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Original Research Article

Myrtus communis extract administration ameliorates ulcerative colitis in rats by reducing inflammatory cytokines, *T-bet/GATA-3* ratio expression, and oxidative stress indices

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Abstract

Objective: Ulcerative colitis (UC) is a chronic, relapsing inflammatory disorder that impacts the large intestine and rectum, and it is characterized by oxidative stress and severe inflammation. Research has shown that *Myrtus communis* possesses antioxidative and anti-inflammatory effects. This study sought to evaluate the impact of *M. communis* therapy on inflammation and oxidative stress in a rat model of experimental colitis.

Materials and methods: A total of 35 male rats were selected and randomly allocated into five groups. Colitis was induced in the rats by administering a 4% acetic acid solution. Four days post-colitis induction, the rats were administered intraperitoneal treatments for the next seven days with either saline, *M. communis leaves ethanolic* extract (at doses of 50 and 100 mg/kg), or sulfasalazine (at a dose of 100 mg/kg).

Results: Colitis caused increased histopathological damage in the colon, along with elevated levels of Malondialdehyde, Myeloperoxidase, Tumor Necrosis Factor α,Interleukine-6 ,Interleukine-1β , and *GATA-3* gene expression. Conversely, it decreased the levels of Superoxide Dismutase, Glutathione Peroxidase, IL-10, and *T-bet* gene expression in the treated rats compared to the control group.

Conclusion: *Myrtus communis* exhibits advantageous benefits on UC via reducing inflammation and oxidative stress.

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Introduction

Ulcerative colitis (UC) is a long-lasting condition that causes inflammation in the gastrointestinal tract, leading to a variety of challenges for those affected (Keshavarzi et al. 2026). This disease leads to inflammation, ulcers, edema, bleeding, and diarrhea (Camacho 2025; Singh and Dulai 2022). On the other hand, the pathogenesis of UC has not yet been precisely defined, and most researchers have confirmed the interaction between genetic, immunological, and environmental factors in its pathogenesis (Bejeshk et al. 2022). Therefore, any treatment that can inhibit the activation of these immunological and inflammatory mechanisms seems to improve the patient's symptoms and reduce inflammatory reactions (Bejeshk et al. 2023b).

Cytokine signaling pathways play a crucial role in developing inflammatory bowel diseases, such as UC. Interleukine-1β, Tumor necrosis factor-α, and Interleukine-6 are essential pro-inflammatory cytokines that play a significant role in the generation of acute inflammation with neutrophilic infiltration of the gastrointestinal mucosa (Nakase et al. 2022). Also, the T-bet/GATA-3 ratio, known as an index of the Th1/Th2 cytokine profile, is impaired following colitis (Okamura et al. 2014a; WU et al. 2017).

Oxidative stress is a key factor in the inflamed tissues associated with UC. Also, oxidative stress in UC can increase the risk of colon cancer (Jena et al. 2012).

Although many treatments for inflammatory bowel disease exist, no effective treatment has been found for this condition. Treatments include aminosalicylates, antibiotics, corticosteroids, and immunosuppressants, all of which have extensive side effects (Min and Monaco 1991).

Traditionally, use of many herbal medicines has been helpful in the treatment of many diseases, while they have fewer side effects (Rajizadeh et al. 2024). *Myrtus communis* is a renowned medical plant (Rajizadeh et al. 2023a). Studies have disclosed that this plant has antioxidant and anti-inflammatory impacts (Esmaeilpour et al. 2023; Rajizadeh et al. 2023b).

Myrtus communis has attracted increasing attention due to its diverse pharmacological properties, particularly its antioxidant, anti-inflammatory, and immunomodulatory activities. Previous studies have demonstrated the protective effects of *M. communis* extracts in experimental colitis, mainly through attenuation of oxidative damage and improvement of histopathological alterations (Khosropour et al. 2019; Li et al. 2025). However, UC is a multifactorial inflammatory disorder involving not only oxidative stress but also dysregulated immune responses and cytokine imbalance. In particular, altered T-helper cell polarization, reflected by transcription factors such as T-bet and GATA-3, plays a critical role in the progression and severity of intestinal inflammation. Despite the promising findings of previous studies, limited evidence is available regarding the potential immunomodulatory mechanisms of *M. communis* in UC, especially with respect to the T-bet/GATA-3 signaling axis and inflammatory cytokine profile. Therefore, the present study aimed to investigate the protective effects of *M. communis* extract in an acetic acid-induced rat model of UC through a comprehensive evaluation of inflammatory cytokines, oxidative stress indices, and T-bet/GATA-3 ratio expression, in addition to conventional histopathological assessments. This approach may provide further insight into the immunological mechanisms underlying the therapeutic potential of *M. communis* in UC.

Materials and Methods

Animals

This study received approval from the Ethical Committee at Bojnurd University of Medical Sciences, Bojnurd, Iran, under code number IR.NKUMS.AEC.1402.001.

