SPANDIDOS PUBLICATIONS

Cytotoxic, apoptotic and genotoxic effects of thymoquinone-oxime derivative on gastric cancer cells: An *in vitro* study

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Abstract. Gastric cancer (GC) remains a notable global health concern, emphasizing the need for novel and effective therapeutic agents. The present study investigated the cytotoxic, genotoxic and apoptotic effects of thymoquinone-oxime (TQ-ox) on human gastric adenocarcinoma AGS cells and assessed its potential to induce DNA damage. Cytotoxicity was evaluated in AGS and normal human gastric epithelial cells (HGEpiCs) using a luminometric ATP assay. Intracellular reactive oxygen species (ROS) and mitochondrial membrane potential (MMP) were measured fluorometrically, whereas intracellular glutathione (GSH) content was determined via a luminometric GSH assay kit. DNA damage was quantified by a comet assay, and apoptosis was assessed by fluorescence microscopy with acridine orange/ethidium bromide (AO/EB) double staining. TQ-ox exhibited dose-dependent cytotoxicity in gastric cells, with AGS cells having a slightly higher sensitivity to TQ-ox treatment than HGEpiCs [half-maximal inhibitory concentration (IC_{50}): 40.29 vs. 46.42 μM]. Treatment with TQ-ox significantly increased intracellular ROS levels and DNA damage, while also inducing a significant depletion of intracellular GSH and a reduction of MMP, indicating an increase in oxidative stress (OS) and mitochondrial dysfunction. AO/EB staining supported a dose-dependent increase in apoptosis of gastric cells at sub-IC_{50} concentrations of TQ-ox. Similarly, comet assay results revealed greater genotoxic effects in AGS cells compared with HGEpiCs, particularly at higher doses of TQ-ox. These findings demonstrated that TQ-ox exerted cytotoxic, pro-apoptotic and genotoxic effects on GC cells, likely mediated by OS, mitochondrial impairment and DNA damage. Taken together, these results provide additional evidence supporting the mechanistic effects of TQ-ox on GC cells and highlighted its potential as a candidate molecule for further preclinical evaluation.

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Introduction

Gastric cancer (GC) ranks fifth worldwide in terms of cancer incidence and mortality (1). The prevalence and high mortality rate of GC make it a notable health issue, and its treatment and management are planned according to the localized or advanced/metastatic status of the disease. Although surgery is the primary treatment option for GC, systemic therapies, such

as chemotherapy, targeted therapy and immunotherapy, have increased in importance. Molecular diagnostic techniques have facilitated genetic studies of GC and the identification of new potential molecular targets. Although notable progress has been made in the treatment of GC, further research and development are needed (2-4).

In previous years, advantages such as low toxicity and cost-effectiveness have increased interest in bioactive compounds as therapeutic agents. Among these, the *Nigella sativa*-derived compound thymoquinone (TQ) inhibits cancer cell proliferation, migration and invasion at different cancer stages (5). In addition, the anticancer effects of TQ have been demonstrated in numerous types of cancer, such as breast, pancreatic and prostate cancer, and it has been suggested that TQ represents a new pharmacological agent that can be used in cancer treatment (5-8).

The potent biological activity of TQ may allow modifications to enhance its activity and applications toward specific targets when modified with oxime derivatives. Owing to the favorable properties of oxime bond formation, such as high efficiency, chemoselectivity, formation in aqueous solvents and water as the only by-product, oxime chemistry has progressed rapidly and has been used chiefly for bioconjugation. In addition, oxime chemistry is a promising field for bioconjugation and eco-friendly polymer synthesis due to its low environmental impact (9-11).

Studies have explored the anticancer effects of TQ and its specific mechanisms on GC. It has been observed that the antitumor effects of TQ increase when combined with chemotherapeutics, such as 5-fluorouracil and cisplatin. These studies have shown that TQ may be a pioneer molecule for treating GC. However, the number of studies investigating the effect of TQ-oxime (TQ-ox) on GC is insufficient (12-16).

The present study aimed to investigate the effects of TQ-ox on the human GC cell line AGS and normal human gastric epithelial cells (HGEpiCs) cell line. Therefore, the present study evaluated the cytotoxic, genotoxic and apoptotic effects of TQ-ox, and its potential to cause DNA damage. Building on our previous studies (8,11,17) investigating TQ-ox in other cancer models, the present study extended that work by examining its cytotoxic, oxidative stress (OS)-mediated, mitochondrial, apoptotic and genotoxic effects specifically in AGS cells in comparison with HGEpiCs within a single integrated experimental framework.

