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TQ-Ox, a novel synthetic derivative of thymoquinone on ovarian cancer cells *in vitro*

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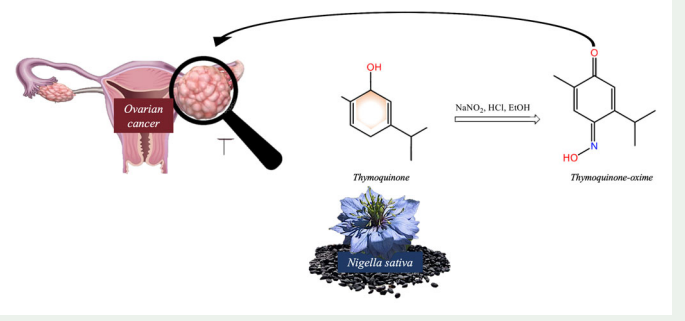
ABSTRACT

There are many studies in the literature on thymoquinone (TQ)-related cancer cells and models, and there is no relevant study investigating the efficacy of the oxime derivative of TQ (TQ-Ox). This study synthesized TQ-Ox and examined its cytotoxic, genotoxic and apoptotic properties in ovarian cancer cells. The structure TQ-Ox was confirmed with NMR. The cytotoxicity by luminometric ATP, intracellular reactive oxygen species (iROS) by fluorometric, intracellular calcium (iCa^{2+}) by fluorometric, mitochondrial membrane potential (MMP) by flow cytometry, glutathione (GSH) levels with GSH/GSSG-Glo assay, DNA damage by comet assay, and apoptosis by acridine orange/ethidium bromide dye were determined. Concentrations of TQ-Ox were statistically increased cytotoxicity, DNA damage, apoptosis, iROS, and iCa^{2+} in a concentration-dependent manner ($p < 0.001$). Besides, MMP and GSH levels also decreased statistically significantly ($p < 0.001$) with increasing concentrations. TQ-Ox would be an effective treatment option by increasing cytotoxicity, genotoxicity, and apoptosis in ovarian carcinoma.



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1. Introduction

Ovarian cancer is one of the leading cancer types, with a relatively high death rate despite 5% in females (Cancer facts and figures 2021). This is mainly because 80% of diagnosis does not happen until stage III or IV because of its vague symptom profile, earning ovarian cancer the name: silent killer (Jayson et al. 2014). Therefore, early detection and prevention strategies are of utmost importance. Conventional therapy is usually surgical resection and chemotherapy, composed of a combination of platinum and nonplatinum drugs. However, drug resistance tends to develop during the treatment, leading to a clinical failure (English et al. 2016). Due to ovarian cancer most likely resulting in death, novel therapeutic approaches are required for clinical application. The resistance to platinum chemotherapy is common in advanced diseases (Muggia 2009). Identifying new drugs to improve platinum response is censorious to prolonging the life of women with resistant diseases.

Thymoquinone (TQ), 2-isopropyl-5-methyl-1,4-benzoquinone, is an active compound acquired from the seeds of *Nigella sativa*. It has many pharmacological uses, such as antimicrobial, antioxidant, and anticancer (El-Far 2015; Atta et al. 2017; El-Far et al. 2018). TQ has been observed to successfully inhibit different stages of cancer when administered on its own, including proliferation, migration, and invasion (Imran et al. 2018). It has also been demonstrated TQ promotes the antitumor effects of currently available antineoplastic agents *in vitro* and *in vivo* in various cancer types (El-Far et al. 2020). The clinical potential of this molecule is being investigated in great detail globally. It has been found that TQ, the active ingredient of the black seed, is effective on different cancer cells. However, there is no relevant data on the effect of the newly synthesized oxime derivative of TQ (TQ-ox) in cancer.

Oximes are organic functional groups with pharmacological properties. Modified oximes have various biological properties such as anti-inflammatory, antimicrobial, antioxidant, and anticancer (Surowiak et al. 2020). In the literature, many oxime derivatives have been designed as cytotoxic agents and have been reported to have good therapeutic effects for various cancer types (Chiou et al. 2015; Qu et al. 2015; Ajduković et al. 2021; Gomes et al. 2021). Schepetkin et al. studied oximes in detail and reported that adding oxime groups to the main skeleton structure increases the biological activity of various natural compounds. They also stated that the reason for the increase in the activity of oximes is that although the presence of a terminal oxime group is required for its activity, the oxime group will also offer a significant advantage in drug design against carbonyl groups due to the presence of two H-bond acceptors and a donor (Schepetkin et al. 2021).

