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Evaluation of the Effect of *Silybum marianum* (L.) Seed Extract on PI3K/Akt/mTOR Pathway and DNA Repair in NRK-52E Cell Line

Silybum Marianum (L.) Tohum Ekstraktının NRK-52E Hücre Serisinde PI3K/Akt/mTOR Yolağına ve DNA Tamirine Etkisinin Değerlendirilmesi

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ABSTRACT

Objective: *Silybum marianum* extract (SME), rich in silymarin and its derivatives, exhibits protective effects against cellular injury. This study aimed to investigate the protective role of SME against H_2O_2-induced genotoxicity and its regulatory effect on the PI3K/Akt/mTOR pathway in rat kidney (NRK-52E) cells, which are sensitive to oxidative stress

Material and Method: Experimental groups were designed as Control, Serum Starvation (SS), SME, H_2O_2 (HP), and SME+HP. SME and H_2O_2 concentrations were determined by MTT assay. Genomic damage was evaluated using the comet assay, and gene expression levels were analyzed by RT-Qpcr.

Results: The proliferative concentration of SME was 25 µg/ml, and the cytotoxic concentration (IC25) of H_2O_2 was 30 µM. HP treatment significantly increased genomic damage compared to the control ($p<0.05$), whereas SME pretreatment markedly reduced this damage ($p<0.05$). Expression levels of TP53 and PARP-1 decreased in the HP group but were upregulated in the SME+HP group. Similarly, PI3K/Akt/mTOR pathway gene expression decreased in the HP group and approached control levels following SME pretreatment.

Conclusion: H_2O_2 at cytotoxic levels induces genomic damage and suppresses PI3K/Akt/mTOR pathway and DNA repair gene expression. SME effectively mitigates oxidative stress–induced genotoxicity and restores normal signaling activity, suggesting its potential as a cellular protective agent. Further in vivo and clinical studies are warranted to validate these findings.

Keywords: *Silybum marianum, silymarin; oxidative stress, genotoxicity, PI3K/Akt/mTOR pathway, NRK-52E*

ÖZET

Giriş: Silimarin ve türevleri açısından zengin olan *Silybum marianum* ekstresi (SME), hücresel hasara karşı koruyucu etkiler göstermektedir. Bu çalışma, oksidatif strese duyarlı olan sıçan böbrek (NRK-52E) hücrelerinde SME'nin H_2O_2 ile indüklenen genotoksisiteye karşı koruyucu rolünü ve PI3K/Akt/mTOR yolağı üzerindeki düzenleyici etkisini araştırmayı amaçlamıştır

Materyal ve Metot: Deney grupları Kontrol, Serum Açlığı (SS), SME, H_2O_2 (HP) ve SME+HP olarak tasarlanmıştır. SME ve H_2O_2 konsantrasyonları MTT testi ile belirlenmiştir. Genomik hasar komet testi ile değerlendirilmiş, gen ekspresyon düzeyleri ise RT-qPCR ile analiz edilmiştir.

Bulgular: SME'nin proliferatif konsantrasyonu 25 µg/ml, H_2O_2'nin sitotoksik konsantrasyonu (IC25) ise 30 µM olarak belirlenmiştir. HP uygulaması kontrol grubuna kıyasla genomik hasarı anlamlı ölçüde artırırken ($p<0,05$), SME ön uygulaması bu hasarı belirgin biçimde azaltmıştır ($p<0,05$). TP53 ve PARP-1 ekspresyon düzeyleri HP grubunda azalmış; ancak SME+HP grubunda yukarı regüle olmuştur. Benzer şekilde, PI3K/Akt/mTOR yolağı gen ekspresyonu HP grubunda azalmış ve SME ön uygulamasının ardından kontrol düzeylerine yaklaşmıştır.

Sonuç: Sitotoksik düzeylerdeki H_2O_2, genomik hasarı indüklemekte ve PI3K/Akt/mTOR yolağı ile DNA onarım genlerinin ekspresyonunu baskılamaktadır. SME, oksidatif stres kaynaklı genotoksisiteyi etkili biçimde hafifletmekte ve normal sinyal aktivitesini yeniden sağlamakta olup hücresel koruyucu bir ajan olarak potansiyel taşıdığını göstermektedir. Bu bulguların doğrulanması için ileri in vivo ve klinik çalışmalara ihtiyaç vardır.

Anahtar kelimeler: *Silybum marianum; silimarin; oksidatif stres; genotoksisite; PI3K/Akt/mTOR yolağı; NRK-52E*

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INTRODUCTION

As a critical mediator of redox sensing, signaling, and homeostasis, the excessive accumulation of hydrogen peroxide can lead to oxidative stress, which in turn may cause mutations in genetic material and impair intracellular signaling pathways (Sies, 2017; Xin et al., 2022). This condition can underlie the development of apoptosis, cancer, neurodegenerative diseases, and chronic inflammatory processes (Ivanov et al., 2024; Checa and Aran, 2020). In particular, the PI3K/AKT/mTOR signaling pathway plays a crucial role in cell proliferation, survival, protein synthesis, and metabolic balance; disruptions in this pathway disrupt the physiological balance of cells and pave the way for various pathologies (Tewari et al., 2022). Moreover, the regulation of DNA repair genes such as TP53 and PARP-1 plays a critical role in the cellular response to oxidative damage (Pazzaglia and Pioli, 2019). Plant-derived natural antioxidants have attracted considerable attention due to their potential to mitigate cellular damage. In this context, *Silybum marianum* (L.), commonly known as milk thistle, is a medicinal plant rich in flavonolignans (Maaloul et al., 2024). Its active component, silymarin, and its derivatives silibinin, silychristin, and silydianin have been shown to exhibit not only hepatoprotective properties but also significant antioxidant and anti-inflammatory effects, protecting against various toxins, particularly in the liver (Gruenwald et al., 2004). According to the literature, silymarin has been reported to activate cellular antioxidant defense mechanisms, regulate protein expression, and reduce cellular damage (Surai, 2015; Iraqi et al., 2025). This study evaluated the regulatory role of *Silybum marianum* extract in mitigating disruptions to the PI3K/AKT/mTOR signaling pathway and genotoxic damage induced by hydrogen peroxide (H_2O_2). The findings demonstrate that *Silybum marianum* exerts protective effects against cellular and genotoxic stress, positively influencing gene expression by modulating intracellular mechanisms.