Materials and methods

TQ-ox synthesis. The TQ-ox used in the present study was not newly synthesized; rather, it was obtained from our previously published work (8). In the prior study, TQ-ox was synthesized by nitrosating carvacrol with $NaNO_2$ in ethanol and hydrochloric acid under an argon atmosphere. The crude product was purified by sequential washing steps, and its purity and identity were confirmed through melting-point determination, thin-layer chromatography analysis, and structural characterization by ^{1}H-NMR, ^{13}C-NMR and elemental analysis.

Cell lines and culture conditions. The present study used the AGS human GC cell line (cat. no. CRL-1739™), which was commercially purchased from the American Type Culture Collection. AGS cells were cultured in F-12K medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin (P/S) (all from Sigma-Aldrich; Merck KGaA). Primary HGEpiCs were obtained from Innoprot (cat. no. P10778). These cells were cultured in epithelial cell medium kit (cat. no. P60106; Innoprot) supplemented with 2% FBS (Sigma-Aldrich; Merck KGaA), 1% epithelial cell growth supplement (included in the epithelial cell medium kit) and 1% P/S (Sigma-Aldrich; Merck KGaA), according to the manufacturer's recommendations.

HGEpiCs were used to determine the effects of the tested compounds on non-cancerous gastric epithelium and to perform a comparative toxicity/selectivity assessment with AGS cells.

Unless otherwise specified, both AGS cells and HGEpiCs were maintained under identical incubation conditions of 37˚C in a humidified atmosphere containing 5% CO_2 and 95% air. All *in vitro* experiments described in the present study including cytotoxicity, intracellular reactive oxygen species (ROS), GSH, mitochondrial membrane potential (MMP), apoptosis and comet assays were also performed under these same incubation conditions. For assessing the effects of TQ-ox on cytotoxicity and intracellular ROS levels, AGS cells and HGEpiCs ($7x10^3$ cells/well) were seeded in 96-well plates; for evaluating the effect of TQ-ox on apoptosis and DNA damage, $5x10^4$/well for each AGS cells and HGEpiCs were seeded in 6-well plates and incubated for 24 h.

Cytotoxic activity. A luminescent ATP test was conducted to determine the cytotoxic effects of TQ-ox. AGS cells and HGEpiCs were seeded at $7x10^3$ cells/well in 96-well plates, which was determined to be the optimal density for these assays. After 24 h of attachment, the cells were incubated with synthesized TQ-ox at concentrations ranging from 2.5 to 100 μM for 24 h at 37˚C in a humidified incubator containing 5% CO_2 and 95% air. To detect ATP concentration, a homogeneous method that indicates the presence of viable cells was performed using a CellTiterGlo® luminescence cell viability kit (Promega Corporation), according to the manufacturer's instructions. Subsequently, measurements were performed after 5 min using a multiplate reader (Synergy™ HTX Flash Multimode Reader; BioTek; Agilent Technologies, Inc.). Luminescence emitted in the presence of ATP was quantified in relative luminescence units. The half-maximal inhibitory concentration (IC_{50}) values of TQ-ox were calculated from dose-response curves.

Intracellular ROS detection. The effect of TQ-ox on intracellular ROS was assessed using the fluorometric method with 2',7'-dichlorodihydrofluorescein diacetate (H_2DCF-DA; Sigma-Aldrich; Merck KGaA) fluorescent dye. AGS cells and HGEpiCs were treated with various concentrations of TQ-ox (2.5-100 μM) for 24 h at 37˚C in a humidified incubator containing 5% CO_2 and 95% air. After incubation, the medium was aspirated, and the wells were washed three times with 1X Dulbecco's PBS (dPBS). Subsequently, 100 μl 10 μM H_2DCF-DA prepared in distilled water was added to each well and the cells were incubated for 30 min at 37˚C. The fluorescence intensity of the resulting DCF was determined using a fluorescence plate reader (Synergy HTX Multi-Mode Reader) at an excitation of 488 nm and an emission of 525 nm. The

results were analyzed relative to the control group, which was treated with 0.1% DMSO.

