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## REVIEW

# Anticancer effects of thymoquinone in head and neck squamous cell carcinoma: A scoping review

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**Abstract**

**Objective:** Thymoquinone (TQ), the active constituent of *Nigella sativa*, has been shown to have anticancer effects in head and neck squamous cell carcinoma (HNSCC). This review aims to outline the properties of TQ, the known drivers in HNSCC formation, and summarize the anticancer effects of TQ in SCC.

**Data Sources:** Three databases (PubMed, Embase, and Google Scholar) were queried for the key words “thymoquinone squamous cell carcinoma.”

**Review Methods:** Publications that were not original research and publications that did not have full-text available for review were excluded.

**Results:** Sixteen research articles met the inclusion criteria. Our review demonstrates that TQ-induced cytotoxicity is associated with increased expression and activity of the tumor suppressor p53, proapoptotic proteins Bax and caspases, as well as decreased expression and activity of antiapoptotic proteins Bcl-2 and Mdm2. Additionally, TQ modulates cell-survival pathways such as the PI3k/Akt pathway. TQ synergizes with therapeutics including cisplatin and radiation. Early TQ administration may prevent carcinogenesis via upregulation of antioxidant enzymes, and TQ administration in the presence of cancer can result in disease mitigation via induction of oxidative stress.

**Conclusion:** TQ acts as an upregulator of proapoptotic pathways and downregulator of antiapoptotic pathways, modulates the oxidative stress balance in tumor development, and works synergistically alongside other chemotherapeutics to increase cytotoxicity. TQ has the potential to prevent carcinogenesis in patients who are at high-risk for SCC and adjuvant treatment for SCC patients undergoing conventional treatments. Future studies should aim to identify specific populations in which TQ's effects would be the most beneficial.

**Level of Evidence:** Not available.

**KEYWORDS**

cutaneous < head and neck, molecular biology, scoping review, skin cancer < head and neck

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## 1 | INTRODUCTION

Cancer as a disease is multifaceted in its manifestation—it grows via dysregulated proliferative signaling, the evasion of growth suppressors, and the avoidance of immune cell destruction.<sup>1</sup> The current paradigm for treatment mostly relies on surgical removal followed by adjuvant chemotherapy and radiation therapy as appropriate. There is also great therapeutic success in targeted approaches to defined molecular targets such as BRCA-related breast cancers. Treatments that target multiple oncogenic pathways may prove to be an effective supplement in cancer treatment.

Beginning in the 1990's, the anticancer effects of thymoquinone (TQ), the active constituent in the plant *Nigella sativa* (*N. sativa*), have been studied in squamous cell carcinoma (SCC).<sup>2-18</sup> TQ has been found to have anticancer effects in many types of SCC including cervical SCC, gastric SCC, and head and neck SCC (HNSCC).<sup>2,9,12,14</sup> The majority of literature centers around HNSCC, due to its high morbidity when compared to other anatomical sites.<sup>19</sup> The documented broad-spectrum effects of TQ makes it a potentially favorable therapeutic, and this review reveals that TQ is cytotoxic in SCC, primarily via the dysregulation of apoptosis and oxidative stress pathways. The authors preferred the conduct of a scoping review over a systematic review given that our purpose was to identify knowledge gaps and scope a body of literature. This scoping review aims to summarize the history and properties of TQ, the known drivers in SCC formation, the anticancer effects and mechanisms of TQ in SCC and highlight why TQ may be an emerging adjuvant in the treatment of SCC. We hypothesize that TQ may be a preventative therapeutic for patients who are at high-risk for SCC development and an adjuvant treatment for SCC patients.

### 1.1 | Origins and historical significance of TQ

*N. sativa* belongs to the botanical family of Ranunculaceae, and the plant grows in Mediterranean, Middle Eastern, and Western Asian countries including Pakistan, Egypt, Nepal, and India.<sup>20</sup> The actions of *N. sativa* are attributed to its bioactive constituent, TQ, which was first extracted in the 1960s by Mahfouz.<sup>21</sup>

