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Protective effect of the essential oil of *Zanthoxylum myriacanthum* var. *pubescens* against dextran sulfate sodium-induced intestinal inflammation in mice

Kai-Long Ji[a,b,1], Xiao-Qing Gan[a,b,1], You-Kai Xu[a], Xiu-Fen Li[a], Juan Guo[a], Mahmoud M. Dahab[a], Ping Zhang[a,*]

[a] *Key Laboratory of Tropical Plant Resources and Sustainable Use, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Menglun 666303, PR China*

[b] *University of Chinese Academy of Sciences, Beijing 100049, PR China*

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ABSTRACT

Background: *Zanthoxylum myriacanthum* var. *pubescens* is an ethnic medicine for digestive disease known as Maqian. A previous report showed that the Maqian fruits essential oil (MQEO) exhibited an NO inhibitory effect on RAW 264.7 cells, but the effect on inflammatory disease *in vivo* remains unknown.
Purpose: To investigate the anti-inflammatory effect of *Z. myriacanthum* var. *pubescens* as potential candidate for the treatment of intestinal inflammation.
Study design: Evaluation of anti-inflammatory effect of MQEO using dextran sulfate sodium (DSS)-induced intestinal inflammation in mice and exploration of the mechanisms with THP-1 cells.
Methods: C57BL/6 mice were provided drinking water containing 3% DSS for 10 days followed by normal drinking water for 3 days. MQEO (35 and 70 mg/kg) were given 5 days before experiments and continued for another 13 days. At the end of experiments, mice were euthanized and colonic tissue was collected to be analyzed by H&E staining, RT-PCR and immunohistochemistry for evaluating the damage of colons, the mRNA levels of IL-1β, IL-6, IL-12p35 and TNF-α, and the expressions of myeloperoxidase (MPO) and matrix metalloproteinase-9 (MMP-9). The LPS-stimulated THP-1 cell line was used for exploring the role of inflammatory markers using ELISA, western blot and flow cytometry methods.
Results: Oral administration of MQEO (35 and 70 mg/kg) markedly attenuated the symptoms of intestinal inflammation, including diarrhea, rectal bleeding, and loss of body weight. It also reduced the shortening of colon length and histopathological damage. The expressions of MPO and MMP-9 and the mRNA levels of pro-inflammatory cytokines (IL-1β, IL-6 and IL-12p35) in colonic tissue significantly decreased after MQEQ treatment. The activation of NF-κB p65 in colonic mucosa was also markedly suppressed. In addition, MQEO significantly suppressed LPS-stimulated production of TNF-α and IL-1β, effectively blocked phosphorylation of IKK and IκB, and dose-dependently reduced LPS-stimulated expression of TLR4 in THP-1 cells at concentrations ranging from 0.01‰ to 0.05‰ (v/v).
Conclusion: MQEO exhibited protective effect against DSS-induced intestinal inflammation and the anti-inflammatory activity may be associated with TLR4 mediated NF-κB signaling pathway, suggesting it might be used as an anti-inflammatory agent.

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Introduction

Zanthoxylum myriacanthum var. *pubescens* is distinguished by its softly hairy rachis, leaves and petiolules. It is endemic to the Xishuangbanna region, China (Huang, 1997) and is known as Maqian in minority Dai area of China (Jia and Li, 2005; Li et al., 2014). Ethnobotanical investigations showed that the fruits of *Z. myriacanthum* var. *pubescens* have a special fragrance and are widely consumed as a spice for cooking by Dai people (Huang, 1997). Its fruits and roots are frequently used as an ethnic

Abbreviations: MQEO, Fruits essential oil of *Zanthoxylum myriacanthum* var. *pubescens*; IBD, Inflammatory bowel disease; UC, Ulcerative colitis; CD, Crohn's disease; DSS, Dextran sulfate sodium; NF-κB, Nuclear factor-κB; TNF-α, Tumor necrosis factor α; IL, Interleukin; MPO, Myeloperoxidase; MMP-9, Matrix metalloproteinase-9; TLR, Toll-like receptors; LPS, Lipopolysaccharides; Dex, Dexamethasone; DAB, 3,3′-Diaminobenzidine; DAI, Disease activity index.

* Corresponding author. Tel.: +8606918713169; fax: +8606918713061.
E-mail address: zhangping@xtbg.org.cn (P. Zhang).

[1] These authors contributed equally to this work.

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medicine for treating abnormal pain, parasite invasion, and centipede bites (Jia and Li, 2005). In addition, it is also recommended for gastrointestinal disorders (Li et al., 2014).

Ulcerative colitis (UC) and Crohn's disease (CD) are the two main types of inflammatory bowel disease (IBD) and characterized by chronic and recurring intestinal inflammation. Although the exact etiology and pathogenesis remain unknown, a series of complex interactions, including environmental factors, infectious microbes, ethnic origin, and genetic susceptibility, are involved in the development of IBD (Baumgart and Carding, 2007). Studies revealed that the mucosal immune system is activated during IBD, which is commonly accompanied by the overproduction of inflammatory cytokines such as tumor necrosis factor α (TNF-α), interleukin-1β (IL-1β), IL-6, and IL-12 (Baumgart and Carding, 2007). Several intracellular pathways, including nuclear factor-κB (NF-κB), were proved to be activated in this disease process and regulated a pleiotropic gene expression (Wong and Tergaonkar, 2009). Membrane-associated toll-like receptors (TLR) are considered as a key receptor for recognition of gut microbes. Lipopolysaccharide (LPS), which is the major component of the outer membrane of Gram-negative bacteria, can be directly recognized by TLR4 and mediates signal pathway transduction resulting in triggering translocation of NF-κB and the secretion of inflammatory cytokine (Płóciennikowska et al., 2015).