Glutathione (GSH) analysis. The luminometric commercially obtained GSH-Glo™ Glutathione Assay luminescence kit (cat. no. V6911; Promega Corporation) was used to analyze intracellular GSH levels, according to the manufacturer's instructions. Both cell lines were seeded at 7.5×10^3 cells/well in white opaque 96-well plates and incubated for 24 h at 37˚C in a humidified incubator containing 5% CO_2 and 95% air. Different TQ-ox concentrations (2.5-100 μM) were applied followed by another incubation for 24 h. Subsequently, GSH solution was added to the cells and after a 5-min incubation, luminescence was measured using a BioTek, Synergy HTX Multi-Mode Reader. To correct for differences in cell number, GSH luminescence values were normalized to ATP levels from parallel wells and compared with the control group.

MMP detection. Both cell lines were treated with different concentrations of TQ-ox (2.5-100 μM) for 24 h at 37˚C in a humidified incubator containing 5% CO_2 and 95% air followed by medium removal and washing with 1X dPBS. Subsequently, 40 nM 3,3'-dihexyloxacarbocyanine iodide (3) (Molecular Probes; Thermo Fisher Scientific, Inc.) was added and the cells were incubated for 15 min at 37˚C in a humidified incubator containing 5% CO_2 and 95% air. Washing was performed three times using 1X dPBS, and fluorescence intensity was measured using an excitation of 484 nm and an emission of 501 nm using a fluorescence plate reader (BioTek, Synergy HTX Multi-Mode Reader). The results were analyzed and compared with those of the control group.

Apoptosis analysis via acridine orange/ethidium bromide (AO/EB) double staining. AO/EB double staining was used to analyze apoptosis-related nuclear morphological changes, as described in a study reported by McGahon *et al* (18). Based on the cytotoxicity assay results, sub-cytotoxic concentrations of TQ-ox close to the IC_{50} value were selected to evaluate mechanistic apoptotic responses without inducing extensive necrotic cell death. Therefore, apoptotic experiments were conducted using 5-40 μM TQ-ox, and both AGS cells and HGEpiCs were incubated with TQ-ox for 24 h at 37˚C in a humidified incubator containing 5% CO_2 and 95% air prior to staining. The treated cells were stained with 1:1 AO/EB double stain for 5 min at room temperature. A total of ~50 individual cells per TQ-ox concentration were analyzed to quantify apoptotic morphology and ensure reliable statistical evaluation using ImageJ V1.54 software (National Institutes of Health). Apoptotic and necrotic cells were then visualized under a fluorescence microscope (Nikon Eclipse Ts2; Nikon Corporation) and images of stained cells were captured.

DNA damage assessment via comet assay. Based on the cytotoxicity assay results, sub-cytotoxic concentrations of TQ-ox close to the IC_{50} value (5-40 μM) were selected to evaluate genotoxic responses without inducing extensive necrotic cell death. After treatment with TQ-ox for 24 h at 37˚C in a humidified incubator containing 5% CO_2 and 95% air, DNA damage was assessed using the alkaline single-cell gel electrophoresis method described in a previous study reported by Singh *et al* (19). Briefly, both cell lines were detached with 0.25% trypsin/EDTA, washed twice with 1X dPBS and resuspended in 0.7% low-melting agarose. The suspension was layered onto slides that had been pre-coated with 1% normal-melting agarose and air-dried at room temperature (22-24˚C) for 1 h. The slides were then kept at 4˚C for 15 min to allow solidification. Slides were subsequently incubated for ≥4 h in lysis buffer containing 50 mM Tris-HCl, 150 mM NaCl, and 1 mM EDTA (pH 10). All reagents were obtained from Sigma-Aldrich (Merck KGaA). After lysis, the slides were electrophoresed in alkaline buffer (pH 13) at 300 mA for 20 min.

After electrophoresis, the slides were stained with 5 mg/ml EB for 5 min at room temperature and examined under a fluorescence microscope at an excitation of 546 nm. A total of ~50 individual cells per TQ-ox condition were analyzed using the Comet Assay IV software (Instem), and images of stained cells were captured. DNA damage was quantified as the percentage of DNA in the comet tail.

Ethics statement. The present study involved only in vitro experiments using commercially available human cell lines and did not include human participants or animal subjects. Ethics approval was obtained from the Hamidiye Scientific Research Ethics Committee of the University of Health Sciences (Istanbul, Türkiye; approval no. 2024/15-15/33-24/760).

