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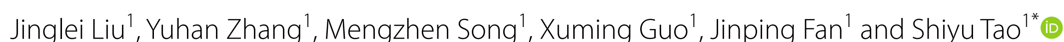
Taurodeoxycholic acid alleviates diquat-induced intestinal barrier function injury in mice through the upregulation of Nrf2-mediated signaling pathway



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Abstract

Oxidative stress is an important contributor to gastrointestinal diseases in multiple ways. Taurodeoxycholic acid (TDCA) is a metabolite of bile acids and has anti-inflammatory and protective effects on the intestinal tract. However, whether TDCA can alleviate oxidative stress in the intestine is still unclear. Here, we investigated the effects of TDCA on diquat-induced oxidative stress in the jejunum and its mechanism. The results revealed that TDCA increased the concentrations of antioxidant enzymes in the serum, jejunal tissue and intestinal epithelial cells of the mice, as did the expression of tight junction-associated proteins and the Nrf2 protein in the jejunal epithelial tissue and intestinal epithelial cells. We then explored the mechanism of Nrf2 with ML385 (a specific Nrf2 inhibitor). The results showed that after ML385 treatment, the levels of antioxidant enzymes were significantly decreased in the serum, jejunum, and intestinal epithelial tissues of the mice. The expression of tight junction proteins in jejunum epithelial tissues and intestinal epithelial cells was also decreased. In conclusion, our study suggests that TDCA alleviates oxidative stress to improve intestinal barrier function through the Nrf2-mediated signaling pathway. These findings help elucidate the role of TDCA in protecting the intestinal barrier and its mechanism of action, providing insights for the prevention and treatment of intestinal diseases caused by oxidative stress.

Keywords Taurodeoxycholic acid, Diquat, Jejunum, Oxidative stress, Nrf2

Introduction

Oxidative stress is a common phenomenon caused by an imbalance between oxidants and antioxidants in organisms, which may result in damage to some or all biological systems (Forman and Zhang 2021). Oxidative stress (OS) is an important factor that contributes to various digestive diseases, such as gastroduodenal ulcers, gastrointestinal cancers, and inflammatory bowel disease. OS can lead to or accelerate the development of diseases in a direct or indirect manner (Bhattacharyya et al. 2014; Forman and Zhang 2021; Sahoo et al. 2023). The intestinal mucosa is the protective barrier of the intestinal tract. Tight junctions between intestinal epithelial cells, such as transmembrane proteins (occludin and claudin family) and peripheral membrane proteins (ZO-1), play a vital role in maintaining the normal function of the intestinal barrier. Under most conditions, the intestinal tract has natural defense mechanisms against oxidative stress. However, under conditions such as exacerbated ROS production, the permeability of the intestinal epithelial barrier is increased by disrupting the tight junctions

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between the intestinal epithelium, which in turn leads to intestinal lesions (Diaz de Barboza et al. 2017; Tang et al. 2023; Zhao et al. 2021). Therefore, alleviating oxidative stress through various pathways is vital for maintaining intestinal barrier function and curing intestinal diseases.

Gut microbes metabolize some of the primary bile acids produced by the liver into secondary bile acids (Wahlstrom et al. 2016), regulating the function of the intestinal tract and other organs and further mediating some physiological processes, such as metabolism, immunity, and inflammation. It has been reported that primary bile acid chenodeoxycholic acid (CDCA) promotes cell proliferation and intestinal development by hindering apoptosis and increasing the cellular antioxidant capacity (Xu et al. 2022a). The secondary bile acids ursodeoxycholic acid (UDCA) and glycoursodeoxycholic acid (GUDCA) protect brain nerves by decreasing apoptosis and mitigating oxidative stress (Huang et al. 2022). Tauroursodeoxycholic acid (TUDCA) protects intestinal health by strengthening the intestinal barrier and immune system (Song et al. 2022). Taurodeoxycholic acid (TDCA) is one of the metabolites of bile acid (Bai et al. 2022) and is a conjugate of deoxycholic acid and taurine, which is often in the form of a sodium salt (Chiang 2003). Most studies have demonstrated that TDCA protects intestinal health by maintaining the height of the intestinal villi. TDCA is also beneficial in relieving intestinal inflammation (Perrone et al. 2010; Zahiri et al. 2011; Zou et al. 2023). However, it is still unclear whether TDCA has a mitigating effect on intestinal oxidative stress, and the mechanism of action of TDCA in mitigating intestinal oxidative stress remains elusive.

This study aimed to investigate the mitigating effect of TDCA on oxidative stress in mouse jejunum and intestinal epithelial cells via diquat-induced oxidative stress both in mouse tissue and in a mouse intestinal epithelial cell line as a model. The potential mechanism by which TDCA attenuates diquat-induced intestinal oxidative stress was further explored via the Nrf2-specific inhibitor ML385.

Results

Effects of different concentrations of TDCA on diquat-induced changes in the intestinal morphology of the jejunum in mice

To explore the effect of TDCA on intestinal oxidative stress, we treated mice with three concentrations of TDCA, 5 mg/kg, 10 mg/kg, 15 mg/kg, and treated with diquat on day 10 (Fig. 1A). The height of the jejunal villi significantly decreased in diquat (DQ) group compared to the control (CON) group ($P<0.001$). There was no difference in the depth of the crypts or the ratio of jejunal villus height to crypt depth ($P>0.05$) between the DQ group and the CON group. Compared with the DQ group, the 10 mg/kg ($P<0.01$) and 15 mg/kg ($P<0.001$) TDCA groups presented significantly greater jejunal villus heights, whereas no change was observed in the 5 mg/kg TDCA group ($P>0.05$). None of the three TDCA concentrations significantly affected jejunal crypt depth or the ratio of villus height to crypt depth ($P>0.05$) (Fig. 1B-E).

Effects of different concentrations of TDCA on oxidation and antioxidant indices in the serum and jejunum of mice

To reveal the effects of different concentrations of TDCA on oxidative indices, we examined the oxidative and antioxidative indices in the serum and jejunal tissues of the mice. The results of the analysis indicated that, compared with that in the CON group, the malondialdehyde (MDA) level in the serum (Fig. 2A-E) was significantly greater in the DQ group ($P<0.0001$). The contents of superoxide dismutase (SOD) ($P<0.01$), glutathione peroxidase (GSH-Px) ($P<0.0001$), catalase (CAT) ($P<0.0001$) and total antioxidant capacity (T-AOC) ($P<0.0001$) decreased significantly in the DQ group compared to the CON group. Compared with those in the DQ group, the serum levels of MDA, SOD and CAT in the DQ+TDCA (5) group did not differ ($P>0.05$). Compared to the DQ group, there was a significant increase in the levels of GSH-Px ($P<0.001$ in 5 mg/kg TDCA; $P<0.0001$ in 10 mg/kg TDCA) and T-AOC ($P<0.05$) in the 5 mg/kg and 10 mg/kg TDCA-treated mice, whereas there was a significant decrease in the serum levels of MDA ($P<0.01$) and a significant increase in the levels of SOD ($P<0.05$), GSH-Px ($P<0.0001$), CAT ($P<0.05$), and T-AOC ($P<0.0001$) in the 15 mg/kg TDCA-treated mice.