The animal farm of Kerman University of Medical Sciences obtained 35 male Wistar rats, each weighing approximately 250 g. The animals were kept in optimal conditions and the rats had the free access to food and water and randomly assigned to one of five groups, each including seven rats: 1) Control group (Con): healthy rats receiving saline intraperitoneally as a vehicle; 2) Colitis group: rats induced with colitis through rectal administration of acetic acid and supplied with saline intraperitoneally as a vehicle; 3) Colitis/ *M. communis* at dose 50 mg/kg (M50) group: colitis-induced rats administered with 50 mg/kg of *M. communis* extract via intraperitoneal injection (Berendika et al. 2022); 4) Colitis/*M. communis* at 100 mg/kg (M100) group (Berendika et al. 2022): colitis-induced rats administered with 100 mg/kg of *M. communis* extract via intraperitoneal injection; and 5) Positive control group: colitis-induced rats administered with 100 mg/kg of sulfasalazine via intraperitoneal injection.

Colitis induction

The rats were fasted for 36 hr while having access to water to clear their guts. Under mild anesthesia via Ketamine/Xylazine I.P injection, a 2-mm diameter, 8-cm long plastic tube was used to administer 4% acetic acid intrarectally. In order to prevent any potential leakage, each animal was held in a prone position for 5 min and then housed individually in wire cages for 24 hr. Colitis developed in the rats after 4 days. After induction, the rats received *M. communis* extract at two doses for seven consecutive days intraperitoneally (Keshavarzi et al. 2026).

Preparation of *M. communis* ethanolic extract

Myrtus communis was collected from the Darkesh region of North Khorasan in the spring season and its voucher number is MP.1292. The leaves of this plant were used in this study. The soaking method was used for extraction. In this method, 100 g of plant powder was soaked in ethanol for 48 hr. Then, the solutions were filtered and transferred to a rotary to separate the solvent, and then the obtained extract was dried in a 40℃ oven. The rotary evaporation device, or vacuum distillation, creates a vacuum and centrifugal force that causes evaporation and complete solvent removal from the laboratory samples. Significant efficiency in the solvent evaporation process and reducing the time of the separation process are among the advantages of the rotary evaporation device.

Colon weight to length ratio

On the final day, animals were euthanized using a ketamine/xylazine (100/10 mg/kg) overdose (Via Intraperitoneal injection). An 8 cm segment of the distal colon was then excised, opened lengthwise, cleaned with saline, and weighed to determine a tissue edema index (weight-to-length ratio) (Bejeshk et al. 2023b).

Microscopic examination

Colonic tissue specimens were fixed in 10% neutral-buffered formalin, processed routinely, and embedded in paraffin. Sections of 3–4 µm thickness were prepared using a microtome and stained with hematoxylin and eosin (H&E). Histopathological evaluation was performed under a light microscope by a pathologist who was blinded to the experimental groups. Colonic tissue damage was assessed semi-quantitatively based on inflammatory cell infiltration, epithelial damage, and mucosal architecture disruption. Histological changes were graded as follows: 0 (no inflammation), 1 (mild inflammation with minimal infiltration), 2 (moderate inflammation with noticeable infiltration and partial mucosal damage), and 3 (severe inflammation with extensive infiltration, epithelial destruction, and marked tissue damage), according to a modified histological scoring system (Bejeshk et al. 2023b).

Biochemical assessment

The colonic tissue was combined with a phosphate buffer for analytical purposes. After centrifugation, the supernatant was obtained to analyze Malondialdehyde (MDA) via the TBARS method, total antioxidant capacity (TAC) using the FRAP method, and catalase levels. The catalase activity was assessed using a suitable kit from Navandsalamat Co. The myeloperoxidase (MPO) activity was assessed using the suitable kit from Navandsalamat Co. This research evaluated the concentrations of TNF-α, IL-6, IL-10, and IL-1β concentrations in colon tissue using an enzyme-linked immunosorbent test (ELISA) per the manufacturer's instructions (Karmania Pars Gene Co).

Quantitative real-time PCR (qPCR)

The Parstous RNA extraction kit (A101231, Mashhad, Iran) was applied to extract total RNA from colon tissue. cDNA synthesis for all factors was performed with the same kit (Easy cDNA Synthesis kit; Parstous, A101161, Mashhad, Iran) but with a different running program. Real-time PCR was done utilizing RealQ Plus 2x Master Mix Green (Amplicon, Denmark) in a Step OnePlus instrument (Applied Biosciences). All procedures for all factors were the same according to the manufacturers of the kit and instrument. The primers utilized for real-time PCR are detailed in Table 1. *GAPDH* served as the internal control.

Table 1. Sequence of primers used in Real-Time PCR

Name	Sequence (5'-3')	Length
T-bet F	TCTTCCCTCCCAGCAGCCTAC	20
T-bet R	CAGTACCATCTCGCCGCCAC	21
GATA3 F	AAGTTCAACCAGCACCAGAC	21
GATA3 R	TCCACCAAGACTACATCCACA	20
GAPDH F	TTCACCTATGCCACCCTCAT	21
GAPDH R	ACTGCTCCCTTCTCACTCTCC	21

Disease activity index (DAI)

Daily assessments of body weight, stool consistency, and the presence and characteristics of blood in the stool were conducted during the therapy period. Disease activity was evaluated utilizing a composite disease activity index (DAI), calculated as the aggregate of weighted scores for percentage of body weight loss (0: <1%, 1: 1–5%, 2: 6–10%, 3: 10–15%, 4: >15%), stool consistency (0: normal, 1: slightly loose, 2: loose, 3: watery diarrhea), and rectal bleeding (0: absent, 1: hemoccult-positive, 2: bloody, 3: grossly bloody) (Abdel-Razek et al. 2024).

