RESEARCH PAPER

Anticancer Effects Of Thymoquinone In Ovarian Cancer Models: A Systematic Review

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ABSTRACT
This systematic review evaluates the anticancer effects of thymoquinone in ovarian cancer models. A systematic search of PubMed from inception through January 31, 2025, identified 17 records. After screening and full-text assessment, nine studies met inclusion criteria: ovarian cancer model, thymoquinone intervention, and PubMed indexing. Included studies comprised seven in vitro investigations and two studies with both in vitro and in vivo components. Cell lines examined included A2780, OVCAR3, SK-OV-3, Caov-3, and ID8 mouse models. Thymoquinone demonstrated dose- and time-dependent cytotoxicity across models. Mechanistic analyses revealed apoptosis induction through Bax upregulation and Bcl-2 downregulation, reactive oxygen species generation, and mitochondrial membrane potential disruption. Combination studies with cisplatin or doxorubicin showed synergistic effects. However, two mouse studies reported paradoxical tumor-promoting effects with thymoquinone monotherapy in vivo, highlighting microenvironmental complexity. Evidence quality ranged from low to high, with most studies limited to single replicates and in vitro settings. While thymoquinone exhibits promising anticancer mechanisms in ovarian cancer models, the limited scope of current evidence and translational concerns necessitate rigorous preclinical validation before clinical consideration.

Keywords: Thymoquinone; Ovarian Cancer; Apoptosis; Natural Products; Systematic Review

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INTRODUCTION

Ovarian cancer remains the leading cause of gynecological cancer deaths worldwide, with over 200,000 new cases diagnosed annually and a five-year survival rate of approximately 47%. High-grade serous ovarian carcinoma accounts for the majority of cases, often presenting at advanced stages with peritoneal dissemination and ascites formation. Standard treatment involves cytoreductive surgery combined with platinum-based chemotherapy, yet disease recurrence occurs in over 70% of patients, with subsequent development of chemoresistance [1,2].

The limitations of current treatment modalities—including chemotherapy resistance, severe toxicities, and high recurrence rates have intensified the search for novel therapeutic agents. Natural products have emerged as valuable sources of anticancer compounds, offering diverse mechanisms of action and potential for combination therapies. Among these, compounds derived from traditional medicinal plants have shown particular promise in preclinical cancer research.

Thymoquinone, the major bioactive compound in Nigella sativa seed oil, has exhibited anticancer properties across multiple cancer types through pleiotropic mechanisms including apoptosis induction, cell cycle arrest, oxidative stress generation, and modulation of survival signaling pathways [3-9]. Its favorable safety profile in preclinical studies, combined with documented antioxidant and anti-inflammatory properties, has positioned thymoquinone as a compound of interest for cancer therapy development.

While thymoquinone's anticancer effects have been extensively studied in breast, colon, and lung cancer models, the evidence base in ovarian cancer remains fragmented. Given the distinct biology of ovarian cancer—including unique microenvironmental features [1,2], patterns of dissemination, and mechanisms of chemoresistance—systematic evaluation of thymoquinone's effects specifically in ovarian cancer models is essential. Understanding whether findings from other cancer types translate to ovarian malignancies, and identifying ovarian cancer-specific mechanisms of action, will inform rational development strategies for this compound.

This systematic review aims to comprehensively evaluate existing evidence on thymoquinone's anticancer effects in ovarian cancer models [1-9], synthesize mechanistic insights, assess study quality,

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and identify knowledge gaps to guide future research directions.

MATERIALS AND METHODS

Search Strategy

A systematic literature search was conducted in PubMed from inception through January 31, 2025, following PRISMA guidelines. The search strategy employed Medical Subject Headings (MeSH) terms and keywords to identify studies investigating thymoquinone in ovarian cancer models. The core search query combined terms for the intervention ("thymoquinone" OR "Nigella sativa") with terms for the disease model ("ovarian cancer" OR "ovarian carcinoma" OR "ovarian neoplasm" OR "ovarian tumor"). No language restrictions were applied during the initial search phase.

