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# Marjoram oil attenuates oxidative stress and improves colonic epithelial barrier function in dextran sulfate sodium-induced ulcerative colitis in Balb/c mice

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**ORIGINAL ARTICLE**

## Abstract

In this study, we explored the effects of marjoram oil (MO) on dextran sulfate sodium (DSS)-induced colitis in Balb/c mice. Marjoram oil was found to significantly reduce the severity of DSS-induced colonic inflammation, as reflected by improved disease activity index, prevented colon length shortening, lower histopathological score, decreased myeloperoxidase activity, and reduced interlukin 6 (IL-6) levels. Moreover, marjoram oil pretreatment enhanced the colonic epithelial integrity by decreasing paracellular permeability. Marjoram oil was found to clearly reduce the colonic levels of thiobarbituric acid reactive substances assay and enhance the activity of superoxide dismutase, catalase, and glutathione sulfhydryl content. Marjoram oil could exert a protective effect on ulcerative colitis through its anti-inflammatory and antioxidant properties.

*Keywords:* dextran sulfate sodium, experimental colitis, Origanum majorana, oxidative stress, inflammation, Ussing chamber

## Introduction

Inflammatory bowel disease (IBD) is a long-term gastrointestinal disorder marked by recurrent inflammation and extensive mucosal destruction in the intestine (Mijan and Lim, 2018). Histologically, extensive ulcerated regions with significant neutrophil infiltration and epithelial cell necrosis characterize ulcerative colitis (UC) (Wang *et al*., 2019). Although the cause of IBD is unknown, various factors are known to have a role in its development, including breakdown of the epithelial barrier, a decrease in commensal bacteria tolerance, immunologic alterations, oxidative stress, and an increase in inflammatory mediators (Liu *et al*., 2017; Goyal *et al.*, 2014). Immunological cell infiltration into the gut's lamina propria, as well as uncontrolled synthesis of pro-inflammatory mediators and reactive oxygen species (ROS) in colonic tissues, contributes to the breakdown of intestinal barrier integrity and an increased inflammation of the mucosa (Maloy and Powrie, 2011).

An overproduction of ROS causes lipid peroxidation which reduces cellular antioxidant capacity and leads to severe colonic inflammation (Tahan *et al.*, 2011). Endogenous antioxidants can ordinarily protect the intestinal mucosa from oxidative damage (Mandalari *et al.*, 2011). Nevertheless, inflammation increases the demand for these antioxidants, resulting in a pro-oxidant–antioxidant imbalance, resulting in mucosal damage (Mandalari *et al.*, 2011; Zhu and Li, 2012).

Myeloperoxidase (MPO) activity is another indicator of the severity of ulcerative colitis. In colitis, MPO is widely

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used as a marker of oxidative stress and gastrointestinal inflammation (Klebanoff *et al.*, 2005). A previous investigation demonstrated that inflammatory cytokine-caused apoptosis was found to have a deleterious impact on the structure of adherent junction and epithelial barrier functions (Su *et al.*, 2013).

Animal models of IBD are suitable for studying the pathogenesis of the disease and developing new drugs. Dextran sulfate sodium (DSS)-provoked acute colitis is among the most regularly adopted methods (Bang and Lichtenberger, 2016) because of its great homogeneity, lesions' reproducibility predominantly in the distal colon, and similarity to clinical IBD (Valatas *et al.*, 2013).

Currently, several therapies, such as nonsteroidal anti-inflammatory medications, corticosteroids, immune-suppressing medications, and biological agents are being investigated (Baakhtari *et al.*, 2018; Yang *et al.*, 2015). Nevertheless, homeostasis and maintenance of intestinal barrier are rarely emphasized in conventional pharmacotherapy, and interest in integrative and complementary medicines is growing (Larussa *et al.*, 2017). Patients with IBD frequently use complementary and alternative medicines (CAMs) (Cheifetz *et al.*, 2017; Lin and Cheifetz, 2018), which may include plants or herbal items, probiotics, and prebiotics (Cheifetz *et al.*, 2017). Thus, in IBD patients, use of CAM, particularly herbal medicines, is increasing as a safe anti-oxidant/anti-inflammatory therapeutic option (Wan *et al.*, 2014).

Owing to their high phenolic content, plants belonging to family *Lamiaceae* are studied extensively as natural antioxidant sources (Trouillas *et al.*, 2003). Marjoram (*Origanum majorana*) is the most important member of family *Lamiaceae*, which includes a wide variety of aromatic herbs and shrubs and volatile oil plants (Paterson *et al.*, 2006).

Polyphenols, such as flavonoids, having several therapeutic properties are found in abundance in *Origanum majorana* (Goel and Vasudeva, 2015). Leaves of Origanum majorana are found to exhibit antioxidant (Duletic *et al.*, 2018; Erenler *et al.*, 2016), antimicrobial (Della Pepa *et al.*, 2019; Guerra-Boone *et al.*, 2015), anti-neurodegenerative (Della Pepa *et al.*, 2019; Duletic *et al.*, 2018; Erenler *et al.*, 2016; Guerra-Boone *et al.*, 2015), hepatoprotective (Mossa *et al.*, 2013), cardioprotective (Ramadan *et al.*, 2013), antiulcer (Al-Howiriny *et al.*, 2009), anti-inflammatory (Arranz *et al.*, 2015), and anticancer properties (Al Benhalilou *et al.*, 2019 Dhaheri *et al.*, 2013). Origanum majorana has also demonstrated positive effects in acute infectious diarrhoea (Makrane *et al.*, 2018). Recent studies have demonstrated that essential oil of Origanum majorana suppressed the development of colon cancer cells through p38 mitogen-activated protein kinase (MAPK)-provoked apoptosis *in vitro* and *in vivo (*Athamneh *et al.*, 2020).

To date, no scientific data exist on the effect of Origanum majorana on intestinal inflammation. Therefore, the purpose of our study was to evaluate the possible protective effect of Origanum majorana on DSS-provoked colitis in Balb/c mice.

## Materials and Methods

### Chemicals

Marjoram oil was obtained from commercial CAP Pharm (EL Captain Company, Egypt). Dextran sulfate sodium (40 kDa), fluorescein isothiocyanate conjugated dextran (FITC-Dextran; catalog number: FD4 [4 kDa]), and mouse interlukin 6 (IL-6) enzyme-linked-immunosorbent serologic assay (ELISA) kit were purchased from Sigma Aldrich (France). The remaining solvents and chemicals were obtained from Sigma Chemical Co. (St. Louis, MO, USA).

Marjoram oil composition was analyzed using Perkin Elmer Clarus 500 gas chromatograph capillary column HP-5 (30 m × 0.25 mm ID, 0.25-µm film thickness), coupled to Clarus 500 mass spectrometer (GC-MS). More details about the chemical composition of marjoram oil were reported earlier by Bouayad Debbagh *et al.* (2021).

### Experimental animals

Male Balb/c mice, 8-week old, were kept at the University of Oran's animal facility with a 12-h light–dark cycle at 23 ± 2°C. Animals had free access to commercial standard diet and water. All experimental procedures involving animals were approved by the current Algerian legislation covering the protection of animals.

### Colitis induction and design of treatment

Animals were divided into the following four groups, with eight mice in each group: control group (Ctrl) received tap water; DSS group (DSS) received DSS 4% (40 kDa; Sigma-Aldrich) in their drinking water *ad libitum* for 7 days; marjoram oil group (M) received 0.5-mL/kg body weight per os (by gavage) of marjoram oil for 14 day; and marjoram oil + DSS group (M+DSS), in which mice received MO (0.5 mL/kg body weight) orally for 14 day and were exposed to DSS 4% for 7 days (day 15–21) (Figure 1).