The 5-aminosalicylates, glucocorticosteroids, and immunomodulators have been commonly used for the treatment of IBD, however, they possesses a series of side effects and can cause related complications, such as diabetes mellitus, osteonecrosis, and hepatic fibrosis or cirrhosis (Baumgart and Sandborn, 2007). Natural products derived from medicinal plants or herbs have been developed as an important complementary treatment for IBD (Gilardi et al., 2014; Rahimi et al., 2010). Although the fruits essential oil of *Z. myriacanthum var. pubescens* (MQEO) was reported to exhibit an NO inhibitory effect on LPS-stimulated RAW 264.7 cells (Li et al., 2014), the effect on inflammatory disease *in vivo* has not been identified. In the present study, we investigated the anti-inflammatory effect *in vivo* against dextran sulfate sodium (DSS)-induced intestinal inflammation in mice and explored related mechanisms with the THP-1 cell line.

Material and methods

Animals

Female C57BL/6 mice (6–8 weeks, 20–25 g) were purchased from Wei Tong Li Hua Experimental Animal Technical Corporation, Beijing, China. Animals were housed under a 12-h light/dark cycle in constant temperature and humidity with access to food and water *ad libitum* and were acclimatized to the experimental room for one week before testing. All experimental procedures were approved by the ethics committee on laboratory animal use of Xishuangbanna Tropical Botanical Garden (XTBG), Chinese Academy of Sciences.

Plant material

The fruits of *Z. myriacanthum* var. *pubescens* were collected from a market in Mengla, Yunnan Province, China in December 2014. The botanical authentication was carried out by one of the authors You-Kai Xu. A voucher specimen (HITBC_152673) was deposited at the herbarium of XTBG.

Preparation and analysis of MQEO

MQEO was extracted using hydrodistillation method (Li et al., 2013). The analysis of MQEO was performed using an Agilent 7890A gas chromatograph equipped with an Agilent 5975 inert mass selective detector (mass rang, 20–500 m/z; EI ionization potential, 70 eV) and an FID detector. The column is HP-5 (5% phenyl methyl siloxan) capillary column (50 m × 0.32 mm; film thickness, 0.52 μm) and helium was used as the carrier gas with the flow rate of 2.2 ml/min. Injector and FID detector temperatures were both 250 °C. The oven temperature was programmed rising from 50 to 100 °C at 2 °C/min, and held isothermal at 100 °C for 20 min; then increased to 250 °C at 10 °C/min. one microliter sample was injected manually.

Antibodies and reagents

Anti-β-actin (42 kDa), anti-NF-κB p65 (65 kDa), anti-IκB (39 KDa), anti-Phospho-IκB (40 kDa), anti-IKKβ (87 kDa) and anti-Phospho-IKKα/β (IKKα, 85 kDa; IKK-β, 87 kDa) antibodies were Cell Signaling Technology products, while anti-myeloperoxidase (MPO) and anti-matrix metalloproteinase-9 (MMP-9) antibodies were purchased from Abcam. Horseradish peroxidase (HRP) conjugated secondary antibody and 3, 3′-diaminobenzidine (DAB) were from Proteintech. In addition, DSS (36–50 kDa) (MP Biochemicals, USA), d-limonene (purity ≥ 98%), lipopolysaccharides (LPS), and dexamethasone (Dex) (Sigma-Aldrich, USA) were also used in this study.

Induction of intestinal inflammation and treatments

Mice were randomized into groups. To induce acute intestinal inflammation, mice were provided with drinking water containing 3% (w/v) DSS for 10 days followed by normal drinking water for 3 days (Wirtz et al., 2007). Mice in the vehicle and DSS groups were daily administered orally with vehicle solution (0.2% Tween-80, Tianjin Chemical and Industry Factory, China), while other groups were treated with MQEO (35 and 70 mg/kg) dissolved in vehicle solution. Drugs and vehicle solution were given 5 days before administration of DSS and continued until the end of experiments. The disease activity index (DAI) including body weight, stool consistency and fecal blood levels were monitored daily and calculated as previously described (Cooper et al., 1993) (Table S1). At the end of experiments, mice were sacrificed and colons were collected. Colon length was measured and the distal part was excised for histological assessment with H&E staining. The degree of lesion was scored according to the previous report (Wu et al., 2014) (Table S2).

RNA extraction and quantitative RT-PCR analysis

Total RNA was extracted from colonic tissues using Trizol reagent (Invitrogen, USA) according to the manufacturer's instruction. Complementary DNA (cDNA) was reverse transcribed from 1 μg RNA using PrimeScript RT reagent kit (TaKaRa, Japan). RT-PCR detection of gene expression was performed using a SYBR *Premix Ex Taq* II kit (TaKaRa, Japan) on the LightCycler 480 II system of the fluorescent quantitative PCR (Roche, Switzerland). The thermocycler conditions were: initial denaturation at 95 °C for 30 s, 40 cycles of 95 °C for 10 s, and 58 °C for 1 min. The sequences of primers (Table S3) were synthesized by BGI (Shenzhen, China). The expression of each gene was normalized to the reference gene, β-actin, and calculated relative to the vehicle group according to the $2^{-\Delta\Delta Ct}$ method.