Statistical analysis. Statistical analyses were performed using the Statistical Package for the Social Sciences version 28.0 (IBM Corp.). The Shapiro-Wilk test was used to assess the normality of the data distribution. The results of statistical analyses are presented as the mean ± standard deviation from at least four independent experiments. Statistical comparisons were performed separately for each cell line, with each treatment concentration compared only to the corresponding vehicle control. Comparisons among >2 groups were conducted using one-way analysis of variance followed by Tukey's post hoc test. IC_{50} values were calculated via nonlinear regression analysis (GraphPad Prism; Dotmatics). $P<0.05$ was considered to indicate a statistically significant difference.

Results

Cytotoxic activity and intracellular ROS. According to the results of the ATP assay for detecting cytotoxicity, TQ-ox showed a dose-dependent cytotoxic effect on AGS cells after 24 h of treatment. When compared with the 0.1% DMSO control group, AGS cell viability showed a statistically significant reduction beginning at 5 and maintained up to 20 μM ($P<0.01$). This effect became more pronounced at 30 μM ($P<0.001$), demonstrating a clear dose-dependent effect, with marked cytotoxicity observed at concentrations ≥40 μM ($P<0.001$) (Fig. 1A). A similar dose-dependent decline was observed in HGEpiCs, where viability started to significantly decrease at 5 μM ($P<0.05$), became more pronounced at 10 μM ($P<0.01$), and reached strong significance at concentrations ≥20 μM ($P<0.001$). At the highest concentrations (80-100 μM), both cell lines showed a marked reduction in viability, with viability dropping to 20-30% of control levels ($P<0.001$).

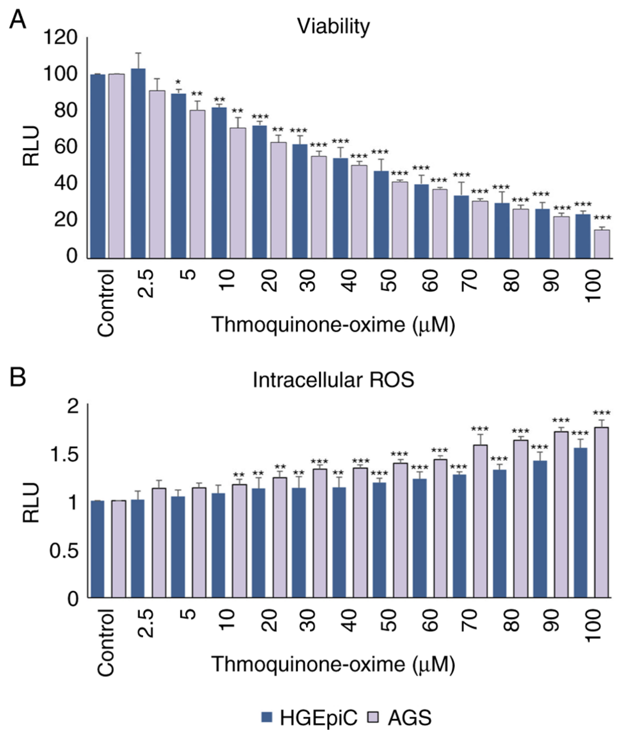

Figure 1. (A) Cell viability decreases as the dose increases. (B) Intracellular ROS levels increase significantly as the thymoquinone-oxime concentration increases in both cell lines. Statistical significance was analyzed using one-way analysis of variance with Tukey's post hoc test. *P<0.05, **P<0.01 and ***P<0.001 vs. control. RLU, relative luminescence units; ROS, reactive oxygen species; RFU, relative fluorescence units; HGEpiC, human gastric epithelial cell.

