Thymoquinone-chemotherapeutic combinations: new regimen to combat cancer and cancer stem cells

Ali H. El-Far, Mohamed A. Tantawy, Soad K. Al Jaouni & Shaker A. Mousa

Naunyn-Schmiedeberg's Archives of Pharmacology

ISSN 0028-1298

Naunyn-Schmiedeberg's Arch Pharmacol
DOI 10.1007/s00210-020-01898-y

Springer

Naunyn-Schmiedeberg's Archives of Pharmacology
https://doi.org/10.1007/s00210-020-01898-y

REVIEW ARTICLE

Thymoquinone-chemotherapeutic combinations: new regimen to combat cancer and cancer stem cells

Ali H. El-Far[1] · Mohamed A. Tantawy[2,3] · Soad K. Al Jaouni[4] · Shaker A. Mousa[5]

Received: 23 October 2019 / Accepted: 6 May 2020

Abstract

Cancer is a worldwide disease that causes millions of cases of mortality and morbidity. The major problem associated with the cancer is its resistance to conventional therapy and a high relapse rate. The use of chemotherapy to treat cancer began at the start of the twentieth century with attempts to control cancer. In time advance, many cancer chemotherapeutic agents have been developed for cancer treatment with different mechanisms of action including the alkylating agents, antimetabolites, antimicrotubule, topoisomerase inhibitors, and cytotoxic antibiotics, all of which have toxic effects toward normal cells in the body. Here, we reviewed chemotherapeutics' anticancer role potentiation and safety by thymoquinone (TQ) alone or in combination with the most common therapeutic drugs. Our search was done through PubMed, Science Direct, Springer Link, Taylor & Francis Online, Wiley Online Library, Nature publication group, SAGE Journals, and Web of Science databases. We recognized that TQ-chemotherapeutics combination increased chemo-modulation to the anticancer effect of different chemotherapeutics and protected the normal body cells from the toxic injuries that are induced by chemotherapeutics based on its antioxidant power. Moreover, the current study investigates the possible combinatory effect of TQ and chemotherapeutics to control cancer stem cells through molecular docking targeting of wingless/integrated (Wnt) and Hedgehog (Hh). We found that TQ modulates the Wnt and Hh pathways, by binding with tankyrase-2 and smoothened 7TM receptor, respectively, more efficiently than most chemotherapeutics drugs, while methotrexate showed high-binding affinity compared with TQ. Therefore, we encourage researchers to investigate the chemo-modulatory potential and protective effects of TQ in combination with chemotherapeutics for either cancer or cancer stem cell treatment.

Keywords Thymoquinone · Anticancer potential · Chemotherapeutic safety · Cancer stem cells · Molecular docking

Introduction

Cancer is a disease that leads to many deaths worldwide. Cancer types are classified based on the tissue affected: carcinoma (derived from epithelial tissues), sarcoma (soft tissues and bone), glioma (brain), leukemia, and lymphoma (hematopoietic and lymphatic tissues). Among all these types, carcinomas are the most frequent malignant tumors (Parkin et al. 2001).

Ali H. El-Far and Mohamed A. Tantawy equally contributed as first authors.

✉ Ali H. El-Far
ali.elfar@damanhour.edu.eg

✉ Mohamed A. Tantawy
mohamed_tantawy@daad-alumni.de

Soad K. Al Jaouni
saljaouni@kau.edu.sa

Shaker A. Mousa
shaker.mousa@acphs.edu

[1] Department of Biochemistry, Faculty of Veterinary Medicine, Damanhour University, Damanhour 22511, Egypt

[2] Department of Hormones, Medical Research Division, National Research Center, Dokki, Giza 12622, Egypt

[3] Stem Cells Lab, Center of Excellence for Advanced Sciences, National Research Centre, Dokki, Giza 12622, Egypt

[4] Department of Pediatric Hematology/Oncology, King Abdulaziz University Hospital and Scientific Chair of Yousef Abdul Latif Jameel of Prophetic Medicine Application, Faculty of Medicine, King Abdulaziz University, Jeddah 21589, Saudi Arabia

[5] Pharmaceutical Research Institute, Albany College of Pharmacy and Health Sciences, Rensselaer, NY 12144, USA

Published online: 26 May 2020

Numerous studies have been done to control cancer progression using chemotherapy agents (Alfarouk et al. 2015) of different types and mechanisms of action as represented in Table 1. Besides the beneficial role of chemotherapy in cancer treatment, it induces injuries to some normal cells such as the cells of bone marrow, cells lining the gastrointestinal and reproductive tracts, and hair follicles (Lundqvist et al. 2015). Therefore, researchers try to find drugs that potentiate the anticancer effect of chemotherapeutic agents and protect against their toxicities.

Thymoquinone (TQ) is the main active constituent of *Nigella sativa* seeds that has numerous pharmacological activities including anticancer, antioxidant, and antimicrobial (Fig. 1) (El-Far 2015; Atta et al. 2017; El-Far et al. 2018). TQ induces apoptosis in various cancer cell types through numerous cellular targets such as phosphorylation of nuclear factor-κB (NF-κB) and decreasing the extracellular signal–regulated kinases 1/2 (ERK1/2) and phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K) activities. Also, TQ reduced cancer metastasis through activation of c-Jun N-terminal kinases and p38 (Imran et al. 2018). Possible mechanisms of TQ anticancer effects against different cancer types were fully discussed in our previous review (El-Far 2015).

The current review sheds light on the beneficial effect of TQ combinations on the commonly used chemotherapeutic drugs based on data retrieved from published research articles. We also searched the literature for research on the protective role of TQ against chemotherapeutics' toxicities to normal cells in treated animals or patients.

Thymoquinone combination with chemotherapeutic agents

The possible mechanisms of chemo-modulatory and protective effects of TQ and different chemotherapeutic drug combinations in treatment of various cancer types are stated in Table 2 and discussed below in alphabetical order.

5-Fluorouracil

Chemo-modulatory effects 5-Fluorouracil (5-FU) is a drug of choice for colorectal, breast, and aerodigestive tract cancers (Longley et al. 2003). 5-FU is an analogue of uracil inducing disruption in RNA synthesis (Wohlhueter et al. 1980; Houghton et al. 1995). Several studies were done to potentiate the anticancer effect of 5-FU using natural products (Lei et al. 2012; Kensara et al. 2016; Sarman et al. 2016; Liu et al. 2018). TQ was found to potentiate the anticancer effect of 5-FU in azoxymethane-induced colorectal cancer in rats by downregulation of the gene expression of wingless/integrated (Wnt), β-catenin, NF-κB, cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS), and thiobarbituric acid reactive substances (TBRAS) levels, while it upregulated the expression of antitumorigenesis dickkopf–related protein-1 (DKK-1), cyclin-dependent kinase inhibitor-1A (CDNK-1A), transforming growth factor-beta 1 (TGF-β1), transforming growth factor beta receptor II (TGFβRII), Smad4, and glutathione peroxidase (GPx) (Kensara et al. 2016). Sarman et al. (2016) studied the effect of TQ and oxaliplatin (OXA)/5-FU combination on osteosarcoma cell line (MG63). TQ had potential benefits in preventing the OXA and 5-FU-induced toxicities. Also, TQ/5-FU combined treatment induced apoptosis of human gastric cancer cells lines by enhancing caspase-3 and caspase-9 activities (Lei et al. 2012). Indeed, downregulation of Wnt/ß-catenin and PI3K/protein kinase B (AKT) pathways were stated in human colon carcinoma HCT116 and HT29 (Ndreshkjana et al. 2019). In the same manner, TQ/5-FU combination significantly reduced the number of squamous cell carcinoma cell line (FaDu) (Williams et al. 2014).

Protective effects 5-FU may cause some side effects including myelosuppression, mucositis, nausea, emesis, hand-foot syndrome, and mainly toxic cardiac reactions (Steger et al. 2012). We did not find published research studies investigating the protective effect of TQ against 5-FU toxicity.

Bleomycin

Protective effects Bleomycin (BLM) is an antibiotic agent with antitumor activity; it was extracted from the fungus *Streptomyces verticillus* by Umezewa in 1966 (Reinert et al. 2013). Interestingly, the lungs and skin are the targets of BLM toxicity because they have the lowest levels of the BLM hydrolase that metabolizes BLM to non-toxic molecules (Ferrando et al. 1997). BLM administration in rats significantly increased the levels of lactate dehydrogenase (LDH), total protein, and mucin in bronchoalveolar lavage, while in TQ-BLM combination, these parameters were significantly decreased in comparison with BLM-treated rats. Also, TQ significantly increased superoxide dismutase (SOD) and glutathione-*S*-transferase (GST) activities along with downregulation of NF-κB (El-Khouly et al. 2012). The antioxidant activity of TQ is the main mechanism by which TQ protects against BLM toxicity.

Bortezomib

Chemo-modulatory effects Bortezomib (BTZ) is a proteasome inhibitor that upregulates phorbol-12-myristate-13-acetate-induced protein 1 (NOXA), a pro-apoptotic protein inducing cancer cell death. Also, BTZ induced suppression of the NF-κB antiapoptotic signaling (Chen et al. 2011). Siveen et al. (2013) studied the potential effect of TQ on the BTZ anticancer effect against multiple myeloma cell lines through activation of caspase-3, resulting in the cleavage of poly (ADP-ribose) polymerase (PARP). In addition, the authors stated

Table 1 Types and mechanisms of action of chemotherapeutic agents

Types	General mechanism of action	Chemotherapeutic agents	References
Alkylating agents	Covalently binds to DNA via their alkyl group inducing apoptosis.	*Nitrogen mustards*: mechlorethamine, cyclophosphamide, melphalan, chlorambucil, ifosfamide, and busulfan. *Nitrosoureas*: *N*-nitroso-*N*-methylurea, carmustine, lomustine, and semustine, fotemustine, and streptozotocin. *Tetrazines*: dacarbazine, mitozolomide, and temozolomide. *Aziridines*: thiotepa, mitomycin, and diaziquone. *Cisplatin and derivatives*: cisplatin, carboplatin, and oxaliplatin.	Damia and D'Incalci (1998); Lind (2008)
Antimetabolites	Molecules that enter the DNA and RNA synthesis instead of their building blocks.	*Antifolates*: methotrexate and pemetrexed. *Fluoropyrimidines*: fluorouracil and capecitabine. *Deoxynucleoside analogues*: cytarabine, gemcitabine, decitabine, azacitidine, fludarabine, nelarabine, cladribine, clofarabine, and pentostatin. *Thiopurines*: thioguanine and mercaptopurine.	Damia and D'Incalci (1998); Lind (2008); Parker (2009)
Antimicrotubule	Plant-derived chemicals that block cell division by preventing microtubule function.	*Vinca alkaloids*: vincristine and vinblastine. *Taxanes*: paclitaxel and docetaxel.	Heijden et al. (2004); Yue et al. (2010)
Topoisomerase inhibitors	Drugs that affect the activities of topoisomerase I and topoisomerase II.	*Topoisomerase I inhibitors*: camptothecin, irinotecan, and topotecan. *Topoisomerase II poisons*: etoposide, doxorubicin, mitoxantrone, and teniposide. *Topoisomerase II blockers*: novobiocin, merbarone, and aclarubicin	Malhotra and Perry (2003); Nitiss (2009)
Cytotoxic antibiotics	Interrupt cell division.	*Anthracyclines*: doxorubicin and daunorubicin. *Anthracyclines derivatives*: epirubicin and idarubicin. Bleomycin. Actinomycin.	Sobell (1985); Azambuja et al. (2005); Dong et al. (2019)

that TQ potentiated the antitumor effects of BTZ in a mouse model by downregulation of Ki-67, vascular endothelial growth factor (VEGF), B cell lymphoma 2 (Bcl2), and p65 expressions. These studies revealed that TQ/BTZ successfully induced the pro-apoptotic and suppressed the apoptotic molecules leading to cancer control.

Protective effects BTZ is an antineoplastic drug that reversibly inhibits the mammalian 26S proteasome and interacts with the NF-κB system persuading cell cycle arrest. Unfortunately, it induced neurotoxicity as stated by Schiff et al. (2009). We encourage researchers to study the potential effect of TQ on BTZ's anticancer activity and protection against BTZ's neurotoxicity.

Cisplatin

Chemo-modulatory effects Cisplatin (cis-diamminedichloroplatinum II, CDDP) was the first chemotherapeutic drug, discovered in 1965 (Nicholson et al. 2013). CDDP-based combination therapy has been stated to result in a higher response than that of CDDP single-agent therapy (Moon et al. 2018). TQ-CDDP combination potentiated the CDDP cellular apoptosis against human esophageal carcinoma (Eca-109) cell line via blocking of Janus kinase 2 (JAK2)/signal transducer and activator of transcription 3 (STAT3) signaling pathway (Hu et al. 2017b). Combined treatment of CDDP with TQ represented a significant tumor suppression effect superior to single agents through a significant increase in the levels of phosphatase and tensin homolog (PTEN) and obvious decreases in p-AKT, cyclin D1, and P-glycoprotein (P-gp). In vivo results showed that combination of TQ and CDDP represented a more effective antitumor agent than either agent alone in a xenograft tumor mouse model (Ma et al. 2017). Their combination has downregulated NF-κB expression in small cell lung cancer (SCLC) cell lines (Jafri et al. 2010). Another in vitro study of Alaufi et al. (2017) stated that TQ-CDDP combination showed more apoptosis to head and neck squamous cell carcinoma cells (UMSCC-14C) by a percentage of 99.3 ± 1.2%. Furthermore, TQ treatment promoted CDDP-induced pH2AX

expression in mouse ovarian cancer cells (ID8-NGL) and in tumors in C57BL/6 mice (Wilson et al. 2015).