Statistical analysis

All data were analyzed using GraphPad Prism software (Version 10.0, GraphPad Software, San Diego, CA, USA). The normality of data distribution was assessed using the Shapiro–Wilk test. Following confirmation of normality, one-way analysis of variance (one-way ANOVA) was applied to compare differences among experimental groups for all measured parameters, including inflammatory cytokines, oxidative stress indices and *T-bet/GATA-3* gene expression. When significant differences were detected, Tukey's post hoc multiple comparison test was performed to identify intergroup differences. Data are presented as mean ± standard error of the mean (SEM), and a p-value of <0.05 was considered statistically significant.

Results

The effects of *M. communis* extract on inflammatory cytokines following colitis in rats

The findings indicated that concentrations of TNFα, IL-1β, and IL-6 were significantly increased in the colonic tissue of rats with colitis relative to healthy rats (p<0.001). The concentration of IL-10, an anti-inflammatory agent, was decreased in the colonic tissue of rats with colitis relative to healthy rats (p<0.001). Administration of *M. communis* extract at doses of 50 and 100 mg/kg significantly reduced proinflammatory cytokine levels (TNFα, IL1β, and IL-6) and increased the anti-inflammatory cytokine IL-10 in colon tissue compared to the colitis group, with effects comparable to those of sulfasalazine (Figure 1).

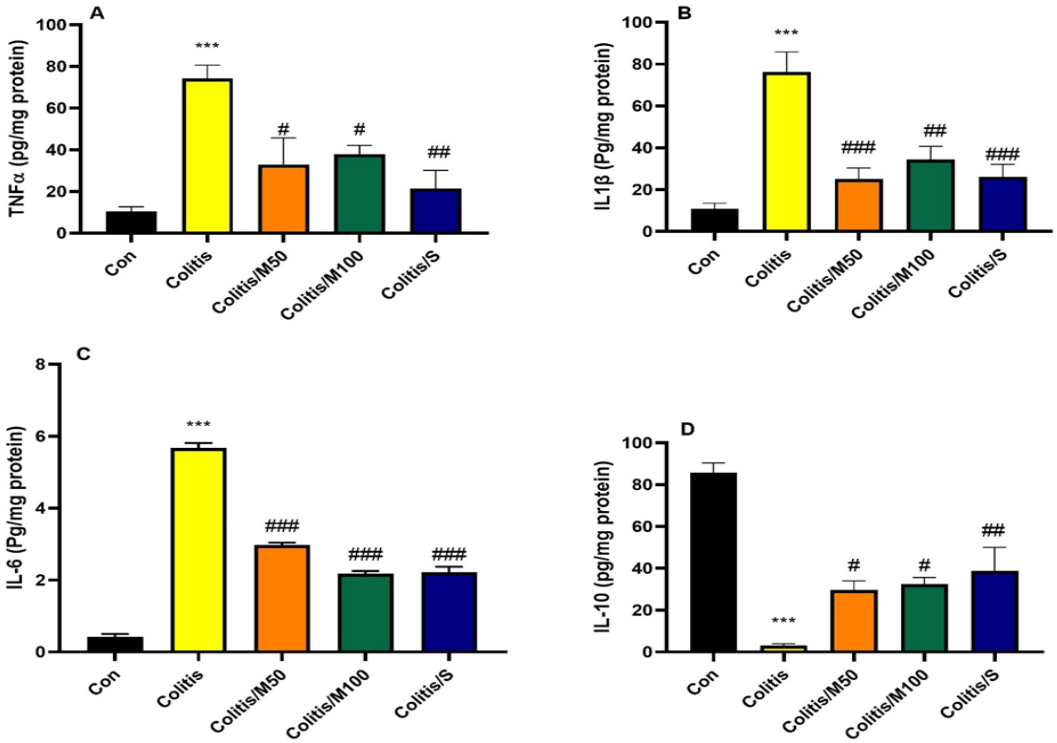

Figure 1. The impact of *Myrtus communis* extract at doses of 50 and 100 mg/kg on inflammatory cytokines within the colon tissue of rats suffering from colitis. Data are presented as mean±SEM.***p<0.001 vs control (Con). #p<0.05 , ##p<0.01 and ###p<0.001 vs colitis. M50: *Myrtus communis* at dose of 50 mg/kg. M100: *Myrtus communis* at dose of 100 mg/kg. S : sulfasalazine.

The effects of *M. communis* extract on GATA3 and T-bet expression following colitis in rats

The *GATA-3* gene expression significantly elevated in the colitis group compared to the control group (p<0.001). The injection of *M. communis* at 50 and 100 mg/kg diminished the expression of *GATA-3* in comparison to the colitis rats (p<0.05). The expression of *T-bet* was significantly reduced in the colitis group compared to the control group (p<0.001). In comparison to the colitis group, the treatment of *M. communis* at 50 and 100 mg/kg significantly elevated *T-bet* (p<0.001) (Figure 2).