This study planned that the therapeutic effect will be further increased by adding an oxime functional group to the TQ compound with high anticancer activity. For this reason, we synthesized the oxime derivative of TQ, which possesses both lipophilic and hydrophilic characteristics. We investigated its cytotoxic, genotoxic, apoptotic, and inflammatory properties in ovarian cancer cells and healthy ovarian cancer cell lines.

2. Results and discussion

Chemotherapy for cancer treatment is one of the most commonly used therapeutic modalities, but its therapeutic outcome is often unsatisfactory due to its severe adverse effects (Abbas and Rehman 2018). Therefore, developing new antitumor agents with little toxicity to the host is crucial. The TQ-Ox compound, which will be formed by chemical synthesis from TQ, the active ingredient of the black seed, will be investigated for the first time on ovarian cancer. Although there are many studies in the literature on thymoquinone-related cancer cells and models, there is no relevant study investigating the efficacy of TQ-Ox to be synthesized against ovarian cancer cells to increase the effectiveness of thymoquinone. In this study, we synthesized the oxime derivative of TQ, which possesses both lipophilic and hydrophilic characteristics. We tested for its cytotoxic, genotoxic, apoptotic and inflammatory properties in ovarian cancer cells and healthy ovarian cell lines.

In the ^{1}H-NMR spectrum of the TQ-Ox, aromatic protons were observed in the range of 7.44–6.26 ppm, in aliphatic protons were observed in the range of 2.91 and 1.04 ppm. The hydroxyl group proton was observed at 13.39 ppm. ^{13}C-NMR spectra of TQ-Ox reveal 9 carbon peaks containing 6 aromatic carbon peaks observed between 186-118 ppm in the aromatic region and 3 methyl carbon peaks between 27 and 17 ppm in the aliphatic region. The elemental analysis result of the TQ-Ox supports synthesized structures. Consequently, ^{1}H and ^{13}C-NMR data and the elemental analysis results of the thymoquinone-oxime established the proposed structures.

Cisplatin and Taxol, the chemotherapeutic agents used in the study, are agents used to treat many types of cancer, including ovarian cancer (Dasari and Tchounwou 2014). Studies have shown that TQ sensitizes ovarian cancer cells *in vitro* models to the cytotoxic effect of cisplatin and that its inhibitory effect of TQ is even better than cisplatin (Wilson et al. 2015; Liu et al. 2017). In addition, in another study, it was thought that the combination of TQ and Taxol has *in vivo* therapeutic potential and that TQ could sensitize cancer cells to Taxol in TQ-mediated apoptosis of cancer cells, extrinsic apoptosis (S¸akalar et al. 2016). This study evaluated the efficacy of the oxime derivative of TQ and current chemotherapeutic agents Cisplatin and Taxol against ovarian cancer and healthy cell lines. The highest dose of TQ-Ox (100 μM) had the same cell viability effect with Cisplatin (40 μM) and Taxol (20 μM) against cancer cell lines. In addition, the IC_{50} values in SKOV-3 and CHO-K1 cell lines are 34.46 μM and 65.71 μM, respectively; and proved that TQ-Ox induces cell death more potently in the cancer cell line.

A mechanism through which active substances exert their effect is iROS activity. Moderate levels of ROS are effective in protecting cells against infectious agents. As a result of overproduction, cells cannot adequately eliminate these excesses, and 'oxidative stress' occurs. ROS severely damages cell membranes and other structures such as protein, lipid, and DNA (Pham-Huy et al. 2008). Taha et al. found that TQ acted as a catalyst in generating more ROS in a concentration-dependent manner that increased the level of ROS in the CAOV3 cancer cell line (Taha et al. 2016). Figures S2B and S2D show that TQ-Ox concentrations between 2.5 and 100 μM have significantly increased the iROS and iCa^{2+} production. Intracellular Ca^{2+} homeostasis is a signaling mechanism controlling many cellular functions and increased in cells induce the

proapoptotic mechanism (Hajnóczky and Csordás 2010). Increasing TQ-Ox concentrations increased Ca^{2+} concentrations in SKOV-3 and CHO-K1 cells concentration-dependent. TQ-Ox can induce the iROS activity in SKOV-3 cells more efficiently than in CHO-K1 healthy cells in a concentration-dependent manner ($p < 0.001$).

