



Investigation of Apoptotic Effects of Thymoquinone on Glioblastoma Cells

Pınar Çetinalp¹ · Öykü Gönül Geyik² · Senanur Malcanlı² · Sevgin Değirmencioğlu³ ·
Sevda Tanrikulu Küçük¹ · Hikmet Koçak⁴ · Engin Ulukaya⁵

Received: 3 February 2025 / Revised: 28 February 2025 / Accepted: 14 March 2025 / Published online: 14 April 2025
© The Author(s) 2025

Abstract

Background Glioblastoma multiforme (GBM) is the most aggressive and fatal primary brain tumor, characterized by rapid proliferation, resistance to therapy, and poor prognosis. Current treatment strategies, including surgery, radiotherapy, and chemotherapy, have limited efficacy and significant side effects. Phytotherapeutic agents such as thymoquinone (TQ), the major bioactive compound of *Nigella sativa*, have gained attention for their potential anticancer properties. TQ has been reported to exhibit pro-apoptotic, anti-inflammatory, and anti-proliferative effects in various cancer models.

Objective This study aimed to investigate the apoptotic effects of TQ on U87 human glioblastoma cells by evaluating its impact on cell viability, migration, colony formation, and the expression of key genes and proteins involved in apoptosis and inflammation.

Methods U87 glioblastoma cells were treated with increasing concentrations of TQ (10–100 μ M) for 24, 48, and 72 h. Cell viability was assessed using the Sulforhodamine B (SRB) assay. A scratch assay was performed to evaluate cell migration, and a colony formation assay was used to assess clonogenic potential. Quantitative real-time PCR (qRT-PCR) and Western blotting were conducted to analyze the expression levels of genes and proteins associated with apoptosis, inflammation, and cell cycle regulation.

Results TQ treatment significantly reduced U87 cell viability in a time- and dose-dependent manner, with IC₅₀ values of 75 μ M at 24 h, 45 μ M at 48 h, and 36 μ M at 72 h. The scratch assay demonstrated that TQ had no significant effect on U87 cell migration. Colony formation was inhibited at 10 μ M after 24 h. qRT-PCR analysis revealed that at 50 μ M TQ, caspase-3 and Bax mRNA levels were significantly upregulated. At 75 μ M, caspase-9, caspase-3, Bax, and p21 mRNA levels increased, while caspase-8, Bcl-2, and Akt1 expression decreased. Western blot analysis showed increased expression of cleaved caspase-3, p21, and ATG5 proteins, along with decreased expression of caspase-8 and TNF- α at 24 h.

Conclusion TQ exhibits potent pro-apoptotic effects in U87 glioblastoma cells by modulating key apoptotic and inflammatory pathways, including the PI3K/AKT signaling axis. These findings suggest that TQ may serve as a promising adjuvant therapeutic agent for GBM. Further studies are required to elucidate its full molecular mechanisms and potential clinical applications.

Keywords Glioblastoma multiforme · Thymoquinone · Apoptosis · Cell viability · PI3K/AKT signaling

✉ Pınar Çetinalp
pinarcetinalp@outlook.com

¹ Department of Biochemistry, Faculty of Medicine,
Demiroglu Bilim University, Büyükdere Cad. No:120/1
34394 Esentepe-Şişli, Istanbul, Turkey

² Department of Medical Biology, Faculty of Medicine, Istinie
University, Istanbul, Turkey

³ Department of Biochemistry, Faculty of Medicine, Kırklareli
University, Kırklareli, Turkey

⁴ Department of Medical Education, Faculty of Medicine,
Istinie University, Istanbul, Turkey

⁵ Department of Biochemistry, Faculty of Medicine, Istinie
University, Istanbul, Turkey

1 Introduction

Cancer, a disease marked by an abnormal and uncontrolled cell proliferation, is major public health problem, and the second leading cause of mortality in the world after cardiovascular diseases. Glioblastoma multiforme (GBM) is the most common, aggressive, and fatal primary brain tumor, classified as a grade IV astrocytoma by the World Health Organization (WHO) [1–6]. Despite advances in surgery, radiotherapy, and alkylating agent-based chemotherapy, GBM remains highly resistant to treatment, with a poor prognosis and high recurrence rates. Chemotherapeutic drugs, while essential, have significant limitations due to their non-specific targeting of both normal and cancerous cells, leading to severe side effects. In recent years phytotherapeutic sources have been investigated as an alternative to chemotherapeutic cancer drugs [7]. One of these, *Nigella sativa* (black seed or black cumin) is a flowering plant native to South and Southwest Asia and mostly found in North Africa, the Middle East, and Southern Europe [8, 9]. Most research has investigated its medical treatment effects, on many different types of cancer. Investigation continues on the pathways through which it exerts its anti-cancer, anti-inflammatory and anti-neoplastic effects. The major bioactive component of the volatile oil of *N. sativa* seeds, thymoquinone (TQ) (2-isopropyl-5-methyl-1,4-benzoquinone, $C_{10}H_{12}O_2$; molecular weight: 164.2 g/mol) has anti-proliferative, pro-apoptotic, anti-angiogenic, anti-neoplastic and antioxidant effects and the molecular and cellular mechanisms by which it exerts these effects are being investigated. [10, 11]. Recent studies have shown that the pro-apoptotic properties of TQ reduce cancer cell survival in various cancer cell lines such as canine osteosarcoma, human colon carcinoma, breast adenocarcinoma, ovarian adenocarcinoma and colorectal adenocarcinoma [12–14]. TQ has been reported to initiate cancer cell apoptosis through signalling pathways that induce autophagy, DNA damage, telomeric attrition and immunoregulation. [15]. GBM is characterized by its ability to evade apoptosis and sustain uncontrolled proliferation, which is largely driven by the dysregulation of key signaling pathways. A number of potential mechanisms for apoptosis of cancer cells have been identified through targeting of intrinsic and extrinsic pathways via protein–protein interactions. Apoptosis has two main pathways: intrinsic (mitochondrial) and extrinsic (death receptor associated). Mitochondria has a main part in the intrinsic pathway. The extrinsic pathway is mediated by death receptors play a major risk on the cell surface. The main regulatory proteins in both pathways are caspases [16–19]. The main regulatory proteins in both pathways are caspases [16–18]. Potential mechanisms for apoptosis of cancer cells have been

