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# Hydro-alcoholic extract of *Dracocephalum moldavica* for treatment of ulcerative colitis in Wistar rats

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**Abstract**

**Background and objective:** The prevalence of ulcerative colitis, a condition characterized by inflammation in the intestinal wall, has been increasing. *Dracocephalum moldavica* has anti-inflammatory, antioxidant, and immunomodulatory properties. The current study aimed to evaluate the therapeutic effects of *Dracocephalum moldavica* on ulcerative colitis in Wistar rats.

**Materials and methods:** Thirty-two male Wistar rats were divided into four groups for this study. Group 1(control) received only phosphate buffer saline, group 2 was exposed to acetic acid to develop ulcerative colitis, group 3 was the rats with ulcerative colitis treated with *Dracocephalum moldavica* extract at concentration of 30 mg/kg/day, and group 4 was the rats with ulcerative colitis treated by prednisolone at concentration of 4 mg/kg/day. After ten consecutive days, the rats were euthanized humanely, and their intestinal tissue was thoroughly examined to measure the levels of inflammatory mediators and oxidative stress indices.

**Results and conclusion:** Treatment with *Dracocephalum moldavica* showed more advantage than treatment with prednisolone in restoring the overall antioxidant capacity of the colonic specimens in rats with induced colitis. Although malondialdehyde levels decreased, the total protein content of colonic homogenates increased in both treatment groups, albeit not significantly. Moreover, *Dracocephalum moldavica* extract showed a significant reduction in TNF-α, IL-6, and IFNɣ cytokines compared to prednisolone. The stories of myeloperoxidase and nitric oxide were significantly decreased in the colons of rats treated with *Dracocephalum Moldavia***,** surpassing the effects observed in the prednisolone group. The findings of this study suggest that the use of *Dracocephalum moldavica* extract as an herbal remedy has excellent potential in alleviating inflammation in rat models of ulcerative colitis. The positive roles of *Dracocephalum moldavica* extract are attributed to its direct antioxidant, anti-inflammatory, and immunomodulatory effects.

*Keywords*: *Dracocephalum Moldavica*, Hydro-alcoholic extract, Immunomodulator, Inflammation, Ulcerative colitis

**Abbreviations:** IFNɣ (interferon-gamma); TNFα (tumor necrosis factor alpha); IL- (interleukin-)

## 1. Introduction

Immunostimulants are substances that stimulate the immune system, while immunosuppressor compounds

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weaken a particular component, leading to a reduction in immunological responses. Medicinal plants can impact the immune system. The body's defense against pathogens is crucial, and the B lymphocyte plays a significant role in humoral immunity. This cell helps eliminate infectious agents by producing antibodies, neutralizing toxins, facilitating phagocytosis, activating complement, and creating positive feedback. In addition to humoral immunity, cellular immunity is vital in defending the body against pathogens. It directly targets microbes, parasites, and cells infected with viruses and other pathogens. Before introducing the mentioned components, innate immunity served as the foundation for activating both arms of the immune system. It primarily combats pathogens and infectious agents. Based on their composition, medicinal plants can influence any of these areas and exhibit immunomodulatory effects, categorizing them as either immunostimulants or immunosuppressors [1].

Medicinal plants and their derived compounds are widely recognized as effective treatment options with minimal side effects worldwide. These plants help treat and manage various diseases and have fewer adverse effects than chemical drugs [2]. According to statistical data, approximately 25% of medications available today are derived from chemical substances found in plants. [3]. *Dracocephalum moldavica* is an annual herb that has about 60 species. Distributed mainly in temperate regions of Asia [4]. *D. moldavica*, a perennial herb from the Lamiaceae (Labiatae) family, has various traditional uses in Iran. Badershoo is known for its culinary properties and effectiveness in treating stomach and liver disorders, headaches, and congestion. Additionally, *D. moldavica* is a cardiotonic agent in Iranian folk medicine. In vitro studies have demonstrated the anti-*Helicobacter pylori* activity of *D. moldavica*'s total extract. Furthermore, this plant possesses sedative, carminative, skin anti-inflammatory (when used topically), spasmolytic, anti-microbial, and fungicidal effects, as reported in pharmacological studies [5].

