) developed a novel liquid and solid SNEDDS containing thymoquinone and curcumin with increased drug loading and dissolution rate. Other important *in-vitro* and *in-vivo* experimental models involved in the thymoquinone-mediated anticancer activities have been shown in Tables 1 and 2.

## 4. Nanotechnological intervention for lung and oral cancer treatment

The nano drug carriers (1–100 nm) have a uniform shape and size and provide the enhanced surface charges to carry drugs of our interest to the targeted destination. Thymoquinone is a potent anti-cancer therapeutic compound affecting multiple signaling pathways, but it has therapeutic limitations due to its poor solubility in the aqueous medium. Nanotechnology expands the application by enhancing the bioavailability and effectiveness, reducing the toxicity, and facilitating the delivery at the specific site. Other advantages of thymoquinone nanoformulations are targeted delivery with sustained release, biodegradability and biocompatibility of nanomaterial, protection of thymoquinone from degradation, prolonged circulation, and enhanced permeability retention (EPR) effect. Oral liquid thymoquinone formulations enhanced the solubility, photo-stability, and 6-fold increase of oral bioavailability (N. T. et al., 2016).

*In-vivo* studies on rats reported hepatic and renal toxicity with high doses of thymoquinone (*Acute and subchronic toxicity of thymoquinone in mice - Badary*, 1998). Liposomes encapsulate biological active compounds that are released at specific sites, thus limiting the associated side effects. Therefore, liposomes act as a solubilizing agent and carry thymoquinone, which enhances their bioavailability and uptake by cells.

Khan A (PubMed, n.d.) proposed a liposomal-based nanoparticle formulation of thymoquinone against aryl hydrocarbon receptor (AhR)-induced cancer in *in-vitro* and *in-vivo* lung cancer models.

A study by HM (2019) evaluated the anti-cancer effects of thymoquinone encapsulated liposomes and thymoquinone-curcumin-encapsulated liposomes on the A549 cancer cells and found the inhibition of cancer cell proliferation by enhancing cell uptake and inducing oxidative stress damage-causing DNA damage and cell apoptosis when treated with TQ-curcumin-rhodamine B-encapsulated DPPC liposome. The application of PEGylated PLGA thymoquinone nanoparticles in NSCLC cells showed improved delivery, thereby extending the therapeutic avenues of thymoquinone. U. P. (2019) investigated the transferrin receptor, which is over-expressed in NSCLC A549 cells, and developed PEGylated PLGA thymoquinone NP with transferrin (TF-TQ-Np). This facilitates the internalization of thymoquinone and acts as an anti-tumorigenic agent through the modulation of miR-34a and miR-16.

