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Sophora flavescens Containing-QYJD Formula Activates Nrf2 Anti-Oxidant Response, Blocks Cellular Transformation and Protects Against DSS-Induced Colitis in Mouse Model

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Abstract

Qu-Yu-Jie-Du decoction (QYJD) is a commercially available traditional Chinese medicine (TCM). It is an aqueous extract of a Chinese herbal formula primarily consisting of eight TCM herbs: *Taraxacum campylodes* G.E. Haglund, *Coix lacryma-jobi* L., *Smilax glabra* Roxb., *Sanguisorba officinalis* L, *Styphnolobium japonicum* (L.) Schott, *Prunus persica* (L.) Batsch, *Sophora flavescens* Aiton, and *Eupolyphaga sinensis* Walker. Matrine and oxymatrine are two of the major phytochemical constituents of QYJD. Inflammation and oxidative stress are strongly associated with colon carcinogenesis. Colorectal cancer (CRC) is the third most common type of cancer. Therefore, cancer chemopreventive agents targeting CRC are urgently needed. This study was conducted to investigate the potential anticancer effects and the underlying mechanisms of QYJD and its active constituents, matrine and oxymatrine, in human colon cancer HT29 cells and in a dextran sulfate sodium (DSS)-induced colitis mouse model. QYJD and matrine effectively inhibited the proliferation and anchorage-independent growth of HT29 cells in a dose-dependent manner. QYJD and matrine also induced an Nrf2-mediated anti-oxidant response element-luciferase activity and upregulated the Nrf2-mediated anti-oxidative stress genes HO-1 and NQO1 at both the mRNA and protein levels. In the DSS-induced colitis mouse model, QYJD reduced the disease activity index (DAI) and alleviated colonic shortening. Elevated Nrf2 and HO-1 mRNA levels were also observed in QYJD-treated mice. These findings showed that QYJD could elicit anti-inflammatory and anti-oxidative stress response *in vitro* in a cell line and *in vivo* in a DSS-induced colitis mouse model. These responses may contribute to the overall anticolon cancer effect of QYJD.

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Keywords

Qu-Yu-Jie-Du Decoction; Colitis; Colon Cancer; Matrine; Oxymatrine; Nrf2

Introduction

Colorectal cancer (CRC) is the third most common malignancy in males and the second most common cancer worldwide (Arnold *et al.*, 2017). Inflammatory bowel disease (IBD), a chronic and relapsing inflammatory disease of the intestinal tract, is associated with an increased risk of CRC (Tanaka, 2012). Given the progressive nature and poor prognosis of CRC, new therapeutic strategies, such as prevention, are urgently needed (Kummar and Doroshow, 2011).

Traditional Chinese medicine (TCM) has received worldwide attention because these treatments show remarkable potency and few side effects. Thus, TCM therapeutics can be used in chemoprevention strategies and as potential alternatives for various types of diseases, including cancers.

Qu-Yu-Jie-Du decoction (QYJD) is an aqueous extract of a Chinese herbal mixture consisting of eight Chinese medicinal herbs: 20.8% of *Taraxacum campylodes* G.E. Haglund (Herba Taraxaci; Pu Gong Ying), 20.8% of *Coix lacryma-jobi* L. (Semen coicis; coix seed), 16.7% of *Smilax glabra* Roxb., 10.4% of *Sanguisorba officinalis* L. (garden burnet root; Radix Sanguisorbae), 10.4% of *Styphnolobium japonicum* (L.) Schott (flos sophorae immaturus), 8.3% of *Prunus persica* (L.) Batsch (Tao Ren; Semen Persicae), 8.3% of *Sophora flavescens* Aiton (Ku Shen; Sophora Flavescens) and 4.2% of *Eupolyphaga sinensis* Walker (Tu Bie Chong; Eupolyphaga seu steleophaga). It is a modified form of Xia Yu Xue decoction, which was first described in the Synopsis of Prescriptions of The Golden Chamber 200 AD. The classic recipe includes three mixed herbs, *Rheum palmatum* L., *Prunus persica* (L.) Batsch and *Eupolyphaga sinensis* Walker and has been used as an effective therapy for abdominal pain and oligomenorrhea caused by blood stasis. Studies have shown that it can ameliorate intestinal epithelial damage through targeting the inflammatory and anti-oxidant signaling pathways (Liu *et al.*, 2015; Ma *et al.*, 2017).

The optimized formula, QYJD, has been commonly used to treat many diseases, including cancers, in China for many years with few adverse effects. Lian *et al.* found that QYJD could improve the micro-environment of ova in patients with endometriosis by reducing the expression of TNF and IL-6 (Li *et al.*, 2008; Lian *et al.*, 2009). QYJD, in combination with endocrine therapy, has also been demonstrated to be able to (i) improve life quality of advanced prostate cancer patients; (ii) reduce adverse reactions of Western medicine treatment; and (iii) improve immune system functions (Jia *et al.*, 2013). A previously published clinical study has revealed that QYJD significantly improved the survival outcomes and reduced the side effects of chemotherapy in hepatocellular carcinoma (HCC) patients (Zhao *et al.*, 2017). In recent years, many studies have been conducted to investigate the beneficial effects of QYJD, individually or in combination with other therapeutic drugs, in CRC patients. Two clinical studies have demonstrated that QYJD can reduce adverse effects of chemotherapy with FOLFOX4 and 5-fluorouracil and improve the quality of life in