Recent pharmacological research has demonstrated that the active constituents found in *D. moldavica* safeguard ischemic myocardium and mitigate the harm caused by oxygen-free radicals on platelets in individuals suffering from coronary heart disease [4]. In the past few years, the analysis of drug composition has uncovered that *D. moldavica* primarily consists of volatile oil, trace elements, terpenoids, flavonoids, and polysaccharides. Flavonoids are considered to be among the vital active constituents [6]. The subjects who consumed a high amount of flavonoids showed a decreased likelihood of developing coronary heart disease, as indicated by epidemiological research [7]. The effects of flavonoids are characterized by their protective properties, such as antioxidative, anti-inflammatory, and antithrombotic activities. Additionally, these compounds play a role in reducing blood fat levels and inhibiting platelet aggregation. Nevertheless, the specific pharmacological action mechanism through which total flavonoids from *D. moldavica* affect atherosclerosis remains unknown[4].

This herbaceous plant has been reported to contain active components with anti-inflammatory and antioxidant effects, such as hydroxycinnamic acids, flavonoids, and rosmarinic acid [4]. Rosmarinic acid has also been reported to significantly inhibit lung cell apoptosis and decrease the level of p53 in lipopolysaccharide-induced septic mice by inhibiting the activation of the GRP78/IRE1alpha/JNK pathway [8]. Additionally, rosmarinic acid has been shown to downregulate TNF-a, IL-6, and HMGB-1 levels in lipopolysaccharide-induced RAW 264.7 cells by inhibiting the IkB kinase pathway [9]. For these reasons, this plant seems suitable for treating inflammatory diseases such as autoimmune diseases.

Ulcerative colitis is a recurrent and unexplained syndrome that falls under the category of inflammatory bowel disorders (IBD). The precise cause of ulcerative colitis remains uncertain to this day. Ulcerative colitis is characterized by an increased production of harmful free radicals, a reduced ability to counteract their effects through antioxidants, and the presence of pro-inflammatory cytokines. The excessive presence of reactive oxygen species and subsequent lipid peroxidation decrease cellular

antioxidant capacity, resulting in inflammation in the colon. The development of ulcerative colitis is heavily influenced by severe inflammation and oxidative stress. Common symptoms of ulcerative colitis include diarrhea, bloody stools, abdominal pain, and ulceration in the mucous lining of the large intestine [10]. Due to the rise in the occurrence and prevalence of ulcerative colitis globally, it is widely recognized as a global ailment [11]. Commonly, its initial therapy involves the use of NSAIDs, corticosteroids, and immunosuppressants. However, these medications come with undesirable side effects such as allergies, nausea, and lymphoma [12]. With these explanations, this study investigates the effect of *D. moldavica* on the process and treatment of experimental ulcerative colitis in rats.

## 2. Material and method

### 2.1. Reagents

Fetal calf serum and Dulbecco's modified medium (DMEM) were obtained from GIBCO/Life Technologies Inc. (Gaithersburg, MD). Zist Chemi Co. (Tehran, Iran) provided a total protein assay kit. RNX-Plus solution for RNA isolation was procured from DENAzistAsia(Mashhad, Iran). SYBR Premix Ex TaqII and cDNA reverse transcription kits were purchased from TAKARA (Takara Biomedical Ltd., China). Myeloperoxidase and nitric oxide assay kits were prepared by Navand-Salamat Corporation (NavandSalamat, Urmia, Iran). Other reagents were purchased from Sigma-Aldrich (St. Louis, MO).

### 2.2. Preparation of extract from *D. moldavica*

The fresh plant was washed after collection. The plants were dried in the shade. Then, they were ground in a mortar and kept in 50% ethanol for 48 h. The resulting mixture was passed through a cloth filter and then filter paper. The resulting material was placed in a rotary for 24 h, and the thick liquid obtained was transferred to a plate to remove water and kept inside the oven until drying. Finally, 1 g of the extract was dissolved in 10 ml of water.

### 2.3. Animals

Male Wistar albino rats (150-200 g) were obtained from the Urmia University animal house care center and housed there. The animals were kept with a 12-h light/dark cycle and had a standard pellet sand and water diet. Before the trial's start, the animals' adaptation period took (room temperature 25 ±5 °C) for ten days. All the procedures were performed according to the standard animal experimentation protocol of the ethics committee of the Urmia University for Animal Studies (AECVU-182-2018).