Immunohistochemical analysis

Immunohistochemical analysis of colon sections was performed according to methods previously described (Wu et al., 2014). Paraffin-embedded slices of colon tissue (4 μm) were incubated

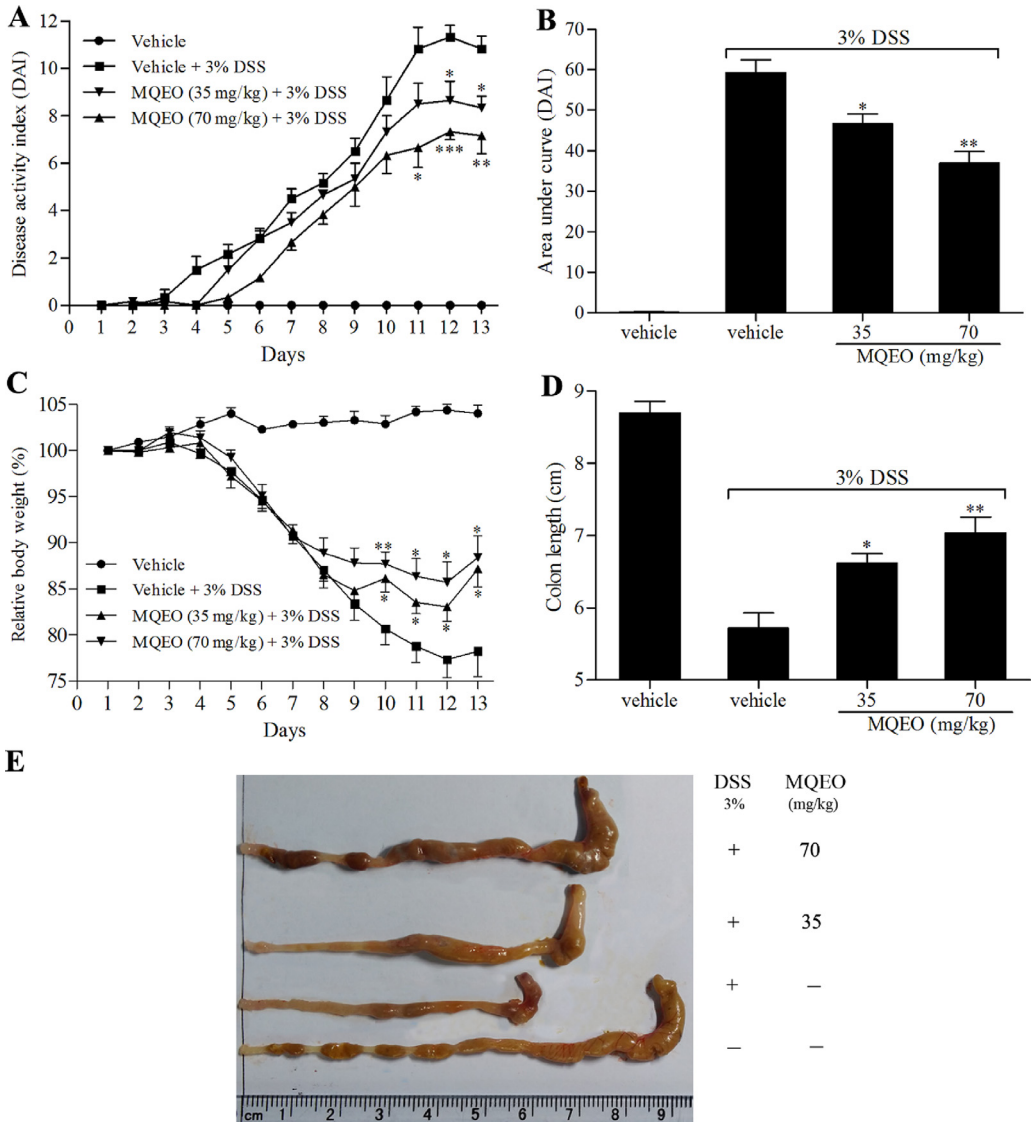

Fig. 1. Effects of pre-treatment with MQEO on the changes in disease activity index (DAI) (A–B), body weight (C) and colon length (D–E) of mice with DSS-induced intestinal inflammation. Data are mean ± SEM ($n = 6–8$) ($^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. DSS group).

Table 1
Composition of the volatile compounds identified in the essential oil of *Zanthoxylum myriacanthum* var. *pubescens* fruits.

Compounds	R_t (min)	RI (cal)	RI (lit)	Composition (%)
1,3-Dimethyl-benzene	17.84	881	–	0.59
α-Pinene	22.39	938	939	7.06
β-Pinene	26.38	992	979	3.92
α-Phellandrene	27.83	1008	1002	17.97
3-Carene	28.34	1013	1011	0.54
o-Cymene	29.61	1024	1026	5.76
Limonene	30.14	1029	1029	31.20
β-Phellandrene	30.26	1030	1029	20.31
cis-β-Ocimene	30.53	1033	1037	2.95
trans-β-Ocimene	31.65	1044	1050	3.52
α-Terpineol	49.61	1197	1188	0.36
n-Decanal	50.32	1208	1201	0.66
Acetic acid octyl ester	50.58	1214	–	0.69
Dodecanal	57.60	1414	1408	0.29
β-Caryophyllene	58.33	1448	1419	0.70
Germacrene D	59.61	1505	1485	0.61
Total				97.13

Rt (min): retention time; RI (cal): Linear retention indices determined relative to a series of *n*-alkanes (C8–C30) on the *HP-5* capillary column; RI (lit): literature indices.

overnight with anti-NF-κB p65, anti-MPO and anti-MMP-9 primary antibodies at 4 °C. Then washed with PBS and incubated with HRP-conjugated secondary antibody for 1 h at room temperature. After washing with PBS again, the slices were developed using DAB as a chromogen and counter-stained with hematoxylin. Images were acquired using a Leica DM2500 system (Lecia Microsystems, GER).

Anti-inflammatory activity in THP-1 cells

THP-1 cells were obtained from the Kunming cell bank of the Chinese Academy of Sciences (Kunming, China). Cells were pretreated with MQEO (v/v, 0.01‰, 0.02‰, 0.04‰, and 0.05‰), limonene (v/v, 0.01‰, 0.02‰ and 0.04‰) or Dex (5 μM) for 30 min before LPS (1 μg/ml) stimulation for 24 h. Supernatant was collected and the production of TNF-α and IL-1β was assayed using ELISA kits (BD Biosciences, USA) according to the manufacturer's instructions.

