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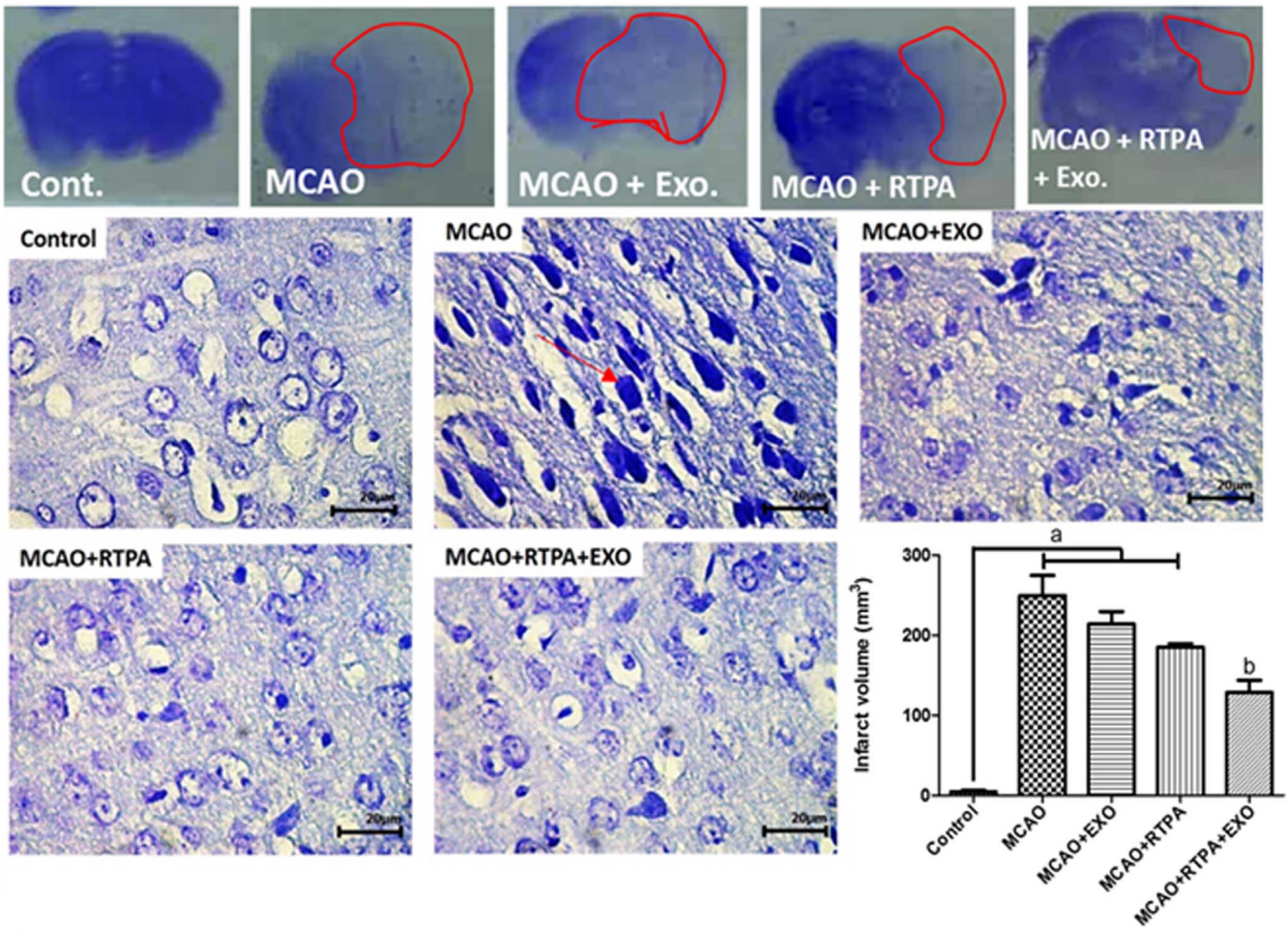

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AIM AND SCOPES
Journal of Cellular Neuroscience and Oxidative Stress is an online journal that publishes original research articles, reviews and short reviews on the molecular basis of biophysical, physiological and pharmacological processes that regulate cellular function, and the control or alteration of these processes by the action of receptors, neurotransmitters, second messengers, cation, anions, drugs or disease.

