Shikonin attenuates H_2O_2-induced oxidative injury in HT29 cells via antioxidant activities and the inhibition of mitochondrial pathway-mediated apoptosis

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Abstract. Shikonin, a natural naphthoquinone extracted from the roots of *Lithospermumery throrhizon*, possesses multiple pharmacological properties, including antioxidant, anti-inflammatory and antitumor effects. It has been hypothesized that the properties of shikonin are associated with its oxygen free radical scavenging abilities. However, the mechanism underlying the antioxidant activity of shikonin is not completely understood. The aim of the present study was to investigate the effect of shikonin against H_2O_2-induced oxidative injury in HT29 cells and to explore the underlying molecular mechanism. The concentration and duration of H_2O_2 treatment to cause maximal damage, and the effects of shikonin (2.5, 5 or 10 μg/ml) on the activity of H_2O_2-induced HT29 cells were determined by MTT assay. The apoptotic rate in HT29 cells was determined by annexin V/propidium iodide staining. HT29 cell cycle alteration was also analyzed by propidium iodide staining. Reactive oxygen species (ROS) production was assessed by monitoring 2',7'-dichlorofluorescin in diacetate fluorescence. Mitochondrial membrane potentials were determined by JC-1 staining. The activities of malondialdehyde, superoxide dismutase, caspase-9 and caspase-3 were measured using spectrophotometric assays. The expression levels of Bcl-2, Bax and cytochrome *c* were determined by western blotting. The results suggested that shikonin increased cell viability, reduced cell apoptosis and increased the proliferation index in H_2O_2-treated HT29 cells. Shikonin also significantly inhibited increases in intracellular reactive oxygen species (ROS), restored the mitochondrial membrane potential, prevented the release of lactic dehydrogenase and decreased the levels of superoxide dismutase and malondialdehyde in H_2O_2-induced HT29 cells. Furthermore, shikonin significantly decreased caspase-9 and caspase-3 activities, increased Bcl-2 expression and decreased Bax and cytochrome *c* expression levels in H_2O_2-induced HT29 cells. The results indicated that shikonin protected against H_2O_2-induced oxidative injury by removing ROS, ameliorating mitochondrial dysfunction, attenuating DNA oxidative damage and inhibiting mitochondrial pathway-mediated apoptosis.

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Introduction

Oxidative stress is caused by an imbalance between the production of reactive oxygen species (ROS) and the antioxidant capacity of cellular antioxidants in biological systems (1). Excessive oxidative stress is related to the pathogenesis of various diseases, including neurodegenerative diseases, tumors and inflammation (2,3). Previous studies have demonstrated that increased levels of ROS production lead to the development of several chronic intestinal inflammatory diseases (4-6). In particular, increased ROS production is typically associated with the pathogenesis of inflammatory bowel disease, which is characterized by chronic inflammation in human gastrointestinal disease (7,8). Therefore, inhibiting oxidative stress-induced injury may serve as an important therapeutic strategy.

Shikonin, a natural naphthoquinone extracted from the roots of the traditional Chinese medicine *Lithospermumery throrhizon*, possesses multiple pharmacological properties, including antioxidant, anti-inflammatory, antiviral, enhanced immunity, antifertility and antitumor effects (9-13). Numerous studies have demonstrated that shikonin displayed efficient antioxidative activities against various types of ROS (14,15). Guo *et al* (2) reported that shikonin attenuated acetaminophen-induced acute liver injury via inhibition of oxidative stress. Several studies indicated that shikonin displayed significant protective effects in brain and hepatic ischemia/reperfusion injury by reducing ROS (16-19). However, the potential antioxidant mechanism underlying shikonin activity is not completely understood.

The present study aimed to investigate the effects of shikonin against H_2O_2-induced oxidative stress injury in human intestinal epithelial cells and to explore the underlying molecular mechanism. In many studies, human colon cancer cells were used as oxidative damage models (20,21). Therefore, HT29 human colon cancer cells were selected to construct oxidative damage models in the present study.

Materials and methods

Cell culture and treatment. HT29 cells were purchased from American Type Culture Collection. The cell line was established at the Memorial Sloan Kettering Cancer Center and was authenticated using STR profiling. Cells were cultured in RPMI-1640 medium (Gibco; Thermo Fisher Scientific, Inc.) supplemented with 10% FBS (Gibco; Thermo Fisher Scientific, Inc.) and 1% penicillin-streptomycin (Gibco; Thermo Fisher Scientific, Inc.) in a humidified atmosphere with 5% CO_2 at 37˚C. Cells ($5x10^4$-10^5 cells/ml) were harvested and used for subsequent experiments. Cells were divided into six groups for treatment: A control group, cells cultured in medium without H_2O_2 and shikonin; DMSO group, cells cultured in medium with 0.1% DMSO; and H_2O_2 group, cells cultured in medium with 800 μM H_2O_2 (Sigma-Aldrich; Merck KGaA) all of which were cultured for 24 h at 37˚C; and 3 shikonin groups, cells pretreated with 2.5, 5 or 10 μg/ml shikonin (Shanghai Yuan Ye Biotechnology Co., Ltd.; purity ≥98%) for 6 h at 37˚C, as previously described (22,23), and then co-treated with 800 μM H_2O_2 for 24 h at 37˚C.

MTT assay. For the MTT assay, HT29 cells ($5x10^3$) were seeded in 96-well plates and cultured for 24 h at 37˚C. cells were treated with H_2O_2 (25-1,600 μM) for 4, 8, 12 or 24 h at 37˚C, or treated with shikonin (2.5, 5, 10, 25, 50, 100 and 200 μg/ml) for 24 h at 37˚C. Subsequently, cells were incubated with 0.5 mg/ml MTT (Sigma-Aldrich; Merck K Ga A) for an additional 4 h at 37˚C. The supernatant was discarded and 100 μl DMSO was added to each well to dissolve the formazan crystals. Absorbance was measured at a wavelength of 490 nm using a microplate reader. The results are presented as a percentage of the control.