Flow cytometry

Cells were treated with MQEO as indicated above then collected, washed twice with PBS, and re-suspended in 100 μl of

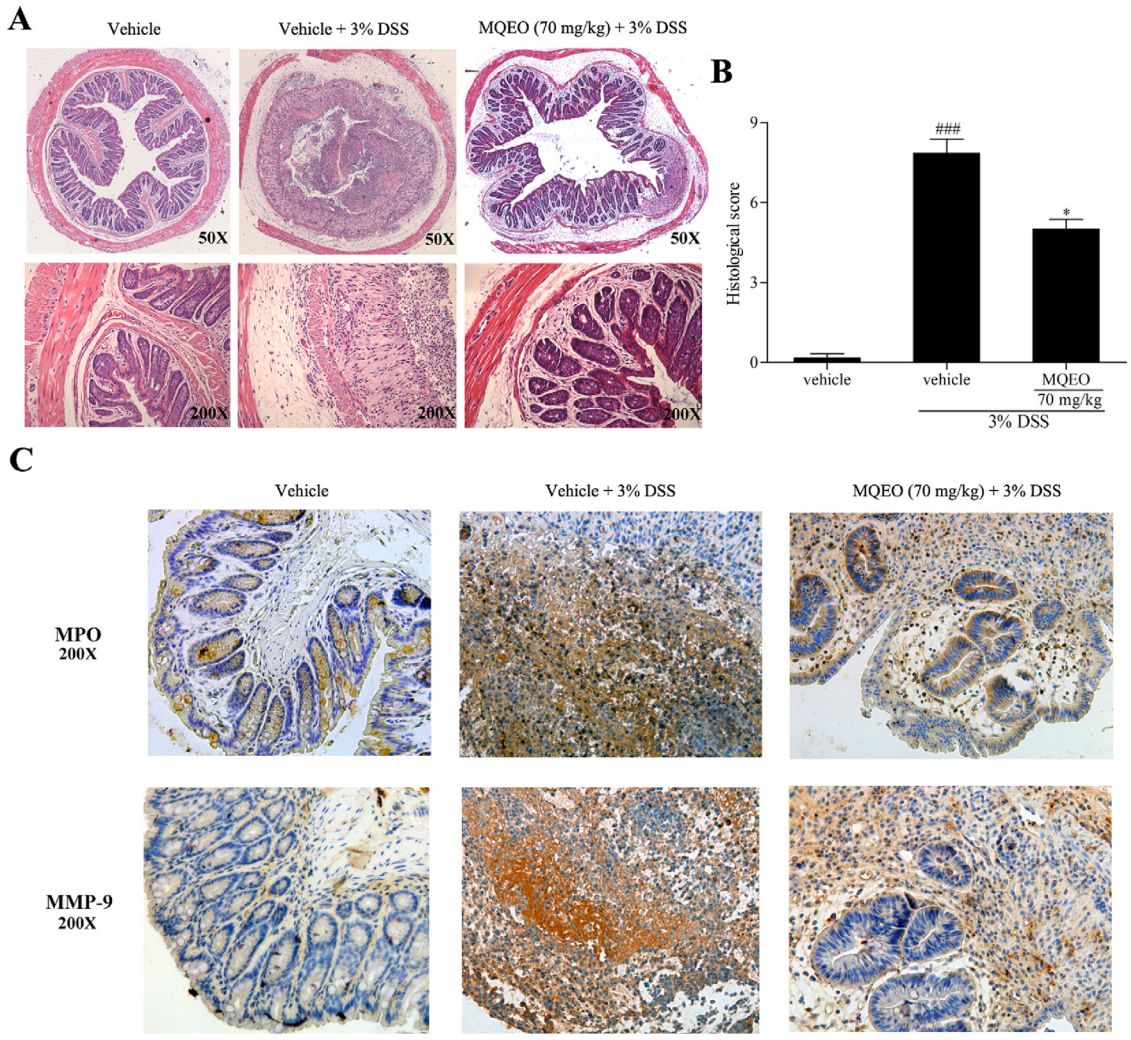

Fig. 2. Effects of pre-treatment with MQEO on the changes of histological section (A), histological score (B) and inflammatory markers (MPO and MMP-9) expressions (C) in colonic tissue of mice with DSS-induced intestinal inflammation. Data are mean ± SEM ($n = 6$) ($^{###}P < 0.001$ vs. vehicle group; $^{*}P < 0.05$ vs. DSS group).

human Fc receptor binding inhibitor (Affymetrix, USA) at 4 °C for 20 min. Subsequently, washed with PBS containing 2% FBS, and incubated with PE-conjugated anti-human CD284 (TLR4) (Affymetrix, USA) for 30 min at 4 °C. After being washed twice with PBS, cells were re-suspended in 400 μl of PBS containing 2% FBS and analyzed on a BD FACSVerse flow cytometer.

Western blot analysis

After treatment with MQEO as indicated above, cells were collected and lysed in a RIPA lysis buffer (Vazyme, China) with protease inhibitors at 4 °C. The concentration of cell protein lysates was determined using a Micro BCA Protein Assay Kit (Bytotime, China). Thirty to fifty micrograms of each sample was mixed with 5× SDS loading buffer and heated at 100 °C for 10 min before being resolved by SDS-polyacrylamide gel electrophoresis. Proteins were transferred to a PVDF membrane and blocked for 1 h with TBS buffer containing 5% non-fat milk, then incubated overnight with primary antibodies at 4 °C. After thorough washes, membranes were incubated with HRP-coupled secondary antibody for 1 h. Protein bands were detected using a chemiluminescence reagent (Millipore, USA) by the ChemiDoc XRS+ Imaging System (Bio-Rad, USA) and quantified by Image-Pro Plus software. Densitometry values were normalized to β-actin.