Areas of particular interest are four topics. They are;

A- Ion Channels (Na^+- K^+ Channels, Cl^- channels, Ca^{2+} channels, ADP-Ribose and metabolism of NAD^+, Patch-Clamp applications)

B- Oxidative Stress (Antioxidant vitamins, antioxidant enzymes, metabolism of nitric oxide, oxidative stress, biophysics, biochemistry and physiology of free oxygen radicals)

C- Interaction Between Oxidative Stress and Ion Channels in Neuroscience
(Effects of the oxidative stress on the activation of the voltage sensitive cation channels, effect of ADP-Ribose and NAD^+ on activation of the cation channels which are sensitive to voltage, effect of the oxidative stress on activation of the TRP channels in neurodegenerative diseases such Parkinson's and Alzheimer's diseases)

D- Gene and Oxidative Stress
(Gene abnormalities. Interaction between gene and free radicals. Gene anomalies and iron. Role of radiation and cancer on gene polymorphism)

READERSHIP

Biophysics	Biochemistry
Biology	Biomedical Engineering
Pharmacology	PhysiologyGenetics
Cardiology	Neurology
Oncology	Psychiatry
Neuroscience	Neuropharmacology

Keywords
Ion channels, cell biochemistry, biophysics, calcium signaling, cellular function, cellular physiology, metabolism, apoptosis, lipid peroxidation, nitric oxide, ageing, antioxidants, neuropathy, traumatic brain injury, pain, spinal cord injury, Alzheimer's Disease, Parkinson's Disease.

RESEARCH ARTICLE

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The treatment of thymoquinone increased expression levels of apoptotic and oxidative genes in the NRK-52E rat kidney epithelial cell line

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List of Abbreviations;

CASP3, *caspase 3;* ***CASP8,*** *caspase 8;* ***CASP9,*** *caspase 9;* ***Bax,*** *Bcl-2 associated X;* ***Bcl-2****, Bcl-2 apoptosis regulator;* ***GPX1,*** *glutathione peroxidase 1;* ***SOD1,*** *superoxide dismutase 1;* ***NCF1,*** *neutrophil cytosolic factor 1;* ***ROS,*** *reactive oxigene species;* ***MDA,*** *malondialdehyde;* ***TQ,*** *Thymoquinone;* ***MTT,*** *3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide assay;* ***TQ_P,*** *TQ proliferative concentration;* ***TQ_{IC50},*** *TQ IC50 concentration;* ***RT-qPCR,*** *reverse transcription quantitative real-time PCR*

Abstract

This study was aimed to show the effects of Thymoquinone (TQ), the active ingredient of Nigella sativa, on apoptotic and oxidative pathways in kidney cells, depending on concentration and time. For this purpose, the NRK-52E rat kidney epithelial cell line was used.

To determine the IC50 and proliferative values of TQ, the MTT cell viability test was performed at 24 and 48 hours. The proliferative (TQP-10μM) and toxic (TQIC50-60 μM) concentrations were determined. The expression of apoptotic (caspase 3 (CASP3), caspase 8 (CASP8), caspase 9 (CASP9), Bax (Bcl-2 associated X), Bcl-2 (Bcl-2 apoptosis regulator)) genes and oxidative (glutathione peroxidase 1(GPX1)), superoxide dismutase 1 (SOD1), neutrophil cytosolic factor 1 (NCF1)) genes were determined as concentration and time-dependent, in the samples taken by RT-qPCR at the concentrations and times dependent.

According to the results of this analysis, GPX1 gene was significantly up-regulated in Tp compared to the control gene at 24 hours, SOD1 gene and NCF1 gene did not change in TIC50. Apoptotic genes were found to be limited up-regulated at both concentrations at 24 hours. At 48 hours, GPX1 was up-regulated at TQP concentration and SOD1 at TQIC50 concentration. The NCF1 gene was significantly expressed at TPIC50 at only 48 hours. Apoptotic genes were limitedly upregulated at TQP concentration at 48 hours, while significant upregulation of all apoptotic genes was found at TQIC50 concentration.

In conclusion, GPx increased significantly at Tp at the 24th hour, SOD1 at TQIC50 concentration, and NCF gene increased significantly at the toxic concentration at the 48th hour. It was revealed that the effect of the apoptotic pathway on TQ-dependent cell death was limited at the 24th hour, while apoptotic cell death at toxic concentrations occurred externally at the 48th hour.