In the abovementioned studies, TQ significantly potentiated the anticancer effect of CDDP through upregulation of apoptotic pathways, cell cycle arrest, and DNA double-strand breaks.

Protective effects Numerous adverse effects have been associated with CDDP medication, particularly nephrotoxicity. Possible mechanisms of CDDP side effects on the kidneys, liver, gastrointestinal tract, and heart are due to reactive oxygen species (ROS) generation (Choi et al. 2015). TQ has protected from CDDP-induced nephrotoxicity by significant decreases in elevated serum urea and creatinine that were increased due to CDDP (Badary et al. 1997). Similarly, CDDP increased plasma neutrophil gelatinase–associated lipocalin (NGAL) and interleukin-6 (IL-6) levels. TQ significantly abrogated the CDDP nephrotoxicity by restoration of these parameters near to their normal levels (Ali et al. 2015). Another study by Farooqui et al. (2017) reported alterations induced in rats' kidney function and the curative effect of TQ. TQ in three different doses (0.5, 1.5, and 3 mg/kg, orally) before and after a single-dose CDDP significantly induced recovery of serum creatinine levels. Also, Ulu et al. (2012) stated that CDDP severely affected the renal transporter and renal efficacy through upregulation of multidrug resistance–associated proteins (MRP2 and MRP4), 8-isoprostane, and malondialdehyde (MDA), while the expression of organic anion transporters (OAT1 and OAT3) and organic cation transporters (OCT1 and OCT2) was significantly reduced. In TQ-CDDP combination, the levels of MRP2 and MRP4 proteins were decreased.

CDDP significantly decreased the hepatic antioxidant status, reduced glutathione (GSH) contents, significantly increased hepatic MDA levels, and upregulated hepatic NF-κB-p65, inflammatory tumor necrosis factor-α (TNF-α), iNOS, and interleukin-1beta (IL-1β). TQ attenuated the CDDP-induced oxidative stress by activation of GPx and GST along with significant decreases in MDA levels and TNF-α, iNOS, and IL-1β expressions (Al-Malki and Sayed 2014).

Another study of Adalı et al. (2016) found the protective potential of TQ against the cardiomyopathic alterations induced by CDDP in rats. The authors reported the beneficial importance of TQ in attenuation of myocardial fibers congestion, edema, and pyknotic nuclei induced by CDDP. In addition, TQ's protective role was determined in CDDP-induced alterations in the activities of brush border membrane, carbohydrate metabolism, and antioxidant defense enzymes in rat intestine (Shahid et al. 2017). Also, TQ significantly increased neuronal cell viability in vitro compared with CDDP-treated cells (Üstün et al. 2018). From this study, we can conclude that TQ is a promising drug that protects against CDDP-induced neurotoxicity. Recently, a natural product mix of *Costus speciosus*, *Fumaria indica*, *Cichorium intybus*, and TQ (CFCT) ameliorated cisplatin-induced hepatorenal injuries in rats via contributing to the antioxidant defense system including SOD, catalase (CAT), GPx, and GST (Abuzinadah and Ahmad 2020).

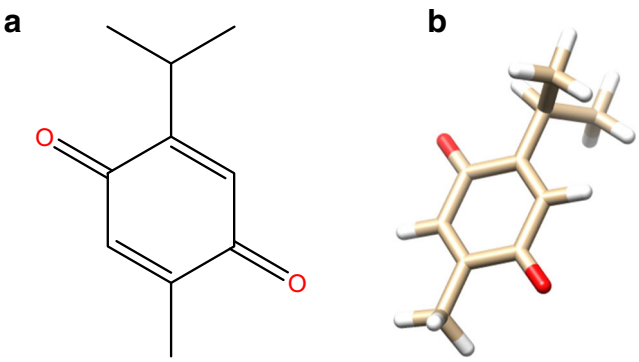

Fig. 1 2D and 3D structures of thymoquinone. **a** 2D was generated using ChemDraw® Ultra program (version 8.0 April 23, 2003). **b** 3D structure was generated using UCSF Chimera (version 1.11.2)

Cyclophosphamide

Chemo-modulatory effects Cyclophosphamide (CTX) is a commonly used drug for treatment of numerous cancers (Fraiser et al. 1991). TQ-CTX (0.5 mM–20 μM) combination significantly induced cell cycle arrest of Her2+ SKBR-3 and Her2− MDA-MB-231 breast cancer cell lines (Khan et al. 2019). Khan and collegues stated that TQ-CTX combination significantly decreased the AKT-phosphorylation and upregulated the PTEN. Furthermore, they reported a significant decrease in cyclin D1 led to cell cycle arrest.

Protective effects CTX causes a wide spectrum of toxicities including damage to the bladder, immunosuppression, alopecia, and cardiotoxicity (Fraiser et al. 1991). For these reasons, some studies have explored the TQ protection of CTX-associated toxicities. Alenzi et al. (2010) observed significant increases in serum liver enzymes activities, bilirubin, urea, creatinine, triacylglycerol, cholesterol, and low-density lipoprotein (LDL)-cholesterol in CTX-treated rats. Treatment of rats with TQ-CTX induced a significant reduction in CTX toxicity. In the same manner, TQ or liposomal formulation of TQ defeated the hepatotoxicity of CTX through enhancement of antioxidant status including SOD and CAT activities in mice. Concomitantly, the extent of liver injuries was decreased and associated with significant declines in elevated serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and bilirubin (Laskar et al. 2016).

CTX resulted in a significant increase in MDA and total nitrate/nitrite while decreasing the GSH, GPx, CAT, and adenosine triphosphate levels in the rats' heart tissue. The elevated

oxidative stress led to heart injuries with elevations of creatine kinase isoform MB (CK-MB), LDH, cholesterol, triacylglycerol, creatinine, urea, and TNF-α. Interestingly, supplementation of CTX-treated rats with TQ approximately reversed the biochemical alterations to their control values. Also, TQ supplementation attenuated CTX-induced cardiotoxicity by the maintenance of heart antioxidant potential (Nagi et al. 2011). In the same manner, TQ significantly potentiated the antioxidant status of CTX-treated animals, leading to significant reduction in oxidative stress and inflammatory markers in rat lung (Suddek et al. 2012), mouse bladder (Gore et al. 2016), and human lymphocytes (Yuksel et al. 2017).

Docetaxel

Chemo-modulatory effects Docetaxel (DTX) is an antimicrotubule cytotoxic agent discovered in the mid-1990s, derived from the needles of yew trees. DTX is a cytotoxic agent for numerous tumor types such as breast, lung, prostate, stomach, head, and neck cancers (Rochigneux et al. 2018). However, DTX has numerous side effects including neuropathy. Therefore, researchers have tried to find a combinatory drug that potentiates DTX cytotoxicity for cancer cells and reduces its side effects. Dirican et al. (2014) studied the effect of TQ-DTX combination against human prostate cancer cell line (DU-145) and found a significant inhibition in PI3K/AKT pathway led to cancer apoptosis.

TQ-DTX combination induced apoptosis in prostate cancer cells (DU145 and C4-2B) via inhibition of the PI3K/AKT signaling pathway and increased the expression of pro-apoptotic markers (Bcl2-associated X protein (Bax) and BH3 interacting-domain death agonist (Bid)) along with caspase-3 and PARP, while decreased the expression of antiapoptotic marker, Bcl-xL (Singh et al. 2019).

TQ-DTX nanoencapsulation formula re-sensitized resistant triple negative breast cancer cell lines (MDA-MB-231) to the anticancer effects of DTX. Also, this nanoencapsulation significantly inhibited cell migration and increased apoptosis of both MCF-7 and MDA-MB-231 (Zafar et al. 2020b). Another coencapsulation of TQ and DTX into PEGylated liposomes was studied by Odeh et al. (2019). The authors showed that the combination of TQ-DTX significantly induced cytotoxicity compared with TQ and DTX alone. Furthermore, Zafar et al. (2020a) optimized low molecular weight chitosan (CS)-grafted lipid nanocapsules for the codelivery of TQ and DTX to treat drug-resistant breast cancer. Nanocapsules had significantly higher cytotoxicity against MCF-7 and MDA-MB-231.

Protective effects DTX has been utilized in the treatment of tumors. Unfortunately, DTX is one of the cytotoxic agents that induces side effects on normal body cells (Syrigou et al. 2011) such as severe abdominal toxicity as recorded by de Matteis et al. (2000). TQ-DTX nanoencapsulation normalized the blood biochemical and histological changes in kidney and liver tissue sections compared with DTX-treated mice (Zafar et al. 2020b).

Doxorubicin

Chemo-modulatory effects Doxorubicin (DOX) is a widely used chemotherapeutic agent for treatment of several carcinomas, sarcomas, and hematological cancers (Carvalho et al. 2009). Therefore, numerous studies have been done to investigate its combination with TQ to potentiate the cytotoxicity of DOX and alleviate its toxicity. El-Ashmawy et al. (2017) studied the effect of TQ-DOX combination against solid Ehrlich carcinoma (SEC)–bearing mice compared with conventional drugs and reported that TQ-induced apoptosis was accompanying with the activation of caspases and PARP cleavage in breast cancer (MCF-7)/DOX cells. Moreover, TQ treatment upregulated Bax and PTEN and downregulated Bcl2 proteins with inhibition of AKT phosphorylation in DOX-treated MCF-7 (Arafa et al. 2011). Also, Effenberger-Neidnicht and Schobert (2010) stated that TQ potentiated the anticancer effect of DOX-treated leukemia (HL-60), melanoma (518A2), colon (HT-29), cervix (KB-V1), and MCF-7 cell lines.

Fatfat et al. (2019) studied the effect of TQ-DOX combination and found significant inhibitions of the viability HTLV-1 positive (HuT-102) and HTLV-1 negative (Jurkat) CD4+ malignant T cell lines and increased sub-G1 cells compared with DOX or TQ alone. Ibiyeye et al. (2019) observed significant increases in MDA-MB-231 apoptosis and reduction of their migration and invasion when treated with TQ-DOX-loaded cockle shell–derived aragonite calcium carbonate nanoparticles compared with free drugs. This may be due to the increased bioavailability of TQ-DOX by nanoencapsulation. In 2020, the same authors repeated the same experiment on MBA-MD-231 cells and observed the same results in addition to inhibition of 3D sphere formation compared with free TQ and DOX (Ibiyeye and Zuki 2020). Recently, we introduced a new idea for cancer control that based on induction of senescence of HCT116 and MCF-7 with 0.1 μM of DOX and at day 5 of DOX treatment, DOX-treated cells were treated with TQ (50 μM) to determine their senolytic effect (El-Far et al. 2020). TQ induced a significant apoptosis of DOX-treated cells compared with the corresponding proliferative cells. Finally, the abovementioned studies of TQ and DOX indicate the sensitivity of cancer cells to DOX-TQ compared with DOX alone.

Protective effects DOX is a chemotherapeutic agent used for breast cancer, leukemia, and lymphoma treatment. However, DOX application is limited due to its severe cardiotoxicity (Cusack et al. 1995) that is mediated by ROS (Šimůnek

Table 2 Possible mechanisms of chemo-modulatory and protective effects alleviation of TQ for different chemotherapeutic drugs

