ORİJİNAL ARAŞTIRMA ORIGINAL RESEARCH

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The Effects of Thymoquinone, a Bioactive Compound of Nigella Sativa, in Combination with Cisplatin on the Viability of HeLa Cervical Cancer Cells

Nigella Sativa'nın Biyoaktif Bileşeni Timokinonun Sisplatinle Kombinasyonunun HeLa Serviks Kanser Hücre Canlılığına Etkileri

Songül YILMAZ[a], Merve BECİT[b], Sevtap AYDIN DİLSİZ[a]

[a]Department of Pharmaceutical Toxicology, Hacettepe University Faculty of Pharmacy, Ankara, Türkiye,
[b]Department of Pharmaceutical Toxicology,Afyonkarahisar University of Health Sciences, Faculty of Pharmacy, Afyon, Türkiye

ABSTRACT Objective: Thymoquinone, a monoterpene molecule which is derived from the volatile oil of Nigella sativa L. seed known as black seed "çörek otu" in Turkey, is a bioactive compound. Although it is consumed as food, it is claimed that thymoquinone has a wide range of effects, from its anticancer effect to its antiallergic effect, together with its main antioxidant property. Thymoquinone is thought to play an important role in the prevention and treatment of various types of cancers. Cisplatin, a platinum-based anticancer agent, has been used for many years in the treatment of solid tumors such as ovarian, cervix, testis, prostate, bladder and lung cancers despite its toxic effects. The use of plant extracts in combination with chemotherapeutic drugs in order to reduce the side effects of drugs used in chemotherapy and to increase their intra-body effects is the focus of research. The aim of this study is to reveal the cytotoxic effects of the combined use of thymoquinone, which is known to have important biological effects and whose importance has increased in recent studies, together with the anticancer drug cisplatin, on cervical cancer cells (HeLa). **Material and Methods:** HeLa cervical carcinoma cells provided from the American Type Culture Collection were used. The effects of thymoquinone in the concentration range of 1.95 µM-1000 µM and cisplatin in the concentration range of 0.49 µM - 250 µM on HeLa cell viability were measured by MTT method after 24 and 48 hours of incubation. After determining the IC50 values of each substance alone, the effects of combinations of thymoquinone with IC50 doses of cisplatin on cell viability were determined by MTT method after 24 and 48 hours of incubation. **Results:** In HeLa cells, for 24 h and 48 h incubations, IC50 values of thymoquinone were found to be 143.7 µM and 67.5 µM, respectively and IC50 values of cisplatin were found to be 20.3 µM and 12.9 µM, respectively. According to the study on the effects of the combined administration of thymoquinone on cisplatin cytotoxicity. Thymoquinone in the concentration range of 7.8-250 µM for 24 hours of incubation, statistically decreased the IC50 value of cisplatin (20 µM) in a dose-dependent manner (20.6%, 33.3%, 46.8%, 56.5%, 70.8%, 84.2% for 7.8 µM, 15.6 µM, 31.3 µM, 62.5 µM, 125 µM, 250 µM, respectively) in HeLa cells. Thymoquinone in the concentration range of 15.68-250 µM for 48 hours of incubation, statistically decreased the IC50 value of cisplatin in a dose-dependent manner (41.6%, 44.2%, 62.2%, 71.1%, 81.9% for 15.6 µM, 31.3 µM, 62.5 µM, 125 µM, 250 µM, respectively). **Conclusion:** In conclusion, our findings show that the combination of thymoquinone with cisplatin can increase the cytotoxicity of cisplatin in HeLa cells, and therefore it is thought that thymoquinone may increase the anticancer effect of cisplatin; however, to confirm their clinical use and to determine its interactions with anticancer drugs, the advanced in vitro and in vivo studies are needed.