CRC patients (Lai *et al.*, 2012; Zhou, 2013). Another clinical study by Zhang compared the prognostic benefits between QYJD, radiofrequency ablation (RFA) and the combination of these two and found that combination therapy of QYJD and RFA had more potential in improving the quality of life in CRC patients, compared to the other two therapies (Zhang, 2012). A clinical study by Zhou *et al.* also reported that QYJD in combination with capecitabine could improve quality of life and reduce the adverse effects of chemotherapy in CRC patients (Zhou *et al.*, 2016). In addition, cell line and animal xenograft studies have shown that QYJD can inhibit proliferation and induce apoptosis in Human cancer HT29 cells (Liu *et al.*, 2016; Zhao *et al.*, 2017). However, the underlying mechanisms of QYJD remain unclear. Matrine and oxymatrine are two major alkaloid components of QYJD. Matrine and oxymatrine have been widely used in China due to their extensive range of pharmacological effects, including anti-inflammatory and anti-oxidative properties. Thus, the antitumor activity of matrine and oxymatrine has attracted increasing attention (Li *et al.*, 2010; Liu *et al.*, 2010; Taylor *et al.*, 2010; Yu *et al.*, 2011). However, whether matrine and oxymatrine can inhibit the proliferation of human colon cancer HT29 cells and the underlying molecular mechanisms are unclear. Therefore, we aimed to investigate the antitumor effect of QYJD, matrine and oxymatrine in HT29 cells and to further elucidate the molecular mechanisms involved in its antineoplastic activities.

Anti-oxidant response elements (AREs) are present in the promoter region of genes activated by Nuclear Factor, Erythroid 2 Like 2 (NFE2L2 or Nrf2), which include phase II detoxification/anti-oxidant enzymes, such as heme oxygenase 1 (HO-1), NADPH quinone oxidoreductase 1 (NQO1) and glutathione *S*-transferase; these enzymes maintain the normal redox state of the body and cells. Therefore, Nrf2 expression can reduce the organ damage induced by electrophilic reagents. In a relaxed state, Nrf2 is present in the cyto-plasm in association with Kelch-like ECH-associated protein 1 (Keap1). Disturbance of the interaction between Nrf2 and Keap1, including covalent or oxidative modification of cysteine thiols in Keap1 by electrophiles or oxidative stress, results in Nrf2 release and its translocation into the nucleus (Cheng *et al.*, 2016). Many phase II enzymes as well as some detoxifying enzymes, such as glutathione S-transferase, peroxiredoxin1, γ-glutamate cysteine ligase, HO-1 and NQO1, are inducible and activated by Nrf2 (Darley-Usmar *et al.*, 1996; Giudice and Montella, 2006; Kim *et al.*, 2007; Park *et al.*, 2013). Nrf2 binding to the ARE sequence in genes encoding phase II/anti-oxidant enzymes causes transcriptional activation of these genes, resulting in removal of reactive oxygen species or toxic chemicals.

Exposure to dextran sulfate sodium (DSS) leads to intestinal permeability and mucosal changes in the ileum and colon, which may also contribute to induction of oxidative stress (Dawson *et al.*, 2010). Oxidative stress is closely linked to inflammation, which promotes many diseases, including cancers (Mantovani *et al.*, 2008; Cheng *et al.*, 2016). Therefore, we studied the anti-inflammatory activity of QYJD in DSS-induced colitis models and investigated whether its anti-oxidant effect was associated with the Nrf2 pathway.

Materials and Methods

Reagents

QYJD is a commercially available TCM decoction. It was kindly provided by Kangmei Pharmaceutical (Puning, China). The formula was extracted with water and freeze-dried into powder. Quality and quantity analyses of the aqueous extract were performed with LC/MS. Dimethyl sulfoxide (DMSO), matrine (M5319) and oxymatrine (O0891) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sulforaphane (SFN) was purchased from LKT Laboratories (St. Paul, MN). DSS (36–50 kDa) was purchased from MP Bio-medicals (Solon, OH, USA). Testosterone (> 98% purity) was purchased from Nacalai Tesque (Kyoto, Japan) and was used as an internal standard (IS). Acetonitrile, dichlor-omethane and formic acid were HPLC grade. Ultrapure water was used for all analyses. All the other chemicals and reagents commercially available were of the highest analytical grade. Primary and secondary antibodies were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Primers for qPCR were purchased from Integrated DNA Technologies (Coralville, IA).

Identification of Matrine and Oxymatrine in QYJD by LC/MS

The following conditions were used to analyze matrine and oxymatrine: system, Agilent 1290 Infinity LC system (Agilent Technologies, USA), which consists of a solvent degasser, a binary pump, an auto-sampler and a column oven; column, Agilent ZORBAX RRHD SB-C18 (100 × 3 mm, 1.8 μm); mobile phase A, 0.1% formic acid in water; mobile phase B, 100% acetonitrile; flow rate, 0.3 mL/min; wavelengths, 210 nm for matrine and oxymatrine and 243 nm for testosterone; injection volume, 10 μL; MS/MS detector, Agilent 6540 quadrupole-time of flight mass spectrometer, used in combination with an Agilent 1290 Infinity ultra-performance liquid chromatography system. Samples were analyzed using Dual AJSESI (Agilent Technologies) in the positive model. Data were collected and analyzed by Quantitative Analysis Software (version B.06.00, Agilent Technologies).

Cell Culture and Cell Viability Assay

HT29 cells were purchased from the American Type Culture Collection (ATCC). A stably transfected single clone HepG2-ARE-C8 (HepG2-C8) cell line was previously established in our laboratory using the pARE-TI-luciferase reporter gene (Yu *et al.*, 2000). Both cell lines were routinely maintained in Dulbecco's modified Eagle's medium (Gibco, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (Gibco) at 37°C in a humidified 5% CO_2 atmosphere. Cells were seeded in 96-well plates at a density of 1×10^4 cells/well. Twenty-four hours later, cells were treated with different doses of QYJD, matrine and oxymatrine.

After treatment for 48 h, cell viability was tested using the Cell Titer 96 Aqueous Nonradioactive Cell Proliferation Assay kit (Promega Corp., Madison, WI). The absorbance was read at 490 nm on an Infinite 200 PRO microplate reader (Tecan Systems, San Jose, CA).

Colony Formation Assay

HT29 cells (5×10^3 cells/well) were transferred to 1 mL of basal medium Eagle (BME) containing 0.33% agar over 3 mL of BME containing 0.5% agar with 10% FBS in 6-well

plates. Both the top and the bottom layers contained different concentrations of QYJD in each well. The cells were maintained in soft agar at 37°C in a humidified 5% CO_2 atmosphere for 14 days. The colonies were photographed using a computerized microscope system (Nikon Eclipse E600) with Nikon ACT-1 software (Version 2.20) and counted using ImageJ Software (Version 1.47k).