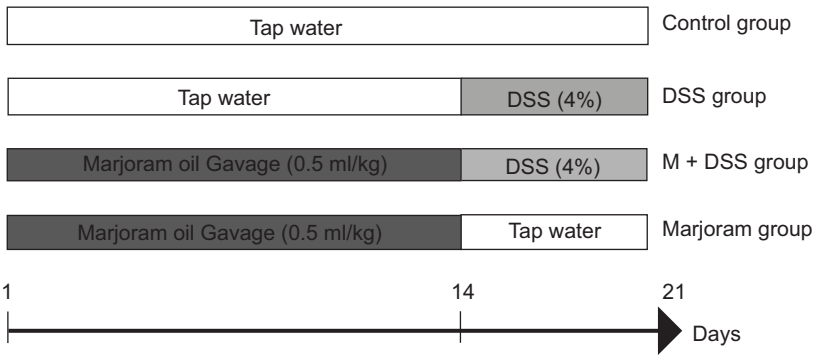


**Figure 1. Experimental protocol of the study.**

### Clinical colitis severity assessment

The disease activity index (DAI) was measured daily on the basis of clinical manifestations of colitis (Cooper *et al.*, 1993) comprising body weight, faecal bleeding, stool consistency, and diarrhoea (Table 1).

### Analysis of colon inflammation

The animals were sacrificed after 21 days of treatment. Blood was collected and the plasma was kept at −80°C for assays. The colon was excised and washed with saline solution. The length and weight of colon was measured. A 5-mm section of the distal colon was put in freshly prepared 10% (w/v) formaldehyde (pH 7.0), processed, and paraffin-embedded for histopathological evaluation. The mid-distal portion (30–60% region) was used for electrophysiological studies. The remaining colon tissue was kept at −80°C for additional biochemical analysis.

### *Ex vivo* experiments: Ussing chamber assay

After sacrifice, distal colon was removed and excised through mesenteric border. Colonic tissue samples were immediately placed in Ussing chambers as sheets (Physiologic Instrument, San Diego, CA) with an exposed area of 0.2 $cm^2$. Both sides were filled with Ringer solution (5 mL), oxygenated continuously (95% $O_2$ and 5% $CO_2$), and maintained at 37°C. The mucosal-to-serosal flux of FITC-Dextran with a 4-kDa mean molecular mass (FD4) was measured across colonic samples to determine paracellular permeability.

Colonic paracellular permeability was determined by measuring mucosal-to-serosal flux of FITC-Dextran across colonic samples. After a stabilization period of 15–25 min, the mucosal reservoir was injected with FD4 at a concentration of 2.2 mg/mL. For measuring FD4, basolateral samples (500 µL replaced by appropriate 500-µL buffer solution) were taken for 90 min at 30-min intervals for recording electrophysiological parameters. The concentration of FD-4 was determined using a fluorescence microplate reader with a 520-nm emission wavelength and a 490-nm excitation wavelength (Perkin-Elmer, Waltham, MA, USA). The apparent permeability coefficient (Papp) was calculated for this period as follows:

$$Papp\ (cm/s \times 10^{-6}) = dc/dt \times (V/(A \times C_0)),$$

where dc/dt is the change in serosal concentration during 60–90 min (mol/L/s) period, $C_0$ is the initial marker concentration in mucosal reservoir (mol/L), "V" is the volume of serosal reservoir ($cm^3$), and "A" is the exposed intestinal area in the chamber ($cm^2$).

### Histological assessment of colitis

Distal colon sections were taken and fixed in phosphate-buffered formalin (10%), and then dried and paraffin-embedded. For light microscopic examination, these samples were stained with hematoxylin and eosin (H&E). Histological damage was determined using the scoring system of Stillie and Stadnyk (2009).

### Measurement of IL-6 level

Level of IL-6 in plasma samples was measured in duplicate using commercially mouse IL-6 ELISA kit (Sigma-Aldrich) according to manufacturer's instructions.

### Myeloperoxidase activity assay

The MPO activity is defined as the quantity of enzyme that degraded 1 µmol of $H_2O_2$ per minute. It was measured in units per milligram of protein. In this study, the MPO activity was measured in proximal colon

**Table 1. Disease activity index (DAI) score (Cooper *et al.*, 1993) (n = 8).**

| Score | Weight loss | Stool consistency | Bleeding |
|---|---|---|---|
| 0 | None | Normal | No bleeding |
| 1 | 1–5% | – | – |
| 2 | 5–10% | Loose stools | Slight bleeding |
| 3 | 10–15% | – | – |
| 4 | More than 15% | Watery diarrhoea | Gross bleeding |

tissue according to Lenoir *et al.* (2011). In a 50-mM phosphate buffer with hexadecyl trimethyl ammonium bromide (0.5%), a section of colon was homogenized in ice with a T25 Ultra-Turrax system (HTAB; IKA, Staufen, Germany). The homogenate was sonicated in ice for 30 s prior to centrifugation at 3,200 g for 10 min at 4°C. The supernatants were frozen and thawed thrice prior to being centrifuged for 10 min at 3,200×g at 4°C.

In microcuves, 50 µL of supernatants were combined with 1 mL of phosphate buffer (10 mM), 500 µL of 0.22% guaiacol, and 10 µL of $H_2O_2$ (0.3 %). After 3 min, absorbance was measured at every minute for 20 min at 470 nm. The study was performed in triplicate.

### *In vivo* antioxidant activity

*Homogenization of tissues and quantification of proteins*

In ice cold water, frozen tissue specimens were weighed (range 100 mg) and homogenized with 0.9-mL potassium phosphate buffer (0.12 mmol/L, pH = 7.4).

The samples were centrifuged twice at 3,000×g for 20 min at 4°C. Superoxide dismutase, catalase, glutathione peroxidase (Gpx) activities, and reduced glutathione level were determined in all supernatants. Protein content was measured according to the method described by Lowry *et al.* (1951).

*Thiobarbituric acid reactive substances assay (TBARS)*

Lipid peroxidation was measured by the method of Salih *et al.* (1987), In which 2 g of colon was homogenized in 30 mL of trichloroacetic acid (10%) and 200 µL of butyl hydroxyl toluene. Samples were centrifuged for 10 min at 3,500 rpm. Thiobarbituric acid (0.02 M), 5 mL, was then added to 5 mL of supernatant and the mixture was heated for 30 min at 100°C. Absorbance of the supernatant was read against blank specimen at 532 nm. TBARS was expressed in nanomole (nmol) of MDA (malondialdehyde) per gram of tissue.

Catalase (CAT) activity was determined according to the method of Aebi (1984), in which 20-µL of supernatant was mixed with 1.8 mL of 100 mmol/L phosphate buffer (pH 7.4) and 200-µL $H_2O_2$ (50 mM). Following a decrease in absorbance at 240 nm, CAT activity was measured spectrophotometrically in units/milligram of protein, compared to a standard curve.

Superoxide dismutase (SOD) was determined according to the method described by Marklund and Marklund (1974). The mixture included 970 µL of Tris-HCl (50 mM, pH 8.2) and 15 µL of supernatant. The reaction began by adding 15 µL of pyrogallol (12-mM pyrogallol in 1-mM HCl) to the mixture. A control was prepared from the same mixture by adding 985-µL Tris-HCl instead of 15-µL of pyrogallol. Change in absorbance was measured during a 5-min period at a wavelength of 325 nm, and variation in absorbance per minute was calculated and expressed per milligram of protein, corresponding to the quantity of enzyme that suppressed auto-oxidation of pyrogallol by 50%.

Glutathione peroxidase was determined by Rodrigues *et al.* (2002) method, in which 200 µL of tissue homogenate was added to a solution of Tris-HCl (0.4 M, pH 7), 0.1-mL sodium azide (10 mM), and 0.2-mL reduced glutathione (1 mM) followed by 0.1 mL of 0.2-mM hydrogen peroxide. After a 10-min incubation period at room temperature, the mixture was centrifuged at 1,200×g for 10 min after adding 0.4 mL of 10% trichloroacetic acid. After this, 0.2 mL of supernatant and 0.1 mL of Ellman's reagent were combined, and the absorbance was measured at 340 nm.