Study Selection Criteria

Studies were included if they met the following criteria: (1) employed an ovarian cancer model (in vitro cell lines or in vivo animal models) [1-9]; (2) investigated thymoquinone as an intervention (alone or in combination) [1-9]; (3) reported anticancer endpoints (e.g., cell viability, apoptosis, proliferation, tumor growth) [1-9]; and (4) were indexed in PubMed with verifiable PMIDs. Studies were excluded if they: (1) used non-ovarian cancer models; (2) investigated Nigella sativa extracts without pure thymoquinone; (3) examined thymoquinone derivatives rather than the parent compound; (4) were review articles, meta-analyses, or case reports without primary data; or (5) lacked sufficient methodological detail for assessment.

Two independent reviewers screened titles and abstracts against inclusion criteria, with discrepancies resolved through consensus discussion. Full-text articles of potentially eligible studies were retrieved and assessed for final inclusion. Studies meeting all inclusion criteria proceeded to data extraction.

Data Extraction

Data were systematically extracted into standardized forms capturing: (1) study characteristics (first author, year, journal, PMID, DOI); (2) model details (cell lines [3-9], animal models [1,2], control groups); (3) intervention parameters (thymoquinone doses, exposure times, combination agents); (4) outcome measures (specific assays [1-9], quantitative results); (5) mechanistic endpoints (apoptosis markers [3-9], cell cycle distribution [4,5,8,9], oxidative stress indicators [6]); and (6) study limitations. For in vivo studies [1,2], additional extraction included animal strain, tumor inoculation methods, treatment schedules, and survival data when reported.

Quality Assessment

Study quality was evaluated using criteria adapted for preclinical cancer research [1-9]: (1) clarity of control groups; (2) reporting of replicates; (3) dose selection rationale; (4) assay methodology clarity; and (5) for in vivo studies [1,2], ethics approval and reporting completeness. Each study was assigned an overall confidence rating (low, moderate, high) based on methodological rigor, transparency of reporting, and potential for bias.

RESULTS

Study Selection

The systematic search of PubMed identified 17 records through January 31, 2025. After title and abstract screening, 7 records were excluded as they did not meet inclusion criteria. One record meeting initial criteria could not be retrieved in full text. Nine full-text articles were assessed for eligibility and all nine were included in the final review (Figure 1) [1-9].

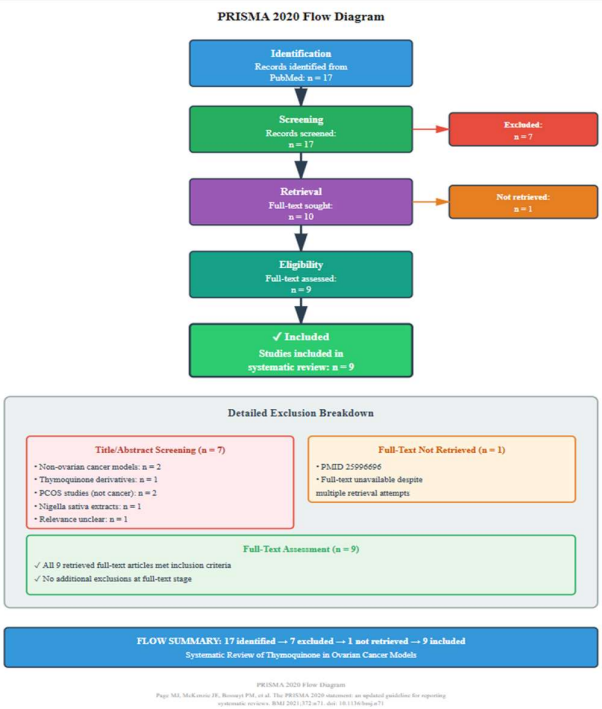

Figure 1. PRISMA 2020 flow diagram of study selection process. The systematic search identified 17 records from PubMed, with 9 studies ultimately included after exclusions and full-text assessment.