MATERIAL AND METHOD

Preparation of *Silybum marianum* Seed Extract 100 g of *Silybum marianum* seeds were washed, dried, ground into powder, and extracted with 70% methanol at 100°C using a magnetic stirrer with heating for 24 hours in a dark environment at room temperature. After filtration through Whatman paper, the methanol was gently evaporated at 40°C using a rotary evaporator. The remaining aqueous residue was aliquoted (5 ml each) into 10 ml tubes, frozen at –80°C, and lyophilized at –55°C. The dry extract was stored at –20°C for use in cell culture (Asaker, 2011)

Cell Experiment Validation

Cell Culture

The study materials comprised NRK-52E cell line (ATCC® CRL-3435™), passage number. NRK-52E (Normal Rat Kidney epithelial) cell line used in this study was obtained commercially from the American Type Culture Collection (ATCC). Cells were used at passage between passages 12-20 for all experiments. The identity and quality of the NRK-52E cells were confirmed prior to use. Morphological examination was performed routinely to verify characteristic epithelial cell morphology. Screening for potential microbial or cross-contaminations was also conducted, and no contamination was detected. Since the cells were acquired from an internationally recognized cell bank that performs cell line authentication, and were maintained in our laboratory for less than six months following receipt, no further re-authentication was required according to journal guidelines. The cells were cultured in vitro in a medium (DMEM high glucose, Cat-No: 11965092) containing, 10 % fetal bovine serum (Biological Industries, Certified FBS), 1 % penicillin/streptomycin (Capricorn, Cat-No: PSG-B), 1% L-glutamine(Capricorn, Cat-No:GLN-B), at 37 ◦C, 95 % humidity, 5 % CO2. For MTT, 10^5 cells per well were seeded in plates (96 wells). For the study groups, 10^6 cells were seeded per flask (25 cm^2). The cells were allowed to adhere to the surface overnight. At the end of 24 hours, plant was treated to the cells according to the MTT result and the concentrations in the study groups.

MTT Assay

In cell culture studies, IC_{25} (quarter maximal inhibitory concentration), i.e. the cytotoxic concentration that inhibits the proliferation of 25% of the cells, is used to determine the active substance or extract with the best potential. *Silybum marianum* extract dissolved in the stock

solution DMSO (Dimethyl sulfoxide). Final concentrations, SME(0, 25, 50, 75, 100, 125, 150, 175, 200, 225 µg/ml) that DMSO ratio to have nontoxic (≤0.005) effect and hydrogen peroxide (0 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 µM) were prepared by dilution with medium. The concentrations were applied to the cells at 24 h. MTT viability test was performed at the end of the determination. Proliferative concentration . of SME and, IC_{25} of HP value were determined. The study was conducted in five groups: a control group (control), a Serum Starvation (SS) group, a Hydrogen Peroxide-IC_{25} (HP) group, a *Silybum marianum* Extract (SME) group, and an incubation with both SME and HP (SME+HP) group, as shown in Table 1. Cell lines without extract application were considered as the control group.

Table 1. Study groups

No	Group	Medium	SME	HP	
1	Control	+	-	-	The group that was subjected to 8 hours of starvation followed by 6 hours of incubation in normal medium
2	Serum Starvation (SS)	+	-	-	The group that was starved for 8 hours in FBS-free medium
3	Hydrogen Peroxide (HP)	+	-	+	The group that was starved for 8 hours and then cultured for 6 hours in normal medium supplemented with HP to induce DNA damage.
4	Silybum Marianum Extract (SME)	+	+	-	The group that was starved for 8 hours and then cultured for 6 hours in normal medium supplemented with SM
5	SME+HP	+	+	+	The group subjected to 8 hours of starvation, followed by 2 hours of incubation in normal medium with SME, and then 6 hours of incubation with both SME and HP

Comet Assay

The cells collected in the study were applied on gel-covered slides according to the Comet protocol and smeared. The prepared slides were run with the electrophoresis method. The prepared slides were examined under a fluorescent microscope to investigate DNA damage and the tail moment it created. After this, 6 images were taken to include all area of the slides. In these images, DNA damage (%) and tail moment (%) were calculated using ImageJ software (Yılmaz et al., 2021).

RNA extraction and cDNA isolation

Following the administration, the cells were detached from the surface of the flask through the utilization of the trypsinization technique. The cells that were gathered were subjected to centrifugation in order to eliminate the supernatant. Subsequently, the cells were rinsed with sterile phosphate-buffered saline (PBS) and subjected to another round of centrifugation to remove the supernatant. The total RNA was extracted from the cells using the Trizol Reagent-chloroform (GeneAll, 301-001) technique. The quantification and assessment of the integrity of the acquired messenger RNAs (mRNAs) were conducted using a spectrophotometer and agarose gel electrophoresis. Following the completion of accurate measurement, the process of cDNA synthesis was carried out using the High-Capacity cDNA Reverse Transcription isolation kit (Applied Biosystems™ Cat: 4368814, Lithuania).