Reduced glutathione (GSH) levels in colon tissues were assessed according to the method described by Sedlak and Lindsay (1968). GSH reacts with Dithiobis-2-nitrobenzoic acid (DTNB) to produce thio-2-nitrobenzoic acid, which has a wavelength of 412 nm. For measuring it, 1 mL of tissue homogenate (100-mg sample diluted with 0.9 mL of 0.12 mmol/L phosphate buffer, pH 7.2) was combined with 800 µL of ice-cold distilled water and 200 µL of trichloroacetic acid (50%) and incubated for 15 min; 400 µL of supernatant was mixed with 800 µL of Tris buffer (0.4 mol/L, pH 8.9) and 20-µL DTNB reagent (0.01 mol/L) after centrifugation at 1,200×g for 15 min. After 5 min of incubation, the absorbance of the reaction mixture was measured at 412 nm against a blank reagent.

### Statistical analysis

Data were presented as mean ± standard error of mean (SEM). Results were examined by one-way ANOVA followed by Fisher's least significant difference (LSD) test

(Statistica Stat Soft, Maisons-Alfort, France); $p < 0.05$ was considered as statistically significant.

## Results

### Marjoram oil improved DSS-induced colitis

Mice exposed to DSS-induced colitis, as evidenced by severe anorexia, weight loss (Figure 2A), reduction in DAI score from day 5 until the last experimental day (Figure 2B), decreased colon weight (Figure 3B), and drab hair color. The length of colon in DSS-treated mice was significantly shorter by 43.69% ($p < 0.001$) than that in mice of both control and marjoram groups (Figure 3A), indicating the successful establishment of mice colitis.

Pre-treatment of colitic mice with marjoram oil showed a reduction in the severity of colitis as evidenced by a decreased weight loss, prevented colon shortening, and reduced DAI score on day 8 ($p < 0.05$). The length of

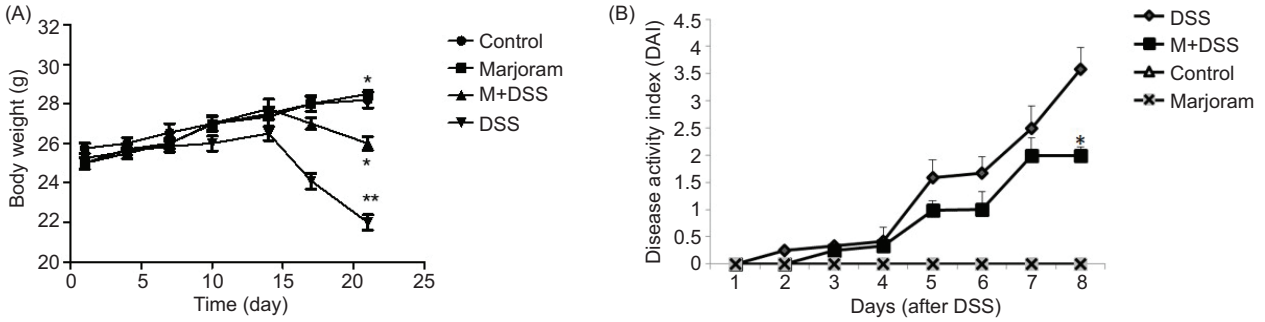


**Figure 2. Marjoram oil attenuated clinical signs of dextran sulfate sodium (DSS)-induced colitis in mice. (A) Body weight changes *$p < 0.05$, **$p < 0.01$ J21 *vs* J14. (B) Scores of disease activity index (DAI) in marjoram pre-treated mice and colitic (DSS) mice monitored every day. Data are expressed as the mean ± SD (n = 6 mice/groups). *$p < 0.05$ compared with DSS.**

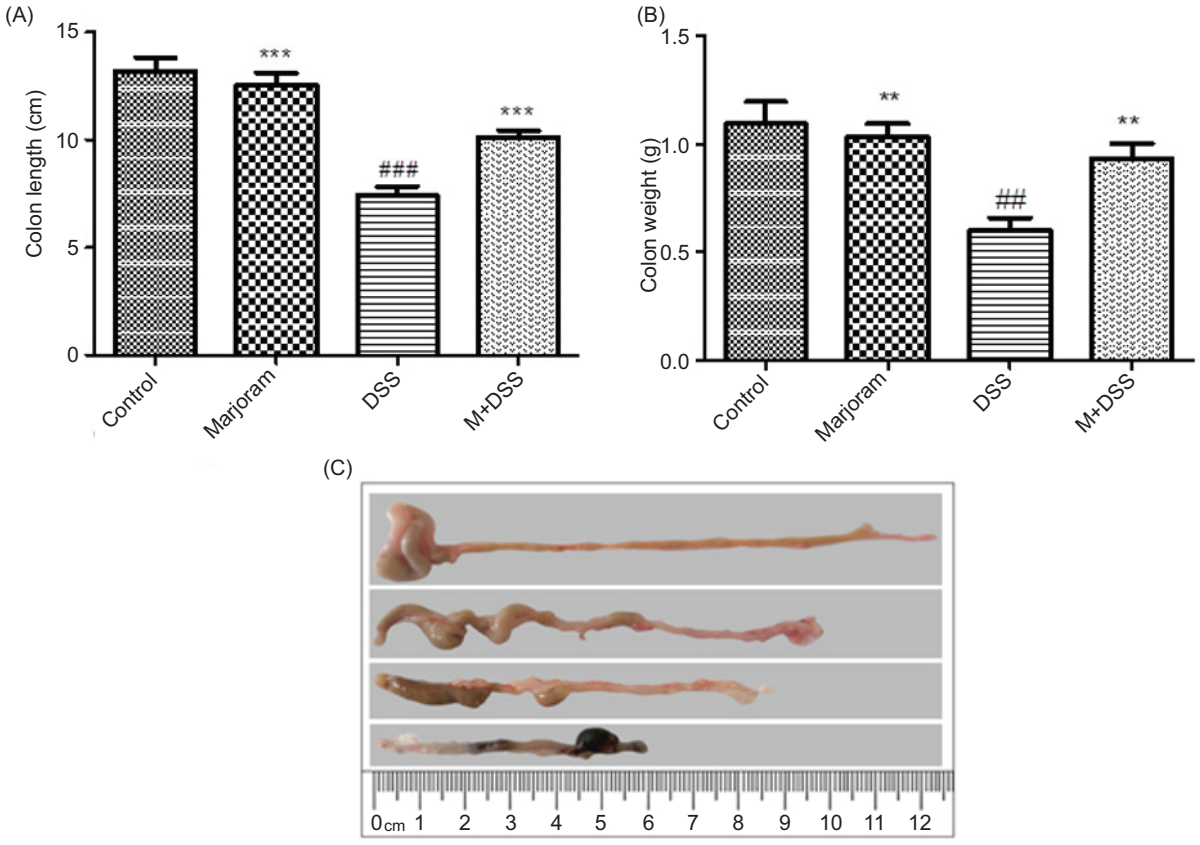


**Figure 3. (A) Colon length. (B) Colon weight. (C) Colon macroscopic analysis. Data are expressed as mean ± SD (n = 6 mice/groups). ##$p < 0.01$, ###$p < 0.001$, compared with the control group; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, compared with the DSS group.**

colon was significantly restored (27%) in the (M+DSS) group, compared to the DSS group ($p < 0.001$).

### Marjoram oil alleviated DSS-induced colonic cellular permeability

After DSS treatment, FITC-dextran permeation was significantly higher, compared to both control and marjoram groups ($p < 0.05$). However, mice from the (M+DSS) group possessed a lower colonic permeability to FITC-dextran ($p < 0.05$; Figure 4A). To determine the functional viability and integrity of colonic epithelium under *ex vivo* conditions, transepithelial ion conductance measurements were done for 120 min. As illustrated in Figure 4B, transepithelial ion conductance in colonic tissues from DSS group was considerably ($p < 0.05$) higher than that of the control group, indicating that colonic permeability to ions was relatively increased in colitic group. In contrast, the (M+DSS) group showed a significant recovery ($p < 0.01$) in transepithelial ion conductance. These results suggested that marjoram oil could decrease paracellular permeability by maintaining colonic epithelial integrity.