Keywords: Apoptotic gene expression; Kidney cells; Oxidative gene expression; Thymoquinone

Introduction

In addition to the many beneficial effects of thymoquinone (TQ), it is also recommended as a preventive and therapeutic in many diseases. These effects of TQ have been investigated in many experimental in vivo and in vitro studies and it is the major compound of N. sativa seeds. The molecular mechanisms of these beneficial effects of TQ are the most important subject of these studies. Signaling pathways in cellular metabolism provide important data about cellular metabolism. Apoptotic and oxidative pathways are important cellular mechanisms and their relationship with many important diseases has been demonstrated. Genes involved in apoptotic and oxidative pathways are important in cell metabolism and, accordingly, in the etiology of many diseases (Chu et al., 2014; Ayuob et al., 2020; Guo et al., 2020; Alkis et al., 2021).

TQ is known to improve the negative effects of oxidative stress and increase blood antioxidant capacity and antioxidant enzyme activity. *N. sativa* and TQ reduce oxidative stress by a mechanism that includes des up-regulation of antioxidant enzymes and molecules such as glutathione peroxidase (GPX), superoxide dismutase (SOD), glutathione reductase (GR), catalase (CAT) reduced glutathione (GSH), followed by a decrease in reactive oxygen species (ROS) and malondialdehyde (MDA) levels (Rastad et al., 2016; Kuzay, 2019; Hannan et al., 2021b).

It has been reported that TQ has a dual effect in which it can act as both a pro-oxidant and an antioxidant in a concentration-dependent manner, acting as an antioxidant at low concentrations, while having pro-oxidant properties at higher concentrations (Mahmoud and Abdelrazek, 2019).

TQ antioxidant property can be converted to thymohydroquinone, which has cytoprotective properties inside the cell. In cancer cells, the pro-oxidant property of TQ occurs in the presence of metal ions, including copper and iron, which induce the conversion of TQ to semiquinone. This leads to the generation of reactive oxygen species (ROS), which causes DNA damage and induction of cellular apoptosis (Mahmoud and Abdelrazek, 2019; Chae et al., 2020).

This study was planned with the aim of demonstrating the effects of TQ on apoptotic and oxidative pathways in kidney cells, depending on concentration and time, of which many beneficial effects have been revealed by scientific studies in recent years, and its effect on the main genes related to apoptotic and oxidative pathways was investigated.

Materials and methods

Cell culture

NRK-52E (ATCC® CRL-1571™) rat kidney epithelial cell line was used as material in the study. NRK-52E cells obtained from our cell culture unit were systematically passaged in an appropriate medium *in vitro* condition (RPMI 1640 medium containing 10% FBS, 1% L-Glutamine, 1% penicillin/streptomycin, 5%CO_2, and 95% humidity, incubation at 37ºC).

The determination of toxic concentration and incubation time of TQ by using the cell viability (MTT) test

It was aimed to create TQ-integrated cytotoxicity with TQ incubation at different intensities and different times in the NRK-52E cell line. TQ master stock concentration dissolved in DMSO (DMSO ≤0.05) for MTT cell viability test for detection of TQ_{IC50} other at 24- and 48-hours final concentrations 1 µM, 5 µM, 10 µM, 20 µM, 30 µM, 40 µM, 50µM, 60 µM, 70 µM, 80 µM, 90µM, 100

µM were prepared by diluting in 12 different concentrations of cell medium.

In 96-well culture plates, 4 wells for each different concentration and 7,000 cells were seeded in each well.

The optical densities of the cells were determined in the ELISA device at a wavelength of 570 nm. According to the detected absorbance values, the IC50 and proliferation concentrations of TQ were determined and the percentages of viability were determined (Figure 1).750000 cells were seeded in each flask in the study groups. Study groups were set up to cross between control and experimental groups and these groups.

RNA extraction

The procedures after this step continued on a cold medium. The cells belonging to the groups were lifted with trypsin and collected. After washing the cells with cold PBS, mRNA was obtained using the TRIzol reagent protocol (Chomczynski and Mackey 1995). Measurement was made in a nanodrop to determine purity and quantity.

