doi.org/10.36721/PJPS.2023.36.4.REG.1039-1046.1

Effect of thymoquinone on endoplasmic reticulum (ER) stress in NRK-52e cells

Muhammed Celebi[1], Semiha Dede[2]* and Ayşe Usta[3]
[1]Van Yuzuncu Yil University, Institute of Health Sciences, Van, Turkiye
[2]Van Yuzuncu Yil University, Faculty of Veterinary Medicine, Biochemistry Department, Van, Turkiye
[3]Van Yuzuncu Yil University, Science Faculty, Chemistry Department, Van, Turkiye

Abstract: Thymoquinone (TQ), the active component of *Nigella sativa*, has many beneficial effects. The endoplasmic reticulum involved in the quality control of protein translocation and folding can vary under different conditions, the phenomenon of causing the accumulation of unfolded or misfolded proteins within the ER lumen is termed ER stress. This *in vitro* study was planned to investigate the effect of TQ on ER stress at proliferative (Tp) and toxic (TQ_{IC50}) concentrations on NRK-52E cells at 24th, 48th hours. The expression of important genes in the ER stress pathway (ATF4, ATF6, BIP, CHOP, IRE1, XBP1, PERK) was analyzed. Expression of all genes except CHOP and XBPI increased at 24 hours and BIP at 48 hours for Tp. In the IC50, the CHOP and XBPI gene expressions increased at the 24th hour, and the CHOP and ATF4 genes increased at the 48th hour. As a result, it was determined that the expression of ER stress genes had significant changes with the TQ induction, depending on time and concentration, especially in the proliferative concentration. It is thought that TQ may have varying effects on healthy kidney cells, and it is important to investigate the mechanism of this effect in further studies.

Keywords: Thymoquinone, endoplasmic reticulum stress, kidney, *in vitro*.

INTRODUCTION

Herbal medicines have attracted attention and are being used more and more as an alternative and supplement to chemical medicines in recent years. *Nigella sativa* and its most important chemical composition, TQ, have been researched for this purpose, and many studies have been carried out. (Usta *et al*., 2018a; Usta *et al*., 2018b, Yüksek, 2021). *Nigella sativa* seeds have been used traditionally for centuries in the treatment of various diseases, such as in the Middle East, Asia, and our country. TQ, the main component of *Nigella sativa*, is a potent inducer of apoptosis in cancer cells, has an antioxidant effect, is anti-diabetic, antitumoral, and anticancerogenic, is anti-inflammatory, anti-allergic, antibacterial, antifungal, and antiparasitic (Kurt *et al*., 2014; Majdalawieh *et al*., 2017; Mollazadeh *et al*., 2017; Ullah *et al*., 2017; Usta and Dede, 2017; Goel *et al*., 2018; Ansary *et al*., 2021).

In addition, it is thought that TQ has a nephroprotective effect by improving many damages that cause nephrotoxicity due to its antioxidant, antiproliferative and proapoptotic activities (Mollazadeh *et al*., 2017; Hosseinian *et al*., 2019; Özer *et al*. 2020; Ansary *et al*., 2021).

The endoplasmic reticulum (ER) is a network system found in the cytoplasm of cells. It provides for the exchange of substances between cells and the production of substances in some regions. ER stress occurs when the balance between the protein folding capacity of the ER and the processed protein load increases in the direction of misfolded or unfolded protein. Infections, ambient temperature, oxidative stress, etc. are factors that affect the folding of proteins. These causes trigger the stress response, ER stress genes are activated, and eventually, cell death occurs. There have been studies showing that ER stress is involved in the etiology of many diseases (Manalo and Medina, 2018; Almanza *et al*., 2019; Chadwick and Lajoie, 2019; Kara and Oztas, 2019; Tatar and Tatar, 2019; Sicari *et al*., 2020; Lee and Lee, 2022).

Excessive ER stress leads to apoptosis, causing ischemic acute kidney injury and thus various kidney diseases. Therefore, approaches to reduce ER stress with various pharmacological agents have been investigated to prevent kidney damage and develop new treatment strategies (Ricciardi and Gnudi, 2020; Li and Chen, 2021; Ni *et al*., 2021).

This study was planned to investigate the time-dependent effect of TQ, which is known to have many beneficial effects, on normal kidney cells at beneficial (proliferative) and toxic (IC50) concentrations, under *in vitro* conditions. Gene expressions (ATF4, ATF6, BIP, CHOP, IRE1, PERK, XBP1) that play a role in ER stress at beneficial (proliferative) and toxic (IC50) concentrations of TQ were investigated by RT-qPCR, at 24 and 48 hours.

**Corresponding author:* e-mail: sdede@yyu.edu.tr

MATERIALS AND METHODS

Cell culture

Rat kidney epithelial NRK-52E (ATCC® CRL-1571™) cells were used as study material. Cells were cultured *in vitro* in RPMI 1640 as described previously (Korkmaz *et al*., 2022).