### Marjoram oil ameliorated DSS-induced histopathological damage

Representative results and microscopic scores are illustrated in Figure 5. The DSS-treated mice had severe epithelial damage, including important cellular invasion into the lamina propria and colon mucosa, goblet cell depletion, thickening of the mucosa, and total architecture alteration, leading to a high microscopic damage score (Figure 5B). However, pre-treatment of DSS-treated mice with marjoram oil reduced infiltration of inflammatory cells with a minimum decrease of epithelial cells, resulting in a significantly reduced microscopic damage score when compared to colitic mice. These results suggested the effective protection rendered by marjoram oil to counter DSS-induced colitis.

### Marjoram oil decreased pro-inflammatory cytokine levels

As shown in Figure 6, induction of colitis by DSS produced a marked increase in IL-6 level ($p < 0.01$). However, pre-treatment with marjoram oil significantly reduced the IL-6 content in plasma ($p < 0.05$) when compared to the DSS group.

### Marjoram oil reduced colonic myeloperoxidase activity

In the DSS group, colonic inflammation resulted in an increase in the activity of MPO in comparison to the control group ($p < 0.001$), reflecting the enhanced neutrophil infiltration that characterized this inflammatory process. However, pre-treatment of DSS-treated group with marjoram oil significantly reduced the colonic activity of MPO, compared to colitic animals ($p < 0.001$, vs the DSS group; Figure 7).

### Marjoram oil decreased oxidative stress in DSS-induced colitis

In the DSS group, substantial oxidative stress was generated in the colonic mucosa as evidenced by a marked increase in TBARS (Figure 8) levels and a significant decrease in SOD and CAT activity in the colon (Figures 9A and 9B). GSH content in the colon of the DSS-treated mice was relatively lesser than that in the control and marjoram oil groups ($p < 0.01$; Figure 9D). Interestingly, marjoram oil reversed the loss of SOD

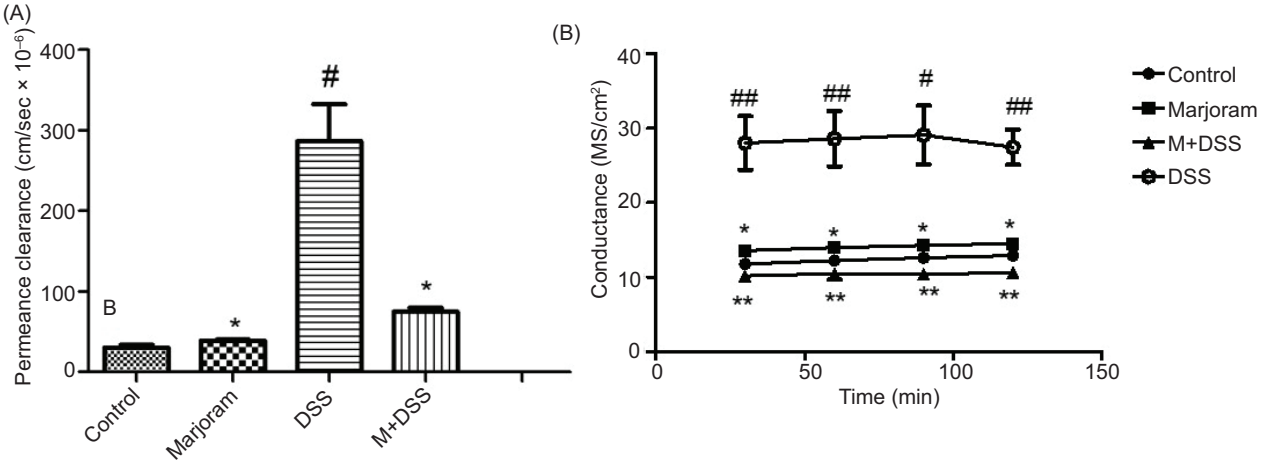


**Figure 4. Analysis of basal gut permeability in male Balb/c mice. (A) Colonic permeability to FITC-dextran (FD4) was measured in Ussing chamber. (B) Transepithelial ion conductance was measured in the colon. Bars indicate mean and SD. n = 6/group. (*$p < 0.05$, **$p < 0.01$ compared to DSS group; #$p < 0.05$, ##$p < 0.01$ compared to control group).**

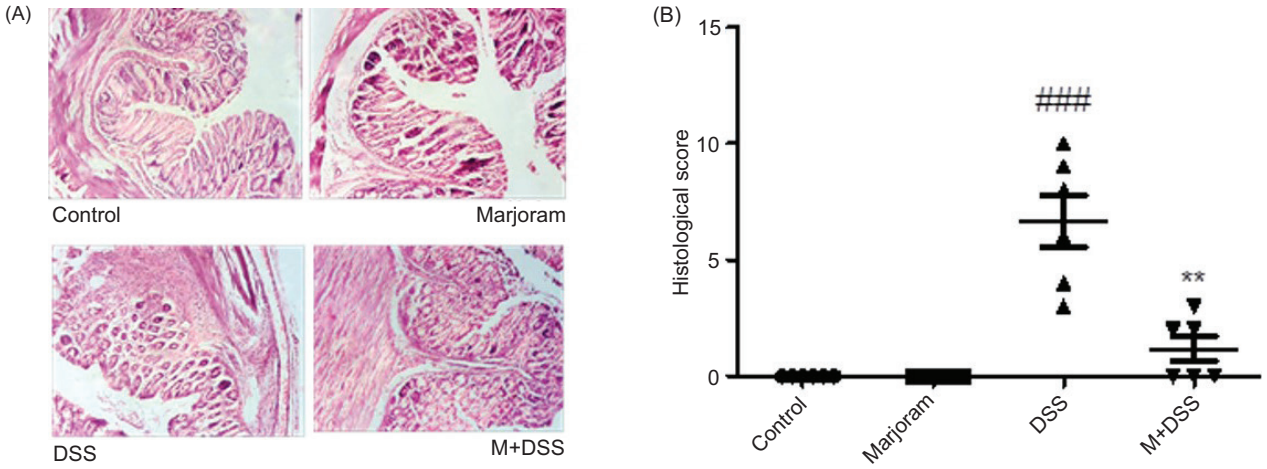


**Figure 5. Effects of marjoram oil on histological damage in DSS-induced mice colitis. (A) Histological appearance of colonic tissue sections (H&E, G×10). (B) Changes in histological score. Data are expressed as the mean ± SD (n = 6). **$p < 0.01$ is significantly different from the DSS group, and ###$p < 0.001$ is significantly different from the control group.**

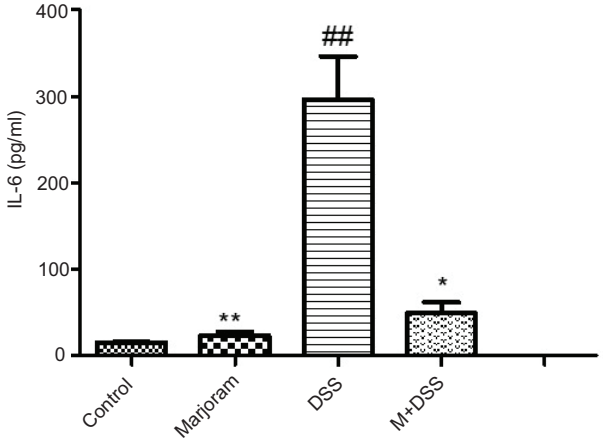


**Figure 6. Effect of marjoram oil on plasma levels of IL-6 in mice treated with 4% DSS. Statistical analysis was performed using one-way Anova. Values are mean ± SD (n = 6 for each group). *Indicates significant differences (*$p < 0.05$, **$p < 0.01$) compared with DSS group. ##$p < 0.01$ compared to control.**

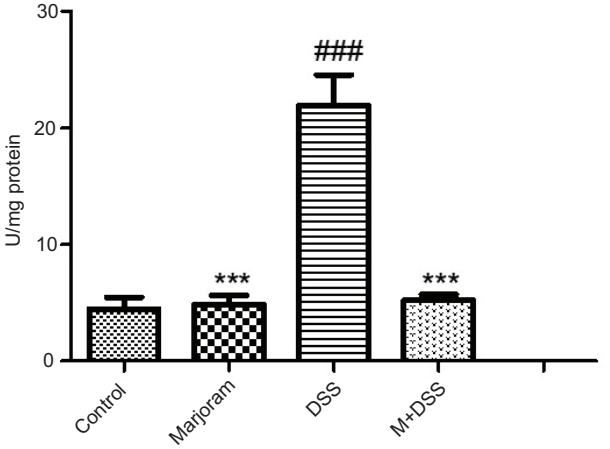


**Figure 7. Colonic myeloperoxidase activity. Data represent mean ± SD (n = 6 per group), ###$p < 0.001$ compared with control group; ***$p < 0.001$ compared with DSS group.**

and CAT activity ($p < 0.01$) and increased GSH content, compared to the DSS group. Furthermore, colonic levels of TBARS were reduced in colitic mice pre-treated with marjoram oil. However, no significant changes in glutathione peroxidase activity were observed in the colonic tissues of different groups (Figure 9C).