Real Time-qPCR analysis

The mRNA transcription levels of the target genes were assessed by utilizing the complementary DNAs (cDNAs) acquired. The optimal conditions for primer design for each gene were found. The reverse transcription quantitative polymerase chain reaction (RT-qPCR) was conducted using the ROTOR-GENE Q instrument manufactured by Qiagen in Germany. To ascertain the patterns of gene expression, the transcription levels of several key genes involved in the PI3K/Akt/mTOR pathway (ERBB2, AKT1, PI3K and mTOR), the TP53 and PARP-1 genes, a marker for DNA damage, were assessed. Actin Beta (ACTB) was used as a control gene in expression

analysis. SYBR Green master mix (ENZO Life Science cat: ENZ-NUC104–0200) was used for amplification detection in the study. The group differences were compared with the number of fold changes of control expression. Using ACTB as an internal reference, the following procedure was employed to perform a two-step PCR amplification: 95 °C for 5 min (1 cycle), 95 °C for 20 s, 60 °C (Binding temperature varied according to primers) for 60 s (40 cycles), and stored at 4 °C. Each sample was repeated in 6 independent replicates, with one ct (cycle threshold) determined at the beginning of the logarithmic phase of the amplifications. In expression analysis, the products of genes were determined by $2^{-\Delta\Delta Ct}$ values (Livak and Schmittgen, 2001). The necessary primers are listed in Table 2.

Table 2. Primary sequence of target genes.

Gen names	F (5′-3′)	R (5′-3′)
ERBB2	ATGCTCATCGCTCACAACCA	AACTCCTCCCTTCAGGATCTC
AKT1	CTCCTCAAGAATGATGGCACC	ACTCAAACTCGTTCATGGTC
PI3K	GGAGAACTATGAACAACCTGTG	CATCTTCCAGTAACGTAGGCAG
TP53	CTGGACGACAGGCAGACTTT	GTCCCGTCCCAGAAGATTCC
PARP-1	ACCAGCAGCAAGTACCATCT	TTCCTTTCGGCTGGGATTCT
ACTB	CTCCTCAAGGATGGCACC	GCTCATTGTAGAAAGTGTGGT
mTOR	GCCATTGCCAGCCTCATTG	GAAAGTGTCCCCTGCCATTG

Statistical analysis

The statistical analyses were conducted using the SPSS version 22.0 software package. Data normality was assessed using the Shapiro–Wilk test, and all variables were found to be normally distributed ($p>0.05$). Accordingly, parametric tests were used for all analyses. Variance across multiple groups was evaluated using one-way ANOVA followed by a post-hoc Bonferroni test. All parameters are reported as mean ± standard deviation. Statistical significance was considered at $p < 0.05$.

RESULTS

SME promoted proliferation in NRK-52E cells

SME was treated to the cell line at different concentrations for 24 h. According to the MTT assay results, cell viability showed an approximately 15% increase at 25 μg/mL compared to the control group. At concentrations of 50, 75, 100, 125, and 150 μg/mL, no significant change in viability was observed relative to the control. However, starting from 175 μg/mL, cell viability began to decrease in a concentration-dependent manner. At the highest concentration (350 μg/mL), cell viability was reduced by up to 64%, as shown in Fig. 1.

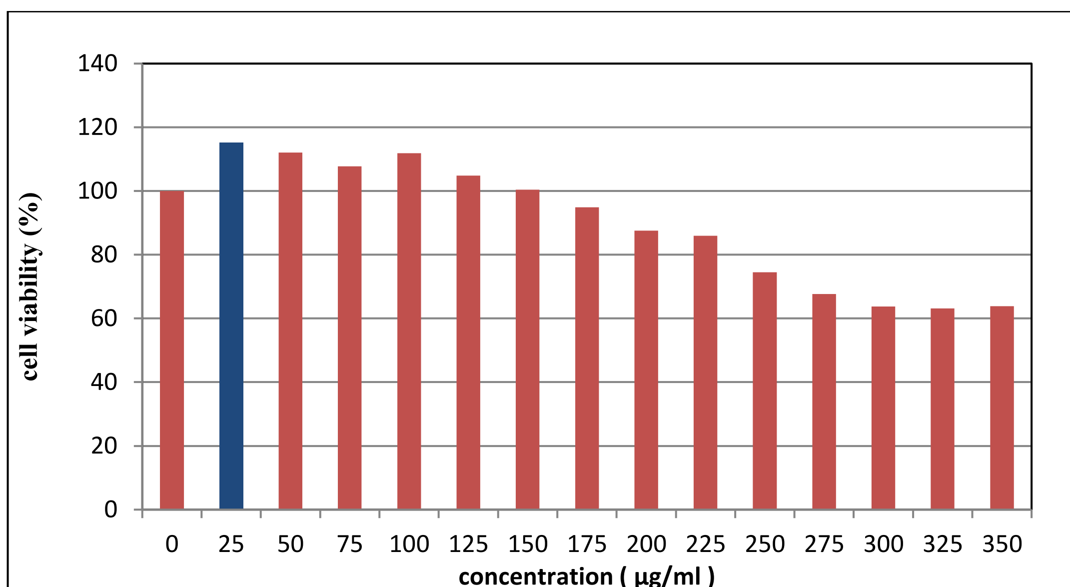

Figure 1 Cell viability test to SME: MTT Assay as a result of the administration of different concentrations of SME in the NRK-52E cell line at the 24th hour. The dark blue bar represents the proliferative concentration of SME, which is highlighted separately to distinguish it from the other concentration groups.