ARE-Luciferase Reporter Assay

The HepG2-C8 cell line was established as described previously (Yu *et al.*, 2000). HepG2-C8 cells were seeded in 6-well plates at a density of 1×10^5 cells/well. After overnight incubation, cells were cultured in DMEM containing 10% FBS and different concentrations of compounds for 12 h. The luciferase activity was measured according to the manufacturer's instructions. Briefly, after drug treatment, cells were washed twice with ice-cold PBS and harvested in reporter lysis buffer. The homogenates were centrifuged at 13, 000× g for 5 min at 4°C. A 10-μl supernatant was assayed for luciferase activity using a TD-20/20 luminometer (Turner Designs, Sunnyvale, CA). Luciferase activity was normalized to protein concentration. The data are presented as fold change relative to the control group (no treatment).

RNA Isolation and Reverse Transcription Quantitative PCR (RT-qPCR)

HT29 cells were seeded in 6-well plates overnight and treated with compounds for 24 h. After treatment, the cells were washed with ice-cold PBS and harvested. Total RNA was extracted using an RNeasy Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. Reverse transcription was carried out using the SuperScript III First-Strand Synthesis System (Invitrogen, Carlsbad, CA, USA). qPCR was performed using the Power SYBR Green PCR Master Mix (Applied Biosystems, Carlsbad, CA, USA) on an ABI7900HT system (Applied Biosystems). Relative mRNA expression levels were determined using the ΔΔCt method.

Preparation of Protein Lysate and Western Blot Analysis

After being treated for 24 h, cells were washed with ice-cold PBS and harvested with 200 μL of a lysis buffer containing 10 mM Tris-HCl (pH 7.4), 50 mM sodium chloride, 30 mM Na-PPi, 50 mM sodium fluoride, 100 μM sodium orthovanadate, 2 mM iodoacetic acid, 5 mM $ZnCl_2$, 1 mM phenylmethylsulfonyl fluoride (PMSF) and 0.5% Triton X-100. Cell lysates were homogenized by passing through a 23-gauge needle three times and incubated on ice for 30 min. The homogenate was centrifuged at 13, 000× g for 15 min at 4°C. Then, 25 μg of total protein, as determined by a Bio-Rad protein assay, was mixed with 4× loading buffer and preheated at 95°C for 3 min. The samples were then loaded on a 10% sodium dodecyl sulfate polyacrylamide gel (SDS-PAGE) and run at 200 V. The proteins were transferred onto a polyvinylidene difluoride membrane using a semidry transfer system (Fisher, Pittsburgh, PA). The membrane was blocked with 5% BSA solution for 1 h at room temperature and then incubated overnight at 4°C with primary antibody (1:1,000 dilution). After hybridization with primary antibody, the membrane was washed with TBST (Tris-buffered saline and Tween 20) three times, then incubated with secondary antibody (1:5,000 dilution) for 45 min at room temperature and washed with TBST three times. The protein

bands were visualized with the SuperSignal chemi-luminescent substrate (Thermo Fisher Scientific) in a Bio-Rad ChemiDoc XRS system and semiquantified using ImageJ Software.

Animals and Experimental Procedures

Four-week-old male C57BL/6J mice were purchased from the Jackson Laboratory (Bar Harbor, ME, USA) and were maintained in a controlled environment (20–22°C, 12-h light and 12-h dark cycles, and 45–55% relative humidity) at the Rutgers Animal Care Facility. The AIN-93M diet was purchased from ResearchDiet Inc. (New Brunswick, NJ, USA). The diet and water were provided *ad libitum*. All animal experiments were performed under the relevant animal protocol (01–016), which was approved by the Institutional Animal Care and Use Committee (IACUC) of Rutgers University. Upon arrival, mice were randomly divided into five groups ($n = 10$): control, DSS and three DSS+QYJD groups (3 QYJD doses: 1, 2 or 4 g per kg body weight) as shown in Fig. 5A. Mice were administered different doses of QYJD solution or saline by oral gavage three times per week from weeks 4 through 8. For induction of acute colitis, mice were exposed to 1.2% DSS (Affymetrix, USA) in drinking water for one week, starting at 7 weeks of age. Disease activity index (DAI) and body weight were measured weekly. At the end of the experiment, mice were sacrificed, the colons were removed and the length of the colons was measured. All the colons were flushed with ice-cold saline to remove feces and were opened longitudinally on filter paper. Colon epithelial cells were isolated using a previously described method. Briefly, tissues were scraped using a glass slide on ice, and the mucosal scrapings were resuspended in ice-cold PBS. Cells were collected by centrifuging and used for molecular analyses.

Statistical Analysis

All statistical analyses were performed using the Statistical Package for Social Sciences (SPSS), Version 22. Data are presented as the mean ± SD. Statistical analysis was performed using one-way ANOVA. P values less than 0.05 were considered statistically significant.

Results

Matrine and Oxymatrine Contents in the QYJD Formula

Matrine and oxymatrine are the major constituents of aqueous extract of *Sophora flavescens Aiton* and *Kushen*. The molecular structures and molecular weights are shown in Fig. 1A. The LC/MS chromatogram of QYJD is shown in Fig. 1B. We identified two peaks corresponding to matrine and oxymatrine at elution times of approximately 5 and 6 min based on the chromatograms of pure matrine and oxymatrine run in the same condition. The mass spectra of matrine and oxymatrine are shown in Fig. 1C. The mass-to-charge ratios (m/z) 249.2 and 265.2 were observed in previous peaks, confirming that they were protonated forms of matrine and oxymatrine, respectively. The concentrations of matrine and oxymatrine in 200 mg/mL of QYJD were 339 μM and 231 μM, respectively, as determined by HPLC (data not shown).