determined through targeting the intrinsic and extrinsic pathways via protein-to-protein interactions have been identified. Extrinsic pathways are mediated by the TNF receptor superfamily; cell binding to the receptor activates caspase 8, which in turn activates caspases 3, 6, and 7 as apoptotic executioner enzymes. In the intrinsic pathway, BCL-2 stimulates p53 protein and p53 then stimulates p21 protein. When cytochrome C is released from the mitochondria into cytosol, p53 stimulates caspases 9 (is one of the initiator caspase in the intrinsic pathway) [19]. In the tumor microenvironment, inflammatory cells secrete pro-inflammatory and oncogenic factors such as reactive oxygen species (ROS), nitric oxide (NO), cytokines such as interleukins, tumor necrosis factor- α (TNF- α), growth factors and chemokines, all of which promote carcinogenesis [20]. Additionally, a significant association exists between cancer cells and inflammatory cells that activate several molecular signaling cascades as nuclear factor kappa B (NF- κ B), mitogen-activated protein kinase (MAPK) and phosphoinositide-3-kinase (PI3K) [20–22]. Among these, nuclear factor kappa B (NF- κ B), mitogen-activated protein kinase (MAPK), phosphoinositide-3-kinase (PI3K), and protein kinase B (AKT) pathways play crucial roles in GBM progression, therapy resistance, and inflammation [20]. NF- κ B Pathway: NF- κ B is a master regulator of inflammation and cell survival, commonly activated in GBM. It promotes tumor progression by upregulating pro-inflammatory cytokines, growth factors, and anti-apoptotic proteins. TQ has been shown to inhibit NF- κ B activation, thereby reducing inflammation-induced tumor progression and sensitizing cancer cells to apoptosis [20]. MAPK Pathway: The MAPK signaling cascade is involved in cellular responses to stress and mitogenic stimuli. It consists of three major subfamilies: extracellular signal-regulated kinases (ERKs), c-Jun N-terminal kinases (JNKs), and p38 MAPKs. TQ has been reported to modulate MAPK signaling, particularly by activating JNK and p38 pathways, leading to increased apoptosis and reduced GBM cell survival [21, 22]. PI3K/AKT Pathway: The PI3K/AKT pathway is one of the most frequently dysregulated signaling networks in GBM, playing a central role in cell survival, proliferation, and therapy resistance. Overactivation of AKT promotes tumor growth by inhibiting pro-apoptotic proteins and enhancing GBM cell survival. TQ has been shown to suppress PI3K/AKT signaling, leading to reduced cell viability, induction of apoptosis, and inhibition of GBM proliferation [23]. Autophagy and ATG5: Autophagy is a double-edged sword in cancer, either promoting survival or facilitating cell death depending on the context. TQ has been linked to autophagy regulation through the upregulation of autophagy-related gene 5 (ATG5), which plays a role in tumor suppression by enhancing apoptotic signaling [24–26].

2 Materials and Methods

2.1 Cell Culture

U87 (HTB-14, ATCC) glioblastoma cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal bovine serum (FBS), 1% (2 mM) L-glutamine (L-Gln) and 1% (100 U/mL/100 µg/mL) penicillin/streptomycin (complete medium) at 37°C in a humid incubator under 5% CO₂ pressure. Thymoquinone (TQ) (Sigma #274,666) was dissolved in dimethyl sulfoxide (DMSO) to a 600 mM solution. Different concentrations were prepared in complete medium. The highest concentration of DMSO equivalent in complete medium was used as the vehicle control.

2.2 Cell Viability Assay

Sulforhodamine B (SRB) assay was used to evaluate the effect of TQ on the viability of U87 glioblastoma cells [27]. 5×10^3 U87 cells/well were seeded in 96-well plates/100 µL of medium per well. Cells in the test wells were incubated overnight and treated with increasing concentrations of TQ. After 24, 48 and 72 h of incubation, 50 µL 50% (w/v) trichloroacetic acid (TCA) solution was added per well and cells were fixed at 4 °C for 1 h. After 5 consecutive washes with distilled water to remove TCA, 50 µL of SRB solution was added to each well and incubated in the dark for 30 min at room temperature. Wells were washed with 1% acetic acid solution to remove unbound dye. The dye bound to proteins was solubilized with 150 µL/well of 10 mM tris base (pH: 10.0) and the plate was shaken at approximately 150 rpm for 10 min. Spectrophotometric measurements followed at 564 nm and 690 nm. The absorbance values at 564 nm were subtracted from the absorbance values at 690 nm.

2.3 Horizontal Migration Assay

Scratch Assay evaluated the TQ's effect on U87 cell horizontal migration [28]. 500,000 cells/well were seeded in 24-well plates in 500 µL of medium per well. When cells were observed to be approximately 80% confluent, straight scratches were made by 200 µL sterile pipette tips. The media were removed and wells were rinsed with sterile 1×PBS. Then, increasing concentrations of TQ were administered a final volume of 500 µL/per well. Microscopy images established baseline ("time point 0"). The same area was imaged at 24- and 48- hour time points. The migratory abilities of the cells were calculated using ImageJ FIJI [3] and visualized using GraphPad Prism v9 software.

2.4 Colony Formation Assay

The colony formation assay evaluated TQ's effect on U87 cell colonogenicity [29]. 250 cells/well were seeded in 24-well plates in 500 µL of medium per well. After overnight incubation, cells in the test wells were treated with increasing concentrations of TQ in 500 µL medium. Plates were then incubated at 37 °C, 5% CO₂ for colony formation. When colonies of at least 50 cells per cluster were observed, the medium were removed from the wells. The wells were rinsed with 1×PBS. An acetic acid–methanol solution (1:3) was added to the wells and incubated at 4 °C for 15 min. At the end of incubation, the wells were carefully washed once again with 1×PBS. 500 µL/well of 0.2% crystal violet (in 2% EtOH) was added to the wells and incubated for 10 min in the dark at room temperature.

2.5 Quantification of mRNA Expression in Cells Using qRT-PCR

To quantify mRNA expression in cells, total RNAs were first isolated from cells using RNeasy RT solution (MRC, USA), following the manufacturer's instructions. RNA concentration and purity were determined using NanoDrop 2000 (Thermo Scientific, USA) by pipetting 1 µL of RNA samples into the device for determination of A_{260/280} and A_{260/230} ratios. Before reverse transcription, RNA concentrations were equalized. Reverse transcription was carried out using the Script cDNA Synthesis Kit (Jena Bioscience, Germany) according to the kit protocol, and resulting cDNA was amplified by qRT-PCR using qPCR GreenMaster with the UNG Kit (Jena Bioscience, Germany). Real-time conditions were performed on the CFX-96 Real Time PCR System (Bio-Rad, USA) with the following protocol: 50 °C for 2 min, 95 °C for 2 min, followed by 35 cycles of 95 °C for 15 s, 51 °C for 20 s-(for INPPL1, TNFα, Bcl-2, Akt1, MAPK1, COX-2 and PPARγ) and 60 °C for 20 s-(for Cas-9, Cas-3, Cas-8, PIK3CB, TP53, Bax, P21, Atg-5 and GAPDH) and 72 °C for 30 s. Melting curve analysis was also done for all primers. The relative mRNA transcript levels were calculated using the $2^{-\Delta\Delta C_t}$ method and the relative expression of each gene was normalized to that of GAPDH. Primers were obtained from LGC Biosearch Technologies (Denmark). All measurements were performed in triplicate. The specific primers used are shown in Table 1.

2.6 Western Blotting

Western blot evaluated TQ's effect on U87 cell expression of specific proteins involved in cell death, proliferation and growth. At the end of the drug treatment period, the cells were trypsinized, collected in 1.5 mL centrifuge tubes and centrifuged at 300 g for 5 min. The supernatant was then

