European Journal of Pharmacology 811 (2017) 240–248

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Full length article

Methylsulfonylmethane is effective against gastric mucosal injury

Keyvan Amirshahrokhi[a,*], Ali-Reza Khalili[b]

[a] Department of Pharmacology, School of Pharmacy, Ardabil University of Medical Sciences, Ardabil, Iran
[b] Division of Pathology, Imam Hospital, Ardabil University of Medical Sciences, Ardabil, Iran

ARTICLE INFO

Keywords:
Methylsulfonylmethane
Gastric ulcer
Oxidative stress
Inflammation
Ethanol

ABSTRACT

Methylsulfonylmethane (MSM) is a natural organosulfur compound has been widely used as a dietary supplement. MSM has protective effects against various disorders through its anti-inflammatory and antioxidant properties however the effect of MSM on gastric mucosal injury remains unclear. The aim of the present study is to determine whether MSM has beneficial effects on ethanol/HCl-induced gastric ulcer in mice. Macroscopic and histopathological evaluation of gastric mucosa revealed that ethanol/HCl administration produced apparent mucosal injuries, while pretreatment with MSM (200 and 400 mg/kg, orally) could effectively protect gastric mucosa against the injuries caused by acidified ethanol. MSM significantly increased the levels of glutathione (GSH), catalase (CAT) and prostaglandin E_2 (PGE_2), and decreased the levels of malondialdehyde (MDA), myeloperoxidase (MPO), carbonyl protein, and nitric oxide (NO) in gastric tissues compared with those in the ethanol group. MSM suppressed gastric inflammation by reducing the levels of proinflammatory cytokines tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6, monocyte chemoattractant protein (MCP)-1 and matrix metalloproteinase (MMP)-9. Moreover, pretreatment of mice with MSM decreased the expression of nuclear factor kappa B (NF-κB) as a key regulator of inflammation in gastric mucosa. Taken together, these data suggest that MSM is able to decrease the severity of ethanol/HCl-induced gastric mucosal injury through inhibition of oxidative stress and inflammation.

1. Introduction

Peptic ulcer is a chronic multifactorial disease of the gastrointestinal tract that affects the mucosal lining of the stomach. The pathophysiology of gastric mucosal injury results from an imbalance between defensive factors, such as gastric mucus, bicarbonate and prostaglandin E, and aggressive factors such as gastric acid, pepsin, non-steroidal anti-inflammatory drugs (NSAIDs), alcohol and Helicobacter pylori infection (Pérez et al., 2017). It has been demonstrated that alcohol consumption causes gastric mucosal injury through direct effects such as gastric epithelial cell damage and indirect effects such as the recruitment of leukocytes and induction of inflammation and oxidative stress (Kang et al., 2017). Ethanol metabolism results in the production of large amounts of reactive oxygen species (ROS) including the superoxide radicals, hydrogen peroxide and peroxynitrite. Ethanol also significantly diminishes the levels of antioxidant agents that can remove ROS. The imbalance between the formation and removal of free radicals plays an important role in the pathogenesis of gastric mucosal damage (Yu et al., 2014; Amirshahrokhi and Khalili, 2016a). It has also been shown that infiltration of neutrophils into the gastric mucosa and release of proinflammatory cytokines such as TNF-α, IL-1β, IL-6 and chemokine MCP-1 have a critical role in the development of gastric mucosal inflammation and injury (Amirshahrokhi and Khalili, 2015a; Watanabe et al., 2004). Nuclear factor-kappa B (NF-κB) is an important transcription factor that regulates inflammatory processes in gastric mucosal damage (Arab et al., 2015). Prostaglandin E (PGE) has been shown to protect gastric mucosa from gastric irritants and the decreased formation of PGE aggravates peptic ulcer disease (Hoshino et al., 2003). In the recent few years, many alternative medicines from natural resources are considered for treatment of gastric ulcer disease (Boeing et al., 2016; Sidahmed et al., 2013).

Methylsulfonylmethane (MSM) or dimethyl sulfone is a natural organosulfur compound (Fig. 1A) found in many green plants, fruits, vegetables and grains. MSM is widely used as a dietary supplement in worldwide and is believed to be nontoxic to humans. MSM is considered as an important source of sulfur for the production of the sulfur-containing amino acids methionine and cysteine. Clinical and experimental studies have shown potential preventive and therapeutic effects of this compound. MSM has not been approved by FDA however it has no toxicity in human studies and is generally recognized as safe by the FDA. MSM has been shown to possess strong antioxidant and anti-inflammatory activities in many studies (Amirshahrokhi and

* Corresponding author.
E-mail addresses: k.amirshahrokhi@arums.ac.ir, k.amirshahrokhi@pharmacy.arums.ac.ir (K. Amirshahrokhi).

http://dx.doi.org/10.1016/j.ejphar.2017.06.034
Received 28 March 2017; Received in revised form 22 June 2017; Accepted 26 June 2017
Available online 28 June 2017

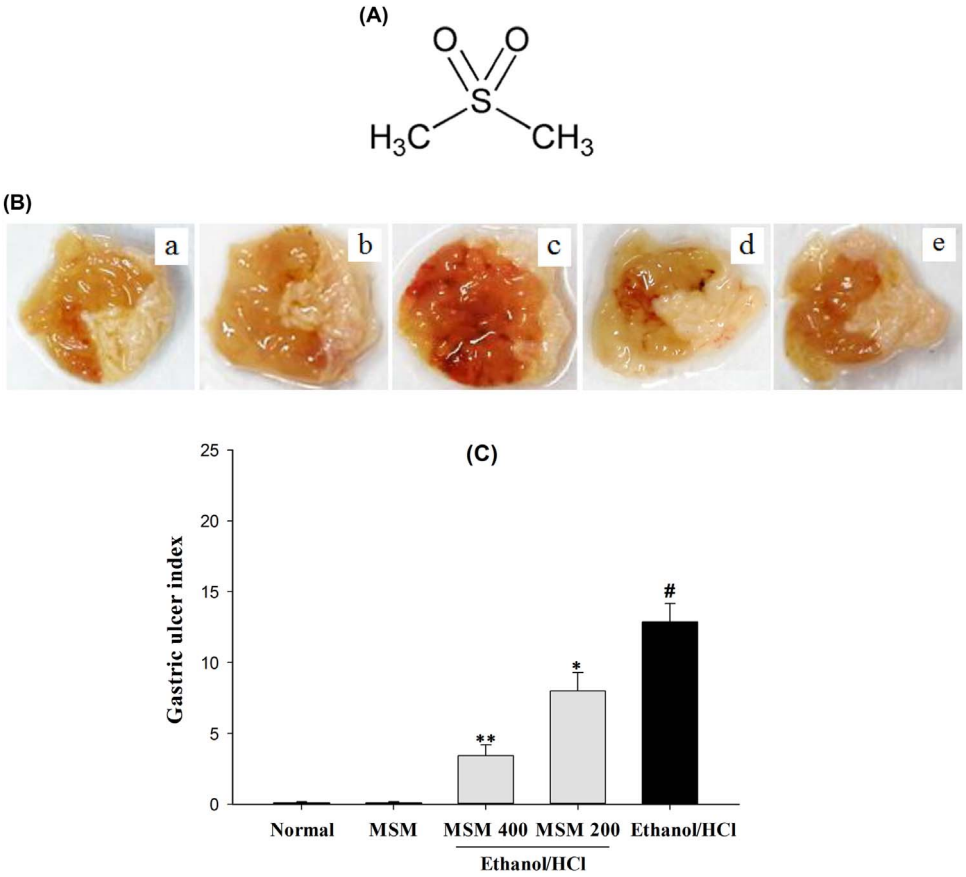

Fig. 1. (A) Chemical structure of MSM (B) Effect of MSM on the macroscopic appearance of the gastric mucosa in ethanol/HCl-induced gastric mucosal lesions in mice. (a) Normal, (b) MSM (400 mg/kg) only, (c) ethanol/HCl, (d) MSM (200 mg/kg) + ethanol/HCl and (e) MSM (400 mg/kg) + ethanol/HCl. (C) gastric ulcer index in each experimental group. The results are the mean ulcer score ± S.E.M. (n = 8). $^{\#}P < 0.001$ compared with normal group; $^{*}P < 0.05$, $^{**}P < 0.01$ compared with ethanol/HCl control group.