Keywords: Thymoquinone; cisplatin; cytotoxicity; HeLa cells

ÖZET Amaç: Türkiye'de çörek otu olarak bilinen Nigella sativa L. tohumlarının uçucu yağından elde edilen monoterpen bir molekül olan timokinon biyoaktif bir bileşiktir. Gıda olarak tüketilmekle beraber, timokinonun başlıca antioksidan özelliği ile birlikte antikanser etkisinden, antialerjik etkisine kadar çok geniş bir etki yelpazesine sahip olduğu ileri sürülmektedir. Timokinonun çeşitli türdeki kanserlerin önlenmesi ve tedavisinde önemli bir rol oynayabileceği düşünülmektedir. Over, serviks, testis, prostat, mesane, akciğer kanserleri gibi katı tümörlerin tedavisinde platin bazlı bir antikanser ajanı olan sisplatin, toksik etkileri olsa da uzun yıllardır kullanılmaktadır. Kemoterapide kullanılan ilaçların yan etkilerini azaltmak ve vücut içi etkilerini arttırmak amacıyla kemoterapi ilaçları ile birlikte kombine olarak bitki ekstraktlarının kullanımı araştırmaların ilgi odağı olmaktadır. Bu çalışmanın amacı önemli biyolojik etkileri olduğu bilinen ve son yapılan çalışmalarda önemi gittikçe artan timokinonun antikanser ilaç olan sisplatinle birlikte kombine kullanımının serviks kanser hücreleri (HeLa) üzerindeki sitotoksik etkilerini ortaya çıkarmaktadır. **Gereç ve Yöntemler:** American Type Culture Collection'dan sağlanan HeLa servikal karsinom hücreleri kullanıldı. 1,95 µM- 1000 µM konsantrasyon aralığında timokinon ve 0.49 µM - 250 µM konsantrasyon aralığında sisplatinin HeLa hücre canlılığı üzerindeki etkileri 24 ve 48 saatlik inkübasyondan sonra MTT yöntemi ile ölçüldü. Her bir maddenin tek başına IC50 değerleri belirlendikten sonra 24 ve 48 saatlik inkübasyonda timokinonun sisplatinin IC50 dozu ile kombinasyonlarının hücre canlılığı üzerine etkileri MTT yöntemi ile belirlendi. **Bulgular:** HeLa hücrelerinde 24 ve 48 saatlik inkübasyonlarda timokinonun IC50 değerleri sırasıyla 143.7 µM ve 67.5 µM, sisplatinin IC50 değerleri sırasıyla 20.3 µM ve 12.9 µM olarak bulundu. Timokinonun kombine uygulamasının sisplatin sitotoksisitesi üzerindeki etkileri üzerine yapılan araştırmaya göre, HeLa hücrelerinde, 7.8 µM - 250 µM konsantrasyon aralığında 24 saatlik inkübasyon için timokinon, sisplatinin IC50 değerini (20 µM) doz bağımlı olarak (7.8 µM, 15.6 µM, 31.3 µM, 62.5 µM, 125 µM ve 250 µM için sırasıyla %20.6, %33.3, %46.8, %56.5, %70.8 ve %84.2) istatistiksel anlamlı azalttı ($p<0.05$). Timokinon 15.68-250 µM konsantrasyon aralığında 48 saatlik inkübasyon için sisplatinin IC50 değerini doz bağımlı olarak (15.6 µM, 31.3 µM, 62.5 µM, 125 µM ve 250 µM için sırasıyla %41.6, %44.2, %62.2, %71.1 ve %81.9) istatistiksel anlamlı azalttı ($p<0.05$). **Sonuç:** Sonuç olarak, bulgularımız HeLa hücrelerinde timokinonun sisplatinle kombinasyonun sisplatin sitotoksisitesini arttırabileceğini göstermektedir ve dolayısıyla timokinonun seviks kanser hücresinde sisplatinin antikanser etkisini arttırabileceği düşünülmektedir; ancak bunların klinik kullanımlarını doğrulamak ve antikanser ilaçlarla etkileşimlerini belirlemek için ileri in vitro ve in vivo çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: Timokinon; sisplatin; sitotoksisite; HeLa hücre hattı

Correspondence: Sevtap AYDIN DİLSİZ
Department of Pharmaceutical Toxicology, Hacettepe University Faculty of Pharmacy, Ankara, Türkiye
E-mail: sevtapay@hacettepe.edu.tr

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Cancer is one of the most common diseases and its spread is increasing in the next century. Breast and cervical cancer are the two most common cancer types in women, although they are among the leading causes of death in the world. The struggle on cancer in the last fifty years has led to an important development in the emergence of new treatment options against cancer. However, despite the emergence of many treatment options such as chemotherapeutic agents, it has been observed that options such as intelligent drugs, which are found to be effective in the treatment of some cancer types, have not changed much. Due to the high cost and potential toxicity of pharmacological treatments and chemotherapy in the long-term treatment of cancer, research has turned to non-pharmaceutical products that are naturally occurring, have high potency and have a lower risk of side effects than pharmaceutical ingredients. Experimental studies have shown that natural components and plant extracts have anticancer potential in various bioanalysis and animal models. In fact, it is stated that products consumed as nutrients such as spices in various parts of the world are used in the protection and healing of many diseases, including cancer, apart from their flavoring, coloring or preservative properties.[1,2]