Complementary DNA (cDNA) Isolation

For using the obtained mRNAs in expression analysis in RT-qPCR, cDNA synthesis was carried out. cDNA was obtained by using the protocol recommended by the Wizbio brand kit (WizScript, cat. no: W2211).

Quantitative Real-time Polymerase Chain Reaction (RT-qPCR) Analysis

In this study, the expression of oxidative stress pathway (GPX1, SOD1, NCF1) and apoptotic pathway (CASP3, CASP8, CASP9, Bax, Bcl-2) genes were investigated.

GPX1 belongs to the glutathione peroxidase family, which catalyzes the reduction of hydroperoxides and hydrogen peroxide (H_2O_2) by glutathione, thereby protecting cells against oxidative damage (Anonymous 1). SOD1 (superoxide dismutase 1) is responsible for destroying free superoxide radicals (Anonymous 2). NCF1 (neutrophil cytosolic factor 1), this oxidase is a multicomponent enzyme that is activated to produce superoxide anion (Anonymous 3).

CASP3 plays a central role in the executive phase of apoptosis (Anonymous 4). CASP8, CASP9, sequential activation of caspases plays a central role in the executive phase of cell apoptosis (Anonymous 5, Anonymous 6). Bax functions as anti- or pro-apoptotic regulators involved in cellular activities. The relationship and ratio of Bax to Bcl-2 also determine the survival or death of a cell following an apoptotic stimulus (Anonymous 7). Bcl-2 blocks the apoptotic death of some cells (Anonymous 8).

GAPDH (glyceraldehyde-3-phosphate dehydro genase) was used as the control gene. One Ct (cycle threshold) was determined as the beginning of the logarithmic phase of the amplifications.

The forward (F) and reverse (R) primers of the target genes were obtained from NCBI (www.ncbi.nlm.nih.gov/refseq/) and Primer3web version 4.1.0 (http://primer3.ut.ee/). The RT-qPCR reactions of target genes were optimized before the study. The primary gene sequences for each gene are given in *Table 1*. The genes were designed and purchased (Atlas Biyoteknoloji, Ankara, Turkiye).

Table 1. *Primary gene sequences for each gene*

GEN	FORWARD (5'-3')	REVERSE (5'-3')
GPX1	TCCACCGGTATGCCTTCTC	TCTCTTCATTCTTGCCATTCTCC
SOD1	GCTTCTGTCGTCTCCTTGCT	CATGCTCGCCTTCAGTTAATCC
NCF1	GTCGGAGAAGGTGGTCTACAG	CGATAGGTCTGAAGGATGATGG
Bax	TGCTACAGGGTTTCATCCAG	ATCCACATCAGCAATCATCC
Bcl-2	ACCAGAATCAAGTGTTCGTC	TCTTCATCTCCAGTATCCCACTC
CASP3	CGAAACTCTTCATCATTCAGG	GAGCATTGACACAATACACGG
CASP8	GATGTCCTGGTGCTATTTCAGAG	CCTCCTTGTCCATGTCTTCTG
CASP9	TCTCACACCAGAAACACCCA	GTCGTTCTTCACCTCCACCA
GAPDH	AACCCATCACCATCTTCCAG	GCCATCCACAGTCTTCTGAG

At the RT-qPCR stage, SYBR green master mix (WizPure, Cat. No: w1711) was used in the study. The reaction contents were the same for both the target and control (housekeeping) genes. The stages of the method were followed based on the protocol of this kit as summarized.

The target genes' products were assessed with the method $2^{-\Delta\Delta Ct}$ (Livak and Schmittgen, 2011). Intergroup differences were assessed by comparison to the increase–decrease fold changes of the expression of the control gene.

Statistical Analysis

Descriptive statistics of the discussed properties are presented as median, mean, standard deviation, minimum, and maximum. To determine whether or not there was a significant difference among the groups based on these properties, Kruskal–Wallis test was used. Dunnet multiple comparisons test was used to determine the source of the difference. In the calculations, the level of statistical significance was taken as 5%, and the SPSS (ver. 22) statistical package software was used for the analyses.