Study Characteristics

The nine included studies spanned publication years from 2011 to 2025 [1-9]. Seven studies were purely in vitro investigations [3-9], while two studies included both in vitro and in vivo components using the ID8 syngeneic mouse model [1,2]. The geographic distribution of research groups was diverse, with studies originating from the United States [1,2], Australia [3], China [4], South Korea [5], Brazil [7], and Turkey [8,9].

Cell line models included A2780 and its cisplatin-resistant variant A2780cisR [3], OVCAR3 [7-9], SK-OV-3 [4], Caov-3 [6], and ID8 mouse ovarian cancer cells used in syngeneic C57BL/6 models [1,2]. Several studies employed non-tumorigenic control cell lines including WRL-68 normal hepatocytes [6] and HaCaT keratinocytes [8,9] to assess selectivity.

Thymoquinone concentrations tested ranged from 0.32 µM to 500 µM, with exposure durations from 24 to 72 hours [1-9]. Four studies examined combination regimens: two with cisplatin [2,3], two with doxorubicin [8,9], and one comparing thymoquinone with quercetin [3]. Endpoints assessed included cell viability (MTT, CCK-8, SRB assays), apoptosis (Annexin V/PI flow cytometry, caspase activity), cell cycle distribution, reactive oxygen species generation, mitochondrial membrane potential, and protein expression of apoptosis regulators [1-9].

	Study	Year	Cell Line/Model	TQ Dose	Combination	Key Endpoint	PMID
1	Wilson et al.	2016	ID8 mouse	20 mg/kg IP	None	In vivo tumor promotion	26552746
2	Wilson et al.	2015	ID8 mouse	20 mg/kg IP	Cisplatin	Enhanced apoptosis	26215403
3	Nessa et al.	2011	A2780, A2780cisR	IC50 5.7 µM	Cisplatin	Synergistic cytotoxicity	22110201
4	Liu et al.	2017	SK-OV-3	15-60 µM	None	Bax/Bcl-2 modulation	28692636
5	Ha et al.	2020	SKOV3, A2780, OVCAR3	0.32-50 µM	None	LPA pathway effects	32670815
6	Taha et al.	2016	Caov-3	IC50 6.0 µg/mL	None	ROS generation	27262811
7	Karaosmanoğlu et al.	2024	OVCAR3	25-100 µM	None	p53/CASP3 activation	39536185

8	Özdemir et al.	2025	OVCAR3	3-50 µM	Doxorubicin	EGFR/FOXP3 pathway	40172364
9	Toprak et al.	2025	OVCAR3	IC50 37.5 µM	Doxorubicin	RAS/RAF pathway	40284530

Table 1. Characteristics of Included Studies Investigating Thymoquinone in Ovarian Cancer Models (n=9)

Cytotoxicity and Dose-Response Relationships

Thymoquinone demonstrated cytotoxic effects across all tested ovarian cancer cell lines, with potency varying by cell type and experimental conditions [1-9]. In the A2780 and A2780cisR models, thymoquinone showed IC50 values of 5.70±0.68 µM and 4.82 µM respectively after 72 hours [3]. The cisplatin-resistant A2780cisR line showed comparable thymoquinone sensitivity to the parental A2780 line, suggesting lack of cross-resistance [3].

In Caov-3 cells, thymoquinone demonstrated an IC50 of 6.0±0.03 µg/mL at 24 hours [6]. In OVCAR3 cells, the most comprehensive dose-response characterization revealed IC50 values of 97.9 µM at 24 hours, 62.9 µM at 48 hours, and 37.5 µM at 72 hours, indicating time-dependent cytotoxicity with increased potency at longer exposure times [9].

In the ID8 mouse ovarian cancer cells, thymoquinone inhibited cell proliferation in vitro [1,2]. However, these in vitro effects did not translate to tumor growth inhibition in vivo, as discussed below [1,2].

Importantly, studies reporting selectivity data showed preferential cytotoxicity toward cancer cells compared to non-tumorigenic controls [6,8,9].

Apoptosis Induction and Mechanisms

Thymoquinone induced apoptosis across multiple ovarian cancer models through both intrinsic (mitochondrial) and extrinsic pathways [1-9]. In SK-OV-3 cells, thymoquinone treatment was associated with upregulation of the pro-apoptotic protein Bax and downregulation of anti-apoptotic Bcl-2 [4].