Drugs	Effects	Mechanisms	Animal or cells/organ	References
5-Fluorouracil (5-FU	Chemo-modulatory	↑Caspase-3, ↑caspase-9	Human gastric cancer cells lines (BGC-823, SGC-7901, MGC-803, and HGC-27)	Lei et al. (2012)
		↑Cytotoxicity	Human squamous cell carcinoma cell line (FaDu)	Williams et al. (2014)
		↑Apoptosis	Osteosarcoma (MG63)	Sarman et al. (2016)
		↑DKK-1, ↑CDNK-1A, ↑TGF-β1, ↑TGF-βRII, ↑Smad4, ↑GPx ↓Wnt, ↓β-catenin, ↓NF-κB, ↓COX-2, ↓iNOS, ↓VEGF, ↓TBRAS	Azoxymethane-induced colorectal cancer in rats	Kensara et al. (2016)
		↓Wnt/ß-catenin, ↓PI3K/AKT	Human colon carcinoma (HCT116 and HT29)	Ndreshkjana et al. (2019)
Bleomycin (BLM)	Protective	↑SOD, ↑GST ↓NF-κB	Rat/lung	El-Khouly et al. (2012)
Bortezomib (BTZ)	Chemo-modulatory	↑Caspase-3, ↑PARP cleavage ↓Ki-67, ↓VEGF, ↓Bcl2, ↓p65	Multiple myeloma cell lines/xenograft mouse model.	Siveen et al. (2013)
Cisplatin (CDDP)	Chemo-modulatory	↓NF-κB	Small cell lung cancer (SCLC) cell lines	Jafri et al. (2010)
		↑pH2AX ↓NF-κB	Mouse ovarian cancer cells (ID8-NGL)/ C57BL/6 mice	Wilson et al. (2015)
		↑PTEN, ↑Bax, ↑Cyt c, ↑AIF, ↑cleaved caspase 9, ↑cleaved caspase 3 ↓p-AKT, ↓CyclinD1, ↓P-gp, ↓Bcl2, ↓procaspase-9, ↓procaspase-3	Xenograft tumor mouse model /Human gastric cancer cells lines (SGC-7901, HGC-27, and MGC-803)	Ma et al. (2017)
		↑Apoptosis	Head and neck squamous cell carcinoma cells (UMSCC-14C)	Alaufi et al. (2017)
		↓JAK2/STAT3	Human esophageal carcinoma cells (Eca-109)	Hu et al. (2017b)
	Protective	↓Urea, ↓creatinine	Rat/kidneys	Badary et al. (1997)
		↑OCT1, ↑OCT2, ↑OAT1, ↑OAT3 ↓8-isoprostane, ↓MDA	Rat/kidneys	Ulu et al. (2012)
		↑GST, ↑GPx ↓NF-κB, ↓MDA, ↓TNF-α, ↓iNOS, ↓IL-1β	Rat/liver	Al-Malki and Sayed (2014)
		↓NGAL, ↓IL-6, ↓CRP, ↓creatinine, ↓urea	Rat/kidneys	Ali et al. (2015)
		↑Bcl2	Rat/heart	Adalı et al. (2016)
		↑Brush border membrane (BBM) marker enzymes activities and antioxidant defense enzymes	Rat/intestine	Shahid et al. (2017)
		↑Brush border membrane (BBM) marker enzymes activities and antioxidant defense system in renal cortex and medulla	Rat/kidneys	Farooqui et al. (2017)
		↑SOD, ↑CAT, ↑GPx, ↑GST ↓MDA	Rats/liver and kidneys	Abuzinadah and Ahmad (2020)
Cyclophosphamide (CTX)	Chemo-modulatory	↑PTEN ↓AKT, cyclin D1	Breast cancer cells (Her2+ SKBR-3 and Her2- MDA-231)	Khan et al. (2019)
	Protective	↓ALT, ↓AST, ↓bilirubin, ↓urea, ↓creatinine, ↓triacylglycerol, ↓cholesterol, ↓LDL-C, ↓MDA	Rat/liver	Alenzi et al. (2010)
		↓CK, ↓LDH, ↓cholesterol, ↓triacylglycerol, ↓creatinine, ↓urea, ↓TNF-α	Rat/heart	Nagi et al. (2011)
		↑GSH, ↑SOD↓LDH, ↓TNF-α, ↓TBARS	Rat/lung	Suddek et al. (2012)
		↑Nrf2, ↑GSH, ↑CAT, ↑SOD	Mice/bladder	Gore et al. (2016)
		↑SOD, ↑CAT ↓AST, ↓ALT, ↓bilirubin	Mice/liver	Laskar et al. (2016)

Table 2 (continued)

Drugs	Effects	Mechanisms	Animal or cells/organ	References
		↓DNA strand breaks	Human lymphocytes	Yuksel et al. (2017)
Docetaxel (DTX)	Chemo-modulatory	↓PI3K/AKT	Human prostate cancer cells (DU-145)	Dirican et al. (2014)
		↑Apoptosis	Breast cancer cell (MCF-7)	Odeh et al. (2019)
		↑Bax, ↑Bid, ↑caspase-3, ↑PARP ↓PI3K/AKT	Prostate cancer cells (DU145 and C4-2B)	Singh et al. (2019)
		↑Apoptosis	Breast cancer cell (MCF-7 and MDA-MB-231)	Zafar et al. (2020b)
		↑Apoptosis	Breast cancer cell (MCF-7 and MDA-MB-231)	Zafar et al. (2020a)
	Protective	Normalized the blood biochemical and histological changes	Mice/liver and kidneys	Zafar et al. (2020b)
Doxorubicin (DOX)	Chemo-modulatory	↑Apoptosis	Human cells of leukemia (HL-60), melanoma (518A2), colon (HT-29), cervix (KB-V1), breast (MCF-7) cancers	Effenberger-Neidnicht and Schobert (2010)
		↑Bax, ↑PARP cleavage, ↑PTEN ↓Bcl2	Breast cancer cell (MCF-7).	(Arafa et al. 2011)
		↑P53 ↓Bcl2	Solid Ehrlich carcinoma (SEC)-bearing mice	(El-Ashmawy et al. 2017)
		↑Apoptosis	HTLV-1 positive (HuT-102) and HTLV-1 negative (Jurkat)	(Fatfat et al. 2019)
		↑Apoptosis ↓migration, ↓invasion	Breast cancer cell line (MDA-MB-231)	(Ibiyeye et al. 2019)
		↑Apoptosis ↓migration, ↓invasion, ↓3D sphere formation	Breast cancer cell line (MDA-MB-231)	(Ibiyeye and Zuki 2020)
		↑Apoptosis	Colon cancer cell (HCT116) Breast cancer cell (MCF-7)	(El-Far et al. 2020)
	Protective	↓LDH, ↓CK-MB	Mice/heart	(al-Shabanah et al. 1998)
		↑ NPSH, ↑CAT	Rat/kidneys	(Badary et al. 2000)
		↑SOD, ↑GST, ↑IL-10, ↑Nrf2 ↓MDA, ↓TNF-α, ↓IL-6, ↓NOX-4	Rat/kidneys	Elsherbiny and El-Sherbiny (2014)
		↓LDH, ↓CK-MB	Mice/heart	El-Ashmawy et al. (2017)
		↑GSH, ↑SOD, ↑CAT ↓MDA	Mice/kidneys	Zidan et al. (2018)
		↑CAT, ↑SOD, ↑GR, ↑GPx, ↑GST ↓IL-2, ↓LDH, ↓CK-MB	Mice/heart	Alam et al. (2018a)
Gemcitabine (GEM)	Chemo-modulatory	↓NF-κB	Pancreatic cancer cells (HPAC, BxPC-3, Panc-1, Panc-28, COLO 357, and L3.6pl)	Kaseb et al. (2008)
		↓NF-κB, ↓Bcl2, ↓XIAP, ↓survivin, ↓COX-2	Human pancreatic cancer cell lines (BxPC-3 and HPAC). Human pancreatic ductal epithelial cells and COLO-357.	Banerjee et al. (2009)
		↑PTEN, ↑caspase-3, ↑caspase-9, ↑Bax, ↑Cyt c ↓Notch1, ↓NICD, ↓p65, ↓Bcl2, ↓Bcl-xL, ↓XIAP, ↓AKT/mTOR/S6	Pancreatic cancer (PANC-1)	Mu et al. (2014)
		↑Apoptosis ↓PKM2	Pancreatic cancer (MIA PaCa-2 and PANC-1) cells	Pandita et al. (2014)
		↑Apoptosis, ↑necrosis, ↑autophagy	Breast adenocarcinoma (MCF-7) and ductal carcinoma (T47D) cells	Bashmail et al. (2018)
Ifosfamide (IFO)	Protective	↑GSH	Rat/kidneys	Badary (1999)

Table 2 (continued)

Drugs	Effects	Mechanisms	Animal or cells/organ	References
		↓Creatinine, ↓urea, ↓MDA		
Methotrexate (MTX)	Protective	Improves testicular histopathology	Mice/testis	Gökçe et al. (2010)
		↑Bcl2 ↓p53, ↓caspase 8, ↓caspase 3, ↓caspase 9, ↓Bax	Mice/testis	Sheikhbahaei et al. (2016)
		↑GSH, ↑CAT ↓MDA, ↓iNOS, ↓COX-2, ↓NF-κB, ↓caspase 3	Rat/intestine	El-Sheikh et al. (2016)
Oxaliplatin (OXA)	Chemo-modulatory	↓NF-κB	Pancreatic cancer cells (HPAC, BxPC-3, Panc-1 and MDA Panc-28, COLO 357, L3.6pl)	Kaseb et al. (2008)
		↓NF-κB, ↓Bcl2, ↓XIAP, ↓survivin, ↓COX-2	Human pancreatic cancer cell lines BxPC-3 and HPAC. Human pancreatic ductal epithelial cells and COLO-357	Banerjee et al. (2009)
Paclitaxel (PTX)	Chemo-modulatory	↑Apoptosis, ↑autophagy	Breast cancer cells (MCF-7 and T47D)	Bashmail et al. (2020)
		↑Caspase-3, ↑caspase-7, ↑caspase-12, ↑PARP cleavage ↓p65, ↓AKT1	Mice/triple-negative breast cancer cell line	Şakalar et al. (2015)
		↑Apoptosis	Breast cancer cells (MCF-7)	Soni et al. (2015)
Tamoxifen (TAM)	Chemo-modulatory	↑Bax, ↑AIF, ↑Cyt c, ↑p-27, ↑caspase-9, ↑PARP cleavage ↓Bcl-xL, ↓Bcl2, ↓XIAP	Human breast cancer cell lines (MCF-7, MDA-MB-231, MDA-MB-468, T-47D, NIH/3T3 and HaCaT)	Rajput et al. (2013)
		↑Apoptosis	Estrogen positive (MCF-7) Estrogen negative (MDA-MB-231)	Ganji-Harsini et al. (2016)
	Protective	↑GSH, ↑SOD ↓ALT, ↓AST, ↓ALP, ↓GGT, ↓total bilirubin, ↓MDA, ↓TNF-α	Rat/heart	Suddek (2014)
Temozolomide (TMZ)	Chemo-modulatory	↑Apoptosis	Human glioblastoma multiforme cell line (U87MG)	Pazhouhi et al. (2016)
		↑Apoptosis	Human glioblastoma multiforme cell line (U87MG)	Khazaei and Pazhouhi (2017)
Topotecan (TPT)	Chemo-modulatory	↑Bax/Bcl2, ↑p53, ↑caspase-3, ↑caspase-9	Leukemia (U937) cells	Khalife et al. (2014)
		↑Apoptosis	Human colorectal cancer cells	Khalife et al. (2016)

AIF apoptosis inducing factor, *AKT* protein kinase B, *ALP* alkaline phosphatase, *ALT* alanine aminotransferase, *AST* aspartate aminotransferase, *Bax* Bcl2-associated X protein, *Bcl2* B cell lymphoma 2, *Bid* BH3 interacting-domain death agonist, *CAT* catalase, *CDNK-1A* cyclin-dependent kinase inhibitor 1A, *CK* creatine kinase, *CK-MB* creatine kinase isoform MB, *COX-2* cyclooxygenase-2, *CRP* C-reactive protein, *Cyt c* cytochrome c, *DKK-1* antitumorigenesis dickkopf–related protein-1, *GGT* gamma-glutamyl transferase, *GPx* glutathione peroxidase, *GR* glutathione reductase, *GSH* reduced glutathione, *GST* glutathione-*S*-transferase, *IL-10* interleukin-10, *IL-1β* interleukin-1beta, *IL-2* interleukin-2, *IL-6* interleukin-6, *iNOS* inducible nitric oxide synthase, *JAK2* Janus kinase 2, *LDH* lactate dehydrogenase, *LDL-C* low-density lipoprotein-cholesterol, *MDA* malondialdehyde, *mTOR* mammalian target of rapamycin, *NF-κB* nuclear factor-κB, *NGAL* neutrophil gelatinase-associated lipocalin, *NICD* Notch intracellular domain, *NPSH* non-protein sulfhydryl, *NOX-4* NADPH oxidase 4, *Nrf2* nuclear factor erythroid-2 related factor 2, *OAT1* organic anion transporters 1, *OAT3* organic anion transporters 3, *OCT1* organic cation transporters 1, *OCT2* organic cation transporters 2, *PARP* poly (ADP-ribose) polymerase, *P-gp* P-glycoprotein, *PI3K* phosphatidylinositol-4,5-bisphosphate 3-kinase, *PKM2* pyruvate kinase M2, *PTEN* phosphatase and tensin homolog, *SOD* superoxide dismutase, *STAT3* signal transducer and activator of transcription 3, *TBARS* thiobarbituric acid reactive substances, *TGF-β1* transforming growth factor beta 1, *TGF-βRII* transforming growth factor beta receptor II, *TNF-α* tumor necrosis factor-α, *VEGF* vascular endothelial growth factor, *Wnt* wingless/integrated, *XIAP* X-linked inhibitor of apoptosis protein, ↑ increase or upregulation, ↓ decrease or downregulation

et al. 2009). Therefore, the use of antioxidants in combination with DOX is of great importance to alleviate its toxicities. al-Shabanah et al. (1998) reported that TQ potentially ameliorated cardiotoxicity induced by DOX through significant reductions in serum elevated LDH and CK-MB levels, which means that TQ decreased cardiac injuries. In addition, the histopathological assessment of cardiac tissue evidenced the cardioprotective effect of TQ against DOX medication in mice. Also, El-Ashmawy et al. (2017) determined significant reductions in LDH and CK-MB in DOX-treated mice due to TQ. The elevated serum levels of cardiac markers came from the oxidative injuries induced by DOX in cardiac tissues. But TQ successfully counteracted the oxidative stress by significant enhancements of the cardiac CAT, SOD, GPx, GR, and GST that scavenged and chelated the ROS that was generated by DOX in cardiac tissues (Alam et al. 2018a).