Results

MTT Results

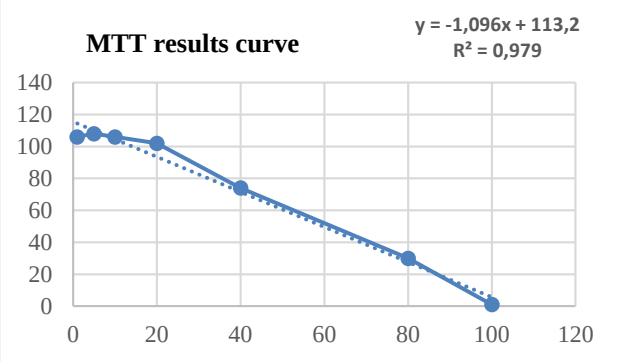

Figure 1*. MTT viability test column and slope plot obtained as a result of TQadministered at different concentrations on the NRK-52E cell line*

MTT cell viability test was performed to determine the proliferative and IC50 values of TQ at the 24th and 48th hours. Proliferative (TQP-10µM)) and toxic (TQIC50-60µM) concentrations were detected (Figure 1).

Gene Expression Results

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

Table 2. *The oxidative gene expressions at 24t^{h} hour.*

Genes	TQ_P	TQ_{IC50}	P
GPX1	7.41±0.87	1.59±0.28	0.004
SOD1	1.31±0.34	5.87±0.43	0.000
NCF1	1.39±0.14	1.74±0.27	0.389

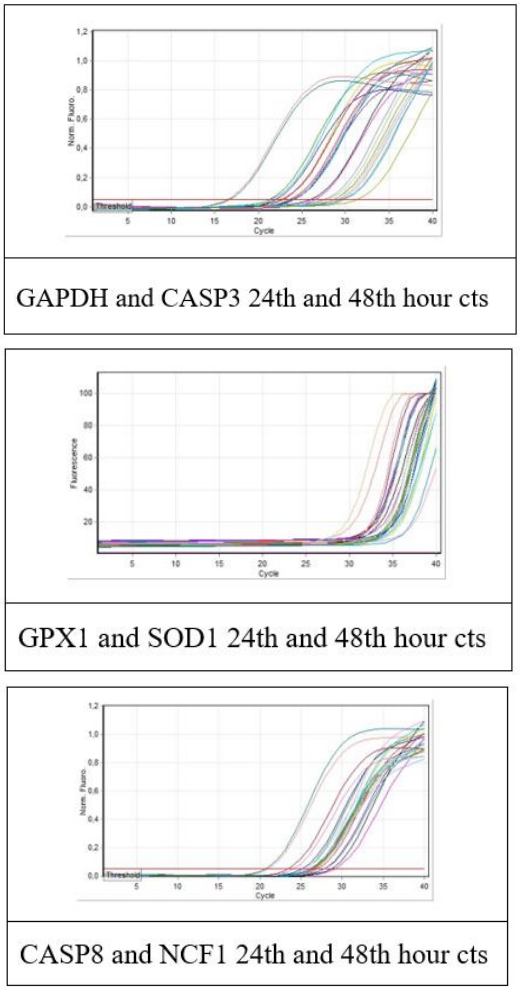

Figure 2. *Melting curve plot of the products obtained for each target gene*

Compared with the control gene, GPX1 was 7.41-fold up-regulated at 24 hours at the TQ proliferative concentration, while TQIC50 was only 1.5-fold up-regulated at the concentration. SOD1 was up-regulated 1.313 times at the TQ proliferative concentration and 5.873 times at the TQIC50 concentration. The NCF1 gene was up-regular 1.397 and 1.740 at 24 hours at both concentrations. In proliferative concentration-applied cells, the oxidative NCF1 gene did not change depending on the concentration at the 24th hour ($p \geq 0.05$). Gpx1 was less up-regulated and SOD1 was more up-regulated at high concentrations.

Table 3. *Apoptotic gene expressions at 24t^{h} hours*

Genes	TQ_P	TQ_{IC50}	P
Bax	1.09±0.13	1.61±0.13	0.058
Bcl-2	3.87±0.52	1.65±0.11	0.012
Bax/Bcl-2	0.28	0.98	0.000
CASP3	1.06±0.04	2.08±0.42	0.062
CASP8	0.77±0.06	1.64±0.39	0.051
CASP9	1.17±0.15	1.65±0.08	0.049

Compared with the control gene, all apoptotic genes were found to be slightly up-regulated at high concentrations The Bax/Bcl-2ratio increased significantly at the toxic concentration. CASP8 was less down-regulated at the TQ_P concentration. Apart from this, in cells treated with proliferative and IC50 concentration TQ, the apoptotic pathway gene expressions were concentration-dependent at 24 hours. It was observed that they were up-regulated by 1.05-3.87 times. The concentration-related changes of the genes were found to be statistically significant ($p \leq 0.05$) for the Bcl-2, CAP8, and CASP9 genes.