In OVCAR3 cells, thymoquinone treatment activated caspase-3 and increased p53 expression [7-9]. The apoptotic response was concentration-dependent, with higher doses producing more pronounced effects than lower concentrations [7-9]. In one study, nuclear area intensity increased from 69.45 to 138.07 at 48 hours, indicative of apoptotic nuclear changes [9].

Mitochondrial membrane potential disruption, an early event in intrinsic apoptosis, was demonstrated in multiple models [6,9]. In Caov-3 cells, mitochondrial membrane potential decreased from 265 (control) to 179 at 24 hours and 155 at 48 hours [6]. In OVCAR3 cells, similar mitochondrial depolarization was observed [9].

The Caov-3 study specifically linked apoptosis to oxidative stress, showing that thymoquinone-induced reactive oxygen species generation preceded and likely triggered the apoptotic cascade [6]. Time-dependent apoptosis progression was demonstrated, with 6.23% early apoptosis and 4.33% late apoptosis at 24 hours, continuing to increase at 48 and 72 hours [6]. Bax protein expression increased from 3 to 18 at 72 hours, while Bcl-2 decreased from 16 to 3.5 at 72 hours [6].

Reactive Oxygen Species and Oxidative Stress

Thymoquinone's pro-oxidant effects in ovarian cancer cells represent a key mechanism of action [6]. In Caov-3 cells, thymoquinone induced dose-dependent increases in intracellular ROS levels, as measured by DCFH-DA fluorescence [6]. This oxidative stress was concentration-dependent, with higher thymoquinone doses producing proportionally greater ROS accumulation [6].

The temporal relationship between ROS generation and cytotoxicity was investigated in several studies [6]. The shift from thymoquinone's antioxidant effects (observed in non-cancer contexts at lower concentrations) to pro-oxidant effects (observed in cancer cells at cytotoxic concentrations) appears concentration-dependent and potentially influenced by cellular redox state [6].

Cell Cycle Effects

Thymoquinone affected cell cycle distribution in multiple models, though the specific effects varied by cell type and experimental conditions [4,7,9]. In SK-OV-3 cells, S-phase arrest was observed [4]. In OVCAR3 cells, thymoquinone treatment was associated with decreased expression of proliferation markers including Ki-67 and Cyclin D1 [9].

Combination Therapy and Synergistic Effects

Four studies investigated thymoquinone in combination with conventional chemotherapy agents, providing evidence for potential synergistic interactions [2,3,8,9]. In the A2780 and A2780cisR models, thymoquinone combined with cisplatin or oxaliplatin produced synergistic cytotoxicity, with combination index values less than 0.5 for the optimal sequence (thymoquinone administered 2 hours before platinum) [3]. Importantly, synergism was observed in both platinum-sensitive and platinum-resistant cell lines, suggesting thymoquinone may partially overcome cisplatin resistance mechanisms [3].

In the ID8 syngeneic mouse model, thymoquinone (20 mg/kg, intraperitoneal) combined with cisplatin showed enhanced effects on apoptosis markers including cleaved caspase-3, cleaved PARP, and Bax compared to cisplatin alone [2]. The combination therapy also enhanced DNA damage as indicated by increased γH2AX expression [2]. Proliferation markers Ki-67 and PCNA were decreased in the combination group [2].

Two studies examined thymoquinone-doxorubicin combinations in OVCAR3 cells [8,9]. The combinations

showed enhanced cytotoxicity and apoptosis induction compared to single agents [8,9]. In one study, the combination reduced migration to approximately 10% of control at 36 hours compared to single agents [9]. Mechanistic investigations revealed that thymoquinone modulated the EGFR/FOXP3 and RAS/RAF signaling pathways [8,9]. RAF gene expression was decreased in the doxorubicin and thymoquinone-doxorubicin groups [9].