Bohlooli, 2013). It has been reported that MSM improves symptoms of pain and physical function in patients with knee osteoarthritis (Kim et al., 2006). MSM has been shown to protect against obesity-induced metabolic disorders, constipation, hyperacidity, mucous-membrane inflammation, intestinal cystitis, colitis and liver damages through its anti-inflammatory and antioxidant properties (Sousa-Lima et al., 2016; Parcell, 2002; Amirshahrokhi et al., 2011).

We hypothesized that, because of its antioxidant and anti-inflammatory effects, MSM may prevent gastric mucosal injury. In this study, we investigated the possible effectiveness and the mechanisms of action of MSM on ethanol/HCl-induced gastric ulcer in mice.

2. Material and methods

2.1. Animals

Experiments were performed on Swiss albino mice weighing 25–30 g. Animals were kept in our animal house under controlled conditions and allowed free access to water and a standard diet. Mice were fasted overnight with free access to water before the induction of gastric ulcers. Animal experiments were conducted in accordance with the National Institute of Health's Guide for the Care and Use of Laboratory Animals (NIH publication 85-23, revised 1996). Study protocol (1395.129) was approved by the animal ethics committee.

2.2. Materials

2,4-dinitrophenylhydrazine, guanidine hydrochloride, trichloroacetic acid, potassium phosphate and Tris–HCl, 5,5′-Dithiobis-2-nitrobenzoic acid, tetramethylbenzidine, thiobarbituric acid, glutathione reduced, 1,1,3,3-tetraethoxypropane, hydrochloric acid, hydrogen peroxide and methylsulfonylmethane were purchased from Sigma-Aldrich. Protease inhibitors (Complete Mini tablets) were purchased from Roche (Germany).

2.3. Experimental design

Administration of ethanol/HCl has long been used as an experimental model to induce gastric mucosal lesions in mice. This model has many clinical and pathophysiologic similarities to human peptic ulcer disease (Lu et al., 2014; Oliveira et al., 2012). The animals were randomly divided into five groups (8 mice per group). The first group served as normal control group and received vehicle only (0.9% saline, orally). The second group of mice was given MSM (400 mg/kg, orally) alone. The third group of mice was given a single dose of acidified ethanol orally. The fourth group was given MSM (200 mg/kg, orally) and acidified ethanol. The fifth group was given MSM (400 mg/kg, orally) and acidified ethanol. MSM was dissolved in saline and administered by gavage 1 h before ulcer induction. After pretreatment with MSM the animals received acidified ethanol (60% ethanol/0.15 M HCl, 0.1 ml/10 g body weight, orally) to induce gastric mucosal damage. The dose of MSM used in this study was selected based on our preliminary experiments.

2.4. Sample collection

One hour after administration of ethanol/HCl all animals were anesthetized with ketamine and xylazine and their stomachs were immediately removed. The stomachs were opened along the greater curvature and rinsed with cold saline solution to remove the gastric

contents and blood clots. Each gastric tissue sample was cut into two parts; one part was fixed in formalin for the histological analysis and another part was frozen in liquid nitrogen and kept at − 80 °C for biochemical analysis.

2.5. Determination of gastric ulcer index

The degree of ethanol/HCl induced gastric mucosal injury was expressed as ulcer index (UI) (Oliveira et al., 2012) Ulcer index was measured based on the number and length of the lesions by using following formula: UI = Σ (A) + (2B) + (3C) (A is the number of small lesions up to 1 mm; B is the number of lesions up to 3 mm; C is the number of linear lesions greater than 3 mm). The mean UI was calculated for each group and statistically analyzed.

2.6. Histopathological analysis

Gastric biopsies were fixed in 10% formalin solution and embedded in paraffin. The paraffin blocks were cut into sections of 4 μm thickness and were stained with hematoxylin and eosin (H & E). The histological evaluation was performed under light microscopy by an expert pathologist. Gastric microscopic damage was scored according to the criteria as described previously (Laine et al., 1988): (1) mucosal edema (score 0–4), (2) Hemorrhage (score 0–4), (3) inflammatory cell infiltration (score 0–3) and (4) epithelial cell loss (score 0–3).

2.7. Preparation of gastric tissue homogenate

The gastric tissue samples were homogenized in ice-cold Tris–HCl buffer (pH 7.4, containing protease inhibitor cocktail) using a homogenizer (Heidolph, Germany). The homogenized samples were centrifuged at 20,000 g for 20 min in a refrigerated centrifuge at 4 °C. The supernatants were collected and stored at − 80 °C for biochemical examination.

2.8. Measurement of reduced glutathione (GSH)

Gastric GSH levels were determined by a modified Ellman's method. Briefly, 50 μl of 10% trichloroacetic acid (TCA) was added to 50 μl of the supernatant to precipitate the proteins. After shaking, the mixtures were centrifuged at 10,000 g at 4 °C for 5 min and the supernatants were separated from the pellets. 50 μl of the resulting supernatants were added to 150 μl of phosphate buffer (0.2 M, pH 7.6, 1 mM EDTA) and 1 mM DTNB (5,5′-Dithiobis-2-nitrobenzoic acid). Absorbance was measured at 412 nm using a microplate reader and results were expressed as μmol/mg protein.

2.9. Measurement of catalase (CAT)

Catalase activity of each sample was measured according to the modified method of Aebi (1984). Briefly, a 10 μl of each supernatant was added to a cuvette containing 0.5 ml of phosphate buffer (50 mM, pH 7). The reaction was initiated by the addition of 0.5 ml hydrogen peroxide (30 mM) to the reaction mixture and the change in absorbance was measured at 240 nm against a blank containing phosphate buffer by using a spectrophotometer (Shimadzu UV-1800, Japan). One unit of enzyme activity was defined as the amount of enzyme that catalyzes the decomposition of 1 mM hydrogen peroxide per minute.

2.10. Measurement of malondialdehyde (MDA) levels

Malondialdehyde is one of the end-products of lipid peroxidation and commonly used as a reliable biomarker of lipid peroxidation process in tissues. The levels of MDA in the gastric tissues were determined using the thiobarbituric acid (TBA) method. Briefly, 100 μl of the supernatant was added to a reaction mixture containing 100 μl of 20% (w/v) TCA and 200 μl of TBA (0.1 M). The reaction mixture was incubated for 1 h at 90 °C. After cooling, the samples were centrifuged at 10,000 g for 5 min and the supernatants were collected. The absorbance value of the supernatants was measured at 532 nm on a 96-well microplate reader (BioTek, USA). The results were expressed as μmol/mg protein.

