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Original Article

Effects of thymoquinone and etoposide combination on cell viability and genotoxicity in human cervical cancer hela cells

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ABSTRACT

Background and Aims: It is thought that thymoquinone might have a crucial role in preventing DNA damage, regulating DNA repair mechanisms, and inhibiting the formation of a cancer. Studies on the cytotoxic and genotoxic effects of thymoquinone together with etoposide in cervical carcinoma cells (HeLa) are not adequate. The objective of this study is to evaluate the effect of combinations with thymoquinone on etoposide cytotoxicity and genotoxicity in HeLa cells.
Methods: Cytotoxicity was evaluated by MTT assay and genotoxicity was determined by Comet assay.
Results: The IC_{50} values of thymoquinone were 233.6 µM and 145.5 µM, and the IC_{50} values of etoposide were 167.3 µM and 52.7 µM for 24 and 48 h, respectively. Thymoquinone significantly decreased the approximate IC_{50} value of etoposide in doses of 15.63 µM and above for 24 h and 31.5 µM and above for 48 h in a dose-dependent manner. 0.1-5 µM thymoquinone and 1 µM etoposide alone did not cause DNA damage, but at higher doses increased DNA damage significantly in a dose-dependent manner. Thymoquinone significantly reduced DNA damage induced by 10 µM etoposide at the doses of 0.1-10 µM.
Conclusion: Our results show that thymoquinone might increase the cytotoxic and genotoxic effects of etoposide in HeLa cells at high doses and reduce DNA damage at low doses that are not cytotoxic, which suggests that etoposide may increase its anticancer effect at high doses, but comprehensive studies are needed on this subject. This study is a preliminary study and will contribute to the development of new treatment strategies.
Keywords: Thymoquinone, etoposide, cytotoxicity, genotoxicity, comet assay, HeLa cells

INTRODUCTION

Cancer is a leading cause of death, and it is among the global problems affecting public health and the economy. Cervical cancer ranks fourth in cancer-related deaths in women, according to the Global Cancer Observatory (GLOBOCAN) database (Sung et al., 2021). Radiotherapy and chemotherapy, capable of improving patients' survival considerably, are used in the treatment of cervical cancer (Green, Kirwan, & Tierney, 2001). Multiple drug regimens are preferred in chemotherapy due to drug resistance and drug-induced toxicity limit treatment. Nowadays, the combination of cisplatin and etoposide is one of the common chemotherapy regimens used (Salvo, Gonzalez Martin, Gonzales, & Frumovitz, 2019; Kluska & Wozniak, 2021).

Studies on this topic started to increase due to the positive effects of phytochemicals in cancer treatment. Current studies suggest that combinational chemotherapy of phytochemicals having different anticancer mechanisms may be successful (Xiaofei

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et al., 2021). The predominant macromolecular effect of etoposide, a semi-synthetic derivative of podophyllotoxin, is the inhibition of DNA-topoisomerase II enzyme or the induction of DNA strand breaks by the formation of free radicals (PubChem, 2022). Its mechanism of action is primarily in the late S and G2 phases of the cell cycle. It inhibits cell cycle progression at a premitotic phase (late S and G2), probably via inhibition of DNA synthesis. Myelosuppression is the dose-limiting toxicity of etoposide. It can cause many side effects including nausea, vomiting, diarrhea or constipation, abdominal pain, weakness, alopecia, and vision problems (Sinkule, 1984). Cisplatin, one of the most commonly used drugs for cancer chemotherapy, has a very high potential for drug toxicity. Some of the well-known adverse reactions to this drug include nausea, vomiting, renal toxicity, ototoxicity, peripheral neuropathy, hypersensitivity reactions and electrolyte disturbances. Some of the rarer reactions include hypocalcemia, headache, salivation, and dizziness (Surendiran et al., 2010).