Early and late apoptosis detection assay. Early and late apoptosis was measured using a FITC-conjugated annexin V and propidium iodide kit (BD Biosciences). Cells ($1x10^6$) were trypsinized, washed with PBS and resuspended in 1Xbinding buffer. Subsequently, 5 μl FITC-conjugated annexin V and 5 μl propidium iodide were added to 100 μl cell suspension. Following incubation for 15 min at room temperature in the dark, apoptosis was analyzed via flow cytometry (EXPO32 ADC; Epics XL-MCL; Beckman Coulter, Inc.).

Cell cycle assay. Cells ($1x10^6$) were trypsinized with 0.25% trypsin-EDTA at room temperature for 24 h and washed three times in PBS. Cell cycle distribution was detected using a Cycle test Plus DNA reagent kit (BD Biosciences). The percentage of cells in each cell cycle phase (G_0/G_1, S and G_2/M) was calculated via flow cytometry (Epics XL-MCL; Beckman Coulter, Inc.). The software used was the inbuilt software provided with the flow cytometer. Proliferation index [PI;PI (%)=S phase (%) + G_2/M phase (%)].

ROS measurement. Intracellular ROS levels were measured using a ROS assay kit (cat. no. S0033M; Beyotime Institute of Biotechnology) according to the manufacturer's instructions. Briefly, cells ($1x10^6$) were collected and incubated for 20 min in 500 μl 2',7'-dichlorofluorescin diacetate fluorescence (DCFH-DA) solution (10 μM) at 37˚C in the dark. Following washing with PBS, cells were resuspended in 500 μl PBS and analyzed via flow cytometry (EXPO32 ADC, Epics XL-MCL; Beckman Coulter, Inc.).

Levels of malondialdehyde (MDA) and superoxide dismutase (SOD) assays. Cells ($1x10^6$) were collected and centrifuged at 10,000 x g for 10 min at 4˚C. The levels of SOD and MDA in the supernatant were measured using SOD (cat. no. S0101S) and MDA (cat. no. S0131S) assay kits (both Beyotime Institute of Biotechnology), respectively, according to the manufacturer's protocol.

Lactate dehydrogenase (LDH) activity assay. Cell membrane integrity was determined using an LDH assay. LDH levels in the cell medium from treated cells were determined using an LDH assay kit (cat. no. C0016; Beyotime Institute of Biotechnology) according to the manufacturer's protocol. Absorbance was measured at a wavelength of 490 nm using a microplate reader. LDH levels were calculated according to the following formula: LDH (%)=(sample-blank)/(control-blank) x100.

Mitochondrial membrane potential assay. To assess mitochondrial integrity, the mitochondrial membrane potential assay kit with JC-1 (cat. no. C2006; Beyotime Institute of Biotechnology) was used. Cells ($1x10^6$) were resuspended in 500 μl medium, followed by addition of 500 μl JC-1 dye for 20 min at 37˚C. Cells were rinsed twice with JC-1 dye buffer. The fluorescent signal in cells was calculated by performing flow cytometry (EXPO32 ADC; Epics XL-MCL; Beckman Coulter, Inc.).

Caspase-3 and caspase-9 activity assays. Cells ($1x10^6$) were digested with trypsin and harvested by centrifugation at 1,000 x g for 5 min at 4˚C. Caspase-3 and caspase-9 activities were measured using caspase-3 (cat. no. BC3830) and caspase-9 (cat. no. BC3890) activity detection kits (Beijing Solarbio Science & Technology Co., Ltd.) according to the manufacturer's protocol. Caspase-3 and caspase-9 activities are presented as U/mg protein.

Western blotting. Total protein was extracted from cells using RIPA buffer (Beyotime Institute of Biotechnology) containing 1 mM PMSF (Beyotime Institute of Biotechnology) and phosphatase inhibitor for 30 min on ice. Total protein was quantified using a bicinchoninic acid protein assay kit (Beyotime Institute of Biotechnology). Equal amounts of protein (20 μg) were separated via 10% SDS-PAGE and transferred to 0.45 μm PVDF membranes. After 5% nonfat milk blocking at room temperature for 2 h, the membranes were incubated with primary antibodies targeted against:

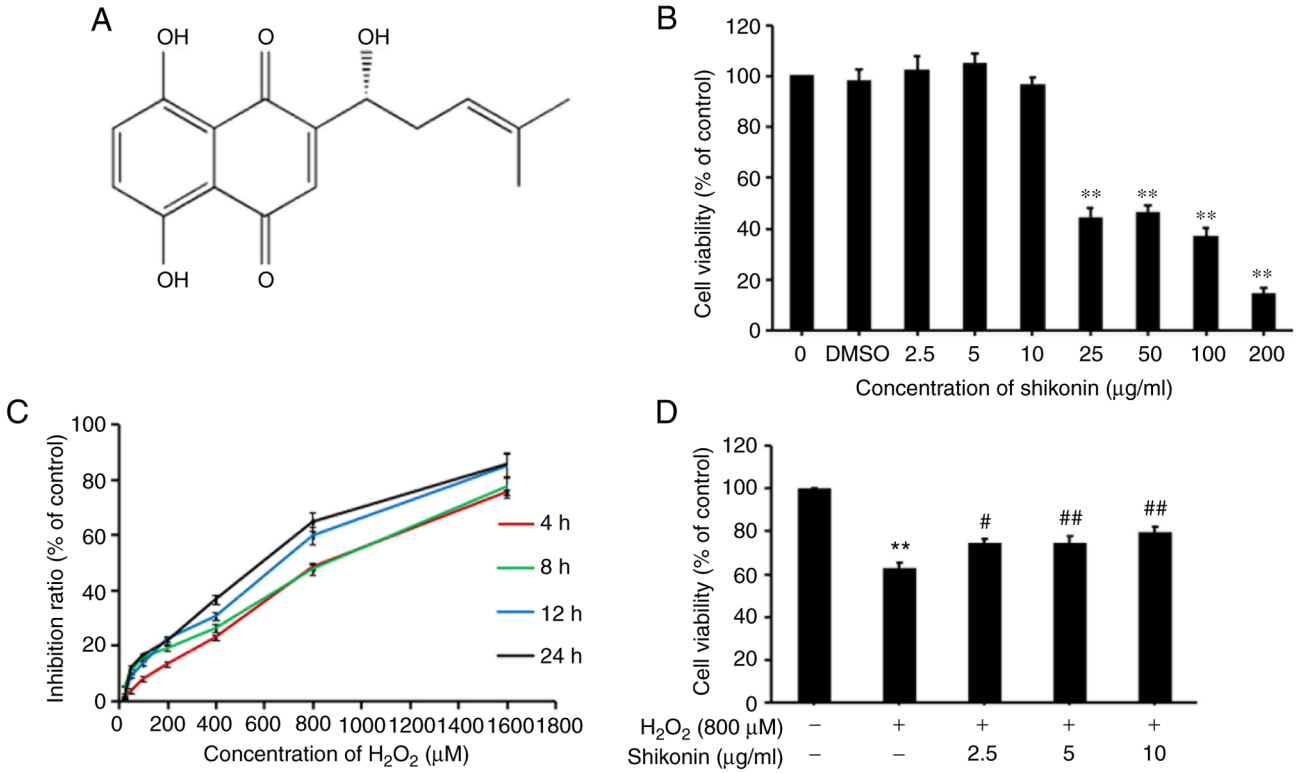

Figure 1. Effects of shikonin and H_2O_2 on cell viability. (A) The chemical structure of shikonin. (B) Cells were treated with different concentrations of shikonin for 24 h. (C) Cells were exposed to different concentrations of H_2O_2 for 4, 8, 12 and 24 h. (D) Cells were pretreated with different concentrations of shikonin for 6 h and then treated with 800 μM H_2O_2 for 24 h. Data are presented as the mean ± SD from at least three independent experiments. **P<0.01 vs. control; $^{\#}$P<0.05 and $^{\#\#}$P<0.01 vs. H_2O_2.

Cytochrome *c* (cat. no. 4280; Cell Signaling Technology, Inc.; 1:1,000), Bax (cat. no. 5023; Cell Signaling Technology, Inc.; 1:1,000), Bcl-2 (cat. no. 3498; Cell Signaling Technology, Inc.; 1:1,000) and β-actin (cat. no. 4970; Cell Signaling Technology, Inc.; 1:1,000) at 4˚C overnight. Following primary incubation, the membranes were incubated with a horseradish peroxidase-conjugated polymer-tagged secondary antibody (cat. no. 7074; Cell Signaling Technology, Inc.; 1:5,000) for 2 h at room temperature. Protein bands were visualized using ECL reagent (Thermo Fisher Scientific, Inc.) and protein expression was semi-quantified using Image J software (National Institutes of Health, version 1.8.0) with β-actin as the loading control.

Statistical analysis. Data are presented as the mean ± SD. All experiments were performed in triplicate. Statistical analyses were performed using SPSS software (version 19.0; IBM Corp.). Comparisons among multiple groups were analyzed using the one-way analysis of variance (ANOVA) test and Dunnett's post hoc test. P<0.05 was considered to indicate a statistically significant difference.

Results

Shikonin attenuates H_2O_2-induced decreases in HT29 cell viability. To identify suitable H_2O_2 and shikonin concentrations and durations of action, an MTT assay was performed to evaluate cell cytotoxicity. The results indicated that H_2O_2 caused marked cytotoxicity in HT29 cells at 800 μM compared with 0 μM, and when cells treated with 800 μM H_2O_2, the cell inhibition rates at 4, 8, 12 and 24 h were 22.91, 26.12, 30.30 and 36.64%, respectively (Fig. 1C). Therefore, cells treated with 800 μM H_2O_2 for 24 h were selected for subsequent experiments. Subsequently, the cytotoxic effect of shikonin at different concentrations was investigated by performing an MTT assay. Compared with the 0 μg/ml shikonin, no significant cytotoxic effect was observed in cells treated with 2.5, 5 and 10 μg/ml shikonin alone. Therefore, three concentrations of shikonin were used in subsequent experiments (Fig. 1B). Finally, to assess the effects of shikonin against H_2O_2-induced cytotoxicity, HT29 cells were pretreated with different concentrations of shikonin (2.5, 5 and 10 μg/ml) for 6 h, and then co-treated with 800 μM H_2O_2 for 24 h. The results indicated that the cell viability of the shikonin group gradually increased compared with the H_2O_2 group in a dose-dependent manner, suggesting that shikonin reversed H_2O_2-induced decreases in cell viability (Fig. 1D).

Shikonin attenuates H_2O_2-induced HT29 cell apoptosis. To assess the protective effects of shikonin against H_2O_2-induced cell apoptosis, flow cytometry was performed to detect apoptosis following Annexin V and propidium iodide staining (Fig. 2). Compared with the control group, the percentage of total apoptotic cells was significantly increased in the H_2O_2 group. By contrast, the shikonin groups displayed a significantly decreased percentage of apoptotic cells compared with the H_2O_2 group, indicating that shikonin inhibited H_2O_2-induced cell apoptosis.