Cytotoxicity (MTT cell viability) test

NRK-52E cells were seeded at 7000 cells per culture plate under appropriate conditions. Cells were incubated for 24 hours at 37°C in a CO2 incubator. After incubation, the medium on the cells was removed, and TQ prepared at different concentrations using cell medium was added. TQ master stock concentration was dissolved in DMSO. It was prepared in cell medium at different final concentrations (1µM-100µM). TQ prepared at these concentrations was applied to the cells. After 24 and 48 hours, 100µl of cell medium (with 10µl of MTT solution) was added to each well and incubated. Then, MTT lysis solution (100µl) was added to each well, and the optical densities of the cells were read (570 nm). TQ IC50 and proliferation concentrations were determined according to the absorbance values obtained. While determining the percentage of cell viability, the viability of the control group was evaluated as 100%. Thus, proliferative and IC50 values of TQ at 24 and 48 hours were determined by MTT cell viability test (Korkmaz *et al*., 2022).

Preparation of study groups

The applications to be made to the study groups are summarized in table 1.

RNA extraction

Cells were centrifuged, and the medium was removed. 1ml of cold PBS (phosphate buffer solution) was added to the underlying cell lysate, and the cells were well suspended. This mixture was transferred to a new sterile tube and centrifuged at 300xg for 5 minutes, and the supernatant was discarded. By adding 1ml of cold trizol reagent to the tube, the cell was homogenized and centrifuged at 3500 rpm for 10 minutes. The supernatant collected on top was transferred to a new tube. Cold chloroform was added to new tubes, incubated, and centrifuged at 12000xg for 15 minutes.

Table 1: Study plan

	Groups	Application
1	Control (C)	Cell medium
2	TQp	Proliferative concentration
3	TQ_{IC50}	IC_{50}concentration

750000 cells per flask were seeded for the study groups. Study groups were prepared as control and experimental groups, with the crossover between these groups.

The upper clear phase was carefully removed, transferred to a new sterile tube, and mixed well by adding isopropyl alcohol. It was centrifuged at 12000 x g for 10 minutes. The supernatant was completely discarded, and the remaining RNA was washed by vortexing with 75% ethanol. It was centrifuged at 7500 x g for 5 minutes. The remaining pellet was dried in a laminar cabinet for 15 minutes, leaving the tube open. RNA was dissolved in 30-50µl of water and stored at -80°C for the cDNA step (Chomczynski and Mackey, 1995).

cDNA (Complementary DNA) synthesis

To be used for gene expression analysis in real-time PCR, cDNA synthesis from the obtained RNAs was performed according to the protocol (WizScript, Cat No. W2211, Wizbio).

Real-time polymerase chain reaction (RT-qPCR)

In this study, BIP (including immunoglobulin heavy chain-binding protein), XBP1 (X-box binding protein 1), CHOP (C/EBP [CCAAT/enhancer-binding protein]-homologous protein), PERK (PKR-like endoplasmic reticulum kinase), IRE1 (inositol-requiring 1), ATF6 (activating transcription factor 6), ATF4 (activating transcription factor 4) genes were expressed. The primer list of target genes is given in table 2. Evaluation of target gene products was performed according to the 2-ΔΔCt method (Livak and Schmittgen, 2011). Differences between groups were evaluated according to the comparison of the increase-decrease fold changes in the expression of the control gene.

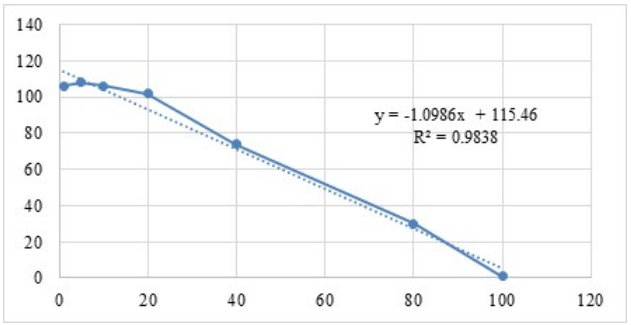

Fig. 1: MTT results (24th. 48th h)

Data analysis

CT values were exported to an Excel file. CT values, Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), were used as reference gene. The fold change was calculated using the delta delta CT data analysis method. Differences were considered significant when $p<0.05$ (Yezdani *et al.,* 2016).

Paired sample t-test was used to determine whether there was a significant difference between groups according to time and concentration. The statistical significance level was taken as 5% in the calculations (SPSS ver. 22).