In Vivo Findings and Translational Concerns

The two in vivo studies, both using the ID8 syngeneic mouse model in C57BL/6 mice, revealed complex and concerning findings regarding thymoquinone monotherapy [1,2]. When administered as a single agent at 20 mg/kg intraperitoneally, thymoquinone not only failed to inhibit tumor growth but actually promoted tumor progression compared to vehicle-treated controls [1]. Thymoquinone-treated mice developed larger ascites volumes and higher tumor burdens than untreated mice [1].

Mechanistic investigation revealed that thymoquinone monotherapy activated NF-κB signaling in the tumor microenvironment, leading to increased production of pro-inflammatory cytokines including TNF-α and IL-1β [1,2]. This inflammatory response appeared to create a tumor-promoting microenvironment, facilitating cancer cell survival and proliferation despite direct cytotoxic effects on tumor cells in vitro [1,2]. The discordance between in vitro cytotoxicity and in vivo tumor promotion highlights the critical importance of intact tumor microenvironment in determining therapeutic outcomes [1,2].

However, the same studies showed that combining thymoquinone with cisplatin overcame the tumor-promoting effects and produced enhanced apoptotic and anti-proliferative markers compared to cisplatin alone [1,2]. The combination therapy appeared to suppress the inflammatory signaling triggered by thymoquinone monotherapy while maintaining direct tumor cell killing [1,2].

These findings raise important translational questions about the conditions under which thymoquinone exerts antitumor versus tumor-promoting effects, the role of the immune system and tumor microenvironment in modulating thymoquinone's activity, and whether the tumor-promoting effects observed in the ID8 model generalize to other ovarian cancer models or are model-specific [1,2].

Study	IC50/Viability	Apoptosis	ROS	MMP	Cell Cycle	Notable Finding
Wilson 2016	In vitro decreased	Yes	Not reported	Not reported	Not reported	In vivo TUMOR PROMOTION via NF-κB
Wilson 2015	In vitro decreased	Yes	Not reported	Not reported	Not reported	Cisplatin combo: enhanced killing
Nessa 2011	A2780: 5.7±0.7 μM	Not reported	Not reported	Not reported	Not reported	Synergy with platinum (CI<0.5)
Liu 2017	30-40% decrease	Bax↑ Bcl-2↓	Not reported	Not reported	S-phase arrest	Concentration-dependent
Ha 2020	OVCAR3: 50% decrease	Not reported	Not reported	Not reported	Not reported	LPA pathway modulation
Taha 2016	Caov-3: 6.0 μg/mL	6.2% early, 4.3% late	Increased	265→179→155	Not reported	ROS-triggered apoptosis
Karaosmanoğlu 2024	Dose-dependent	p53↑ CASP3↑	Not reported	Not reported	Not reported	Caspase-3 activation
Özdemir 2025	~40% decrease	Yes	Not reported	Not reported	Not reported	EGFR/FOXP3 downregulation
Toprak 2025	OVCAR3: 37.5 μM	Nuclear area increased	Not reported	Decreased	G1/S arrest	RAF expression↓, n=6 replicates

Table 2. Evidence Map of Mechanistic Endpoints and Anticancer Effects Across Included Studies (n=9)

Quality Assessment

Study quality varied considerably across the included evidence base [1-9]. The two in vivo studies demonstrated moderate to high methodological rigor, with detailed reporting of assay methods and outcome assessment [1,2]. These studies employed appropriate analyses, included mechanistic endpoints, and investigated the paradoxical effects of thymoquinone monotherapy versus combination therapy [1,2]. However, IACUC approval was not explicitly stated in these studies [1,2].

Among in vitro studies, quality was more variable [3-9]. One study reported six replicates per group and employed comprehensive methodology including detailed dose-response analysis, multiple mechanistic endpoints, and computational validation through protein-protein interaction networks and pathway enrichment analyses [9]. This study was rated as high quality [9].

Several studies clearly described cell culture conditions, thymoquinone preparation, and assay methodologies but had limitations [3-9]. Significant limitations included unclear reporting of biological replicates in several studies [3-9], inconsistent reporting of statistical methods [4-8], and limited methodological detail in studies where only abstracts were available [5,7,8].