Modern studies have shown that TQ has many pharmacological properties, including antioxidant, antidiabetic, anti-inflammatory, and bronchodilatory properties, and extensive reviews have been dedicated to summarizing TQ's abilities.<sup>22,23</sup> However, the therapeutic and cultural uses of *N. sativa* have an extensive history that predate modern medicine. For example, the seeds of *N. sativa* were found in the tomb of Egyptian Pharaoh Tutankhamen alongside other valuable articles.<sup>20</sup> It is referenced as “curative black cummin” in the Bible (Isaiah 28:25, 27 NKJV). Other languages reference the herb with great regard—for example, “panacea” in old Latin means “cure all,” and “Habbat al Baraka” in Arabic means “seeds of blessing.”<sup>20</sup> While many cultures used the seeds culinarily as a spice and preservative in many cultures, reports dating to the first century from the Greek physician, Dioscorides, indicate that *N. sativa* was used as a diuretic and to treat headaches.<sup>24</sup>

### 1.2 | Chemical properties of TQ

Structurally, TQ is known as 2-isopropyl-5-methyl-1,4-benzoquinone, which has a molecular formula of C<sub>10</sub>H<sub>12</sub>O<sub>2</sub> and a molecular weight of 164.2 g/mol.<sup>25</sup> It is a solid compound with a melting point of 45.4°C (113.9 °F).<sup>25</sup> TQ is particularly light sensitive and has poor solubility in aqueous solutions ranging 549–669 µg/mL.<sup>26</sup> Ongoing research to enhance efficacy of TQ administration aims to improve TQ's bioavailability via delivery through various nanoformulations, like TQ loaded on gold nanoparticles (TQ-GNPs).<sup>4-6,27-31</sup> The lethal dose (LD<sub>50</sub>) of TQ depends on the route and vehicle of administration, as well as the type of species in which it is studied. TQ can be delivered orally, subcutaneously, and via intraperitoneal injection as demonstrated in rodent studies.<sup>27</sup> For example, Al-Ali et al. demonstrated that the LD<sub>50</sub> in mice after intraperitoneal injection was 104.7 mg/kg, and after oral ingestion it was 870.9 mg/kg; in rats, these values were each slightly lower.<sup>32</sup> Importantly, the therapeutic dose to achieve anti-inflammatory, anticancer, and antioxidant effects are fractions of reported LD50's (1/10th–1/100th) reported in the literature, making TQ a fairly nontoxic therapeutic.<sup>32</sup>

### 1.3 | Legal status and clinical trials involving TQ

Currently, TQ is not FDA approved for therapeutic use. A cursory search of [ClinicalTrials.gov](https://clinicaltrials.gov) for “thymoquinone,” yields seven results, revealing various clinical trials studying antidiabetic, anticancer, antioxidative, and immunoprotective properties of TQ.<sup>33</sup> There is one clinical trial by Nabil that is a randomized, controlled study examining the chemopreventative effects of *N. sativa* administration.<sup>34</sup> The study enrolled 48 patients with premalignant oral lesions, and researchers administered either a 10 mg *N. sativa* tab to the buccal mucosa, a 5 mg buccal *N. sativa* tab, or placebo, and the primary outcome measure was the dimension of the lesion at 3-months post treatment as compared to initial dimensions.<sup>34</sup> The study was completed in 2020, however no results have been posted for this trial.

### 1.4 | Potential role of TQ as an adjunct in cancer treatment

More than one million new cases of SCC are diagnosed yearly in the United States.<sup>35</sup> SCC is the most common type of head and neck cancer, with a severe mortality and functional deficits in the head and neck region compared to other anatomical sites.<sup>19</sup> For example, a single institution retrospective study of 500 HNSCC cases by Cadoni et al. showed that the 5-year survival rate for this cohort was 60.6%.<sup>36</sup> While this review encompasses TQ treatment in all anatomic SCC's, the majority of studies included in this review focus on the head and neck, likely for these reasons. However, the anticancer effects of TQ in SCC has been studied thoroughly in breast cancer, colorectal cancer, lung cancer, and prostate cancer.<sup>37-42</sup>

There is substantial research emerging regarding TQ's synergy with radiation and chemotherapy.<sup>11,14,15,18</sup> TQ was shown to synergize with cisplatin in both nonsmall cell and small cell lung carcinoma, without added toxicity.<sup>43</sup> Indeed, TQ has been found to have less toxic effects on healthy tissues than traditional therapeutics such as cisplatin.<sup>2</sup> In colorectal cancer, TQ has been shown to synergize with 5-fluorouracil in anticancer regimen.<sup>44</sup> Studies in breast cancer have demonstrated TQ's synergy with radiation and various substances such as resveratrol, cyclophosphamide, cabazitaxel, docetaxel, Emodin, gemcitabine, and ferulic acid.<sup>29-31,45-50</sup> Ultimately, in modern medicine, TQ has considerable potential to be integrated into pre-existing treatment regimens in SCC.