Table 2: The primers

Genes	L (5' to 3')	R (5' to 3')
BIP	rn-BIP-100bp-ID25617-F	ACCAAGACATTTGCCCCAGA
	rn-BIP-100bp-ID25617-R	CAACTGCATGGGTGACCTTC
XBP1	rn-XBP1-177bp-ID289754-F	CAGCAAGTGGTGGATTTGGA
	rn-XBP1-177bp-ID289754-R	CCTTACTCCATTCCCCTTGGA
CHOP	rn-CHOP-103bp-ID29467-F	AACCTGAGGAGAGAGAAACCG
	rn-CHOP-103bp-ID29467-R	TCATACCAGGCTTCCAGCTC
PERK	rn-PERK-162bp-ID29702-F	CTGAAGGACGAAAGCACAGAC
	rn-PERK-162bp-ID29702-R	ACAGGAAATGCCCACTGAGA
IRE1	rn-IRE1-265bp-ID498013-F	TGCGCAGGTGCAATGAC
	rn-IRE1-265bp-ID498013-R	GTAAAGGGAAGTTTCGTCAGGC
ATF6	rn-ATF6-217bp-ID304962-F	AGCCCCTCATTAACACGACA
	rn-ATF6-217bp-ID304962-R	TCACTCCCAGAATTCCTACTGATG
ATF4	rn-ATF4-135bp-ID79255-F	AGACACCGGCAAGGAGGAT
	rn-ATF4-135bp-ID79255-R	AACGTGGCCAAAAGCTCATC
GAPDH	rn-GAPDH-77bp-ID24383-F	AGTGCCAGCCTCGTCTCATA
	rn-GAPDH-77bp-ID24383-R	GGTAACCAGGCGTCCGATAC

RESULTS

MTT results

TQ concentrations determined for the 24th and 48th hours as a result of the MTT test are given in table 3.

Table 3: Determined TQ concentrations

	24th hours	48th hours
TQp (μM)	10	10
TQ_{IC50} (μM)	60	60

The determination of TQ concentrations

ERS gene expression results

The Ct plot and melting curve results obtained in RT-qPCR analyses for all target genes are given in Fig. 2. These plots show that the primers specifically designed for each target gene bind appropriately, and the regions are amplified (fig. 2).

Gene expression results obtained at 24 and 48 hours at Tp and TIC50 concentrations are summarized in tables 4-7.

Table 4: Time-dependent variation of TQ_{IC50} concentration

Genes	24 (X±SD)	48 (X±SD)	P
ATF4	5.032±0.132	0.207±0.038	≤0.001
ATF6	0.593±0.030	4.517±0.343	≤0.001
BIP	0.300±0.035	0.198±0.033	≤0.005
CHOP	4.517±0.279	15.000±1.414	≤0.001
IRE1	55.333±8.042	1.028±0.091	≤0.001
PERK	0.598±0.036	0.210±0.034	≤0.001
XBP1	8.000±0.369	0.698±0.034	≤0.001

Table 5: Time-dependent variation of Tp concentration

Genes	24	48	P
ATF4	0.598±0.037	0.910±0.071	≤0.001
ATF6	0.298±0.035	12.933±0.779	≤0.001
BIP	0.503±0.035	0.987±0.046	≤0.001
CHOP	6.467±0.463	59.167±3.312	≤0.001
IRE1	2.000±0.261	1.000±0.071	≤0.001
PERK	0.2000±0.034	14.750±1.541	≤0.001
XBP1	9.167±0.931	1.517±0.136	≤0.001

Up and down regulation of gene expression was done according to the control gene. It was determined that the ATF4 gene was down-regulated 0.6 times in the TQp group and 0.2 times in the TQ_{IC50} group at the 24th hour. At 48 hours, it up regulated 5 times in the TQp group and down regulated 0.9 times in TQIC50 group.

It was determined that the ATF6 gene expression in the TQp group decreased by 0.3 at the 24th hour, and decreased by 0.6 times in the TQ_{IC50} group. At 48 hours, a 13-fold increase in the TQp group and a 4.5-times increase in TQ_{IC50} were detected.

The expression level of the BIP gene was down-regulated by 0.5 times in the TQp group and 0.3 times in the TQ_{IC50} group for the 24th hour. There was a change in the TQp (0.99) and TQ_{IC50} (0.2) groups for the 48th hour. The CHOP gene was upregulated 6.5 times in the TQp group, 4.5 times in the TQ_{IC50} group at 24 hours. There was an increase in 48 hours, 59 times in the TQp group and 15 times in the $TQ_{IC50.}$

The expression level of the IRE1 gene was 2 times up-regulated in the TQp group at the 24th hour and 55-fold at

the 48th hour. However, there was no change in the TQ_{IC50} group at the 24th and 48th hours. The PERK gene was down-regulated by 0.2-fold in the TQp group and 0.6-fold in the TQ_{IC50} group at the 24th hour. For the 48th hour, it was found to be 15 times up-regulated in the TQp group and 0.2 times up-regulated in the TQ_{IC50}.