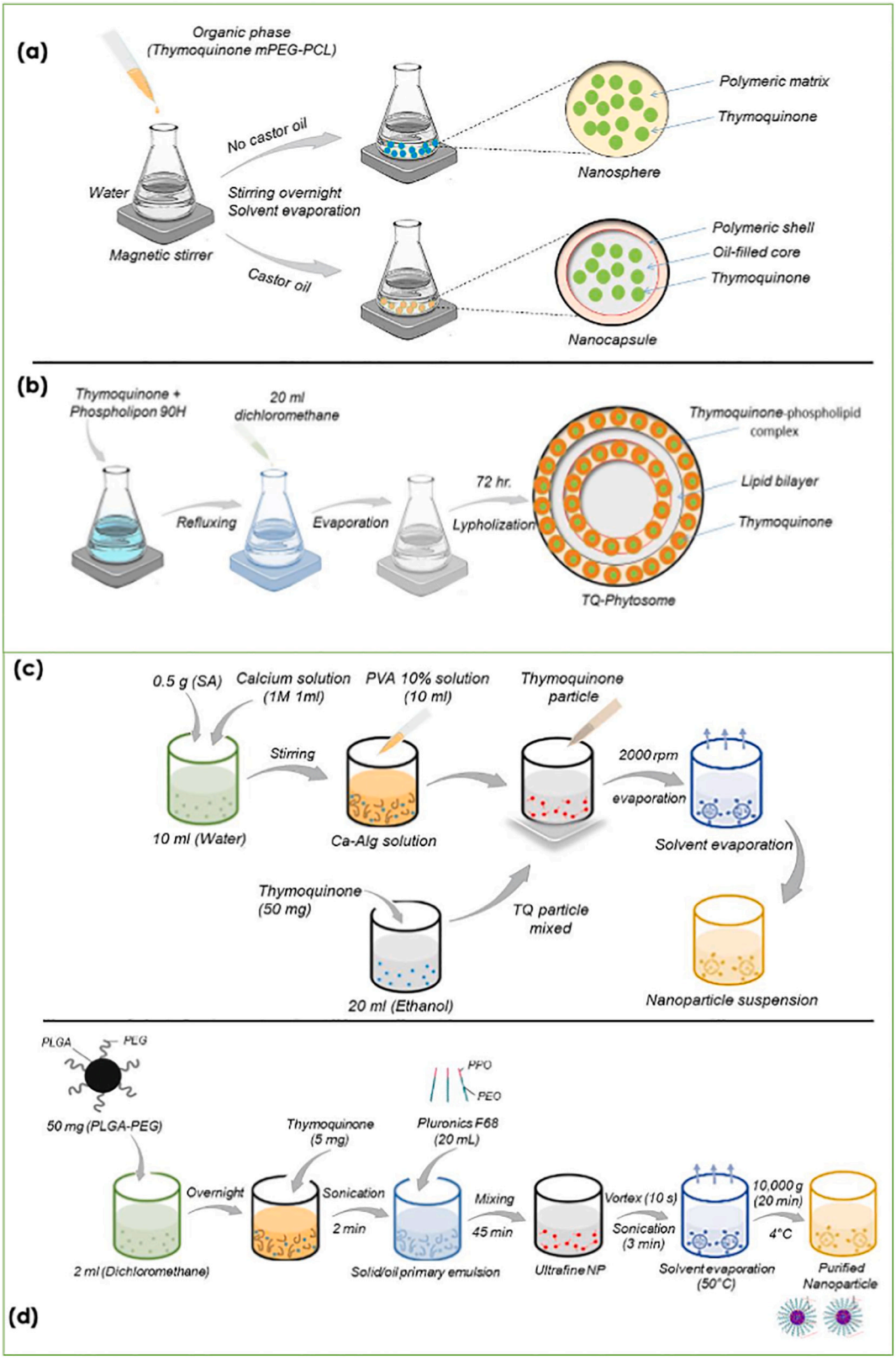


**Fig. 3.** Schematic depicting different methods of thymoquinone nanoformulation. a) Thymoquinone-encapsulated NP using mPEG-PCL copolymers forming F2-NC and NS using nano-precipitation technique; b) thymoquinone-phytosome nanoformulation prepared by combining refluxing with anti-solvent precipitation; c) thymoquinone loaded Ca-alg-PVA carrier was prepared by emulsion solvent evaporation method; d) thymoquinone-PLGA-PF68 nanoparticle formulations formed by emulsion–solvent evaporation technique forming hydrophobic core while intact thymoquinone is located within the core.

### 4.1. Preparation and chemistry behind nanoformulations

Nanotechnology-based drug delivery systems are emerging for their wider applications. Various researchers have developed different nanocarriers for efficient delivery of thymoquinone, such as liposomes, niosomes, nanoparticles, and nanostructured lipid carriers (Fig. 3). S. S. et al. (2020) employed the nanoprecipitation technique to develop thymoquinone-encapsulated nanoparticles using methoxy poly (ethylene glycol)-b-polycaprolactone (mPEG-PCL) copolymers. Reservoir-type nanocapsules (F2-NC) and matrix-type nanospheres (NS) were prepared with 60% thymoquinone loading and sustained *in-vitro* drug release efficiency. A pilot pharmacokinetic study with F2-NC in a murine model revealed a 1.3 fold increase in bioavailability compared to free thymoquinone. A novel drug delivery strategy based on phytosome utilizes the hydrogen bond interaction between the bioactive compound and polar section of phospholipid. This novel formulation enhances the oral bioavailability of the active phytoconstituents (Lu et al., 2019). Alhakamy et al. (2020) developed thymoquinone-phytosome by combining refluxing with anti-solvent precipitation. The $IC_{50}$ value of thymoquinone-phytosomes against the A549 cell line was evaluated as 4.31 ± 2.21 μM showed dose-dependent cytotoxicity. Elevated ROS generation accompanied with caspase-3 activation induced increased apoptotic induction and cell necrosis was reported in A549 cells. Loss of mitochondrial membrane potential due to ROS-based activation of Akt may be the reason for the increased apoptotic activity.