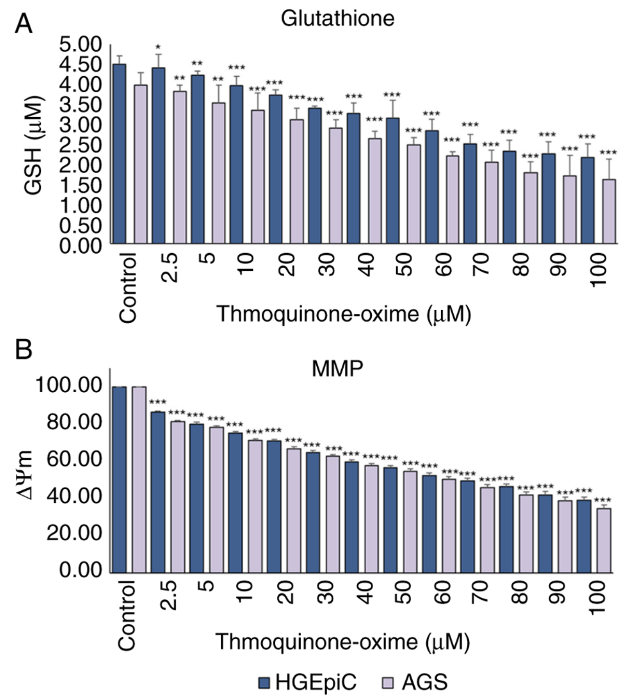

Figure 2. Effects of thymoquinone-oxime treatment on (A) GSH (μM) levels and (B) MMP (ΔΨm). Statistical significance was analyzed using one-way analysis of variance with Tukey's post hoc test. *P<0.05, **P<0.01 and ***P<0.001 vs. control. HGEpiC, human gastric epithelial cell; GSH, glutathione; MMP, mitochondrial membrane potential.

A fluorescent H_2DCF-DA probe was used to assess intracellular ROS levels in each cell line. A dose-dependent increase in ROS production was observed in AGS cells, becoming statistically significant at 10 μM (P<0.01) and highly significant at ≥30 μM (P<0.001) compared with the control group (Fig. 1B). HGEpiCs showed a similar but slightly attenuated pattern, ROS levels were significantly increased at 20 μM (P<0.01), and were strongly elevated at 50-100 μM (P<0.001).

IC_{50} values were determined via nonlinear regression analysis. Based on the cytotoxicity assay, AGS cells demonstrated slightly higher sensitivity to TQ-ox compared with HGEpiCs (IC_{50}, 40.29 vs. 46.42 μM). Therefore, apoptosis and comet assays were conducted using sub-cytotoxic concentrations near the IC_{50} value (5-40 μM) to evaluate biologically relevant apoptotic and genotoxic responses rather than non-specific necrotic cell death.

Intracellular GSH levels and MMP. GSH levels were decreased in TQ-ox-treated HGEpiCs and AGS cells in a dose-dependent manner. In HGEpiCs, GSH levels showed a modest but significant reduction beginning at 2.5 μM (P<0.05), followed by a more pronounced decline at 5 μM (P<0.01), and a highly significant decrease at concentrations ≥10 μM (P<0.001). In AGS cells, a significant decrease in GSH levels was observed, starting at 2.5 and 5 μM (P<0.01), becoming stronger at 10 μM (P<0.001) (Fig. 2A). These results indicated that TQ-ox weakened the intracellular antioxidant defense system and disrupted GSH redox balance.

TQ-ox also induced a significant dose-dependent decrease in MMP in HGEpiCs and AGS cells. From a low TQ-ox concentration of 2.5 μM, MMP was significantly decreased compared with that in the control group (P<0.001). This decrease became more pronounced as the dose increased; MMP levels decreased by ~60% of the initial values after treatment with 100 μM TQ-ox (Fig. 2B).

Apoptosis analysis via AO/EB double staining. The potential of TQ-ox to induce apoptosis in cancer cells was evaluated using the AO/EB double staining assay. The apoptotic effects of TQ-ox doses below IC_{50} were detected by incubating cancer cells with TQ-ox for 24 h followed by fluorescence microscopy. In AGS cells, the data obtained showed that apoptosis significantly increased in a dose-dependent manner compared with that the control group (P<0.001). In HGEpiCs, apoptosis percentage levels showed a modest but significant increase at 5 μM (P<0.01), and a highly significant increase at concentrations ≥10 μM (P<0.001). Representative images of AGS cells and HGEpiCs revealed distinct morphological differences among the experimental groups (Figs. S1 and S2). After staining with AO/EB, viable and healthy cells fluoresced green, whereas apoptotic cells appeared orange and necrotic cells appeared red due to chromatin density and nuclear fragmentation. Fig. 3 shows the apoptotic values as the mean percentage of the ratio of apoptotic cells to total cells at each dose point.