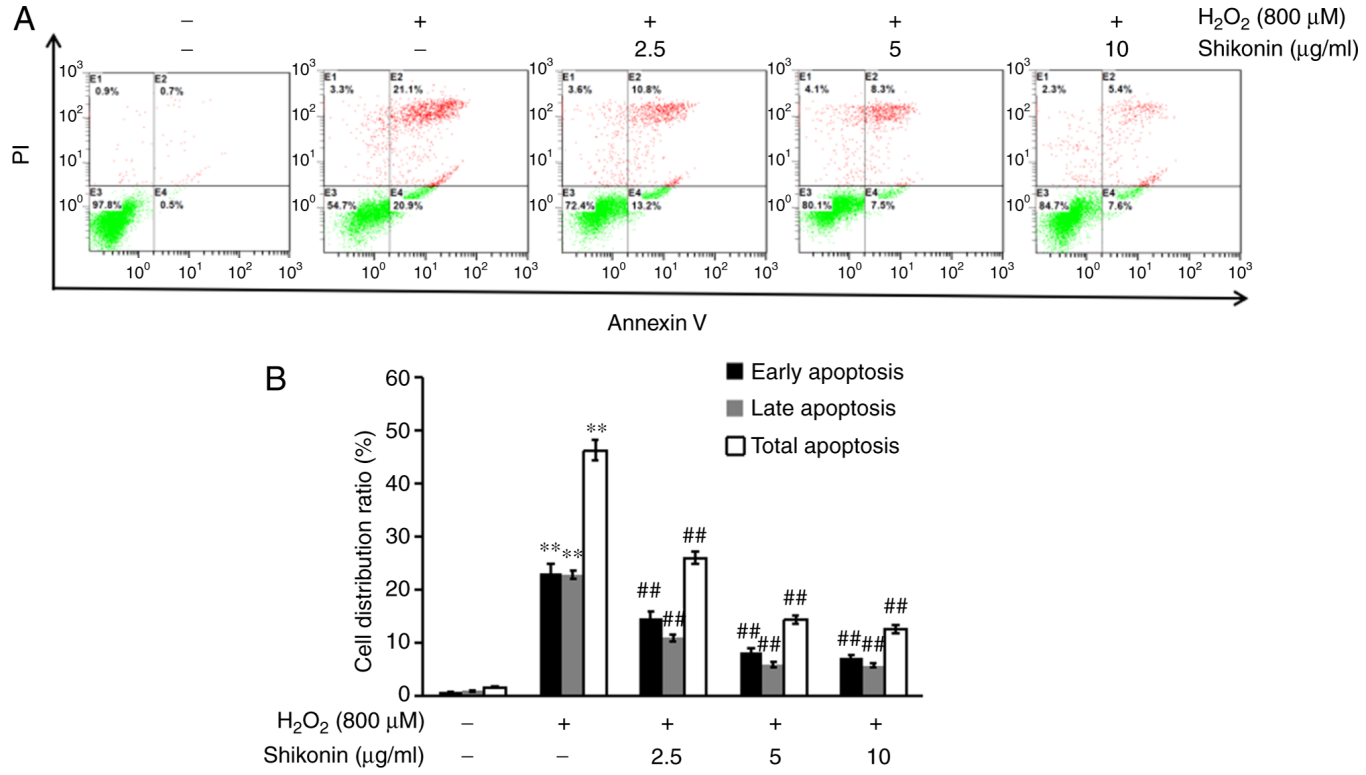

Figure 2. Effect of shikonin (2.5, 5 and 10 μg/ml) on H_2O_2-induced cell apoptosis. HT29 cell apoptosis was (A) assessed via flow cytometry and (B) quantified. Data are presented as the mean ± SD from at least three independent experiments. **P<0.01 vs. control; $^{\#\#}$P<0.01 vs. H_2O_2.