Compared with the CON group, the DQ group presented significantly greater MDA levels ($P<0.0001$) and significantly lower SOD ($P<0.01$), GSH-Px ($P<0.01$), CAT ($P<0.0001$) and T-AOC ($P<0.001$) levels in the jejunum (Fig. 2F-J). Compared with that in the DQ group, the MDA level in the DQ+TDCA (5) group was significantly lower ($P<0.01$). There was no change in the content of SOD, GSH-Px, CAT or T-AOC in the jejunum of 5 mg/kg TDCA-treated mice compared to DQ group ($P>0.05$). In the jejunum of 10 mg/kg TDCA-treated mice, the content of MDA was significantly decreased ($P<0.05$), the contents of SOD and T-AOC were unchanged ($P>0.05$), and the contents of GSH-Px ($P<0.05$) and CAT ($P<0.05$) were significantly increased compared to DQ group. In 15 mg/kg TDCA-treated mice, there was a significant decrease in MDA ($P<0.0001$) and a significant increase in SOD ($P<0.01$), GSH-Px ($P<0.01$), CAT ($P<0.01$) and T-AOC ($P<0.001$) in the jejunum compared to DQ group (Fig. 2F-J). In summary, 15 mg/kg TDCA was the most

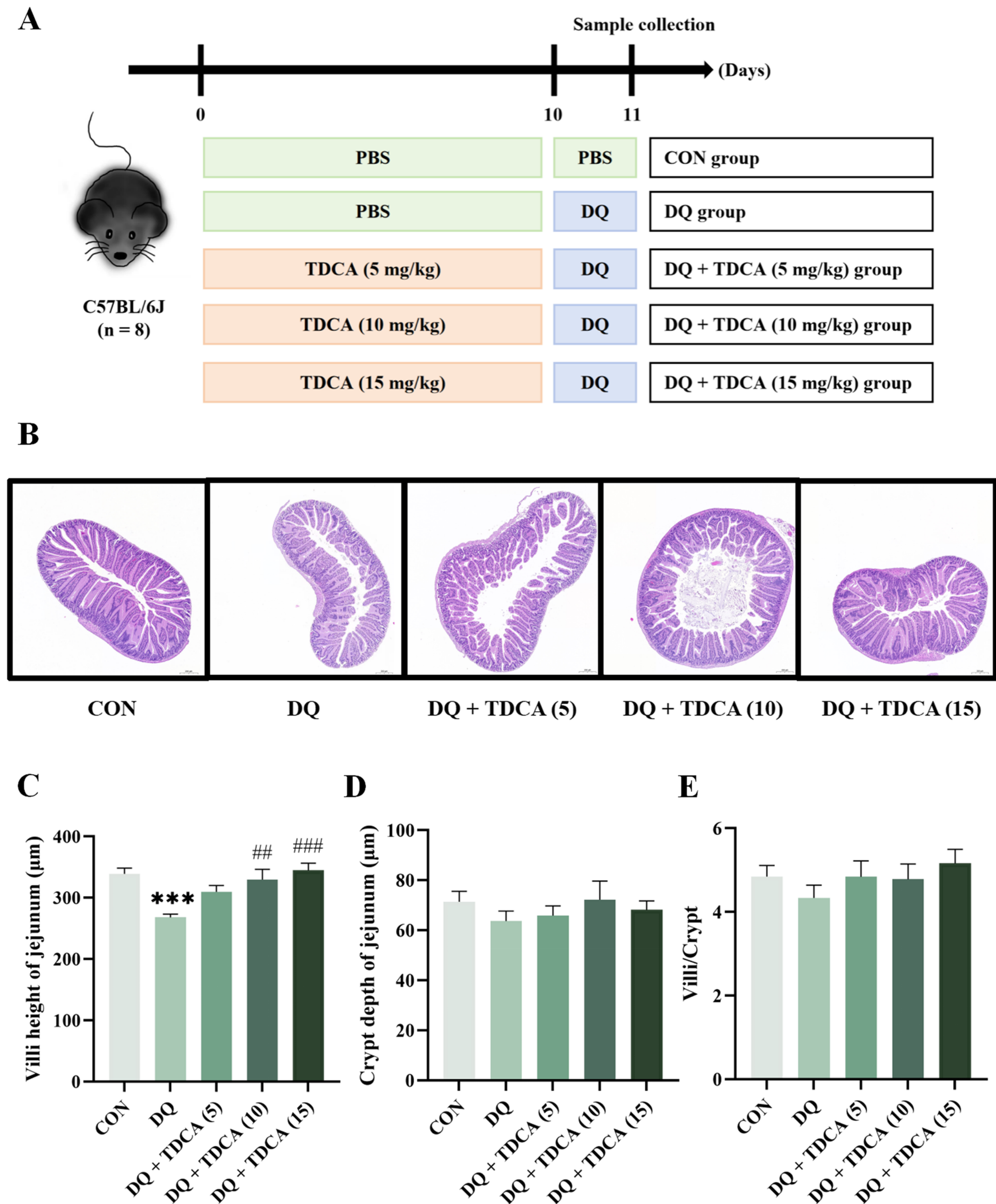


Fig. 1 Effects of different concentrations of TDCA on diquat-induced changes in the intestinal morphology of the jejunum in mice. **A** Experimental grouping scheme. **B** H&E staining of the jejunum. **C**-**E** Jejunal villus height, jejunal crypt depth and ratio of villus height to crypt depth in each group. $n = 8$ for B-E. All the data are presented as the means ± SEMs. When the DQ group was compared with the CON group, ***$P < 0.001$; when the DQ + TDCA group was compared with the DQ group, ##$P < 0.01$, ###$P < 0.001$. CON, control; DQ, diquat; TDCA, taurodeoxycholic acid; TDCA (5) indicates treatment with 5 mg/kg TDCA; TDCA (10) indicates treatment with 10 mg/kg TDCA; and TDCA (15) indicates treatment with 15 mg/kg TDCA

effective and mitigating concentration that was selected for subsequent experiments.

Effects of TDCA on diquat-induced oxidative stress in the jejunum and the expression of intestinal barrier function-related proteins in mice

To investigate the effect of TDCA on intestinal barrier damage caused by diquat in mice, we examined jejunal Nrf2 protein and intestinal interepithelial tight junction protein levels in mice. The results of the analysis revealed that the protein levels of Nrf2 ($P<0.0001$), ZO-1 ($P<0.01$) and Occludin ($P<0.001$) were significantly lower in the DQ group than in the CON group. The protein levels of Nrf2 ($P<0.0001$), ZO-1 ($P<0.01$) and Occludin ($P<0.001$) were significantly greater in the DQ+TDCA (15) group than in the DQ group (Fig. 3).

Effects of different concentrations of TDCA on diquat-induced oxidative stress and barrier function in intestinal epithelial cells in vitro

To explore the effect of TDCA on oxidative stress in intestinal epithelial cells, we treated MODE-K cells with three concentrations of TDCA, 10 μM, 30 μM and 50 μM, for 24 h and then treated them with diquat for 6 h (Fig. 4A). To investigate the effects of TDCA on the oxidative indices of intestinal epithelial cells, we examined the intracellular oxidative indices, and the results revealed that, compared with those in the CON group, the MDA level in the DQ group was significantly greater ($P<0.01$) (Fig. 4B-F). The contents of SOD ($P<0.05$), GSH-Px ($P<0.05$), CAT ($P<0.05$) and T-AOC ($P<0.05$) significantly decreased in the DQ group compared to the CON group. Compared with those in the DQ group, the contents of MDA, SOD, GSH-Px, CAT and T-AOC in the 10 μM TDCA-treated MODE-K group did not differ ($P>0.05$). In 30 μM TDCA-treated MODE-K cells, compared to the DQ group, there was no change in the contents of MDA, SOD, CAT or T-AOC ($P>0.05$), and the content of GSH-Px significantly increased ($P<0.05$). In 50 μM TDCA-treated MODE-K cells, compared to the DQ group, the MDA level was significantly decreased ($P<0.05$), and the contents of SOD, GSH-Px, CAT and T-AOC were significantly increased ($P<0.05$). In summary, we chose 50 μM TDCA for subsequent experiments.

To investigate the protective effect of TDCA on intestinal epithelial cells, we examined the levels of the Nrf2 protein and tight junction protein. The results of the analysis indicated that (Fig. 4G–J) the protein levels of Nrf2 ($P<0.001$), ZO-1 ($P<0.0001$) and Occludin ($P<0.0001$) were significantly lower in the DQ group than in the CON group. The protein levels of Nrf2 ($P<0.01$), ZO-1 ($P<0.0001$) and Occludin ($P<0.0001$) were significantly greater in the DQ + TDCA (50) group than in the DQ group.