It was suggested that thymoquinone, isolated from *Nigella sativa L.* (Ranunculaceae), may show anticancer effects by regulating different molecular targets in various cancer cells (Hafiza & Latifah, 2014). The suggested action mechanisms of thymoquinone in the anticancer treatment include increasing the production of reactive oxygen species, regulation of apoptosis, genotoxicity and inhibition of tumor angiogenesis (Shoieb, El-gayyar, Dudrick, Bell, & Tithof, 2003; El-Mahdy, Zhu, Wang, Wani, & Wani, 2005; Woo, Kumar, Sethi, & Tan, 2012; Racoma, Meisen, Wang, Kaur, & Wani, 2013). Thymoquinone was shown to inhibit proliferation, induce apoptosis and have a chemosensitizing effect by suppressing signal transducer and activator of transcription-3 activation in human multiple myeloma cells (Li, Rajendran, & Sethi, 2010). Some of the tumor suppressor genes and proteins (p53, PTEN, p21, p27, BRCA1) were found to be overexpressed or activated by thymoquinone. Moreover, thymoquinone was determined to inhibit some oncogenic signaling molecules and pathways, phosphoinositide 3 kinase (PI3K)/Akt and mitogen-activated protein kinase (MAPK)/ERK (Gali-Muhtasib, Abou Kheir, Kheir, Darwiche, & Crooks, 2004; Rahmani, Alzohairy, Khan, & Aly, 2014; Diricanet al., 2015). Recent studies revealed that thymoquinone can modulate epigenetic mechanisms, such as changing histone acetylation and deacetylation. Thymoquinone can also change genetic expression of various non-coding RNAs such as miRNA and lncRNA, which are considered key parts of cellular epigenetics (Khan, Tania, & Fu 2019).

A number of well-characterized chemotherapeutic drugs as well as several natural products with anticancer or chemopreventive properties are topoisomerase II poisons. Thymoquinone was reported to have the activity of human topoisomerase IIα due to the similarities to known topoisomerase II poisons. Results indicate that purified thymoquinone, black seed extract, and black seed oil all increase levels of enzyme-mediated DNA cleavage. This is thought to be responsible for its anticancer properties. These enzymes modulate levels of torsional stress in the genetic material and remove knots and tangles from the genome. They function by creating a transient double-strand break in one double helix and passing a separate intact DNA segment through the opening. To maintain genomic integrity while the DNA is cleaved, type II topoisomerases covalently attach to the newly generated 5′ termini of the cleaved helix. This covalent enzyme-cleaved DNA complex is known as the cleavage complex (Ashley & Osheroff, 2014).

It seems that research studies should focus on the discovery of innovative drug strategies to improve treatment outcomes in chemotherapy (Pucci, Martinelli, & Ciofani, 2019). It is claimed that thymoquinone might have an important role in preventing DNA damage, regulating DNA repair mechanisms, and inhibiting carcinogenesis. There are limited studies on the cytotoxic and genotoxic effects of thymoquinone in case of using in combination with etoposide in cervical cancer. The objective of this study is to determine the effects of thymoquinone combinations on etoposide cytotoxicity and genotoxicity in cervical cancer cell lines (HeLa cells) by MTT and alkaline Comet assay, respectively.

MATERIAL AND METHODS

Chemicals

The chemicals used in the experiments were purchased from the following suppliers: etoposide from Koçak Farma (Turkey); dimethyl sulfoxide (DMSO), ethanol, ethidium bromide (EtBr), L-glutamine, fetal bovine serum (FBS), low melting point (LMPA) agarose, 3- (4,5-dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide (MTT), normal melting point (NMPA) agarose, sodium bicarbonate, thymoquinone, trypan blue, trypsin–EDTA, and Dulbecco's phosphate-buffered saline (PBS) from Sigma (St. Louis, MO, USA); Dulbecco's modified Eagle's medium (DMEM) and penicillin-streptomycin from Biowest (France); millipore filters from Millipore (Billerica, MA, USA); all other plastic materials from Corning (Corning Inc., NY, USA). The purity of thymoquinone is ≥ 98.5%.

Cell culture

HeLa cells were obtained from the American Type Culture Collection (ATCC; Rockville, MD, USA). The cells were cultured in DMEM containing low glucose (1000 mg/L) and sodium bicarbonate. The media were supplemented with 10% heat-inactivated FBS, 2mM L-glutamine and 1% penicillin-streptomycin solution (10000 units of penicillin and 10 mg of streptomycin in 0.9% NaCl) in a humidified atmosphere of 5% CO_2 and 95% air at 37°C. The cells were sub-cultured in 75 cm cell culture flasks. The culture medium was changed every 3 days. The passage numbers used in our study for the cell lines were between passage 18 and passage 20.