Rats injected with a single intravenous dose of 6 mg per kg DOX had a severe nephrotic syndrome that detected by significant increases in triacylglycerol, total cholesterol, and lipid peroxides in kidneys tissues, while non-protein sulfhydryl (NPSH) content and CAT activity were significantly decreased (Badary et al. 2000). Elsherbiny and El-Sherbiny (2014) stated that DOX-induced nephrotoxicity in rats was mediated by elevated oxidative stress and inflammatory process. DOX enhanced renal MDA with significant decreases in the SOD and GST activities. In addition, DOX treatment induced significant increases in renal levels of TNF-α, IL-6, and NADPH oxidase 4 (NOX-4). TQ overcame the DOX-induced nephrotoxicity through a restoration of antioxidant potentials of renal tissues. The same results were obtained by Zidan et al. (2018) in mice. TQ successfully alleviated the DOX-cardiotoxicity and nephrotoxicity through enhancement of cellular antioxidant potential.

Gemcitabine

Chemo-modulatory effects Gemcitabine (GEM), 2′,2′-difluorodeoxycytidine, is a cytidine analogue that has been used clinically to treat various solid cancers (Hertel et al. 1990). It is a nucleoside analogue that inhibits DNA synthesis by inhibition of DNA polymerase and ribonucleotide reductase, resulting in cancer apoptosis (Aapro et al. 1998). TQ-GEM combination studies are listed in Table 2. Treatment of pancreatic cancer (PANC-1) with TQ-GEM combination led to suppressions of Notch1, Notch intracellular domain (NICD), and AKT/mammalian target of rapamycin (mTOR)/S6 pathways and upregulated PTEN. Moreover, TQ pretreatment and GEM treatment also induced downregulation of antiapoptotic Bcl2, Bcl-xL, X-linked inhibitor of apoptosis protein (XIAP), and upregulation and activation of pro-apoptotic molecules including caspase-3, caspase-9, and Bax (Mu et al. 2014). TQ-GEM combination downregulated the pyruvate kinase M2 (PKM2) that led to inhibition of pancreatic cell lines' (MIA PaCa-2 and PANC-1) proliferation and induced their apoptosis (Pandita et al. 2014). The same effect was stated by Banerjee et al. (2009) who observed downregulation of NF-κB, Bcl2 family, and NF-κB-dependent antiapoptotic genes (XIAP, survivin, and COX-2) in pancreatic cancer cells (BxPC-3 and HPAC) exposed to TQ with GEM or OXA that induced cellular apoptosis. In another study, TQ pretreatment of pancreatic cancer cells (HPAC, BxPC-3, Panc-1 and MDA Panc-28, COLO 357, and L3.6pl) before GEM or OXA downregulated NF-κB (Kaseb et al. 2008).

These studies reflect promise in pancreatic cancer treatment by TQ and GEM combination through upregulation of intrinsic and extrinsic pathways of apoptosis in addition to suppression of Noch1 and NF-κB pathways. Furthermore, TQ showed promising chemo-modulatory effects on GEM against breast adenocarcinoma (MCF-7) and ductal carcinoma (T47D) cells through inducing apoptosis, necrosis, and autophagy (Bashmail et al. 2018).

Protective effects GEM is a well-known chemotherapy that has side effects including bone marrow suppression, liver and kidney problems (Alam et al. 2018b). TQ protection against GEM toxicity has not been investigated.

Ifosfamide

Protective effects Ifosfamide (IFO) is a widespread chemotherapeutic agent, but it can be associated with heart failure, encephalopathy, and hemorrhagic cystitis. Mesna drug administration can control only the urothelial toxicity of IFO (Klastersky 2003). Badary (1999) studied the induction of Fanconi syndrome in rats with a daily injection of IFO at a dose of 50 mg/kg/i.p. for 5 days. TQ supplementation of rats by a dose of 5 mg/kg/day for 5 days ameliorated the renal damage induced by IFO by restoration of renal GSH depletion and MDA accumulation due to IFO. Thus, it is very important to study the protective effect of TQ against IFO toxicity.

Methotrexate

Protective effects Methotrexate (MTX) blocks DNA synthesis by interfering with the metabolism of folic acid. MTX competitively inhibits dihydrofolate reductase that converts dihydrofolate to tetrahydrofolate that is used for thymidine and purines biosynthesis (Howard et al. 2016). However, MTX is accompanied by toxicity that leads to renal dysfunction, myelosuppression, mucositis, dermatologic toxicity, and hepatotoxicity. Therefore, many trials have been done to overcome MTX-associated toxicity. El-Sheikh et al. (2016) observed the intestinal injuries in MTX-treated rats. They exhibited decreases in intestinal GSH concentration and CAT activity, along with a significant rise in MDA level compared

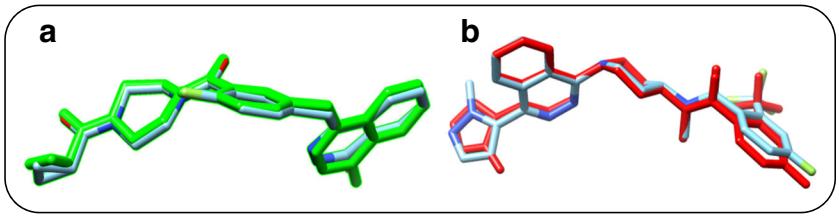

Fig. 2 Docking validation by redocking the cocrystal ligands to their corresponding receptors. The original conformation of each cocrystal ligands is displayed in **a** green stick in the case of tankyrase-2 protein and **b** red stick in the case of smoothened 7TM receptor, while docked poses are represented in gray stick. The root means square deviation (RMSD) was calculated between the original and docked poses of the cocrystal ligands, and it was less than 2 Å.

with the control group. At the molecular level, MTX induced upregulation of intestinal iNOS, COX-2, NF-κB, and caspase 3. TQ significantly reversed the MTX-induced intestinal alteration by significant declines in oxidative/nitrosative stress and apoptotic markers. Another study was done by Sheikhbahaei and colleagues on testicular germ cells. They found that TQ protects the germ cells against MTX-mediated apoptosis by downregulation of p53, caspase 8, caspase 3, caspase 9, and Bax genes while upregulating Bcl2 (Sheikhbahaei et al. 2016). Also, TQ alleviated the histopathological variations induced by MTX in male mice (Gökçe et al. 2010).

Paclitaxel

Chemo-modulatory effects Paclitaxel (PTX) is a taxane member anticancer drug that selectively arrests the cell cycle in G2/M phase (Barbuti and Chen 2015). Many trials have been done to determine the benefits of TQ and PTX anticancer drug combinations. Şakalar et al. (2015) stated that in a triple-negative breast cancer cell line treated with TQ-PTX, genes involved in apoptosis cascade, p53 signaling, and JAK-STAT signaling were differentially expressed. The anticancer effect of PTX against MCF-7 cells was potentiated when PTX was encapsulated with TQ in nanoparticles. Therefore, the authors proposed the usage of drug-loaded TQ-PTX nanoparticles in breast cancer treatment (Soni et al. 2015). Recently, TQ and PTX combination was investigated by Bashmail et al. (2020) against MCF-7 and T47D breast cancer cell lines. TQ significantly increased the apoptotic/necrotic cell death percentages in T47D and significantly induced autophagy in MCF-7 cells after combination with PTX.

Table 3 Results of molecular docking of thymoquinone (TQ) and standard chemotherapeutic drugs against cancer stem cell (CSC) pathways

Drugs	Free energy of binding (Kcal/mol)	
	Wnt	Hedgehog
Methotrexate (MTX)	− 9.6	− 10.1
Thymoquinone (TQ)	− 7.8	− 7.2
Doxorubicin (DOX)	− 9.5	− 5.0
Tamoxifen (TAM)	− 9.5	− 5.2
Paclitaxel (PTX)	− 8.7	− 2.5
Temozolomide (TMZ)	− 8.1	− 7.2
Topotecan (TPT)	− 8.1	− 7.2
Gemcitabine (GEM)	− 7.4	− 7.5
Bleomycin (BLM)	− 7.1	− 2.6
Cyclophosphamide (CTX)	− 6.5	− 6.0
Docetaxel (DTX)	− 6.5	− 6.0
5-fluorouracil (5-FU)	− 5.8	− 5.2

Protective effects PTX is a platinum-based therapy belongs to taxanes with microtubule stabilizing ability (Kampan et al. 2015). However, it may lead to peripheral neurotoxicity especially with high dose (Gordon et al. 1997). The role of TQ's protection against PTX toxicity has not been studied to date.

Tamoxifen

Chemo-modulatory effects Tamoxifen (TAM), a selective estrogen receptor modulator, may control breast cancer proliferation by estrogen receptor antagonism and other mechanisms (Liu et al. 2014). TQ synergizes TAM by mechanisms other than estrogen receptor antagonism as presented in Table 2. TQ-TAM combinations lowered XIAP expression in MCF-7, MDA-MB-231, MDA-MB-468, T-47D, NIH/3T3, and HaCaT cell lines along with inhibition of PI3K/AKT pathway. Downregulation of AKT expression regulated the downstream effectors such as Bcl-xL, Bcl2, and upregulated Bax, as well as apoptosis inducing factor (AIF), cytochrome c, and p-27 expressions (Rajput et al. 2013). A remarkable apoptotic index was observed in combination treatment of either estrogen positive MCF-7 or estrogen negative MDA-MB-231 lines with TQ-TAM (Ganji-Harsini et al. 2016). This novel combination is considered as a promising tool in breast cancer treatment.

Protective effects TAM is a standard chemotherapeutic for breast cancer treatment. Unfortunately, it induces several serious side effects, mainly hepatotoxicity. Suddek (2014) evaluated the effect of TQ on TAM-induced hepatotoxicity in

female rats. TQ significantly decreased hepatic GSH depletion and lipid peroxidation products accumulation. TQ normalized the activity of SOD and inhibited the rise in TNF-α. The authors concluded that TQ protects against TAM-induced hepatotoxicity, which was also confirmed with histopathological analysis of liver sections.

Temozolomide

Chemo-modulatory effects Temozolomide (TMZ), an alkylating agent, is used for melanoma, glioma, and neuroendocrine tumors treatment. Studies listed in Table 2 are examples of TMZ combination with TQ. TQ enhanced the anticancer activity of TMZ by significantly decreasing nitric oxide (NO) production (Pazhouhi et al. 2016) and cell viability (Khazaei and Pazhouhi 2017) of human glioblastoma multiforme cell line (U87MG). Therefore, the combination of TMZ and TQ can be a good plan for the treatment of glioblastoma.

Protective effects TMZ treatment induced liver toxicity associated with significant increases in AST and ALT along with anorexia, alopecia, itching, skin rash, and lung infection (Bae et al. 2014). The protective role of TQ against TMZ toxicity has not been studied to date.

Topotecan

Chemo-modulatory effects Topotecan (TPT) is an anticancer drug for treatment of glioma, small cell lung cancer, prostate cancer, neuroblastoma, leukemia (Hodroj et al. 2018), and ovarian cancers (Mobus et al. 2001). Khalife and colleagues studied the TQ-TPT combination anticancer synergism against leukemia (U937) (Khalife et al. 2014) and human colorectal cancer cells (Khalife et al. 2016). The authors concluded that TQ effectively increased the anticancer effect of TPT against U937 by increasing the levels of Bax/Bcl2, p53, caspase-3, and caspase -9 expressions.

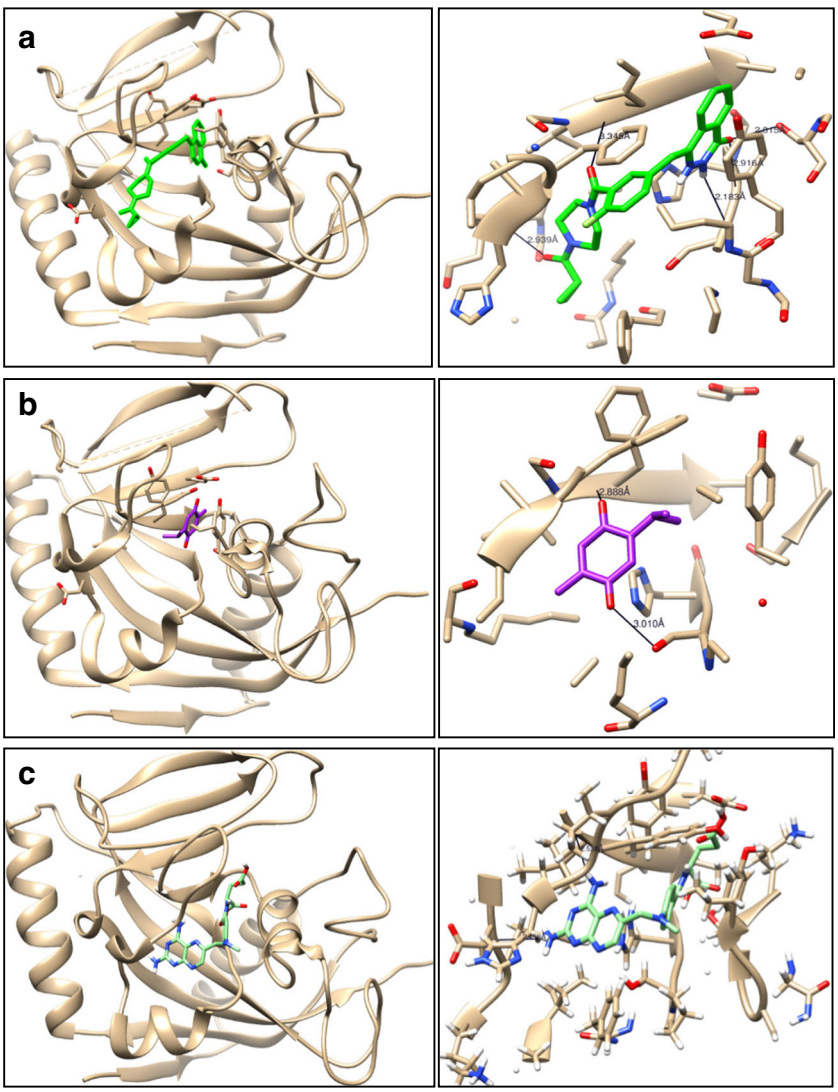

Fig. 3 3D configuration (Left figures) and hydrogen bond formation (right figures) between tested compounds. **a** Reference ligand. **b** Thymoquinone (TQ). **c** Methotrexate with tankyrase-2 receptor

Protective effects In clinical trials, hematologic toxicity has been the main TPT toxicity (Armstrong and O'Reilly 1998). The protective effect of TQ against TPT toxicity has not been studied yet.