QYJD Inhibited HT29 Cell Proliferation and Anchorage-Independent Growth

HT-29 cells were incubated with different concentrations of QYJD, matrine and oxymatrine for 48 h. A dose-dependent decrease in cell viability was observed in the QYJD- and matrine-treated groups. Cell viability was decreased to approximately 50% by 5 mg/mL QYJD or 3 mM matrine treatment. In contrast, no inhibitory effect on cell viability was observed in HT29 cells treated with oxymatrine (Fig. 2A).

For analysis of the effect of QYJD on anchorage-independent growth of HT29 cells and the potential role of Nrf2 in inhibiting anchorage-independent growth, wild-type (shMock) and Nrf2 knockdown (shNrf2) HT29 cells were treated with QYJD at three concentrations — 0.15, 0.23 and 0.31 mg/mL. Based on the previously determined matrine and oxymatrine contents in QYJD, as described in Sec. 3.1, the equivalent concentrations of matrine were 0.26, 0.40 and 0.50 μM, respectively. In addition, the equivalent concentrations of oxymatrine were 0.18, 0.27, and 0.36 μM, respectively. QYJD significantly inhibited the colony formation of wild-type HT29 cells in a dose-dependent manner. However, the inhibitory effect of QYJD on Nrf2 knockout HT29 cells was not as dramatic as in wild-type cells (Fig. 2B).

Induction of ARE-Luciferase Activity by QYJD, Matrine and Oxymatrine

To test the transcriptional activation of AREs by QYJD, matrine and oxymatrine, were treated HepG2-C8 cells with QYJD, matrine and oxymatrine for 12 h, and then, the ARE-luciferase reporter activities were measured (Chen *et al.*, 2000). Figure 3A shows that QYJD and matrine induced ARE-luciferase activity in HepG2-C8 cells with different potencies, while oxymatrine did not induce this activity. QYJD at 30 mg/mL strongly increased ARE-luciferase activity by 8 times compared to vehicle (0.1% DMSO) treatment. Matrine at a concentration of 1.6 mM modestly increased the activity approximately 4 times higher than vehicle treatment.

mRNA Expression of Nrf2 and Its Downstream Genes Induced by QYJD, Matrine and Oxymatrine

To confirm that QYJD, matrine and oxymatrine can induce endogenous Nrf2 and its downstream genes in HT29 cells, we conducted qPCR to quantify the mRNA expression levels of these genes (Figs. 3B–3D). The mRNA expression of Nrf2 was slightly increased by QYJD and matrine treatment. However, the differences were not statistically significant. The mRNA expression of HO-1 was significantly increased by QYJD at 5 mg/mL, while other treatments did not significantly change the mRNA level. Both QYJD at 5 mg/mL and matrine at 0.6 mM increased the mRNA expression of NQO1, but the differences were not statistically significant.

Protein Expression of NRF2 and Nrf2 Target Genes Induced by QYJD, Matrine and Oxymatrine

We then tested the protein level of NRF2 and its target enzymes in HT29 cells treated with QYJD, matrine and oxymatrine. Blots in Fig. 4A are representative of three independent experiments. We used SFN, a well-known Nrf2 activator, as a positive control (Lin *et al.*, 2008; Kong *et al.*, 2013). As shown in Fig. 4B, QYJD and matrine significantly increased

the NRF2 protein levels, while oxymatrine had no effect. The inductions by QYJD and matrine were comparable to that by SFN. Figure 4C shows that only QYJD and SFN significantly increased the protein level of HO1, and they had similar potencies. Matrine at 0.4 mM increased the protein level of NQO1 by approximately 3.5-fold compared to vehicle (0.1% DMSO) treatment, while the other treatments did not have similar effects (Fig. 4D). Thus, we observed that the protein levels of Nrf2, HO-1 and NQO1 generally matched their mRNA expression levels.

QYJD Inhibited DSS-Induced Colitis in Mice through Activation of Nrf2 Signaling Pathways

Mice were fed QYJD by oral gavage three weeks prior to DSS administration to test the protective effects of QYJD against DSS-induced colitis. The experimental design of the animal study is shown in Fig. 5A. At the end of the study, the DAI and colon length were measured for each mouse. As shown in Fig. 5B, the average colon length in the DSS group was significantly shorter than in the control group, and QYJD significantly attenuated the colon shortening caused by DSS. End-point DAI is shown in Fig. 5C. A notable increase in DAI was observed in the DSS-treated group, and QYJD administration at 1 g/kg body weight reduced the DAI. However, the DAI in the other two QYJD administration groups was not significantly different than the DSS-treated group. Colon epithelial cells from all groups were used to determine the mRNA expression of Nrf2 and its downstream genes. As shown in Fig. 5D, QYJD administration increased mRNA expression of Nrf2 and HO-1, which could potentially contribute to the protective role of QYJD against DSS-induced colitis in mice.

Discussion

Patients with IBD and inflammatory disorders are at high risk of developing CRC. Understanding the etiology of these diseases is essential for improving the currently available strategies to treat IBD and, more importantly, to prevent CRC. Nrf2, a transcription factor with anti-inflammation and anti-oxidative stress activities, protects intestinal integrity through regulation of proinflammatory cytokines and induction of phase II detoxifying enzymes (Khor *et al.*, 2006). Elucidation of the molecular mechanisms of TCMs as an alternative for therapy and prevention of CRC has become increasingly important (Guo *et al.*, 2018). Our previous study suggested that QYJD was effective in improving the quality of life of CRC patients and in suppressing tumor growth. Oxidative stress and inflammation are closely associated with mucosal erosions and a variety of gastrointestinal diseases, such as Crohn’s disease and ulcerative colitis (Pravda, 2005; Rezaie *et al.*, 2007). As one of the hallmarks of cancer, inflammation is frequently involved in various types of cancers, such as mammary, prostate and colon cancers. Nrf2, the key to the cellular anti-oxidative response, has been shown to suppress carcinogenesis, especially in the early stages. The anti-oxidant and antitumor properties of TCMs have been widely reported in cultured cells, animal models and humans. To further elucidate the mechanisms, we studied the antiproliferative and anti-oxidant effects of QYJD and its major constituents, matrine and oxymatrine. Mechanistically, our results demonstrated that QYJD and matrine significantly inhibited the proliferation of HT29 cells. Furthermore, the antineoplastic effect of QYJD was shown by its inhibition of anchorage-independent growth of CRC cells. Treatment with QYJD and