SME prevented the cytotoxicity of HP HP was administered to the cells at various concentrations, both alone and in combination with 25 µg/ml SME, for 24 hours. Based on the MTT assay results, cell viability decreased in a dose-dependent manner with increasing HP concentrations. The IC_{25}, indicating a 25% reduction in cell viability, was calculated to be 30 µM. Co-treatment with SME effectively reduced HP-induced cytotoxicity at concentrations of 10, 20, and 30 µM. However, at higher concentrations, SME did not demonstrate a protective effect, as shown in Fig. 2. The images of the study groups also demonstrate a parallel pattern in cell density, similar to the MTT results, as shown in Fig. 3
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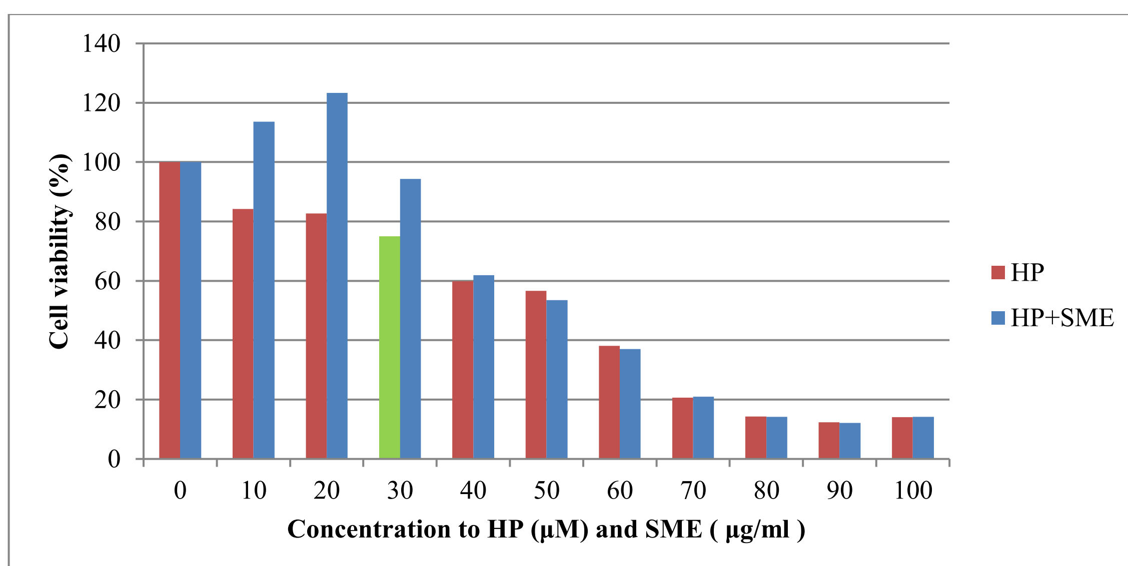

Figure 2 Cell viability test to HP and HP+SME: MTT Assay as a result of the administration of different concentrations of HP and treated 25 µg/ml SME in the NRK-52E cell line at the 24th hour. The green bar represents the IC_{25} concentration of hydrogen peroxide (H_2O_2), which is highlighted separately to distinguish it from the other concentrations.

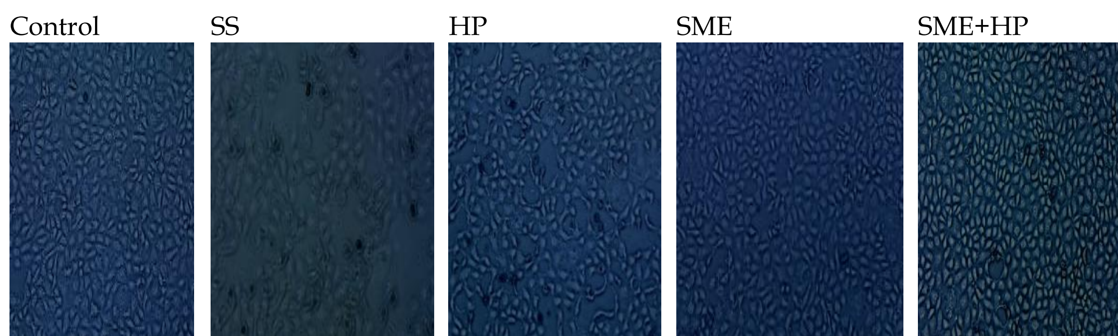

Figure 3. Representative microscope images (4× magnification) of the study groups: control, serum starvation (SS), hydrogen peroxide-IC_{25} (HP), *Silybum marianum* extract (SME), and combined treatment (SME+HP).

SME reversed H_2O_2-induced suppression of PI3K/AKT/mTOR pathway genes in NRK-52E cells

A significant downregulation ($p < 0.05$) of ERBB2 mRNA expression was observed in the HP group relative to the control group. A statistically significant difference was also observed between the SS and control groups, although the magnitude of this difference was relatively modest. In contrast, ERBB2 expression in the SME group was significantly higher than in the HP group ($p < 0.05$), with no significant difference observed relative to the control group. PI3K mRNA expression levels were significantly downregulated in both the SS and HP groups compared to the control group ($p < 0.05$). The SME group showed a significant upregulation of PI3K expression compared to both the control and HP groups ($p < 0.05$). Additionally, PI3K expression in the SME+HP group was significantly increased compared to the HP group ($p < 0.05$). No significant differences in AKT1 mRNA expression levels were observed among the control, SS, and HP groups. However, the SME group exhibited significantly higher AKT1 expression compared to both the control and HP groups ($p < 0.05$). Similarly, AKT1 expression in the SME+HP group was also significantly elevated relative to both the HP and control groups ($p < 0.05$). In the evaluation of mTOR gene mRNA expression levels, a statistically significant decrease was observed in the SS and HP groups compared to the control group ($p < 0.05$). In contrast, mTOR expression was significantly upregulated in the SME group relative to the control group ($p < 0.05$). Furthermore, the SME+HP group exhibited a significant increase in mTOR expression compared to the HP group alone ($p < 0.05$), as show in Fig. 4.