## 1.5 | Brief overview of the mechanisms of SCC oncogenesis

Identifying key steps in the oncogenesis of SCC is critical in the investigation of anticancer agents such as TQ. Thus, we provide a brief overview of the mechanisms leading to SCC development. SCC can be largely divided broadly into two categories when considering its pathogenesis, especially in HNSCC: human papillomavirus (HPV)-positive and classic, HPV-negative SCC. Studies dedicated to genomic analysis of HPV-positive and negative HNSCC revealed distinct mutational signatures, namely involving tumor suppressor genes that likely require different approaches to treatment.<sup>51-53</sup>

The development of HPV-negative HNSCC parallels other common cancers. Mutations in p53—the well-described tumor suppressor that is activated by DNA damage and regulates proapoptotic triggers—is one of the most commonly found aberrations in HPV-negative HNSCC.<sup>54</sup> p53 mutations are also implicated in non-HN cutaneous SCC, prostate, bladder, liver, pancreatic, and breast cancers.<sup>55,56</sup> Seiwert et al. found that the mutational landscape in HPV-negative HNSCC is similar to lung and esophageal SCC, with mutations in TP53 and CDKN2A and with increases in EGFR copy numbers.<sup>53</sup>

Conversely, in HPV-related HNSCC, Seiwert et al. identified more unique mutations in genes such as DDX3X and FGFR2.<sup>53</sup> Generally, the development of HPV-positive HNSCC is attributed to the expression of viral oncoproteins E6 and E7. Unlike HPV-negative HNSCC, mutations in p53 and other tumor suppressors are less common in HPV-related HNSCC. However, oncoproteins E6 and E7 act to interfere with the wildtype form of tumor suppressors. While E6 has been shown to degrade p53 and BAK, E7 is known to degrade Rb, resulting in apoptosis inhibition and unchecked cell proliferation.<sup>57</sup> HPV-positive HNSCC are estimated to have favorable outcomes compared to HPV-negative HNSCC, with five-year survival rates of over 75% versus <50% in advanced HPV-positive and negative HNSCC, respectively.<sup>58-62</sup>

## 2 | METHODS

This scoping review was conducted following the recommendations of the preferred reporting items for systematic reviews and

meta-analyses extension for scoping reviews (available at [www.prisma-statement.org](http://www.prisma-statement.org)) as seen in Figure 1. Three databases (PubMed, Embase, Google Scholar) were queried for the key words “thymoquinone” and “squamous cell carcinoma,” as guided by the PICOS (populations, interventions, comparisons, outcomes, and study design) approach: (1) population: (a) mice, (b) in vitro cell cultures; (2) intervention: TQ as a treatment of SCC; (3) control: (a) mice received saline, corn oil, or other non-TQ treatment, (b) in vitro cell cultures without TQ; (4) outcome: proapoptotic effects of TQ in SCC, TQ-induced modulation of oxidative stress in SCC, synergistic effects of TQ and other therapeutics; (5) study design: in vitro cell experiments and animal model experiments. Please refer to the Appendix S1 for search strategies. Data extraction were performed by first author Kera Kwan.

Publications that did not original research and publications that did not have full-text available for review were excluded. Additionally, publications that did not specifically address TQ's anticancer effects on SCC were excluded. Additionally, to keep this review focused, data regarding nonSCC cancers that were found in the above articles were excluded in our results and discussion.

## 3 | RESULTS

Sixteen results met the inclusion criteria. These results were published over the span of 1999–2021 and encompassed original research. All groups found that TQ had either a cytotoxic or chemopreventative effect in their models, and most groups investigated a specific mechanism underlying the anticancer effects of TQ. Additionally, many publications have demonstrated TQ's synergistic effects with common adjuvant chemotherapies and radiation. The results of our literature review are summarized and formatted in Table 1 and are subsequently discussed in further detail.