The effects of *M. communis* extract on oxidative stress

The data revealed that MDA levels (p<0.001) and MPO levels (p<0.01) were significantly increased, but TAC levels (p<0.001) and catalase activity were reduced in the colitis group relative to the control group. Nonetheless, the administration of *M. communis* 50 and 100 mg/kg was able to normalize the values of the specified parameters (Figure 3).

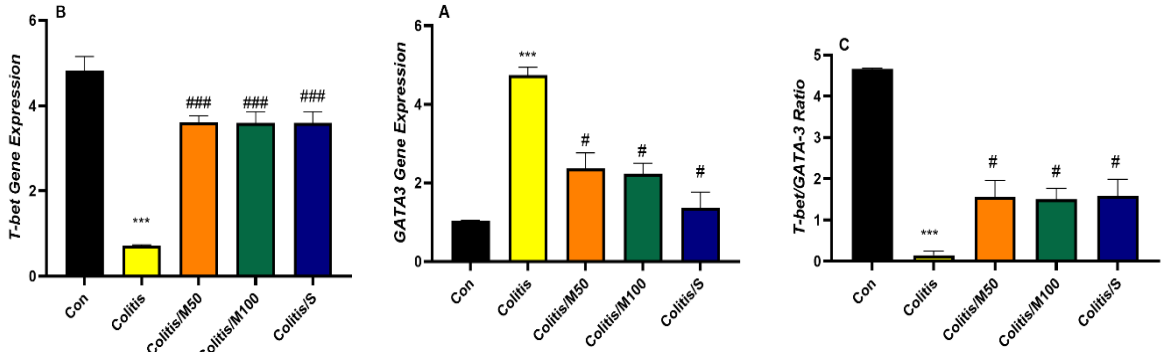

Figure 2. The impact of *Myrtus communis* extract at doses of 50 and 100 mg/kg on the expression of *GATA-3* and *T-bet* in the colonic tissue of rats suffering from colitis. Data are presented as mean±SEM.***p<0.001 vs control (Con). #p<0.05 and ###p<0.001 vs colitis. M50: *Myrtus communis* at dose of 50 mg/kg. M100: *Myrtus communis* at dose of 100 mg/kg. S: sulfasalazine.