Thymoquinone targeting cancer stem cells

Cancer stem cells

Cancer stem cells (CSCs) are a small tumor cell population with high self-renewal capabilities and a greater degree of resistance to traditional chemotherapy and radiotherapy (Han et al. 2013). These kinds of tumor initiating cells are a major driving force for the failure of conventional cancer therapies, relapse, and high mortality rate associated with this disease (Singh and Settleman 2010). Pressingly, in some tumor forms, increased CSC progenitors are closely related to invasiveness, elevated tumor stage, and worse clinical outcome (Hu et al. 2017a). CSCs can be segregated from nearly all cancers on the basis of specific cell surface markers exhibited by these cells (Insan and Jaitak 2014).

CSCs have similar features and are significantly sustained by a number of signaling pathways including Wnt, Hedgehog (Hh), Notch, and PI3K/AKT/mTOR (Moselhy et al. 2015). These pathways are associated in essence with self-renewal, differentiation, drug resistance, and pervasiveness of CSC. These pathways were therefore considered potential hits for therapeutic intervention and ultimately cancer treatment.

Wnt signaling cascade is indeed commonly implicated in many types of cancer (Pu et al. 2017). Tankyrases (TNKS) are thought to be crucial contributors in the Wnt signaling pathway as they have the ability to increase β-catenin by decreasing axis expression to boost Wnt signal pathway (Chen et al. 2009; Huang et al. 2009). In addition, TNKS enhances the length of telomeres by ribosylation of telomeric repeat factor 1 (TRF1), resulting in inappropriately normal cells division, leading to cancer cell transformation (Counter et al. 1994; Folini et al. 2009). Several reports showed that inhibition of TNKS could prevent the massive expansion of cancer cells (Chang et al. 2009; Kirubakaran et al. 2014). Consequently, TNKS inhibition was reported as an encouraging and potent therapeutic approach to a variety of cancers (Zhu et al. 2015).

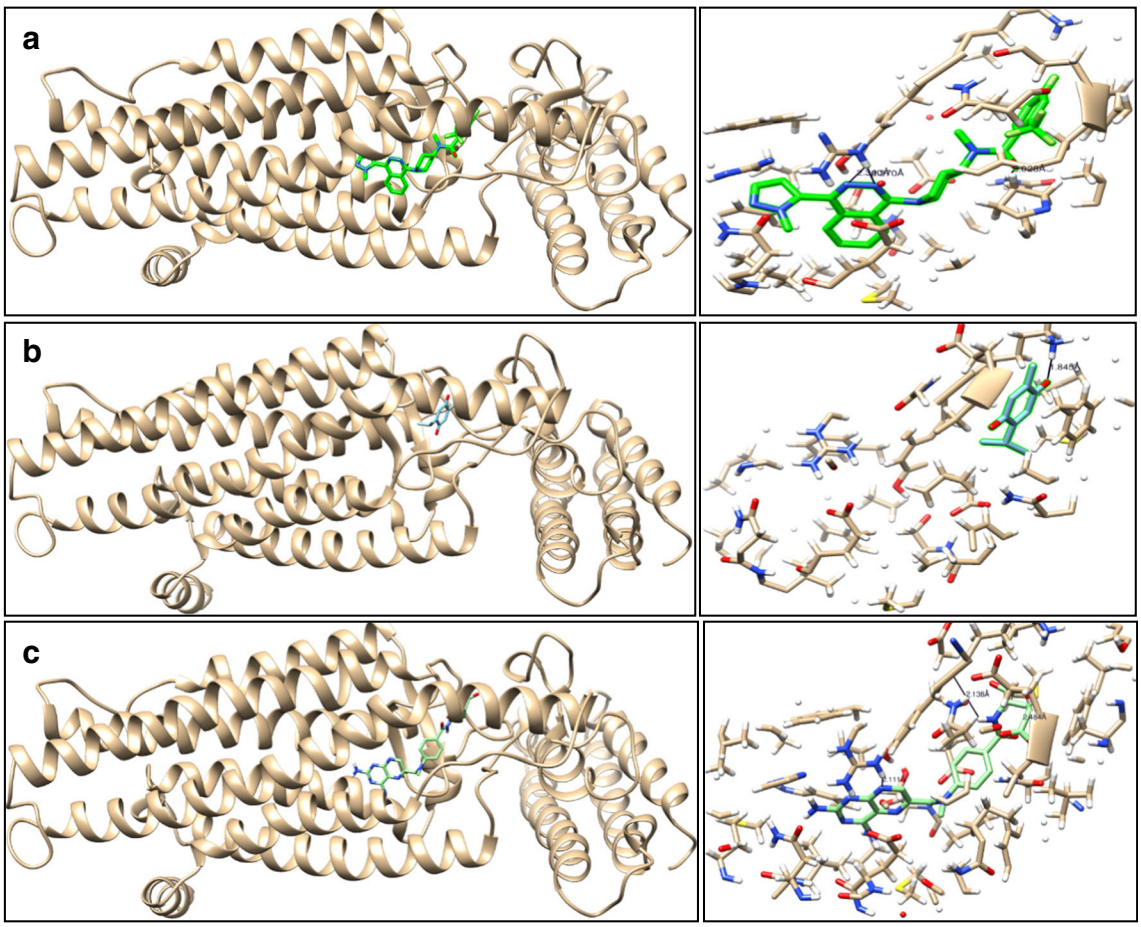

Fig. 4 3D configuration (left figures) and hydrogen bond formation (right figures) between tested compounds. **a** Reference ligand. **b** Thymoquinone (TQ). **c** Methotrexate with smoothened 7TM receptor

Hh pathway is an evolutionary pathway that usually controls the development of tissue by regulating cell fate, expansion, and differentiation (Insan and Jaitak 2014). Its dysregulation has been found to be correlated significantly with multiple types of malignancies along with its intervention in CSC preservation (Merchant and Matsui 2010). Moreover, the Hh pathway includes dynamic interaction between multiple pathways, leading to increasing the possibility for drug resistance (Sengupta et al. 2007). As a targeting strategy for cancer and eradication of CSCs, different targeting techniques have been discovered to control or inhibit Hh pathways, which can be generally listed as Hh protein inhibitors, human smoothened 7TM (Smo) inhibitors, and Gli protein inhibitors (Peukert and Miller-Moslin 2010).

Collectively, numerous targeting CSC strategies hold hope for a long-term treatment and eventually eradication for malignant tumors. Nevertheless, there seem to be no drugs in clinical use to date that deliberately target CSCs. The exploration of CSC-specific drugs is challenging due to various factors that include CSC genotypical heterogeneity and instability, which presents enormous challenges to target these populations specifically (Grichnik 2006). Many natural products have potential effects in sensitizing CSCs to conventional chemotherapy by modulating molecular signaling that governs the stemness properties in a wide range of cancer types (Moselhy et al. 2015).

TQ has a multitargeting ability because it can interact with a broad spectrum of tumorigenic processes by inhibiting malignant transformation and arresting cancer propagation. In addition, TQ can precisely sensitize cancer cells to traditional chemo- and radiotherapies and at the same time mitigate toxic effects in normal cells (Mostofa et al. 2017). Therefore, effective dual-target therapies against CSCs and differentiated tumor cells can be achieved by combining TQ with traditional therapy, and this may counteract tumor growth-related multidrug resistance.

Targeting cancer stem cells signaling pathways by combination of Thymoquinone and chemotherapeutic drugs

To investigate the possible binding of TQ and other chemotherapeutic drugs to Wnt (TNKS 2) and Hh (Smo) signaling pathways, we employed a molecular docking investigation according to our previous publications (El-Kady et al. 2019). Briefly, Chemsketch software (http:/www.acdlabs.com/resources/freeware/) was used to construct the structures of all tested compounds. The structures were prepared using VEGAZZ program for energy minimization (Pedretti et al. 2004). The 3D molecular target structures were retrieved from the Protein Data Bank (PDB) (www.rcsb.org): for this analysis, the X-ray crystal structure of TNKS 2 (PDB ID: 3U9Y) and the human smooth 7TM receptor (PDB ID: 4JKV) were used (Fig. 2). The molecular target preparation steps were done by removing heteroatoms (water and ions), adding polar hydrogen, and assignment of charge. The active sites were defined around the bound cocrystal ligands using grid boxes of appropriate sizes. The docking study and visualization were performed using Autodock vina (Trott and Olson 2010) and Chimera for visualization (Pettersen et al. 2004).

We could see that TQ could modulate and interact with both proteins more efficiently (indicated by low free energy of binding) than most of the chemotherapeutic drugs, except for methotrexate (Table 3; Figs. 3 and 4). Our results were supported by Bashmail and coworkers' finding, as they could show that TQ augments the effect of GEM against breast cancer stem cells ($CD44^{(+)}/CD24^{(-)/(low)}$) (Bashmail et al. 2018). Also, TQ and 5-FU combination targeted the stem cell genes' signature in colorectal cancer cells, leading to an effective eradication of colorectal cancer cells (Ndreshkjana et al. 2019).

Conclusion and recommendations

TQ is a promising drug that potentiates the anticancer activities of various chemotherapeutic agents and can defeat their toxicity to other normal tissues. TQ protects against hepatic, cardiac, renal, neural, and intestinal toxicities of chemotherapeutic agents through enhancement of antioxidant potentials of these organs via significant increases in SOD, CAT, GPx, glutathione reductase (GR), and GST. These enzymes constitute the enzymatic antioxidant cellular defense where SOD converts the toxic superoxide anion to a less toxic H_2O_2. Consequently, the cell can defeat H_2O_2 by CAT and GPx, while GST neutralizes the cellular lipid peroxides in the presence of GSH. GR is responsible for regeneration of GSH from GSSG for antioxidant defense (Birben et al. 2012).

TQ targets CSCs and overcomes chemotherapy resistance associated with this important cell population. To date, researchers have investigated the anticancer potentiation and toxicity alleviation of chemotherapeutics by TQ mostly separately in studies.

Finally, we encourage researchers to investigate both effects in the same experiment of animal models beside in vitro studies to determine the actual doses, frequencies, and metabolism of TQ actions. In addition, we recommend investigating the following ideas:

1) Combinatory effect of TQ on the anticancer effect of other important chemotherapeutic agents that are present in Table 1.
2) Chemotherapeutic agent anticancer potentiation and toxicity alleviation by TQ in the same experiment after in vitro model.

3) TQ-5-FU combination for the treatment of colorectal cancer. Also, the protective role of TQ against 5-FU toxicity.
4) TQ-BLM combination against the gastric, testicular, head and neck, cervix, and vulva cancers. Also, the protective role of TQ against BLM toxicity especially to the lung. Moreover, the effect of TQ on BLM hydrolase especially in the lung and skin.
5) TQ-BTZ combination effect on the NF-κB pathway. Also, the protective role of TQ against BTZ neurotoxicity.
6) TQ-CTX combination effect against various cancer types.
7) TQ-GEM combination effect in the treatment of ovarian, breast, bladder, and non-small cell lung cancer. Also, the protective role of TQ against GEM toxicity.
8) TQ-IFO combination effect in the treatment of several cancer types and study the protective role of TQ against IFO toxicity.
9) TQ-MTX combination effect in the treatment of various cancer types and study the protective role of TQ against MTX toxicity especially intestine.
10) TQ-TAM combination effect in the treatment of breast cancer concerning estrogen receptor antagonist and other pathways such as PI3K/AKT/mTOR.
11) TQ-TMZ combination effect in the treatment of glioma and neuroendocrine tumors.
12) TQ-TPT combination effect in the treatment of neuroblastoma, glioma, prostate, and ovarian cancers.

Acknowledgments The authors give many thanks to Dr. Kelly A. Keating (Pharmaceutical Research Institute, Albany College of Pharmacy and Health Sciences) for her editing and formatting of the manuscript.