matrine activated ARE-luciferase activity and Nrf2 and its downstream genes. Western blotting analysis revealed that QYJD increased the protein levels of Nrf2 and HO-1. We also demonstrated the chemopreventive role of QYJD in DSS-induced ulcerative colitis, which is considered a precancerous lesion. The results demonstrated that QYJD could attenuate colon shortening and lead to clinical improvement in mice with DSS-induced acute colitis, indicating the therapeutic potential of QYJD. We further analyzed the molecular mechanism underlying the QYJD-mediated suppression of colonic inflammation. The intestinal epithelium sits at the interface between an organism and its luminal environment and is thus susceptible to oxidative damage induced by luminal oxidants (Carriere *et al.*, 2001). During the preparation of this manuscript, Chen *et al.* published a paper where they found oxymatrine protected against DSS-induced colitis in mouse by inhibiting the PI3K/Akt signaling pathway (Chen *et al.*, 2017). In 2016, a paper by Liu *et al.* suggested that oxymatrine synergistically enhanced anticancer activity of oxaliplatin by inhibiting the PI3K/Akt/mTOR signaling pathway in colon cancer HT29 and SW480 cells and in a mouse xenograft model (Liu *et al.*, 2016). In our present study, we show that oxymatrine and matrine exert their anticancer effects by activating the Nrf2/ARE anti-oxidative stress pathway in cells and the QYJD formula could also active the Nrf2/ARE anti-oxidative stress pathway in DSS-induced mouse colitis model. These findings suggest that QYJD is a potential therapeutic for CRC.

Acknowledgments

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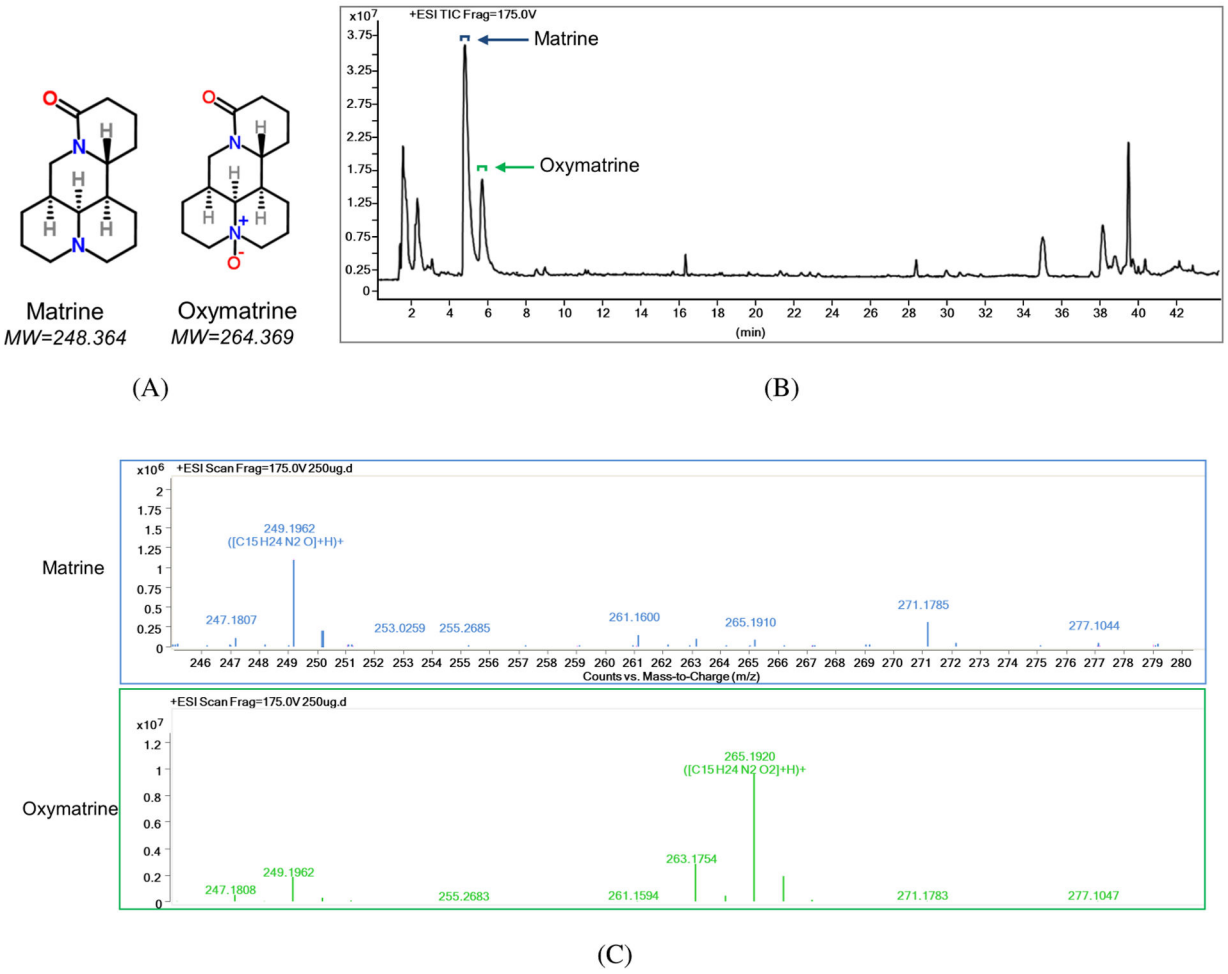

Figure 1.