TDCA alleviates diquat-induced jejunal intestinal morphological injury in mice through the Nrf2 pathway

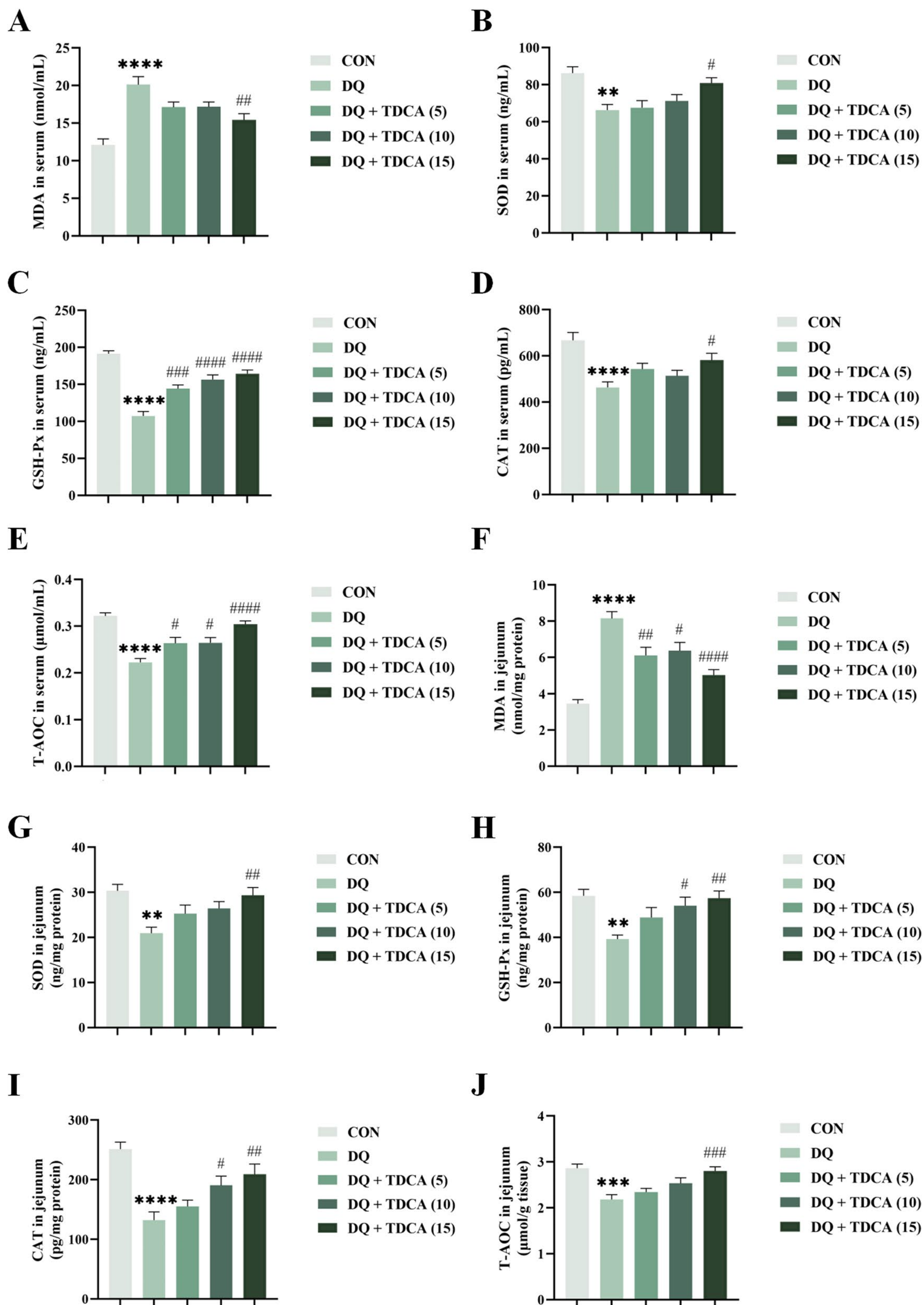
To investigate whether TDCA alleviates jejunal intestinal morphology changes in mice through the Nrf2 pathway, we treated the mice with 15 mg/kg TDCA or 15 mg/kg TDCA+ML385 (an Nrf2-specific inhibitor) and then treated them with diquat on day 10 (Fig. 5A). The jejunal villus height was significantly lower ($P<0.01$) in the DQ group than in the CON group (Fig. 5B-E). There was no change in the depth of the crypt or the ratio of jejunal villus height to crypt depth between CON group and DQ group ($P>0.05$). In contrast to the DQ group, TDCA significantly elevated jejunal villus height in the DQ + TDCA (15) group ($P<0.01$), and there was no change in crypt depth or the ratio of jejunal villus height to crypt depth in the DQ + TDCA (15) group ($P>0.05$). Compared with those in the DQ+TDCA (15) group, no significant changes were found in jejunal villus height, crypt depth or the ratio of jejunal villus height to crypt depth in the DQ+ML385 + TDCA (15) group ($P>0.05$).

TDCA alleviates diquat-induced oxidative stress in the serum and jejunum of mice through the Nrf2 pathway

To explore the effects of TDCA on various oxidative and antioxidant indices through the Nrf2 pathway, we examined the oxidative and antioxidant indices in the serum and jejunal tissues of the mice in each group. Compared with those in the CON group, the MDA level was significantly increased ($P<0.0001$), and the SOD

(See figure on next page.)

Fig. 2 Effects of different concentrations of TDCA on oxidation and antioxidant indices in the serum and jejunum of mice. **A**-**E** MDA, SOD, GSH-Px, CAT, and T-AOC in the serum of each group after DQ induction with 5 mg/kg, 10 mg/kg, or 15 mg/kg TDCA. **F**-**J** MDA, SOD, GSH-Px, CAT, and T-AOC in jejunal tissues from each group after DQ induction with 5 mg/kg, 10 mg/kg, or 15 mg/kg TDCA. $n=8$. All the data are presented as the means ± SEMs. When the DQ group was compared with the CON group, **$P<0.01$, ***$P<0.001$, and ****$P<0.0001$; when the DQ+TDCA group was compared with the DQ group, #$P<0.05$, ##$P<0.01$, ###$P<0.001$, and ####$P<0.0001$. CON, control; DQ, diquat; TDCA, taurodeoxycholic acid; MDA, malondialdehyde; SOD, superoxide dismutase; GSH-Px, glutathione peroxidase; CAT, catalase; T-AOC, total antioxidant capacity; TDCA (5) indicates treatment with 5 mg/kg TDCA; TDCA (10) indicates treatment with 10 mg/kg TDCA; and TDCA (15) indicates treatment with 15 mg/kg TDCA



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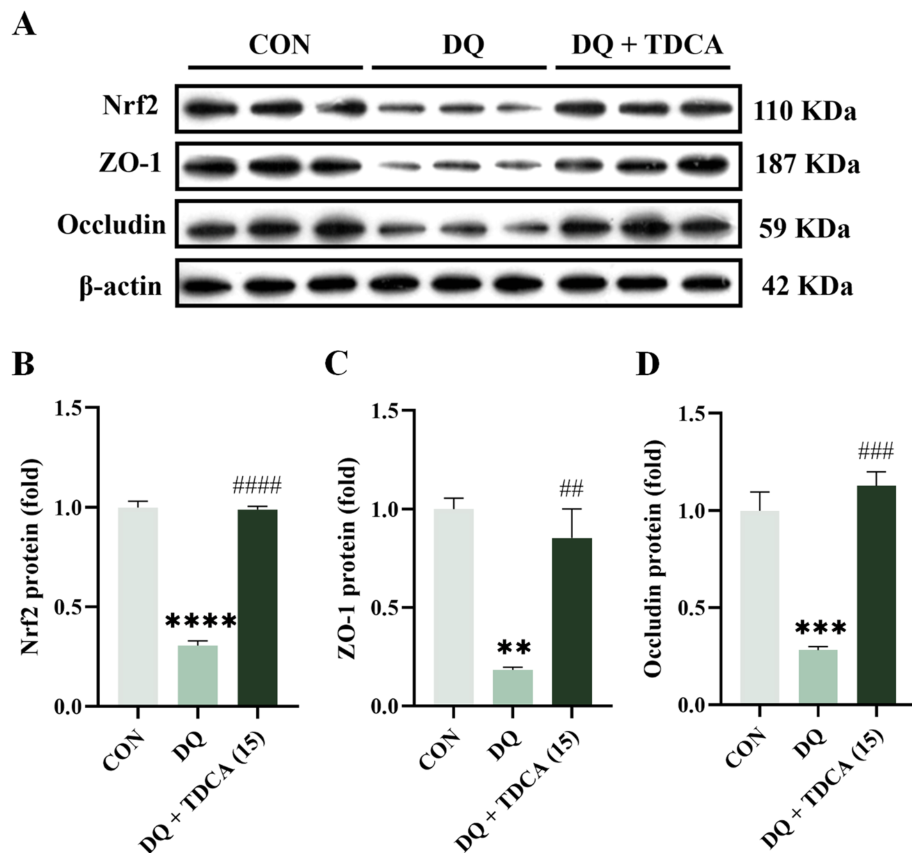