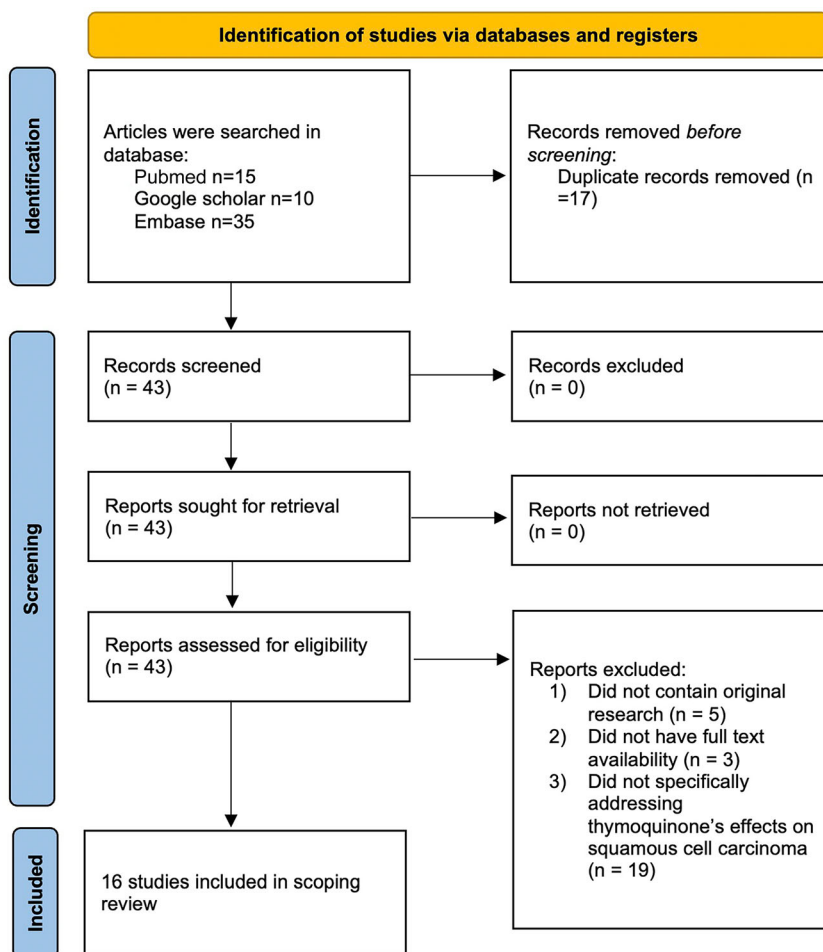
## 4 | DISCUSSION

Based on the results of this review, TQ may be a preventative therapeutic for patients who are at high-risk for SCC development and adjuvant treatment for SCC patients receiving other conventional treatment. Specifically, we have reviewed here that TQ acts as a prominent upregulator of proapoptotic pathways and downregulator of antiapoptotic pathways, modulates the delicate balance of oxidative stress in tumor development, and works synergistically alongside other chemotherapeutics to increase cytotoxicity while reducing damage to healthy tissues.

### 4.1 | Pro-apoptotic effects of TQ in SCC

The ability of cancer to evade programmed cell death contributes to oncogenic proliferation.<sup>63</sup> The studies examined in this review demonstrate that TQ induces apoptosis in SCC.<sup>2,7,8,11,13-17</sup> Several of these studies suggest apoptotic mechanisms via TQ-induced changes

FIGURE 1 PRISMA flow diagram.



in expression or activity of various proapoptotic and antiapoptotic markers and receptors. Before discussing the details of TQ's apoptotic effects, we summarize the interaction of notable apoptotic markers below.

p53 is perhaps one of the most widely known apoptotic antigen. It is a tumor suppressor transcription factor activated by DNA damage and oncogenes that regulates proapoptotic triggers in the intrinsic pathway involving Bax as well as antiapoptotic regulatory proteins such as Bcl-2.<sup>64</sup> Bax is an oligomer that is responsible for pore formation in the mitochondrial membrane, which is key in creating conduits for the passage of proapoptotic factors such as cytochrome c into the cytoplasm.<sup>65</sup> Then, cytosolic cytochrome c activity results in eventual activation of caspases, which carry out the proteolysis that directly results in apoptotic cell death.<sup>66</sup> Many mediators act in opposition to the above proapoptotic factors. For example, Bcl-2 is a well-studied prosurvival protein that inhibits the activities of proapoptotic proteins such as Bax, and Mdm2 oncoprotein antagonizes p53.<sup>65,67</sup>