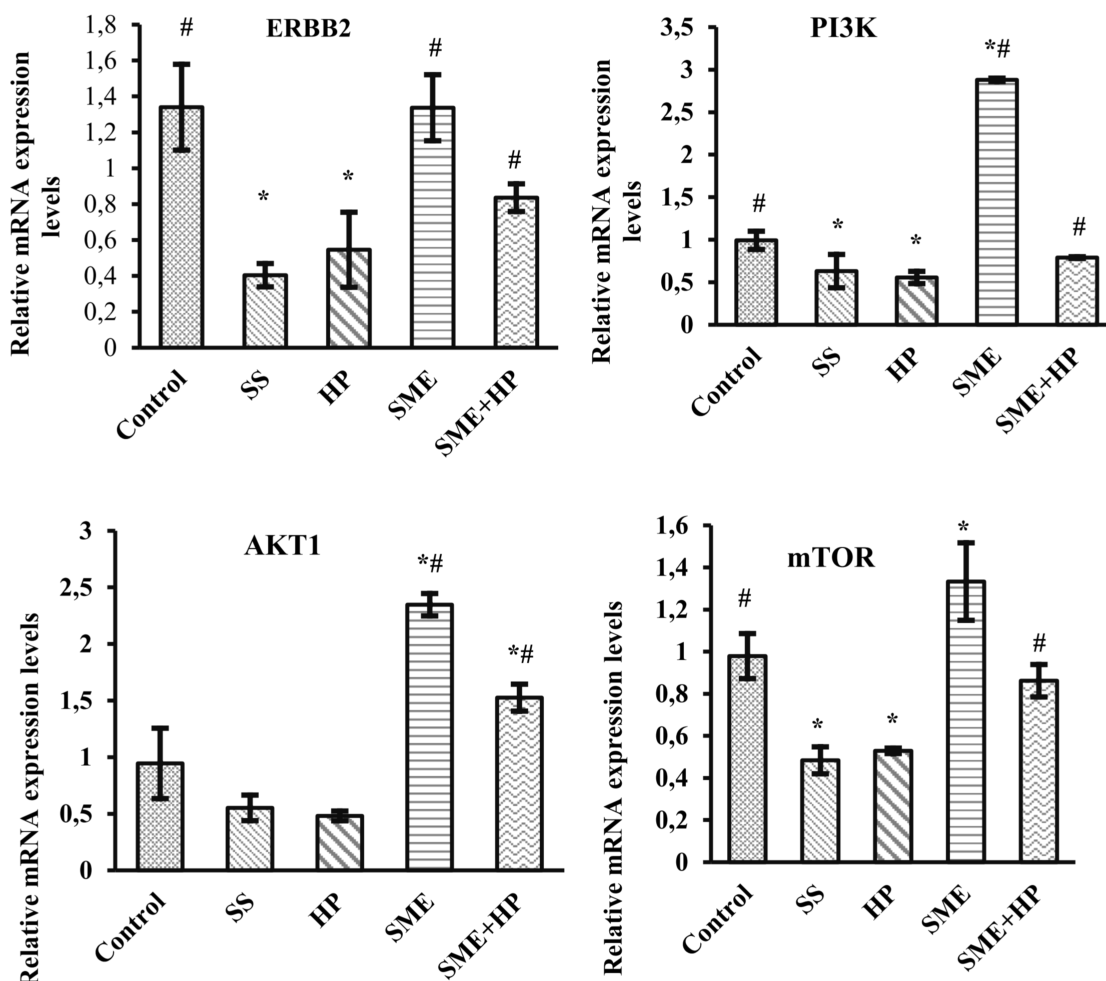

Figure 4 The mRNA transcript levels of ERBB2, PI3K, AKT1 and mTOR in the NRK-52E cell line: Three independent experiments were performed and data represented mean ± standard deviation. * indicates a significant difference (p<0.05) compared to the control group. # indicates a significant difference (p<0.05) compared to the HP group.

SME reversed H_2O_2-induced changes in TP53 and PARP-1 expression

TP53 mRNA levels exhibited a non-significant decrease in the SS and HP groups compared to the control group. In contrast, a statistically significant increase was observed in the SME+HP group relative to both the control and HP groups ($p < 0.05$). PARP-1 mRNA expression was significantly reduced in both the SS and HP groups compared to the control group ($p < 0.05$). Although the SME group also exhibited a significant decrease relative to the control group, its expression levels were significantly higher than those of the HP group ($p < 0.05$). Similarly, the SME+HP group showed a significant reduction in PARP-1 mRNA expression compared to the control group, yet its levels remained significantly higher than those observed in the HP group ($p <0.05$), as show in Fig. 5.