Table 6: Difference between concentrations at 24th hour

Genes	TQp (X±SD)	TIC50 (X±SD)	P
ATF4	0.598±0.037	5.032±0.132	≤0.001
ATF6	0.298±0.035	0.593±0.030	≤0.001
BIP	0.503±0.035	0.300±0.035	≥0.05
CHOP	6.467±0.463	4.517±0.279	≤0.005
IRE1	2.000±0.261	55.333±8.042	≤0.001
PERK	0.2000±0.034	0.598±0.036	≤0.005
XBP1	9.167±0.931	8.000±0.369	≥0.05

Table 7: Difference between concentrations at 48th hour

Genes	TQp (X±SD)	TIC50 (X±SD)	P
ATF4	0.910±0.071	0.207±0.038	≤0.001
ATF6	12.933±0.779	4.517±0.343	≤0.001
BIP	0.987±0.046	0.198±0.033	≤0.005
CHOP	59.167±3.312	15.000±1.414	≤0.001
IRE1	1.000±0.071	1.028±0.091	≤0.001
PERK	14.750±1.541	0.210±0.034	≤0.001
XBP1	1.517±0.136	0.698±0.034	≤0.001

*Significant in comparison to other groups ($p \leq 0.05$). X. mean; SD. Standard deviation

The XBP1 gene was expressed 9 times more in the TQp group and 8 times more in the TQ_{IC50} group at the 24th hour. At 48 hours, it was found to be 1.5 times up-regulated in the TQp group, and 0.7 times up-regulated in TQ_{IC50}.

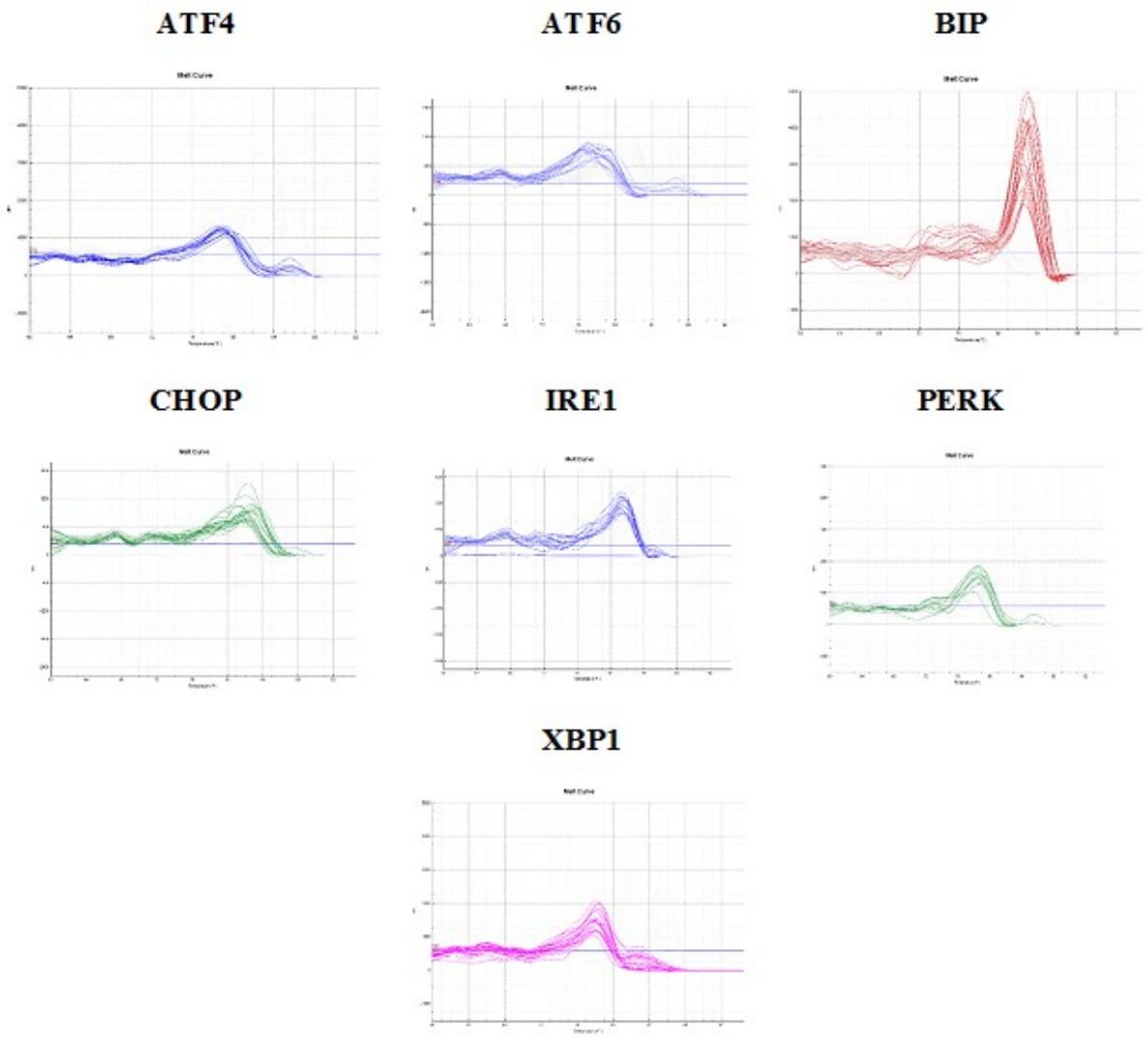

Fig. 2: Melting curve plot of the products obtained for each target gene.