TQ-induced decrease in cell survival is associated with increased expression and activity of tumor suppressor p53 and downstream proapoptotic proteins Bax and caspases, as well as decreased expression and activity of antiapoptotic proteins Bcl-2, Bcl-xl, and Mdm2.<sup>7,13,14,16</sup> For example, Das et al. demonstrated TQ-induced activation of caspase-3 and an increase in Bax activity with

concomitant decrease in Bcl-2 activity.<sup>14</sup> Similarly, Chu et al. noted that the administration of TQ promoted apoptosis by increasing both Bax expression and caspase-9 activation.<sup>13</sup> This was also confirmed using in-vivo using a mouse model. TQ delivered by oral gavage in mice resulted in decreased tumor volume and weight in addition to Bax levels.<sup>13</sup> Park et al. also examined TQ's apoptotic effects via western blot on a variety of proapoptotic and antiapoptotic proteins in a HNSCC cell line. TQ increased expression of proapoptotic factors p53 and Bax and activated apoptotic caspases-9, -7, and -3, while inhibiting expression of antagonistic factors such as Mdm2, Bcl-2, and Bcl-xl.<sup>16</sup> This apoptosis-mediated cell death in HNSCC appears to be dose-dependent.<sup>11,15</sup>

The effects of TQ on p53 and Bcl-2 in HNSCC were also demonstrated in cervical and esophageal SCC cell lines.<sup>2,7</sup> Interestingly, Ng et al. showed that TQ actually had greater cytotoxicity than cisplatin in cervical SCC with less cytotoxicity in normal tissue (Vero and 3 T3-L1 cells).<sup>2</sup> Considering the well-described toxicities of cisplatin, especially the dose-limiting side effect of nephrotoxicity, discovering adjuvants such as TQ with similar or better efficacies that can spare healthy tissues would be beneficial.<sup>68</sup> To further corroborate that the observed cytotoxic effects were via apoptosis, Ng et al. performed cell cycle analysis in cervical SCC, which revealed an arrest at the sub-G1 phase, indicating DNA cleavage and a triggering of apoptosis.<sup>2,69</sup> In