## Discussion

One of the two main types of IBDs is ulcerative colitis, increasing rapidly in prevalence globally (Kokkinidis *et al.*, 2017). Anti-inflammatory medications, including corticosteroids and 5-amino salicylic acid and its derivatives, are frequently used to treat IBD. In contrast, because of the development of additional oxidative stress, these medications are considered as a double-edged sword, as they cause hepatotoxicity and other adverse reactions (Uko *et al.*, 2012). For this reason, natural products have garnered substantial research interest and are being investigated extensively. Our study explored the preventive effects of marjoram oil as a therapeutic strategy for acute ulcerative colitis caused by DSS.

In this study, a colitis model was established with mice being fed with 4% DSS solution for 7 days. DAI score was used as an indicator to evaluate the gravity of gastrointestinal disease in experimental groups. Our results

showed that the DSS-treated mice displayed abnormal physiological conditions with an increased clinical score (DAI), indicating the earlier onset of colitis symptoms, such as diarrhoea, bloody stools, and reduced body weight as reported by Cooper *et al.* (1993). Higher DAI score was related to smaller colon length and elevated histopathological score. DSS is a heparin-like polysaccharide. It is considered to cause mucosal damage and inflammation initially through a direct toxic influence on intestinal epithelial cells by disrupting mucosal barrier to cause colitis (Perse and Cerar, 2012). DSS also causes indirect damage because of modifications in resident bacteria (Okayasu *et al.*, 1990) followed by the mustering and activation of inflammatory cells and the upregulation of inflammatory mediators, resulting in severe colitis (Lewellyn *et al.*, 2017).

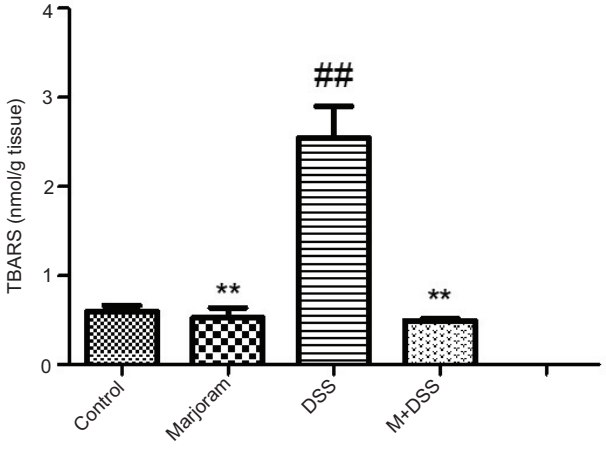


**Figure 8. Effect of marjoram oil on the TBARS levels in colonic tissue of DSS-treated mice (n = 6). Values are expressed as mean ± SD and analyzed using one-way analysis of variance followed by Anova. (##$p < 0.01$, compared to control; **$p < 0.01$ compared with DSS group).**

The clinical symptoms of IBD induced by DSS were lessened by marjoram oil. Administration of marjoram oil to colitic mice resulted in a considerably reduced symptoms of the disease, such as diarrhoea, bloody stools, and weight loss, which were evidenced by a lower DAI score. In accordance with previous results reported by Al Howiriny *et al. (*2009), histopathological damage of the gastric mucosa was significantly prevented by administering marjoram extract.

IBD is characterized by inflammation that affects the integrity of epithelial barrier (Landy *et al.*, 2016). Tissue

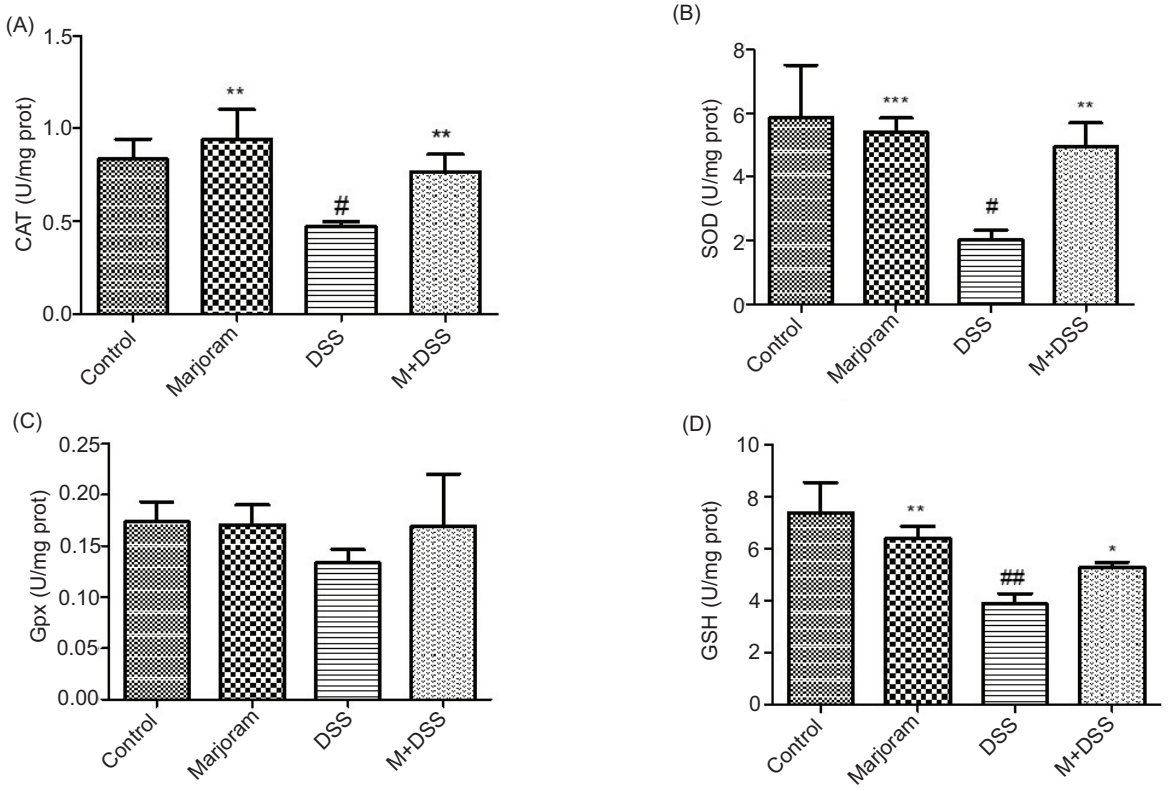


**Figure 9. Effect of marjoram oil on (A) catalase, (B) superoxide dismutase, (C) glutathione peroxidise, and (D) total glutathione activities in the colonic tissues of DSS-treated mice (n = 6). Values are expressed as mean ± SD and analyzed using one way analysis of variance followed by ANOVA. #$p < 0.05$, ##$p < 0.01$, ###$p < 0.001$, compared to control; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, compared with the DSS group).**

conductance or its reciprocal transepithelial electrical resistance is considered as a sensitive biomarker of intestinal barrier integrity and its function (Catanzaro *et al.*, 2015). Changes in transcellular and paracellular ion transport through the epithelium of colonic mucosa, or a disruption in the functional integrity of intercellular tight junctions, could explain an increase in electrical conductance (Mlodzik-Danielewicz and Tyrakowski, 2005).