Data analysis and statistics

All analyses were conducted using GraphPad Prism 5.0 software. Data were expressed as means ± SEM. Statistical analysis was performed using One-way ANOVA followed by Dunnett's T3 test and a two-tailed Student's *t*-test. Differences with $P < 0.05$ were considered to be statistically significant.

Results

Chemical composition of MQEO

MQEO was obtained as yellowish oil with 4% yield (w/w on the basis of the weight of dry material). Sixteen compounds were identified with a predominance of monoterpenes (Table 1). The main constituents were limonene (31.20%), β-phellandrene (20.31%), α-phellandrene (17.97%), α-pinene (7.06%), o-cymene (5.76%), trans-β-ocimene (3.52%), β-pinene (3.92%) and cis-β-ocimene (2.95%) (Table 1).

MQEO attenuated DSS-induced intestinal inflammation

After oral administration of 3% DSS solution for 10 days, test mice in the DSS group soon developed severe symptoms of clinical

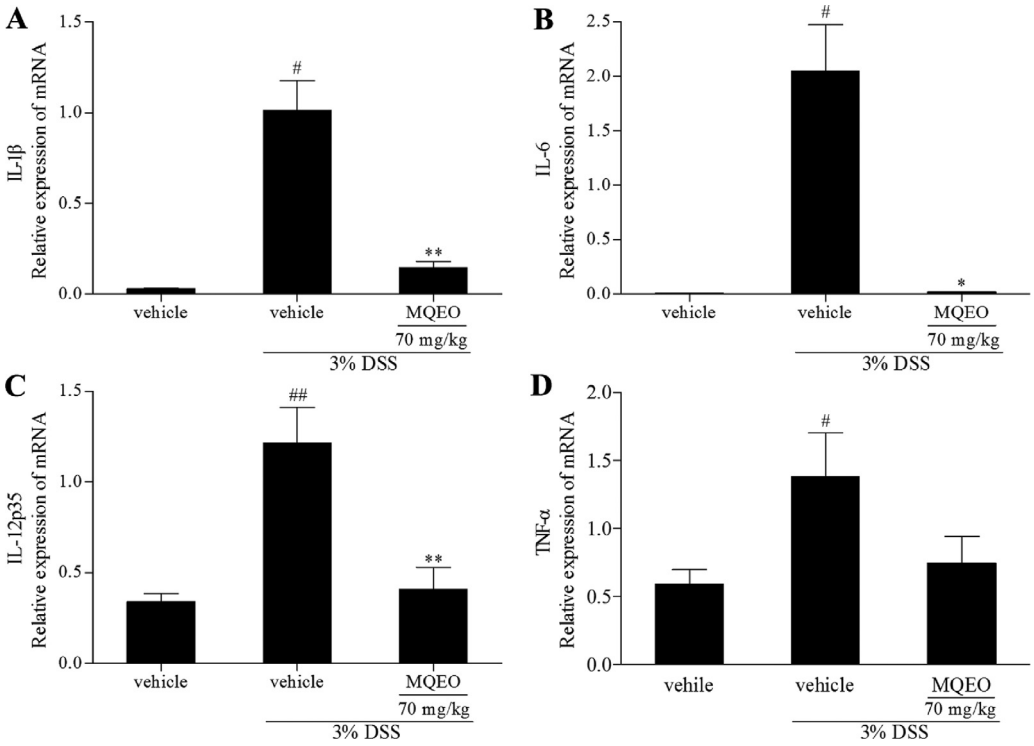

Fig. 3. Effects of pre-treatment with MQEO on reduced mRNA levels of cytokines IL-1β (A), IL-6 (B), IL-12p35 (C) and TNF-α (D) in colonic tissue of mice with DSS-induced intestinal inflammation. Data are mean ± SEM (n = 4–5) ($^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs. vehicle group; $^{**}P < 0.01$ vs. DSS group).

intestinal inflammation, including marked diarrhea, rectal bleeding, and loss of body weight, which resulted in an increase in the DAI from day 4 and a rapid decrease of relative body weight (%) compared with vehicle group (Fig. 1A–C). In order to determine the effect against intestinal inflammation, MQEO was administered orally at the doses of 35 and 70 mg/kg. Interestingly, both treatments significantly attenuated the loss of body weight from day 10 to 13 (Fig. 1C). Daily monitored results showed that both diarrhea and gross bleeding were ameliorated and the DAI scores significantly decreased on day 11, 12 and 13 (Fig. 1A and B). The shortening of colon length is another important symptomatic parameter of DSS-induced intestinal inflammation. As shown in Fig. 1D and E, MQEO treatment effectively reduced the shortening of colon length in test mice.

MQEO reduced colon microscopic damage and the expressions of MPO and MMP-9 in DSS-induced intestinal inflammation

Colon histopathology was evaluated by H&E staining (Fig. 2). In vehicle group, the colons presented the normal architecture of mucosa with complete crypts and a small number of lamina propria mononuclear cells (Fig. 2A). However, severe damage to crypts and epithelia with extensive cellular infiltration into the lamina propria and sub-mucosa in the colon tissue appeared after DSS treatment. Moreover, the architecture of the section was completely destroyed, resulting in a higher score than the control group (Fig. 2B). The sections of MQEO (70 mg/kg) treated mice showed less crypt destruction and cellular infiltration than the DSS group (Fig. 2A). The histological scores were also substantially decreased after MQEO treatment (Fig. 2B). In addition, we tested the expressions of inflammatory markers MPO and MMP-9 in the colon tissue by immunohistochemical staining (Fig. 2C). MQEO (70 mg/kg) treatment also markedly reduced the expressions of MPO and MMP-9 compared with the DSS group.