Fig. 3 Effects of TDCA on diquat-induced oxidative stress in the jejunum and the expression of intestinal barrier function-related proteins in mice. **A** Nrf2, ZO-1, and Occludin protein expression was analyzed via western blotting in the CON, DQ, and DQ+TDCA (15) groups. **B**-**D** Expression of the Nrf2, ZO-1 and Occludin proteins in each group. $n=3$. All the data are presented as the means ± SEMs. When the DQ group was compared with the CON group, ** $P<0.01$, *** $P<0.001$, **** $P<0.0001$; when the DQ+TDCA (15) group was compared with the DQ group, ## $P<0.01$, ### $P<0.001$, #### $P<0.0001$. CON, control; DQ, diquat; TDCA, taurodeoxycholic acid; Nrf2, nuclear factor erythroid 2-related factor 2; TDCA (15) indicates treatment with 15 mg/kg TDCA

($P<0.001$), GSH-Px ($P<0.0001$), CAT ($P<0.0001$), and T-AOC ($P<0.0001$) levels were significantly decreased in the serum of the DQ group (Fig. 6A-E). In contrast to those in the serum of DQ group, the MDA level was significantly lower ($P<0.001$), and the contents of SOD ($P<0.01$), GSH-Px ($P<0.0001$), CAT ($P<0.01$), and T-AOC ($P<0.0001$) were significantly greater in the DQ + TDCA (15) group. Compared with those in the serum of DQ + TDCA (15) group, the MDA level increased significantly ($P<0.05$), and the contents of SOD ($P<0.05$), GSH-Px ($P<0.0001$), CAT ($P<0.01$), and T-AOC ($P<0.0001$) decreased significantly in the DQ + ML385 + TDCA (15) group.

Compared with the CON group, the DQ group presented significantly greater MDA levels ($P<0.0001$) and significantly lower SOD ($P<0.01$), GSH-Px ($P<0.0001$),

(See figure on next page.)

Fig. 4 Effects of different concentrations of TDCA on diquat-induced oxidative stress and barrier function in intestinal epithelial cells in vitro. **A** Experimental grouping scheme. **B**-**F** MDA, SOD, GSH-Px, CAT, and T-AOC contents of MODE-K cells in each group. **G** Nrf2, ZO-1, and Occludin protein expression was analyzed via western blotting in the CON, DQ, and DQ+TDCA (50) groups. **H**-**J** Expression of the Nrf2, ZO-1 and Occludin proteins in each group. $n=3$ for B-J. All the data are presented as the means ± SEMs. When the DQ group was compared with the CON group, * $P<0.05$, ** $P<0.01$, *** $P<0.001$, **** $P<0.0001$; when the DQ+TDCA group was compared with the DQ group, # $P<0.05$, ## $P<0.01$, #### $P<0.0001$. CON, control; DQ, diquat; TDCA, taurodeoxycholic acid; MDA, malondialdehyde; SOD, superoxide dismutase; GSH-Px, glutathione peroxidase; CAT, catalase; T-AOC, total antioxidant capacity; Nrf2, nuclear factor erythroid 2-related factor 2; TDCA (10) indicates treatment with 10 μM TDCA; TDCA (30) indicates treatment with 30 μM TDCA; and TDCA (50) indicates treatment with 50 μM TDCA

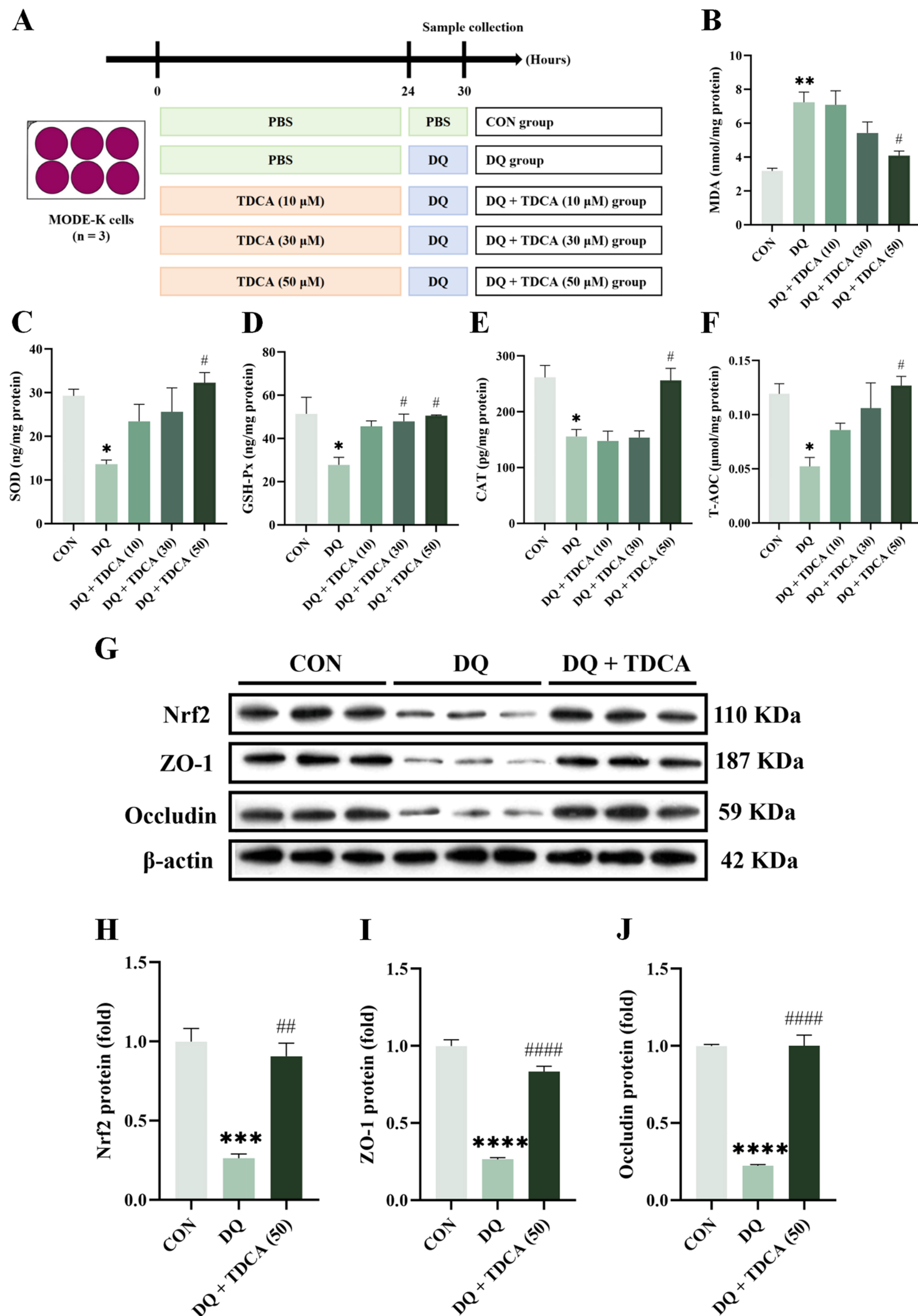


Fig. 4 (See legend on previous page.)