2.11. Measurement of carbonyl proteins

Protein carbonylation is widely used as a biomarker of oxidative damage to proteins, and reflects cellular damage induced by ROS. The content of carbonyl proteins in the gastric tissue was measured by reaction with 2,4-dinitrophenylhydrazine (DNPH) (Amirshahrokhi and Khalili, 2016b). Briefly, 50 μl of the sample was added to 100 μl of 10 mM DNPH in 2 M HCl and incubated at room temperature for 1 h. Then, 100 μl of 20% TCA was added to the mixture and centrifuged at 15,000 g for 5 min. The supernatants were discarded, and the pellets were washed with 1 ml ethanol-ethyl acetate (1:1 v/v) to remove excess DNPH. The final pellets were resuspended in 150 μl of 3 M guanidine hydrochloride in 10 mM potassium phosphate (pH 2.3). The absorbance of the solutions was measured at 370 nm and the level of carbonyl proteins was calculated using the molar extinction coefficient of 22 mM^{-1} cm^{-1}. The results were expressed as nmol carbonyl per mg protein.

2.12. Measurement of prostaglandin E_2 (PGE_2)

The levels of PGE_2 in the gastric tissues were measured using a PGE_2 assay kit (Enzo Life Sciences) according to the manufacturer's protocol. The results were expressed as pg/mg protein.

2.13. Measurement of nitric oxide (NO)

The concentrations of nitrite and nitrate as an index of NO production in the gastric tissue were assessed. The levels of NO were measured using a Nitric Oxide Assay Kit (Enzo Life Sciences) and the results were expressed as μmol/mg protein.

2.14. Measurement of myeloperoxidase (MPO)

The activity of MPO in the gastric tissues was determined by measuring the oxidation of tetramethylbenzidine (TMB). Briefly, 50 μl of the specimens was added to 50 μl of TMB solution (15 mM). The reaction was started by adding 100 μl of H_2O_2 (25 mM) diluted in phosphate buffer (50 mM, pH 5.4). The rate of change in absorbance was measured at 370 nm using a microplate reader. One unit of MPO activity was defined as the quantity of enzyme degrading 1 μmol of hydrogen peroxide per min at 25 °C. The results were expressed as U MPO/mg protein.

2.15. Measurement of inflammatory cytokines and chemokine

The levels of pro-inflammatory cytokines TNF-α, IL-1β, IL-6 and chemokine MCP-1 in the gastric tissue were determined by using mouse ELISA kits from eBioscience according to the manufacturer's instructions. The results were expressed as pg/mg protein.

2.16. Measurement of matrix metalloproteinase-9 (MMP-9)

The plasma levels of MMP-9 were measured by using an ELISA kit (Mouse Total MMP-9 Quantikine ELISA Kit, R & D Systems) according to the manufacturer's instructions. The plasma levels of MMP-9 were presented as ng/ml.

2.17. Quantitative real-time PCR analysis of NF-κB in lung tissue

Total RNA was extracted from gastric tissue of each group using TRIzol Reagent (Roche). To avoid DNA contamination, The RNA samples were treated with DNase I. Synthesis of the cDNA was carried out using a First Strand cDNA Synthesis Kit (Thermo Scientific, USA) according to the manufacturer's instructions. The cDNA samples in each group were then used as the template for SYBR Green I-based real-time PCR using the LightCycler (Roche). Thermal cycling conditions were as follows: an initial denaturation at 94 °C for 5 min, followed by 40 cycles of 10 s of denaturing at 94 °C, 15 s of annealing at 59 °C, and 15 s of extension at 72 °C. The sequences of primers used were as follows: NF-κB forward, ACCTTTGCTGGAAACACACC and reverse, ATGGCCTCGGAAGTTTCTTT; GAPDH forward, GTTACCAGGGCT and reverse, GGGTTTCCCGTT.

2.18. Statistical analysis

Data were expressed as mean ± S.E.M. Results were analyzed statistically by one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test. Differences between groups were considered to be significant when $P < 0.05$.

3. Results

3.1. Gross evaluation of gastric tissue

Macroscopic appearances of the gastric mucosa and gastric ulcer index are shown in Fig. 1B and C, respectively. Mice in the normal control and MSM only-treated groups showed intact stomach without any lesions. Mice treated with ethanol/HCl showed extensive hemorrhagic necrosis in the glandular part of the gastric mucosa. Pretreatment of mice with MSM (200 and 400 mg/kg) significantly reduced ethanol-induced gastric mucosal lesions and ulcer index as compared to ethanol/HCl group. Our preliminary experiment showed that the threshold dose of MSM for a significant inhibitory effect against ethanol/HCl-induced gastric mucosal lesions was 200 mg/kg. With low doses of MSM, the gastric mucosal damage and ulcer index were not decreased significantly compared with the ethanol/HCl group.

3.2. Histopathological examination of gastric tissue

Histopathological appearances of the gastric mucosa and total pathological scores are shown in Fig. 2A and B, respectively. No histological changes were observed in gastric specimens taken from mice in the normal and MSM only-treated groups. Oral administration of acidified ethanol induced severe gastric mucosal damage, characterized by disruption of glandular structure, loss of epithelial cells, infiltration of inflammatory cells, submucosal edema, and hemorrhage. Pretreatment of mice with MSM (200 and 400 mg/kg) significantly reduced these histopathological changes caused by ethanol/HCl and preserved mucosal epithelial integrity and glandular structure. As shown in Fig. 2B, Animals pretreated with MSM (200 and 400 mg/kg) showed less histopathological scores when compared with the ethanol/HCl group.

3.3. Effects of MSM on the levels of GSH, CAT and PGE_2

As shown in Fig. 3, oxidative stress induced by ethanol/HCl leads to the depletion of GSH and CAT and inhibition of PGE_2 production in the gastric tissue when compared with those in the normal control group. Pretreatment with MSM (200 and 400 mg/kg) significantly restored the levels of GSH, CAT activity and PGE_2 production in the gastric tissue as compared with those in the ethanol/HCl group. These effects show the antioxidant and gastroprotective activity of MSM in this experimental model.

3.4. Effects of MSM on the levels of MDA and protein carbonyl

Administration of ethanol/HCl significantly increased the levels of MDA as a marker of lipid peroxidation and protein carbonyl as a marker of protein oxidation in the gastric tissue in comparison to the normal group (Table 1). However, pretreatment with MSM at dose of 200 and 400 mg/kg significantly prevented ethanol/HCl induced increase of MDA and protein carbonyl levels ($P < 0.01$).

3.5. Effects of MSM on the levels of NO and MPO

Oral administration of ethanol/HCl significantly increased NO production and MPO activity in the gastric tissue as compared to the to the normal control group (Fig. 4A and B). Pretreatment with MSM at dose of 200 and 400 mg/kg significantly reduced the formation of NO and the level of MPO in the gastric tissue compared with the ethanol/HCl group.

3.6. Effects of MSM on the levels of pro-inflammatory cytokines

We examined the effects of MSM on the production of proinflammatory cytokines TNF-α, IL-1β, IL-6 and chemokine MCP-1 in the gastric tissue. As shown in Fig. 5, Ethanol/HCl administration produced a significant increase in the levels of TNF-α, IL-1β, IL-6 and MCP-1 as compared to the normal control group ($P < 0.001$). However, MSM pretreatment (200 and 400 mg/kg) significantly inhibited the upregulation of these inflammatory mediators. These results demonstrate remarkable anti-inflammatory properties of MSM.

3.7. Effects of MSM on the levels of MMP-9

Oral administration of ethanol/HCl significantly increased the levels of MMP-9 as a marker of inflammation in the gastric epithelial cells (Fig. 6). Administration of MSM at dose of 200 and 400 mg/kg significantly inhibited the ethanol/HCl-induced increase in MMP-9 production ($P < 0.05$). This result shows that gastroprotective effect of MSM in mice is related to a reduction in MMP-9 production.