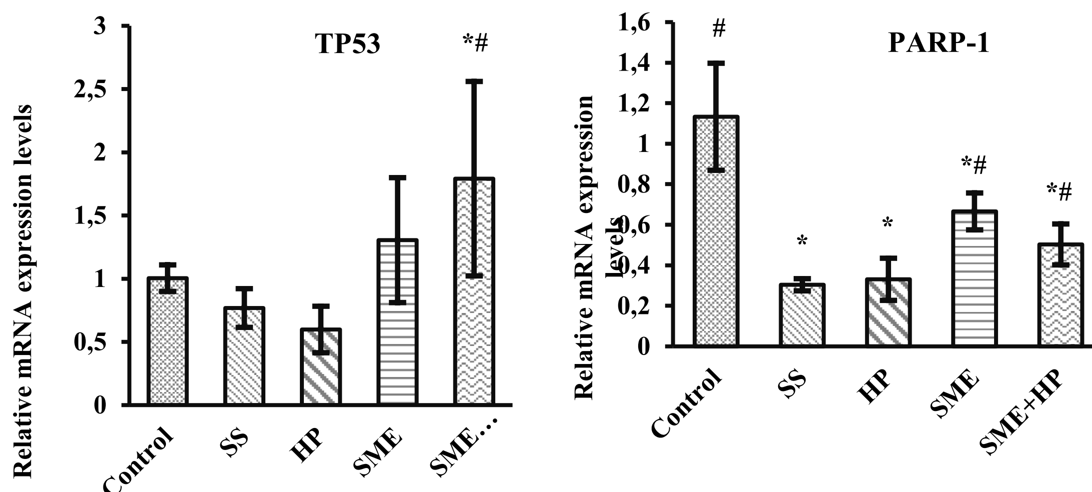

Figure 5 The mRNA transcript levels of TP53 and PARP-1 in the NRK-52E cell line: Three independent experiments were performed and data represented mean ± standard deviation. * indicates a significant difference (p<0.05) compared to the control group. # indicates a significant difference (p<0.05) compared to the HP group.

SME modulated genotoxic effects of H_2O_2 in NRK-52E cells

Figure 6 summarizes the statistical analysis of DNA damage indicators, including DNA damage and tail moment, measured with the Comet assay. The experimental groups displayed statistically significant differences in both DNA damage and tail moment values ($p < 0.05$). In particular, both parameters differed significantly between the control and HP groups ($p < 0.05$). Researchers also detected similar differences between the SA and HP groups, as well as between the SME and HP groups ($p < 0.05$). Treatment with both SME and HP produced statistically significant differences compared to the control and HP groups ($p < 0.05$). Notably, DNA damage in the SME+HP group was lower than in the HP-only group (Fig.6). DNA damage was assessed in all experimental groups, as shown in Fig. 7.

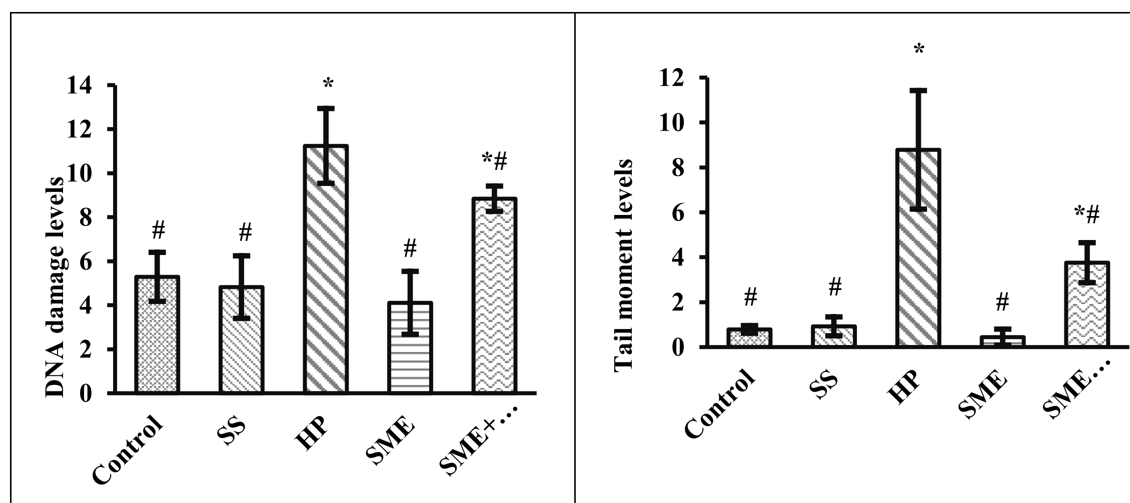

Figure 6. DNA damage and tail moment levels in the NRK-52E cell line at the 24th hour: Indicatest he measurement value of 6 cell images randomly selected on the slides from different zones were performed and data represented mean ± standard deviation. * indicates a significant difference (p<0.05) compared to the control group. # indicates a significant difference (p<0.05) compared to the HP group.