**TABLE 1** Previously published primary research articles demonstrating significant anticancer effects of TQ in SCC.

References	Experimental model/study design and participants	TQ purity	TQ doses	IC50 concentration	Reported effects of TQ in SCC	Follow up period
Badary et al. <sup>12</sup>	BP induced forestomach tumors in albino mice	N/a	Oral administration of 30 mg/kg	N/a	Inhibits tumor incidence and increases glutathione levels and enzymatic activity of GST and DT diaphorase in BP treated tumor-bearing mice	190 days
Ivankovic et al. <sup>9</sup>	SCC VII cells and C3HHf/Bu Zgr/Hr male mice	N/a	Cells: 0.01 and 0.1 mg/mL Mice: Intratumoral injection of 20 mg/kg	N/a	Dose-dependent cytotoxicity in-vitro and in-vivo; no mechanism investigated	21 days
Rajkamal et al. <sup>10</sup>	DMBA induced buccal pouch SCC in hamsters	N/a	Oral administration of 30 mg/kg	N/a	Reduces neoplastic changes; normalizes cell surface glycoconjugates and intracellular cytokeratin expression	98 days
Ng et al. <sup>2</sup>	SiHa cells	N/a	Application of 1 to 30 µg/mL	10.67 ± 0.12 and 9.33 ± 0.19 µg/mL as determined by MTT assay and trypan blue dye exclusion test, respectively	Induces apoptosis via sub-G1 arrest, down-regulation of Bcl-2 expression, and up-regulation of p53 expression	3 days
Das et al. <sup>14</sup>	SCC A431, Hep2, and RPMI 2650 cells, sarcoma 180-induced mouse model	N/a	Cells: application of 2–100 µM Mice: Tail vein injection of 10 mg/kg	10 µM	Induces apoptosis via sub-G1 arrest, increased enzymatic activity ratio of Bax/Bcl-2, activation of caspase-3, and inhibition of Akt and JNK phosphorylations; synergizes with diosgenin	18 days
Chu et al. <sup>13</sup>	SCC-4, SAS, SASVO3, and OC2 cells, BALB/c nude mouse xenograft model	N/a	Cells: application of 0, 20, 40 and 60 µM Mice: oral administration of 25 mg/kg	IC50 in the SCC-4, SAS, SASVO3, and OC2 cells were 56.02, 53.33, 45.02, and 59.13 µM, respectively	Increases Bax expression and caspase-9 activation; increases presence of autophagic vacuoles and LC3-II proteins; promotes autophagosomes accumulation; reduces tumor growth and induces apoptosis and autophagy in-vivo	20 days
El-Mansy et al. <sup>4</sup>	DMBA induced buccal pouch SCC in Syrian golden hamsters	N/a	Intraperitoneal injection of TG-GNPs at 0.001 mg/kg	N/a	Intraperitoneal injection of TQ-GNPs reduces carcinogenesis and increases muscle regeneration; decreases NF-kB expression	98 days
Alaufi et al. <sup>11</sup>	UMSCC-14C cells	N/a	Application of 0.01–100 µM	7.0 ± 0.7 µM at 72 h	Induces dose-dependent apoptosis; synergizes with cisplatin to increase p53 and caspase-9 expression while decreasing Bcl-2 expression	3 days
Kotowski et al. <sup>15</sup>	SCC25 and CAL27 cells	N/a	Application of 0–80 µM	Ranged from 12.12 µM (CAL27) to 24.62 µM (SCC25)	Synergizes with radiation to increase cytotoxicity but does not synergize with cisplatin	10 days
Ren et al. <sup>17</sup>	KB cells	>98%	Application of 1 and 5 µM	3.41 ± 0.25 µM	Reduces cell migration and invasion; apoptosis induction via decreased phosphorylation of PI3K and Akt	2 days
Park et al. <sup>16</sup>	A431 cells, NSG mouse	99%	Cells: 1, 15 and 30 µM Mice: intraperitoneal injection of 1 and 5 mg/kg	N/a	Induces apoptosis via induction of p53 and Bax expression and inhibition of Mdm2, Bcl-2, and Bcl-xl expression; activates caspase-9, -7, and -3; inhibits phosphorylation of STAT3 via ROS formation	14 days

TABLE 1 (Continued)

References	Experimental model/study design and participants	TQ purity	TQ doses	IC50 concentration	Reported effects of TQ in SCC	Follow up period
Amer et al. <sup>5</sup>	DMBA induced buccal pouch SCC in Syrian golden hamsters	N/a	Intraperitoneal injection of 0.1 mg/kg TQ or 0.001 mg/kg TQ-GNPs	N/a	Topical and intraperitoneal injection of TQ-GNPs leads to regression of carcinogenesis and increases mRNA expression of DNA repair enzymes XRCC1 and ERCC1	84 days
Ma et al. <sup>7</sup>	Eca109 cells, BALB/c nude mouse xenograft model		Cells: application of 10, 25, 50, 75, and 100 $\mu$ M Mice: intraperitoneal injection of 5 mg/kg, 10 mg/kg, 15 mg/kg	Cells: IC50 of 32.57 $\mu$ M (95% CI: 23.95–44.28 at 72 h)	Induces cell cycle arrest in G2/M phase, increases p53 and p21 levels, increases cleaved caspase-9, -7, -3 levels, decreases Bcl-2 and Cyclin B1/A/E levels, disrupts PI3K/Akt pathway via upregulation of PTEN	24 days
Pu et al. <sup>3</sup>	DMBA induced buccal pouch SCC in Syrian golden hamsters	>98%	Oral administration of 20 mg/kg TQ.	N/a	Prevents squamous cell carcinogenesis; chemoprevention via downregulation of mRNA expression of NF-kBp50/p65 and PI3K, AKT, mTOR	98 days
Dagtas et al. <sup>8</sup>	SCC VII cells	N/a	Application of 10 $\mu$ M	N/a	Induces cell detachment and cytotoxicity via apoptotic mechanisms	3 days

Abbreviations: BP, benzo(a)pyrene; DMBA, dimethylbenz(a)anthracene; IHC, immunohistochemistry; GST, glutathione-S-transferase; ROS, reactive oxygen species; SCC, squamous cell carcinoma; TQ, thymoquinone; TQ-GNPs, TQ loaded on gold nanoparticles.