DNA damage analysis via comet assay. AGS cells and HGEpiCs were incubated with TQ-ox doses below its IC_{50} values to assess genotoxic damage. In the comet assay, damaged DNA

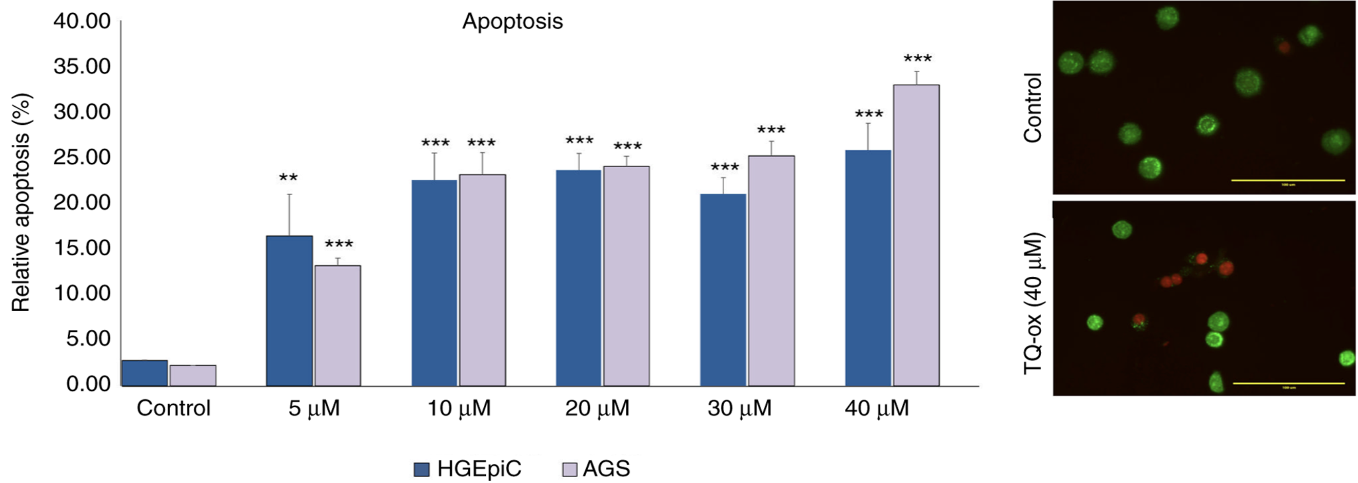

Figure 3. Effect of TQ-ox on apoptosis in HGEpiCs and AGS cells. Statistical significance was analyzed using one-way analysis of variance with Tukey's post hoc test. **P<0.01 and ***P<0.001 vs. control. Representative fluorescence microscopy images of acridine orange/ethidium bromide-stained cells showing live (green) and apoptotic (red/orange) cells in the control and 40 μM TQ-ox treated AGS groups. A total of ~50 cells were analyzed per condition. Scale bar, 100 μm. HGEpiC, human gastric epithelial cell; TQ-ox, thymoquinone-oxime.