Table 1 Primers used for qRT-PCR

Gene Name	Gene bank #	Primer Sequences
INPPL1	NM_001567.4	Forward 5'-GGGAGGGAGAGCACCTTAATA-3' Reverse 5'-GACCAGGAGACAGTGAGAACTA-3'
Caspase-9	NM_032996.3	Forward 5'-CACAGGGTCTGCTCTTTCTC-3' Reverse 5'-CATTTCATCTGTCCCTCTTCCTC-3'
Caspase-3	NM_004346.4	Forward 5'-CCTACAGCCCATTTCTCCATAC-3' Reverse 5'-GCTTCACTTTCTTACTTGGCG-3'
Caspase-8	NM_001228.4	Forward 5'-TTGACCACGACCTTTGAAGAG-3' Reverse 5'-AGCAGATGAAGCAGTCCATG-3'
TNFA	NM_000594.4	Forward 5'-CCAGGGACCTCTCTAATCA-3' Reverse 5'-TCAGCTTGAGGGTTTGCTAC-3'
Bcl-2	XM_047437733.1	Forward 5'-CAGGATAACGGAGGCTGGGATG-3' Reverse 5'-AGAAATCAAACAGAGGCCGCA-3'
PIK3CB	NM_006219.3	Forward 5'-ATTCTTCATTTCCAGGGCTG-3' Reverse 5'-TCACCATTCTCCAAACCACTG-3'
AKT1	NM_001382433.1	Forward 5'-TTCCATCCTGCTAATCTGGGG-3' Reverse 5'-GTCCATGGTGTTCCTACCCA-3'
TP53	NM_001276761.3	Forward 5'-CTTCCATTTGCTTTGTCCCG-3' Reverse 5'-CATCTCCCAAACATCCCTCAC-3'
MAPK1	NM_002745.5	Forward 5'-GTCCTTCGTTATGTTCCCCAG-3' Reverse 5'-TGACATCCCCATTCAACTCG-3'
BAX	NM_002745.5	Forward 5'-TTGCTTCAGGGTTTCATCCA-3' Reverse 5'-ACACTCGCTCAGCTTCTTG-3'
P21	NM_001374511.1	Forward 5'-CGGAACAAGGAGTCAGACATT-3' Reverse 5'-AGTGCCAGGAAAGACAACACTAC-3'
ATG5	NM_001286108.2	Forward 5'-GTATCATCCACAGCCAACA-3' Reverse 5'-AAGGGTGACATGCTCTGATAAA-3'
PPARG	NM_138712.5	Forward 5'-AGGATGCAAGGGTTTCTTCCG-3' Reverse 5'-CCGCCAACAGCTTCTCCTTC-3'
COX2	NM_000963.4	Forward 5'-GCTTCCATTGACCAGAGCAG-3' Reverse 5'-CTCCACAGCATCGATGTCAC-3'
GAPDH	NM_002046.7	Forward 5'-ACCCAGAAGACTGTGGATGG-3' Reverse 5'-TCAGCTCAGGGATGACCTTG-3'

removed and the pellet washed twice with $1 \times$ PBS. Lysis solution containing protease/phosphatase inhibitor was added to the cell pellet and pipetted until the pellet was completely dissolved. The suspension was incubated at -20°C for 2 min and then vortexed for 1 min. This freeze/vortex cycle was repeated 6 times. At the end of this period, the samples were centrifuged at $14,000\text{ g}$ for 20 min at 4°C . At the end of centrifugation, the supernatant was transferred to new tubes and the pellets were discarded. Protein concentration was determined using the Bicinchoninic acid kit for protein determination (Sigma-Aldrich, BCA1). Protein

lysates were resuspended with Laemmli loading buffer and heated at 95°C for 5 min. Equal amounts of protein ($30\text{ }\mu\text{g}$ protein/lane) were subjected to SDS-PAGE at 12% concentration for protein separation and electrotransferred to polyvinylidene difluoride (PVDF) membranes. Membranes were blocked with 5% dry milk in TBS containing 0.1% Tween-20 (TBS-T). After of blocking, the membrane was exposed to primer antibody (1:1000 dilution) overnight at 4°C with constant shaking, followed by horseradish peroxidase-conjugated anti-rabbit secondary antibody (1:3000 dilution). Bands were detected using an ImageQuant LAS

500 imaging system (GE Healthcare, Chicago, IL, USA). Beta-actin antibody was the internal (loading) control. Membrane stripping was performed using manually prepared mild stripping buffer before loading control (beta-actin) is tested. Semi-quantitative densitometric analysis of the protein blots was performed using Image J FIJI software [30].

2.7 Statistical Analysis

Results were presented as means $\pm$ SD of three experiments. Statistical analyses were performed using GraphPad Prism v9 software (San Diego, CA). Kruskal–Wallis was used to compare quantitative data among the groups. If the results of the Kruskal–Wallis were significant, Dunn’s Multiple Comparison Test was used to compare groups’ means. $P < 0.05$ was considered as statistically significant.

3 Results

3.1 Thymoquinone Reduces U87 Glioblastoma Cell Viability in a Time- and Dose-Dependent Manner

The SRB assay evaluated the effects of TQ’s at concentrations of 10–100 μ M for 24, 48 and 72 h on the viability of U87 glioblastoma cells. The results showed that TQ initially decreased the viability of U87 cells at 50 μ M at 24 h (Fig. 1). IC_{50} values at each time point were determined accordingly: 75 μ M for 24 h, 45 μ M for 48 h, 36 μ M for 72 h. The 50 μ M TQ treatment for 24 h was selected for subsequent experiments.

3.2 Thymoquinone Does Not Affect the Migration Ability of U87 Glioblastoma Cells

A scratch assay was performed to determine the effect of TQ on the migration of U87 cells at a concentration range of 10–100 μ M. The wells were imaged at 0, 24 and 48-h time points. At 48 h, it was observed that the scratch was closed in the untreated group. TQ had no effect on the migratory ability of U87 cells at 48 h (Fig. 2).

3.3 Thymoquinone Inhibits Colony Formation in U87 Glioblastoma Cells

The colony formation assay evaluated the effect of TQ on colonogenicity in U87 cells at concentrations of 10–100 μ M for 24, 48 and 72 h. Colonogenicity decreased in 10 μ M at the 24-h TQ treatment time point compared to control (Fig. 3).

3.4 Thymoquinone Modulates Apoptotic and Survival-Related Gene Expression in U87 Cells in a Dose-Dependent Manner

Real-time PCR was performed to determine the dose-dependent effect of TQ on the gene expression levels of the following genes: caspase-9, caspase-3, caspase-8, p53, bax, p21, atg5, TNF- α , bcl2, and Akt1 in U87 cells. When the 25 μ M concentration of TQ was compared with the control group, no significant change was observed in caspase-9, caspase-3, caspase-8, p53, bax, p21, atg5, TNF- α , bcl2, and Akt1 mRNA levels. When the 50 μ M concentration of TQ was compared with the control group, there is a significant increase in caspase-3 and bax mRNA levels. When the 75 μ M concentration of TQ was compared with the control

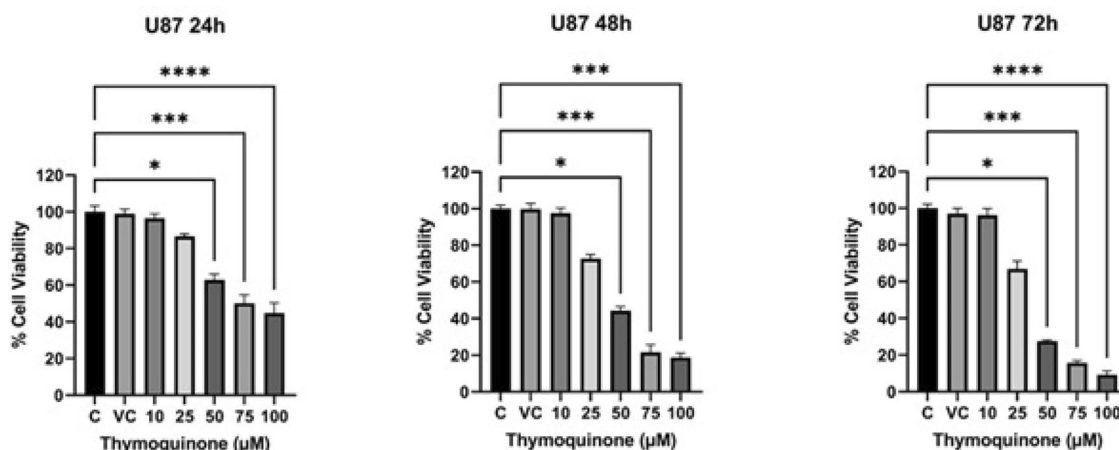


Fig. 1 The effect of TQ’s at concentrations of 10–100 μ M for 24, 48 and 72 h on the viability of U87 glioblastoma cells. Untreated cell viability was considered as 100%, and the other groups were calculated

accordingly. (C control, VC vehicle control) Data are expressed as mean $\pm$ SD. * $p < 0.05$, *** $p < 0.001$ in comparison to control