Nigella sativa L. known as "black seed" is an annual plant from the Ranunculaceae family, grown in the Mediterranean, Pakistan, and India countries in the world and is cultivated in many countries of the world, especially in the Eastern Mediterranean countries. Black seed is also used in the pharmaceutical field, as well as nutritionally by adding it to foods such as tea, coffee, bread. Although black seed oil is native to Arabian and Mediterranean countries, it has been used traditionally for thousands of years in the Mediterranean, India, Asia, Middle and far East countries as a spice, food preservative or as a daily natural preservative and therapeutic option. Studies have shown that 36-38% of black cumin seeds consist of seed oils and the main content of essential oil is thymoquinone, dithymoquinone, thymohydroquinone and thymol. The major bioactive component of black cumin essential oil is thymoquinone, and its activity depends on its antioxidant properties. Studies have reported that thymoquinone has a wide range of protective and/or therapeutic properties. It has been shown to have antimicrobial and anti-inflammatory effects as well as protective and therapeutic effects on especially on cancer and kidney, immune system, gastrointestinal system, cardiovascular system.[3-7]

Cisplatin, a well-known antineoplastic drug from the class of alkylating agents, is used in carcinomas, germ cell tumors and lymphomas and sarcomas. It is frequently preferred in especially in ovarian, breast, brain, testis, head and neck solid tumors, soft tissue, bone, muscle and blood vessel sarcomas. Cisplatin can be used alone or in combination with other drugs or supplements to increase its effect during treatment, to minimize the cytotoxicity especially in the kidney and drug resistance.[8,9]

As shown in the studies, there are not enough studies on the effect of thymoquinone, which has been used in traditional treatment for centuries, together with cisplatin. In this study, the effects of thymoquinone on the cytotoxicity of cisplatin in human cervical cancer (HeLa) cell line was investigated using methyl thiazole tetrazolium (MTT) method. In our study, after determining the effects of certain concentrations of thymoquinone and cisplatin on HeLa cell viability at different incubation times, the effect on cisplatin cytotoxicity following the combined administration of thymoquinone and cisplatin was evaluated. The results of the study are expected to provide information about the use of the combination of thymoquinone and cisplatin in the treatment of cervical carcinoma.

MATERIALS AND METHODS

MATERIALS

The chemicals were purchased from the suppliers: cisplatin (Koçak Farma, Turkey); dimethyl sulfoxide (DMSO), Dublecco's modified Eagle's medium (DMEM), ethanol, fetal bovine serum (FBS), MTT, penicillin-streptomycin, trypan blue, trypsin–EDTA, Dulbecco's phosphate buffered saline (PBS) from Sigma (St. Louis, MO, USA); milli-pore filters from Millipore (Billerica, MA, USA), all other plastic materials from Corning-(Corning Inc., NY, USA). Thy-

moquinone was purchased from Sigma (St. Louis, MO, USA).

CELL CULTURE

HeLa cells were provided from the American Type Culture Collection (ATCC; Rockville, MD, USA). The cells were grown in DMEM containing low glucose (1 g/L) and sodium pyruvate. The media were supplemented with 10% FBS, 2mM L-glutamine and 1% penicillin-streptomycin solution (10000 units of penicillin and 10 mg of streptomycin in 0.9 % NaCl) at 37°C in 5% CO_2 incubators. The cells were subcultured in 75 cm^2 cell culture flasks. The culture medium was changed every 3-4 days. The passage numbers used in our study for both cell lines were between passage 15 and 17.

DETERMINATION OF CYTOTOXICITY

The effects of thymoquinone and cisplatin and their combination on cell viability were determined by MTT assay.[10] According to the cell viability data, IC_{50} was estimated. The cytotoxic profiles of thymoquinone on the IC_{50} of cisplatin were evaluated in a wide range of doses in HeLa cells. Cells were seeded 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 stock solution of thymoquinone was freshly prepared in PBS with 1% DMSO and filtered with milli-pore filters (0.20 µm). The cells were treated with cisplatin (0.49-250 µM), thymoquinone (1.95-1000 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 (1%). 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 200 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.

STATISTICAL ANALYSIS

All experiments were carried out in quadruplicate. The results were given as the mean ± standard deviation. The statistical analysis was performed with software programs *"SPSS 10.5"* (Statistical Package for the Social Sciences, Chicago, IL, USA). The means of data were compared by the one-way variance analysis test and post hoc analysis of group differences was performed by least significant difference (LSD) test. A *p* values less than 0.05 were considered as statistically significant.

RESULTS

The effects of thymoquinone at the concentration of 1.95-1000 µM on HeLa cell viability measured by MTT method after 24 and 48 hours of incubation are given in Figure 1 and Figure 2. For 24 hours incubation, it was observed that thymoquinone did not cause a significant cytotoxic effect at the concentration of 1.95-31.25 µM when compared to the negative control in HeLa cells, but it significantly reduced cell viability in a dose-dependent manner at the concentrations of 62.5 µM and above ($p<0.05$). The IC_{50} value of thymoquinone in HeLa cells was 143.7 µM for 24-hour incubation.