The matrine and oxymatrine contents in QYJD. (A) The molecular structure and molecular weight of matrine and oxymatrine. (B) LC/MS chromatogram of QYJD. The peaks for matrine and oxymatrine are indicated by an arrow. Top square brackets indicate the range of peaks for displaying the mass spectra. (C) Mass spectra of matrine (upper) and oxymatrine (lower). The mass-to-charge (m/z) ratios of all detected peaks are shown above each peak.

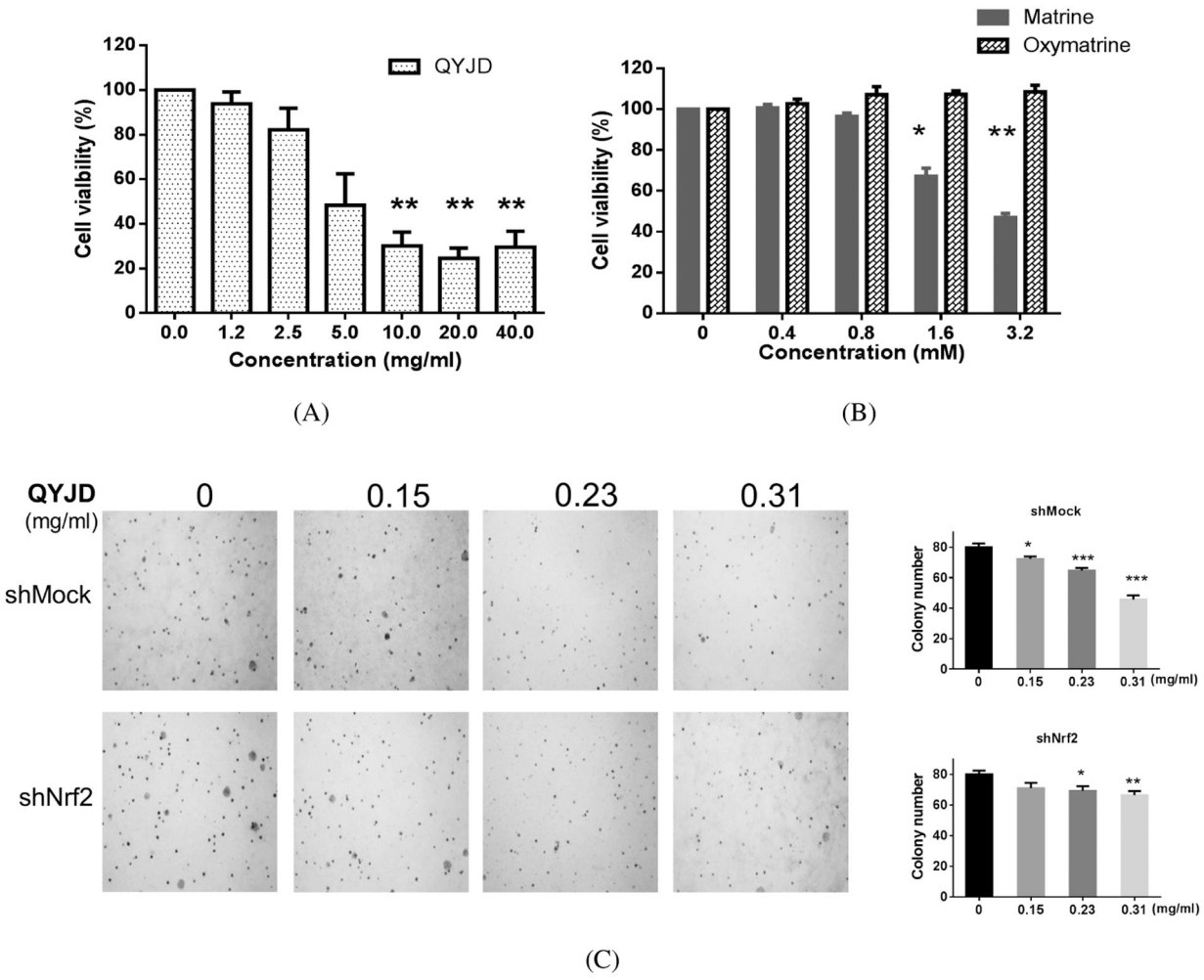

Figure 2.

Cell viability and anchorage-independent growth of HT29 cells are inhibited by QYJD treatment. (A, B) Bar graphs showing cell viability of HT29 cells after treatment with different concentrations of QYJD, matrine and oxymatrine for 24 h. Note that the concentrations are in mg/mL for QYJD and mM for matrine and oxymatrine. (C) Colony formation of wild-type and Nrf2 knockout HT29 cells. Representative colony images of wild-type (shMock) and Nrf2 knockout (shNrf2) HT29 cells treated with different concentrations of QYJD (left) and data from three independent experiments (right). All experiments were performed three times. *$P < 0{:}05$, **$P < 0{:}01$, ***$P < 0{:}001$, one-way ANOVA; compared to the vehicle-treated group.