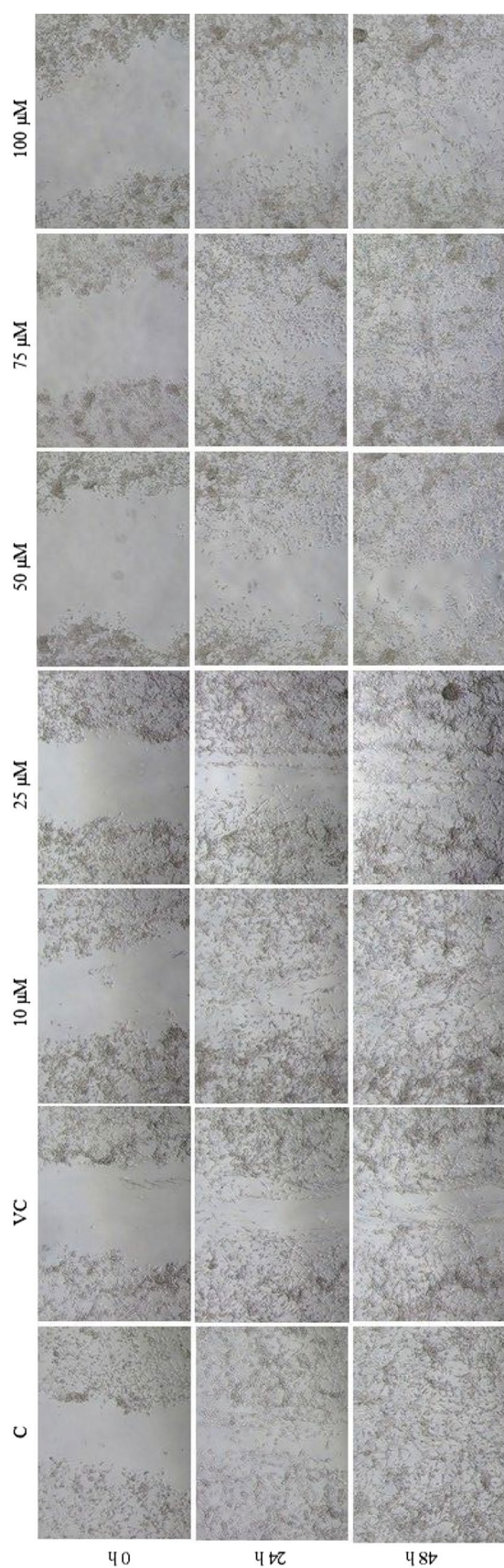


Fig. 2 The effect of TQ on the migration of U87 cells at a concentration range of 10–100 μM . (C control, VC vehicle control)

group, there was a significant increase in cas-9, cas-3, bax, p21, mRNA levels, and a significant decrease in caspase-8, bcl2 and Akt1 mRNA levels (Fig. 4).

3.5 Thymoquinone Regulates Key Apoptotic and Inflammatory Proteins in U87 Glioblastoma Cells

Western blotting was performed to determine the effect of TQ on the expression of specific proteins involved in PI3K/AKT signaling pathway, cell cycle and cell death in U87 cells. In particular, the expression of cleaved caspase-3, caspase-8, p21, TNF α and atg5 proteins was determined. Increased expression of caspase-3, p21 and atg5 protein, decreased expression of caspase-8 and TNF α were observed at the 24 h (Fig. 5).

4 Discussion

Unfortunately, many people are diagnosed and die from cancer, which is the second leading cause of death after myocardial infarction. GBM is the most common malignant, aggressive and fatal cancer of brain tumors in the central nervous system [1–5]. In recent years cancer survivors are increasing, due to advances in early detection with new treatment strategies. In addition to surgery, chemotherapy and radiotherapy, some phytotherapeutic resources are used as adjuncts in treatment because they are less toxic and have fewer side effects [7].

One of these *Nigella sativa* (black cumin) and its active ingredient TQ has been studied in many different cell lines especially cancer cells. In addition to its anti-angiogenesis, antioxidant, anti-apoptotic and anti-proliferative roles, TQ has also been reported to have anti-cancer effects and inhibit tumor growth [8, 31–40].

In this study, we detected the first significant decrease in U87 cells at 24 h at a dose of 50 μM TQ using the SRB assay, which is independent of cellular metabolic activity, to determine cell viability [27]. Similar to our results, TQ decreased the cell viability of U87 cells at 24 h when the IC50 value was approximately 50 μM . [41, 42].

The Scratch Assay was performed to determine the effect of thymoquinone on the migration of U87 glioblastoma cells [28]. In this study, we observed that the scratch in the control group closed after 48 h. TQ was found to have no effect on the migration of U87 cells. This suggests that TQ may interfere with cell adhesion. TQ was found to dose-dependently reduce the migration rate of U87 cells and TQ was observed to induce a rounded cellular shape in glioblastoma cells [42].

We observed that colonogenicity was reduced at 24 h and 10 μM TQ dose and no cancer cells were observed at doses higher than 10 μM . TQ has been shown to suppress

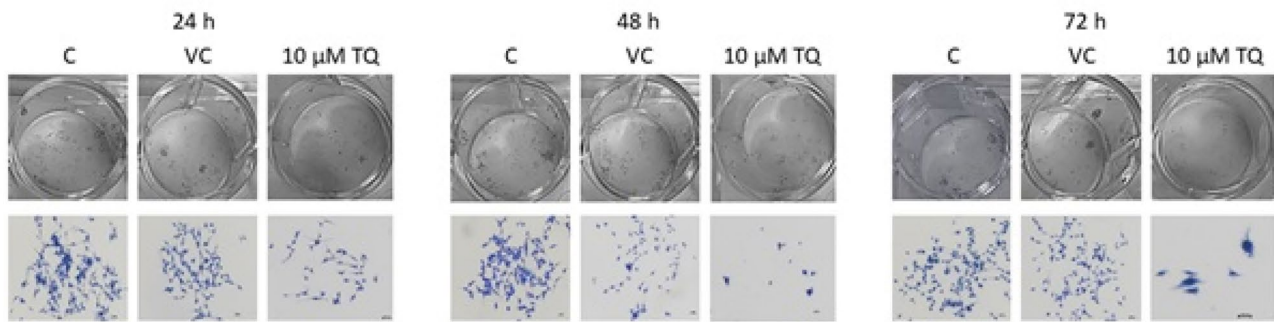


Fig. 3 The effect of TQ on colonogenicity in U737 cells at 24 h, 48 h and 72 h time points. (C Control, VC Vehicle Control)

Fig. 4 The effect of TQ on cell cycle, cell death, and cell signaling pathway genes in 24 h time point. GAPDH was used as an internal (loading) control. Data are expressed as mean $\pm$ SD. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared to control