Determination of cytotoxicity

The effects of thymoquinone and etoposide and their combination on cell viability were determined by MTT assay (Mosmann, 1983; Hansen, Nielsen, & Berg, 1989). According to the cell viability data, $IC_{50}$was estimated. Cells were plated in 96-well plates containing 200 µL medium at a density of 1×10^4 cells/well and incubated to adhere to the plate for 24 h. The number of cells was calculated by trypan blue dye exclusion. The stock solution of thymoquinone was freshly prepared in PBS with DMSO and filtered with millipore filters (0.20 µm). DMSO concentration did not exceed 0.5% (v/v) in medium.

The cells were treated with etoposide (25-400 µM), thymoquinone (3.91-1000 µM), or the combination at the related culture medium for 24 h and 48 h. Negative control experiments were carried out with the culture medium containing DMSO (0.5%) or PBS (1%), for thymoquinone and etoposide, respectively. At the end of the incubation, 5 mg/mL MTT solution was added to each well and incubated for another 4 h at 37°C in the dark. Then the medium was discarded. The formazan crystals were dissolved in 100 µL of DMSO and absorbance of each sample was detected at 570 nm using the microplate reader (SpectraMax M2, Molecular Devices Limited, Berkshire, UK). The percentage of cell viability was calculated using the formula: "Percentage of cell viability = (The absorbance of sample/ control) x 100". The cytotoxic concentration that killed cells by 50% (IC_{50}) was determined from absorbance versus concentration curve.

Determination of genotoxicity

The genotoxicity of thymoquinone and etoposide were measured in HeLa cells using Comet assay. The basic alkaline technique described by Singh, McCoy, Tice, & Schneider (1988) which is a fast and easy technique widely used in the detection of single cell DNA damage, was used for the detection of DNA damage in the cells (Collins, Dobson, Dusinka, Kennedy, & Stetina, 1997; Becit & Aydın Dilsiz, 2020). Cells were plated in 96-well plates containing 200 µL medium at a density of 1×10^4 cells/well using trypan blue dye exclusion and incubated to adhere to the plate for 24 h. HeLa cells were incubated with thymoquinone (0,1-100 µM) and etoposide (1-50 µM) at non-cytotoxic doses for 1 h (preincubation). Moreover, 0.5% DMSO was applied as a negative control. According to the Comet results obtained; the combination of 0.1-50 µM thymoquinone with 5 µM etoposide was also studied. After treatment, the cells were trypsinized and washed. The cell pellets (~1×10^4 cells) were then suspended in 50 µL PBS to reach 1×10^4 cells/50 µL. The cell suspension mixed with 1% LMPA were then embedded on slides precoated with a layer of 1% NMPA. The slides were allowed to solidify on ice for 5 min. The cover slips were then removed. All slides were immersed in cold lysing solution (pH 10) for a minimum of 1 h at 4°C. The slides containing the cells were removed from the lysing solution, drained, and then placed in a horizontal gel electrophoresis tank filled with freshly prepared alkaline electrophoresis solution (300 mmol/L NaOH, 1 mmol/ EDTA-2Na, pH 13.0) for 20 min at 4°C to allow unwinding of the DNA and expression of DNA damage. Electrophoresis was then conducted at 4°C for 20 min at 25 V/300 mA. The slides were neutralized at room temperature by washing 3 times in neutralization buffer (0.4 mol/L Tris-HCl, pH 7.5) for 15 min. After neutralization, the slides were then incubated in 50%, 75%, and 99% of ethanol for 5 min successively. Before reading, the slides were left to dry for at least 1 day. All these steps were performed in the dark to avoid additional DNA damage. The dried microscope slides were stained with EtBr (20 µg/mL in distilled water, 30 µL/slide) and covered with a cover glass prior to analysis with a fluorescence microscope (Leica DM1000, Wetzlar, Germany) equipped with an excitation filter of 515- 560 nm. The microscope was connected to a charge-coupled device camera and a personal computer-based analysis system (Comet Analysis Software, Version 3.0, Kinetic Imaging Ltd., Liverpool, UK) to determine the extent of DNA damage after electrophoretic migration of the DNA fragments in the agarose gel. In order to visualize the DNA damage, the slides were examined at 400X. For each condition, 100 randomly selected comets from each of two replicate slides were scored (without knowledge of the group codes). DNA damage parameters were expressed as DNA tail intensity %.