MQEO decreased the mRNA expressions of IL-1β, IL-6, IL-12p35 and TNF-α in colon

Many pro-inflammatory cytokines, such as IL-1β, IL-6, IL-12p35 and TNF-α, are involved in the initiation and development of IBD. The mRNA levels of those cytokines in colon were evaluated using the quantitative RT-PCR method. After oral administration with DSS, the relative mRNA expressions of IL-1β, IL-6, IL-12p35 and TNF-α were significantly elevated in the DSS group (Fig. 3). MQEO (70 mg/kg) treatments effectively inhibited the mRNA expressions of IL-1β, IL-6 and IL-12p35 (Figs. 3A-C). In addition, the mRNA level of TNF-α also showed a trend of decrease after MQEO treatment (70 mg/kg) compared with the DSS group (Fig. 3D). Consistent with mRNA expression data, treatment of DSS significantly increased protein levels of IL-1β and TNF-α in the colonic tissue (Fig. S1). MQEO treatment significantly suppressed IL-1β production and only showed a trend of suppression of TNF-α production. These data were well correlated with the protective effect of MQEO against intestinal inflammation.

MQEO suppressed TNF-α and IL-1β secretions in LPS-stimulated THP-1 cells

To study the anti-inflammatory mechanisms of MQEO, THP-1 cells were used and the inhibitory effects on LPS-stimulated production of inflammatory cytokines were determined by ELISA. As shown in Fig. 4, LPS treatment led to a marked up-regulation of the levels of TNF-α and IL-1β. However, after treatment with MQEO (v/v, 0.01‰, 0.02‰, 0.04‰ and 0.05‰), the up-regulation was significantly inhibited in a dose-dependent manner (Fig. 4A, C and E) and the inhibition rates were 60 and 82% of TNF-α and 46 and 48% of IL-1β at 0.04‰ and 0.05‰ (v/v), respectively. Limonene is the major component of MQEO (Table 1) and the inhibition rates against LPS-stimulated production of TNF-α and IL-1β were only 41% of TNF-α and 30% of IL-1β at 0.04‰ (v/v)

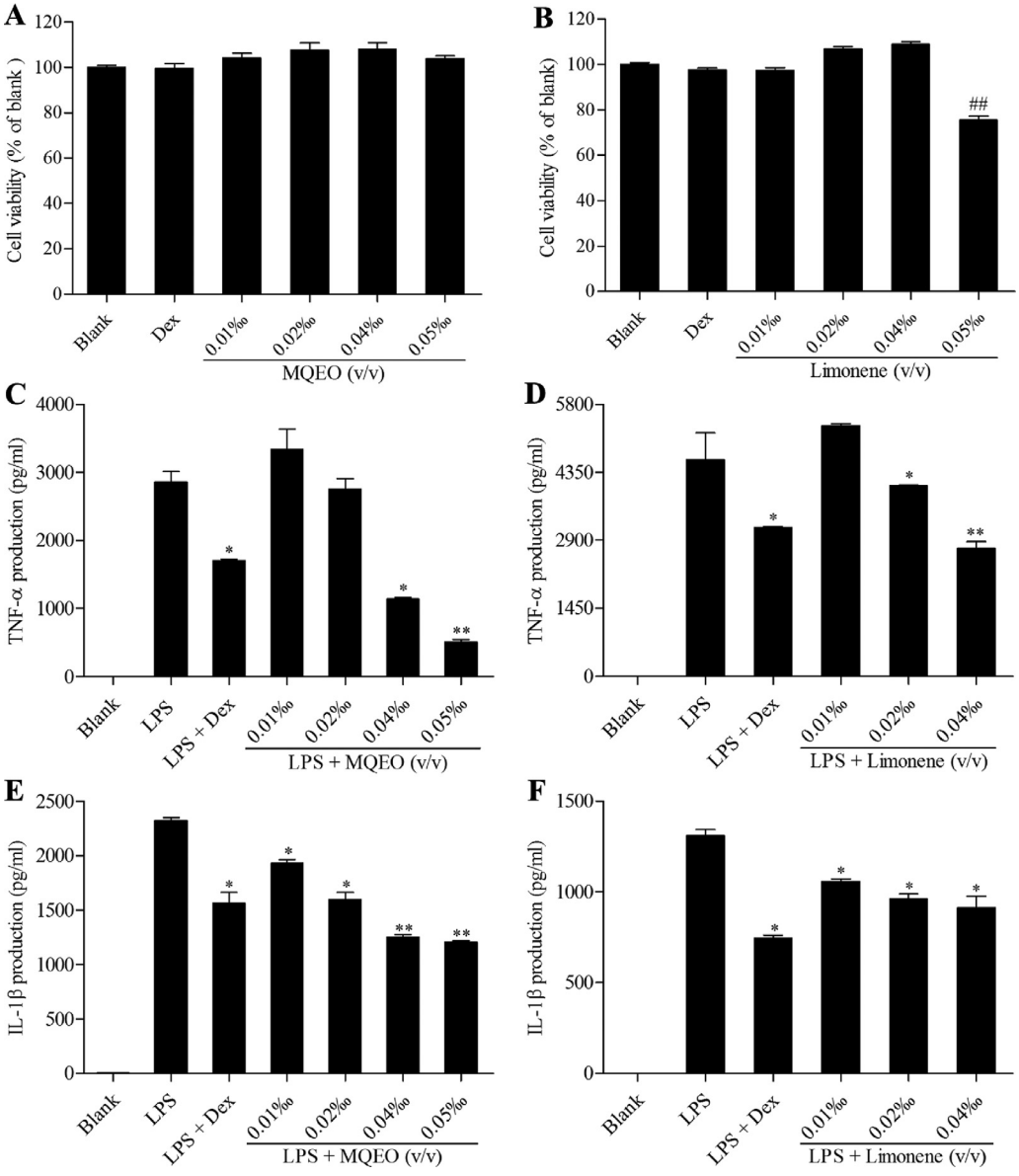

Fig. 4. Effects of MQEO and limonene on cell viability (A–B), TNF-α (C–D) and IL-1β (E–F) levels in LPS-stimulated THP-1 cells. Dex (5 μM) was treated as a positive control. Data are mean ± SD ($n = 3$) ($^{\#\#}P < 0.01$ vs. blank group; $^{*}P < 0.05$, $^{**}P < 0.01$ vs. LPS group).