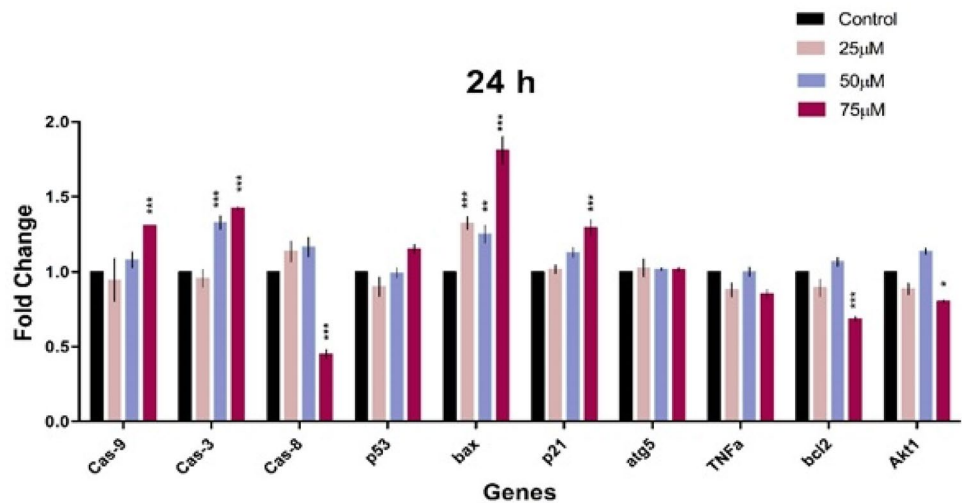
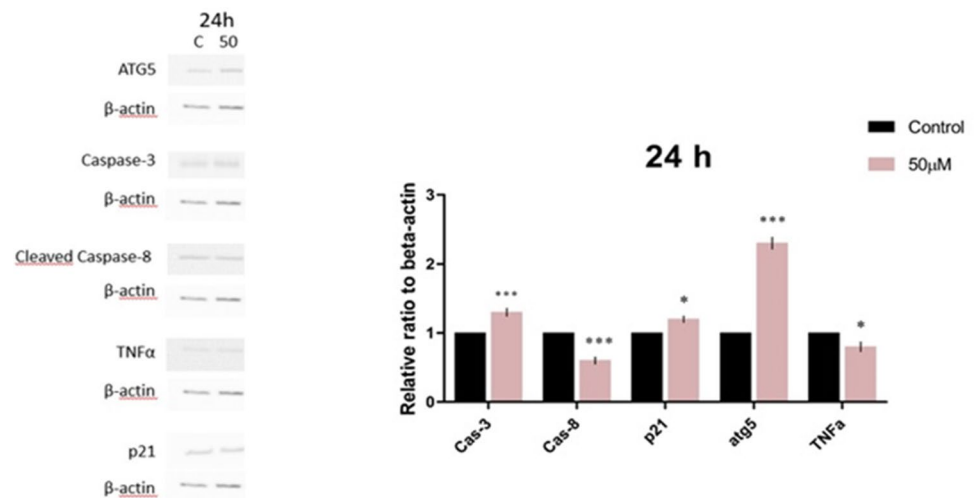


Fig. 5 The effect of TQ on the expression of cleaved caspase-8, caspase-3, p21, atg5 and TNF α proteins in U737 cells. The beta-actin antibody was the internal (loading) control. Data are expressed as mean $\pm$ SD. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ in comparison to control



the growth of human glioblastoma cells and inhibit colony formation in U737 cells [29].

Researches are still ongoing on to understand how TQ exerts its anti-cancer, anti-inflammatory and antineoplastic effects by suppressing pro-apoptotic factors and/

or activating anti-apoptotic factors in cancer cells. TQ also regulates some proteins that belong to either receptor-dependent (extrinsic way) or mitochondrial dependent (intrinsic way) apoptotic pathways [43–46].

TQ is reported to induce apoptosis in cancer cells through generating reactive oxygen species (ROS), DNA damage, telomeric attrition, immunomodulation, regulating signalling pathways and induction of autophagy. It also regulates epithelial to mesenchymal transition (EMT) and inhibits cancer metastasis. In normal cell damage, it shows anti-oxidant and anti-apoptotic effects by reducing oxidative stress [9–11, 40].

Although TQ and its apoptotic effects have been shown in some studies before, more studies are needed to understand the effect of apoptotic and anti-cancer mechanisms.

TQ inhibits cancer cells by apoptotic and autophagic pathways. In a study TQ caused apoptosis due to decreased levels of anti-apoptotic BCL-2 and p53 genes, increased levels of caspases and BAX genes in MCF-7 breast cancer cells [41].

Intrinsic apoptosis is stimulated by genotoxic damage to some organelles in the cell or by oncogene-induced excessive mutagenic stimulation. BAX genes stimulate apoptosis by binding BCL-2 family proteins BAX and BAK lead to release of cytochrome c from mitochondria into the cytoplasm and this activates caspase cascade by activation of caspase 9, active caspase-9 activates caspase-3, caspase 6 and 7 respectively [41].

In a study, TQ was shown to induce both extrinsic and intrinsic apoptotic pathways of brain tumor medulloblastoma [36].

It has been reported that TQ regulates miRNAs in signalling pathways where cancer cells exert effects such as apoptosis, proliferation, angiogenesis and metastasis.

TQ causes an intracellular increase in the levels of apoptotic proteins such as p53 and Bax of GBM cells [47, 48]. It has been found to downregulate Bcl-2 and upregulate Bax, caspase 9 caspase 7 and caspase 3 in acute lymphoblastic leukemia and in gliomas (CEM-ss) [49, 50] In colon cancer cells (HCT116), TQ induced intrinsic apoptosis by upregulating of Bax and downregulating of Bcl-2 [51]. Thymoquinone has shown to activate apoptosis by regulating Bcl-2 and Bax genes in ovarian cancer cells and the potential advantage of TQ for ovarian cancer suggested future clinical trials to confirm its effectiveness [52, 53].

We detected decreasing levels of Bcl-2 at 75 μ M TQ dose and increasing levels of Bax in 25, 50 and 75 μ M using Real Time PCR at the 24th hour.

We also found significantly increasing levels of caspase 9 and caspase 3 at 75 μ M TQ and caspase 3 at 50 TQ dose using Real Time PCR. We also detected increasing levels of caspase 3 at 50 μ M TQ dose at 24 h using Western Blotting. TQ is showed to induce on dependent and independent apoptosis mechanisms in various cancer types [31, 42, 43].

It is also found to induce caspase-independent autophagy by upregulating the p53 gene [33, 54]. In a study leucemic cell were incubated with different TQ concentrations for 24h

and apoptotic and anti-proliferative effect of TQ was shown by detecting p53, p21 and Bcl-2 α [55].

We did not find a significant difference in p53 but we did find a significant increase level in p21 at 75 μ M TQ dose using Real Time PCR and at 50 μ M TQ dose using Western Blotting at the 24th hours.

The extrinsic pathway of apoptosis begins with the binding of FasL and TNF- α to their receptor on the membrane and subsequent activation of caspase-8. Active caspase-8 activates caspase-3, caspase 6 and 7 respectively [20]. In a study with acute lymphoblastic leukemia (CEM-ss) TQ was shown to activate caspase 8 and caspase 3 [49]. We found significantly decreased levels of caspase 8 at 75 TQ dose using Real Time PCR. and at 50 μ M TQ dose using Western blotting at 24 h.