CAT ($P<0.001$), and T-AOC ($P<0.0001$) levels in the jejunum (Fig. 6F-J). Compared with those in the jejunum of DQ group, the MDA level was significantly lower ($P<0.001$), and the contents of SOD ($P<0.05$), GSH-Px ($P<0.0001$), CAT ($P<0.01$) and T-AOC ($P<0.0001$) were significantly greater in the DQ + TDCA (15) group. Compared with those in the jejunum of DQ +TDCA (15) group, the MDA levels were significantly greater ($P<0.05$). The contents of SOD ($P<0.05$), GSH-Px ($P<0.001$), CAT ($P<0.01$) and T-AOC ($P<0.0001$) decreased significantly in theDQ+ML385+TDCA (15) group compared to the DQ + TDCA (15) group.

TDCA alleviates diquat-induced intestinal barrier injury in mice through the Nrf2 pathway

To investigate the effect of TDCA on intestinal barrier integrity through the Nrf2 pathway, we examined jejunal interepithelial tight junction proteins in mice. The results of the analysis (Fig. 7) revealed that the protein levels of ZO-1 ($P<0.0001$) and Occludin ($P<0.0001$) were significantly greater in the DQ + TDCA group than in the DQ group. ZO-1 protein ($P<0.001$) and Occludin protein ($P<0.0001$) levels were significantly lower in theDQ+ML385+TDCA group than in the DQ + TDCA group.

TDCA alleviates diquat-induced oxidative stress and barrier injury in intestinal epithelial cells through the Nrf2 pathway in vitro

To investigate the effects of TDCA on the oxidative stress response and barrier function of intestinal epithelial cells via Nrf2, we treated MODE-K cells with TDCA or ML385+TDCA for 24 h and then treated them with diquat for 6 h (Fig. 8A). To explore the effects of TDCA on the oxidative indices of intestinal epithelial cells through the Nrf2 pathway, we tested the oxidative and antioxidative indices of the cells separately. The results (Fig. 8B-F) revealed that, compared with that in the CON group, the MDA level was significantly greater in the DQ group ($P<0.001$). The contents of SOD ($P<0.001$), GSH-Px ($P<0.01$), CAT ($P<0.01$), and T-AOC ($P<0.0001$) significantly decreased in the DQ group compared to CON group. In contrast to those in the DQ group, the MDA level was significantly lower ($P<0.01$), and the contents of SOD ($P<0.01$), GSH-Px ($P<0.01$), CAT ($P<0.01$) and T-AOC ($P<0.0001$) were significantly greater in the DQ+TDCA (50) group. Compared with those in the DQ+TDCA (50) group, the MDA level increased significantly ($P<0.01$), and the contents of SOD ($P<0.05$), GSH-Px ($P<0.05$), CAT ($P<0.05$), and T-AOC ($P<0.001$) decreased significantly in the DQ+ML385 + TDCA (50) group. The same trend was observed for all the indices in the DQ group and the DQ+ML385 + TDCA (50) group.

To investigate the protective effect of TDCA on intestinal epithelial cells through the Nrf2 pathway, we examined the levels of intercellular tight junction proteins and the Nrf2 protein. The analysis results (Fig. 8G-I) revealed that the protein levels of ZO-1 and Occludin were significantly greater ($P<0.0001$) in the DQ+TDCA group than in the DQ group. Compared with those in the DQ+TDCA group, ZO-1 and Occludin protein levels were significantly lower ($P<0.0001$) in the DQ+ML385 + TDCA group.

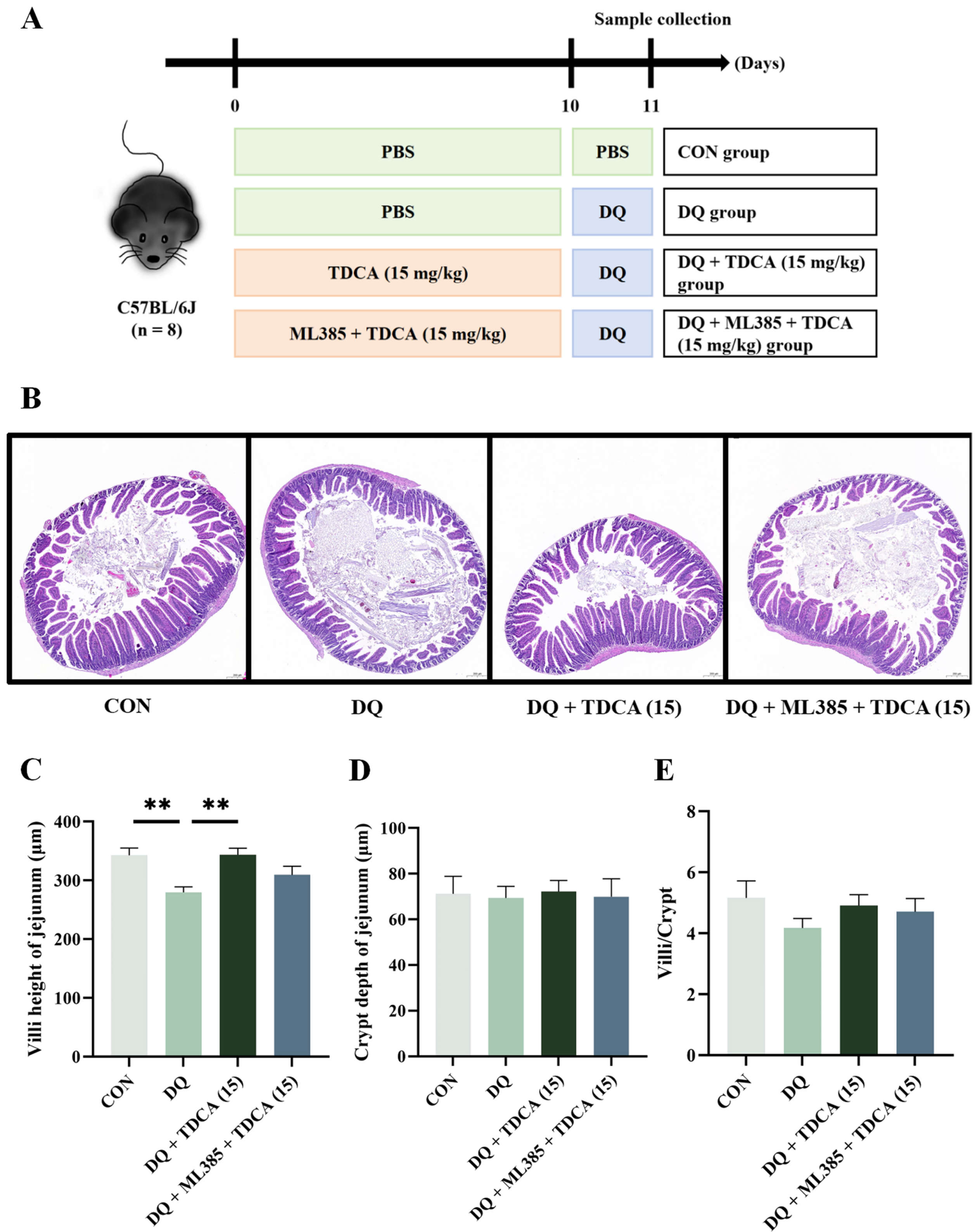
Discussion

The complex mechanism of oxidative stress is rooted in the intricate interplay between one or multiple oxidative systems, ultimately triggering the production and accumulation of ROS. This induces oxidative injury that destroys the intestinal mucosa, which in turn causes different degrees of intestinal damage, leading to the development of various types of intestinal diseases (Bhattacharyya et al. 2014; Yun et al. 2022). TDCA is a conjugate of taurine and deoxycholic acid that can protect the integrity of the intestinal mucosa (Chiang 2003; He et al. 2023). Nrf2 is an important transcription factor involved in the regulation of oxidative stress, which has a significant effect on the occurrence and development of several diseases (Zhang et al. 2022a). In this study, we used diquat to establish an oxidative stress model to investigate the role and mechanism of TDCA in intestinal injury and alleviation of oxidative stress. The experimental results showed that pretreatment with TDCA could alleviate diquat-induced oxidative stress and improve intestinal barrier function through the Nrf2-mediated signaling pathway.