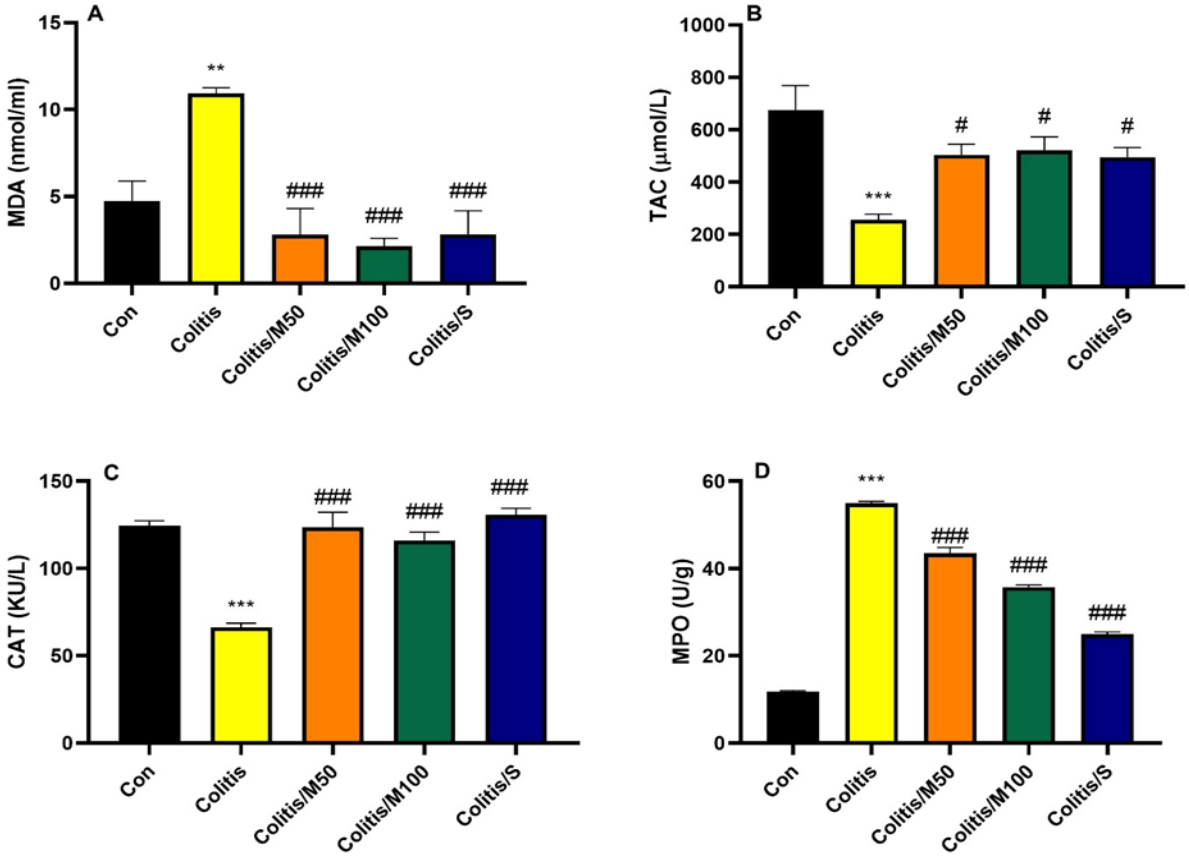

Figure 3. The impact of *Myrtus communis* extract at doses of 50 and 100 mg/kg on the levels of MDA, TAC, CAT, and MPO in the colon tissue of rats suffering from colitis. Data are presented as mean±SEM.***p<0.001 vs control (Con). #p<0.05 and ###p<0.001 vs colitis. M50: *Myrtus communis* at dose of 50 mg/kg. M100: *Myrtus communis* at dose of 100 mg/kg. S: sulfasalazine.

The effects of *M. communis* extract on histological changes following colitis in rats

Histopathological examination of colonic tissues revealed marked differences among the experimental groups (Figure 4). The control group exhibited normal colonic histoarchitecture with intact mucosal epithelium, regular crypt organization, and no evident inflammatory cell infiltration (Figure 4A). In contrast, the colitis group demonstrated severe histopathological alterations characterized by disruption of mucosal architecture, epithelial damage, inflammatory cell infiltration, and tissue disorganization, indicating substantial

colonic injury induced by acetic acid (Figure 4B). Treatment with *M. communis* extract markedly ameliorated these histological abnormalities. The 50 mg/kg treatment group showed partial restoration of mucosal structure with reduced inflammatory infiltration and improved tissue organization (Figure 4C), while the 100 mg/kg treatment group exhibited further histological improvement, with more preserved mucosal integrity and reduced pathological alterations compared with the untreated colitis group (Figure 4D). Similarly, the sulfasalazine-treated group demonstrated considerable recovery of colonic histological features and attenuation of inflammatory damage (Figure 4E). Consistent with microscopic observations, pathological change scores were significantly increased in the colitis group compared with the control group ($p<0.001$), whereas treatment with *M. communis* extract and sulfasalazine significantly reduced histopathological scores compared with the untreated colitis group ($p<0.01$).