There are antioxidant enzyme systems in the body that work to reduce cellular deaths and metabolic disturbances, to eliminate the effects of factors that cause oxidative stress, and to ensure that the cell remains in its optimal conditions, one of these is reduced GSH (Correa and Houghton 2007). High levels of GSH have been observed in cancer, particularly in the breast and ovary (Gamcsik et al. 2012). TQ-Ox reduced GSH levels in cancer cell line SKOV-3 and healthy cell line CHO-K1. After 24 h of incubation with TQ-Ox concentrations between 2.5 and 40 μM, GSH levels were found to decrease statistically. In particular, this decrease caused especially the cancer cells to be more susceptible to the effects of iROS. The produced ROS impair the external mitochondrial potential by releasing death-promoting proteins, and this causes mitochondrial dysfunction (DeHart et al. 2018). It has been suggested that mitochondrial apoptosis is induced by TQ and is central to the apoptotic pathway (Ly et al. 2003).

Differential concentrations of TQ-Ox below IC_{50} concentrations were studied with the comet assay method to evaluate genotoxic damage in cancer and healthy cell lines. TQ has numerous cellular effects in tumor cells, including promoting DNA damage through the generation of ROS (Ahmad et al. 2013; Rahmani et al. 2014). A study on the melanoma cell line showed that TQ induces iROS generation due to its pro-oxidant effect, which causes DNA damage and subsequent apoptosis (Hatiboglu et al. 2018). Increasing TQ-Ox concentrations increased DNA damage statistically ($p < 0.001$), see Figure S3A. Comet image micrographs of SKOV-3 DNA with increasing damage are presented in Figure S3B. Damaged DNA had bright nuclei and comet-like nuclei, while undamaged DNAs were large and round.

Reactive oxygen species are constantly produced by the cell's normal oxygen metabolism and can cause oxidative damage to biomolecules, causing the cell to undergo apoptosis (Gopalakrishnan et al. 2012). A study of TQ derivative TQFL12 on a panel of TNBC cells and a mouse model known as triple-negative breast cancer (TNBC) found that TNBC cells were sensitive to TQFL12 treatment and TQFL12 affected cancer cell migration and invasion *in vitro* and *in vivo* (Wei et al. 2021). We determined that apoptosis was occurring in cells using the AO/EB double staining method. A concentration-dependent statistically significant decrease ($p < 0.001$) was observed using a DiOC6(3) fluorescence probe after incubation of TQ-Ox in cells for 24 h to assess MMP, see Figure S4A. To clarify whether the diverse concentrations of TQ-Ox under IC_{50} concentrations cause apoptosis in cancer SKOV-3 and healthy CHO-K1cell lines after 24 h of incubation. From the results, it was found that apoptosis increased in cells in a concentration-dependent manner. The apoptosis rate in cancer cells was higher than in healthy cells, see Figure S4B. The amount of % apoptosis increased statistically significantly ($p < 0.001$) as the concentration increased. At each concentration, at least 100 cells were counted, and the number of apoptotic cells of SKOV-3 was calculated semi-quantitatively, see Figure S4C. In parallel with the literature, a concentration-dependent, statistically significant decrease was observed using the DiOC6(3) fluorescence

probe after incubation of TQ-Ox in cells for 24 h to assess MMP. The obtained MMP levels were decreased in both ovarian cancer and healthy cell lines.

3. Characterization methods

3.1. Materials and equipment

All chemicals used were of reagent grade quality. Carvacrol and sodium nitrite ($NaNO_2$) were purchased from Sigma-Aldrich and used as received. The solvents were purified, dried, and stored over 4 Å molecular sieves. Fura-2AM, trypsin-EDTA, 2′,7′-dichlorodihydrofluorescein diacetate (H_2DCF-DA), acridine orange/ethidium bromide (AO/EB) double stain, low-melting agarose, normal melting agarose, and lysis solution. McCoy's 5A, F-12K, fetal bovine serum (FBS), and penicillin/streptomycin (P/S) were obtained from Gibco Invitrogen Corporation (Carlsbad, CA, USA).