Statistical analysis

The statistical analysis was performed with SPSS 10.5 (SPSS, Chicago, IL, USA). The means of data were compared by One-way variance analysis test (ANOVA) and post hoc analysis of group differences was performed by least significant difference (LSD) test. All experiments were carried out four times at different times. The results were presented as the mean ± standard deviation (SD). A p value of less than 0.05 was considered statistically significant.

RESULTS

Cytotoxic effects of thymoquinone and etoposide in HeLa cells

Thymoquinone did not show significant cytotoxic effect at the doses of 3.91-125 µM and at the doses of 3.91-62.5 µM when compared to the negative control (0.5% DMSO) after 24 h and 48 h of treatments, respectively; however, the cell viabilities were significantly decreased above 250 µM and 125 µM doses of thymoquinone ($p<0.05$) after 24 h and 48 h of treatments, respectively, in a dose-dependent manner. The IC_{50} values of thymoquinone were 233.6 µM and 145.5 µM after 24 h and 48 h of treatments, respectively (Figure 1).

Etoposide did not cause significant cytotoxic effect at the doses of 25 µM and 50 µM and at the doses of 25 µM when com-

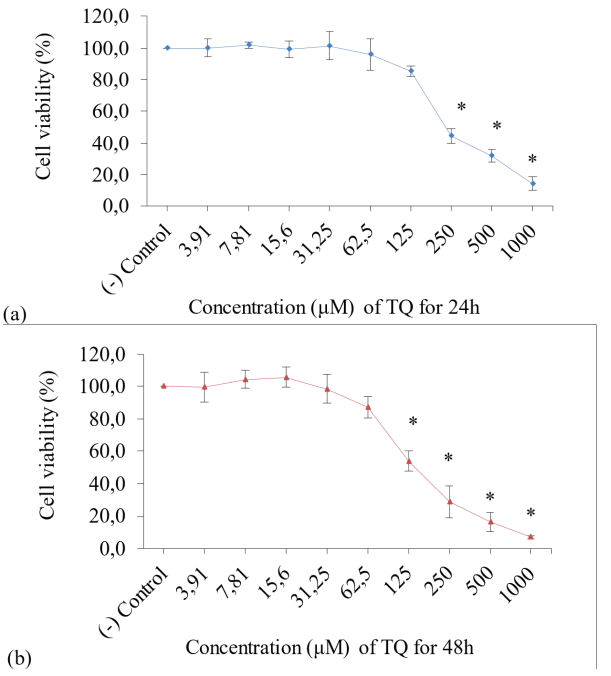

Figure 1. Effect of thymoquinone on HeLa cell viability for 24 h (a) and 48 h (b).*$p<0.05$, compared to negative control (0.5% DMSO). TQ: thymoquinone.

pared to the negative control after 24 h and 48 h of treatments, respectively; however, the cell viabilities were significantly decreased above 50 µM and 100 µM of etoposide ($p<0.05$) in a dose-dependent manner after 24 h and 48 h of treatments, respectively. The IC_{50} value of etoposide were 167.3 µM and 52.7 µM after 24 h and 48 h of treatments, respectively (Figure 2).