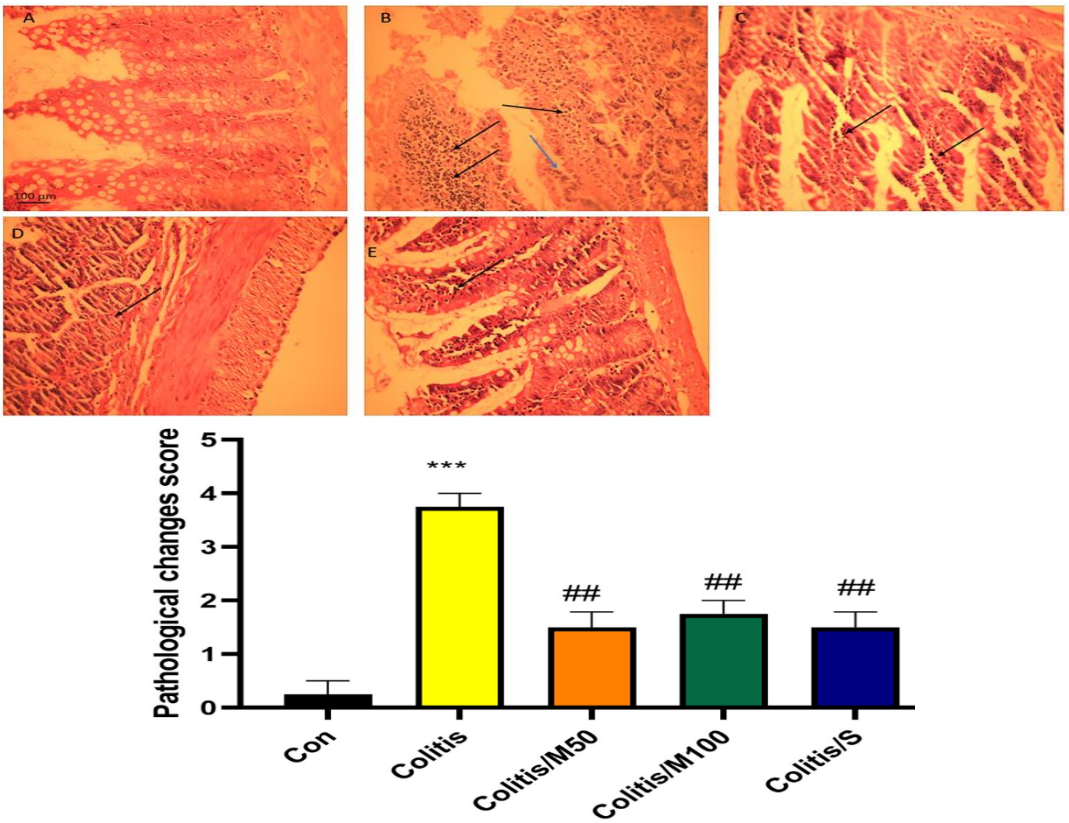

Figure 4. The impact of *Myrtus communis* extract administered at doses of 50 and 100 mg/kg on the histological alterations in the colon tissue of rats suffering from colitis. The micrographs showed the microscopic views of colon tissue in various groups. A(control), B (colitis), C (colitis+M50), D (colitis+M100) and E (colitis+sulfasalazine). Data are presented as mean±SEM.***$p<0.001$ vs control (Con). ##$p<0.01$ vs colitis. M50 : *Myrtus communis* at dose of 50 mg/kg. M100 : *Myrtus communis* at dose of 100 mg/kg. S : sulfasalazine. The magnification is 40x.Scale bar : 100µm Black arrows showed inflammation and blue arrow showed mucosal damage.

The effects of *M. communis* extract on DAI following colitis in rats

As illustrated in Figure 5, induction of colitis significantly elevated the Disease Activity Index (DAI) in the untreated colitis group compared with the control group ($p<0.001$), reflecting severe clinical manifestations of colitis. In contrast, treatment with *M. communis* extract significantly reduced DAI scores at both 50 and 100 mg/kg doses compared with the untreated colitis group, indicating improvement in colitis-associated symptoms. The reduction in DAI suggests

attenuation of disease severity, including clinical manifestations such as body weight loss, stool abnormalities, and rectal bleeding. The sulfasalazine-treated group also demonstrated a pronounced decrease in DAI scores, showing the strongest protective effect among treatment groups. These findings support the beneficial effects of *M. communis* on the clinical progression of experimental UC.

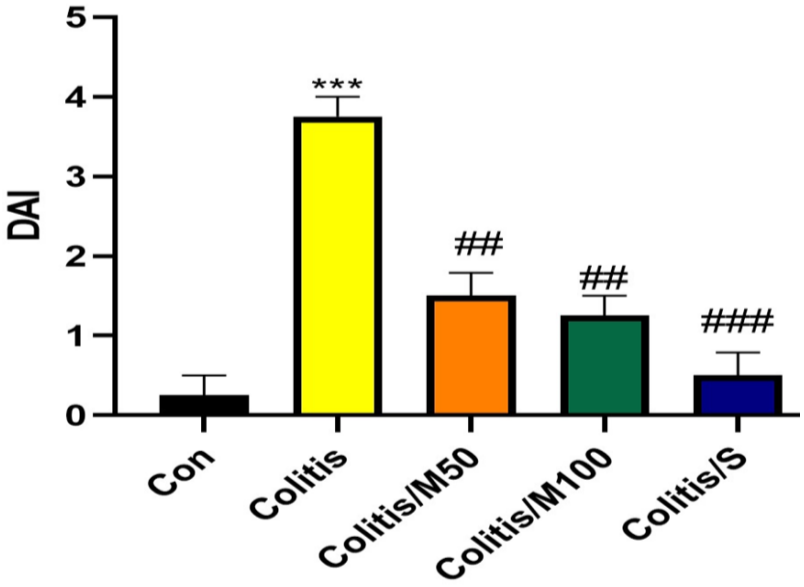

Figure 5. The effects of *Myrtus communis* extract in doses of 50 and 100 mg/kg on DAI in the colon tissue of rats with colitis. Data are presented as mean±SEM.***p<0.001 vs control (Con). ##p<0.01 vs colitis. M50 : *Myrtus communis* at dose of 50 mg/kg.M100 : *Myrtus communis* at dose of 100 mg/kg. S : sulfasalazine.

Discussion

This study aimed to examine the impact of *M. communis* extract on the inflammatory and oxidative effects of experimental colitis in rat colon tissue. Our data revealed elevated levels of inflammatory cytokines, including TNFα, IL-1β, and IL-6, alongside a decrease in the anti-inflammatory cytokine IL-10 after colitis. Additionally, *GATA-3* gene expression was elevated, while *T-bet* gene expression was decreased in colitis animals. Moreover, oxidative stress escalated after colitis. Administering *M. communis* at 50 and 100 mg/kg doses mitigated inflammation and oxidative stress in the colonic tissue of rats afflicted with colitis.

Chronic inflammation can result in the persistent activation of epithelial cells and the infiltration of immune cells and soluble mediators, which is essential in the pathogenesis of UC (Yao et al. 2019). Tumor necrosis factor alpha (TNFα) is an essential proinflammatory mediator implicated in inflammatory bowel disease (IBD) pathophysiology. In studies of people with UC, elevated levels of TNFα have been documented (Danese et al. 2013; Pugliese et al. 2017). Interleukin-1 beta (IL-1β) is a crucial cytokine that stimulates the inflammatory response in colitis and has been markedly increased in colitis models (Abdel-Razek et al. 2024; Zhang et al. 2023). Furthermore, IL-6, a proinflammatory cytokine, has been detected in colon tissue and serum samples from patients with UC (Chen et al. 2015; Li et al. 2010). In addition, it has been shown that the level of IL-10, as an anti-inflammatory factor, is reduced in colitis (Bejeshk et al. 2022; Wang et al. 2019). Our results are consistent with the mentioned reports. Our findings revealed that *M. communis* could reduce TNFα, IL-1β, and IL-6 and increase IL-10 in the colon tissue of rats following colitis.