Table 4. *Oxidative gene expressions at 48th hour*

Genes	TQ_P	TQ_{IC}	P
GPX1	7.22±0.98	1.58±0.25	0.006
SOD1	0.03±0.01	3.62±0.63	0.002
NCF1	1.68±0.23	5.45±0.47	0.002

Compared to the control gene, GPX1 was 7,215-fold up-regulated at 48 hours at the TQ proliferative concentration, while TQIC50 was 1.578-fold up-regulated at the concentration. While SOD1 was 0.03-fold down-regulated at the TQ proliferative concentration, it was up-regulated by 3.623 at the TQIC50 concentration. The NCF1 gene was found to be up-regulated at a rate of 1.677 and 5.452 times at each TQP concentration. In proliferative and IC50-concentration TQ-treated cells, gene expressions affecting the oxidative system were concentration-dependently decreased at the 48th hour, while GPX1 decreased, while SOD and NCF1 genes were significantly up-regulated ($p \leq 0.05$).

Table 5*. The apoptotic gene expressions at 48th hour*

Genes	TQ_P	TQ_{IC}	P
Bax	1.95±0.06	10.83±0.34	0.000
Bcl-2	2.06±0.36	5.31±0.50	0.001
Bax/Bcl-2	0.56	2.04	0.000
CASP3	1.62±0.31	11.33±0.86	0.000
CASP8	1.03±0.15	10.29±0.69	0.000
CASP9	1.46±0.31	3.01±0.12	0.005

When compared with the control gene, Bax was nearly 2 times and other genes were 1.033-2.06 times up-regulated at the TQ_P concentration at the 48th hour. It was observed that all genes were significantly up-regulated at the TQ_{IC50} concentration compared to the proliferative concentration ($p \leq 0.05$).

Table 6. *Time dependent gene expressions at TQ_P concentration*

	GPX1	SOD1	NCF1	Bcl-2	Bax	CASP3	CASP8	CASP9
TQ_P24	7.41±0.87	1.31±0.34	1.39±0.14	3.87±0.52	1.09±0.13	1.06±0.04	0.77±0.06	1.17±0.15
TQ_P48	7.22±0.98	0.03±0.01	1.67±0.23	2.06±0.36	1.95±0.06	1.62±0.31	1.033±0.15	1.46±0.31
p	0.907	0.012	0.413	0.009	0.005	0.106	0.142	0.460

Table 7. *Time dependent gene expressions at TQ_{IC50} concentration*

	GPX1	SOD1	NCF1	Bcl-2	Bax	CASP3	CASP8	CASP9
TQIC24	1.59±0.28	5.87±0.43	1.74±0.27	1.65±0.11	1.61±0.13	2.08±0.42	1.64±0.39	1.65±0.08
TQIC48	1.58±0.25	3.62±0.63	5.45±0.47	5.31±0.50	10.83±0.34	11.33±0.86	10.29±0.69	3.01±0.12
P	0.972	0.036	0.003	0.001	0.000	0.000	0.000	0.001

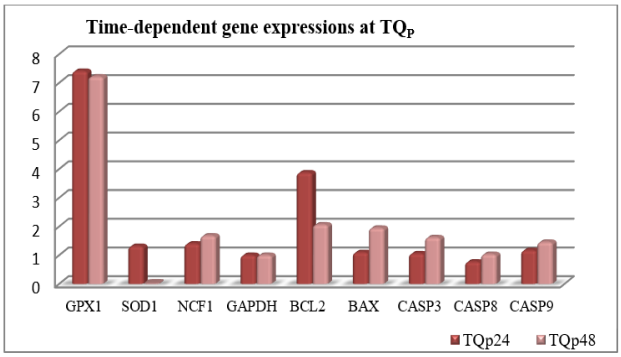

Figure 3. *Graph of time-dependent gene expressions at TQ_P concentration*

At 24 and 48 hours of proliferative TQ concentration, SOD and Bcl-2 expressions decreased significantly ($p \leq 0.05$), while other gene expressions did not change depending on time (Table 6, Figure 3).