In the present study, DSS was found to increase the electrical conductivity and cellular permeability to FITC-dextran. Our results were in line with the study conducted by Kitajima *et al.* (1999), which showed that DSS enhanced colonic mucosal permeability, resulting in the alteration of inflammatory reactions and mucosal barrier function. Indeed, loss of intestinal barrier function in the DSS colitis model was related to changes in tight junction protein distribution (Uko *et al.*, 2012). The impairment of intestinal barrier function induced by DSS in our study was clearly diminished by marjoram oil in colitic mice. This amelioration was related to a decrease in IL-6 level, which plays an integral role in developing IBD (Helieh *et al.*, 2013; Mazur-Bialy *et al.*, 2017). Our data supported the previously reported anti-inflammatory properties of marjoram oil. Arranz *et al.* (2015) indicated that sabinene hydrate and terpineol in sweet marjoram essential oil inhibited the production of proinflammatory cytokines.

In the current study, the status of inflammation in colitic animals was confirmed by an increase in MPO activity, a specific biomarker of inflammation in colonic mucosa (Malle *et al.*, 2007). However, pre-treatment with marjoram oil significantly reduced the colonic MPO activity. MPO is highly expressed in neutrophils, but less so in monocytes and certain macrophages (Malle *et al.*, 2007). Its activity is linearly related to neutrophil infiltration (Iba *et al.*, 2003). Bhattacharyya *et al.* (2014) showed that MPO was able to catalyse the generation of ROS.

When ROS formation surpasses the antioxidant capability of cells, cellular macromolecules are destroyed. This oxidative stress is hypothesized to have a role in the development of several human diseases, particularly ulcerative colitis (Mekkioui and Djerdjouri, 2012; Xu *et al.*, 2009). It was reported that DSS-treated mice exhibited a significant increase in lipid peroxidation (Amirshahrokhi *et al.*, 2011; Yan *et al.*, 2015). This was in line with the results of our research, in which TBARS concentrations were higher in colitic mice than in other groups. Interestingly, pre-treatment with marjoram oil resulted in a considerable reduction of TBARS level, demonstrating the antioxidant activity of marjoram oil. This result confirmed the results of our recent study, in which marjoram oil demonstrated a strong *in vitro* antioxidant power (Bouayad Debbagh *et al.*, 2021).

Glutathione sulfhydryl is thought to be the first line of defense against oxidative stress and inflammatory cascades (Zhu and Li, 2012). GSH is the main reducing agent for colonic epithelial cells; it is frequently reported to diminish during severe DSS-induced colitis (Oz *et al.*, 2005; Wardman *et al.*, 2007). Hence, in agreement with the above findings, we discovered lower GSH levels in the colitic group.

Enzymatic antioxidants are another line of oxidative defense system. In the current study, the activity of CAT and SOD was reduced in the colonic tissues of colitic group. Similar to what was observed in our study and in human IBD patients (Mueller *et al.*, 2013), several studies have revealed that when rodents are exposed to DSS, enzymatic antioxidants are depleted, suggesting enhanced oxidative stress-mediated colon injury (Nishiyama *et al.*, 2012; Pandurangan *et al.*, 2015; Xing *et al.*, 2013).

## Conclusions

Our data suggested that marjoram oil lessened DSS-induced experimental colitis as evidenced by reduced severity of ulcerative colitis, preventing oxidative damage, reducing neutrophil infiltration, and inhibiting IL-6 production. Importantly, marjoram oil preserved the intestinal barrier function from damage caused by inflammatory stimuli.

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## Conflict of interest

The authors declared no conflict of interest with respect to research, authorship, and/or publication of this article.

## References

Aebi, H. 1984. Catalase *in vitro*. Methods Enzymol. 105:121–126. https://doi.org/10.1016/S0076-6879(84)05016-3

Al Dhaheri, Y., Attoub, S., Arafat, K., Abuqamar. S., Viallet, J., Saleh, A., Al Agha, H., Eid, A. and Iratni, R. 2013. Anti-metastatic and anti-tumor growth effects of *Origanum majorana* on highly metastatic human breast cancer cells: Inhibition of NF_B signaling and reduction of nitric oxide

production. PLoS One 8(7):e68808. https://doi.org/10.1371/journal.pone.0068808

Al-Howiriny, T., Alsheikh, A., Alqasoumi, S., Al-Yahya, M., El Tahir, K. and Rafatullah, S. 2009. Protective effect of *Origanum majorana* L. "marjoram" on various models of gastric mucosal injury in rats. Am. J. Chin. Med. 37(3):531–545. https://doi.org/10.1142/s0192415x0900703x

Amirshahrokhi, K., Bohlooli, S. and Chinifroush, M.M. 2011. The effect of methylsulfonylmethane on the experimental colitis in the rat. Toxicol Appl Pharmacol. 253(3):197–202. https://doi.org/10.1016/j.taap.2011.03.017

Arranz, E., Jaime, L., López de las Hazas, M.C., Reglero, G. and Santoyo, S. 2015. Supercritical fluid extraction as an alternative process to obtain essential oils with anti-inflammatory properties from marjoram and sweet basil. Indu Crops Prod. 67:121–129. https://doi.org/10.1016/j.indcrop.2015.01.012

Athamneh, K., Alneyadi, A., Alsamri, H., Alrashedi, A., Palakott, A., El-Tarabily, K.A., Eid, A.H., Al Dhaheri, Y. and Iratni, R. 2020. Origanum majorana essential oil triggers p38 MAPK-mediated protective autophagy, apoptosis, and caspase-dependent cleavage of P70S6K in colorectal cancer cells. Biomolecules. 10(3):412. http://dx.doi.org/10.3390/biom10030412

Baakhtari, S., McCombie, A., BokkelHuinink, S., Irving, P., Siegel, C.A., Mulde, R., Mulder, C.J. and Gearry, R. 2018. Observational study of perspectives of inflammatory bowel disease patients concerning the use of corticosteroids. Dig Dis (Basel). 36(1):33–39. https://doi.org/10.1159/000478772

Bang, B. and Lichtenberger, L.M. 2016. Methods of inducing inflammatory bowel disease in mice. Curr Protoc Pharmacol. 72(5):1–42. https://doi.org/10.1002/0471141755.ph0558s72

Benhalilou, N., Alsamri, H., Alneyadi, A., Athamneh, K., Alrashedi, A., Altamimi, N., Al Dhaheri, Y., Eid, A.H. and Iratni, R., 2019. *Origanum majorana* ethanolic extract promotes colorectal cancer cell death by triggering abortive autophagy and activation of the extrinsic apoptotic pathway. Front Oncol. 9:795. https://doi.org/10.3389/fonc.2019.00795

Bhattacharyya, A., Chattopadhyay, R., Mitra, S. and Crowe, S.E. 2014. Oxidative stress: an essential factor in the pathogenesis of gastrointestinal mucosal diseases. Physiol Rev. 94(2):329–354. https://doi.org/10.1152/physrev.00040.2012

Bouayad Debbagh, I., Grar, H., Saidi, D., Kheroua, O. and Kaddouri, H. 2021. Phytochemical analysis and in vitro antioxidant activities of marjoram oil. Biosci Res.18(4):2888–2894.

Catanzaro, D., Rancan, S., Orso, G., Dall'Acqua, S., Brun, P., Giron, M.C., Carrara, M., Castagliulo, I., Ragazzi, E., Caparrotta, L. and Montopoli, M. 2015. Boswellia serrata preserves intestinal epithelial barrier from oxidative and inflammatory damage. PLoS One.10(5):e0125375. https://dx.doi.org/10.1371%2Fjournal.pone.0125375

Cheifetz, AS., Gianoti, R., Luber, R and Gibson, P.R. 2017. Complementary and alternative medicines used by patients with inflammatory bowel diseases. Gastroenterology. 152(2):415–429. https://doi.org/10.1053/j.gastro.2016.10.004

Cooper, H.S., Murthy, S.N., Shah, R.S. and Sedergran, D.J. 1993. Clinicopathologic study of dextran sulfate sodium experimental murine colitis. Lab Invest. 69(2):238–249. PMID: 8350599.