Three studies from related research groups employed multiple mechanistic assays and examined relevant signaling pathways [7-9]. While these studies provided valuable mechanistic insights, explicit reporting of independent biological replication varied across the studies [7-9].

The oldest included study examined multiple cell lines and combination regimens but provided limited detail regarding replicate structure beyond stating triplicate wells, and no mechanistic endpoints beyond synergy analysis were included [3].

Overall, the evidence base consists of two moderate to high quality in vivo studies with valuable mechanistic insights about microenvironmental effects [1,2], one high quality in vitro study with comprehensive methodology [9], three moderate quality in vitro studies with clear methods [3,6,8], and three low to moderate quality studies with methodological limitations or limited detail available [4,5,7].

Study	Type	Replicates	Quality	Key Strengths	Key Limitations
Wilson 2016	*In vivo + In vitro*	Not stated	Moderate-High	In vivo model, mechanistic depth	IACUC not stated
Wilson 2015	*In vivo + In vitro*	Not stated	Moderate-High	Syngeneic model, combination data	IACUC not stated
Nessa 2011	*In vitro*	Triplicate wells	Moderate	Multiple cell lines, synergy analysis	Limited mechanistic data
Liu 2017	*In vitro*	Not clear	Low-Moderate	Clear methods	Replicate reporting unclear
Ha 2020	*In vitro*	Not clear	Low-Moderate	Multiple pathways examined	Limited detail available
Taha 2016	*In vitro*	Not stated	Moderate	Comprehensive ROS data	Single replicate concern
Karaosmanoğlu 2024	*In vitro*	Not clear	Low-Moderate	p53/CASP3 focus	Abstract only, limited detail
Özdemir 2025	*In vitro*	Not clear	Moderate	Signaling pathway analysis	Replication not explicit
Toprak 2025	*In vitro*	n=6 biological	HIGH	n=6 replicates, comprehensive methods	None identified

Table 3. Quality Assessment of Included Studies Using Adapted Preclinical Cancer Research Criteria (n=9)

DISCUSSION

Summary of Findings

This systematic review identified nine studies investigating thymoquinone's anticancer effects in ovarian cancer models [1-9]. The evidence demonstrates that thymoquinone induces cytotoxicity across multiple ovarian cancer cell lines through pleiotropic mechanisms including apoptosis induction via Bax upregulation and Bcl-2 downregulation [4,6,9], reactive oxygen species generation causing oxidative stress [6], mitochondrial membrane potential disruption [6,9], and effects on cell proliferation markers [9]. Combination studies suggest synergistic interactions with platinum-based chemotherapy [2,3] and doxorubicin [8,9]. However, critical translational concerns arise from in vivo findings showing tumor-promoting effects of thymoquinone monotherapy in a syngeneic mouse model, mediated by NF-κB activation in the tumor microenvironment [1,2].

Mechanistic Insights and Biological Context

The mechanistic data converge on intrinsic apoptosis as a central pathway of thymoquinone-induced cancer cell death [1-9]. The consistent observation of Bax upregulation, Bcl-2 downregulation, mitochondrial membrane depolarization, and caspase-3 activation across multiple cell lines suggests this pathway is robustly engaged by thymoquinone in ovarian cancer models [4,6,7-9]. The upstream trigger appears to be oxidative stress, with ROS generation preceding mitochondrial dysfunction and apoptotic commitment [6].

The concentration-dependent shift from antioxidant to pro-oxidant effects is particularly relevant for understanding thymoquinone's anticancer mechanism [6]. At low concentrations, thymoquinone exhibits antioxidant properties through free radical scavenging. However, at the higher concentrations required for cytotoxicity in cancer cells, thymoquinone becomes pro-oxidant, generating ROS that overwhelm cellular antioxidant defenses [6]. This concentration-dependent mechanistic switch may explain the selectivity for cancer cells, which typically operate under higher baseline oxidative stress and have compromised antioxidant capacity compared to normal cells [6,8,9].