3.8. Effects of MSM on the mRNA expression level of NF-kB

Examination of NF-κB mRNA expression by real-time PCR revealed that the mRNA expression level of NF-κB in the gastric tissues of mice treated with ethanol/HCl significantly increased when compared with normal control mice ($P < 0.01$) (Fig. 7). Pretreatment with MSM at both doses (200 and 400 mg/kg) significantly decreased the ethanol/HCl-induced increase in NF-κB mRNA expression ($P < 0.05$).

4. Discussion

Peptic ulcer disease is one of the most prevalent gastrointestinal disorders. Many studies have been carried out to find out effective alternative drugs from natural resources for treatment of peptic ulcer disease. In the present study, we demonstrated that MSM treatment significantly reduced gastric mucosal damage induced by ethanol/HCl. To evaluate the possible mechanisms responsible for the gastroprotective effect of MSM, we determined the oxidative and inflammatory markers in gastric tissue.

Previous studies showed that ROS play an important role in the initiation and progression of gastric mucosal damage. Various exogenous agents such as ethanol can lead to excessive production of ROS in gastric tissue. Oxygen derived molecules are able to induce lipid peroxidation chain reactions. Lipid peroxidation process in gastric epithelial cells disturbs the integrity and permeability of cell membranes, which leads to cell death and promotes the formation of gastric mucosal ulcers. Oxygen-derived free radicals can cause modification of the peptide chain, protein cross-linking and oxidation of amino acids in

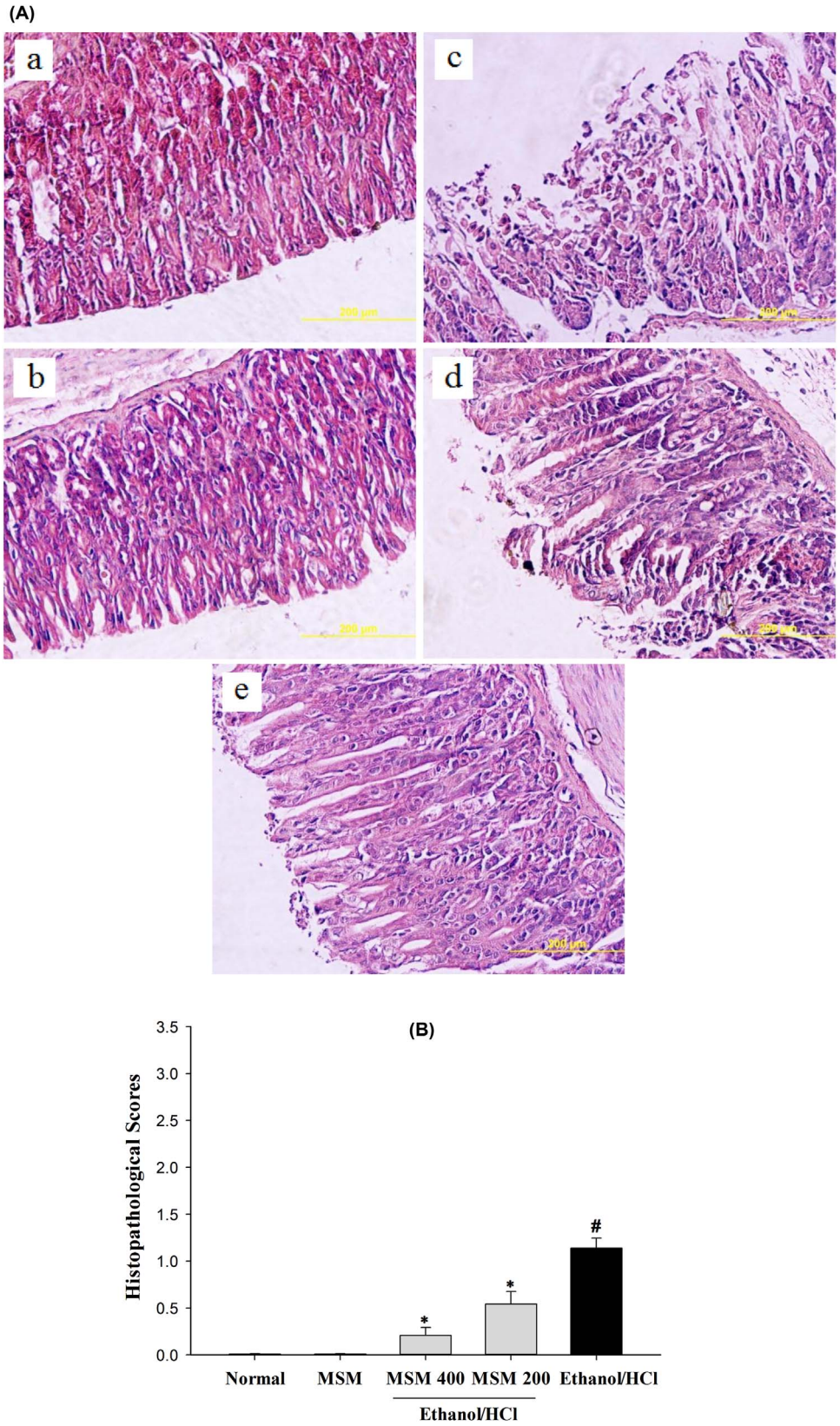

Fig. 2. (A) Histopathological appearance of gastric tissues in (a) normal, (b) MSM only (c) ethanol/HCl, (d) MSM (200 mg/kg) + ethanol/HCl and (e) MSM (400 mg/kg) + ethanol/HCl groups. Mice treated with ethanol/HCl orally showed loss of epithelial cells, edema, neutrophil infiltration and hemorrhage. Pretreatment with MSM (200 and 400 mg/kg) decreased the histological alterations induced by ethanol/HCl. The gastric tissue sections were analyzed by H & E staining (magnification is ×400). (B) Total pathological scores in the gastric mucosa. The results are presented as the mean of the total score ± S.E.M. (n = 8). $^{\#}P < 0.001$ compared with normal group; $^{*}P < 0.05$ compared with ethanol/HCl group.