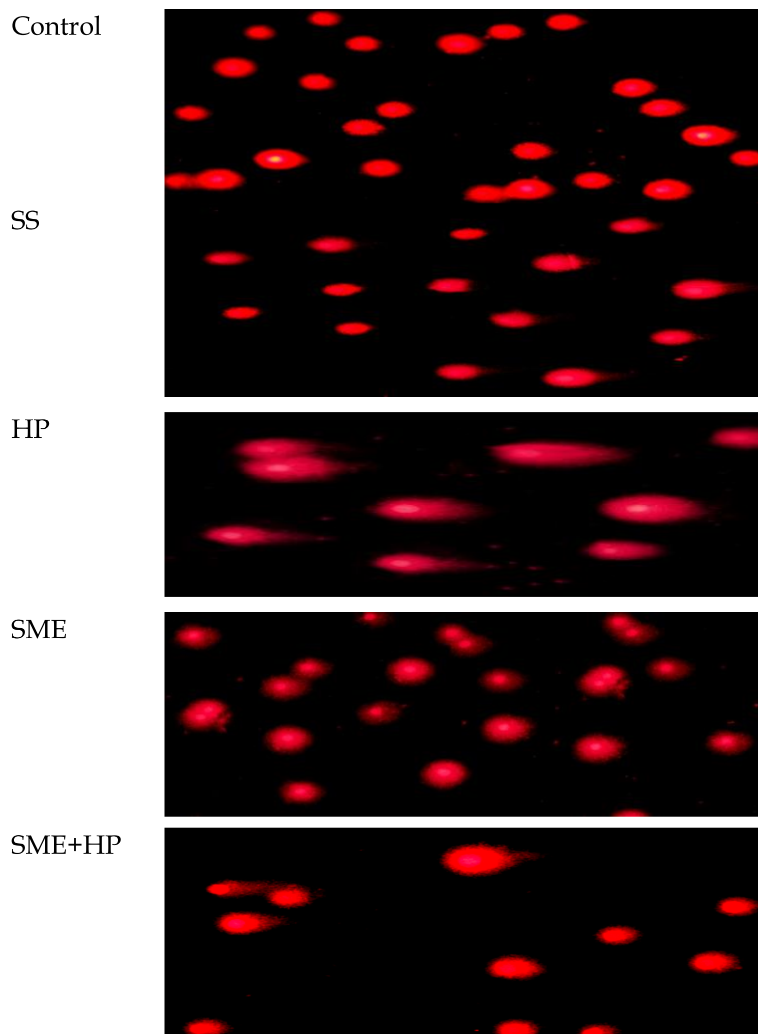

Fig. 7 Representative images of the comet assay for the experimental groups, analyzed using ImageJ software: control, serum starvation (SS), hydrogen peroxide-IC25 (HP), Silybum marianum extract (SME), and combined treatment (SME+HP).

DISCUSSION

In a previous study, the total extract of silymarin was shown to exhibit low levels of cytotoxicity in normal hepatocytes at concentrations ranging from 0 to 250 µg/mL. The cytotoxic effects were found to be minimal at concentrations up to 125 µg/mL. The IC_{50} value was calculated to be approximately 264 µg/mL, indicating that silymarin possesses a low toxicity profile. However, at a concentration of 250 µg/mL, the cytotoxic effect of the total silymarin extract became more pronounced, suggesting that the active compounds within the extract may exert their effects through different biological mechanisms (Yassin et al., 2022). The effects of silibinin and its vanadium complex, oxidovanadium (IV), were evaluated on the HT-29 colon cancer cell line using the MTT assay, and both compounds were observed to inhibit tumor cell viability (Leon et al., 2015). Similarly, in another study conducted on the Ramos cancer cell line, silymarin was shown to induce apoptosis, as evidenced by a decrease in cell viability in the MTT assay (Ranjbar et al., 2020). Hydrogen peroxide (H_2O_2) is commonly used in cytotoxicity studies as a model agent to induce oxidative stress in cells (Sayın, 2010). Oxidative stress caused by H_2O_2 can lead to various forms of cellular damage, including DNA strand breaks and base modifications (Jomova et al., 2025). Several studies have demonstrated the protective effects of natural compounds against H_2O_2-induced oxidative stress in various cell lines. For example, gossypin was shown to significantly attenuate

oxidative stress in murine L929 fibroblast cells and maintain fibroblast viability after exposure to H_2O_2 (Çınar, 2020). Oxyresveratrol also demonstrated protective effects in L929 cells by increasing cell viability under H_2O_2-induced oxidative conditions (Teş, 2021). Another study investigated the application of a pulsed electromagnetic field (PEMF) with a frequency of 75 Hz in a human neuroblastoma cell line after high-dose H_2O_2 treatment. It was shown to mitigate the harmful effects of oxidative stress by modulating intracellular glutathione levels (Gökçek-Saraç et al., 2023). In conclusion, the evidence supports the use of phytochemicals and physical modalities as effective protectants against oxidative stress, offering valuable insights for the advancement of novel therapeutic approaches.

In parallel with these findings, our study revealed that H_2O_2 exerted a dose-dependent cytotoxic effect on NRK-52E renal epithelial cells following 24 hours of exposure, as determined by MTT assay. Notably, co-treatment with a proliferative concentration of *Silybum marianum* extract significantly reduced the cytotoxic effects induced by H_2O_2. These results suggest that *Silybum marianum* extract exhibits a protective role against oxidative stress-mediated cellular damage. Although numerous studies have reported the antioxidant and cytoprotective effects of various phytochemicals, there is a lack of direct evidence in the literature regarding the protective potential of *Silybum marianum* specifically against H_2O_2-induced cytotoxicity. Therefore, our findings contribute novel insights in this context and suggest that further studies employing different cell lines and experimental models are warranted to more comprehensively evaluate the cytoprotective capacity of *Silybum marianum* extract. Based on our data, it can be concluded that *Silybum marianum*, when applied at a proliferative concentration, confers a protective effect against H_2O_2-induced cell death and reduced proliferation in NRK-52E cells.

Among the various reactive oxygen species, H_2O_2 is known to play a significant role, as nearly all sources of oxidative stress generate it, can freely diffuse across tissues, and can modulate signaling pathways and alter the homeostasis of ions such as calcium and iron. (Jin et al., 2000; Yang et al., 2001). Wang et al. investigated the effects of the natural antioxidant flavonoids icariin and silibinin on HUVECs under in vitro conditions by exposing the cells to 750 µM H_2O_2 for 18 hours. Using an LDH release assay, they demonstrated that both icariin and silibinin protected the cell line against H_2O_2-induced cell death and effectively reduced LDH release.

In this study, H_2O_2 was administered to the study groups at an IC_{25} concentration. DNA damage was assessed using the comet assay by evaluating both "DNA damage" and "tail moment" parameters. The analysis revealed that H_2O_2 significantly increased DNA damage in both parameters, consistent with previous findings reported in the literature. Furthermore, co-treatment with silymarin seed extract alongside H_2O_2 resulted in a marked reduction in these damage indicators. This suggests that silymarin may exert a protective or reparative effect against H_2O_2-induced DNA damage. No in vivo or in vitro studies directly addressing the role of silymarin in preventing or repairing H_2O_2-mediated DNA damage were identified. However, Katiyar et al. demonstrated that silymarin effectively protected genomic stability in epidermal cells exposed to UV radiation. These findings suggest a broader genoprotective potential of silymarin under oxidative stress conditions.