3.2. TQ-OX syntheses

TQ-Ox was purified successively by washing with water and hexane. The melting point was found at 155 °C, and the homogeneity of the products was tested in each step by thin-layer chromatography (TLC Silica gel $60F_{254}$).

The TQ-Ox was synthesized *via* nitrosation of the thymol, according to Uliana et al. (2014). As a starting compound, commercially available thymol was used. The TQ-Ox was prepared by the nitrosation reaction of the thymol in the presence of $NaNO_2$ in ethanol and hydrogen chloride (HCl) at room temperature under an argon atmosphere. The crude product was purified by washing with hexane. After well-purification, TQ-Ox was characterized by ^{1}H-NMR, ^{13}C-NMR, MALDI-TOF MS and elemental analysis.

Yield: 2.64 g (67%), m.p.: 155 °C. Anal. Calc. for $C_{10}H_{13}NO_2$: C, 67.02; H, 7.31; N, 7.82%, found: C, 67.01; H, 7.29; N, 7.79%. ^{1}H-NMR (*d*-DMSO, δ, ppm) 13.39 (s, 1H), 7.44 (s, 1H), 6.26 (s, 1H), 2.91 (m, 1H), 2.15 (s, 3H), 1.04 ($J = 8.0$ Hz, 6H). ^{13}C-NMR (*d*-DMSO, δ, ppm) 186.38, 149.91, 147.93, 146.65, 128.61, 118.32, 26.70, 21.92, and 17.18. MALDI-TOF (*m/z*): Calc. 179.22, found as 368.01 $[M + CHCA]^+$, 352.378 $[M - OH + CHCA]^+$ and 391.02 $[M + CHCA + Na]^+$ in Figure S1.

3.3. Spectroscopic methods

^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker Avance III 500 MHz Three-channel NMR spectrometer. Elemental analysis was performed by the Instrumental Analysis Laboratory of the TUBITAK, Marmara Research Centre. Mass spectra were recorded on Bruker Micro flex LT MALDI-TOF MS spectrometer using α-cyano-4-hydroxycinnamic acid (CHCA) as MALDI matrix.

3.4. Cell culture

Ovarian carcinoma human cell line SKOV-3 (HTB-77^{TM}) and ovary healthy cell line CHO-K1 (CCL-61^{TM}) were commercially purchased from American Type Culture Collection (ATCC). The primary medium is McCoy's 5A for SKOV-3 and F-12K for CHO-

K1 cell lines. The culture medium contained a primary medium supplemented with 10% FBS and 1% P/S antibiotic. The cell lines were incubated at 37 °C with saturated moisture in 5% CO_2.

3.5. Cytotoxicity

The cytotoxicity of TQ-Ox and chemotherapeutic drugs Taxol and Cisplatin on the cell lines SKOV-3 and CHO-K1 was measured by luminescent cell viability kit ATP assay (CellTiter-Glo®). Cells were added to 96-well plates seeded (7×10^3 cells/well). After 24 h, TQ-Ox and chemotherapeutic drug concentrations (2–100 μM) were added and incubated for 24 h at 37 °C in 5% CO_2. After incubation, the ATP solution was added and measured using Varioskan Flash Multimode Reader (Thermo Scientific, Waltham). The light emitted in the presence of ATP was expressed as relative luminescence units (RLU). The maximum growth inhibitory concentration (IC_{50}) values were calculated using nonlinear regression analysis from dose-response curves.

3.6. Intracellular reactive oxygen species (iROS)

Intracellular ROS levels were examined using the H_2DCF-DA fluorescent dye. Cell lines were incubated with TQ-Ox concentrations (2–100 μM) for 24 h. The medium was washed three times with aspirated 1× dPBS. After adding 5 μM H_2DCF-DA, the plate was incubated at 37 °C for 30 min in the dark. Intracellular ROS capacity was measured with a fluorimeter using the Varioskan Flash Multi-Mode Reader (Therma Scientific, Waltham) and expressed as relative fluorescence units (RFU).