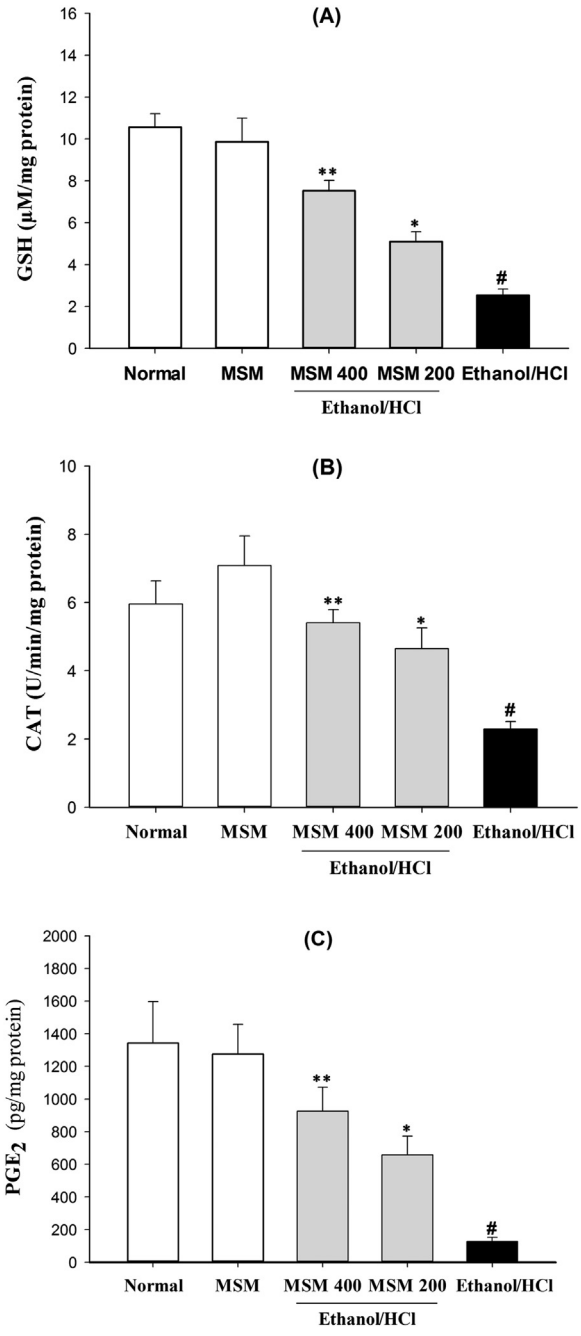

Fig. 3. Effect of MSM on ethanol/HCl-induced changes in the levels of (A) GSH, (B) CAT and (C) PGE_2. Pretreatment with MSM (200 and 400 mg/kg) significantly restored the levels of GSH, CAT and PGE_2 in the gastric tissue compared with the ethanol/HCl group. Data are means ± S.E.M. (n = 8). $^{\#}P < 0.001$ compared with normal group; $^{*}P < 0.05$, $^{**}P < 0.01$ compared with ethanol/HCl group.

Table 1
Effects of MSM on the gastric levels of MDA and protein carbonyl.

Groups	**MDA** (μM/mg protein)	**Protein carbonyl** (nM/mg protein)
Normal	0.097 ± 0.003	3.34 ± 0.25
MSM	0.06 ± 0.009	2.73 ± 0.61
Ethanol/HCl	0.24 ± 0.019[a]	9.13 ± 0.72[a]
MSM (200) + ethanol/HCl	0.158 ± 0.02[b]	6.15 ± 0.64[b]
MSM (400) + ethanol/HCl	0.15 ± 0.01[b]	4.62 ± 0.6[b]

Data are means ± S.E.M. (n = 8).
[a] $P < 0.001$ compared with normal group.
[b] $P < 0.01$ compared with ethanol/HCl group.

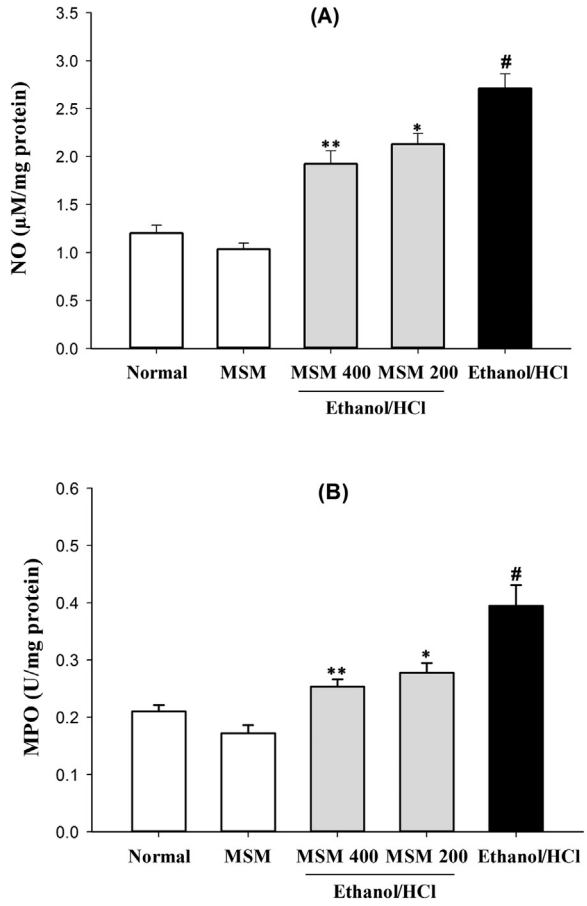

Fig. 4. Effects of MSM on the NO production (A) and MPO activity (B) in the gastric tissue. Pretreatment with MSM (200 and 400 mg/kg) remarkably reduced the production of NO and MPO activity in the gastric tissue compared with the ethanol/HCl group. Data are means ± S.E.M. (n = 8). $^{\#}P < 0.001$ compared with normal group; $^{*}P < 0.05$, $^{**}P < 0.001$ compared with ethanol/HCl group.

proteins and lead to the formation of protein carbonyl derivatives (Pérez et al., 2017). An increase in lipid peroxidation and carbonyl proteins was demonstrated in the experimental model of gastric mucosal ulcers (Liu et al., 2016). In agreement with other studies, our results showed that ethanol administration significantly increased the production of MDA and carbonyl proteins in the gastric tissue. However, pretreatment with MSM significantly prevented lipid peroxidation and protein carbonylation process induced by ethanol. MSM has been shown to decrease the plasma levels of MDA and protein carbonyl in human (Nakhostin-Roohi et al., 2011). These findings confirm the antioxidant properties of MSM and therefore, its antioxidant effects may represent an important mechanism by which MSM prevents gastric mucosal damage induced by ethanol.

GSH as thiol-containing tripeptide is one of the crucial cellular antioxidants, which is considered as an important intracellular defense against oxidative damage induced by ROS. The gastric mucosa possesses high levels of GSH which provides additional protection against gastric acid and the oxidative compounds present in the diet (Pérez et al., 2017). GSH is a potential scavenger of $O_2^{\bullet-}$, H_2O_2 and $HO^{\bullet}$ and reduction of GSH renders the gastric epithelial cells more susceptible to damage induced by oxidative compounds. Catalase is an antioxidant enzyme that exerts a remarkable protective role in the gastric epithelium against mucosal injury and inflammation. It has been shown that ethanol-induced gastric damage is associated with a significant decrease of GSH and catalase levels in the gastric tissue (Antonisamy et al., 2015; Amirshahrokhi and Khalili, 2016a). In the present study, our results showed that administration of ethanol/HCl significantly decreased the levels of GSH and catalase activity in the