In line with our results, research indicates that the essential oil of *M. communis* reduces inflammation in rats by lowering serum levels of IL-6 and TNF-α (Maxia et al. 2011). Tumen et al demonstrated that the *M. communis* berries extract has a wound-healing role through anti-inflammatory effects (Tumen et al. 2017). Two studies showed that the administration of *M. communis* orally for 3 and 4 days had ameliorating effects on colitis in rats (Khosropour et al. 2019; Sen et al. 2017). The main differences between the mentioned and current research include the route of administration, duration of administration, and measured factors. On the other hand, myrtenol is a main ingredient in *M. communis* extract, and many investigations showed the anti-inflammatory effects of myrtenol in various organs such as the lung (Rajizadeh et al. 2023b; Rajizadeh et al. 2023c) and brain (Bejeshk et al. 2023a; Esmaeilpour et al. 2023; Rajizadeh et al. 2023a) and kidney (Amirteimoury 2023).

T-bet and *GATA-3* are known as Th1 and Th2 lineage commitment transcription factors (Okamura et al. 2014a). Several investigations revealed that the *GATA-3* expression is high and the T-bet expression is low in experimental colitis (Ohtani et al. 2010; Seidelin et al. 2015b). The results indicate that the disparity between Th1 and Th2 cells in colonic tissues could play a role in UC (WU et al. 2017). Our results also showed a similar pattern.

Not many studies have been conducted on the effects of *M. communis* on *T-bet* and *GATA-3* so far, and this is one aspect of the novelty of this work. It seems that part of the anti-inflammatory effects of *M. communis* following colitis is due to the modulation of *T-bet* and *GATA-3* gene expression.

Oxidative stress is another significant factor that contributes to tissue damage in UC (Mitani et al. 2017). Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are byproducts of oxidative stress that adversely affect the organization of membranes as well as the protein structures and DNA bases (Kruidenier et al. 2003). MDA is a byproduct of polyunsaturated fatty acid oxidation and serves as an oxidative stress marker, alongside myeloperoxidase (MPO). Catalase, on the other hand, mitigates oxidative stress by scavenging oxygen radicals (Margaritelis et al. 2015). MPO is an indicator of neutrophil infiltration, reflecting the progression of acute inflammation (Mustafa et al. 2006). In UC, the accumulation of neutrophils in the inflamed intestinal mucosa is significant. Neutrophil granules contain various enzymes, including myeloperoxidase, which are crucial for bacterial defense. Upon activation, these granules release enzymes, some of which are proteolytic, along with cytotoxic oxygen metabolites. Consequently, activated neutrophils can cause tissue damage at inflammation sites (Bejeshk et al. 2022; Masoodi et al. 2011). Our findings also showed that oxidative stress and MPO levels in the colitis group were higher than those of the control group.

In UC and experimental colitis models, dysregulation of *T-bet* and *GATA-3* plays a central role in the imbalance of T helper cell responses and the associated cytokine profile. A reduction in T-bet expression has been linked to impaired Th1-mediated immune regulation, particularly decreased Interferon gamma (IFN-γ) signaling, while increased *GATA-3* gene expression promotes Th2 polarization with enhanced production of IL-4, IL-5, and IL-13. This shift in transcription factor expression contributes to a disturbed Th1/Th2 equilibrium within the inflamed colonic mucosa, which is reflected by elevated pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6 and reduced anti-inflammatory cytokines like IL-10. Such cytokine dysregulation further sustains mucosal inflammation and tissue injury in colitis. These findings are consistent with previous studies reporting altered *T-bet/GATA-3* gene expression and cytokine imbalance in experimental colitis and human UC

(Okamura et al. 2014b; Seidelin et al. 2015a).

The anti-oxidative effects of *M. communis* is well known (Alipour et al. 2014; Dabbaghi et al. 2023).Many studies showed that *M. communis* could reduce MDA in skin (Ozcan et al. 2019), lung (Samareh Fekri et al. 2018) , colon (Sen et al. 2017) and small intestine (Ozcan et al. 2020) injuries. Anti-oxidative and anti-inflammatory effects of some other herbal medicines, such as *Dracocephalum kotschyi* (Keshavarzi et al. 2022b), Walnut (*Juglans regia*) (Keshavarzi et al. 2022a; Keshavarzi et al. 2019), and *Mumijo* (*Asphaltum punjabinum*) (Shahrokhi et al. 2015) are also reported in animal models of UC. Also, it has been shown that *M. communis* could diminish MPO activity (Maxia et al. 2011; Sen et al. 2017). Our results also revealed the anti-oxidative activity of *M. communis*.