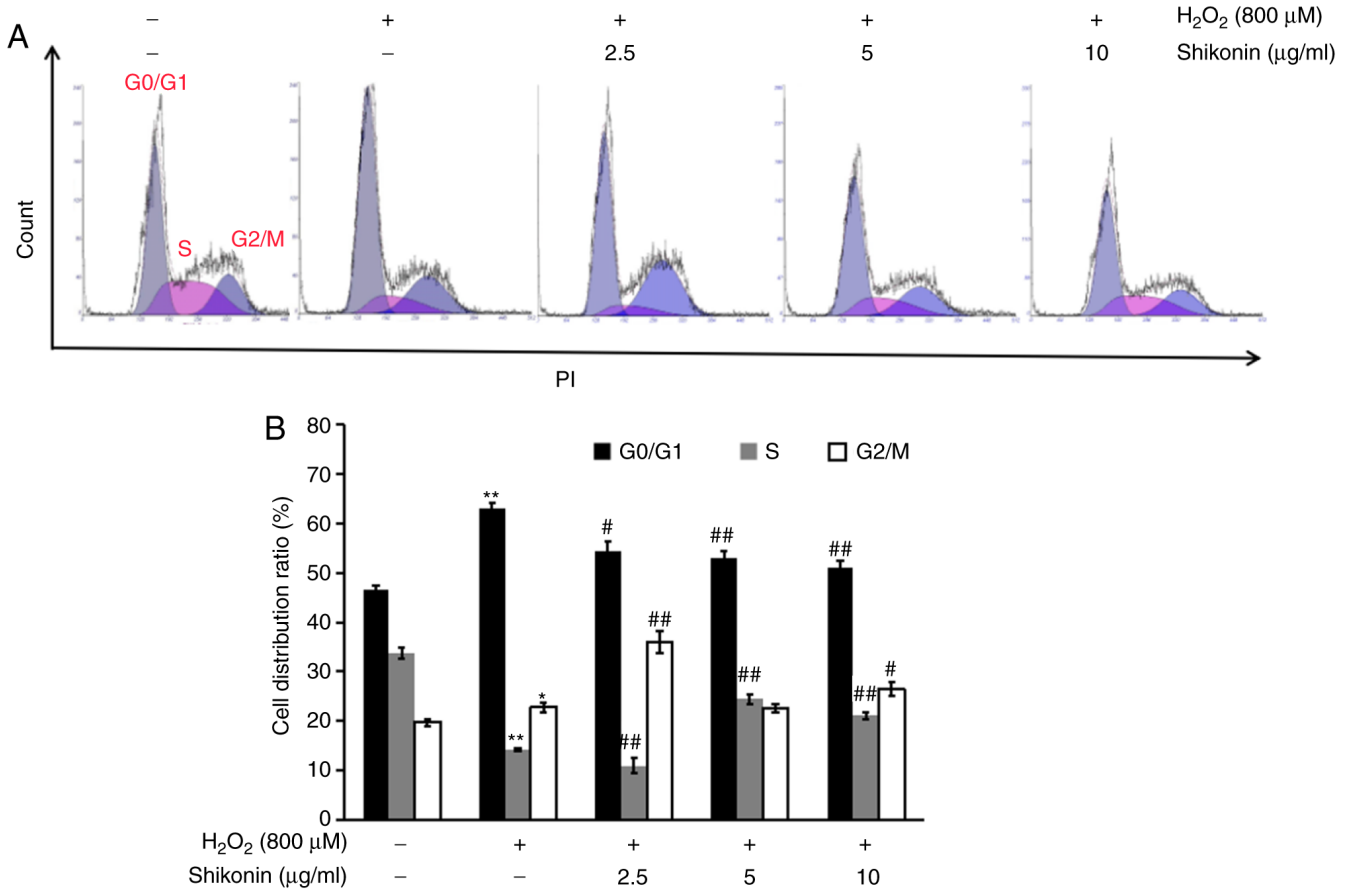

Figure 3. Effects of shikonin (2.5, 5 and 10 μg/ml) on H_2O_2-induced G_0/G_1 cell cycle arrest in HT29 cells. HT29 cell cycle distribution was (A) assessed via flow cytometry and (B) quantified. Data are presented as the mean ± SD from at least three independent experiments. *P<0.05 and **P<0.01 vs. control; $^{\#}$P<0.05 and $^{\#\#}$P<0.01 vs. H_2O_2.