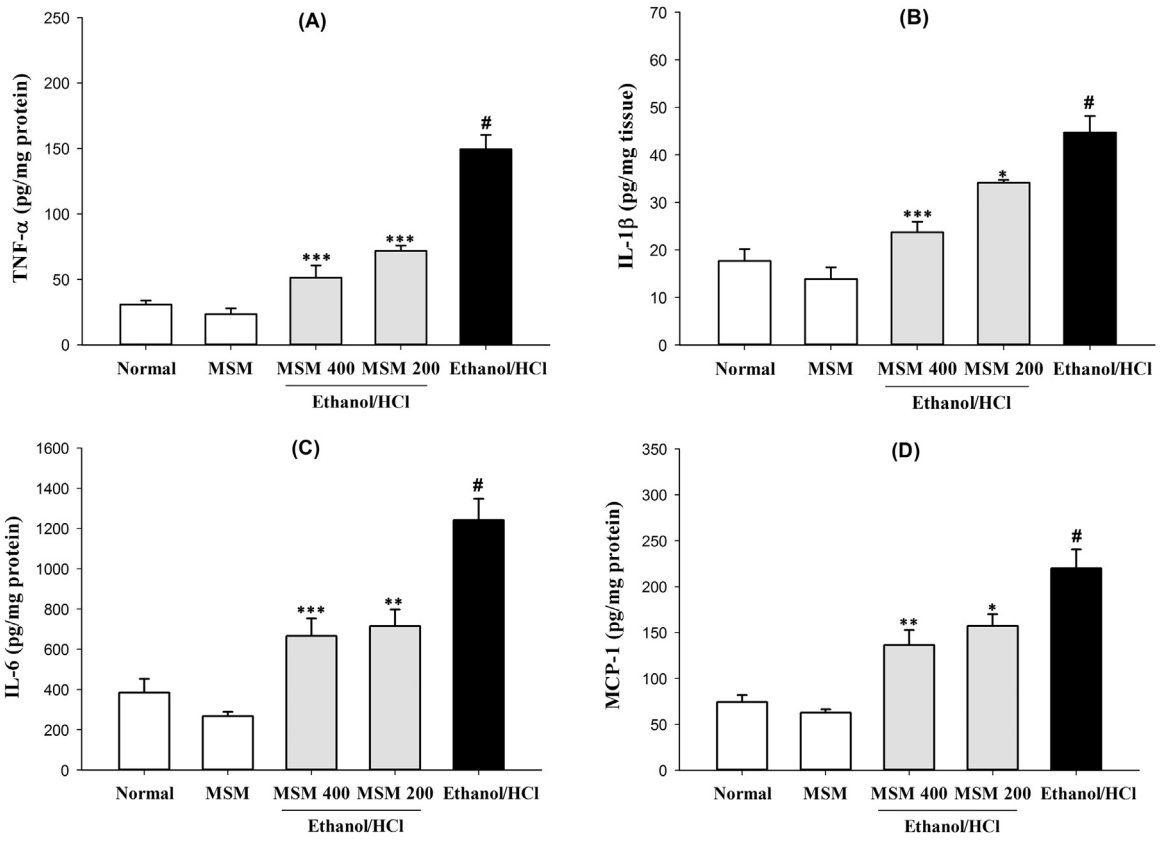

Fig. 5. Effect of MSM on (A) TNF-α, (B) IL-1β, (C) IL-6 and (D) MCP-1 production in gastric tissue of mice. Oral administration of ethanol/HCl increased the levels of TNF-α, IL-1β, IL-6 and MCP-1 in gastric tissue, whereas pretreatment with MSM (200 and 400 mg/kg) prevented the ethanol/HCl-induced increase in TNF-α, IL-1β, IL-6 and MCP-1. Data are means ± S.E.M. (n = 8). $^{\#}P < 0.001$ compared with normal group; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with ethanol/HCl group.

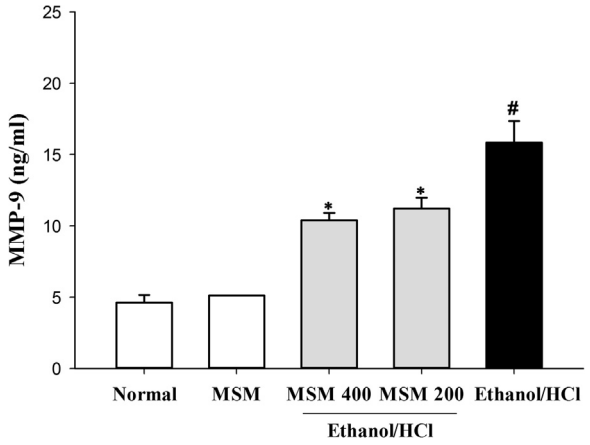

Fig. 6. Effect of MSM on the plasma levels of MMP-9. Pretreatment with MSM inhibited the ethanol/HCl-induced increase in MMP-9 levels. Data are means ± S.E.M. (n = 8). $^{\#}P < 0.001$ compared with normal group; $^{*}P < 0.05$, compared with ethanol/HCl group.

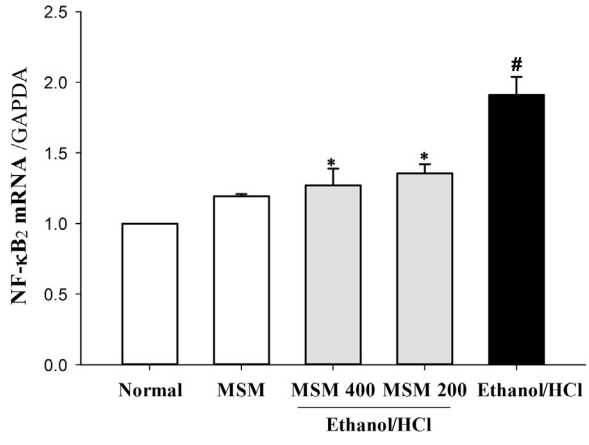

Fig. 7. Effect of MSM pretreatment on ethanol/HCl-induced increase in NF-κB2 mRNA expression in gastric tissue. The mRNA levels were normalized by the expression of GAPDH. Data are means ± S.E.M. of three experiments. $^{\#}P < 0.01$ compared with normal group; $^{*}P < 0.05$ compared with ethanol/HCl group.

gastric tissue. We suggest that, the reduction of GSH and catalase levels may result from their consumption because of neutralization of ethanol-induced ROS and toxic products in the gastric tissue. MSM pretreatment inhibited the depletion of GSH and restored catalase activity in the gastric tissue of mice exposed to ethanol/HCl. It has been reported that chronic MSM administration increases liver glutathione content and shows hepatoprotective effect against chemically-induced oxidant stress (DiSilvestro et al., 2008). In our study, we propose that MSM may increase the synthesis of GSH and then it is able to improve survival of cells by preventing GSH depletion.

PGE_2 is an endogenous gastroprotective mediator that reduces acid secretion, stimulates mucus and bicarbonate secretion and accelerates ulcer healing. PGE_2 can also increase gastric mucosal blood flow and the resistance of epithelial cells to damage induced by exposure to various noxious substances such as ethanol and NSAIDs (Wallace, 2008; El-Maraghy et al., 2015). In our study, ethanol/HCl administration resulted in a significant decrease in the gastric levels of PGE_2 however MSM pretreatment was able to increase the levels of PGE_2.

NO, as an important biological mediator, plays a dual role in gastric mucosa; at physiological concentrations, NO protects against gastric mucosal injury; while at excessive production, it triggers tissue damage (Elliott and Wallace, 1998; Das and Vasudevan, 2007). It has been shown that oral administration of ethanol increases inducible nitric

oxide synthase (iNOS) expression in the gastric tissue, leading to NO overproduction and subsequently gastric mucosal cell damage (Yu et al., 2014). Under pathophysiological conditions, NO causes cell injury through direct cytotoxic effects and free radicals generation (Amirshahrokhi and Khalili, 2015b). In the present study, our findings showed that oral administration of ethanol results in an increase in the levels of NO in the gastric tissue while pretreatment with MSM significantly reduced ethanol-induced NO production. This finding suggests that inhibition of NO-mediated cytotoxicity is probably involved in the gastroprotective action of MSM. In agreement with this finding, it has been reported that MSM is able to reduce NO production in various cells (Kim et al., 2015; Karabay et al., 2014).