H_2O_2 plays a central role in cellular oxidative stress and interacts intricately with the tumor suppressor gene TP53, which mediates both protective and harmful responses to stress conditions. Under low levels of oxidative stress, TP53 protects the cell by upregulating the expression of antioxidant genes. However, under severe oxidative stress, p53 activates pro-oxidative genes and suppresses the expression of antioxidant genes, leading to an increase in reactive oxygen species (ROS) and apoptosis (Liu and Xu, 2011). In our study, it was revealed that H_2O_2 reduced TP53 mRNA expression. These results are consistent with previous literature demonstrating reduced TP53 expression due to oxidative stress induced by high H_2O_2 concentrations. H_2O_2 activates TP53 via various mechanisms, and this activation can trigger various cellular responses such as cell cycle arrest, DNA repair, or apoptosis.

H_2O_2-induced oxidative stress is known to activate PARP-1 due to DNA damage, potentially leading to cellular energy depletion and cell death (Mathews and Berk, 2008).

However, under certain conditions, H_2O_2 has also been reported to reduce PARP-1 expression. For instance, during the differentiation of C2C12 myoblasts, PARP-1 levels decline, correlating with increased resistance to oxidative stress (Oláh et al., 2015). In our study, we found that H_2O_2 exposure decreased PARP-1 expression while increasing DNA damage in NRK-52E cells. Co-treatment with *Silybum marianum* extract restored PARP-1 expression and markedly reduced DNA damage. These findings suggest that H_2O_2 may affect PARP-1 expression in a cell type- or context-dependent manner. While the literature lacks direct reports on silymarin reversing H_2O_2-induced PARP-1 suppression, our findings suggest a possible modulatory role, warranting further investigation.

The PI3K/AKT/mTOR signaling pathway is a highly conserved, key transduction network present in all higher eukaryotic cells, promoting cell survival, growth, and proliferation in response to external stimuli (Glaviano et al., 2023). Liu et al. (2020) reported that exposure to cytotoxic concentrations of H_2O_2 in H9c2 cardiac cells downregulated gene expression of components within the PI3K/AKT/mTOR signaling pathway. A recent study demonstrated that taxifolin, a constituent of silimarin, inhibits the mTOR/PI3K signaling pathway and promotes autophagy in glioblastoma multiforme cells (Sharma et al., 2025).

In this study, exposure of NRK-52E cells to cytotoxic concentrations of H_2O_2 resulted in a significant downregulation of mRNA expression of key genes within the PI3K/AKT/mTOR signaling axis. This finding is consistent with previous reports, such as Liu et al. [2020], which showed that H_2O_2 treatment of H9c2 cardiac cells similarly suppressed the expression of genes involved in the PI3K/AKT/mTOR signaling pathway. Remarkably, pretreatment of the cells with *Silybum marianum* extract before H_2O_2 exposure partially restored mRNA expression, approaching the levels of untreated controls. These observations suggest a modulating effect of *Silybum marianum* components on the oxidative stress-induced disruption of critical survival pathways, which is consistent with previous findings that compounds such as taxifolin can inhibit mTOR/PI3K signaling while promoting protective mechanisms, such as autophagy, in cancer cells (Sharma et al., 2025).

This study demonstrated that *Silybum marianum* extract effectively mitigates H_2O_2-induced cytotoxicity and DNA damage in NRK-52E renal epithelial cells. Consistent with prior research, H_2O_2 caused dose-dependent decreases in cell viability and increased DNA strand breaks, whereas co-treatment with the extract significantly alleviated these detrimental effects. Notably, the extract restored PARP-1 expression and partially reversed the downregulation of PI3K/AKT/mTOR pathway genes, suggesting modulation of key survival mechanisms under oxidative stress. These findings extend the known antioxidant and genoprotective roles of silymarin to renal cells, an area less explored compared to hepatic and cancer models. Nevertheless, this study has limitations. It was conducted in vitro using a single cell line, which may not fully capture the complexity of in vivo oxidative stress responses. The precise molecular interactions responsible for the observed effects, including the modulation of TP53 and PARP-1, remain to be clarified. Additionally, identifying the active compounds within the extract would enhance understanding of its protective mechanisms. Future studies involving diverse models and detailed mechanistic approaches are necessary to confirm and expand upon these results.

Conclusion

In conclusion, *Silybum marianum* extract shows promise as a protective agent against oxidative stress-induced damage in renal epithelial cells by reducing cytotoxicity, DNA damage, and disruption of survival signaling pathways. These results provide a foundation for further investigation into its therapeutic potential for oxidative stress-related renal disorders. Comprehensive *in vivo* studies and mechanistic analyses are needed to fully elucidate its efficacy and mode of action.

Ethical Approval

This study was approved by the Animal Researches Local Ethic Committee of Van Yüzüncü Yıl University (Turkey) at its meeting dated 01 June 2023 (Decision No: 2023/07-01).

Author Contributions

Veysel Yüksek: conceptualization, data curation, formal analysis, investigation, methodology, validation, visualization, writing – original draft, resources, writing – review and

editing, supervision, writing – review and editing. Feyzanur Ceran: data curation, investigation, formal analysis, investigation, validation, methodology, validation resources, writing – review and editing writing – review and editing.

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Conflicts of Interest
The authors declare no conflicts of interest.

Data Availability Statement
The data that support the findings of this study are available from the corresponding author upon reasonable request.

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