DISCUSSION

TQ has been shown to affect numerous molecular and signalling pathways in many inflammatory and degenerative diseases, including cancer. There are many studies on the effect of TQ on ER stress-mediated apoptosis (Zhang *et al*., 2018; Liou *et al*., 2019; Landucci *et al*., 2021).

ER stress plays a role in the emergence of various kidney diseases. There are studies suggesting that the endoplasmic reticulum (ER) plays a role in the emergence of acute kidney injury (ARF) and, leads to unfolded protein response (UPR) or ER stress. Various kidney diseases such as inflammation, ischemia-reperfusion, genetic mutations of kidney proteins, proteinuria, diabetic nephropathy caused by cyclosporine A treatment, renal fibrosis, and kidney damage have been reported to occur due to ER stress-induced apoptosis (Taniguchi and Yoshida, 2015, Maekawa and Inagi, 2017, Yan *et al*., 2018, Mo *et al*., 2019).

ER stress-induced ATF6, PERK and, IREI signaling pathways initiate pathways that promote cell survival. In addition, in cases where ER stress is chronically prolonged, it also causes cellular dysfunction and, thus, the induction of apoptosis pathways (Sarvani *et al*., 2017; Adams *et al*., 2019).

Genes that are important in the occurrence of ER stress are UPR genes, BIP (including immunoglobulin heavy chain-binding protein), XBP1 (X-box binding protein 1), CHOP (C/EBP [CCAAT/enhancer-binding protein]-homologous protein), IRE1 (inositol-requiring 1), ATF6 (activating transcription factor 6), and ATF4 (activating transcription factor 4) (Limonta *et al*., 2019; Tatar and Tatar, 2019; Nakada *et al.,* 2021).

Induction of ER stress can be both cytoprotective and, cytotoxic by activating apoptosis in the cell (Cybulsky, 2017). In fact, the PERK-ATF4-CHOP pathway of the ER stress response is proapoptotic in some kidney diseases. Thus, the ER stress response protects against some kidney diseases. Removal of unfolded proteins by autophagy is also protective for some ER stress-induced kidney diseases (Taniguchi and Yoshida, 2015; Wei *et al*., 2021). As a matter of fact, in the present study, it was determined that CHOP and, XBPI gene expressions were significantly increased in the kidney epithelial cells of TQ at both proliferative and, IC50 concentrations at 24 hours. Other genes were found to be slightly down-regulated. On the other hand, IRE1 increased its expression 2-fold only at the proliferative concentration.

At the 48th hour, it was observed that all genes were up-regulated at the TQp concentration, and even the CHOP and IRE1 concentration increased more than 50 times. Although CHOP and ATF6 were significantly increased at TQ_{IC50} concentration, other genes were found to be slightly down-regulated.

Some genetic mutations are also considered to cause kidney diseases by inducing ER stress, protein misfolding, and disruption of protein traffic (Park *et al*., 2019). Therefore, normalization of ER stress using various pharmacological agents for the treatment of kidney diseases may be promising to prevent or arrest the progression of kidney disease (Cybulsky, 2017). This stress signal network may be a target for interventions aimed at improving CKD (Maekawa and Inagi, 2017). Compounds that can mimic ER stress inhibitors, may provide regulatory effects on ER stress-induced apoptosis. Studies have shown that some substances used as preservatives may be important as potential therapeutic agents by inhibiting ER stress (Liu *et al*., 2018; Mo *et al*., 2019; Tatar and Tatar, 2019; Jeon Gómez-Sierra *et al*., 2020).

In this study, it is thought that the time-dependent increases in TQp concentration may activate the mitochondrial pathway of apoptosis by stimulating the ER stress response in the healthy cell line. There are studies where TQ is used as a preventative against kidney disorders. It has been shown to have anti-inflammatory and antioxidant properties in animal and *in vitro* models, especially against various kidney diseases caused by inflammation and oxidative stress (Jalili *et al*., 2017; Shaterzadeh-Yazdi *et al*., 2018; Dera *et al*., 2019b; Hosseinian *et al.,* 2019; Aslan *et al*., 2020b; Hashem *et al*., 2020; Özer *et al*., 2020). The protective effects of TQ are thought to be due to increased antioxidant capacities and the mediation of the reduction of ER stress and apoptosis (Bouhlel *et al*. 2018).

Studies have been conducted to show that thymoquinone, known for its antioxidant, anti-inflammatory, and renal protective effects, may cause nephropathy. TQ increases cell viability up to a certain concentration. An increase in the causes of kidney damage (Yuksek, 201), mitochondrial damage (Stelmashook *et al.*, 2020). Oral administration of TQ to rats can cause liver and kidney damage at certain concentrations (60 mg/kg) (Kurt *et al*., 2014). Although black cumin and its bioactive components are relatively well tolerated, in some cases they can lead to oxidative stress and disrupt signaling pathways (Hannan *et al*., 2021). It has been reported that TQ has strong cytotoxicity, especially in cancer cells, and a weaker killing effect against normal cells (Alaufi *et al*, 2017).