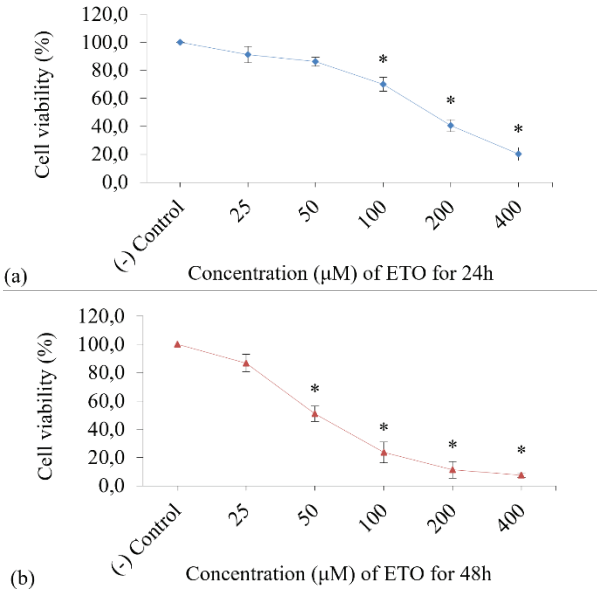

Figure 2. Effect of etoposide on HeLa cell viability for 24 h (a) and 48 h (b).*p<0.05, compared to negative control (0.5% DMSO). ETO: etoposide.

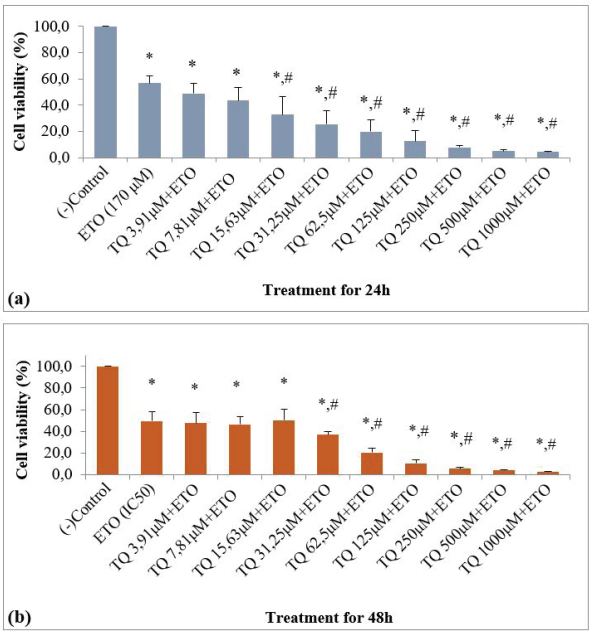

Figure 3. Effect of thymoquinone on etoposide cytotoxicity at 24 h (a) and 48 h (b)on HeLa cells. *p<0.05, compared to negative control (0.5% DMSO); #p<0.05, compared to etoposide (IC50: 170 µM for 24h and 50 µM for 48 h) as positive control. ETO: etoposide; TQ: thymoquinone.

Effects of thymoquinone on etoposide cytotoxicity in HeLa cells

As shown in Figure 3 (a), thymoquinone did not change the IC_{50} value of etoposide (170 µM, approximately) at the concentration ranges of 3.91-7.81 µM after 24 h of treatment; however, the IC_{50} value of etoposide was significantly reduced at the concentration of 15.63 µM and above of thymoquinone (1.73, 2.22, 2.88, 4.50, 7.31, 11.69, 12.72 fold for 15.63 µM, 31,3 µM, 62,5 µM, 125 µM, 250 µM, 500 µM and 1000 µM, respectively) when compared to the negative control after 24 h of treatment ($p<0.05$). As shown in Figure 3 (b), thymoquinone did not change the IC_{50} value of etoposide (50 µM, approximately) at the concentration ranges of 3.91-15.63 µM after 48 h of treatment; however, the IC_{50} value of etoposide was significantly reduced at the concentration of 31.25 µM and above of thymoquinone (1.34, 2.44, 4.80, 9.32, 12.84, 12.37 fold for 31,3 µM, 62,5 µM, 125 µM, 250 µM, 500 µM and 1000 µM, respectively) when compared to the negative control after 48 h of treatment ($p<0.05$). There was no significant difference in cell viability at 48 hours of exposure compared to 24 hours of exposure. It was determined that cell viability did not change in a time dependent manner (Figure 3 a and b).

Effects of thymoquinone on etoposide genotoxicity in HeLa Cells

The genotoxicity of etoposide at non-cytotoxic (1-50 µM) doses in HeLa cells are given in Figure 4. It was found that etoposide did not significantly change DNA damage at 1 µM concentration ($p>0.05$), but increased DNA damage significantly at 5-50 µM doses ($p<0.05$) when compared to the negative control.