Della Pepa, T., Elshafie, H.S., Capasso, R., DeFeo, V., Camele, I., Nazzaro, F., Scognamiglio, M.R. and Caputo, L. 2019. Antimicrobial and phytotoxic activity of *Origanum heracleoticum* and *O. majorana* essential oils growing in Cilento (Southern Italy). Molecules. 24(14):2576. https://doi.org/10.3390/molecules24142576

Duletic, S., AlimpićAradski, A., Kolarevic, S., Vuković-Gačić, B., Oaldje, M., Živkovi´c, J., Šavikin, K. and Marin, P. 2018. Antineurodegenerative, antioxidant and antibacterial activities and phenolic components of *Origanum majorana* L. (Lamiaceae) extracts. J Appl Bot Food Qual. 91:126–134. http://dx.doi.org/10.5073/JABFQ.2018.091.018

Erenler, R., Sen, O., Aksit, H., Demirtas, I., Yaglioglu, A.S., Elmastas, M. and Telci, I. 2016. Isolation and identification of chemical constituents from *Origanum majorana* and investigation of antiproliferative and antioxidant activities. J Sci Food Agric. 96(3):822–836. https://doi.org/10.1002/jsfa.7155

Guerra-Boone, L., Alvarez-Roman, R. and Salazar-Aranda, R. 2015. Antimicrobial and antioxidant activities and chemical characterization of essential oils of Thymus vulgaris, Rosmarinus officinalis, and Origanum majorana from northeastern Mexico. Pak J Pharm Sci. 28(1):363–369. PMID: 25631514.21.

Goel, P. and Vasudeva, N. 2015. *Origanum majorana* L. – phytopharmacological review. Indian J Nat Prod Res. 6:261–267. https://op.niscpr.res.in/index.php/IJNPR/article/view/6800/590

Goyal, N., Rana, A., Ahlawat, A., Bijjem, K.R.V. and Kumar, P. 2014. Animal models of inflammatory bowel disease. Rev Inflammopharmacol. 22(4):219–233. https://doi.org/10.1007/s10787-014-0207-y

Helieh, O., Cheresa, T., Willem, J.S. and Villiers, D. 2013. Green tea polyphenols and sulfasalazine have parallel anti-inflammatory properties in colitis models. Front Immunol. 5(4):132. https://doi.org/10.3389/fimmu.2013.00132

Iba, Y., Sugimoto, Y., Kamei, C. and Masukawa, T. 2003. Possible role of mucosal mast cells in the recovery process of colitis induced by dextran sulfate sodium in rats. Int Immunopharmacol. 3(4):485–491. https://doi.org/10.1016/s1567–5769(02)00299-0

Kitajima, S., Takuma, S. and Morimoto, M. 1999. Changes in colonic mucosal permeability in mouse colitis induced with dextran sulfate sodium. Exp Anim. 48(3):137–143. https://doi.org/10.1538/expanim.48.137

Klebanoff, S.J. 2005. Myeloperoxidase: friend and foe. J Leukoc Bio. 77(5):598–625. https://doi.org/10.1189/jlb.1204697

Kokkinidis, D.G., Bosdelekidou, E.E. and Iliopoulou, S.M. 2017. Emerging treatments for ulcerative colitis: a systematic review. Scand J Gastroenterol. 52(9):923–931. https://doi.org/10.1080/00365521.2017.1326163

Landy, J., Ronde, E., English, N., Clark, S.K., Hart, A.L., Knight, S., Ciclitira, P. J. and Al-Hassi, H.O. 2016. Tight junctions in inflammatory bowel diseases and inflammatory bowel disease associated colorectal cancer. World J. Gastroenterol. 22(11):3117–3126. https://doi.org/10.3748/wjg.v22.i11.3117

Larussa, T., Imeneo, M. and Luzza, F. 2017. Potential role of nutraceutical compounds in inflammatory bowel disease. World J. Gastroenterol. 23(14):2483–2492. https://doi.org/10.3748/wjg.v23.i14.2483

Lenoir, L., Rossary, A., Joubert-Zakeyh, J., Vergnaud-Gauduchon, J., Farges, M-C., Fraisse, D., Texier, O., Lamaison, J.L., Vasson, M.P. and Felgines, C. 2011. Lemon verbena infusion consumption attenuates oxidative stress in dextran sulfate sodium-induced colitis in the rat. Dig Dis Sci. 56(12):3534–3545. https://doi.org/10.1007/s10620-011-1784-x

Lewellyn, S.R., Britton, G.J., Contijoch, E.J., Mortha, A.J.F., Colombel, J.F.A., Grinspan, A., Clemente, J.C., Merad, M. and Faith, J.J. 2017. Deciphering the combinatorial influence of diet and the microbiota on experimental colitis. BioRxiv. 117929. Epub ahead of print. https://doi.org/10.1101/117929

Lin, SC. and Cheifetz, A.S. 2018. The use of complementary and alternative medicine in patients with inflammatory bowel disease. Gastroenterol Hepatol. 14(7):415–425. PMID: 30166957.

Liu, Y., Wang, X., Hou, Y., Yin, Y., Qiu, Y., Wu, G.and Hu, C.A.A. 2017. Roles of amino acids in preventing and treating intestinal diseases: recent studies with pig models. Amino Acids. 49(8):1277–1291. https://doi.org/10.1007/s00726-017-2450-1

Lowry, O.H., Rose Rough, NJ., Farr, AL. and Randall, RJ. 1951. Protein measurement with the folin phenol reagent. J Biol Chem. 193(1):265–275. https://doi.org/10.1016/S0021-9258(19)52451-6

Makrane, H., Aziz, M., Mekhfi, H, Ziyyat, A., Legssyer, A. and Melhaoui, A. 2018. *Origanum majorana* L. extract exhibit positive cooperative effects on the main mechanisms involved in acute infectious diarrhoea. J Ethnopharmacol. 239:111503. https://doi.org/10.1016/j.jep.2018.09.005

Maloy, K.J. and Powrie, F. 2011. Intestinal homeostasis and its breakdown in inflammatory bowel disease. Nature. 474(7351): 298–306. https://doi.org/10.1038/nature10208

Malle, E., Furtmüller, P.G., Sattler, W. and Obinger, C. 2007. Myeloperoxidase: a target for new drug development? Br J Pharmacol. 152(6):838–854. https://dx.doi.org/10.1038%2Fsj.bjp.0707358

Mandalari, G., Bisignano, C., Genovese, T., Mazzon, E., Wickham, M.S. and Paterniti, I. 2011. Natural almond skin reduced oxidative stress and inflammation in an experimental model of inflammatory bowel disease. Int Immunopharmacol. 11(8):915–24. https://doi.org/10.1016/j.intimp.2011.02.003

Marklund, S.L. and Marklund, G. 1974. Involvement of superoxide anion radical in the autooxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem. 47(3):469–474. https://doi.org/10.1111/j.1432-1033.1974.tb03714.x

Mazur-Bialy, A.I., Bilski, J., Wojcik, D., Brzozowski, B., Surmiak, M., Hubalewska Mazgaj, M., Chmura, A., Magierowski, M., Magierowska, K. and Mach, T. 2017. Beneficial effect of voluntary exercise on experimental colitis in mice fed a high-fat diet: the role of irisin, adiponectin and proinflammatory biomarkers. Nutrients. 9(4):410. https://doi.org/10.3390/nu9040410

Mekkioui, A. and Djerdjouri, B. 2012. N-acetylcysteine improves redox status, mitochondrial dysfunction, mucin-depleted crypts and epithelial hyperplasia in dextran sulfate sodium-induced oxidative colitis in mice. Eur J Pharmacol. 691(1–3):209–217. https://doi.org/10.1016/j.ejphar.2012.06.014

Mijan, A.L. and Lim, B.O. 2018. Diets. Functional foods and nutraceuticals as alternative therapies for inflammatory bowel disease: present status and future trends. World J Gastroenterol. 24(25):2673–2685. https://dx.doi.org/10.3748%2Fwjg.v24.i25.2673

Młodzik-Danielewicz, N. and Tyrakowski, T. 2005. Effects of amiloride and bumetanide on hyperpolarization after movement across the distal colon epithelium. Pharmacol Rep. 57(4): 489–497. PMID: 16129916.