The unfolded protein response (UPR) pathway regulates proteostasis and cell fate through the activity of the transcription factors ATF4, ATF6, and XBP1 (Yang *et al*., 2020). The increase in CHOP in kidney tissue inhibits

and protects against the inflammatory response to kidney damage. ATF4 and CHOP directly induce protein synthesis and genes involved in the UPR. However, under conditions where ATF4 and CHOP increase protein synthesis, oxidative stress and cell death may result (Bujisic *et al*., 2017; Almanza *et al.*, 2019). The fact that TQ increased 5 times at the proliferative concentration at the 48th hour in this study can be considered evidence of the TQ protective effect on kidney tissues.

In this study, the expression levels of genes activated due to ER stress were determined. Based on these results, it has been shown that ER stress occurs in normal kidney cells under normal conditions due to TQ administration. Especially at TQp concentration, activation of ER stress factors, which starts at the 24th hour, peaks at the 48th hour. TQ, which is often used for its protective and therapeutic properties, has been found to activate ER stress factors even at proliferative concentration. It was determined that the activating effect of TQ applied at the IC50 concentration on the expression of ER stress genes was more limited than the proliferative concentration, and this situation continued at the 48th hour.

It has been determined that the genes are up-regulated at the TQp concentration, i.e., while driving the cell to proliferation. It was determined that only ATF6 and CHOP genes increased while killing the cell at the TQ_{IC50} concentration. Here, it was determined that the XBP1 gene was high in the first stage for both concentrations, but decreased at 48 hours and thus behaved differently from other ER stress genes. It is noteworthy that the genes involved in ER stress are activated or decreased by different mechanisms according to TQ concentration and time.

CONCLUSION

As a result, it was determined that the stimulation of ER stress can be effective both cytoprotectively and cytotoxically by activating apoptosis in kidney cells, and TQ can provide protection against some kidney diseases by stimulating ER stress at the appropriate dose. However, it has been concluded that it can be harmful as well as beneficial. It has been observed that TQ, used with the expectation of full benefit, may have varying effects on the kidney, including ER stress, depending on time and concentration. In order to reveal the molecular basis underlying the mechanism of this effect, it is thought that it would be beneficial to carry out further studies, including new and supportive parameters and additional concentrations and times.

ACKNOWLEDGMENTS

The authors would like to thank Van Yuzuncu Yil University Scientific Research Projects Directorate for supporting the project and, Dr. Veysel YÜKSEK for their technical assistance and this research was supported by Van Yuzuncu Yil University Scientific Research Projects Directorate as project number TYL-2019-8502.

REFERENCES

Adams CJ, Kopp MC, Larburu N, Nowak PR and Ali MMU (2019). Structure and molecular mechanism of ER stress signaling by the unfolded protein response signal activator IRE1. *Front MolBiosci.* **6**: 11.

Almanza A, Carlesso A, Chintha C, Creedican S, Doultsinos D, Leuzzi B, Luís A, McCarthy N, Montibeller L, More S, Papaioannou A, Püschel F, Sassano ML, Skoko J, Agostinis P, de Belleroche J, Eriksson LA, Fulda S, Gorman AM, Healy S, Kozlov A, Muñoz-Pinedo C, Rehm M, Chevet E and Samali A (2019). Endoplasmic reticulum stress signalling-from basic mechanisms to clinical applications. *FEBS J.* **286**(2): 241-78.

Ansary J, Giampieri F, Forbes-Hernandez TY, Regolo L, Quinzi D, Gracia Villar S, Garcia Villena E, Tutusaus Pifarre K, Alvarez-Suarez JM, Battino M and Cianciosi D (2021). Nutritional value and preventive role of *Nigella sativa* L. and its main component thymoquinone in cancer: an evidenced-based review of preclinical and clinical studies. *Molecules,* **26**(8): 2108.

Aslan M, Kırımlıoğlu E, Afşar E, Çeker T and Yılmaz Ç (2020b). Increased PUFA levels in kidney epithelial cells in the course of diclofenac toxicity. *Toxicol. In vitro*. **66**: 104836.

Bouhlel A, Bejaoui M, Ben Mosbah I, Hadj Abdallah N, Ribault C, Viel R, Hentati H, Corlu A and Ben Abdennebi H (2018). Thymoquinone protects rat liver after partial hepatectomy under ischaemia/ reperfusion through oxidative stress and endoplasmic reticulum stress prevention. *Clin. Exp. Pharmacol Physiol*. **7**: 12961.

Chadwick SR and Lajoie P (2019). Endoplasmic reticulum stress coping mechanisms and lifespan regulation in health and diseases. *Front. Cell Dev. Biol.,* **7**: 84.

Chomczynski P and Mackey K (1995) Modification of the TRI reagent procedure for isolation of RNA from polysaccharide- and proteoglycan-rich sources. *Biotechniques,* **19**(6): 942-945

Cybulsky AV. Endoplasmic reticulum stress, the unfolded protein response andautophagy in kidney diseases. *Nat Rev Nephrol*. 2017; 13(11):681-96.