3.7. Intracellular calcium (iCa^{2+})

Intracellular Ca^{2+} levels were evaluated using Fura-2 AM fluorescent dye. The cells were incubated with TQ-Ox concentrations (2–40 μM) under IC_{50} and were added in seeded 96-well black opaque plates after 24 h. After the treatment with TQ-Ox concentration, the cells were washed with 1× dPBS. After washing, 5 μM Fura-2 AM was added and incubated for 45 min. After incubation, the fluorescence was measured with an Ex:340nm/Em:380nm fluorescence plate reader device (Varioskan Flash Multi-Mode Reader, Thermo, Waltham, USA). Results were calculated by comparison with ATP with the DMSO control.

3.8. Glutathione

The glutathione (GSH) levels in cell lines SKOV-3 and CHO-K1 were measured and quantified with GSH/GSSG-Glo assay (Promega, USA). The cell lines were seeded into the plate (96 opaque-white). The adhered cells were treated and incubated with TQ-Ox concentrations (2–40 μM) for 24 h. 50 μL glutathione reagent for lyse cell was added to the removed medium after incubation, and the opaque-white plate was incubated at room temperature for 5 min in a shaker. After incubation, 100 μL Luciferin detection reagent was added, and luminescence was measured in Thermo Varioskan

Multimode Flash Reader. The control calculated the results as % relative luminescence (RLU).

3.9. Mitochondrial membrane potential

Mitochondrial membrane potential (MMP) levels of TQ-oxime and chemotherapeutic drugs on ovarian cancer and healthy cell lines were evaluated using a DiOC6(3) (3,3′-dihexyloxacarbocyanine iodide) molecular fluorescence probe in flow cytometry (Rottenberg and Wu 1998). The cells were seeded into a 6-well plate at 37 °C in 5% CO_2 for 24 h. Then the medium of each plate was removed with 0.25% trypsin-EDTA and centrifuged for 5 min at 400×*g* at +4 °C with 1× dPBS. The obtained supernatant was discarded, and the residual pellet was suspended with DiOC6(3). The obtained suspension was incubated for 20 min at 37 °C. Then, the cells were measured by flow cytometry (BD, FACS Canto^{TM}II).

3.10. Comet assay

The genotoxic effects of TQ-Ox on SKOV-3 and CHO-K1 cell lines were performed with a Comet assay modified by Singh et al. (1988). Each cell line was seeded in 6-well plates and incubated for 24 h at 37 °C with doses of TQ-Ox below the IC_{50}. After 24 h, the supernatants of the trypsin-EDTA-enhanced cells were aspirated, and each cell was mixed with 0.6% low melting point agarose (LMA). This mixture was added to 1% coated normal melting point agarose (NMA) slides. Precooled gels were incubated in a lysis buffer at +4 °C for 4 h. After incubation, the slides were washed with cold 1x dPBS and electrophoresed at 4 °C (26 V, 300 mA) to dissolve the DNA. Electrophoresed slides were washed in neutralization buffer and fixed with ethanol. Dried slides were stained with 2 μg/mL ethidium bromide, and the DNA tail percentages were calculated by fluorescence microscopy using Comet assay IV software (Leica DM 1000, Solms).

3.11. Apoptosis with acridine orange/ethidium bromide double staining

After TQ-Ox treatment in cell lines, changes in nuclear morphology were examined with double staining AO/EB described by McGahon et al. (1995). The treated cells were stained with 1:1 AO/EB staining. Morphology changes in nuclear were evaluated, and the percentages of inhibition were interpreted by counting an average of 100 cells with a fluorescent microscope (Leica DMA 1000, Solms).

3.12. Statistical analysis

SPSS version 25.0 program (IBM, Armonk, NY, USA) was used for all statistical analyses. The shapiro-Wilk test was used for normality distribution. Parametric data were expressed as mean ± standard deviation (SD), while non-parametric data were expressed as the interquartile range (IQR). The difference between the two parameters in the groups was calculated by using the Mann–Whitney *U* test. The Kruskal–Wallis test was used to compare more than two independent parameters. The correlation

between two variables was evaluated using the Spearman rank correlation coefficient. By using the chi-square test, the categorical data were evaluated. The $p < 0.05$ was regarded as statistically significant, and all experiments were done in triplicate.