The therapeutic potential of *M. communis* has been attributed to its diverse bioactive constituents, including monoterpenes (such as myrtenol, α-pinene, and 1,8-cineole), flavonoids, tannins, and phenolic acids (Al-Snafi et al. 2024; Giampieri et al. 2020). Previous studies have suggested that these compounds exert anti-inflammatory and antioxidant effects through modulation of oxidative stress pathways and inflammatory signaling cascades, including suppression of NF-κB activation, reduction of pro-inflammatory cytokine production (e.g. TNF-α, IL-1β, and IL-6), and enhancement of endogenous antioxidant defense systems (Amiresmaili et al. 2025; Esmaeilpour et al. 2024; Rajizadeh et al. 2025; Rajizadeh et al. 2023a). In particular, myrtenol has been reported to exhibit anti-inflammatory activity by attenuating oxidative stress and inflammatory mediator production, suggesting a potential mechanistic basis for the protective effects of *M. communis*in UC (Li et al. 2025).

According to our observations, *M. communis* extract administration with both 50 and 100 mg/kg doses could reduce inflammation in the colon tissue of rats with colitis via decreasing the levels of TNFα, IL1β, IL-6, and expression of *GATA-3* gene and also through increasing the level of IL-10 and expression of *T-bet* gene. On the other hand, *M. communis* suppresses oxidative stress in colon tissue by reducing MDA and MPO and increasing TAC and Catalase. So, *M. communis* administration seems to be a promising treatment approach in curating UC (Figure 6).

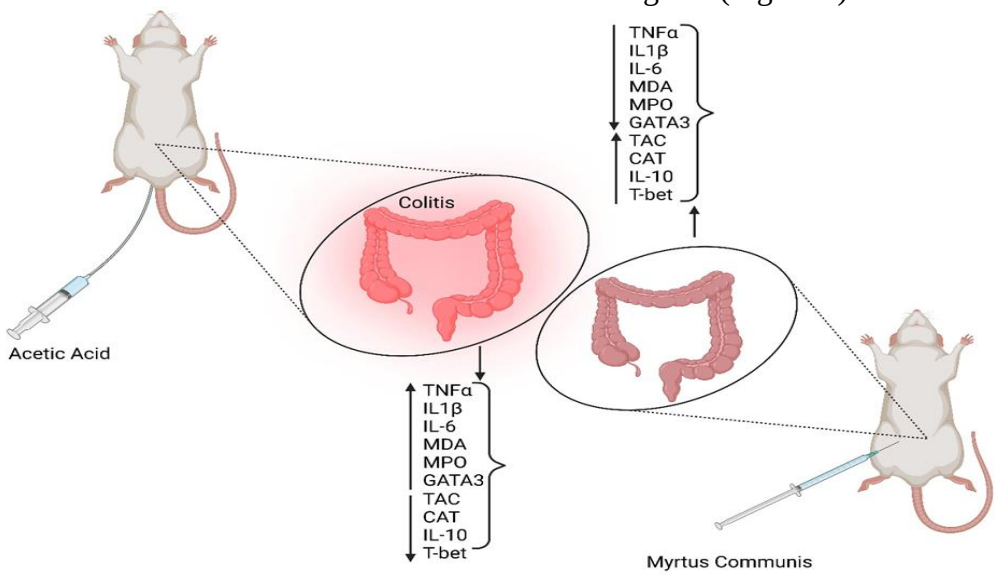

Figure 6.The impact of *Myrtus communis* on UC

Despite the significant findings of this study, several limitations should be acknowledged. First, the present work primarily focused on gene expression changes and biochemical markers without providing protein-level confirmation of key transcription factors such as *T-bet* and *GATA-3*. Therefore, the observed alterations in mRNA expression may not fully reflect functional protein activity. Second, the study lacked mechanistic investigations such as pathway-specific inhibition or knockdown experiments, to directly establish causal relationships between *M. communis* treatment and modulation of inflammatory and oxidative signaling pathways. Additionally, downstream signaling pathways involved in Th1/Th2 differentiation and oxidative stress regulation were not explored in detail. Finally, histological assessment, although supportive, was descriptive and not complemented by quantitative immunohistochemical analysis. Future studies incorporating protein expression analysis, mechanistic pathway validation, and functional immune assays are warranted to better elucidate the precise molecular mechanisms underlying the protective effects of *M. communis* in colitis.

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Conflicts of interest

The authors state that they have no conflicts of interest

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Ethical Considerations

This investigation was verified with the Ethical Committee at Bojnurd University of Medical Sciences, Bojnurd, Iran.

Code of Ethics

IR.NKUMS.AEC.1402.001

Authors' Contributions

Zakieh Keshavarzi: Project administration, Investigation, Formal analysis, Data curation. Atena Alifarsangi: Writing – review & editing, Fatemeh Bagheri: Resources, Methodology, Investigation, Formal analysis, Data curation. Sonia Fathi-karkan: Validation, Formal analysis, Data curation. Zahra Soltani: Methodology.: Conceptualization. Vahid Hajali: Data curation, Investigation, Methodology. Sedigheh Amiresmaili[8:] Project administration, Validation, Visualization, Writing – original draft, Writing – review & editing. Mohammad Amin Rajizadeh: Supervision, Project administration.

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