Dera A and Rajagopalan P (2019). Thymoquinone attenuates phosphorylation of AKT to inhibit kidney cancer cell proliferation. *J. Food Biochem*., **43**(4): e12793.

Goel S and Mishra P (2018). Thymoquinone inhibits biofilm formation and has selective antibacterial activity due to ROS generation. *Appl. Microbiol. Biotechnol*., **102**(4): 1955-1967.

Gómez-Sierra T, Medina-Campos ON, Solano JD, Ibarra-Rubio ME and Pedraza-Chaverri J (2020). Isoliquiritigenin pretreatment induces endoplasmic reticulum stress-mediated hormesis and attenuates cisplatin-induced oxidative stress and damage in LLC-PK1 cells. *Molecules,* **25**(19): 4442.

Hannan MA, Zahan MS, Sarker PP, Moni A, Ha Hand Uddin MJ (2021). Protective effects of black cumin (*Nigella sativa*) and its bioactive constituent, thymoquinone against kidney injury: An aspect on pharmacological insights. *Int. J. Mol. Sci*., **22**(16): 9078.

Hosseinian S, Shahraki S, Ebrahimzadeh Bideskan A, Shafei MN, Sadeghnia HR, Soukhtanloo M, Rahmani F and Khajavi Rad A (2019). Thymoquinone alleviates renal interstitial fibrosis and kidneydys function in rats with unilateral ureteral obstruction. *Phytother. Res*., **33**(8): 2023-33.

Jalili C, Salahshoor MR, Hoseini M, Roshankhah S, Sohrabi M and Shabanizadeh A (2017). Protective effect of thymoquinone against morphine injuries to kidneys of mice. *Iran J. Kidney Dis*., **11**(2): 142-150.

Kara M and Oztas E (2019). Endoplasmic reticulum stress-mediated cell death. *In*: Gali-Muhtasib H and Rahal ON (Eds.), Programmed Cell Death. *IntechOpen*. https://doi.org/10.5772/intechopen.85401.

Korkmaz R, Yüksek V and Dede S (2022). The effects of sodium fluoride (NaF) treatment on the PI3K/Akt signal pathway in NRK-52E cells. *Biol. Trace Elem. Res.,* **200**: 3294-3302.

Kurt E, Dede S and Ragbetli C (2015). The investigations of total antioxidant status and biochemical serum profile in thymoquinone-treated rats. *Afr. J. Trad. Complement. Altern. Med*., **12**(2): 68-72.

Landucci E, Mazzantini C, Buonvicino D, Pellegrini-Giampietro DE and Bergonzi MC (2021). Neuroprotective effects of thymoquinone by the modulation of ER stress and apoptotic pathway in *in vitro* model of excitotoxicity. *Molecules,* **26**(6): 1592.

Lee JH and Lee J (2022). Endoplasmic reticulum (ER) stress and its role in pancreatic β-cell dysfunction and senescence in type 2 diabetes. *Int. J. Mol. Sci*., **23**(9): 4843.

Li C and Chen YM (2021). Endoplasmic reticulum-associated biomarkers for molecular phenotyping of rare kidney disease. *Int. J. Mol. Sci*., **22**(4): 2161.

Limonta P, Moretti RM, Marzagalli M, Fontana F, Raimondi M and MontagnaniMarelli M (2019). Role of endoplasmic reticulum stress in the anticancer activity of natural compounds. *Int. J. Mole. Sci*., **20**(4): 961.

Liou YF, Chen PN, Chu SC, Kao SH, Chang YZ, Hsieh YS and Chang HR (2019). Thymoquinone suppresses the proliferation of renal cell carcinoma cells via reactive oxygen species-induced apoptosis and reduces cell stemness. *Environ. Toxicol*., **34**(4): 1208-1220.

Liu CM, Yang HX, Ma JQ, Yang W, Feng ZJ, Sun JM, Cheng C, Li J and Jiang H (2018). Role of AMPK pathway in lead-induced endoplasmic reticulum stress in kidney and in paeonol-induced protection in mice. *Food Chem. Toxicol*., **122**: 87-94.

Livak KJ and Schmittgen TD (2011) Analysis of relative gene expression data using real-time quantitative PCR and the 2−ΔΔCT method. *Methods,* **25**(4): 402-408.

Maekawa H and Inagi R (2017). Stress signal network between hypoxia and er stress in chronic kidney disease. *Front Physiol*., **8**: 74.

Majdalawieh AF, Fayyad MW and Nasrallah GK (2017). Anti-cancer properties and mechanisms ofaction of thymoquinone, the major active ingredient of *Nigella sativa*. *Crit. Rev. Food SciNutr.,* **57**(18): 3911-3928.

Manalo RVM and Medina PMB (2018). The endoplasmic reticulum stress response in disease pathogenesis and pathophysiology. *Egyptian J. Med. Human Gen*., **19**(2): 59-68.