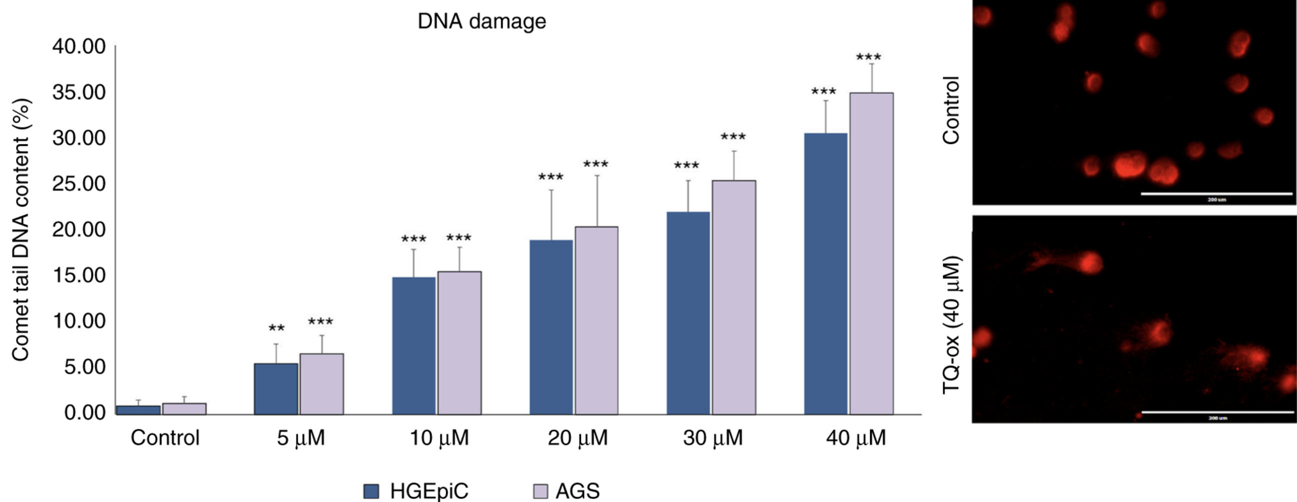

Figure 4. Effect of TQ-ox on DNA damage in HGEpiCs and AGS cells following 24 h of TQ-ox treatment (5-40 μM). Statistical significance was analyzed using one-way analysis of variance with Tukey's post hoc test. **P<0.01 and ***P<0.001 vs. control. Representative fluorescence microscopy images showing nuclei (control) and comet tails (40 μM TQ-ox-treated cells), indicating fragmented DNA. A total of ~50 cells were analyzed per condition. Scale bar, 200 μm. HGEpiC, human gastric epithelial cell; TQ-ox, thymoquinone-oxime.

appears as comet-shaped structures with bright, fragmented nuclei, whereas undamaged DNA appears as large, intact and round nuclei. In the current study, TQ-ox treatment increased the proportion of cells exhibiting comet-like tails, indicating enhanced DNA fragmentation, whereas the nuclei of untreated control cells predominantly remained intact and round. This demonstrated that TQ-ox may induce measurable DNA damage. In addition, a significant increase in percentage tail values was observed with increasing concentrations of TQ-ox in both cell lines (Fig. 4). While DNA damage was relatively low in the control group, the application of TQ-ox starting at 5 μM resulted in a statistically significant increase in DNA damage (P<0.01 and P<0.001), with the highest effect observed at 40 μM. AGS cells exhibited notably greater DNA damage than HGEpiCs, particularly at high doses. Representative comet assay images of AGS cells and HGEpiCs demonstrated distinct differences in DNA damage (tail formation) among the experimental groups (Figs. S3 and S4).

Discussion

The therapeutic uses of oxime compounds for various diseases are currently under investigation (20). The search for synthetic modifications of steroid compounds is a promising strategy for finding new drug candidates. Even subtle changes in the substitution pattern of steroid chemical frameworks can have marked effects on their specific biological activities. In particular, some steroidal oxime compounds and oxime ethers have exhibited notable antioxidant, antibacterial and antitumor properties, highlighting their potential as therapeutic agents (21,22).

The cytotoxic, genotoxic and apoptotic effects of ox-modified TQ were investigated in AGS cells and HGEpiCs.

TQ-ox significantly reduced cell viability in a dose-dependent manner, increased intracellular ROS levels, disrupted MMP and induced DNA damage. These results suggested that TQ-ox exerted its anticancer effects through OS-mediated apoptotic mechanisms and mitochondrial dysfunction.

OS is caused by an imbalance between the antioxidant capacity of the cell and pro-oxidant molecules (18). This imbalance can lead to DNA damage, protein aggregation and digestive system membrane dysfunction (23). By interacting with macromolecules, such as DNA, ROS can disrupt important cellular functions. Oxidative DNA damage may lead to the development of cancer by increasing the risk of mutagenesis (24). Oxime compounds effectively modulate ROS and mediate cellular OS levels. These compounds have been shown to exhibit therapeutic potential against OS-related diseases by regulating ROS production and maintaining redox balance (25).

The addition of oxime groups may enhance the biological effects and therapeutic applications of therapeutic agents by optimizing their interactions with biomolecules (26). A study by Bozali *et al* (17) reported that cytotoxicity, ROS elevation, GSH depletion, mitochondrial dysfunction, DNA damage and apoptosis were notably induced in B16F10 melanoma cells at concentrations of 10-20 μM TQ-ox. Similarly, Güler and Bozali (11) found that TQ-ox induced dose-dependent increases in ROS and Ca^{2+} levels, as well as notable genotoxic and apoptotic responses in HepG2 liver cancer cells. Similar patterns of OS, MMP collapse and DNA damage have been observed in the ovarian carcinoma SKOV-3 cell line (8).