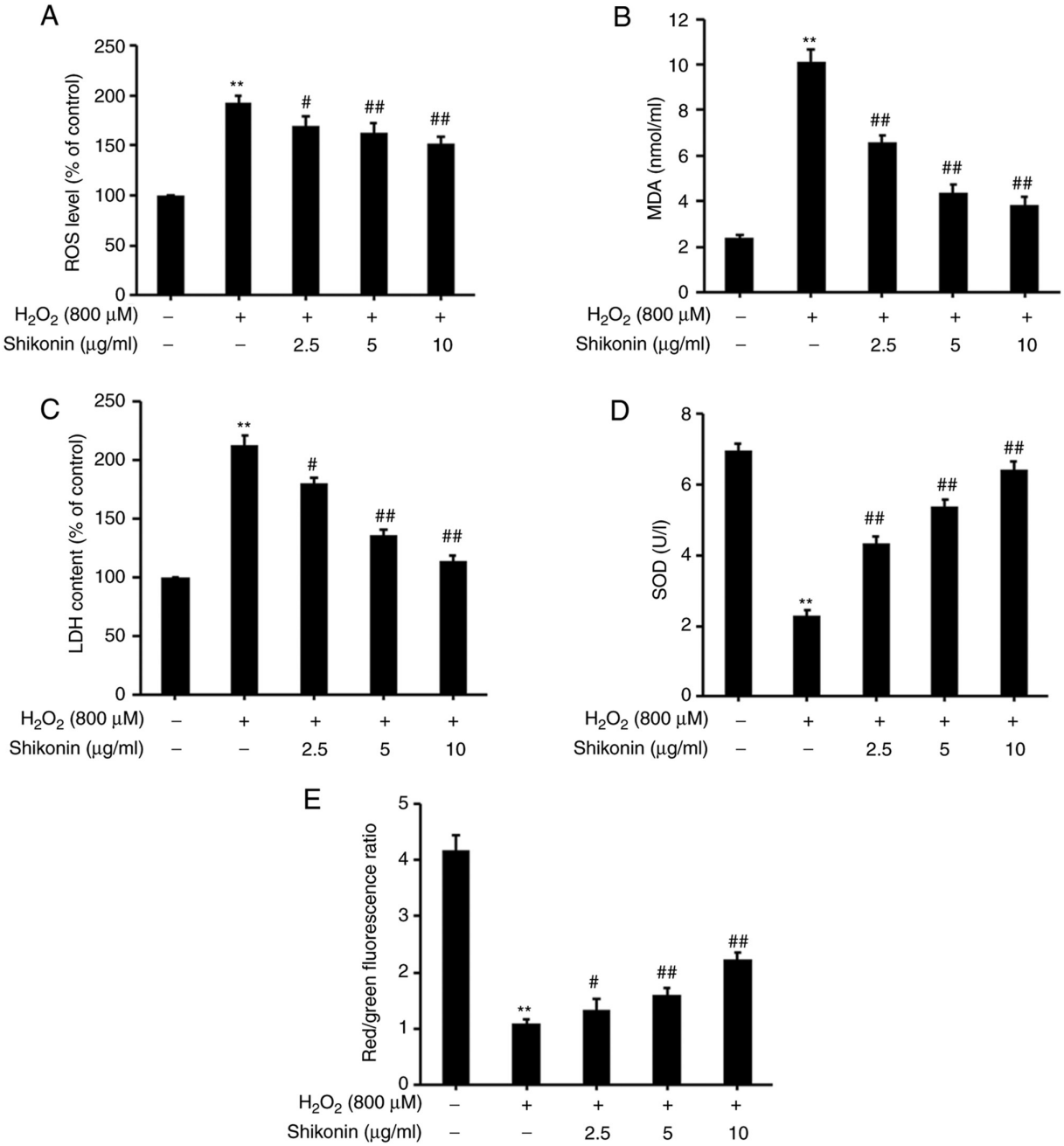

Figure 4. Protective effects of shikonin (2.5, 5 and 10 μg/ml) on H_2O_2-induced oxidative stress injury of HT29 cells. (A) Intracellular ROS levels were assessed via DCFH-DA fluorescence. (B) MDA activity was measured using an MDA assay kit. (C) Extracellular LDH levels were measured using an LDH assay kit. (D) SOD activities were measured using a SOD assay kit. (E) Evaluation of mitochondrial membrane potential was determined by conducting JC-1 staining. Data are presented as the mean ± SD from at least three independent experiments. **P<0.01 vs. control; $^{\#}$P<0.05 and $^{\#\#}$P<0.01 vs. H_2O_2. ROS, reactive oxygen species; DCFH-DA, 2',7'-dichlorofluorescin diacetate fluorescence; MDA, malondialdehyde; LDH, lactate dehydrogenase; SOD, superoxide dismutase.

Shikonin suppresses H_2O_2-induced G_0/G_1 cell cycle arrest in HT29 cells. To investigate the mechanism underlying the effects of shikonin on H_2O_2-mediated inhibition of cell proliferation, the effects of shikonin on regulating the cell cycle in H_2O_2-treated HT29 cells were evaluated by conducting flow cytometry. Compared with the control group, an increased percentage of G_0/G_1 cells and a decreased proliferation index (PI) [PI;PI (%)=S phase (%) + G_2/M phase (%)] were observed in the H_2O_2 group (Fig. 3). The PI gradually increased in a dose-dependent manner in the shikonin groups compared with the H_2O_2 group. The results suggested that shikonin suppressed H_2O_2-induced G_0/G_1 cell cycle arrest.

Shikonin inhibits H_2O_2-induced increases in ROS and MDA levels in HT29 cells. The levels of intracellular ROS as a major oxidant were assessed by conducting a DCFH-DA assay (Fig. 4A). Following exposure to H_2O_2, the levels of intracellular ROS were significantly increased compared with the control group. However, significantly lower levels of intracellular ROS were observed in the shikonin groups compared with the H_2O_2 group.

MDA is a biomarker of oxidative stress (24), and MDA activity was measured using an MDA assay kit (Fig. 4B). Upon H_2O_2 exposure, MDA levels were significantly increased compared with the control group. MDA levels in the shikonin