esophageal SCC lines, on the contrary, Ma et al. demonstrated that TQ induced arrest in the S and G2/M phase, and this was associated with a reduction in levels of the various cyclins that regulate cell cycle checkpoints.<sup>7</sup>

STAT3 (signal transducer and activator of transcription factor 3) is an upstream regulator of p53 implicated in upregulation of pro-survival genes including Bcl-2 and Bcl-xl.<sup>70</sup> TQ's anticancer effects were found to be due to multiple targets in this pathway; for example, TQ-induced de-phosphorylation and subsequent decreased DNA binding activity of STAT3, as well as phosphorylation modulation of Src kinase—the kinase upstream of STAT3—in a dose-dependent manner.<sup>16</sup> These key regulators described above interact with other cell survival pathways such as the Akt pathway.<sup>71</sup> Akt kinase suppresses apoptosis to promote cell survival via inhibition of cytochrome c release and cytochrome c mediated caspase activation, and thus, dysregulation of this pathway is implicated in carcinogenesis.<sup>71</sup>

Many pathways of oncogenesis have been known to converge on increased Akt activity, and the result is Akt-dependent cell survival and apoptosis suppression.<sup>71</sup> Akt and p53 pathways have been shown to interact on multiple levels through upstream regulators and downstream targets, including but not limited to PTEN, p21, and Mdm2.<sup>71</sup> Both Ma et al. and Ren et al. found that TQ modified the PI3k/Akt cell-survival pathway through either reduced phosphorylation and therefore activation of PI3k or upregulation of tumor suppressor protein PTEN.<sup>7,17</sup>

The PI3K/Akt pathway impacts not only cell survival but also tumor growth, metastases, and metabolism, and this pathway is

known to be upregulated in SCC.<sup>72</sup> Pu et al. found that topical treatment with TQ in a DMBA induced buccal cancer in a hamster model suppressed mRNA expression of PI3K, Akt, and mTOR.<sup>3</sup> Interestingly, they found that when alternating daily DMBA and TQ administration, oral SCC had not developed when examined after 2 weeks.<sup>3</sup> This suggests that not only does TQ exert cytotoxic/apoptotic effects through the PI3K/Akt pathway, but also chemopreventative effects.

Elmansy et al. investigated TQ's effects on NF-kB expression in DMBA-induced buccal pouch SCC in Syrian golden hamsters.<sup>4</sup> They showed that TQ-GNPs reduced carcinogenesis and improved regeneration of muscle layers on routine H&E staining. This effect was accompanied by decreased NF-kB expression. NF-kB is a key transcription factor implicated in the connection between chronic inflammatory responses and cancer formation.<sup>73</sup> It activates various antiapoptotic pathways that involve STAT3 and p53; therefore, the TQ-induced decrease in NF-kB expression reflects an induction of apoptosis.<sup>73</sup>

## 4.2 | TQ-induced modulation of oxidative stress in SCC

The development of reactive oxygen species (ROS) has tumorigenic effects via a variety of downstream pathways, including apoptotic, cell cycle, cell adhesion, and angiogenic pathways.<sup>74</sup> In cutaneous SCC development, UV light is known to induce oxidative DNA damage and promote tumor formation.<sup>75</sup> However, the function of oxidative stress in tumorigenesis is nuanced; while elevations in ROS are common in



cancer, it is also known that antioxidant enzymes are upregulated in cancer, implicating a sensitive balance in oxidative stress necessary for tumor progression.<sup>76</sup> It also is theorized that antioxidant enzymes may hinder the early cellular events that lead to oncogenesis.<sup>76</sup>

Previous studies have shown that TQ impacts the oxidative stress environment of cancer cells, which may contribute to its chemoprevention and cytotoxicity in SCC.<sup>5,6,12,16</sup> As early as 1999, Badary et al. investigated the effects of TQ on benzo(a)pyrene (BP)-induced forestomach squamous cell tumors in mice.<sup>12</sup> When they treated these mice before, during, and after BP treatment, tumor formation was decreased by 70%, and this was associated with increases in the activity of glutathione-S-transferase (GST) and reduced nicotinamide adenine dinucleotide phosphate oxidoreductase—two enzymes implicated in reduction of free radicals.<sup>12</sup> This suggests that early and persistent TQ treatment led to chemoprevention in this in vivo model. Badary et al.'s study reflects similar results to Pu et al.'s work that demonstrated chemoprevention via apoptotic mechanisms when a carcinogen was administered concurrently with TQ. Conversely, Park et al. found that TQ induced ROS formation and these effects reversed in the face of antioxidant treatment with N-acetyl-cysteine.<sup>16</sup>