For 48 hours of incubation, it was observed that thymoquinone did not cause a significant cytotoxic effect at the concentration of 1.95-15.62 µM when compared to the negative control in HeLa cells, but it significantly reduced cell viability in a dose-dependent manner at concentrations of 31.25 µM and above

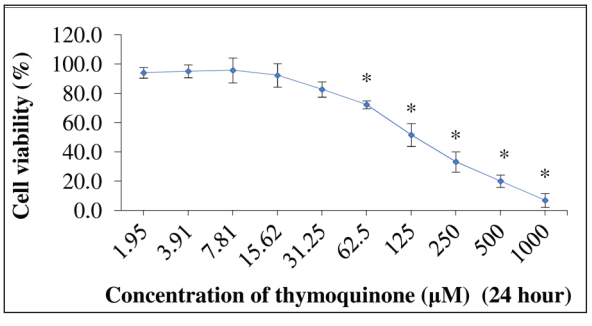

FIGURE 1: Effect of thymoquinone on HeLa cell viability at 24-hour exposure. Results are given as mean ± standard deviation. Cell viability was evaluated relative to the negative control. 1% DMSO was used as negative control. [a]p<0.05, compared with the negative control.

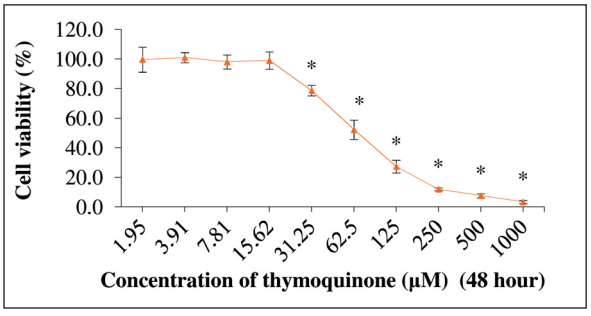

FIGURE 2: Effect of thymoquinone on HeLa cell viability at 48-hour exposure. Results are given as mean ± standard deviation. Cell viability was evaluated relative to the negative control. 1% DMSO was used as negative control. [a]p<0.05, compared with the negative control.

($p<0.05$). The IC_{50} value of thymoquinone in HeLa cells was found to be 67.5 µM for 48-hour incubation.

The effects of cisplatin on HeLa cell viability measured by the MTT method after 24 and 48 hours of incubation at the concentration of 0.49-250 µM are given in Figure 3 and Figure 4. For 24 hours incubation, cisplatin did not cause a significant cytotoxic effect in the concentration range of 0.49-7.81 µM when compared to the negative control in HeLa cells, but it significantly reduced cell viability in a dose-dependent manner at concentrations of 15.62 µM and above ($p<0.05$). The IC_{50} value of cisplatin in HeLa cells was found to be 20.31 µM at 24-hour exposure.

For 48 hours of incubation, cisplatin did not have a significant cytotoxic effect at the concentration of 0.49-3.91 µM when compared to the negative control in HeLa cells, but it significantly reduced cell viability in a dose-dependent manner at concentrations of 7.81 µM and above ($p<0.05$). The IC_{50} value of cisplatin in Hela cells was found to be 12.9 µM at 48 hours of exposure.

The effects of thymoquinone at the concentration of 7.8-250 µM on cisplatin cytotoxicity in HeLa cells at 24- and 48- hours incubation is given in Figure 5. In HeLa cells, thymoquinone at the concentration of 7.8-250 µM for 24 hours of incubation, statistically decreased the IC_{50} value of cisplatin (20 µM) in a dose-dependent manner (20.6%, 33.3%, 46.8%, 56.5%, 70.8%, 84.2% for 7.8 µM, 15.6 µM, 31.3 µM, 62.5 µM, 125 µM, 250 µM, respectively) when compared to the treatment of cisplatin ($p<0.05$).

It was observed that in HeLa cells, thymoquinone at the concentration of 15.6-250 µM for 48 hours of incubation, statistically decreased the IC_{50} value of cisplatin in a dose-dependent manner (41.6%, 44.2%, 62.2%, 71.1%, 81.9% for 15.6 µM, 31.3 µM, 62.5 µM, 125 µM, 250 µM, respectively) when compared to the treatment of cisplatin ($p<0.05$).

DISCUSSION

Nigella sativa seeds, which are in the Ranunculaceae family and known as "*çörek otu*" in our country, are used for nutritional purposes by adding to foods such as tea, coffee, bread, and traditionally such as asthma, cough, bronchitis, inflammation, diabetes, gastrointestinal problems. It has been known to be used for many years in the treatment of various diseases. Thymoquinone is the main pharmacologically active basic compound in the essential oil of black cumin seed.