(Fig. 4B, D and F). Those data also showed a better effect of MQEO than limonene against the LPS-stimulated secretions of TNF-α and IL-1β.

Previous studies have shown that limonene has the potential of anti-inflammatory bioactivities (d'Alessio et al., 2013; Yoon et al., 2010). We therefore tested its activity against DSS-induced intestinal inflammation in mice, but the results including the relative body weight, disease activity index and colon length did not show significant effects at the doses of 14 and 28 mg/kg.

MQEO suppressed IκB and IKK phosphorylation and TLR4 expression in LPS-induced THP-1 cells and reduced NF-κB p65 activation in colon of mice with DSS-induced intestinal inflammation

NF-κB is an important transcription factor in the pathophysiological process of inflammation. For the intestinal inflammation experiment, the expression of NF-κB p65 in the colonic tissue was analyzed by immunohistochemical staining and the levels in nuclear and cytoplasm were largely suppressed after MQEO treatment (70 mg/kg) (Fig. 5E). In THP-1 cells, LPS stimulation led to marked phosphorylation of IκB and IKK as shown by western blotting (Fig. 5A–C). However, the phosphorylation was significantly suppressed after MQEO treatment. In addition, the expression of TLR4 in LPS-stimulated THP-1 cells was evaluated by flow cytometry. The results showed MQEO effectively suppressed LPS-stimulated increase in TLR4 expression in a dose-independent manner (v/v, 0.01‰, 0.02‰, 0.04‰ and 0.05‰) (Fig. 5D).

Discussion

Traditional medicinal herbs are increasingly used for treating IBD (Gilardi et al., 2014; Rahimi et al., 2010). *Z. myriacanthum* var. *pubescens* is a traditional medicinal herb whose fruits are commonly consumed as a spice and used for the treatment of gastrointestinal disorders. A previous report showed that MQEO suppressed LPS-stimulated NO production in RAW 264.7 cells, suggesting its anti-inflammatory activity (Li et al., 2014). However, no reports demonstrated the anti-inflammatory of MQEO *in vivo*.