Mossa, A.T., Refaie, A.A., Ramadan, A. and Bouajila, J. 2013. Amelioration of prallethrin-induced oxidative stress and hepatotoxicity in rat by the administration of *Origanum majorana* essential oil. Biomed Res Int. 2013:859085. https://doi:10.1155/2013/859085

Mueller, K., Blum, N.M. and Mueller, A.S. 2013. Examination of the anti-inflammatory, antioxidant, and xenobiotic- inducing potential of broccoli extract and various essential oils during a Mild DSS-induced colitis in rats. ISRN Gastroenterol. 2013:710856. https://doi.org/10.1155/2013/710856

Nishiyama, Y., Kataoka, T., Yamato, K., Taguchi, T. and Yamaoka, K. 2012. Suppression of dextran sulfate sodium-induced colitis in mice by radon inhalation. Mediators Inflamm. 2012:239617. https://doi.org/10.1155/2012/239617

Okayasu, I., Hatakeyama, S., Yamada, M., Ohkusa, T., Inagaki, Y. and Nakaya, R. 1990. A novel method in the induction of reliable experimental acute and chronic ulcerative colitis in mice. Gastroenterology. 98(3):694–702. https://doi.org/10.1016/0016-5085(90)90290-h

Oz, H.S., Chen, T.S., McClain, C.J. and de Villiers, W.J.S. 2005. Antioxidants as novel therapy in a murine model of colitis. J Nutr Biochem.16(5):297–304. https://doi.org/10.1016/j.jnutbio.2004.09.007

Pandurangan, A.K., Mohebali, N., Norhaizan, M.E. and Looi, C.Y. 2015. Gallic acid attenuates dextran sulfate sodium-induced experimental colitis in BALB/c mice. Drug Des Devel Ther. 9:3923–3934. https://dx.doi.org/10.2147%2FDDDT.S86345

Paterson, J.R., Srivastava, R., Baxter, G.J., Graham, A.B. and Lawrence, J.R. 2006. Salicylic acid content of spices and its implications. J Agric Food Chem. 54(8):2891–2896. https://doi.org/10.1021/jf058158w

Perse, M. and Cerar, A. 2012. Dextran sodium sulphate colitis mouse model: traps and tricks. Biomed Biotechnol. 2012:718617. https://doi.org/10.1155/2012/718617

Ramadan, G., El-Beih, N.M., Arafa, NM. and Zahra, M.M. 2013. Preventive effects of Egyptian sweet marjoram (*Origanum majorana* L.) leaves on hematological changes and cardiotoxicity in isoproterenol-treated albino rats. Cardiovasc Toxicol. 13(2):100–109. https://doi.org/10.1007/s12012-012-9189-4

Rodrigues, M.R.A., Caram˜ao, E.B., Arce, L., R´ıos, A. and Valc´arcel, M. 2002. Determination of monoterpene hydrocarbons and alcohols in *Majorana shortness* Moench by micellar electro kinetic capillary chromatographic. J Agric Food Chem. 50(15):4215–4220. https://doi.org/10.1021/jf011667n

Salih, A.M., Smith, D.M., Price, J.F. and Dawson, L.E. 1987. Modifid extraction 2 thiobarbituric acid method for measuring lipid oxidation in pountry. Poultry Sci. 66(9):1483–1488. https://doi.org/10.3382/ps.0661483

Sedlak, J. and Lindsay, R.H. 1968. Estimation of total, protein-bound, and non-protein sulfhydryl groups in tissue with

Ellman's reagent. Anal Biochem. 25(1):192–205. https://doi.org/10.1016/0003-2697(68)90092-4

Stillie, R. and Stadnyk, A.W. 2009. Role of TNF receptors, TNFR1 and TNFR2 in dextran sodium sulfate-induced colitis. Inflamm Bowel Dis. 15(10):1515–1525. https://doi.org/10.1002/ibd.20951

Su, L., Nalle, S.C., Shen, L., Turner, E.S., Singh, G., Breskin, L.A., Khramtsova, E.A., Khramtsova, G., Tsai, P.Y., Fu, Y.X.C., Abraham, C.J.R. and Turner, J.R. 2013. TNFR2 activates MLCK-dependent tight junction dysregulation to cause apoptosis-mediated barrier loss and experimental colitis. Gastroenterology. 145(2):407–415. https://doi.org/10.1053/j.gastro.2013.04.011

Tahan, G., Aytac, E., Aytekin, H., Gunduz, F., Dogusoy, G., Aydin, S., Tahan, V. and Uzun, H. 2011. Vitamin E has a dual effect of anti-inflammatory and antioxidant activities in acetic acid-induced ulcerative colitis in rats. Can J Surg. 54(5):333–338. https://doi.org/10.1503/cjs.013610

Trouillas, P., Calliste, C.A., Allais, DP., Simon, A., Ma, A., Delage, C. and Duroux, J.L. 2003. Antioxidant, anti-inflammatory and anti-oxidant antiproliferative properties of sixteen water plant extracts used in the Limousin countryside as herbal teas. Food Chem. 80(3):399–407. https://doi.org/10.1016/S0308-8146(02)00282-0

Uko, V., Thangada, S. and Radhakrishnan, K. 2012. Liver disorders in inflammatory bowel disease. Gastroenterol Res Pract. 2012:642923. https://doi.org/10.1155/2012/642923

Valatas, V., Vakas, M. and Kolios, G. 2013. The value of experimental models of colitis in predicting efficacy of biological therapies for inflammatory bowel diseases. Am. J. Physiol. Gastrointest. Liver Physiol. 305(11):763–785. https://doi.org/10.1152/ajpgi.00004.2013

Wan, P., Chen, H., Guo, Y. and Bai, A.P. 2014. Advances in treatment of ulcerative colitis with herbs: from bench to bedside. World J Gastroenterol. 20(39):14099–14104. https://doi.org/10.3748/wjg.v20.i39.14099

Wang, Z., Adachi, S., Kong, L., Watanabe, D., Nakanishi, Y., Ohteki, T., Hoshi, N. and Kodama, Y. 2019. Role of eosinophils in a murine model of inflammatory bowel disease. Biochem Biophys Res Commun. 511(1):99–104. https://doi.org/10.1016/j.bbrc.2019.02.056

Wardman, P. 2007. Fluorescent and luminescent probes for measurement of oxidative and nitrosative species in cells and tissues: progress, pitfalls, and prospects. Free Radic Biomed. 43(7):995–1022. https://doi.org/10.1016/j.freeradbiomed.2007.06.026

Xing, J., You, C., Dong, K., Sun, J., You, H. and Dong, Y. 2013. Ameliorative effects of 3,4-oxo isopropylideneshikimic acid on experimental colitis and their mechanisms in rats. Int Immunopharmacol. 15(3):524–31. https://doi.org/10.1016/j.intimp.2013.02.008

Xu, L., Yang, Z.L., Li, P. and Zhou, Y.Q. 2009. Modulating effect of hesperidin on experimental murine colitis induced by dextran sulfate sodium. Phytomedicine. 16(10):989–995. https://doi.org/10.1016/j.phymed.2009.02.021

Yan, H., Wang, H., Zhang, X., Li, X. and Y.J. 2015. Ascorbic acid ameliorates oxidative stress and inflammation in dextran sulfate sodium induced ulcerative colitis in mice. Int J Clin Exp Med. 8(11):20245–20253. https://www.ncbi.nlm.nih.gov/pubmed/26884937

Yang, B.L., Chen, Y.G. and Gu, Y.F. 2015. Long-term outcome of infliximab combined with surgery for perianal fistulizing Crohn's disease. World J Gastroenterol. 21(8):2475–2482. https://dx.doi.org/10.3748%2Fwjg.v21.i8.2475

Zhu, H. and Li, Y.R. 2012. Oxidative stress and redox signaling mechanisms of inflammatory bowel disease: updated experimental and clinical evidence. Exp Biol Med (Maywood). 237(5):474–480. https://doi.org/10.1258/ebm.2011.011358