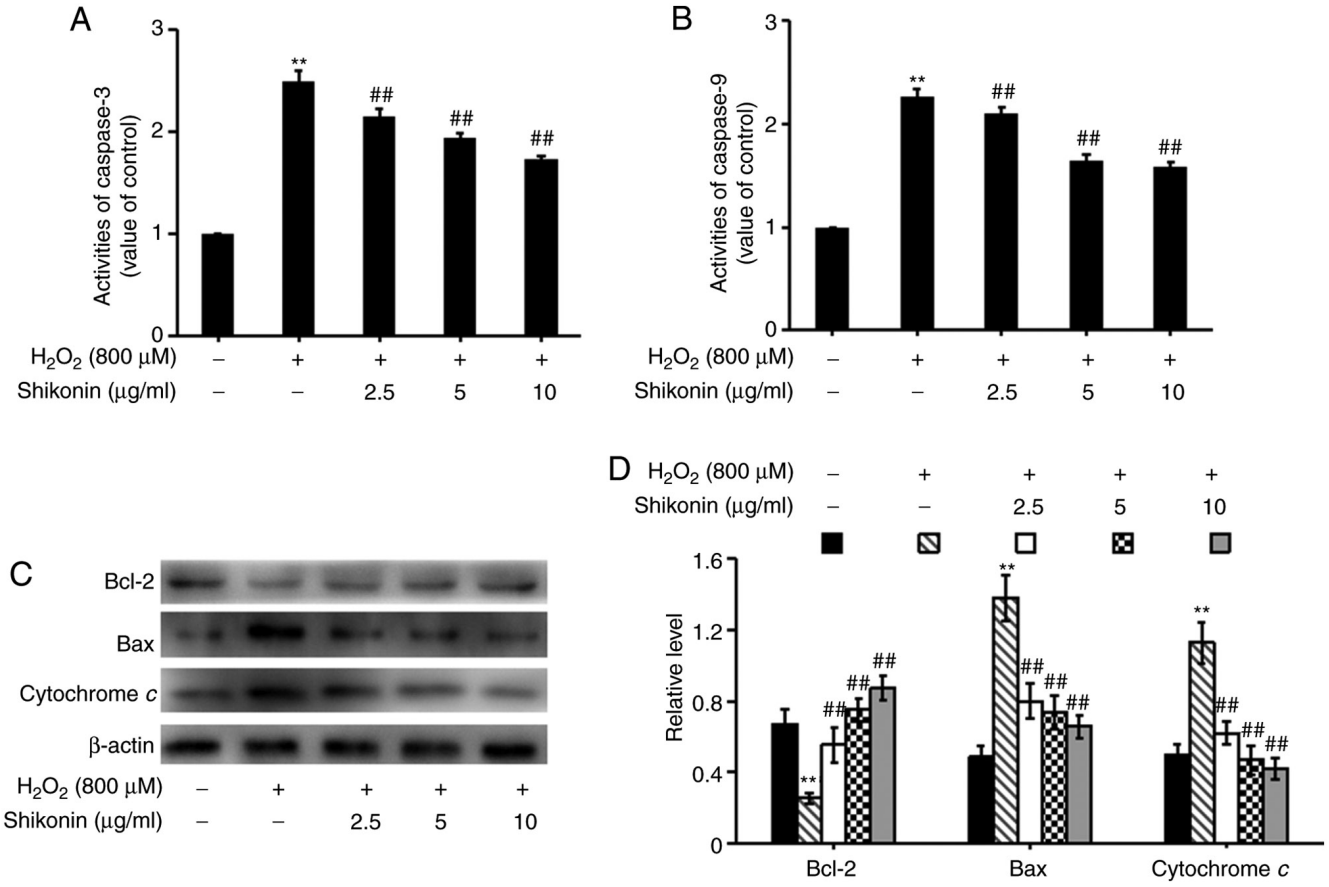

Figure 5. Effects of shikonin (2.5, 5 and 10 μg/ml) on H_2O_2-induced activation of the mitochondria-mediated apoptosis pathway in HT29 cells. (A) Caspase-3 activity was measured using a caspase-3 colorimetric assay kit. (B) Caspase-9 activity was measured using a caspase-9 colorimetric assay kit. (C) Bcl-2, Bax, cytochrome c and β-actin protein expression levels were (C) determined via western blotting and (D) semi-quantified. Data are presented as the mean ± SD from at least three independent experiments. **P<0.01 vs. control; $^{\#\#}$P<0.01 vs. H_2O_2.

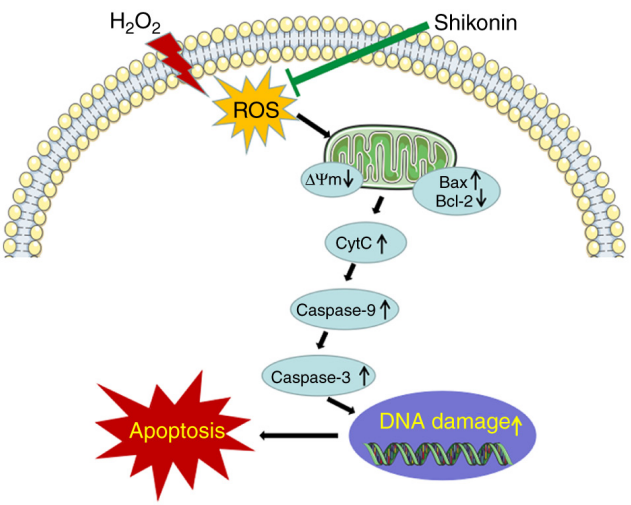

Figure 6. Potential cytoprotective mechanism underlying the effects of shikonin against H_2O_2-induced oxidative injury via elimination of ROS, attenuation of DNA damage and inhibition of mitochondria mediated apoptosis. ROS, reactive oxygen species; CytC, cytochrome *c*.

groups gradually decreased in a dose-dependent manner compared with the H_2O_2 group. The results suggested that shikonin inhibited H_2O_2-induced intracellular ROS and MDA accumulation.

Shikonin prevents H_2O_2-induced LDH release in HT29 cells. LDH, a glycolytic enzyme, has been suggested to be a key indicator of cell membrane integrity (12). The effects of shikonin on extracellular LDH levels were measured using an LDH assay kit (Fig. 4C). LDH levels were significantly increased in H_2O_2-treated cells compared with the control group. LDH levels in the shikonin groups were significantly lower compared with the H_2O_2 group. The results indicated that shikonin prevented H_2O_2-induced LDH release.

Shikonin inhibits H_2O_2-induced decreases in SOD levels in HT29 cells. SOD is an important antioxidant enzyme in the mitochondria and serves as a defense against oxidative stress (25). SOD activity was measured using a SOD assay kit (Fig. 4D). SOD levels were significantly decreased in the H_2O_2 group compared with the control group. Compared with the H_2O_2 group, SOD levels gradually increased with increasing shikonin concentrations, suggesting that shikonin prevented H_2O_2-mediated decreases in SOD levels.

Shikonin reverses H_2O_2-induced decreases in mitochondrial membrane potential in HT29 cells. Mitochondrial dysfunction has been reported to be associated with H_2O_2-inducedcell apoptosis (26). To further investigate whether shikonin was associated with H_2O_2-induced mitochondrial dysfunction, the mitochondrial membrane potential in HT29 cells

was investigated by performing a JC-1 dye assay (Fig. 4E). Mitochondrial membrane potential levels were significantly decreased in HT29 cells exposed to H_2O_2 compared with the control group. However, a gradual increase in mitochondrial membrane potential levels was observed in cells pretreated with 2.5, 5 or 10 μg/ml shikonin compared with the H_2O_2 group. Therefore, the results indicated that shikonin reversed H_2O_2-mediated decreases in mitochondrial membrane potential.

Shikonin protects HT29 cells against oxidative stress via inhibition of the mitochondrial apoptosis pathway. To assess whether the mitochondrial apoptosis pathway was involved in promoting the effects of shikonin on cell apoptosis, caspase-3 and caspase-9 levels were measured by performing caspase assays (Fig. 5A and B), and the expression levels of Bcl-2, Bax and cytochrome *c* were determined via western blotting (Fig. 5C and D). Following exposure to H_2O_2, Bcl-2 expression levels were significantly decreased, whereas the activity levels of caspase-3 and caspase-9, as well as the expression levels of Bax and cytochrome *c* were significantly increased compared with the control group. The opposite effects were observed in H_2O_2-treated cells pretreated with shikonin (2.5, 5 and 10 μg/ml). Therefore, the results indicated that shikonin protected HT29 cells against oxidative stress via inhibiting the mitochondrial apoptosis pathway in a concentration-dependent manner.