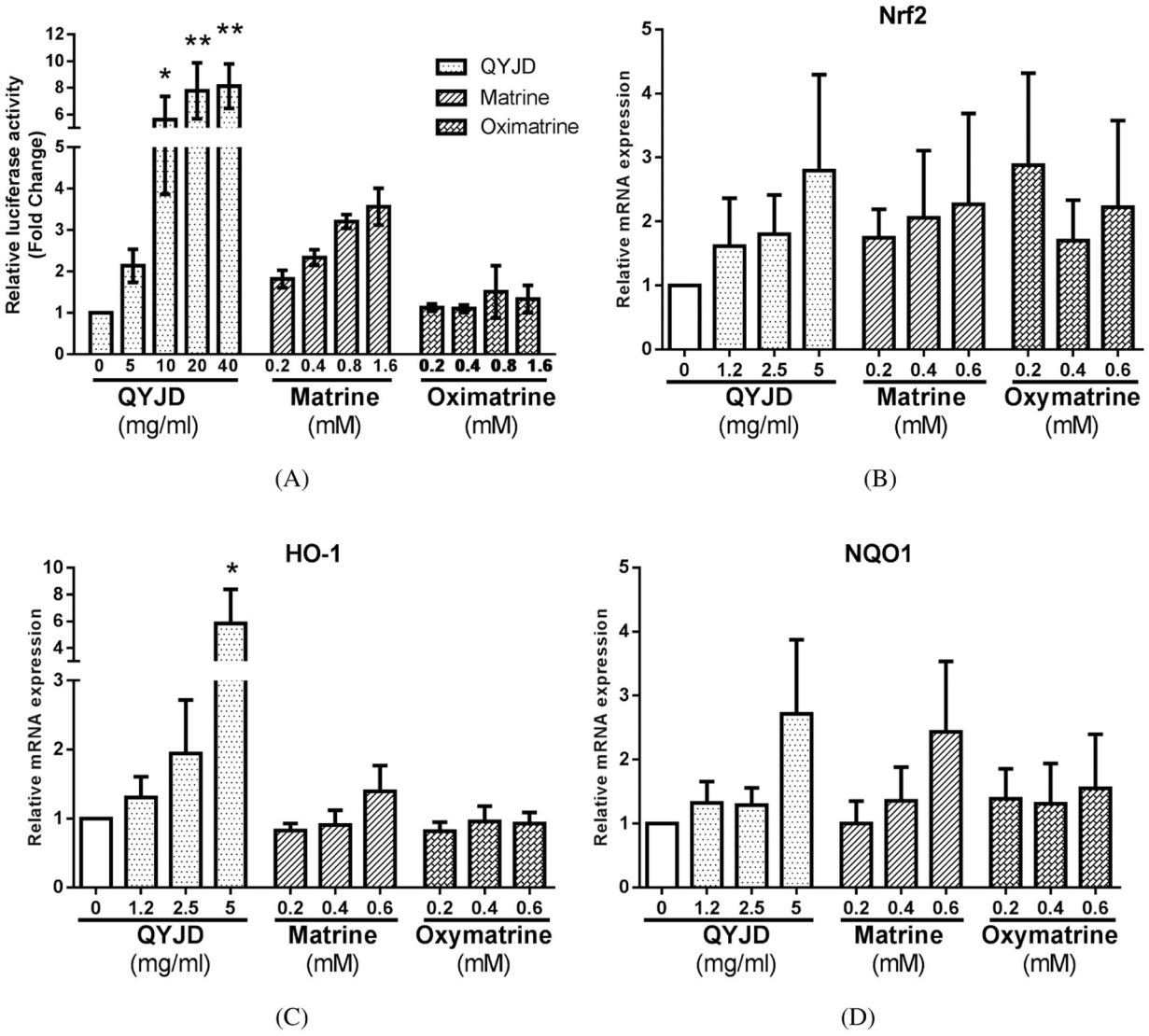

Figure 3.

QYJD induces ARE-luciferase activity in HepG2-C8 cells and activates mRNA expression of Nrf2 and its downstream genes in HT29 cells. (A) Relative luciferase activity induced by QYJD, matrine and oxymatrine in HepG2-C8 cells. Data are presented as a fold change compared to the vehicle (0.1% DMSO)-treated group. (B–D) Relative mRNA expression of Nrf2, HO-1 and NQO1 induced by QYJD, matrine and oxymatrine in HT29 cells. All experiments were performed three times. *P < 0:05, **P < 0:01, one-way ANOVA; compared to the vehicle-treated group.