The infiltration of neutrophils into the gastric mucosa plays an important role in the progression of gastric mucosal inflammation and damage. Activated macrophages and neutrophils release several proinflammatory mediators and ROS during gastric inflammation. MPO is a peroxidase enzyme found in the neutrophils and has been used extensively as a biochemical marker of neutrophil infiltration into the damaged tissue (Amirshahrokhi and Khalili, 2015a; Watanabe et al., 2004). In this study, we observed that ethanol/HCl administration significantly increased the MPO activity in the stomach, indicating gastric mucosal inflammation. Pretreatment with MSM caused a significant reduction of the inflammatory cell infiltration and MPO activity in the gastric tissue. This result reveals that MSM may possess gastroprotective effects through inhibition of gastric mucosal inflammation. Furthermore, the ability of MSM to inhibit MPO activity has been shown in other experimental models (Bohlooli et al., 2013; Amirshahrokhi et al., 2011; Marañón et al., 2008). These findings were also supported by histological analysis of the gastric mucosa. Our histological examination showed that ethanol/HCl administration induced gastric submucosal edema, neutrophil infiltration, loss of epithelial cells and severe hemorrhage, while pretreatment with MSM reduced these histological alterations and pathologic score in the gastric tissue of animals receiving ethanol/HCl.

Many studies have shown that proinflammatory cytokines TNF-α, IL-1β, IL-6 and chemokine MCP-1 are involved in the pathogenesis of gastric mucosal injury. TNF-α plays an important role in the pathogenesis of various gastrointestinal diseases including colitis, gastric cancer and gastritis as well as hepatitis (Amirshahrokhi et al., 2010; Chang et al., 2015; Amirshahrokhi and Khalili, 2016a). TNF-α can induce a cascade of events leading to neutrophil activation, up regulation of adhesion molecules and the production and release of other cytokines and chemokines. TNF-α stimulates immune cells to generate toxic metabolites and reduces gastric microcirculation around ulcerated mucosa and delays its healing (Verma and Kumar, 2016; Arab et al., 2015). IL-1β and IL-6 are potent proinflammatory cytokines synthesized mainly by activated monocytes and macrophages. It has been reported that the levels of these cytokines in serum and gastric tissue correlate with the severity of gastric mucosal inflammation (Li et al., 2014). MCP-1 is a member of the CC chemokine family and plays an important role in the regulation of leukocyte recruitment at sites of inflammation in several diseases. There are many studies demonstrating increased levels of chemokines MCP-1 and IL-8 in gastric mucosal inflammation and damage induced by NSAIDs, ethanol and H. pylori. The main source of MCP-1 is inflammatory cells and increased levels of MCP-1 could be due to an increase in the number of macrophages and neutrophils in injured gastric mucosa (Watanabe et al., 2004; Amirshahrokhi and Khalili, 2016a). In agreement with previous studies, we also showed that the administration of ethanol/HCl caused an increase in the levels of TNF-α, IL-1β, IL-6 and MCP-1 in the gastric tissue and pretreatment with MSM significantly decreased the levels of these proinflammatory mediators. The ability of MSM to inhibit proinflammatory cytokines has been shown in various experimental models (Ahn et al., 2015; Karabay et al., 2014; Sousa-Lima et al., 2016).

NF-κB is an important transcription factor that mediates pivotal inflammatory events in gastric damage induced by various agents including ethanol. NF-κB is a redox-sensitive transcription factor that regulates cellular responses to ROS and inflammatory mediators. Activation of NF-κB induces the expression of TNF-α, IL-1β, IL-6, MCP-1 and several other adhesion molecules. Moreover, NF-κB induces the expression of iNOS, which catalyzes the production of NO in response to a variety of stress conditions (Arab et al., 2015; Amirshahrokhi and Khalili, 2016b; Pérez et al., 2017). It has also been shown that NF-κB is a key mediator to induce inflammatory protein MMP-9 which contributes to gastric inflammation and injury. MMP-9 is one of the most important members of the metalloproteinase family and involves in the dysregulation of extracellular matrix remodeling during gastric ulcer formation. MMP-9 is produced by inflammatory and epithelial cells under the influence of inflammatory mediators and ROS (Li et al., 2014; Swarnakar et al., 2007). In the present study, we demonstrated that ethanol administration induced NF-κB expression and pretreatment with MSM significantly reduced ethanol-induced NF-κB activation in the gastric tissue. Indeed, the gastroprotective effect of MSM in mice is related to a reduction in NF-κB signaling pathway. It has been reported that the inhibitory effect of MSM on NF-κB activation results in the downregulation of mRNA for IL-1, IL-6 and TNF-α (Butawan et al., 2017). Moreover the plasma levels of MMP-9, as a marker of gastric inflammation, were inhibited by MSM pretreatment.

5. Conclusion

The findings of the present study demonstrate that pretreatment with MSM inhibits the oxidative and inflammatory mediators in the gastric tissue and can be used as a potential gastroprotective agent. However clinical use of MSM in gastric mucosal damage in humans should be further investigated.

References

Aebi, H., 1984. Catalase in vitro. Methods Enzymol. 105, 121–126.
Ahn, H., Kim, J., Lee, M.J., Kim, Y.J., Cho, Y.W., Lee, G.S., 2015. Methylsulfonylmethane inhibits NLRP3 inflammasome activation. Cytokine 71, 223–231.
Amirshahrokhi, K., Bohlooli, S., 2013. Effect of methylsulfonylmethane on paraquat-induced acute lung and liver injury in mice. Inflammation 36, 1111–1121.
Amirshahrokhi, K., Khalili, A.R., 2015a. The effect of thalidomide on ethanol-induced gastric mucosal damage in mice: involvement of inflammatory cytokines and nitric oxide. Chem. Biol. Interact. 225, 63–69.
Amirshahrokhi, K., Khalili, A.R., 2015b. Thalidomide ameliorates cisplatin-induced nephrotoxicity by inhibiting renal inflammation in an experimental model. Inflammation 38, 476–484.
Amirshahrokhi, K., Khalili, A.R., 2016a. Gastroprotective effect of 2-mercaptoethane sulfonate against acute gastric mucosal damage induced by ethanol. Int. Immunopharmacol. 34, 183–188.
Amirshahrokhi, K., Khalili, A.R., 2016b. Carvedilol attenuates paraquat-induced lung injury by inhibition of proinflammatory cytokines, chemokine MCP-1, NF-κB activation and oxidative stress mediators. Cytokine 88, 144–153.
Amirshahrokhi, K., Ghazi-khansari, M., Mohammadi-Farani, A., Karimian, G., 2010. Effect of captopril on TNF-α and IL-10 in the livers of bile duct ligated rats. Iran. J. Immunol. 7, 247–251.
Amirshahrokhi, K., Bohlooli, S., Chinifroush, M.M., 2011. The effect of methylsulfonylmethane on the experimental colitis in the rat. Toxicol. Appl. Pharmacol. 253, 197–202.
Antonisamy, P., Duraipandiyan, V., Aravinthan, A., Al-Dhabi, N.A., Ignacimuthu, S., Choi, K.C., Kim, J.H., 2015. Protective effects of friedelin isolated from Azima tetracantha Lam. against ethanol-induced gastric ulcer in rats and possible underlying mechanisms. Eur. J. Pharmacol. 750, 167–175.
Arab, H.H., Salama, S.A., Omar, H.A., Arafa, el-S.A., Maghrabi, I.A., 2015. Diosmin protects against ethanol-induced gastric injury in rats: novel anti-ulcer actions. PLoS One 10, e0122417.
Boeing, T., da Silva, L.M., Somensi, L.B., Cury, B.J., Michels Costa, A.P., Petreanu, M., Niero, R., de Andrade, S.F., 2016. Antiulcer mechanisms of Vernonia condensata Baker: a medicinal plant used in the treatment of gastritis and gastric ulcer. J. Ethnopharmacol. 184, 196–207.
Bohlooli, S., Mohammadi, S., Amirshahrokhi, K., Mirzanejad-Asl, H., Yosefi, M., Mohammadi-Nei, A., Chinifroush, M.M., 2013. Effect of methylsulfonylmethane pretreatment on aceta-minophen induced hepatotoxicity in rats. Iran. J. Basic Med. Sci. 16, 896–900.
Butawan, M., Benjamin, R.L., Bloomer, R.J., 2017. Methylsulfonylmethane: applications and Safety of a Novel Dietary Supplement. Nutrients 9, E290.