The possible mechanism of shikonin. The potential cytoprotective mechanism underlying the effects of shikonin against H_2O_2-induced oxidative injury via elimination of ROS, attenuation of DNA damage and inhibition of mitochondria mediated apoptosis (Fig. 6).

Discussion

The present study investigated the protective effects of shikonin on H_2O_2-induced oxidative stress in HT29 cells and explored the mechanism underlying the antioxidative effects of shikonin. Shikonin protected against H_2O_2-induced injury in HT29 cells. Consistent with previous reports, the results of the MTT assay indicated that H_2O_2 displayed cytotoxicity in HT29 cells in a concentration-dependent manner (23,27). Moreover, the flow cytometry results demonstrated that H_2O_2 decreased the PI and increased apoptosis in HT29 cells compared with the control group. H_2O_2-mediated decreases in HT29 cell viability were significantly reversed following pretreatment with shikonin. In addition, shikonin also increased the PI and attenuated apoptosis in H_2O_2-treated HT29 cells. The results suggested that shikonin exerted a protective effect against H_2O_2-induced oxidative stress in HT29 cells.

ROS is an important antioxidant enzyme in the mitochondria, which has been demonstrated to serve a critical role in DNA oxidative damage and is also a major oxidant *in vivo* (28-32). Under normal physiological conditions, SOD and other antioxidants can scavenge ROS, resulting in a dynamic equilibrium between the generation and removal of ROS (33,34). MDA is a biomarker of oxidative stress that reflects ROS-induced membrane lipid peroxidation (35,36). As cells are damaged, high amounts of ROS accumulate and induce lipid peroxidation on the membrane to produce MDA. MDA can damage the membrane structure, eventually leading to enhanced membrane permeability, increased generation of intracellular enzymes and the release of LDH (37). The results of the present study indicated that shikonin significantly decreased the levels of intracellular ROS and LDH, decreased MDA levels and restored SOD activity in H_2O_2-treated cells. Collectively, the results indicated that shikonin reduced oxidative stress, at least in part via its antioxidant activity and ROS elimination.

Mitochondria are the major physiological sources of ROS, and H_2O_2 may increase oxidative damage by inducing mitochondrial dysfunction, resulting in increased ROS production and induction of mitochondrial membrane potential loss (38,39). In the present study, shikonin significantly inhibited H_2O_2-induced mitochondrial membrane potential loss, indicating that shikonin may display a protective effect against H_2O_2-induced oxidative damage via the mitochondrial pathway.

The mitochondrial, death receptor and endoplasmic reticulum signaling pathways are three major apoptosis signaling pathways that are dependent on caspases (40). In the mitochondria-mediated apoptosis pathway, oxidative stress induces the opening of mitochondrial permeability transition pores. Mitochondria release the apoptotic promoter and cytochrome *c*, which activates the caspase cascade and induces apoptosis (40). The Bcl-2 family, including antiapoptotic regulator Bcl-2, proapoptotic regulator Bax and death proteins, is a major regulator of the mitochondrial apoptotic pathway (41). The antiapoptotic mechanism underlying Bcl-2 is direct antioxidation, whereas Bax is the primary mediator of the mitochondrial apoptosis pathway (42). Activated Bax leads to the release of cytochrome *c* and mediates apoptosis induced by the mitochondrial pathway (43). It has been reported that Bcl-2 upregulation and Bax downregulation can alleviate the occurrence of apoptosis (44). The results of animal experiments also demonstrated that ischemia-reperfusion injury and heart failure can cause Bcl-2 downregulation and increase apoptosis, whereas Bcl-2 expression in myocardial cells is upregulated following ischemic preconditioning treatment (45). Consistent with previous reports, the present study also suggested that shikonin upregulated Bcl-2 expression and downregulated Bax expression in H_2O_2-induced HT29 cells.

Caspases are a family of proteases that serve important roles in the process of apoptosis (46). Caspase-9 is upstream of the apoptotic signal transduction process and activates caspase-3 (40). Caspase-3-mediated protein cleavage is an important component of the molecular mechanism underlying apoptosis. In addition, caspase-3 serves a key role in nuclear apoptosis, including chromatin condensation and DNA fragmentation (40,47). The present study examined the activities of caspase-3 and caspase-9, and the results indicated that the shikonin group displayed significant downregulation of caspase-3 and caspase-9 activities compared with the H_2O_2 group, suggesting that shikonin protected against H_2O_2-induced oxidative damage of HT29 cells from mitochondrial machinery mediated by the apoptotic pathway.

In conclusion, the present study indicated that the protective effects of shikonin against H_2O_2-induced oxidative stress injury were activated at least in part via removing ROS, ameliorating mitochondrial dysfunction, attenuating DNA oxidative

damage and inhibiting mitochondrial pathway-mediated apoptosis. The results suggested a potential mechanism underlying the antioxidant role of shikonin and a new perspective for the rational use of shikonin for the treatment of oxidation damage-associated diseases in the future.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

YZ and AQ designed the study, acquired the data and drafted the manuscript. QH performed the statistical analysis and drafted the manuscript. JZ and LL performed the statistical analysis. JZ revised the manuscript for intellectual content. KC acquired the data. CC designed the study and drafted the manuscript. All authors have read and approved the final manuscript. CC and YZ confirm the authenticity of all the raw data.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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