Mo JS, Choi D, Han YR, Kim N and Jeong HS (2019). Morin has protective potential against ERstress induced apoptosis in renal proximal tubular HK-2 cells. *Biomed. Pharmacother.,* **112**: 108659.

Mollazadeh H, Afshari AR and Hosseinzadeh H (2017). Review on the potential therapeutic roles of *Nigella sativa* in the treatment of patients with cancer: involvement of apoptosis: Black cumin and cancer. *J. Pharmacopuncture,* **20**(3): 158-172.

Nakada EM, Sun R, Fujii U and Martin JG (2021). The impact of endoplasmic reticulum-associated protein modifications, folding and degradation on lung structure and function. *Front Physiol*., **12**: 665622.

Ni L, Yuan C and Wu X (2021). Endoplasmic reticulum stress in diabetic nephrology: Regulation, pathological role, and therapeutic potential. *Oxid. Med. Cell Longev*., **2021**:7277966.

Özer MK, Bilgic S, Armagan I and Savran M (2020). Thymoquinone protection from amikacin induced renal injury in rats. *Biotech. Histochem*., **95**(2): 129-136.

Park SJ, Kim Y and Chen YM (2019). Endoplasmic reticulum stress and monogenic kidney diseases in precision nephrology. *Pediatr. Nephrol*., **34**(9): 1493-500.

Ricciardi CA and Gnudi L (2020). The endoplasmic reticulum stress and the unfolded protein response in kidney disease: Implications for vascular growth factors. *J. Cell Mol. Med*., **24**(22): 12910-12919.

Sarvani C, Sireesh D and Ramkumar KM (2017). Unraveling the role of ER stress inhibitors in the context of metabolic diseases. *Pharmacol. Res*., **119**: 412-21.

Shaterzadeh-Yazdi H, Noorbakhsh MF, Samarghandian S and Farkhondeh T (2018). An overview on renoprotective effects of thymoquinone. *Kidney Dis.,* (Basel). **4**(2): 74-82.

Sicari D, Delaunay-Moisan A, Combettes L, Chevet E and Igbaria A (2020). A guide to assessing

endoplasmic reticulum homeostasis and stress in mammalian systems. *FEBS J*., **287**(1): 27-42.

Stelmashook EV, Chetverikov NS, Golyshev SA, Genrikhs EEandIsaev NK (2020). Thymoquinone induces mitochondrial damage and death of cerebellar granule neurons. *Biochemistry (Mosc)*. **85**(2): 205-212.

Taniguchi H and Yoshida H (2015). Endoplasmic reticulum stress in kidney function and disease. *Curr. Opin. Nephrol. Hypertens,* **24**(4): 345-350.

Tatar M and Tatar T (2019). Endoplasmic reticulum stress and related diseases. *Osmangazi J. Med*., **41**(3): 294-303.

Ullah R, Rehman A, Zafeer MF, Rehman L, Khan YA, Khan MH and Abidi SMA (2017). Anthelmintic potential of thymoquinone and curcumin on *Fasciola gigantica*. *PloS One,* **12**(2): e0171267.

Usta A, Dede S and Çetin S (2018a). The effect of thymoquinone treatment on total oxidant and total antioxidant level in experimental diabetic rats. *Atatürk Üniv. Vet. Bil. Derg*., **13**(1): 84-91.

UstaA, Dede S and Yoruk IH (2018b). The effect of thymoquinone on serum antioxidant vitamin levels in diabetic rats. *Turk J. Vet. Res*., **2**(1): 26-33.

Usta A and Dede S (2017). The effect of thymoquinone on nuclear factor kappa B levels and oxidative DNA damage on experimental diabetic rats. *Pharmacog. Mag*., **13**(3): S458.

Wei XM, Jiang S, Li SS, Sun YS, Wang SH, Liu WC, Wang Z, Wang YP, Zhang R and Li W (2021). Endoplasmic reticulum stress-activated PERK-eIF2α-ATF4 signaling pathway is involved in the ameliorative effects of ginseng polysaccharides against cisplatin-induced nephrotoxicity in mice. *ACS Omega*, **6**(13): 8958-8966.

Wang D, Zou Y, Yu S, Lin S, Li H, Yin Y, Qiu L, Xu T and Wu J (2018). Activation of aldehyde dehydrogenase 2 slows down the progression of atherosclerosis via attenuation of ER stress and apoptosis in smooth muscle cells. *Acta Pharmacol. Sinica*., **39**(1): 48-58.

Yuksek V (2021). Activation of PI3K/AKT/mTOR pathway thymoquinone-induced in NRK-52E cell line. *J. Institute Sci. Technol*., **11**(1): 68-74.

Zhang M, Du H, Huang Z, Zhang P, Yue Y, Wang W, Liu W, Zeng J, Ma J, Chen G, Wang X and Fan J (2018). Thymoquinone induces apoptosis in bladder cancer cell via endoplasmic reticulum stress-dependent mitochondrial pathway. *Chem. Biol. Interact*., **292**: 65-75.