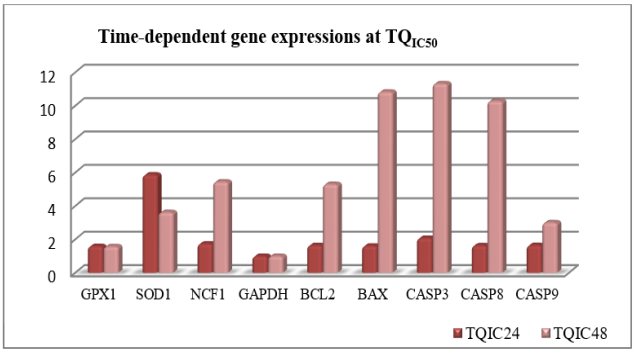

Figure 4. *Graph of time dependent gene expressions at TQ_{IC50} concentarion*

TQ_{IC50} concentration was found to be effective in all genes except GPX1. While SOD1 decreased significantly, all other genes were found to increase significantly at the 48th hour ($p \leq 0.01$) (Table 7, Figure 4).

Discussion

As a phytochemical compound, TQ has a variety of biological effects, including antioxidant, antibacterial, antineoplastic, nephroprotective, hepatoprotective, gastroprotective, neuroprotective, anti-nociceptive, and anti-inflammatory activities (Guo et al., 2020; Hannan et al., 2021a; Hannan et al., 2021b; Talebi et al., 2021). In studies investigating the molecular and clinical mechanisms underlying the kidney protective potential of black seed and TQ, its regulatory roles in antioxidant defense system, NF-κB signaling, caspase pathways and TGF-β, antioxidant, anti-inflammation, anti-apoptosis and antifibrosis. In the current study, it was aimed to investigate the mechanism of beneficial and concentration-dependent toxic effects of TQ on kidney cells. Using the NRK-52 epithelial kidney cell line and applying TQ at various concentrations and times, its effect on the genes involved in the apoptotic and oxidative pathways was determined at the gene expression stage. In addition to elucidating the mechanisms of the beneficial effects of TQ, the possibilities of the results obtained here were also investigated to provide useful information in detecting the destructive mechanisms that may occur due to TQ, especially in the kidney tissue.

Oxidative stress and inflammation play a role in the pathogenesis and progression of kidney diseases. TQ 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. It has also been documented that TQ protects kidney tissue by significantly reducing the side effect of nephrotoxicity associated with various drugs (Shaterzadeh-Yazdi et al.,2018; Oskouei et al., 2018; El-Shemi et al., 2018; Mabrouk, 2018; Abdel-Daim et al., 2020; Dera et al., 2020; Hannan et al., 2021b).

When the expression levels of the genes involved in the oxidant-antioxidant system were compared with the control gene, according to these results, it was determined that the antioxidant genes SOD and GPX genes and NCF1 gene expressions, which is a multicomponent oxidase enzyme activated to produce superoxide anion, were up-regulated at different rates at both concentrations and hours, depending on concentration and time, after TQ administration. The increase in oxidative stress at the 24th hour could be prevented by the effect of concentration-dependent up-regulated antioxidant genes. At 48 hours, the result of increased oxidative stress at the toxic concentration at the 48th hour. Although the SOD gene was relatively low at this concentration compared to the TQ_P concentration, it was concluded that its up-regulation was due to the use of SOD against increased oxidative stress.

It has been shown that TQ treatment reduces oxidative stress markers (superoxide, hydrogen peroxide, and nitric oxide) by promoting antioxidant enzymes and therefore reduces oxidative stress. SOD, CAT, and GSH levels down-regulate pro-oxidant genes and up-regulate antioxidant genes (Hannan et al., 2021a). In a study where

it was reported that TQ had beneficial effects as well as harmful effects on the kidneys depending on the concentration and duration of administration, it was determined that TQ increased cell viability up to a certain concentration and then caused cytotoxicity (Yuksek, 2021).

It has also been reported that long-term administration of TQ alone causes liver toxicity, but does not affect organ function at a tolerable concentration (Ahmad et al., 2019). In another study; it was concluded that after 60 mg/kg TQ administration, this concentration especially affects biochemical liver and kidney function parameters and may be harmful (Kurt et al. 2015). N. sativa, the main source of TQ, has been shown to reduce the development of kidney failure when given prior to the use of nephrotoxic drugs. However, a case of acute renal failure that developed after the use of N. sativa in an individual with diabetes was also presented (Arslan et al. 2013).