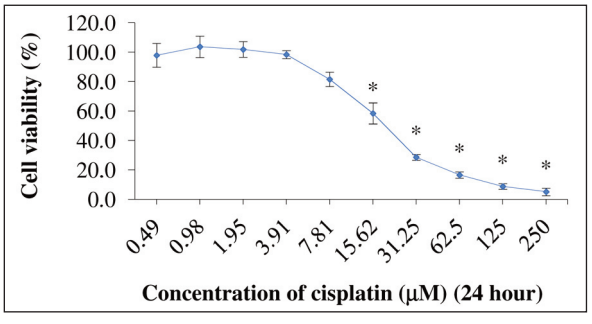

FIGURE 3: Effect of cisplatin on HeLa cell viability at 24-hour exposure. Results are given as mean ± standard deviation. Cell viability was evaluated relative to the negative control. 1% DMSO was used as negative control. ap<0.05, compared with the negative control.

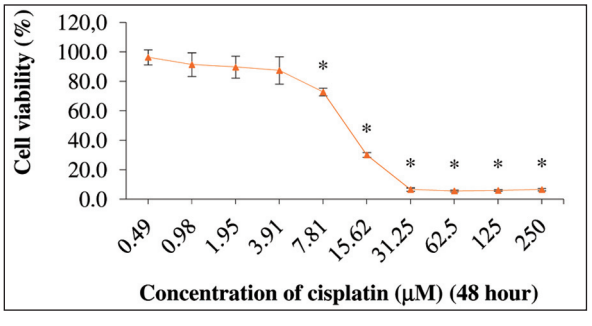

FIGURE 4: Effect of cisplatin on HeLa cell viability at 48-hour exposure. Results are given as mean ± standard deviation. Cell viability was evaluated relative to the negative control. 1% DMSO was used as negative control. ap<0.05, compared with the negative control.

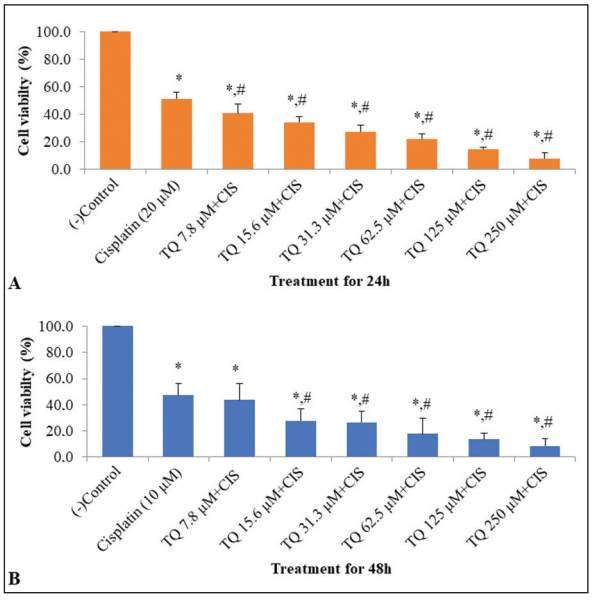

FIGURE 5: Effect of thymoquinone on cisplatin cytotoxicity at 24-hour exposure **(A)** and 48-hour exposure **(B)** in HeLa cells. Results are given as mean ± standard deviation. Cell viability was evaluated relative to the negative control. 1% DMSO was used as negative control. *p<0.05, compared with the negative control. #p<0.05, compared to cisplatin (20 µM and 10 µM, for 24h and 48h, respectively). CIS: cisplatin; TQ: thymoquinone.

It is known that thymoquinone can reduce oxidative stress and increase antioxidant defense by scavenging free oxygen radicals.[4-7]

Studies have reported that thymoquinone may have protective effects on kidney, liver, heart, lung and stomach against oxidative damage, and black cumin seeds and its components have various beneficial effects such as anticarcinogenic, anti-inflammatory, analgesic, hypoglycemic, immune response modulator. Studies have reported that thymoquinone has a wide range of protective and/or therapeutic properties.[7,11,12] Despite these broad effects, studies on the mechanisms of these effects are not sufficient.

Breast and cervical cancers are among the leading causes of death in women worldwide. Due to the high cost and potential toxicity of chemotherapy in cancer treatment, there is increasing interest in naturally occurring phytochemicals, which have high efficacy and have a lower risk of side effects compared to pharmaceutical components. Experimental studies have suggested that natural components and plant extracts have anticancer potential.[1]

Cisplatin is a well-known antineoplastic drug and is frequently used in solid tumors of the ovary, breast, brain, testis, head and neck, as well as sarcomas of soft tissue, bone, muscle and blood vessels. According to the studies, there are not enough studies on the effect of thymoquinone, which has been used in traditional medicine for many years, together with cisplatin. The combined use of plant extracts is being investigated in order to reduce the side effects of drugs used in chemotherapy and to increase their intra-body effects.[13]

In the Western world, ovarian cancer has been defined as the cancer that causes the most deaths from gynecological cancers, possibly due to a poor prognosis or lack of early symptoms.[14] Although platinum-containing drugs such as cisplatin are routinely used in ovarian cancer, side effects and drug resistance developed against them result in a low survival.[14]