A recent study on A549 lung cancer cells demonstrated that TQ-ox induces dose-dependent cytotoxicity through OS, GSH depletion, calcium elevation and MMP loss, leading to apoptosis (27). These findings support the consistent pro-oxidant and pro-apoptotic effects of TQ-ox observed across cancer types and therefore its potential as an anticancer agent. Collectively, the findings of these previous studies underscore that oxime modification of TQ maintains or enhances its pro-oxidant and genotoxic mechanisms, possibly by facilitating increased interactions with mitochondrial and redox-sensitive targets. Consistent with these studies, the findings of the present study demonstrated that TQ-ox exerted similar pro-oxidant and cytotoxic effects in AGS cells and HGEpiCs. The observed dose-dependent increase in intracellular ROS levels, GSH depletion and MMP loss suggested that TQ-ox disrupted redox homeostasis and mitochondrial integrity, ultimately leading to apoptosis. The slightly higher sensitivity of AGS cells compared with normal HGEpiCs reinforced the potential selectivity of TQ-ox toward malignant cells. DNA damage analysis via comet assay revealed a dose-related increase in tail fluorescence intensity, suggesting that TQ-ox exerted genotoxic effects in AGS cells. The observed DNA fragmentation further supported the role of OS and mitochondrial impairment in TQ-ox-induced cytotoxicity. These combined results suggested that TQ-ox triggered cell death via multiple converging mechanisms, including oxidative imbalance, mitochondrial collapse, apoptosis and genotoxic stress.

However, the present study also had some limitations. Primarily, experiments were conducted *in vitro*, and *in vivo* validation is needed to fully characterize the pharmacodynamic and pharmacokinetic properties of TQ-ox. Future studies including additional GC subtypes and animal models will be valuable to further generalize these findings.

In conclusion, the present study revealed that increasing TQ-ox concentrations induced cytotoxicity, genotoxicity and apoptosis in AGS cells and HGEpiCs by increasing intracellular ROS and decreasing cell viability, intracellular GSH levels and MMP. TQ-ox exerted pro-oxidant activity on cancer cells at higher concentrations, triggering cell death mechanisms. While TQ-ox exerted strong pro-oxidant, cytotoxic, apoptotic and genotoxic effects in both AGS cancer cells and HGEpiCs at the same concentrations, the magnitude of these responses differed between the two cell types. AGS cells exhibited a more pronounced and earlier response, characterized by a significant reduction in cell viability and increased oxidative stress at lower concentrations, followed by marked induction of apoptosis and DNA damage at higher doses. By contrast, HGEpiCs displayed a comparatively attenuated response, with significant effects occurring predominantly at higher concentrations of TQ-ox. Overall, these findings indicate that although TQ-ox exerts cytotoxic and genotoxic effects in both normal and cancerous gastric epithelial cells, AGS cells are more susceptible to TQ-ox-induced oxidative stress-mediated damage, suggesting a differential cellular sensitivity between malignant and non-malignant cells. These findings support the therapeutic potential of TQ-ox in GC but highlight the need for further research into its dose-selectivity, molecular mechanisms and *in vivo* efficacy.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

KB, Zİ, NK, TG, MK, YMK, ME, MDa, EA, FT, CA, OS, MDu and EMG were responsible for conceptualization, methodology, formal analysis and investigation of the present study, as well as contributions towards composing the original draft of the manuscript, reviewing and editing the manuscript, and the acquisition of resources and funding. EMG was also responsible for supervision of the present study. KB, Zİ and EMG confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was performed in accordance with the principles of The Declaration of Helsinki and was approved by the Hamidiye Scientific Research Ethics Committee of the University of Health Sciences, Türkiye (approval no. 2024/15 -15/33-24/760).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Use of artificial intelligence tools

During the preparation of this work, AI tools were used to improve the readability and language of the manuscript [Grammarly (app.grammarly.com) and Trinka AI (trinka.ai)] and to translate the manuscript into English [DeepL Translator (deepl.com/en/translator)], and subsequently, the authors revised and edited the content produced by the AI tools as necessary, taking full responsibility for the ultimate content of the present manuscript.

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