Intestinal tissue contains the enzymes and nutrient transporters required for digestion, which is the main part of the digestion and absorption of nutrients and plays a key role in the health of the organism (Mowat

(See figure on next page.)

Fig. 5 TDCA alleviates diquat-induced jejunal intestinal morphological injury in mice through the Nrf2 pathway. **A** Experimental grouping scheme. **B** H&E staining of the jejunum. **C**-**E** Jejunal villus height, jejunal crypt depth and ratio of villus height to crypt depth in each group. $n=8$ for B-E. All the data are presented as the means ± SEMs. When the DQ group was compared to the CON group, $^{**}P<0.01$, and when the DQ+TDCA group was compared to the DQ group, $^{**}P<0.01$. CON, control; DQ, diquat; TDCA, taurodeoxycholic acid; ML385, specific Nrf2 inhibitor; TDCA (15) indicates treatment with 15 mg/kg TDCA



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and Agace 2014). Diquat is a bipyridine herbicide (Basilicata et al. 2022) that is commonly used as an inducer of oxidative stress. Several studies have shown that diquat impairs the integrity of the intestinal epithelial barrier by increasing intestinal epithelial permeability (Song et al. 2017; Xu et al. 2022b; Yin et al. 2015; Zhang et al. 2022b). Similar conclusions emerged from our results: diquat-induced oxidative stress reduces jejunal villus depth and significantly decreases antioxidant enzyme activity and the integrity of tight junctions, causing severe intestinal damage. Moreover, we found that exogenous supplementation with TDCA increased jejunal villus height, crypt depth, and antioxidant enzyme contents, suggesting that TDCA has an ameliorative effect on oxidative stress and a protective effect on oxidative stress-induced jejunal injury. TDCA significantly enhances otoprotection by reducing the activity of extracellular antioxidant pathways, such as those associated with decreased caspase-3 production (Shah et al. 2020; Wong and Ryan 2015). Therefore, we hypothesized that the mitigating effect of TDCA on intestinal oxidative stress is closely related to the activation of antioxidant pathways.

The body has the ability to maintain redox balance by scavenging excessive reactive oxygen species (ROS), hydrogen peroxide (H_2O_2) and other substances through antioxidant enzymes or antioxidants such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) (Balaban et al. 2005; Evans et al. 2002; Hayes et al. 2020; van der Pol et al. 2019). Oxidative stress leads to intracellular lipid peroxidation damage, and malondialdehyde (MDA) is a metabolite of lipid oxidation. The content of MDA reflects the degree of damage. The total antioxidant capacity (T-AOC) is the total antioxidant level of various antioxidants and oxidative enzymes (Xu et al. 2022b). The above oxidative indices are important for evaluating resistance to oxidative stress. Our results revealed that diquat significantly elevated the MDA level and significantly decreased the contents of SOD, GSH-Px, CAT and T-AOC. Moreover, we found that exogenous supplementation with TDCA before diquat stimulation could reduce MDA levels and increase the contents of antioxidant enzymes. These findings suggest that TDCA can mitigate diquat-induced oxidative damage by increasing the contents of antioxidant enzymes.

Nrf2 is a decisive endogenous antioxidant transcription factor that is released from Keap1 and translocated to aggregate when the organism is subjected to oxidative stress, thereby initiating the transcription of several antioxidant genes (Ma 2013; Sies et al. 2017; Xiang et al. 2022). We therefore examined the protein expression of Nrf2. Diquat-induced oxidative stress caused a significant decrease in the Nrf2 protein content in the absence of exogenous supplementation with TDCA. Diquat-induced oxidative stress caused a significant increase in Nrf2 protein after exogenous supplementation with TDCA, and there was no difference from the control group. A preliminary study indicated that TDCA can alleviate oxidative stress by activating Nrf2-mediated signaling pathways. Therefore, we utilized ML385, a specific inhibitor of the Nrf2 protein, in combination with TDCA to further explore whether the alleviating effect of TDCA on oxidative stress is dependent on the Nrf2-mediated signaling pathway. The results of both the animal and cellular experiments revealed that the use of ML385 almost completely eliminated the preventive effect of TDCA. There was little difference in the expression of antioxidant enzymes and other oxidation indices in the serum and jejunal tissues detected by diquat treatment alone versus the cotreatment with diquat, TDCA and ML385. Thus, we further determined that the amelioration of diquat-induced oxidative damage by TDCA is dependent on the Nrf2-mediated signaling pathway.

As mentioned previously, oxidative stress-induced damage increases the permeability of intestinal epithelial cells. The tight junctions between intestinal epithelial cells are a crucial component and structural basis of the mechanical barrier of the intestinal epithelium. ZO-1 and Occludin proteins are the main proteins that maintain the integrity of the mechanical barrier of the intestinal mucosa and determine the permeability of the intestinal tract (Cui et al. 2021; Zhang et al. 2022a). TDCA was found to enhance the recovery of intestinal epithelial cells (He et al. 2023; Perrone et al. 2010). Our current study is consistent with these findings. Both in vitro and in vivo experiments demonstrated that ZO-1 and Occludin protein levels were significantly decreased in the DQ group. However, after exogenous supplementation with TDCA, the expression of tight junction proteins was significantly increased. The above findings suggest that TDCA

(See figure on next page.)

Fig. 6 TDCA alleviates diquat-induced oxidative stress in the serum and jejunum of mice through the Nrf2 pathway. **A**-**E** Serum levels of MDA, SOD, GSH-Px, CAT, and T-AOC in each group. **F**-**J** Expression levels of MDA, SOD, GSH-Px, CAT, and T-AOC in jejunal tissues from each group. $n = 8$. All the data are presented as the means ± SEMs. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$ indicate between-group comparisons. CON, control; DQ, diquat; TDCA, taurodeoxycholic acid; MDA, malondialdehyde; SOD, superoxide dismutase; GSH-Px, glutathione peroxidase; CAT, catalase; T-AOC, total antioxidant capacity; ML385, specific Nrf2 inhibitor; TDCA (15) indicates treatment with 15 mg/kg TDCA