The results of the evaluation of genotoxicity of thymoquinone at non-cytotoxic (0.1-100 µM) doses in HeLa cells using the alkaline Comet assay are given in Figure 4. DNA damage was evaluated in terms of DNA tail intensity. It was found that thymoquinone did not significantly change DNA damage at 1-5 µM doses ($p>0.05$), but increased DNA damage significantly at 10-100 µM doses ($p<0.05$) when compared to the negative control.

Thymoquinone significantly reduced etoposide (10 µM)-induced DNA damage at the doses of 0.1-10 µM ($p<0.05$); however, it induced DNA damage at 50 µM concentration ($p>0.05$) (Figure 5).

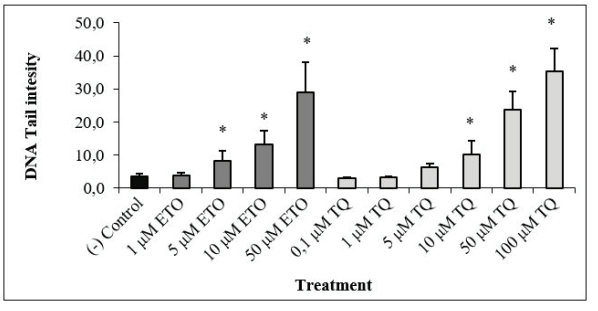

Figure 4. Genotoxicity of thymoquinone and etoposide in HeLa cells. *p<0.05, compared to negative control (0.5% DMSO). ETO: Etoposide, TQ: thymoquinone.

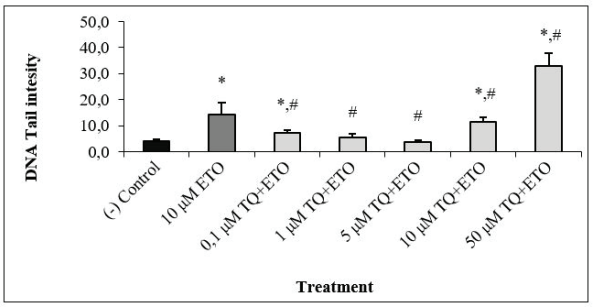

Figure 5. Effect of thymoquinone against etoposide-induced DNA damage in HeLa cells. [a]p<0.05, compared to negative control (0.5% DMSO); [b]p<0.05, compared to etoposide (10 µM) as positive control. ETO: Etoposide, TQ: thymoquinone.

DISCUSSION

Cervical cancer remains a significant cause of morbidity and mortality (Sung et al., 2021). Currently, the combination of cisplatin and etoposide is a commonly used chemotherapy regimen (Salvo et al., 2019). Despite their effectiveness, however, concerns about their adverse effects and drug resistance issues continue. In addition, it is very important to avoid or minimize redundant costs in treatment. Research studies on the combination therapy with phytochemicals are increasing, with a view to increase the effectiveness of cancer treatment and reduce toxicity (Negrette-Guzman, 2019). It is suggested that thymoquinone may be promising in cancer treatment because of its anticancer and chemosensitizing properties (Mahmoud &Abdelrazek, 2019). Studies on the efficiency of thymoquinone along with etoposide in cervical cancer are not sufficient. The aim of this study was to evaluate the cytotoxic and genotoxic effects of the combination of thymoquinone and etoposide on HeLa cells viability.

In our study, the IC_{50} values of thymoquinone in HeLa cells were 233.6 µM and 145.5 µM at 24 and 48 h, respectively, and the IC_{50} values of etoposide were 167.3 µM and 52.7 µM at 24 and 48 h, respectively, by MTT assay. The results show that the cell viability reducing effect of thymoquinone and etoposide in HeLa cells were both in a dose- and time-dependent manner. Thymoquinone significantly decreased the approximate IC_{50} value of etoposide at 15.63 µM and above doses for 24 h and at 31.25 µM and above doses for 48 h, in a dose-dependent manner. Our study shows that thymoquinone can increase the cytotoxic effect of etoposide in HeLa cells. The *in vitro* effect of TQ lowers the IC_{50} of etoposide and it can be interpreted that the necessary etoposide dose for therapy can be decreased, resulting in fewer adverse effects.