AKT1 activates cell growth and survival but inhibites apoptosis of normal cell. The effective anticancer activity of TQ by the inhibition of AKT pathway was detected in different types of cancer cells previously. The anticancer activity of TQ in kidney cancer cells was detected by the inhibition of cell proliferation mediated by the AKT pathway [56]. We detected significant decrease in levels of AKT-1 at 75 μ M TQ dose using Real Time PCR at the 24th hour.

Activation of autophagy often accelerates the death of cells ATG5 is involved in autophagy and responsible for the formation of a supramolecular protein complex [25, 57]. TQ has been shown to increase the expression of the ATG5 gene in breast cancer cells [58]. We found no difference of ATG5 level using Real Time PCR but we did find increase in ATG5 at 50 μ M TQ dose using western blotting at 24 h.

In Myeloid leukemia KBM-5, TQ suppressed TNF- α -induced NF- κ B activation and down-regulated the expression of NF- κ B-regulated antiapoptotic gene products such as Bcl-2 [59]. It also inhibited proinflammatory genes as TNF- α in pancreatic ductal adenocarcinoma cells indicating its role as an inhibitor of proinflammatory pathways [60]. The anti-inflammatory and antimetastatic effects of TQ were evaluated on TNF- α stimulated human breast adenocarcinoma cells (MDA-MB-231 and MDA-MB-468) [61]. We detected decreasing levels of TNF- α at 75 μ M TQ dose using Real Time PCR and at 50 μ M TQ dose in western blotting at 24 h.

When we evaluated the apoptotic, anti-cancer, anti-proliferative, anti-inflammatory and autophagic effects of TQ on U87 GBM cells; we found that TQ increased the gene and protein levels of BAX and caspase 9 in the intrinsic apoptotic pathway, caspase 3 in both intrinsic and extrinsic apoptotic pathways and decreased BCL-2 gene level. We detected decreased levels of caspase 8 in the extrinsic apoptotic pathway. So we thought that TQ was more effective in the intrinsic pathway. Moreover, we found the anti-proliferative effect of TQ by decreasing AKT-1 gene and increasing p21 gene and protein levels.

We also found that it exerted its effect on autophagy by increasing ATG5 gene and protein levels and its anti-inflammatory effect by decreasing the TNF- α gene and protein levels.

The pharmacokinetics of thymoquinone have been studied in a rabbit model, using different doses of TQ application as intraperitoneal, intravenous and oral. intraperitoneal, It has been found that oral administration had the maximum plasma concentration [62].

There are a few invitro studies on the effect of TQ on glioblastoma. In a study genotoxic, apoptotic and cytotoxic effects of different doses of TQ for 24 h on C6 rat glioma cells were investigated. They showed the increasing number of apoptotic cells, also decreased BCL-2 proteins and increased Bax, caspase 3 and caspase 9 proteins [63, 64].

In a study cytotoxic effects of TQ on brain cancer cells SW1088 was determined [65] In another study after TQ administration Glia 36, T98 and U(MG human glioblastoma cells' proliferation inhibition were found [66].

We suggest that in order to understand the relationship of TQ with apoptosis better, it is necessary to work with different cancer cell types and evaluate the expression of other proteins involved in apoptotic pathways.

We also believe that TQ is effective for cancer therapy and it enhances the effect of cancer drugs. But still there isn't enough clinical studies about TQ application. More studies including clinical trials are necessary to confirm its safety and efficiency in the cancer treatments.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s44411-025-00116-6>.

Acknowledgements None

Author Contributions Authors' contributions All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Pınar Çetinalp, Sevgin Değirmencioğlu, Sevdâ Tanırkulu Küçük, Öykü Gönül Geyik, Senanur Malcanlı and Hikmet Koçak. The first draft of the manuscript was written by Pınar Çetinalp and all authors commented on previous versions of the manuscript. Senanur Malcanlı prepared Figs. 1 and 2, Sevgin Değirmencioğlu prepared Table 1 and Figs. 3–5. All authors read and approved the final manuscript.

Funding Open access funding provided by the Scientific and Technological Research Council of Türkiye (TÜBİTAK). This work was supported by the Research Fund of Demiroğlu Bilim University. Project Number: 20200104.

Data Availability No data sets were generated or analysed during the current study.

Declarations

Conflict of Interest The authors declare no competing interests.

Ethics Approval For this type of study formal consent is not required.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

1. Grossman SA, Batara JF. Current management of glioblastoma multiforme. *Semin Oncol*. 2004;31:635–44.
2. Central Brain Tumor Registry of the United States (CTBRUS) Report 2010; <http://www.cbtrus.org/-factsheet/factsheet.html>.
3. Pazhouhi M, Sariri R, Rabzia A, Khazaei M. Thymoquinone synergistically potentiates temozolomide cytotoxicity through the inhibition of autophagy in U87MG cell line. *Iran J Basic Med Sci*. 2016;19(8):890–8.
4. Majdalawieh AF, Fayyad MW, Nasrallah GK. Anti-cancer properties and mechanisms of action of thymoquinone, the major active ingredient of *Nigella sativa*. *Crit Rev Food Sci Nutr*. 2017;57(18):3911–28. <https://doi.org/10.1080/10408398.2016.1277971>.
5. Aloizou A-M, Pateraki G, Siokas V, Mentis A-FA, Liampas I, Lazopoulos G, Kovatsi L, Mitsias PD, Bogdanos DP, Paterakis K. The role of MiRNA-21 in gliomas: hope for a novel therapeutic intervention? *Toxicol Rep*. 2020;7:1514–30.
6. Stupp R, Mason WP, van den Bent MJ, Weller M, Fisher B, Taphoorn MJB. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *New Engl J Med*. 2005;352:987–96.
7. Rajamanickam S, Agarwal R. Natural products and colon cancer: current status and future prospects. *Drug Develop Res*. 2008;69:460–71.
8. Banerjee S, Azmi AS, Padhye S. Structure-activity studies on therapeutic potential of thymoquinone analogs in pancreatic cancer. *Pharm Res*. 2010;27:1146–58.
9. Khan A, Chen H, Tania M, Zhang D. Anticancer activities of *Nigella sativa* (black cumin). *Afr J Tradit Complement Altern Med*. 2011;8:226–32.
10. Aumeeruddy Zakariyyah M, Mahomoodally MF. Combating breast cancer using combination therapy with 3 phytochemicals: piperine, sulforaphane, and thymoquinone. *Cancer*. 2019;125:1600–11.
11. Padhye S, Banerjee S, Ahmad A, Mohammad R, Sarkar FH. From here to eternity—the secret of pharaohs: therapeutic potential of black cumin seeds and beyond. *Canc Ther*. 2008;6:495–510.
12. Shoieb AM, Elgayyar M, Dudrick PS, Bell JL, Tithof PK. In vitro inhibition of growth and induction of apoptosis in cancer cell lines by thymoquinone. *Int J Oncol*. 2003;22:107–13.
13. Imran M, Abdur R, Imtiaz AK, Shahbaz M, Qaisrani TB, Fatmawati S, Abu-Izneid T, Imran A, Ur Rahman K, Gondal TA. Thymoquinone: a novel strategy to combat cancer: a review. *Biomed Pharmacother*. 2018;106:390–402.
14. Taha MME, Sheikh BY, Salim LZA, Mohan S, Khan A, Kamalidehghan B, Ahmadipour F, Abdelwahab SI. Thymoquinone induces apoptosis and increase ROS in ovarian cancer cell line. *Cell*. 2016;62:97–101.