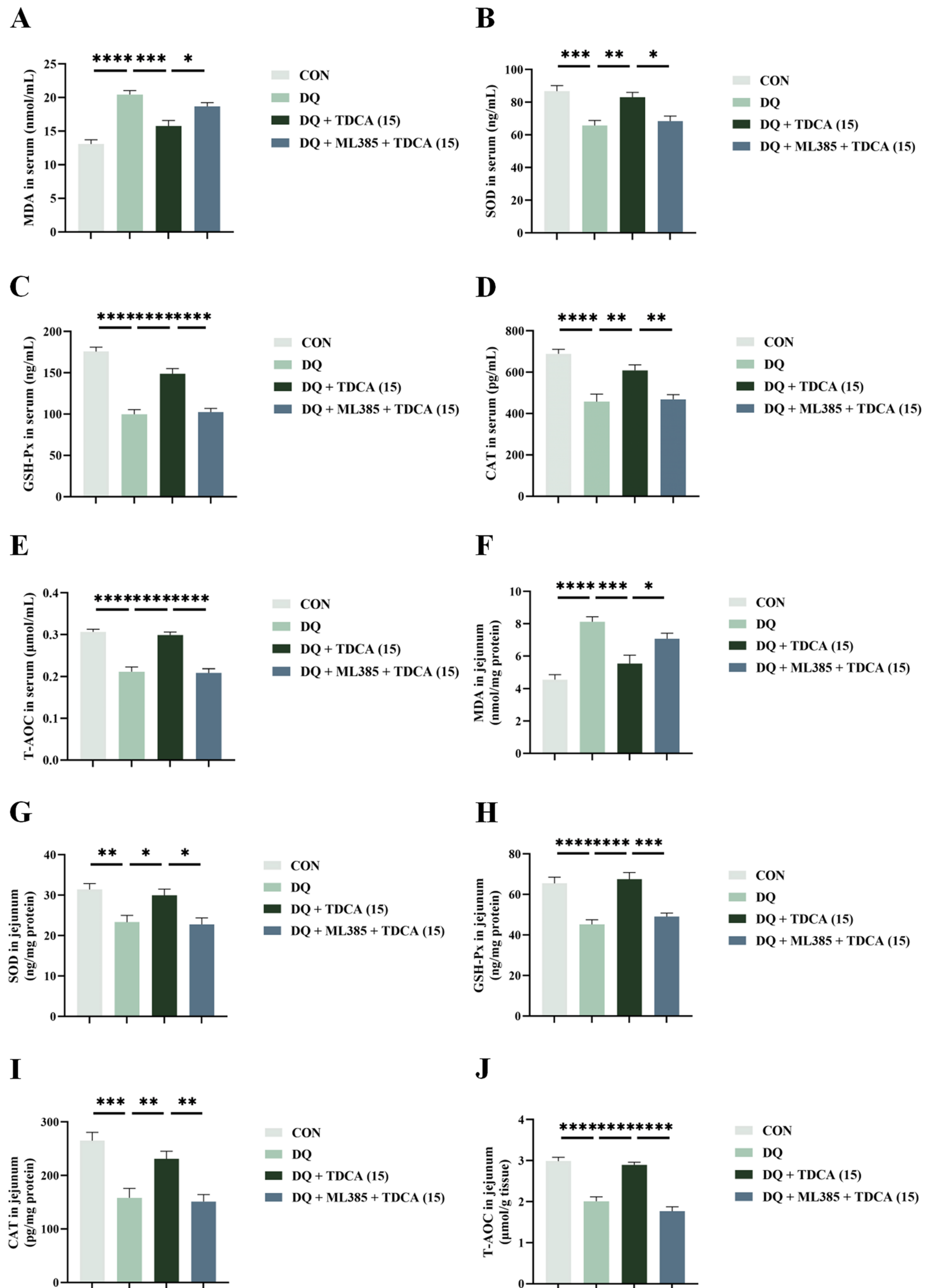

Fig. 6 (See legend on previous page.)

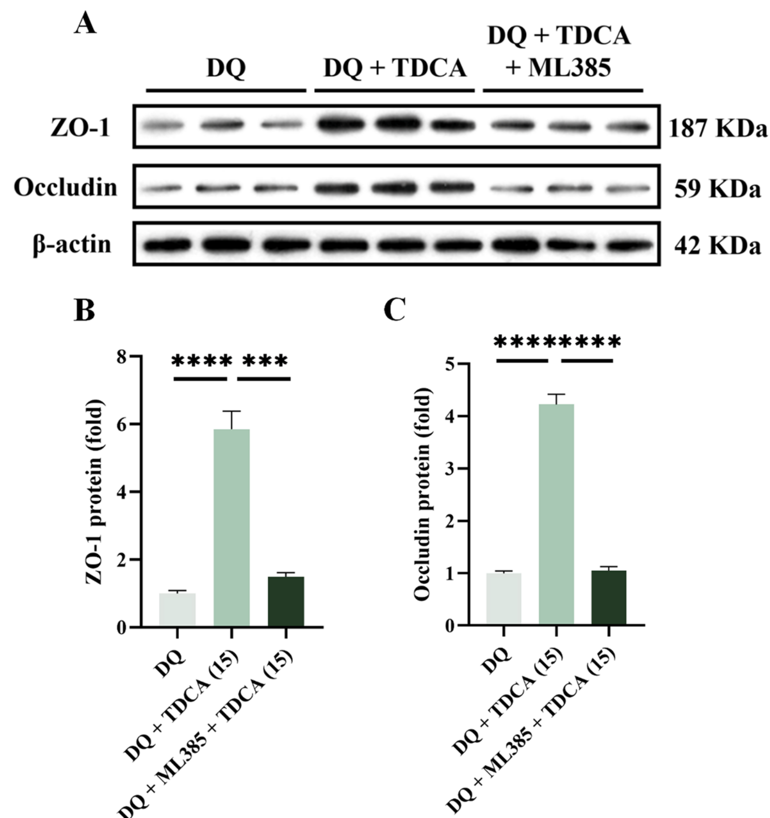

Fig. 7 TDCA alleviates diquat-induced intestinal barrier injury in mice through the Nrf2 pathway. **A** ZO-1 and Occludin protein expression was analyzed by western blot in the CON, DQ + TDCA, and DQ + TDCA + ML385 groups. **B**-**C** ZO-1 and Occludin protein expression in each group. $n = 3$. All the data are presented as the means ± SEMs. $^{***}P < 0.001$, $^{****}P < 0.0001$ indicates between-group comparisons. CON, control; DQ, diquat; TDCA, taurodeoxycholic acid; ML385, specific Nrf2 inhibitor; TDCA (15) indicates treatment with 15 mg/kg TDCA

prevents the oxidative stress-induced increase in cell permeability and thus maintains the integrity of intestinal epithelial cells.

Conclusion

In this study, TDCA enhanced intestinal barrier function by increasing antioxidant enzyme activity, thereby inhibiting diquat-induced intestinal oxidative stress through the activation of the Nrf2 signaling pathway. These findings increase the understanding of the TDCA-mediated pathway in protecting the intestinal barrier and its underlying mechanisms, providing potential strategies for the prevention and treatment of intestinal diseases caused by oxidative stress.

Methods

Chemical

Diquat (6385–62-2, average molecular weight: 344.05) was obtained from Shanghai Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). Taurodeoxycholic acid (207,737–97-1) was purchased from Sigma–Aldrich LLC. (Shanghai, China). ML385 (846,557–71-9) was procured from APExBIO Technology LLC. (Shanghai, China).

(See figure on next page.)

Fig. 8 TDCA alleviates diquat-induced oxidative stress and barrier injury in intestinal epithelial cells through the Nrf2 pathway in vitro. **A** Experimental grouping scheme. **B**-**F** MDA, SOD, GSH-Px, CAT, and T-AOC contents of MODE-K cells in each group. **G** ZO-1 protein and Occludin protein expression was analyzed by western blot in the CON group, DQ + TDCA group and DQ + TDCA + ML385 group. **H**-**I** ZO-1 and Occludin protein expression in each group. $n = 3$ for B-I. All the data are presented as the means ± SEMs. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$ indicate between-group comparisons. CON, control; DQ, diquat; TDCA, taurodeoxycholic acid; MDA, malondialdehyde; SOD, superoxide dismutase; GSH-Px, glutathione peroxidase; CAT, catalase; T-AOC, total antioxidant capacity; ML385, specific Nrf2 inhibitor; TDCA (50) indicates treatment with 50 μM TDCA