Recently, there has been an increased interest in the use of nutritional chemoprotective agents such as phytochemicals, as they have fewer side effects with chemotherapeutic agents to inhibit the growth of cancer cells. Since nutritional components against tumors show their antitumor activities by regulating cell signaling pathways, unlike platinum-containing drugs, it has been predicted that a synergistic effect may occur with the use of platinum-containing drugs and nutritional components together, which will increase antitumor activity and/or compensate for side effects.[15] The possible mechanisms of action of natural components that induce cell death or apoptosis in cervical cancer cells have been demonstrated in many in vitro studies.[16]

Although there are many studies on thymoquinone in the literature, studies combining cisplatin with thymoquinone are insufficient. Our study showing the effects of thymoquinone and cisplatin combination in cervical carcinoma cell line is very important in terms of providing scientific data. In present study, the cytotoxic effects of the combined use of thymoquinone, which is known to have important biological effects and whose importance has increased in recent studies, together with the anticancer drug cisplatin on cervical cancer cells (HeLa)

were investigated by MTT method. In our study, after examining the effects of certain concentrations of thymoquinone and cisplatin on HeLa cell viability, the effects on cisplatin cytotoxicity following the combined administration of thymoquinone and cisplatin were evaluated at different incubation times.

The MTT method is a colorimetric test method that measures mitochondrial enzyme activity and is applied to evaluate cell viability in vitro. It is based on the reduction of MTT and other tetrazolium salts to violet colored formazan crystals that are insoluble in water inside the cell by the respiratory chain and other electron transport systems.[10]

In our cytotoxicity study, HeLa cells were preferred for many reasons, such as their favorable proliferation rate and excellent colony-forming properties, as well as their sensitivity to many chemicals. In addition, this cell line is frequently used today for cancer research.

Many studies have shown that thymoquinone has a selective cytotoxic effect on cancer cells. It is discussed that thymoquinone can selectively kill tumor cells.[12]

In our study, it was determined that thymoquinone dose-dependently decreased cell viability at concentrations of 31.25 µM and above when compared to the negative control, and IC_{50} values were 143.7 µM and 67.5 µM for 24- and 48-hour incubations, respectively, in HeLa cells. According to this study, cytotoxicity of thymoquinone to HeLa cells appears to be both dose-dependent and time-dependent. We also found that cisplatin at the concentrations of 15.2 µM and above for 24-hour incubations; at the concentrations of 7.81 µM and above for 48-hour incubations decreased cell viability statistically in a dose-dependent manner when compared to the negative control and IC_{50} values were 20.31 µM and 12.9 µM for 24-hour and 48-hour incubations, respectively, in HeLa cells.

In an *in vitro* study on thymoquinone, it has been shown that when applied at 1 mM concentration, it causes a rapid cytotoxic effect by causing nuclear shrinkage and plasma membrane bubble formation within minutes in the isolated hepatocytes from Fischer rats. In a study performed at lower concentrations of thymoquinone, it has been shown that 100 µM concentrations of thymoquinone cause cell death within hours and cause acute cytotoxicity within 48 hours at 50 µM and 2 µM concentrations in the hepatocytes. It was observed that thymoquinone at 10 µM and 20 µM concentrations triggered an increase in the level of necrosis compared to the control. Although lower doses are less toxic, it has been observed that it triggers the emergence of toxic effects such as necrosis level, frequency of chromosomal aberrations and micronuclei formation.[17]

In the study of Hafıza et al (2014), the cytotoxic effects of thymoquinone applied at concentrations ranging from 3.0 to 200 µM in different cell lines consisting of HeLa, SiHa 3T3, Vero cells were examined after 24th, 48th and 72nd hours of incubation. The IC_{50} values of thymoquinone in HeLa, SiHa 3T3, Vero cells were 119.2 µM, 87.8 µM, 70.6 µM, 21.8 µM after 24 hours of incubation; 72.1 µM, 52.3 µM, 69.3 µM and 17.7 µM after 48 hours of incubation; 29.6 µM, 23.4 µM, 61.7 µM and 17.4 µM72 hours of incubation, respectively. In this study, in which different cytotoxic effects of thymoquinone emerged in different cells, it was seen that cytotoxicity was time and dose dependent, which is consistent with our findings.[16]

In the study by Ng et al. (2015), the cytotoxicity of thymoquinone-loaded nano-structured lipid carriers in breast and cervical cancer cells (HeLa and SiHa) were observed for 24, 48 and 72 hours. It was reported that the treatment of a thymoquinone-loaded nano-structured lipid carrier caused dose-dependent increasing cytotoxicity, and the number of viable cells was more than 87% as a result of the treatment of the nano-carrier lipid alone.[3]