Author contributions AF, MT, SA, and SM conceived and designed research. AF and MT conducted data collection. AF drafted the paper. MT conducted molecular docking study. AF, MT, SA, and SM analyzed data, and revised the manuscript. AF and MT submitted the manuscript. All authors read and approved the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Abbreviations AIF, Apoptosis inducing factor; AKT, Protein kinase B; ALP, Alkaline phosphatase; ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; Bax, Bcl2-associated X protein; Bcl2, B cell lymphoma 2; Bid, BH3 interacting-domain death agonist; CAT, Catalase; CDNK-1A, Cyclin-dependent kinase inhibitor 1A; CK, Creatine kinase; CK-MB, Creatine kinase isoform MB; COX-2, Cyclooxygenase-2; CRP, C-reactive protein; Cyt c, Cytochrome c; DKK-1, Antitumorigenesis dickkopf–related protein-1; GGT, Gamma-glutamyl transferase; GPx, Glutathione peroxidase; GR, Glutathione reductase; GSH, Reduced glutathione; GST, Glutathione-*S*-transferase; IL-10, Interleukin-10; IL-1β, Interleukin-1beta; IL-2, Interleukin-2; IL-6, Interleukin-6; iNOS, Inducible nitric oxide synthase; JAK2, Janus kinase 2; LDH, Lactate dehydrogenase; LDL-C, Low-density lipoprotein-cholesterol; MDA, Malondialdehyde; mTOR, Mammalian target of rapamycin; NF-κB, Nuclear factor-κB; NGAL, Neutrophil gelatinase-associated lipocalin; NICD, Notch intracellular domain; NPSH, Non-protein sulfhydryl; NOX-4, NADPH oxidase 4; Nrf2, Nuclear factor erythroid-2 related factor 2; OAT1, Organic anion transporters 1; OAT3, Organic anion transporters 3; OCT1, Organic cation transporters 1; OCT2, Organic cation transporters 2; PARP, Poly (ADP-ribose) polymerase; P-gp, P-glycoprotein; PI3K, Phosphatidylinositol-4,5-bisphosphate 3-kinase; PKM2, Pyruvate kinase M2; PTEN, Phosphatase and tensin homolog; SOD, Superoxide dismutase; STAT3, Signal transducer and activator of transcription 3; TBARS, Thiobarbituric acid reactive substances; TGF-β1, Transforming growth factor beta 1; TGF-βRII, Transforming growth factor beta receptor II; TNF-α, Tumor necrosis factor-α; VEGF, Vascular endothelial growth factor; Wnt, Wingless/integrated; XIAP, X-linked inhibitor of apoptosis protein

References

Aapro MS, Martin C, Hatty S (1998) Gemcitabine—a safety review. Anticancer Drugs 9:191–202. https://doi.org/10.1097/00001813-199803000-00001

Abuzinadah MF, Ahmad A (2020) Pharmacological studies on the efficacy of a thymoquinone-containing novel polyherbal formulation against cisplatin-induced hepatorenal toxicity in rats. J Food Biochem 44:e13131. https://doi.org/10.1111/jfbc.13131

Adalı F, Gonul Y, Kocak A, Yuksel Y, Ozkececi G, Ozdemir C, Tunay K, Bozkurt MF, Sen OG (2016) Effects of thymoquinone against cisplatin-induced cardiac injury in rats. Acta Cir Bras 31:271–277. https://doi.org/10.1590/S0102-865020160040000008

Alam MF, Khan G, Safhi MM, Alshahrani S, Siddiqui R, Sivagurunathan Moni S, Anwer T (2018a) Thymoquinone ameliorates doxorubicin-induced cardiotoxicity in Swiss Albino mice by modulating oxidative damage and cellular inflammation. Cardiol Res Pract 2018: 1483041–1483046. https://doi.org/10.1155/2018/1483041

Alam S, Illo C, Ma YT, Punia P (2018b) Gemcitabine-induced cardiotoxicity in patients receiving adjuvant chemotherapy for pancreatic cancer: a case series. Case Rep Oncol 11:221–227. https://doi.org/10.1159/000488139

Alaufi OM, Noorwali A, Zahran F, al-Abd AM, al-Attas S (2017) Cytotoxicity of thymoquinone alone or in combination with cisplatin (CDDP) against oral squamous cell carcinoma in vitro. Sci Rep 7: 13131. https://doi.org/10.1038/s41598-017-13357-5

Alenzi FQ, El-Bolkiny Yel S, Salem ML (2010) Protective effects of Nigella sativa oil and thymoquinone against toxicity induced by the anticancer drug cyclophosphamide. Br J Biomed Sci 67:20–28

Alfarouk KO, Stock C-M, Taylor S, Walsh M, Muddathir AK, Verduzco D, Bashir AHH, Mohammed OY, Elhassan GO, Harguindey S, Reshkin SJ, Ibrahim ME, Rauch C (2015) Resistance to cancer chemotherapy: failure in drug response from ADME to P-gp. Cancer Cell Int 15:71. https://doi.org/10.1186/s12935-015-0221-1

Ali BH, Al Za'abi M, Shalaby A et al (2015) The effect of thymoquinone treatment on the combined renal and pulmonary toxicity of cisplatin and diesel exhaust particles. Exp Biol Med 240:1698–1707. https://doi.org/10.1177/1535370215579013

Al-Malki AL, Sayed AAR (2014) Thymoquinone attenuates cisplatin-induced hepatotoxicity via nuclear factor kappa-β. BMC Complement Altern Med 14:282. https://doi.org/10.1186/1472-6882-14-282

al-Shabanah OA, Badary OA, Nagi MN et al (1998) Thymoquinone protects against doxorubicin-induced cardiotoxicity without compromising its antitumor activity. J Exp Clin Cancer Res 17: 193–198

Arafa E-SA, Zhu Q, Shah ZI, Wani G, Barakat BM, Racoma I, el-Mahdy MA, Wani AA (2011) Thymoquinone up-regulates PTEN expression and induces apoptosis in doxorubicin-resistant human breast cancer cells. Mutat Res Mol Mech Mutagen 706:28–35. https://doi.org/10.1016/j.mrfmmm.2010.10.007

Armstrong D, O'Reilly S (1998) Clinical guidelines for managing topotecan-related hematologic toxicity. Oncologist 3:4–10

Atta MS, Almadaly EA, El-Far AH et al (2017) Thymoquinone defeats diabetes-induced testicular damage in rats targeting antioxidant, inflammatory and aromatase expression. Int J Mol Sci 18:919. https://doi.org/10.3390/ijms18050919

Azambuja E, Fleck JF, Batista RG, Menna Barreto SS (2005) Bleomycin lung toxicity: who are the patients with increased risk? Pulm Pharmacol Ther 18:363–366. https://doi.org/10.1016/j.pupt.2005.01.007

Badary OA (1999) Thymoquinone attenuates ifosfamide-induced Fanconi syndrome in rats and enhances its antitumor activity in mice. J Ethnopharmacol 67:135–142. https://doi.org/10.1016/S0378-8741(98)00242-6

Badary OA, Nagi MN, Al-Shabanah OA et al (1997) Thymoquinone ameliorates the nephrotoxicity induced by cisplatin in rodents and potentiates its antitumor activity. Can J Physiol Pharmacol 75:1356–1361. https://doi.org/10.1139/cjpp-75-12-1356

Badary OA, Abdel-Naim AB, Abdel-Wahab MH, Hamada FM (2000) The influence of thymoquinone on doxorubicin-induced hyperlipidemic nephropathy in rats. Toxicology 143:219–226. https://doi.org/10.1016/S0300-483X(99)00179-1

Bae SH, Park M-J, Lee MM, Kim TM, Lee SH, Cho SY, Kim YH, Kim YJ, Park CK, Kim CY (2014) Toxicity profile of temozolomide in the treatment of 300 malignant glioma patients in Korea. J Korean Med Sci 29:980–984. https://doi.org/10.3346/jkms.2014.29.7.980

Banerjee S, Kaseb AO, Wang Z, Kong D, Mohammad M, Padhye S, Sarkar FH, Mohammad RM (2009) Antitumor activity of gemcitabine and oxaliplatin is augmented by thymoquinone in pancreatic cancer. Cancer Res 69:5575–5583. https://doi.org/10.1158/0008-5472.can-08-4235

Barbuti A, Chen Z-S (2015) Paclitaxel through the ages of anticancer therapy: exploring Its role in chemoresistance and radiation therapy. Cancers (Basel) 7:2360–2371. https://doi.org/10.3390/cancers7040897

Bashmail HA, Alamoudi AA, Noorwali A, Hegazy GA, AJabnoor G, Choudhry H, al-Abd AM (2018) Thymoquinone synergizes gemcitabine anti-breast cancer activity via modulating its apoptotic and autophagic activities. Sci Rep 8:11674. https://doi.org/10.1038/s41598-018-30046-z

Bashmail HA, Alamoudi AA, Noorwali A, Hegazy GA, Ajabnoor GM, al-Abd AM (2020) Thymoquinone enhances paclitaxel anti-breast cancer activity via inhibiting tumor-associated stem cells despite apparent mathematical antagonism. Molecules 25:426. https://doi.org/10.3390/molecules25020426

Birben E, Sahiner UM, Sackesen C, Erzurum S, Kalayci O (2012) Oxidative stress and antioxidant defense. World Allergy Organ. J. 5:9–19

Carvalho C, Santos R, Cardoso S, Correia S, Oliveira P, Santos M, Moreira P (2009) Doxorubicin: the good, the bad and the ugly Effect. Curr Med Chem 16:3267–3285. https://doi.org/10.2174/092986709788803312

Chang P, Coughlin M, Mitchison TJ (2009) Interaction between poly(ADP-ribose) and NuMA contributes to mitotic spindle pole assembly. Mol Biol Cell 21:4575–4585. https://doi.org/10.1091/mbc.e09-06-0477

Chen B, Dodge ME, Tang W, Lu J, Ma Z, Fan CW, Wei S, Hao W, Kilgore J, Williams NS, Roth MG, Amatruda JF, Chen C, Lum L (2009) Small molecule-mediated disruption of Wnt-dependent signaling in tissue regeneration and cancer. Nat Chem Biol 5:100–107. https://doi.org/10.1038/nchembio.137

Chen D, Frezza M, Schmitt S, Kanwar J, P. Dou Q (2011) Bortezomib as the first proteasome inhibitor anticancer drug: current status and future perspectives. Curr Cancer Drug Targets 11:239–253. https://doi.org/10.2174/156800911794519752

Choi Y-M, Kim H-K, Shim W, Anwar MA, Kwon JW, Kwon HK, Kim HJ, Jeong H, Kim HM, Hwang D, Kim HS, Choi S (2015) Mechanism of cisplatin-induced cytotoxicity is correlated to impaired metabolism due to mitochondrial ROS generation. PLoS One 10:e0135083. https://doi.org/10.1371/journal.pone.0135083

Counter CM, Botelho FM, Wang P, Harley CB, Bacchetti S (1994) Stabilization of short telomeres and telomerase activity accompany immortalization of Epstein-Barr virus-transformed Human B Lymphocytes. J Virol 68:3410–3414

Cusack BJ, Young SP, Olson RD (1995) Daunorubicin and daunorubicinol pharmacokinetics in plasma and tissues in the rat. Cancer Chemother Pharmacol 35:213–218. https://doi.org/10.1007/bf00686550

Damia G, D'Incalci M (1998) Mechanisms of resistance to alkylating agents. Cytotechnology 27:165–173. https://doi.org/10.1007/978-94-017-2374-9_10

de Matteis A, Nuzzo F, Rossi E, Landi G, Perrone F (2000) Intestinal side-effects of docetaxel/vinorelbine combination. Lancet 355: 1098–1099. https://doi.org/10.1016/s0140-6736(05)72219-4

Dirican A, Atmaca H, Bozkurt E, Erten C, Karaca B, Uslu R (2014) Novel combination of docetaxel and thymoquinone induces synergistic cytotoxicity and apoptosis in DU-145 human prostate cancer cells by modulating PI3K–AKT pathway. Clin Transl Oncol 17: 145–151. https://doi.org/10.1007/s12094-014-1206-6

Dong Y, Liao H, Yu J, Fu H, Zhao D, Gong K, Wang Q, Duan Y (2019) Incorporation of drug efflux inhibitor and chemotherapeutic agent into an inorganic/organic platform for the effective treatment of multidrug resistant breast cancer. J Nanobiotechnol 17:125. https://doi.org/10.1186/s12951-019-0559-y

Effenberger-Neidnicht K, Schobert R (2010) Combinatorial effects of thymoquinone on the anti-cancer activity of doxorubicin. Cancer Chemother Pharmacol 67:867–874. https://doi.org/10.1007/s00280-010-1386-x

El-Ashmawy NE, Khedr EG, Ebeid E-ZM et al (2017) Enhanced anticancer effect and reduced toxicity of doxorubicin in combination with thymoquinone released from poly-N-acetyl glucosamine nanomatrix in mice bearing solid Ehrlish carcinoma. Eur J Pharm Sci 109:525–532. https://doi.org/10.1016/j.ejps.2017.09.012

El-Far AH (2015) Thymoquinone anticancer discovery: possible mechanisms. Curr Drug Discov Technol 12:80–89

El-Far AH, Al Jaouni SK, Li W, Mousa SA (2018) Protective roles of thymoquinone nanoformulations: potential nanonutraceuticals in human diseases. Nutrients 10:1369

El-Far AH, Darwish NHE, Mousa SA (2020) Senescent colon and breast cancer cells induced by doxorubicin exhibit enhanced sensitivity to curcumin, caffeine, and thymoquinone. Integr Cancer Ther 19:1–14. https://doi.org/10.1177/1534735419901160