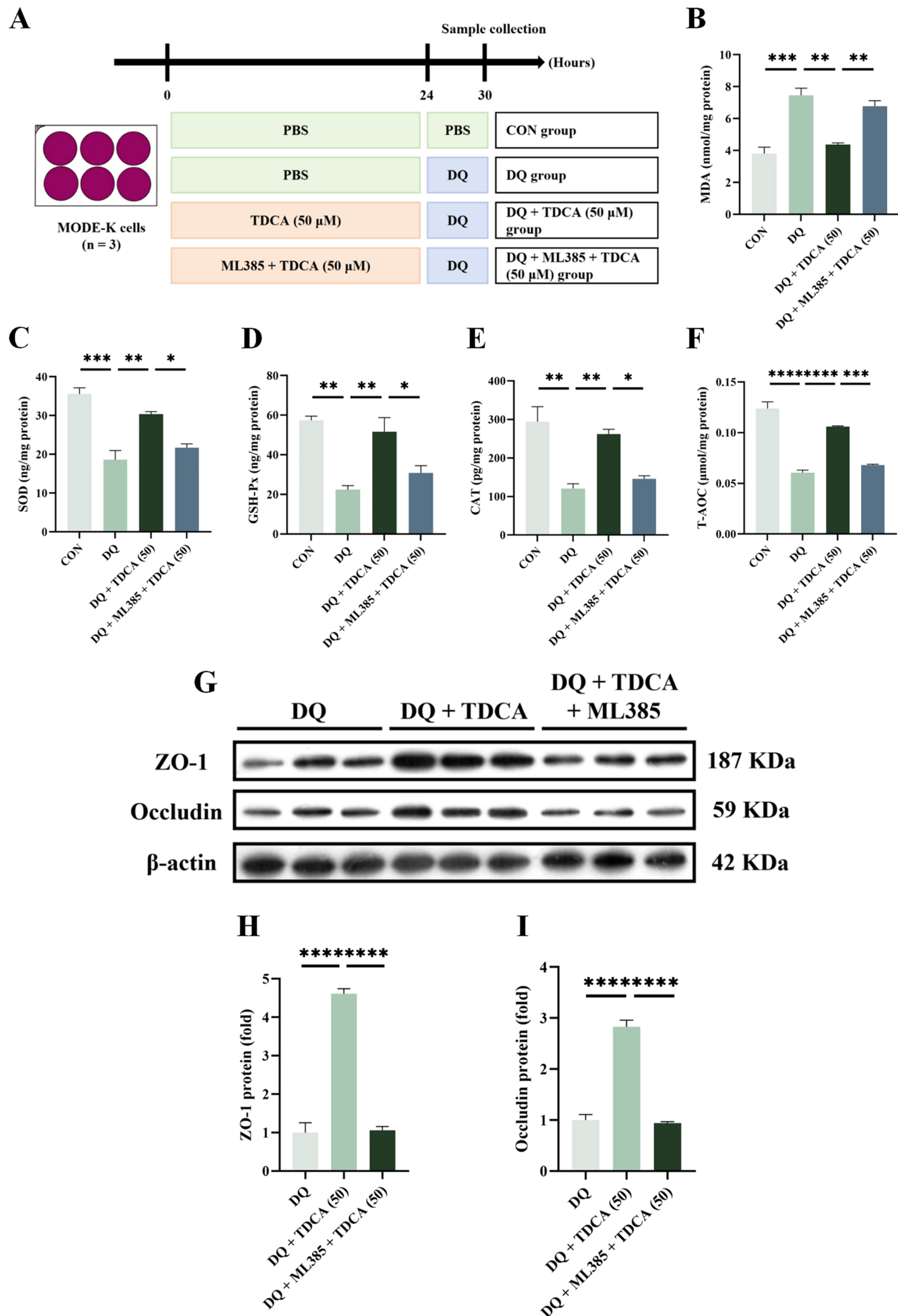

Fig. 8 (See legend on previous page.)

Animal experiments

For animal experiment 1, 40 six-week-old C57BL/6J male mice were randomly divided into five groups ($n = 8$/group). All C57BL/6J mice were obtained from the Experimental Animal Center of Huazhong Agricultural University (Wuhan, China). Every mouse in the control group and diquat group was orally administered 0.2 mL of phosphate-buffered saline (PBS) every day. Then, 0.2 mL of PBS or 25 mg/kg diquat was injected intraperitoneally into the control group or diquat group, respectively, on day 10. In the TDCA + diquat group, the three groups were orally administered 0.2 mL of 5 mg/kg, 10 mg/kg, or 15 mg/kg TDCA daily, and 0.2 mL of 25 mg/kg diquat was injected intraperitoneally on day 10 in the three groups. Bodyweight data were recorded every day. The samples were collected on day 11 and immediately placed in liquid nitrogen for storage at -80°C.

In animal experiment 2, 32 six-week-old C57BL/6J male mice were randomly divided into four groups ($n = 8$/group). The TDCA + diquat group received 15 mg/kg TDCA. In the ML385 + TDCA + diquat group, every mouse was orally administered 0.2 mL of 15 mg/kg TDCA daily, 0.2 mL of 30 mg/kg ML385 by gavage at intervals of 1 day, or 0.2 mL of 25 mg/kg diquat by intraperitoneal injection on day 10, respectively. Bodyweighting and sample storage were the same as those described above for animal experiment 1.

Cell experiments

In cell experiment 1, MODE-K cells were cultured in DMEM + FBS and randomly divided into 5 groups ($n = 3$/group). The MODE-K-cell line was kindly provided by Prof. Wang of China Agricultural University (Beijing, China). For the control group, PBS was added at 24 h. For the diquat group, PBS was added and then 100 μM diquat were added at 24 h. For the three TDCA + diquat groups, 10 μM, 30 μM and 50 μM TDCA were added, and 100 μM diquat was added at 24 h. The samples were collected at 30 h and frozen at -80°C.

For cell experiment 2, MODE-K cells were randomly divided into four groups ($n = 3$/group). The TDCA + diquat group was treated with 50 μM TDCA. The ML385 + TDCA + diquat group was treated with 50 μM TDCA and cocultured with 10 μM ML385. Diquat (100 μM) was added at 24 h, and the subsequent procedures were the same as those in cell experiment 1.

Morphological analysis

Jejunal tissues were fixed with 4% paraformaldehyde and embedded in paraffin, after which 5 μm-thick slices were obtained and stained with hematoxylin and eosin (H&E). Digital images were taken via a light microscope. The height of the jejunal villi and the depth of the crypts were measured via CaseViewer (V. 2022.2). The histopathological score of the jejunal epithelium was determined according to the criteria of our previous study (Tao et al. 2019).

ELISA

A malondialdehyde (MDA) ELISA kit (YJ544883), superoxide dismutase (SOD) ELISA kit (YJ001998), glutathione peroxidase (GSH-Px) assay kit (YJ058194), catalase (CAT) assay kit (YJ037752) and total antioxidant capacity (T-AOC) kit (ml022376) were procured from Shanghai Enzyme-linked Biotechnology Co. Ltd. (Shanghai, China) to determine the levels in mouse serum, jejunum tissue and the MODE-K-cell line according to previous methods (Hu et al. 2018).

Western blotting

Proteins were extracted from the mouse jejunal tissue and MODE-K cells, separated by SDS–PAGE, and then transferred to nitrocellulose membranes (BioTrace, Pall Co., USA). Nitrocellulose membranes were then immersed in blocking buffer, incubated with the primary antibody, washed with Tris-buffered saline with Tween (TBST) and immersed in the secondary antibody dilution (Li et al. 2023). Protein expression was recorded and analyzed with an imaging system (Bio-Rad, USA) and Quantity One software (Bio-Rad, USA), respectively. The following antibodies used in the western blot assays were used: Nrf2 antibody (Proteintech, 16,396–1-AP; 1:1000), ZO-1 antibody (Abcam, ab96587; 1:1000), Occludin antibody (Abcam, Ab167161; 1:1000), and β-actin antibody (CST, 4967; 1:1000).

Statistical analysis

Each set of data was statistically analyzed via GraphPad Prism (V. 9.5.1; San Diego, CA, USA). Comparisons were made via one-way ANOVA and Tukey's post hoc multiple comparison method, and all the results are expressed as the means ± SEMs, with $P < 0.05$ considered statistically significant. The number of times the data are used for statistical analysis is indicated in the figure notes.

Abbreviations

ANOVA	One-way analysis of variance
CAT	Catalase
CON	Control
DMEM	Dulbecco's modified Eagle's medium
DQ	Diquat
FBS	Fetal bovine serum
GSH-Px	Glutathione peroxidase
H&E	Hematoxylin and eosin
MDA	Malondialdehyde
ML385	Specific Nrf2 inhibitor
Nrf2	Nuclear factor erythroid 2-related factor 2
OS	Oxidative stress
PBS	Phosphate buffer saline
ROS	Reactive oxygen species

SOD	Superoxide dismutase
T-AOC	Total antioxidant capacity
TBST	Tris-buffered saline with Tween
TDCA	Taurodeoxycholic acid

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Authors' contributions
The authors' responsibilities were as follows: TSY designed the research; LJL, ZYH, SMZ, GXM and FJP conducted the experiments and analyzed the data; LJL wrote the paper; TSY had primary responsibility for the final content; and all the authors read and approved the final manuscript.

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Availability of data and materials
Not applicable.

Declarations

Ethics approval and consent to participate
The trial was conducted at Huazhong Agricultural University in Wuhan, Hubei Province, China. All animal experiments and sample collection procedures were approved by the Institutional Animal Care and Use Committee of Huazhong Agricultural University (HZAUMO-2023–0315). All methods in this study followed the Health Guidelines for the Care and Use of Laboratory Animals at Huazhong Agricultural University.

Competing interests
The authors declare that they have no competing interests.

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