4. Conclusions

According to the results, we propose that TQ-Ox has cytotoxic, genotoxic, and apoptotic effects on ovarian in a dose-dependent manner. TQ-Ox may have potential as an option for additional targeted therapies, as in ovarian cancer patients. Our findings show that TQ-Ox, a new TQ derivative, has a higher anti-tumour effect on the ovarian cancer cell line. Consequently, it may be a candidate compound for the treatment of ovarian cancer and may have *in vivo* and clinical values needed for this synthesis TQ in further studies.

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References

Abbas Z, Rehman S. 2018. An overview of cancer treatment modalities in neoplasm. London, UK: IntechOpen.

Ahmad A, Husain A, Mujeeb M, Khan SA, Najmi AK, Siddique NA, Damanhouri ZA, Anwar F. 2013. A review on therapeutic potential of *Nigella sativa*: a miracle herb. Asian Pac J Trop Biomed. 3(5):337–352.

Ajduković JJ, Jakimov DS, Rárová L, Strnad M, Dzichenka YU, Usanov S, Škorić DĐ, Jovanović-Šanta SS, Sakač MN. 2021. Novel alkylaminoethyl derivatives of androstane 3-oximes as anticancer candidates: synthesis and evaluation of cytotoxic effects. RSC Adv. 11(59): 37449–37461.

Atta MS, Almadaly EA, El-Far AH, Saleh RM, Assar DH, Al Jaouni SK, Mousa SA. 2017. Thymoquinone defeats diabetes-induced testicular damage in rats targeting antioxidant, inflammatory and aromatase expression. IJMS. 18(5):919.

Cancer facts & figures. 2021. CA Cancer J Clin. NIH National Cancer Institute.

Chiou C-T, Lee W-C, Liao J-H, Cheng J-J, Lin L-C, Chen C-Y, Song J-S, Wu M-H, Shia K-S, Li W-T. 2015. Synthesis and evaluation of 3-ylideneoxindole acetamides as potent anticancer agents. Eur J Med Chem. 98:1–12.

Correa P, Houghton J. 2007. Carcinogenesis of *Helicobacter pylori*. Gastroenterology. 133(2): 659–672.

Dasari S, Tchounwou PB. 2014. Cisplatin in cancer therapy: molecular mechanisms of action. Eur J Pharmacol. 740:364–378.

DeHart DN, Fang D, Heslop K, Li L, Lemasters JJ, Maldonado EN. 2018. Opening of voltage dependent anion channels promotes reactive oxygen species generation, mitochondrial dysfunction and cell death in cancer cells. Biochem Pharmacol. 148:155–162.

El-Far AH. 2015. Thymoquinone anticancer discovery: possible mechanisms. Curr Drug Discov Technol. 12(2):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(10):1369.

El-Far AH, Tantawy MA, Al Jaouni SK, Mousa SA. 2020. Thymoquinone-chemotherapeutic combinations: new regimen to combat cancer and cancer stem cells. Naunyn Schmiedebergs Arch Pharmacol. 393(9):1581–1598.

English DP, Menderes G, Black J, Schwab CL, Santin AD. 2016. Molecular diagnosis and molecular profiling to detect treatment-resistant ovarian cancer. Expert Rev Mol Diagn. 16(7):769–782.

Gamcsik MP, Kasibhatla MS, Teeter SD, Colvin OM. 2012. Glutathione levels in human tumors. Biomarkers 17(8):671–691.

Gomes AR, Pires AS, Abrantes AM, Gonçalves AC, Costa SC, Varela CL, Silva ET, Botelho MF, Roleira FMF. 2021. Design, synthesis, and antitumor activity evaluation of steroidal oximes. Bioorg Med Chem. 46:116360.

Gopalakrishnan B, Nash KM, Velayutham M, Villamena FA. 2012. Detection of nitric oxide and superoxide radical anion by electron paramagnetic resonance spectroscopy from cells using spin traps. JoVE (66):e2810, https://pubmed.ncbi.nlm.nih.gov/22929836/.

Hajnóczky G, Csordás G. 2010. Calcium signalling: fishing out molecules of mitochondrial calcium transport. Curr Biol. 20(20):R888–R891.