15. Asaduzzaman KM, Tania M, Fu S, Fu J. Thymoquinone, as an anticancer molecule: from basic research to clinical investigation. *Oncotarget*. 2017;18(31):51907–19. <https://doi.org/10.18632/oncotarget.17206>.
16. Ahmad A, et al. Thymoquinone (2-Isopropyl-5-methyl-1, 4-benzoquinone) as a chemopreventive/anticancer agent: chemistry and biological effects. *Saudi Pharm*. 2019;8:1113–26. <https://doi.org/10.1016/j.jsps.2019.09.008>.
17. Hassan M, Watari H, Abualmaaty A, Ohba Y, Sakuragi N. Apoptosis and molecular targeting therapy in cancer. *Biomed Res Int*. 2014. <https://doi.org/10.1155/2014/150845>.
18. Khan KH, Blanco-Codecido M, Molife LR. Cancer therapeutics: targeting the apoptotic pathway. *Crit Rev Oncol Hematol*. 2013;90:3200–19. <https://doi.org/10.1016/J.CRITREVONC.2013.12.012>.
19. Singh R, Letai A, Sarosiek K. Regulation of apoptosis in health and disease: the balancing act of BCL-2 family proteins. *Nat Rev Mol Cell Biol*. 2019;20:175–93.
20. Qu X, Tang Y, Hua S. Immunological approaches towards cancer and inflammation: a cross talk. *Front Immunol*. 2018;563(6):1625–37. <https://doi.org/10.3389/fimmu.2018.00563>.
21. Mahmoud YK, Abdelrazek HMA. Cancer: thymoquinone antioxidant/pro-oxidant effect as potential anticancer remedy. *Biomed Pharmacother*. 2019;115: 108783. <https://doi.org/10.1016/j.biopha.2019.108783>.
22. Alam M, Hasan GM, Ansari MM, Sharma R, Yadav DK, Hassan MI. Therapeutic implications and clinical manifestations of thymoquinone. *Phytochemistry*. 2022;200: 113213. <https://doi.org/10.1016/j.phytochem.2022.113213>.
23. Dera A, Rajagopalan PJ. Food biochem thymoquinone attenuates phosphorylation of AKT to inhibit kidney cancer cell proliferation. *J Food Biochem*. 2019;43(4): e12793. <https://doi.org/10.1111/jfbc.12793>.
24. Noda NN, Inagaki F. Mechanisms of autophagy. *Annu Rev Biophys*. 2015;44:101–22. <https://doi.org/10.1146/ANNUREV-BIOPHYS-060414-034248>.
25. Green DR, Levine B. ‘To be or not to be?': How selective autophagy and cell death govern cell fate. *Cell*. 2014;157(1):65. <https://doi.org/10.1016/J.CELL.2014.02.049>.
26. Khazaei MR, Bozorgi M, Khazaei M, Moradi A Computational and in vitro analyses on synergistic effects of paclitaxel and thymoquinone in suppressing invasive breast cancer cells A. *Mol Biol Rep*. 2024. <https://doi.org/10.1007/s11033-024-09328-5>.
27. Vichai V, Kirtikara K. Sulforhodamine B colorimetric assay for cytotoxicity screening. *Nat Protoc*. 2006;1(3):1112–6. <https://doi.org/10.1038/nprot.2006.179>.
28. Liang CC, Park AY, Guan JL. In vitro scratch assay: a convenient and inexpensive method for analysis of cell migration in vitro. *Nat Protoc*. 2007;2(2):329–33. <https://doi.org/10.1038/nprot.2007.30>.
29. Franken NAP, Rodermond HM, Stap J, Haveman J, Bree C. Colonyogenic assay of cells in vitro. *Nat Protoc*. 2006;1(5):2315–9. <https://doi.org/10.1038/nprot.2006.339>.
30. Peterson T. Densitometric analysis using NIH image. *NAVBO eNewsletter*. 1997;16(3):1–5. <https://doi.org/10.1038/nprot.2006.339>.
31. Khan MA, Tania M, Wei et al C. Thymoquinone inhibits cancer metastasis by down regulating TWIST1 expression to reduce epithelial to mesenchymal transition. *Oncotarget*. 2015;6(23):19580–91.
32. Zhu WQ, Wang J, Guo XF, Liu Z, Dong WG thymoquinone inhibits proliferation in gastric cancer via the STAT3 pathway in vivo and in vitro. *World J Gastroenterol*. 2016;22:4149–59.
33. Kundu J, Choi BY, Jeong CH, Kundu JK, Chun KS. Thymoquinone induces apoptosis in human colon cancer HCT116 cells through inactivation of STAT3 by blocking JAK2- and Src mediated phosphorylation of EGF. *Spandidos Publications*. 2014;5(29):821–8.
34. Rajput S, Kumar BN, Dey KK, Pal I, Parekh A, Mandal M. Molecular targeting of Akt by thymoquinone promotes G(1) arrest through translation inhibition of cyclin D1 and induces apoptosis in breast cancer cells. *Life Sci*. 2013;93:783–90.
35. Salim LZ, Mohan S, Othman R, Abdelwahab SI, Kamalidehghan B, Sheikh BY, Ibrahim MY. Thymoquinone induces mitochondria-mediated apoptosis in acute lymphoblastic leukaemia in vitro. *Molecules*. 2013;18:11219–40.
36. Ashour AE, Abd-Allah AR, Korashy HM, Attia SM, Alzahrani AZ, Saquib Q, Bakheet SA, Abdel-Hamied HE, Jamal S, Rishi AK. Thymoquinone suppression of the human hepatocellular carcinoma cell growth involves inhibition of IL-8 expression, elevated levels of TRAIL receptors, oxidative stress and apoptosis. *Mol Cell Biochem*. 2014;389:85–98.
37. El-Baba C, Mahadevan V, Fahlbusch FB, Mohan SS, Rau TT, Gali-Muhtasib H, Schneider-Stock R. Thymoquinone-induced conformational changes of PAK1 interrupt prosurvival MEK-ERK signaling in colorectal cancer. *Mol Cancer*. 2014;13:201.
38. Torres MP, Ponnusamy MP, Chakraborty S, Smith LM, Das S, Arafat HA, Batra SK. Effects of thymoquinone in the expression of mucin 4 in pancreatic cancer cells: implications for the development of novel cancer therapies. *Mol Cancer Ther*. 2010;9:1419–31.
39. Cecarini V, Quassinti L, Di Blasio A, Bonfili L, Bramucci M, Lupidi G, Cuccioloni M, Mozzicafreddo M, Angeletti M, Eleuteri AM. Effects of thymoquinone on isolated and cellular proteasomes. *FEBS J*. 2010;277:2128–41.
40. Butt MS, Imran M, Imran A, Arshad MS, Saeed F, Gondal TA, Shariati MA, Gilani SA, Tufail T, Ahmad I, Rind NA, Mahmoodally MF, Islam S, Mehmood Z. Therapeutic perspective of thymoquinone: a mechanistic treatise. *Food Sci Nutr*. 2021. <https://doi.org/10.1002/fsn3.2070>.
41. Yıldırım İH, Azzawri AA, Duran T. Thymoquinone induces apoptosis via targeting the Bax/BAD and Bcl-2 pathway in breast cancer cells. *Dicle Tıp Dergisi/Dicle Med J*. 2019;46(3):411–7.
42. Yool AJ, et al. Molecular targets for combined therapeutic strategies to limit glioblastoma cell migration and invasion. *Front Pharmacol*. 2020;11:27. <https://doi.org/10.3389/fphar.2020.00358>.
43. Alhmied F, Alammam A, Alsultan B, Alshehri M, Pottot FH. Molecular mechanisms of thymoquinone as anticancer agent. *Comb Chem High Throughput Screen*. 2021;24(10):1644–53. <https://doi.org/10.2174/1386207323999201027225305>.
44. Kwan K, Han AY, Mukdad L, Barragan F, Selim O, Alhiyari Y, St John M. Anticancer effects of thymoquinone in head and neck squamous cell carcinoma: a scoping review. *Laryngoscope Investig Otolaryngol*. 2023;8(4):876–85. <https://doi.org/10.1002/lio2.1097>.
45. Chowdhury FA, Hossain MK, Mostofa AGM, Akbor M, Shahdaat M, Sayeed B. Therapeutic potential of thymoquinone in glioblastoma treatment: targeting major gliomagenesis signaling pathways. *Biomed Res Internat*. 2018. <https://doi.org/10.1155/2018/4010629>.
46. Ashour AE, Ahmed AF, Kumar A, Zoheir KM, AboulSoud MA, Ahmad SF, Attia SM, Abd-Allah AR, Cheryan VT, Rishi AK Thymoquinone inhibits growth of human medulloblastoma cells by inducing oxidative stress and caspase-dependent apoptosis while suppressing NF-κB signaling and IL-8 expression. *Mol Cell Biochem*. 2016;416:141–55.
47. Chowdhury FA, Hossain MK, Mostofa AGM, Akbor MM, Bin SS. Therapeutic potential of thymoquinone in glioblastoma treatment: targeting major gliomagenesis signaling pathways. *Biomed Res Int*. 2018;31:4010629. <https://doi.org/10.1155/2018/4010629>.