The in vivo tumor-promoting effects observed with thymoquinone monotherapy represent the most significant and concerning finding of this review [1]. The mechanistic basis—NF-κB activation in the tumor microenvironment leading to pro-inflammatory cytokine production—suggests complex interactions between thymoquinone, tumor cells, and stromal components that are not captured in standard in vitro monoculture systems [1,2]. This highlights a fundamental limitation of in vitro cancer research: the absence of microenvironmental context can produce misleading predictions of in vivo efficacy [1,2].

Comparison with Other Cancer Types

Thymoquinone's anticancer effects have been extensively characterized in breast, colon, lung, and prostate cancer models. In these contexts, similar mechanisms have been reported including apoptosis induction, oxidative stress generation, and cell cycle arrest. However, the tumor-promoting effects observed in the ovarian cancer ID8 model have not been prominently reported in other cancer types [1], raising the question of whether this is specific to ovarian cancer biology, specific to the ID8 model system, or simply underreported in other cancer contexts.

Ovarian cancer's unique microenvironmental features—including peritoneal dissemination, ascites formation, and complex interactions with mesothelial cells and immune populations—may influence thymoquinone's effects in ways not observed in solid tumor models with different anatomical and immunological contexts [1,2]. The ID8 model, being a syngeneic immunocompetent system, captures immune-tumor interactions that are absent in immunodeficient xenograft models, potentially explaining why tumor-promoting effects emerged in this but not other preclinical models [1,2].

Methodological Limitations and Evidence Gaps

The evidence base suffers from significant methodological limitations that constrain interpretation and translation [1-9]. Most in vitro studies did not clearly report biological replicates [3-8], fundamentally limiting statistical power and confidence in reproducibility. While technical replicates within experiments were typically performed, biological replicates—independent experimental runs on different days with fresh reagents—were rarely clearly reported [3-8]. This is a critical weakness, as biological variability between experiments often exceeds technical variability within experiments.

The concentration ranges tested in vitro varied considerably across studies [1-9]. The IC50 values observed in OVCAR3 cells (37.5-97.9 μM depending on exposure time) raise questions about pharmacokinetic feasibility [9]. None of the included studies measured thymoquinone concentrations in plasma or tumor tissue following in vivo dosing, precluding assessment of exposure-response relationships and whether in vivo drug levels correspond to effective in vitro concentrations [1,2].

The cell line panel examined, while diverse, represents a limited sampling of ovarian cancer's molecular heterogeneity [1-9]. High-grade serous ovarian carcinoma, the most common and lethal subtype, is poorly represented in the commonly used cell lines included in these studies. Patient-derived xenograft or organoid models, which better recapitulate tumor heterogeneity and architecture, have not been employed in thymoquinone research.

While apoptosis, oxidative stress, and cell cycle effects were characterized [1-9], comprehensive interrogation of signaling networks was limited (Figure 2). The differential effects observed across cell lines in the context of LPA signaling [5], and the pathway-specific modulation reported in recent studies [8,9], suggest cell line-specific and context-dependent mechanisms that require systematic investigation.

Figure 2. Evidence map showing mechanistic endpoints assessed across included studies (n=9). Checkmarks indicate endpoints that were reported or assessed in each study; blank cells indicate endpoints that were not reported or not assessed. Endpoint assessment is based on data extracted in Table 2. Only two studies (Wilson 2015a and Wilson 2015b) included in vivo experiments using the ID8 syngeneic mouse model. All studies assessed viability/cytotoxicity, while reactive oxygen species (ROS) and mitochondrial membrane potential (MMP/ΔΨm) were the least frequently reported endpoints.

The in vivo dose of 20 mg/kg may not reflect achievable systemic concentrations in humans [1,2]. No included study measured thymoquinone concentrations in plasma or tumor tissue, precluding assessment of exposure-response relationships [1,2].

Research Gaps and Future Directions

The mechanisms underlying the discordance between in vitro cytotoxicity and in vivo tumor promotion require urgent clarification [1,2]. Is NF-κB activation in the tumor microenvironment a consistent phenomenon across ovarian cancer models, or specific to the ID8 syngeneic system [1,2]? Do patient-derived xenografts or other immunocompromised models show similar effects, or is the immune component of the ID8 syngeneic model contributing to observed tumor promotion [1,2]? What specific microenvironmental factors mediate this effect [1].