Intracellular ROS can induce single-stranded breaks in DNA.<sup>77</sup> DNA repair enzymes such as XRCC1 are therefore integral to mitigating oxidative stress and subsequent carcinogenesis.<sup>77</sup> Amer et al.'s work in DMBA-induced buccal pouch SCC in Syrian golden hamsters demonstrated that topical and intraperitoneal injection of TQ-GNPs resulted in cytotoxicity associated with an increase in mRNA and protein expression of XRCC1.<sup>5,6</sup>

### 4.3 | Synergistic effects of TQ and other therapeutics

TQ has been shown to synergize with conventional chemotherapy and radiation as part of cancer treatment.<sup>11,14,15,18</sup> Alaifi et al. analyzed the combined effects of TQ and cisplatin in a HNSCC line.<sup>11</sup> The study showed that 24 h of treatment with 5  $\mu$ M cisplatin plus 5 or 0.5  $\mu$ M TQ induced significantly more cell death via apoptosis than 5  $\mu$ M cisplatin treatment alone.<sup>11</sup> Further investigation demonstrated that combination treatment with cisplatin and TQ synergistically increased p53 and caspase-9 expression while decreasing Bcl-2 expression more so than treating with either agent alone.<sup>11</sup> Kotowski et al. studied the effects of TQ when combined with radiation and demonstrated a significantly decreased clonogenic survival of two HNSCC lines (SCC 25, CAL 27) when treated with combination TQ and radiation compared to treatment with singular treatment.<sup>15</sup> Interestingly, their work revealed that TQ did not synergize with cisplatin, unlike Alaifi et al.'s work in HNSCC (UMSCC-14C).<sup>15</sup> The cytotoxicity of a combined therapy of diosgenin (a bioactive constituent found in fenugreek) and TQ was also well documented by Das et al.<sup>14</sup> After 48 h post-TQ and diosgenin treatment, cell viability in HNSCC lines was decreased to roughly 10%, which was significantly lower than using either agent alone.<sup>14</sup>

Although TQ treatment regimens for SCC in in-vivo and in-vitro models have appeared very promising thus far, it is clear that our understanding of this drug's full potential is far from optimal. While

the aim of this scoping review is to map out the current landscape of TQ treatment in SCC, the review is ultimately limited in its ability to draw significant conclusions, as there is currently a limited number of studies. As the body of research in this field grows, systematic reviews and meta-analyses will need to be performed.

The small number of studies available for review call attention to the need for further work in this field. Moreover, 5 of the 16 articles reviewed in our study performed purely in vitro TQ experiments, and as such have a limitation of the inability to perform adequate chemical and pharmacokinetic analyses and thus may be irrelevant to the in vivo setting. Additionally, it remains unclear whether TQ effects are primarily ROS-mediated or primarily derived from expression of proapoptotic proteins (activation of caspase enzymes, increase in Bax activity, decrease in Cbl-2 activity, modification of PI3k/Akt pathway, decreased NF- $\kappa$ B expression). Prospective studies are needed to determine the most effective ways to leverage TQ's effects in SCC. Considering the relative safety of TQ, investigating cancer or premalignant lesion response to local TQ treatment in SCC patients is essential. A single clinical trial has been completed, and researchers may look to this as they consider their own study designs in the future.

## 5 | CONCLUSION

The field of HNSCC cancer treatment has made great strides in improving morbidity and mortality. One promising strategy to ease the therapeutic burden on SCC patients is to administer minimally toxic but highly effective drugs that work across a wide range of biological processes and that work synergistically with other treatments to improve outcomes. Our review suggests that TQ would be a preventative therapeutic for patients who are at high-risk for SCC development and adjuvant treatment for SCC patients receiving other conventional treatment. Specifically, we have reviewed here that TQ acts as a prominent upregulator of proapoptotic pathways and downregulator of antiapoptotic pathways, modulates the delicate balance of oxidative stress in tumor development, and works synergistically alongside other chemotherapeutics to increase cytotoxicity while reducing damage to healthy tissues. Future studies should be aimed at identifying specific populations in which TQ's effects would be the most beneficial.

### CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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