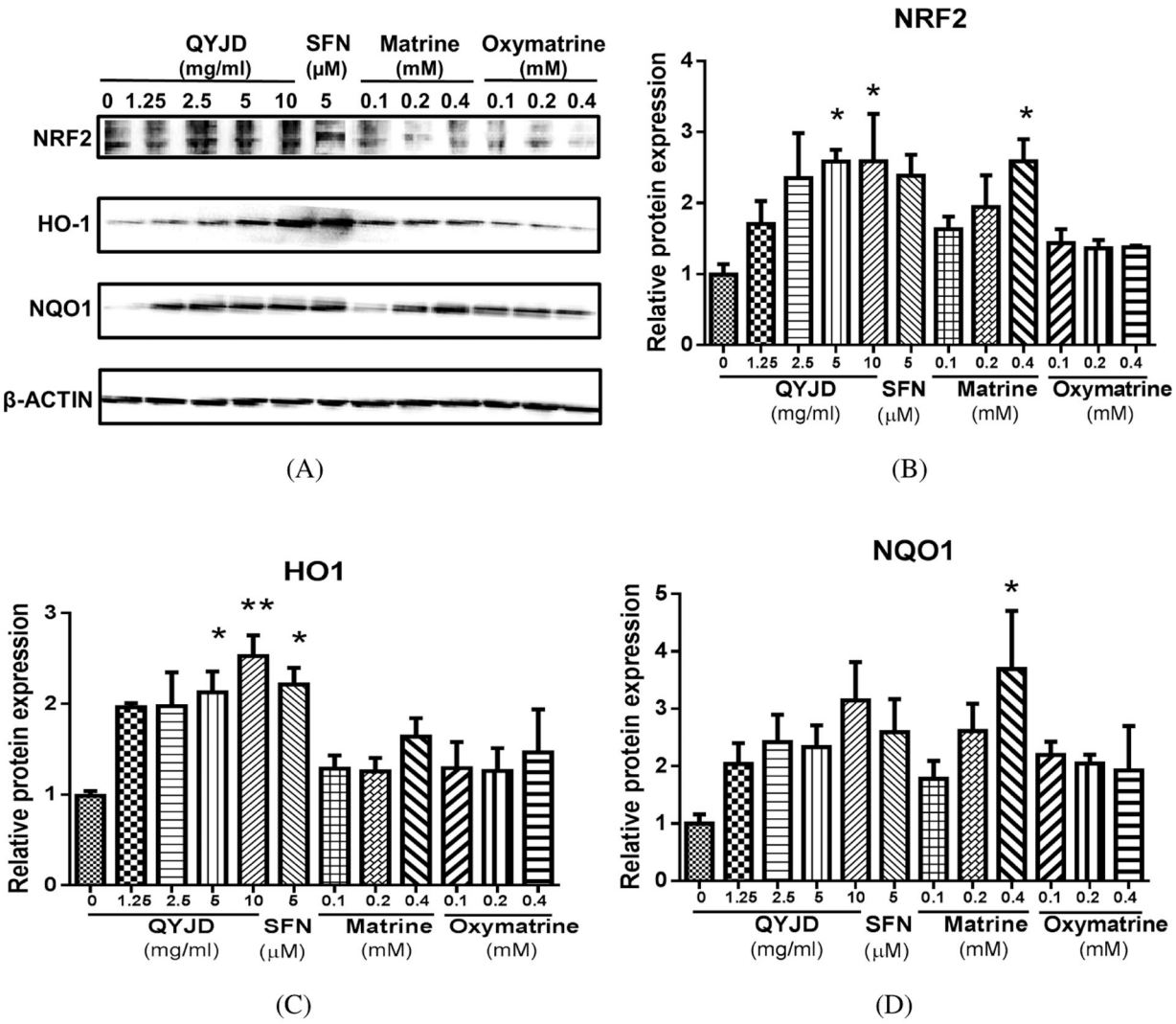

Figure 4.

Protein expression of NRF2 and its target enzymes in HT29 cells treated with QYJD, matrine and oxymatrine. (A) Representative images of Western blots from three independent experiments. Sulforaphane (SFN, 5 μM) was used as a positive control for activating NRF2. β-ACTIN served as a loading control. (B–D) Relative protein levels of Nrf2, HO-1 and NQO1 induced by QYJD, matrine and oxymatrine in HT29 cells. All experiments were performed three times. *$P < 0{:}05$, one-way ANOVA; compared to the vehicle-treated group.

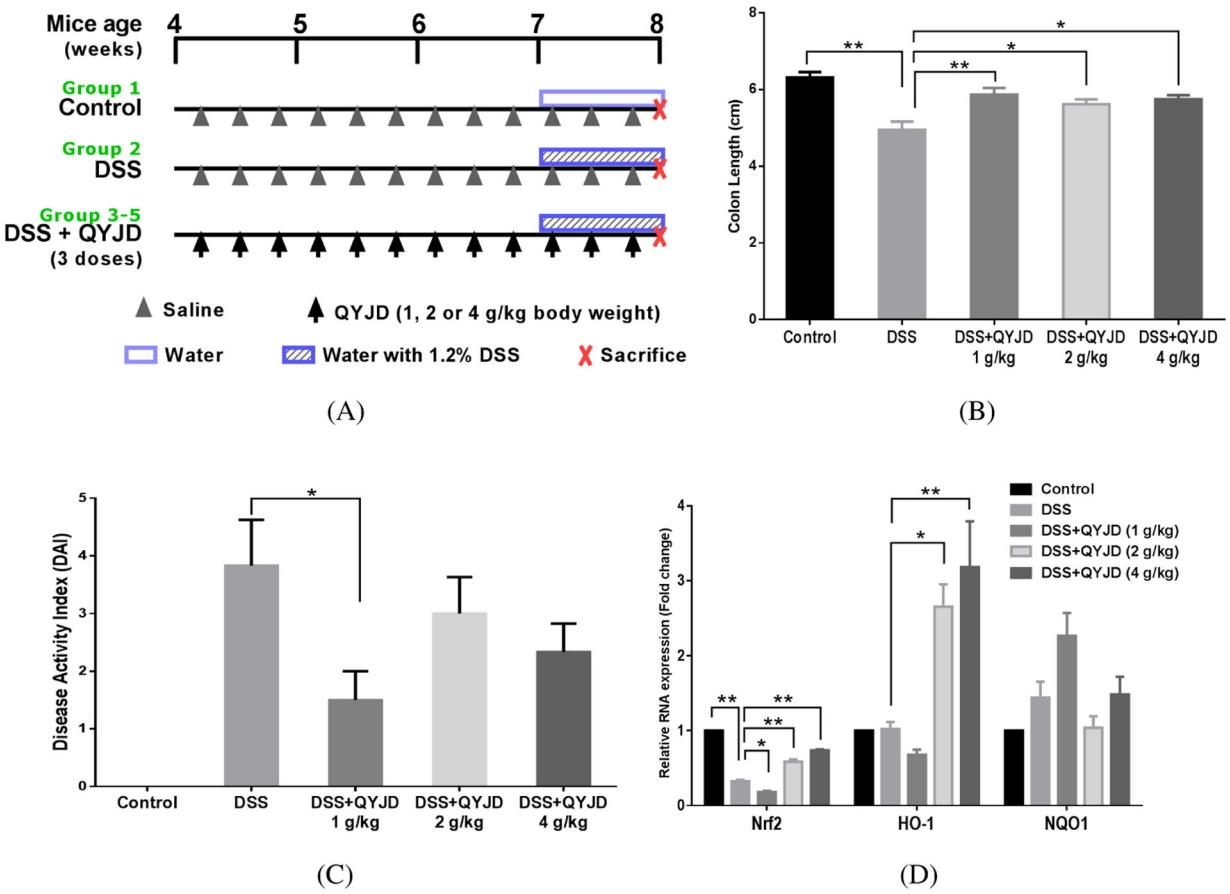

Figure 5.

QYJD attenuates colitis in DSS-induced mice. (A) The experimental design of the animal study. Three doses of QYJD (1, 2 and 4 g/kg body weight) were used in this study. $n = 10$. (B, C) Average colon length and disease activity index (DAI) in each group. (*$P < 0{:}05$, **$P < 0{:}01$, one-way ANOVA; compared to the DSS group.) (D) Relative mRNA expression of Nrf2, HO-1 and NQO1 in colon epithelial cells. Data are shown as an average level of all samples in each group, and experiments were performed three times for each sample. *$P < 0{:}05$, **$P < 0{:}01$, one-way ANOVA; compared to the DSS group.