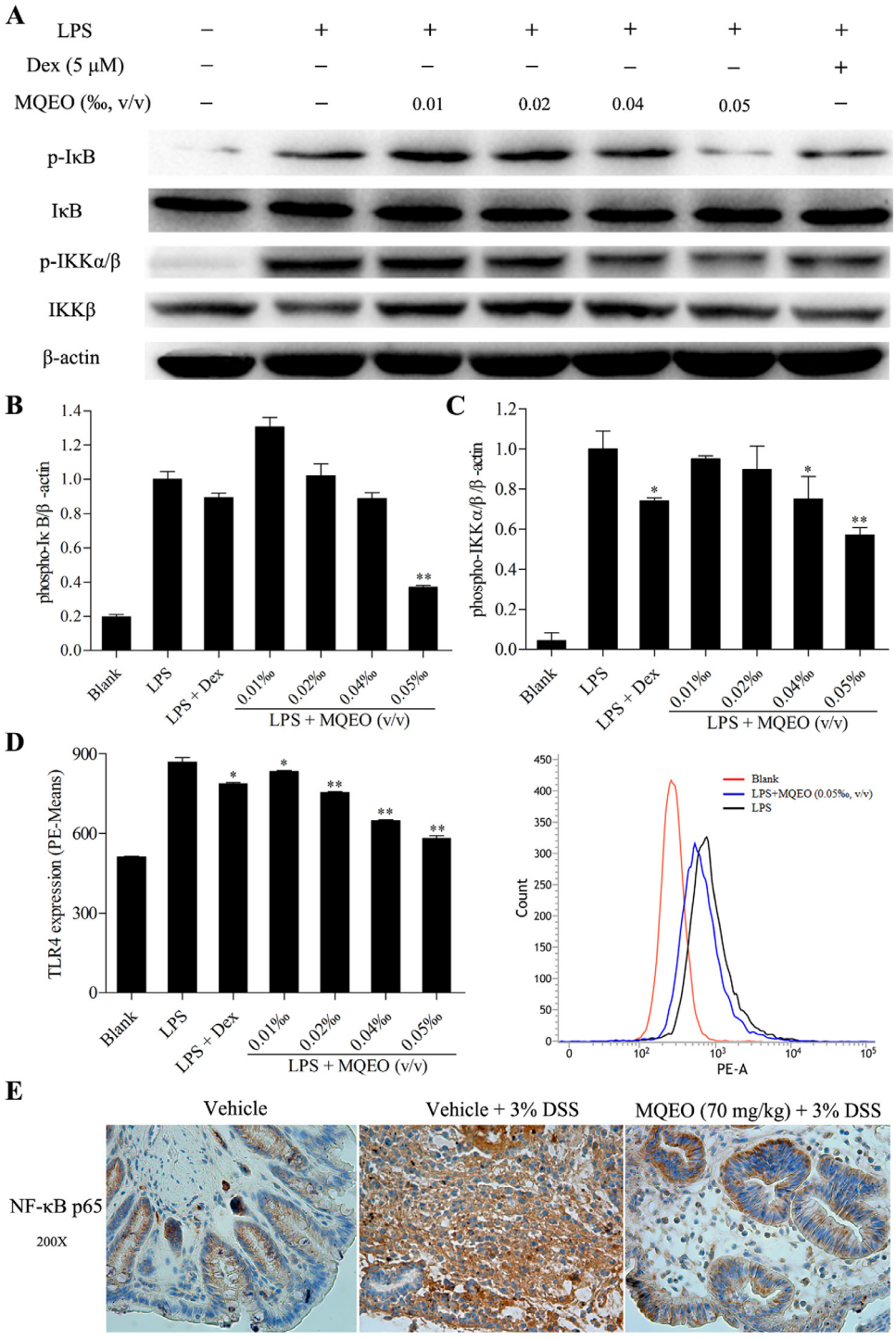

Fig. 5. Effects of MQEO on the expressions of IκB (A, B), IKK (A, C) and TLR4 (D) in LPS-induced THP-1 cells and immunostaining for NF-κB in colonic tissue of mice with DSS-induced intestinal inflammation (E). Data are presented as mean ± SD ($n = 3$) ($^{*}P < 0.05$, $^{**}P < 0.01$ vs. LPS group).

Herein we evaluated its effect in the DSS-induced intestinal inflammation. This is also the first time to reveal the beneficial effects of MQEO in intestinal inflammation.

UC is a chronic inflammatory disease of the gastrointestinal tract which often happens with the dysregulation of the mucosal immune system (Baumgart and Carding, 2007). DSS can directly damage the gut epithelial cells of tested mice, followed by the intestinal inflammation symptoms of diarrhea, rectal bleeding, extensive crypt destruction, infiltration with granulocytes, edema and ulceration (Cooper et al., 1993). Because of the advantages of a simple experimental procedure, high reproducibility, and resemblance to human UC, the DSS-induced intestinal inflammation is therefore reliable for the studies of UC (Wirtz et al., 2007). Using this model, oral administration of MQEO at the doses of 35 and 70 mg/kg ameliorated the specific clinical symptoms induced by DSS, although there was no observation of a significant dose-response. In addition to accompanying a decrease in the DAI score, including loss of body weight, stool consistency and fecal blood, the protective effect of MQEO also prevented the shortening of colon length. Similar intestinal anti-inflammatory activity with

alleviation of the severity and extension of the intestinal injuries in mice is also exhibited by other essential oils, such as thyme aromatic oil, oregano aromatic oil (Bukovská et al., 2007) and cinnamon oil (Bujňáková et al., 2013).

Infiltration of leukocytes into the inflamed tissues is a hallmark of many chronic inflammatory states. The degradation of the extracellular matrix by MMP was shown to involve this process (O' Sullivan et al., 2015). Moreover, the overproduction of reactive oxygen species and nitrogen species in inflamed tissue can induce severe oxidative stress for IBD patients. To reduce this impairment, the redox-sensitive signaling pathway was activated and released related enzymes including MPO (Wu et al., 2014). Immunohistochemical staining showed the high level of MPO and MMP-9 in colon tissue in the DSS-induced intestinal inflammation group and markedly decreased after MQEO treatment. These results are consistent with improvement in histological changes in colon tissue.

Overproduction of inflammatory cytokines is primarily responsible for the inflammation cascade reaction and consequent colon damage in the development of UC (Baumgart and Carding, 2007). Our data also showed MQEO reduced the mRNA expressions of TNF-α, IL-1β, IL-6 and IL-12p35 in DSS-induced intestinal inflammation and this may be related to the inhibition of NF-κB activation. It is well accepted that the NF-κB pathway plays an important role in the maturation of UC (Wong and Tergaonkar, 2009) and the inflammation process, and has been developed as a target for seeking anti-inflammatory agents from natural products. For example, ellagic acid and curcumin, which are naturally occurring plant phenols, exhibited anti-inflammatory activity through NF-κB signaling pathway by preventing IκB degradation and inducing an inhibition of the nuclear translocation of p65 (Kim et al., 2005; Rosillo et al., 2011). In THP-1 cells, MQEO (v/v, 0.01‰–0.05‰) effectively prevented the LPS-stimulated increase in TLR4 expression and markedly blocked the downstream IκB kinase (IKK) and IκB phosphorylation. Moreover, the pro-inflammatory cytokines, including TNF-α and IL-1β, were also suppressed, suggesting the anti-inflammatory effect may be associated with the TLR4 mediated NF-κB signaling pathway.

Identification of the specific and bioactive compounds from medicinal plants has attracted interest due to candidate drug development (Gautam and Jachak, 2009). GC–MS analysis of MQEO (Table 1) has showed that limonene is the major ingredient and it exhibited some anti-inflammatory activity in LPS-stimulated THP-1 cells which was not as good as MQEO. However, limonene alone (14 and 28 mg/kg) did not show beneficial effects against DSS-induced intestinal inflammation. Whether other single compound other than limonene or several ingredient in MQEO together contribute synergistically for the benefits against DSS-induced intestinal inflammation remain to be further investigated. Furthermore, more detail investigations should be performed to further explore the exact mechanism of MQEO for anti-inflammatory activity *in vivo* and *in vitro*.

In summary, we demonstrated that the EO from *Z. myriacanthum* var. *pubescens* fruits possesses healing activity against DSS-induced intestinal inflammation in mice. The anti-inflammatory effect was through inhibiting the overproduction of inflammation mediators and may be associated with TLR4 mediated NF-κB signaling pathway. In addition, our data also support the traditional use of *Z. myriacanthum* var. *pubescens* against digestive disorders and suggest its fruits EO might be a useful therapy for the treatment of human IBD.

Conflict of interest

The authors have declared that there is no conflict of interest.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.phymed.2016.05.006.

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