In several studies, the IC_{50} value of etoposide was observed to be different. The IC_{50} values of etoposide for 48 h incubation of human gastric cancer (BGC-823), HeLa, and lung cancer (A549) cells were reported as 43.74 ± 5.13 µM, 209.90 ± 13.42 µM, and 139.54 ± 7.05 µM, respectively (Xiao et al., 2014). In another study, however, the viability of HeLa cells treated with 50 µM etoposide were found to be 81.6% for 48 h incubation and 37.5% for 72 h incubation (Rello-Varona et al., 2006).

Consistent with our findings, many studies demonstrated that thymoquinone might have a cytotoxic effect on various cancer cells. However, it is apparent that cytotoxic profiles may be different, depending on different methods applied and cell lines used. The IC_{50} values of thymoquinone were determined by the sulforhodamine B assay as 44.8 µM and 35.1 µM, for 24 h of HeLa and hepatocellular carcinoma (HepG2) respectively (ElKhoely et al., 2015). The IC_{50} values of thymoquinone in different cell lines (HeLa, SiHa, 3T3, Vero cells) were determined by MTT assay to be119.2 µM, 87.8 µM, 70.6 µM, and 21.8 µM at 24 h exposure, 72.1 µM, 52.3 µM, 69.3 µM, and 17.7 µM at 48 h exposure, and 29.6 µM, 23.4 µM, 61.7 µM, and 17.4 µM at 72 h exposure, respectively (Hafiza & Latifah, 2014). In a study conducted by MTT assay, IC_{50} values for thymoquinone were determined to be 2.4 µM, 10.3 µM, and 8.3 µM, respectively, for 24 h incubation of different glioblastoma cell lines (T98G, U87MG ve Gli36DEGFR). Thymoquinone was determined to cause enzyme release from lysosomes as well as apoptotic cell death in a p53-independent and caspase-dependent manner (Racoma et al., 2013). In another study using MTT assay, the IC_{50} value of thymoquinone was determined to be 25 µM for 48 h incubation of breast cancer (MCF-7) cells. It was also shown in that study that thymoquinone regulated the expression of apoptosis-related genes, (BAD, bax, and p53) (Yıldırım, Azzawri, & Duran, 2019). It was reported that IC_{50} concentration of thymoquinone in lung (LNM35), liver (HepG2), colon (HT29), melanoma (MDA-MB-435), and breast (MDA-MB-231 and MCF-7) cells for 24 h was 34 µM for HepG2 and between 50 and 78 µMfor other cells, and decreased cell viability dose-dependently. In that study, high doses (100 µM) of thymoquinone were noted to have toxic effects by causing DNA damage and activating mitochondrial-proapoptotic signaling pathways (Attoub et al., 2013). The IC_{50} values of thymoquinone determined by MTT assay and trypan blue dye test after 72 h in human cervical squamous carcinoma (SiHa) cells, were determined as 64 µM and 55.9 µM, respectively (Ng, Yazan, & Ismail, 2011).

We have not seen any *in vitro* studies on thymoquinone combined with etoposide in cervical cancer. Thymoquinone can exhibit a synergistic effect in reducing cell viability with anticancer drugs, such as cisplatin, that can cause DNA-damage (El Nabi et al., 2019; Pucci et al., 2019). The potentially synergistic effect of thymoquinone with topotecan was shown on human colon cancer cell lines. After determining the best combination (40 µM thymoquinone and 0.6 µM topotecan) in the study, thymoquinone was reported to increase the efficiency of topotecan by inhibiting proliferation through mechanisms independent of p53 and Bax/Bcl2 and reducing toxicity (Khalife, Hodroj, Fakhoury, & Rizk, 2016). In another study, the IC_{50} values of gemcitabine, in MCF-7 cells, were shown to be significantly decreased following thymoquinone combination. It was concluded that thymoquinone showed promising chemomodulatory effects on gemcitabine in breast cancer cells by inducing apoptosis, necrosis, and autophagy (Bashmail et al., 2018).