It is the fact that multiple molecular mechanisms of action of thymoquinone is the reducing cancer growth and lifespan include activation of tumor suppressor genes such as PTEN and p21, proinflammation, and inhibition of NF-κB stimulation and reduction of angiogenic stimuli.[18-20]

Some resistance mechanisms to platinum compounds have been described in cancer cells. It has been stated that NF-κB inhibitors potentiate the antitumor activity of various cytotoxic agents, and cisplatin triggers NF-KB and reduces cell death. In the other mechanism, cisplatin induces DNA double-strand breaks, but this effect has been found to decrease in ovarian cancer cells.[21]

In our present study, we investigated the effects of the combined administration of thymoquinone on cisplatin cytotoxicity in HeLa cells. We observed that thymoquinone in the concentration range of 7.8-250 µM statistically decreased the IC_{50} value of cisplatin (20 µM) (20.6%, 33.3%, 46.8%, 56.5%, 70.8%, 84.2% for 7.8 µM, 15.6 µM, 31.3 µM, 62.5 µM, 125 µM, 250 µM, respectively) for 24 hours of incubation ($p<0.05$). Thymoquinone in the concentration range of 15.68-250 µM statistically decreased the IC_{50} value of cisplatin (41.6%, 44.2%, 62.2%, 71.1%, 81.9% for 15.6 µM, 31.3 µM, 62.5 µM, 125 µM, 250 µM, respectively) for 48 hours of incubation ($p<0.05$). Our result shows that thymoquinone increases cisplatin cytotoxicity in HeLa cells both in a dose- and time-dependent manner.

The effect of thymoquinone was investigated in the study using canine osteosarcoma (COS31) and its cisplatin-resistant human mammary adenocarcinoma (MCF7), human ovarian adenocarcinoma (BG-1) and Madin-Darby canine kidney (MDCK) cell lines. In cell lines where thymoquinone applied at concentrations ranging from 0-100 µM at 12-hour, 24-hour and 48- hour, thymoquinone with IC_{50} values between 7.7-101 µM, regardless of concentration, inhibited the proliferation of COS31, COS31/rCDDP, MCF7, BG-1 and MDCK cells. MDCK cells was to be the most resistant to the inhibitory effect of thymoquinone. The IC_{50} value of thymoquinone in MDCK cells was 101 µM, the IC_{50} value in BG-1 cells was 39.65 µM, and the IC_{50} value in MCF7 cells was 10.32 µM. Cisplatin-resistant variant COS31/rCDDP cells were shown to be the most sensitive cell line to the inhibition effect of thymoquinone among all cells, with an IC_{50} value of 7.7 µM, and IC_{50} value of thymoquinone in daughter cell line COS31 cells was 34.8 µM.[22]

It was determined that there was no cell proliferation for 72 hours in SiHa cells exposed to thymoquinone at the concentrations of 1 µg/ml, 3 µg/ml, 10 µg/ml and 30 µg/ml. In SiHa cells to which thymoquinone or cisplatin were treated, a significant decrease was observed in the percentage of viable cells at concentrations of 1 µg/ml - 30 µg/ml at the 24th, 48th and 72nd hours, but it was reported that thymoquinone was less cytotoxic than cisplatin in normal cells.[23]

In the study of Jefri et al. (2010), they used NCI-H460 as the non-small cell lung cancer cell line and NCI-H146 as the small cell lung cancer cell line. Based on IC_{50} values calculated from previous experience, 80 µM and 100 µM thymoquinone and 0.1% DMSO, 125 µM, 2.5 µM and 5.0 µM cisplatin were applied to the cells separately and together at the indicated concentrations, and were followed on the 24th, 48th and 72th hours. It was found that thymoquinone significantly inhibited cell proliferation at all concentrations studied, especially at the 24th hour, and the inhibition effect decreased at the 48th and 72nd hours; it was shown that cisplatin was not as active as thymoquinone at 24 hours, but significantly inhibited cell proliferation at 48 and 72 hours. It was determined that the effect of the combined use of thymoquinone and cisplatin on cell proliferation was higher at 72 hours, up to 89%, than the use of both components alone. In this study, the researchers concluded that thymoquinone and cisplatin exerted a synergistic effect.[24]

Nessa et al. (2011), in their study investigating the synergistic effect of cisplatin and thymoquinone in the A2780 ovarian cell line and its cisplatin-resistant A2780CisR cell line, a synergistic effect was demonstrated after the co-administration of cisplatin and thymoquinone. The concentration of cisplatin applied to the cell lines were 0.26-4.09 µM for A2780 cell line and 1.66-26.52 µM for A2780CisR cell line and the concentration of thymoquinone were 2.28-36.49 µM for A2780 cell line and 1.93-30.83 µM for A2780CisR cell line. In the study design, sequences were determined to show the simultaneous addition of two components and one before the other. After 72 hours of incubation, a weak synergistic effect was

observed for all three sequences in the A2780 cell line, and a stronger synergistic effect was observed in the A2780CisR cell line. It was reported that bolus addition showed the least synergistic effect in both cell lines, while the sequence in which thymoquinone was added first and then cisplatin showed the highest synergistic effect.[25]