Hatiboglu MA, Kocyigit A, Guler EM, Akdur K, Nalli A, Karatas E, Tuzgen S. 2018. Thymoquinone Induces Apoptosis in B16-F10 Melanoma Cell Through Inhibition of p-STAT3 and Inhibits Tumor Growth in a Murine Intracerebral Melanoma Model. World Neurosurg. 114:e182–e190.

Jayson GC, Kohn EC, Kitchener HC, Ledermann JA. 2014. Ovarian cancer. Lancet (London, England). 384(9951):1376–1388.

Liu X, Dong J, Cai W, Pan Y, Li R, Li B. 2017. The effect of thymoquinone on apoptosis of SK-OV-3 ovarian cancer cell by regulation of Bcl-2 and Bax. Int J Gynecol Cancer. 27(8):1596–1601.

Ly JD, Grubb DR, Lawen A. 2003. The mitochondrial membrane potential (deltapsi(m)) in apoptosis; an update. Apoptosis 8(2):115–128.

McGahon AJ, Martin SJ, Bissonnette RP, Mahboubi A, Shi Y, Mogil RJ, Nishioka WK, Green DR. 1995. The end of the (cell) line: methods for the study of apoptosis in vitro. Methods Cell Biol. 46:153–185.

Mran 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.

Muggia F. 2009. Platinum compounds 30 years after the introduction of cisplatin: implications for the treatment of ovarian cancer. Gynecol Oncol. 112(1):275–281.

Pham-Huy LA, He H, Pham-Huy C. 2008. Free radicals, antioxidants in disease and health. Int J Biomed Sci. 4(2):89–96.

Qu H-E, Huang R-Z, Yao G-Y, Li J-L, Ye M-Y, Wang H-S, Liu L. 2015. Synthesis and pharmacological evaluation of novel bisindole derivatives bearing oximes moiety: identification of novel proapoptotic agents. Eur J Med Chem. 95:400–415.

Rahmani A. H, Alzohairy MA, Khan MA, Aly SM. 2014. 2014. Therapeutic implications of black seed and its constituent thymoquinone in the prevention of cancer through inactivation and activation of molecular pathways. Evid Based Complement Alternat Med. 2014:724658.

Rottenberg H, Wu S. 1998. Quantitative assay by flow cytometry of the mitochondrial membrane potential in intact cells. Biochim Biophys Acta. 1404(3):393–404.

Şakalar Ç, İzgi K, İskender B, Sezen S, Aksu H, Çakır M, Kurt B, Turan A, Canatan H. 2016. The combination of thymoquinone and paclitaxel shows anti-tumor activity through the interplay with apoptosis network in triple-negative breast cancer. Tumor Biol. 37(4):4467–4477.

Schepetkin IA, Plotnikov MB, Khlebnikov AI, Plotnikova TM, Quinn MT. 2021. Oximes: novel therapeutics with anticancer and anti-inflammatory potential. Biomolecules 11(6):777.

Singh NP, McCoy MT, Tice RR, Schneider EL. 1988. A simple technique for quantitation of low levels of DNA damage in individual cells. Exp Cell Res. 175(1):184–191.

Surowiak AK, Lochyński S, Strub DJ. 2020. Unsubstituted oximes as potential therapeutic agents. Symmetry 12(4):575.

Taha MM, Sheikh BY, Salim LZ, Mohan S, Khan A, Kamalidehghan B, Ahmadipour F, Abdelwahab SI. 2016. Thymoquinone induces apoptosis and increase ROS in ovarian cancer cell line. Cell Mol Biol (Noisy-le-Grand, France). 62(6):97–101.

Uliana MP, Servilha BM, Alexopoulos O, de Oliveira KT, Tormena CF, Ferreira MA, Brocksom TJ. 2014. The DielseAlder reactions of para-benzoquinone nitrogen-derivatives: an experimental and theoretical study. Tetrahedron 70(39):6963–6973.

Wei C, Zou H, Xiao T, Liu X, Wang Q, Cheng J, Fu S, Peng J, Xie X, Fu J. 2021. TQFL12, a novel synthetic derivative of TQ, inhibits triple-negative breast cancer metastasis and invasion through activating AMPK/ACC pathway. J Cell Mol Med. 25(21):10101–10110.

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.