El-Kady DS, Abd Rabou AA, Tantawy MA et al (2019) Synthesis and evaluation of novel cholestanoheterocyclic steroids as anticancer agents. Appl Biochem Biotechnol 188:1–28. https://doi.org/10.1007/s12010-018-02943-6

El-Khouly D, El-Bakly WM, Awad AS et al (2012) Thymoquinone blocks lung injury and fibrosis by attenuating bleomycin-induced oxidative stress and activation of nuclear factor Kappa-B in rats. Toxicology 302:106–113. https://doi.org/10.1016/j.tox.2012.09.001

El-Sheikh A, Morsy M, Hamouda A (2016) Protective mechanisms of thymoquinone on methotrexate-induced intestinal toxicity in rats. Pharmacogn Mag 12:76. https://doi.org/10.4103/0973-1296.176106

Elsherbiny NM, El-Sherbiny M (2014) Thymoquinone attenuates doxorubicin-induced nephrotoxicity in rats: role of Nrf2 and NOX4. Chem Biol Interact 223:102–108. https://doi.org/10.1016/j.cbi.2014.09.015

Farooqui Z, Shahid F, Khan AA, Khan F (2017) Oral administration of Nigella sativa oil and thymoquinone attenuates long term cisplatin treatment induced toxicity and oxidative damage in rat kidney. Biomed Pharmacother 96:912–923. https://doi.org/10.1016/j.biopha.2017.12.007

Fatfat M, Fakhoury I, Habli Z, Mismar R, Gali-Muhtasib H (2019) Thymoquinone enhances the anticancer activity of doxorubicin against adult T-cell leukemia in vitro and in vivo through ROS-dependent mechanisms. Life Sci 232:116628. https://doi.org/10.1016/j.lfs.2019.116628

Ferrando AA, Pendás AM, Llano E, Velasco G, Lidereau R, López-Otín C (1997) Gene characterization, promoter analysis, and chromosomal localization of human bleomycin hydrolase. J Biol Chem 272:33298–33304. https://doi.org/10.1074/jbc.272.52.33298

Folini M, Gandellini P, Zaffaroni N (2009) Targeting the telosome: therapeutic implications. Biochim Biophys Acta - Mol Basis Dis 1792:309–316. https://doi.org/10.1016/j.bbadis.2009.01.014

Fraiser LH, Kanekal S, Kehrer JP (1991) Cyclophosphamide toxicity. Characterising and avoiding the problem. Drugs 42:781–795

Ganji-Harsini S, Khazaei M, Rashidi Z, Ghanbari A (2016) Thymoquinone could increase the efficacy of tamoxifen induced apoptosis in human breast cancer cells: an in vitro study. Cell J 18:245–254

Gökçe A, Oktar S, Koc A, Yonden Z (2010) Protective effects of thymoquinone against methotrexate-induced testicular injury. Hum Exp Toxicol 30:897–903. https://doi.org/10.1177/0960327110382564

Gordon AN, Stringer CA, Matthews CM, Willis DL, Nemunaitis J (1997) Phase I dose escalation of paclitaxel in patients with advanced ovarian cancer receiving cisplatin: rapid development of neurotoxicity is dose-limiting. J Clin Oncol 15:1965–1973. https://doi.org/10.1200/jco.1997.15.5.1965

Gore PR, Prajapati CP, Mahajan UB, Goyal SN, Belemkar S, Ojha S, Patil CR (2016) Protective effect of thymoquinone against cyclophosphamide-induced hemorrhagic cystitis through inhibiting DNA damage and upregulation of Nrf2 expression. Int J Biol Sci 12:944–953. https://doi.org/10.7150/ijbs.15781

Grichnik JM (2006) Genomic Instability and Tumor Stem Cells. J Invest Dermatol 126:1214–1216. https://doi.org/10.1038/sj.jid.5700240

Han L, Shi S, Gong T, Zhang Z, Sun X (2013) Cancer stem cells: therapeutic implications and perspectives in cancer therapy. Acta Pharm Sin B 3:65–75. https://doi.org/10.1016/j.apsb.2013.02.006

Heijden R, Jacobs D, Snoeijer W, Hallard D, Verpoorte R (2004) The catharanthus alkaloids: pharmacognosy and biotechnology. Curr Med Chem 11:607–628. https://doi.org/10.2174/0929867043455846

Hertel LW, Boder GB, Kroin JS, Rinzel SM, Poore GA, Todd GC, Grindey GB (1990) Evaluation of the antitumor activity of gemcitabine (2',2'-difluoro-2'-deoxycytidine). Cancer Res 50:4417–4422

Hodroj MH, Jardaly A, abi Raad S et al (2018) Andrographolide potentiates the antitumor effect of topotecan in acute myeloid leukemia cells through an intrinsic apoptotic pathway. Cancer Manag Res Vol 10:1079–1088. https://doi.org/10.2147/cmar.s160924

Houghton JA, Tillman DM, Harwood FG (1995) Ratio of 2'-deoxyadenosine-5'-triphosphate/thymidine-5'-triphosphate influences the commitment of human colon carcinoma cells to thymineless death. Clin Cancer Res 1:723–730

Howard SC, McCormick J, Pui CH, Buddington RK, Harvey RD (2016) Preventing and managing toxicities of high-dose methotrexate. Oncologist 21:1471–1482. https://doi.org/10.1634/theoncologist.2015-0164

Hu X, Cong Y, Luo HH, Wu S, Zhao LE, Liu Q, Yang Y (2017a) Cancer stem cells therapeutic target database: the first comprehensive database for therapeutic targets of cancer stem cells. Stem Cells Transl Med 6:331–334. https://doi.org/10.5966/sctm.2015-0289

Hu X, Ma J, Vikash V, Li J, Wu D, Liu Y, Zhang J, Dong W (2017b) Thymoquinone augments cisplatin-induced apoptosis on esophageal carcinoma through mitigating the activation of JAK2/STAT3 pathway. Dig Dis Sci 63:126–134. https://doi.org/10.1007/s10620-017-4856-8

Huang SMA, Mishina YM, Liu S, Cheung A, Stegmeier F, Michaud GA, Charlat O, Wiellette E, Zhang Y, Wiessner S, Hild M, Shi X, Wilson CJ, Mickanin C, Myer V, Fazal A, Tomlinson R, Serluca F, Shao W, Cheng H, Shultz M, Rau C, Schirle M, Schlegl J, Ghidelli S, Fawell S, Lu C, Curtis D, Kirschner MW, Lengauer C, Finan PM, Tallarico JA, Bouwmeester T, Porter JA, Bauer A, Cong F (2009) Tankyrase inhibition stabilizes axin and antagonizes Wnt signalling. Nature 461:614–620. https://doi.org/10.1038/nature08356

Ibiyeye KM, Zuki ABZ (2020) Cockle shell-derived aragonite CaCO3 nanoparticles for co-delivery of doxorubicin and thymoquinone eliminates cancer stem cells. Int J Mol Sci 21:1900. https://doi.org/10.3390/ijms21051900

Ibiyeye KM, Nordin N, Ajat M, Zuki ABZ (2019) Ultrastructural changes and antitumor effects of doxorubicin/thymoquinone-loaded CaCO3 nanoparticles on breast cancer cell line. Front Oncol 9:599. https://doi.org/10.3389/fonc.2019.00599

Imran M, Rauf A, Khan IA, Shahbaz M, Qaisrani TB, Fatmawati S, Abu-Izneid T, Imran A, Rahman KU, Gondal TA (2018) Thymoquinone: a novel strategy to combat cancer: a review. Biomed. Pharmacother. 106:390–402

Insan M, Jaitak V (2014) New approaches to target cancer stem cells: current scenario. Mini-Rev Med Chem 14:20–34. https://doi.org/10.2174/13895575113136660107

Jafri SH, Glass J, Shi R, Zhang S, Prince M, Kleiner-Hancock H (2010) Thymoquinone and cisplatin as a therapeutic combination in lung cancer: in vitro and in vivo. J Exp Clin Cancer Res 29:87. https://doi.org/10.1186/1756-9966-29-87

Kampan NC, Madondo MT, McNally OM et al (2015) Paclitaxel and its evolving role in the management of ovarian cancer. Biomed Res Int 2015:1–21. https://doi.org/10.1155/2015/413076

Kaseb AO, Banerjee S, Wang Z, Sarkar FH, Ramzi M (2008) Enhanced chemosensitivity to gemcitabine and oxaliplatin by thymoquinone in pancreatic cancer. J Clin Oncol 26:15565. https://doi.org/10.1200/jco.2008.26.15_suppl.15565

Kensara O, El-Shemi A, Mohamed A et al (2016) Thymoquinone subdues tumor growth and potentiates the chemopreventive effect of 5-fluorouracil on the early stages of colorectal carcinogenesis in rats. Drug Des Devel Ther 10:2239–2253. https://doi.org/10.2147/dddt.s109721

Khalife R, Stephany E-H, Tarras O, Hodroj MH, Rizk S (2014) Antiproliferative and proapoptotic effects of topotecan in combination with thymoquinone on acute myelogenous leukemia. Clin Lymphoma Myeloma Leuk 14:S46–S55. https://doi.org/10.1016/j.clml.2014.04.014

Khalife R, Hodroj M, Fakhoury R, Rizk S (2016) Thymoquinone from Nigella sativa seeds promotes the antitumor activity of noncytotoxic doses of topotecan in human colorectal cancer cells in vitro. Planta Med 82:312–321. https://doi.org/10.1055/s-0035-1558289

Khan A, Aldebasi YH, Alsuhaibani SA, Khan MA (2019) Thymoquinone augments cyclophosphamide-mediated inhibition of cell proliferation in breast cancer cells. Asian Pacific J Cancer Prev 20:1153–1160. https://doi.org/10.31557/APJCP.2019.20.4.1153

Khazaei M, Pazhouhi M (2017) Temozolomide-mediated apoptotic death is improved by thymoquinone in U87MG cell Line. Cancer Invest 35:225–236. https://doi.org/10.1080/07357907.2017.1289383

Kirubakaran P, Arunkumar P, Premkumar K, Muthusamy K (2014) Sighting of tankyrase inhibitors by structure- and ligand-based screening and in vitro approach. Mol Biosyst 10:2699–2712. https://doi.org/10.1039/c4mb00309h

Klastersky J (2003) Side effects of ifosfamide. Oncology 65:7–10. https://doi.org/10.1159/000073351

Laskar AA, Khan MA, Rahmani AH, Fatima S, Younus H (2016) Thymoquinone, an active constituent of Nigella sativa seeds, binds with bilirubin and protects mice from hyperbilirubinemia and cyclophosphamide-induced hepatotoxicity. Biochimie 127:205–213. https://doi.org/10.1016/j.biochi.2016.05.020

Lei X, Lv X, Liu M, Yang Z, Ji M, Guo X, Dong W (2012) Thymoquinone inhibits growth and augments 5-fluorouracil-induced apoptosis in gastric cancer cells both in vitro and in vivo. Biochem Biophys Res Commun 417:864–868. https://doi.org/10.1016/j.bbrc.2011.12.063

Lind MJ (2008) Principles of cytotoxic chemotherapy. Medicine (Baltimore). 36:19–23

Liu C-Y, Hung M-H, Wang D-S, Chu PY, Su JC, Teng TH, Huang CT, Chao TT, Wang CY, Shiau CW, Tseng LM, Chen KF (2014) Tamoxifen induces apoptosis through cancerous inhibitor of protein phosphatase 2A–dependent phospho-Akt inactivation in estrogen receptor–negative human breast cancer cells. Breast Cancer Res 16:431. https://doi.org/10.1186/s13058-014-0431-9

Liu H, Liu H, Zhou Z, Parise RA, Chu E, Schmitz JC (2018) Herbal formula Huang Qin Ge Gen Tang enhances 5-fluorouracil antitumor activity through modulation of the E2F1/TS pathway. Cell Commun Signal 16:7. https://doi.org/10.1186/s12964-018-0218-1

Longley DB, Harkin DP, Johnston PG (2003) 5-Fluorouracil: mechanisms of action and clinical strategies. Nat Rev Cancer 3:330–338. https://doi.org/10.1038/nrc1074

Lundqvist EÅ, Fujiwara K, Seoud M (2015) Principles of chemotherapy. Int J Gynecol Obstet 131:S146–S149. https://doi.org/10.1016/j.ijgo.2015.06.011

Ma J, Hu X, Li J et al (2017) Enhancing conventional chemotherapy drug cisplatin-induced anti-tumor effects on human gastric cancer cells both in vitro and in vivo by Thymoquinone targeting PTEN gene. Oncotarget 8:85926–85939. https://doi.org/10.18632/oncotarget.20721

Malhotra V, Perry MC (2003) Classical chemotherapy: mechanisms, toxicities and the therapeutic window. Cancer Biol. Ther. 2:S2–S4

Merchant AA, Matsui W (2010) Targeting Hedgehog – a cancer stem cell pathway. Clin Cancer Res 16:3130–3140. https://doi.org/10.1158/1078-0432.CCR-09-2846

Mobus V, Pfaff PN, Volm T et al (2001) Long time therapy with topotecan in patients with recurrence of ovarian carcinoma. Anticancer Res 21:3551–3556