In our study, genotoxicity profiles were evaluated by calculating the DNA tail intensity using the alkaline Comet assay. It was found that thymoquinone (at non-cytotoxic doses) did not sig-

nificantly change DNA damage at doses of 1-5 µM in HeLa cells, but significantly increased DNA damage at doses of 10-100 µM. It was found that etoposide did not significantly change DNA damage at 1 µM concentration, but increased DNA damage significantly at 5-50 µM doses. DNA damage induced by etoposide (5 µM) in HeLa cells significantly diminished at 0.1-10 µM doses of thymoquinone; however, it increased at 50 µM concentration of thymoquinone.

There are many studies on the reducing or preventing effects of thymoquinone concerning DNA damage induced by chemical substances. Khader, Bresgen, & Eckl (2009) suggested that high doses of thymoquinone might cause DNA damage by increasing oxidative stress in hepatocytes, depleting glutathione and reducing antioxidant enzymes. Gurung et al. (2010) reported that, using the Comet assay, thymoquinone did not increase DNA damage in human glioblastoma cell lines at a dose of 25 µM, but significantly increased at a 50 µM dose. They suggested that thymoquinone could induce DNA damage, telomere shortening, and cell death. The cytotoxic and genotoxic effects of thymoquinone (5, 10 and 20 µM) in human peripheral leukocytes were investigated, alone or in combination with doxorubicin (0.15 µg/mL). It was reported that thymoquinone dose-dependently increased apoptotic cell death and DNA damage determined by using the Comet assay, but reduced doxorubicin-induced apoptotic cell death and DNA damage (Al-Shdefat, Abd-ElAziz, & Al-Saikhan, 2014).

Thymoquinone was found to inhibit proliferation and migration of cancer cells by changing some apoptosis-related gene expressions, and it was suggested that thymoquinone might induce apoptosis in cancer cells (Sakalar et al., 2013). Studies claimed that thymoquinone could increase the P53 gene expression level and regulating the Bax/Bcl-2 ratio in SiHa cells (Coutts & La Thangue, 2006) and could occur via the PPAR-g activation pathway (Woo et al., 2012).

According to our results, the cytotoxic effect of etoposide could increase at doses where thymoquinone alone was not toxic in HeLa cells. Thymoquinone achieved this effect at relatively higher (31.25 µM and above) but non-cytotoxic doses, rather than at low doses. Given that thymoquinone has a low bioavailability and a very short half-life, more stable formulations of thymoquinone should be worked on in research studies. There are some limitations of this study. The effects of a triple combination could have been evaluated, by including cisplatin to thymoquinone and etoposide combination, considering the fact that cisplatin and etoposide combination is the most common chemotherapy regimen applied. In this study, in addition to examining the effectiveness of thymoquinone on the cytotoxicity of etoposide in HeLa cells, different possible cellular pathways such as apoptosis, cell cycle checkpoints, and antioxidant defense system could have been examined through more advanced techniques. Although preliminary, our results, demonstrating the complementary role of thymoquinone in improving the therapeutic efficiency of etoposide used in cancer treatment, can be considered to introduce new data to the relevant literature and present promising findings. This present study may be a pioneer for further research.

CONCLUSION

As a result of this study, it was revealed that thymoquinone can increase the cytotoxic and genotoxic effects of etoposide in cervical cancer cells at high doses and reduce DNA damage at low but not cytotoxic doses, suggesting that thymoquinone might increase the anticancer effect of etoposide at high doses. Although thymoquinone was recognized to have a significant role of in terms of reducing side effects, increasing treatment efficiency and reducing treatment costs in the treatment of various human cancers, current data should be supported by further clinical studies. Moreover, low stability of thymoquinone due to its molecular structure should also be considered, and durable formulations of this molecule should be developed. This issue needs to be handled by further studies.

Peer-review: Externally peer-reviewed.

Informed Consent: Written consent was obtained from the participants.

Author Contributions: Conception/Design of Study- S.A.D., H.G.N.Ç.; Data Acquisition- H.G.N.Ç., M.B.K., A.Ç.; Data Analysis/Interpretation- H.G.N.Ç., M.B.K., A.Ç. S.A.D.; Drafting Manuscript- H.G.N.Ç., M.B.K., A.Ç..; Critical Revision of Manuscript- S.A.D., H.G.N.Ç.; Final Approval and Accountability- H.G.N.Ç., M.B.K., A.Ç. S.A.D.

Conflict of Interest: The authors have no conflict of interest to declare.

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