In a study on the toxicity of TQ, the LD50 values given in intraperitoneal injection and oral gavages in mice were 10-15 times and 100-150 times higher than the reported TQ concentrations for its anti-inflammatory, antioxidant, and anti-cancer effects, especially in experimental animals. It was reported to be a relatively safe compound when given orally (Al-Ali et al., 2008).

TQ administration increases antioxidant capacity in the liver, kidney, and brain tissues in a concentration-dependent manner. It exerts beneficial effects as an antioxidant, anti-inflammatory, anti-apoptosis, and anti-necrosis agent through the inhibition of growth factors, biochemical and oxidative stress markers, and regulation of gene expression (Oskouei et al., 2018; Abdel-Daim et al., 2020). It has been reported that TQ can protect against toxicity caused by some substances excreted by the kidneys with its antioxidant and anti-apoptotic effects (Sener et al., 2016; Jalili et al., 2017; Mabrouk, 2018).

TQ has also been found to have anticancer properties and anti-proliferative effects on kidney cancer cells (Dera and Rajagopalan, 2019). TQ exerts anticancer effects by inducing apoptosis by regulating the expression of proapoptotic and anti-apoptotic genes and increasing intracellular ROS production (Chae et al., 2020; Almajali et al., 2021). It has been reported that TQ is effective in inhibiting different cancer stages, by inducing apoptosis, regulating the levels of pro- and anti-apoptotic genes, decreasing ERK1/2 activities, and thus inhibiting metastasis (Imran et al., 2018).

TQ has beneficial effects by triggering ROS and superoxide production and activation of the apoptotic and autophagic cascade (Liou et al., 2019). It is known that TQ can protect against toxicity caused by some substances excreted by the kidneys with its antioxidant and anti-apoptotic effects (Jalili et al., 2017), and it shows its anti-apoptotic properties against kidney damage by preventing the formation of apoptotic cells (Şener et al., 2016).

In comparison with the control gene, anti-apoptotic and apoptotic genes examined in this study were found to be affected by TQ application according to concentration and time. Significant activation of apoptotic pathways was observed at 48 hours at toxic concentrations. It was concluded that the Bax/Bcl-2 ratio increased at the toxic concentration and the CAS3 and CAS8 genes expressions were also activated, the internal pathway was not sufficiently effective, and TQ used the external pathway in cell death at toxic concentrations. Antiapoptotic Bcl-2 gene expression was also increased. According to these results, it was determined that the receptor pathway was more effective than the mitochondrial pathway at the toxic concentration at the 48th hour.

It is reported that TQ administration inhibits nephrotoxicity induced by different chemicals by reducing oxidative damage, apoptosis, and inflammation. It also has a protective effect against kidney damage caused by reperfusion by inhibiting apoptosis and cell proliferation (Hannan et al., 2021a; Hannan et al., 2021b).

As a result, it was determined that GPx at TQ_P concentration and SOD1 at TQ_{IC50} concentration were significantly upregulated at 24 hours, NCF gene did not change significantly and oxidative stress could be prevented. Oxidative stress increased at the toxic concentration at 48 hours and SOD was used as an antioxidant. It was concluded that there was generally no significant change in apoptotic genes at the 24th hour and that TQ at proliferative concentration had antiapoptotic properties. The apoptotic pathways were activated at the toxic concentration at the 48th hour, the internal apoptotic pathway was not efficient enough, and TQ used the external pathway in cell death at toxic concentrations.

It was concluded that the obtained data will be useful in determining the relationship between increasing concentration in healthy kidney cells and TQ, which decreases cell viability over time, and the oxidant and

apoptotic system. For this, it was thought that gene expression analysis, post-translational, and activity levels should also be determined and this was important in elucidating the mechanism of action of TQ.

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Author contribution

Conceptualization, methodology: S.D., R.S.K., Funding acquisition: S.D., Experimental procedures: R.S.K., S.D., A.U. All authors wrote, read and approved the final version of the manuscript.

Conflicts of interest

The authors have no conflicts of interest to declare that are relevant to the content of this article.

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