48. Paramasivam A, Sambantham S, Shabnam J, Raghunandhakumar S, Anandan B, Rajiv R, et al. Anti-cancer effects of thymoquinone in mouse neuroblastoma (Neuro-2a) cells through caspase-3 activation with down-regulation of XIAP. *Toxicol Lett*. 2012;213(2):151–9.
49. Kolli-Bouhafs K, Boukhari A, Abusnina A, Velot E, Gies JP, Lugnier C, Rondé P. thymoquinone reduces migration and invasion of human glioblastoma cells associated with FAK, MMP-2 and MMP-9 down-regulation. *Invest New Drugs*. 2012;30:2121–31.
50. McDonald FE, Ironside JW, Gregor A, Wyatt B, Stewart M, Rye R, Adams J, Potts HWW. The prognostic influence of bcl-2 in malignant glioma. *Br J Cancer*. 2002;86(12):1899–904. <https://doi.org/10.1038/sj.bjc.6600217>.
51. Isaev NK, Chetverikov NS, Stelmashook EV, Genrikhs EE, Khaspekoy LG, Illarioshkin SN. Thymoquinone as a potential neuroprotector in acute and chronic forms of cerebral pathology. *Biochemistry (Mosc)*. 2020;85(2):167–76. <https://doi.org/10.1134/S0006297920020042>.
52. Liu Xiaoli, Dong Jinhu, Cai Wangyu, Pan Ying, Li Ruili, Li Bing. The effect of thymoquinone on apoptosis of SK-OV-3 ovarian cancer cell by regulation of Bcl-2 and bax. *Int J Gynecol Cancer*. 2017;8:1596–601. <https://doi.org/10.1097/IGC.0000000000001064>.
53. Liu Xiaoli, Dong Jinhu, Cai Wangyu, Pan Ying, Li Ruili, Li Bing. The effect of thymoquinone on apoptosis of SK-OV-3 ovarian cancer cell by regulation of Bcl-2 and bax. *Int J Gynecol Cancer*. 2017;27(8):1596–601. <https://doi.org/10.1097/IGC.0000000000001064>.
54. Dastjerdi MN, Mehdiabady EM, Iranpour FG, Bahramian H. Effect of thymoquinone on P53 gene expression and consequence apoptosis in breast cancer cell line. *Int J Prev Med*. 2016;7:66.
55. Diab M, Josiane S, El-Sabban M, Harakeh S. Inhibition of proliferation and induction of apoptosis by thymoquinone via modulation of TGF family, p53, p21 and Bcl-2 α in Leukemic cells. *Anti-Cancer Agents Med Chem*. 2017. <https://doi.org/10.2174/1871520617666170912133054>.
56. Shariati M, Bernstam FM. Targeting AKT for cancer therapy. *Expert Opin Investig Drugs*. 2019;28(11):977–88. <https://doi.org/10.1080/13543784.2019.1676726>.
57. Shojaedini M, Hemadi M, Saki G, Fakhredini F, Khodayar MJ, Khorsandi LJ. Thymoquinone effects on autophagy, apoptosis, and oxidative stress in cisplatin-induced testicular damage in mice. *J Assist Reprod Genet*. 2024;41(7):1881–91.
58. Zhou P, Zhang Z, Liu M, Li P, Zhu Y. Effects of autophagy related gene 5 on tumor development and treatment. *Oncol Rep*. 2023;50(2):155. <https://doi.org/10.3892/or.2023.8592>.
59. Sethi G, Ahn KS, Aggarwal BB. Targeting nuclear factor-kappa B activation pathway by thymoquinone: role in suppression of antiapoptotic gene products and enhancement of apoptosis. *Mol Cancer Res*. 2008;6:1059–70.
60. Chehl N, Chipitsyna G, Gong Q, Yeo CJ, Arafat HA. Antiinflammatory effects of the *Nigella sativa* seed extract, thymoquinone, in pancreatic cancer cells. *HPB (Oxford)*. 2009;11:373–81.
61. Getinet MA, Messeha S, Taka E, Mochona B, Redda KK, Soliman KFA. Thymoquinone inhibition of chemokines in TNF- α -induced inflammatory and metastatic effects in triple-negative breast cancer cells. *Int J Mol Sci*. 2023;24(12):9878. <https://doi.org/10.3390/ijms24129878>.
62. Alkharfy KM, Ahmad A, Khan RM, Al-Shagha WM. Pharmacokinetic plasma behaviors of intravenous and oral bioavailability of thymoquinone in a rabbit model. *Eur J Drug Metab Pharmacokinet*. 2015;40:319–23. <https://doi.org/10.1007/s13318-014-0207-8>.
63. Kurowska N, Madej M, Strzalka-Mrozik B. Thymoquinone: a promising therapeutic agent for the treatment of colorectal cancer. *Curr Issues Mol Biol*. 2023;46(1):121–39. <https://doi.org/10.3390/cimb46010010>.
64. Guler EM, Sisman BH, Kocyigit A, Hatiboglu MA. Investigation of cellular effects of thymoquinone on glioma cell. *Toxicol Rep*. 2021;4(8):162–70. <https://doi.org/10.1016/j.toxrep.2020.12.026>.
65. Shahein Samar A, Aboul-Enein Ahmed M, Higazy Iman M, Faten Abou-Elella, Witold Lojkowski, Ahmed Esam R, Mousa Shaker A, Khaled AbouAitah. Targeted anticancer potential against glioma cells of thymoquinone delivered by mesoporous silica core-shell nanoformulations with pH-dependent release. *Int J Nanomed*. 2019;14:5503. <https://doi.org/10.2147/IJN.S206899>.
66. Racoma Ira O, Hans Meisen Walter, Qi-En Wang, Balveen Kaur, Wani Altaf A. Thymoquinone inhibits autophagy and induces cathepsin-mediated, caspase-independent cell death in glioblastoma cells. *PLoS One*. 2013. <https://doi.org/10.1371/journal.pone.0072882>.

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.