Wilson et al (2015) showed that the combination treatment of thymoquinone with cisplatin induced synergistic anti-tumor effects in cultured ID8-NGL cells (mouse ovarian cancer cells), and reduced tumor burden, proliferative and apoptotic markers in ID8-NGL-derived tumors. Thymoquinone induced cisplatin-mediated cytotoxicity in ovarian cancer cells *in vitro* and in a mouse syngeneic model, DNA damage was responsible for this effect. They concluded that thymoquinone could be a promising therapeutic agent in ovarian carcinoma.[26]

Alaufi et al. (2017) studied the potential cytotoxicity of thymoquinone, cisplatin and their combination against neck squamous cell carcinoma cells (UMSCC-14C) in contrast to normal oral epithelial cells. The IC_{50} of thymoquinone alone in UMSCC-14C cells for 24, 48 and 72 hours were 8.6 µM, 7.0 µM and 7.0 µM, respectively. Cisplatin alone was more potent cytotoxic than thymoquinone against UMSCC-14C cells. The IC_{50} of cisplatin alone in UMSCC-14C cells for 24, 48 and 72 hours were 6.2 µM, 2.8 µM and 2.6 µM, respectively. Combination of thymoquinone improved the cytotoxic effect of cisplatin against UMSCC-14C cells and reduced its IC_{50}'s to be 3.0 µM, 1.6 µM and 1.2µM after exposure for 24, 48 and 72 hours, respectively. The results revealed a dose and time dependent cytotoxic effects and decrease of the viability of UMSCC-14C oral cancer cells in response to thymoquinone treatment. Equitoxic combination of thymoquinone and cisplatin showed synergistic interaction in the cells. However, the combined cytotoxic effect of thymoquinone and CDDP was both concentration- and time-dependent.[27]

Thymoquinone has been reported to cause synergistic cytotoxicity in combination with chemotherapeutic agents such as 5-fluorouracil, doxorubicin, tamoxifen and cisplatin in the cancer cell lines such as gastric cancer cells, human cells of HL-60 leukaemia, 518A2 melanoma, HT-29 colon, KB-V1 cervix, and MCF-7 breast carcinomas as well as multi-drug-resistant variants thereof and on non-malignant human fibroblasts.[28-30]

CONCLUSION

In our study, it has been tried to clarify the subject by revealing the cytotoxicity of thymoquinone, which is one of the plant-derived phenolic compounds that are frequently taken through food, in HeLa cancer cells, its safe use and the effects of reducing or increasing cell viability in its use with cisplatin. With these findings, it is thought that the combined use of thymoquinone and cisplatin will reveal the positive or negative aspects of cancer treatment and add a new perspective to chemotherapy.

The safety of thymoquinone has supported by many clinical studies and *in vivo* and *in vitro* studies. It seems that thymoquinone may play an important role in the prevention and treatment of various human cancers, and the available data should be supported by more clinical studies. Consistent with current literature studies, our study shows that thymoquinone can increase cisplatin cytotoxicity in a dose- and time-dependent manner.

In conclusion, our findings suggest that thymoquinone can increase cisplatin cytotoxicity in HeLa cells. The increasing effect of thymoquinone on the cytotoxicity of cisplatin in HeLa cells is promising in terms of increasing the effectiveness of cisplatin in the chemotherapy of cervical carcinoma and reducing its side effects. However more comprehensive studies are needed to reveal the efficacy and therapeutic importance of thymoquinone together with cisplatin in cancer treatment.

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Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: *Songül Yılmaz, Sevtap Aydın Dilsiz;* ***Design:*** *Songül Yılmaz, Merve Becit, Sevtap Aydın Dilsiz;* ***Control/Supervision:*** *Sevtap Aydın Dilsiz;* ***Data Collection and/or Processing:*** *Songül Yılmaz, Merve Becit, Sevtap Aydın Dilsiz;* ***Analysis and/or Interpretation:*** *Songül Yılmaz, Merve Becit, Sevtap Aydın Dilsiz;* ***Literature Review:*** *Songül Yılmaz, Merve Becit;* ***Writing the Article:*** *Songül Yılmaz, Merve Becit, Sevtap Aydın Dilsiz;* ***Critical Review:*** *Songül Yılmaz, Merve Becit, Sevtap Aydın Dilsiz;* ***References and Fundings:*** *Sevtap Aydın Dilsiz;* ***Materials:*** *Sevtap Aydın Dilsiz.*

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