Chang, X., Luo, F., Jiang, W., Zhu, L., Gao, J., He, H., Wei, T., Gong, S., Yan, T., 2015. Protective activity of salidroside against ethanol-induced gastric ulcer via the MAPK/NF-κB pathway in vivo and in vitro. Int. Immunopharmacol. 28, 604–615.
Das, S.K., Vasudevan, D.M., 2007. Alcohol-induced oxidative stress. Life Sci. 81, 177–187.
DiSilvestro, R.A., DiSilvestro, D.J., DiSilvestro, D.J., 2008. Methylsulfonylmethane (MSM) intake in mice produces elevated liver glutathione and partially protects against carbon tetrachloride-induced liver injury. FASEB J. 22, 445–448.
Elliott, S.N., Wallace, J.L., 1998. Nitric oxide: a regulator of mucosal defense and injury. J. Gastroenterol. 33, 792–803.
El-Maraghy, S.A., Rizk, S.M., Shahin, N.N., 2015. Gastroprotective effect of crocin in ethanol-induced gastric injury in rats. Chem. Biol. Interact. 229, 26–35.
Hoshino, T., Tsutsumi, S., Tomisato, W., Hwang, H.J., Tsuchiya, T., Mizushima, T., 2003. Prostaglandin E2 protects gastric mucosal cells from apoptosis via EP2 and EP4 receptor activation. J. Biol. Chem. 278, 12752–12758.
Kang, G.D., Lee, S.Y., Jang, S.E., Han, M.J., Kim, D.H., 2017. Irisolidone attenuates ethanol-induced gastric injury in mice by inhibiting the infiltration of neutrophils. Mol. Nutr. Food Res. 61.
Karabay, A.Z., Aktan, F., Sunguroğlu, A., Buyukbingol, Z., 2014. Methylsulfonylmethane modulates apoptosis of LPS/IFN-γ-activated RAW 264.7 macrophage-like cells by targeting p53, Bax, Bcl-2, cytochrome c and PARP proteins. Immunopharmacol. Immunotoxicol. 36, 379–389.
Kim, L.S., Axelrod, L.J., Howard, P., Buratovich, N., Waters, R.F., 2006. Efficacy of methylsulfonylmethane (MSM) in osteoarthritis pain of the knee: a pilot clinical trial. Osteoarthr. Cartil. 14, 286–294.
Kim, S.H., Smith, A.J., Tan, J., Shytle, R.D., Giunta, B., 2015. MSM ameliorates HIV-1 Tat induced neuronal oxidative stress via rebalance of the glutathione cycle. Am. J. Transl. Res. 7, 328–338.
Li, W.F., Hao, D.J., Fan, T., Huang, H.M., Yao, H., Niu, X.F., 2014. Protective effect of chelerythrine against ethanol-induced gastric ulcer in mice. Chem. Biol. Interact. 208, 18–27.
Liu, J., Wang, F., Luo, H., Liu, A., Li, K., Li, C., Jiang, Y., 2016. Protective effect of butyrate against ethanol-induced gastric ulcers in mice by promoting the anti-inflammatory, anti-oxidant and mucosal defense mechanisms. Int. Immunopharmacol. 30, 179–187.
Lu, L., Chan, R.L., Luo, X.M., Wu, W.K., Shin, V.Y., Cho, C.H., 2014. Animal models of gastrointestinal inflammation and cancer. Life Sci. 108, 1–6.
Marañón, G., Muñoz-Escassi, B., Manley, W., García, C., Cayado, P., de la Muela, M.S., Olábarri, B., León, R., Vara, E., 2008. The effect of methyl sulphonyl methane supplementation on biomarkers of oxidative stress in sport horses following jumping exercise. Acta Vet. Scand. 50, 45.
Nakhostin-Roohi, B., Barmaki, S., Khoshkhahesh, F., Bohlooli, S., 2011. Effect of chronic supplementation with methylsulfonylmethane on oxidative stress following acute exercise in untrained healthy men. J. Pharm. Pharmacol. 63, 1290–1294.
Oliveira, I.S., da Silva, F.V., Viana, A.F., dos Santos, M.R., Quintans-Júnior, L.J., Martins Mdo, C., Nunes, P.H., Oliveira Fde, A., Oliveira Rde, C., 2012. Gastroprotective activity of carvacrol on experimentally induced gastric lesions in rodents. Naunyn Schmiede. Arch. Pharmacol. 385, 899–908.
Parcell, S., 2002. Sulfur in human nutrition and applications in medicine. Altern. Med. Rev. 7, 22–44.
Pérez, S., Taléns-Visconti, R., Rius-Pérez, S., Finamor, I., Sastre, J., 2017. Redox signaling in the gastrointestinal tract. Free Radic. Biol. Med. 104, 75–103.
Sidahmed, H.M., Hashim, N.M., Amir, J., Abdulla, M.A., Hadi, A.H., Abdelwahab, S.I., Taha, M.M., Hassandarvish, P., The, X., Loke, M.F., Vadivelu, J., Rahmani, M., Mohan, S., 2013. Pyranocycloartobiloxanthone A, a novel gastroprotective compound from Artocarpus obtusus Jarret, against ethanol-induced acute gastric ulcer in vivo. Phytomedicine 20, 834–843.
Sousa-Lima, I., Park, S.Y., Chung, M., Jung, H.J., Kang, M.C., Gaspar, J.M., Seo, J.A., Macedo, M.P., Park, K.S., Mantzoros, C., Lee, S.H., Kim, Y.B., 2016. Methylsulfonylmethane (MSM), an organosulfur compound, is effective against obesity-induced metabolic disorders in mice. Metabolism 65, 1508–1521.
Swarnakar, S., Mishra, A., Ganguly, K., Sharma, A.V., 2007. Matrix metalloproteinase-9 activity and expression is reduced by melatonin during prevention of ethanol-induced gastric ulcer in mice. J. Pineal Res. 43, 56–64.
Verma, S., Kumar, V.L., 2016. Attenuation of gastric mucosal damage by artesunate in rat: modulation of oxidative stress and NFκB mediated signaling. Chem. Biol. Interact. 257, 46–53.
Wallace, J.L., 2008. Prostaglandins, NSAIDs, and gastric mucosal protection: why doesn't the stomach digest itself? Physiol. Rev. 88, 1547–1565.
Watanabe, T., Higuchi, K., Hamaguchi, M., Shiba, M., Tominaga, K., Fujiwara, Y., Matsumoto, T., Arakawa, T., 2004. Monocyte chemotactic protein-1 regulates leukocyte recruitment during gastric ulcer recurrence induced by tumor necrosis factor-alpha. Am. J. Physiol. Gastrointest. Liver Physiol. 287, G919–G928.
Yu, C., Mei, X.T., Zheng, Y.P., Xu, D.H., 2014. Gastroprotective effect of taurine zinc solid dispersions against absolute ethanol-induced gastric lesions is mediated by enhancement of antioxidant activity and endogenous PGE2 production and attenuation of NO production. Eur. J. Pharmacol. 740, 329–336.