Moon JY, Song I-C, Ko YB, Lee HJ (2018) The combination of cisplatin and topotecan as a second-line treatment for patients with advanced/recurrent uterine cervix cancer. Medicine (Baltimore) 97:e0340. https://doi.org/10.1097/md.0000000000010340

Moselhy J, Srinivasan S, Ankem MK, Damodaran C (2015) Natural products that target cancer stem cells. Anticancer Res 35:5773–5788

Mostofa AGM, Hossain MK, Basak D, Bin Sayeed MS (2017) Thymoquinone as a potential adjuvant therapy for cancer treatment: evidence from preclinical studies. Front Pharmacol 8. https://doi.org/10.3389/fphar.2017.00295

Mu G, Zhang L, Li H, Liao Y, Yu HG (2014) Thymoquinone pretreatment overcomes the insensitivity and potentiates the antitumor effect of gemcitabine through abrogation of Notch1, PI3K/Akt/mTOR regulated signaling pathways in pancreatic cancer. Dig Dis Sci 60:1067–1080. https://doi.org/10.1007/s10620-014-3394-x

Nagi MN, Al-Shabanah OA, Hafez MM, Sayed-Ahmed MM (2011) Thymoquinone supplementation attenuates cyclophosphamide-induced cardiotoxicity in rats. J Biochem Mol Toxicol 25:135–142. https://doi.org/10.1002/jbt.20369

Ndreshkjana B, Çapci A, Klein V, Chanvorachote P, Muenzner JK, Huebner K, Steinmann S, Erlenbach-Wuensch K, Geppert CI, Agaimy A, Ballout F, el-Baba C, Gali-Muhtasib H, Roehe AV, Hartmann A, Tsogoeva SB, Schneider-Stock R (2019) Combination of 5-fluorouracil and thymoquinone targets stem cell gene signature in colorectal cancer cells. Cell Death Dis 10:379. https://doi.org/10.1038/s41419-019-1611-4

Nicholson S, Hall E, Harland SJ, Chester JD, Pickering L, Barber J, Elliott T, Thomson A, Burnett S, Cruickshank C, Carrington B, Waters R, Bahl A (2013) Phase II trial of docetaxel, cisplatin and 5FU chemotherapy in locally advanced and metastatic penis cancer (CRUK/09/001). Br J Cancer 109:2554–2559. https://doi.org/10.1038/bjc.2013.620

Nitiss JL (2009) Targeting DNA topoisomerase II in cancer chemotherapy. Nat. Rev. Cancer 9:338–350

Odeh F, Naffa R, Azzam H, Mahmoud IS, Alshaer W, al Bawab A, Ismail S (2019) Co-encapsulation of thymoquinone with docetaxel enhances the encapsulation efficiency into PEGylated liposomes and the chemosensitivity of MCF7 breast cancer cells to docetaxel. Heliyon 5:e02919. https://doi.org/10.1016/j.heliyon.2019.e02919

Pandita A, Kumar B, Manvati S, Vaishnavi S, Singh SK, Bamezai RNK (2014) Synergistic combination of gemcitabine and dietary molecule induces apoptosis in pancreatic cancer cells and down regulates PKM2 expression. PLoS One 9:e107154. https://doi.org/10.1371/journal.pone.0107154

Parker WB (2009) Enzymology of purine and pyrimidine antimetabolites used in the treatment of cancer. Chem Rev 109:2880–2893. https://doi.org/10.1021/cr900028p

Parkin DM, Bray F, Ferlay J, Pisani P (2001) Estimating the world cancer burden: Globocan 2000. Int J Cancer 94:153–156. https://doi.org/10.1002/ijc.1440

Pazhouhi M, Sariri R, Rabzia A, Khazaei M (2016) Thymoquinone synergistically potentiates temozolomide cytotoxicity through the inhibition of autophagy in U87MG cell line. Iran J Basic Med Sci 19:890–898

Pedretti A, Villa L, Vistoli G (2004) VEGA - an open platform to develop chemo-bio-informatics applications, using plug-in architecture and script programming. J Comput Aided Mol Des 18:167–173. https://doi.org/10.1023/B:JCAM.0000035186.90683.f2

Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, Ferrin TE (2004) UCSF Chimera–a visualization system for exploratory research and analysis. J Comput Chem 25:1605–1612. https://doi.org/10.1002/jcc.20084

Peukert S, Miller-Moslin K (2010) Small-molecule inhibitors of the Hedgehog signaling pathway as cancer therapeutics. ChemMedChem 5:500–512. https://doi.org/10.1002/cmdc.201000011

Pu Y, Zhang S, Chang Z, Zhang Y, Wang D, Zhang L, Li Y, Zuo Z (2017) Discovery of new dual binding TNKS inhibitors of Wnt signaling inhibition by pharmacophore modeling, molecular docking and bioassay. Mol Biosyst 13:363–370. https://doi.org/10.1039/C6MB00712K

Rajput S, Kumar BNP, Sarkar S, Das S, Azab B, Santhekadur PK, Das SK, Emdad L, Sarkar D, Fisher PB, Mandal M (2013) Targeted apoptotic effects of thymoquinone and tamoxifen on XIAP mediated Akt regulation in breast cancer. PLoS One 8:e61342. https://doi.org/10.1371/journal.pone.0061342

Reinert T, Baldotto CS d R, Nunes FAP, Scheliga AA d S (2013) Bleomycin-induced lung injury. J Cancer Res 2013:1–9. https://doi.org/10.1155/2013/480608

Rochigneux P, Schleinitz N, Ebbo M, Aymonier M, Pourroy B, Boissier R, Salas S, Deville JL (2018) Acute myositis: an unusual and severe

side effect of docetaxel: a case report and literature review. Anticancer Drugs 29:477–481. https://doi.org/10.1097/cad.0000000000000620

Şakalar Ç, İzgi K, İskender B, Sezen S, Aksu H, Çakır M, Kurt B, Turan A, Canatan H (2015) The combination of thymoquinone and paclitaxel shows anti-tumor activity through the interplay with apoptosis network in triple-negative breast cancer. Tumor Biol 37:4467–4477. https://doi.org/10.1007/s13277-015-4307-0

Sarman H, Bayram R, Benek SB (2016) Anticancer drugs with chemotherapeutic interactions with thymoquinone in osteosarcoma cells. Eur Rev Med Pharmacol Sci 20:1263–1270

Schiff D, Wen PY, van den Bent MJ (2009) Neurological adverse effects caused by cytotoxic and targeted therapies. Nat Rev Clin Oncol 6:596–603. https://doi.org/10.1038/nrclinonc.2009.128

Sengupta A, Banerjee D, Chandra S, Banerji SK, Ghosh R, Roy R, Banerjee S (2007) Deregulation and cross talk among Sonic hedgehog, Wnt, Hox and Notch signaling in chronic myeloid leukemia progression. Leukemia 21:949–955. https://doi.org/10.1038/sj.leu.2404657

Shahid F, Farooqui Z, Abidi S, Parwez I, Khan F (2017) Oral administration of thymoquinone mitigates the effect of cisplatin on brush border membrane enzymes, energy metabolism and antioxidant system in rat intestine. Biomed Pharmacother 94:1111–1120. https://doi.org/10.1016/j.biopha.2017.08.044

Sheikhbahaei F, Khazaei M, Rabzia A et al (2016) Protective effects of thymoquinone against methotrexate-induced germ cell apoptosis in male mice. Int J Fertil Steril 9:541–547. https://doi.org/10.22074/ijfs.2015.4614

Šimůnek T, Štěrba M, Popelová O, Adamcová M, Hrdina R, Geršl V (2009) Anthracycline-induced cardiotoxicity: overview of studies examining the roles of oxidative stress and free cellular iron. Pharmacol Rep 61:154–171. https://doi.org/10.1016/s1734-1140(09)70018-0

Singh A, Settleman J (2010) EMT, cancer stem cells and drug resistance: an emerging axis of evil in the war on cancer. Oncogene 29:4741–4751. https://doi.org/10.1038/onc.2010.215

Singh SK, Apata T, Gordetsky JB, Singh R (2019) Docetaxel combined with thymoquinone induces apoptosis in prostate cancer cells via inhibition of the PI3K/AKT signaling pathway. Cancers (Basel) 11:1390. https://doi.org/10.3390/cancers11091390

Siveen KS, Mustafa N, Li F et al (2013) Thymoquinone overcomes chemoresistance and enhances the anticancer effects of bortezomib through abrogation of NF-κB regulated gene products in multiple myeloma xenograft mouse model. Oncotarget 5:634–648. https://doi.org/10.18632/oncotarget.1596

Sobell HM (1985) Actinomycin and DNA transcription. Proc Natl Acad Sci U S A 82:5328–5331. https://doi.org/10.1073/pnas.82.16.5328

Soni P, Kaur J, Tikoo K (2015) Dual drug-loaded paclitaxel–thymoquinone nanoparticles for effective breast cancer therapy. J Nanoparticle Res 17:18. https://doi.org/10.1007/s11051-014-2821-4

Steger F, Hautmann MG, Kölbl O (2012) 5-FU-induced cardiac toxicity - an underestimated problem in radiooncology? Radiat Oncol 7:212. https://doi.org/10.1186/1748-717x-7-212

Suddek GM (2014) Protective role of thymoquinone against liver damage induced by tamoxifen in female rats. Can J Physiol Pharmacol 92:640–644. https://doi.org/10.1139/cjpp-2014-0148

Suddek GM, Ashry NA, Gameil NM (2012) Thymoquinone attenuates cyclophosphamide-induced pulmonary injury in rats. Inflammopharmacology 21:427–435. https://doi.org/10.1007/s10787-012-0160-6

Syrigou E, Dannos I, Kotteas E, Makrilia N, Tourkantonis I, Dilana K, Gkiozos I, Saif MW, Syrigos KN (2011) Hypersensitivity reactions to docetaxel: retrospective evaluation and development of a desensitization Protocol. Int Arch Allergy Immunol 156:320–324. https://doi.org/10.1159/000324454

Trott O, Olson AJ (2010) AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J Comput Chem 31:455–461. https://doi.org/10.1002/jcc.21334

Ulu R, Dogukan A, Tuzcu M, Gencoglu H, Ulas M, İlhan N, Muqbil I, Mohammad RM, Kucuk O, Sahin K (2012) Regulation of renal organic anion and cation transporters by thymoquinone in cisplatin induced kidney injury. Food Chem Toxicol 50:1675–1679. https://doi.org/10.1016/j.fct.2012.02.082

Üstün R, Oğuz EK, Şeker A, Korkaya H (2018) Thymoquinone prevents cisplatin neurotoxicity in primary DRG neurons. Neurotoxicology 69:68–76. https://doi.org/10.1016/j.neuro.2018.09.001

Williams S, Tucci MA, Benghuzzi HA (2014) The effect of combination treatments of epigallocatechin-3-gallate, thymoquinone, and 5-fluorouracil on fadu nasopharyngeal carcinoma cells. Biomed Sci Instrum 50:361–366

Wilson AJ, Saskowski J, Barham W, Yull F, Khabele D (2015) Thymoquinone enhances cisplatin-response through direct tumor effects in a syngeneic mouse model of ovarian cancer. J Ovarian Res 8:46. https://doi.org/10.1186/s13048-015-0177-8

Wohlhueter RM, McIvor RS, Plagemann PGW (1980) Facilitated transport of uracil and 5-fluorouracil, and permeation of orotic acid into cultured mammalian cells. J Cell Physiol 104:309–319. https://doi.org/10.1002/jcp.1041040305

Yue QX, Liu X, Guo DA (2010) Microtubule-binding natural products for cancer therapy. Planta Med. 76:1037–1043

Yuksel S, Tasdemir S, Korkmaz S (2017) Protective effect of thymoquinone against cyclophosphamide-induced genotoxic damage in human lymphocytes. Bratislava Med J 118:208–211. https://doi.org/10.4149/bll_2017_041

Zafar S, Akhter S, Ahmad I, Hafeez Z, Alam Rizvi MM, Jain GK, Ahmad FJ (2020a) Improved chemotherapeutic efficacy against resistant human breast cancer cells with co-delivery of docetaxel and thymoquinone by chitosan grafted lipid nanocapsules: formulation optimization, in vitro and in vivo studies. Colloids Surfaces B Biointerfaces 186:110603. https://doi.org/10.1016/j.colsurfb.2019.110603

Zafar S, Akhter S, Garg N, Selvapandiyan A, Kumar Jain G, Ahmad FJ (2020b) Co-encapsulation of docetaxel and thymoquinone in mPEG-DSPE-vitamin E TPGS-lipid nanocapsules for breast cancer therapy: formulation optimization and implications on cellular and in vivo toxicity. Eur J Pharm Biopharm 148:10–26. https://doi.org/10.1016/j.ejpb.2019.12.016

Zhu D-F, Zhu G-L, Kong L-M, Bao NM, Zhou L, Nian Y, Qiu MH (2015) Cycloartane glycosides from the roots of Cimicifuga foetida with Wnt signaling pathway inhibitory activity. Nat Products Bioprospect 5:61–67. https://doi.org/10.1007/s13659-015-0053-7

Zidan A-AA, El-Ashmawy NE, Khedr EG et al (2018) Loading of doxorubicin and thymoquinone with F2 gel nanofibers improves the antitumor activity and ameliorates doxorubicin-associated nephrotoxicity. Life Sci 207:461–470. https://doi.org/10.1016/j.lfs.2018.06.008