#### 4.1.1. Thymoquinone loaded calcium alginate and polyvinyl alcohol carrier

The emulsion-solvent evaporation method was used to formulate a thymoquinone loaded Ca-alg-PVA carrier having effective chemotherapeutic potential in oral cancer (Pu et al., 2021). In 7,12-dimethylbenzaanthracene (DMBA) stimulated hamster buccal pouch cancer in the Syrian hamster model, oral administration of 20 mg/kg bw showed restoration of antioxidants, detoxification enzymes, downregulation of NF-κβp50/p65 and PI3K/Akt/mTOR mRNA expression.

### 4.2. Thymoquinone-PLGA-PF68 nanoparticles

TQ-PLGA-PF68 NP's were prepared by encapsulating thymoquinone with polylactic acid/polyglycolic acid (PLGA) and polyethylene glycol (PEG)-500 using the emulsion solvent evaporation method (Noor et al., 2021).

## 5. Thymoquinone: targeting multiple cancers

Thymoquinone exhibits antiproliferative activities against various types of cancerous cells including breast cancer, colon, hepatic cancer, pancreatic, cervical, renal, blood, skin, ovarian, and prostate cancer (A. J et al., 2021). In MDA-MB-468 breast cancer cells, thymoquinone executes antiproliferative activities by promoting apoptosis (p38 phosphorylation), production of ROS, activation of p53, p21, and Bax, decreasing Bcl-2 (CC et al., 2013). Particularly, in human colorectal adenocarcinoma (HT-29) cells, thymoquinone reduces the cellular viability by promoting ROS-dependent apoptosis (caspase 3 and 7 mediated), narcosis (Mc et al., 2017). In the case of the hepatic HepG2 cell line, thymoquinone arrest the cell cycle at G2/M phases and the increased concentration of Bax/Bcl-2 induces the caspase 3 and 9 mediated apoptosis (E. A et al., 2015). Thymoquinone-dependent induction of EMT-regulatory proteins and cancer metastasis have been reported in a dose-dependent manner. Thymoquinone doses increase promoter DNA methylation of the TWIST1 gene in BT549 cells. This results, in thymoquinone-based inhibition of TWIST1 promoter activity and decreases its expression, leading the cancer cell migration, invasion, and metastasis (Ashour et al., 2016; Khan et al., 2015; Tania et al., 2014). Besides, thymoquinone induces the anti-proliferative activity against pancreatic carcinoma (human-HS766T cells) by upregulation of the p21 gene and inhibits the histone deacetylase activity (C. N et al., 2009). In a cervical cancer cell line (C33A), TQ induces the caspase-3 mediated apoptosis and inhibits invasion and migration in CaSki cells, and arrests the cell cycle in SiHa cells at sub-G1 phases (Li et al., 2017). In human T lymphocytes (Jurkat cells), thymoquinone showed anticancer activity by degrading α/β tubulin with upregulation of the p73 tumor suppressor gene (A. M et al., 2012). A reduction in cell viability and enhanced apoptosis have been observed in human melanoma (skin) cancer MDA-MB-435 cells (MA et al., 2015). A short description of other cancer types is mentioned in Table 2 (A. J et al., 2021).

## 6. Conclusions and future perspectives

Cancer causes one of the leading concerns of death worldwide. Advancement in science and technology in the last few decades has been enormous. Phyto-compounds having anti-cancerous and anti-proliferative activity are traditionally in use for treating many diseases. However, exploration of these plant-based compounds and articulating with nanotechnology enhances their utility and effectiveness in combating deadly cancers. Thymoquinone which is known to have anti-cancerous activity has minimum side effects. Because of its poor solubility in an aqueous solution, it can be encapsulated, entrapped, or loaded into different carriers forming various nano-formulations. This enhances the specificity, release efficiency, and targeted application of thymoquinone as an anti-cancerous ingredient. The role of thymoquinone and its various nanoformulations for targeted delivery to tumorigenic cells for improved anti-inflammatory and anticancer potentials. These nanoformulation deliveries of Thymoquinone reported significantly increase the targeting payload and promising upgrades to its anticancer efficacy. However, the mechanistic of nanoformulated thymoquinone impact on cancer cell line is still not fully understood and needs to be investigated. The selective nanoformulations to improve the sensitivity of therapeutic agents are one of the major challenges in cancer treatment that need to be investigated.

## Ethics approval and consent to participate

Not applicable.

## CRediT authorship contribution statement

ST designed the review, drafted the manuscript, and prepared the figures and tables. VT designed the review, drafted the manuscript, and prepared the figures and tables. NR revised the manuscript. SJAI revised the manuscript. PM revised the manuscript. WHS revised the manuscript. All the authors revised, finalized, and approved the submitted version of the manuscript.

## Declaration of competing interest

There is no financial or commercial conflict of interest.

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