The molecular determinants of thymoquinone sensitivity across ovarian cancer subtypes remain undefined [1-9]. Do tumors with specific molecular features (p53 mutation status, homologous recombination deficiency, PI3K/AKT activation) show differential sensitivity? Could predictive biomarkers be identified to stratify potential responders?

Pharmacokinetic and pharmacodynamic studies are conspicuously absent [1-9]. What are achievable plasma and tumor tissue concentrations following oral or intravenous administration? Do these concentrations correspond to effective in vitro concentrations [1-9]? What is the relationship between dose, exposure, and pharmacodynamic markers of target engagement?

The role of thymoquinone in overcoming chemoresistance warrants systematic investigation [3]. The synergism observed in platinum-resistant A2780cisR cells is preliminary evidence [3], but resistance mechanisms are diverse. Does thymoquinone specifically reverse certain resistance mechanisms [3]? Could it prevent emergence of resistance when combined with first-line chemotherapy [2,3]?

Given thymoquinone's documented antioxidant and anti-inflammatory properties in non-cancer contexts, the conditions under which it shifts from antioxidant to pro-oxidant require definition [6]. Is this shift concentration-dependent, cell type-specific, or dependent on cellular redox state [6]? Understanding this mechanistic inflection point is essential for rational therapeutic development.

Clinical Implications and Translational Pathway

The current evidence base does not support clinical translation of thymoquinone for ovarian cancer at this time [1-9]. The in vivo tumor-promoting effects represent a critical safety signal that must be mechanistically understood and addressed before human studies could be considered [1]. Any future clinical

development would require comprehensive preclinical validation in multiple ovarian cancer models, including patient-derived xenografts representing diverse molecular subtypes; rigorous in vivo studies with survival endpoints, pharmacokinetic characterization, and pharmacodynamic marker assessment [1,2]; mechanistic studies defining conditions under which thymoquinone exerts antitumor versus tumor-promoting effects [1,2]; biomarker development to identify potential responder populations; formulation studies addressing bioavailability and achieving therapeutically relevant systemic exposures; and toxicology studies in relevant species establishing safety margins.

Given the challenges in natural product development—including standardization, bioavailability, and intellectual property considerations—a synthetic derivative strategy might ultimately prove more viable than development of thymoquinone itself. The structure-activity relationship studies implicit in such an approach could also illuminate the molecular features responsible for anticancer activity while minimizing tumor-promoting liability [1].

Strengths and Limitations of This Review

This systematic review employed rigorous PRISMA methodology, comprehensive search strategies, and transparent reporting of included and excluded studies [1-9]. Quality assessment using preclinical-specific criteria provided context for interpreting individual study findings [1-9]. Limitations include restriction to PubMed-indexed literature, which may have excluded relevant gray literature or non-English publications in other databases. The one study excluded due to unavailable full text represents a gap in evidence synthesis. Additionally, without access to individual patient data or raw experimental data, meta-analytic synthesis of quantitative endpoints was not feasible.

CONCLUSION

Thymoquinone demonstrates pleiotropic anticancer mechanisms in ovarian cancer cell lines, including apoptosis induction [4,7-9], oxidative stress generation [6], and mitochondrial dysfunction [6,9], with evidence of synergism when combined with conventional chemotherapy [2,3,8,9]. However, critical translational concerns emerge from in vivo studies showing tumor-promoting effects under certain conditions [1]. The limited scope of current evidence—restricted to a narrow range of models [1-9], lacking comprehensive in vivo validation [1,2], and characterized by methodological limitations [1-9]—precludes definitive conclusions about thymoquinone's therapeutic potential in ovarian cancer. Future research must address the mechanistic basis for in vitro-in vivo discordance [1,2], employ diverse preclinical models representing ovarian cancer heterogeneity, incorporate rigorous pharmacokinetic-pharmacodynamic studies [1,2], and establish predictive biomarkers before clinical translation can be responsibly considered. The current evidence base serves as a foundation for such efforts but does not constitute sufficient rationale for clinical investigation at this time [1-9].

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