### 2.4. Acid citric induced ulcerative colitis model in Wistar rat

Male Wistar albino rats (150-200 g) were obtained from the Urmia University animal house care center and housed there. The animals were kept with a 12-h light/dark cycle and had a standard pellet and water ad libitum diet. Before the trial's start, the animals' adaptation period took (room temperature 25 ±5°C) for ten days. All the procedures were performed according to the standard animal experimentation protocol of the ethics committee of the Urmia University for Animal Studies (AECVU-182-2018).

### 2.5. Experimental protocols

Induction of colitis in rats was conducted as designated previously [13]. Briefly, after fasting rats for 24 h, the animals were anesthetized using ether. The catheter was placed in the colon, and a 2 ml solution of 4% acetic acid was installed through the rectum. Rats were assigned to the following groups (n=8): Group 1 was an intact group and received only isotonic saline as the control group (Control), Group 2 receives citric acid to induce ulcerative colitis, Group 3 received acid citric to cause ulcerative colitis and prednisolone 4 mg/kg/day of body weight (UC+Pred), and Group 4 obtained acid citric-induced ulcerative colitis and *D. moldavica* 30 mg/kg/day of body weight (UC+DM). Treatments were started on induction day and continued until ten days; then, rats were sacrificed, and samples for tests were collected. Throughout the experiment, body weight, stool

consistency, and gross bleeding were monitored daily. The disease cumulative score was calculated based on the sum of all factors in Table 1.

Table 1- Scoring system for assessing the severity of ulcerative colitis

| Score | Weight loss | Stool consistency | Blood feces |
|---|---|---|---|
| 0 | Negative | Normal | Negative |
| 1 | 1-9% | Soft | Red |
| 2 | 10-19% | Very soft | Dark red |
| 3 | >20% | Diarrhea | Black |

*The disease cumulative score was calculated as the sum of all scores.

### 2.6. Myeloperoxidase and nitric oxide enzyme activity determination

Myeloperoxidase level in the colonic homogenates was monitored like a protocol defined previously [14]. In brief, 10 µl of the homogenized colon was combined with 110 µl of a tetramethyl benzidine solution (2.9 mM tetramethyl benzidine in 14.5% dimethyl sulfoxide and 150 phosphate buffer saline at pH 5.4), along with 80 µl of 0.75 mM hydrogen peroxide. The optical density at 450 nm was immediately measured. Following 15 min incubation at 37 °C, 50 µl of 2 M sulfuric acid was added to stop the reaction. The absorbance at 450 nm was then determined using a spectrophotometer. Internal standards of horseradish peroxidase (10 µl at concentrations of 2.5 and 25 milliunits/ml) were utilized. The activity of myeloperoxidase was calculated by comparing the absorbance to the horseradish peroxidase standard curve. The results were expressed as milliunits per milliliter (mg/ml). The level of nitric oxide in the colonic homogenates was measured with the colorimetric method using the commercial kit (NavandSalamat, Urmia, Iran).) by manufacturer instruction.

### 2.7. Malondialdehyde

Determination of malondialdehyde level in the colonic homogenates followed a similar protocol as described by AlRejaie et al. [15]. In brief, 2.5 ml of reaction buffer consisting of 0.37% thiobarbituric acid, 0.25 M HCl, and 15% trichloroacetic acid in a 1:1:1 ratio was mixed with 100 µl of colon homogenate. The mixture was then heated at 96 ºC for 60 min. After cooling, it was centrifuged at 3500 ×g for 10 min. The absorbance of the supernatant at 540 nm was measured to determine malondialdehyde levels. The results were reported as nM of malondialdehyde per mg of protein.

### 2.8. Total protein levels assessment in colonic tissues

The determination of total protein levels in colonic homogenates was conducted using the pyrogallol red-molybdate method, following the guidelines provided by the manufacturer [16].

### 2.9. Real-time PCR

To monitor TNF-α, IFN-γ, IL-6, and IL-4 levels, the Intestine tissue was subjected to total RNA extraction using the RNX-Plus solution, following the instructtions provided by the manufacturer. The extracted RNA was then utilized to synthesize complementary DNA. PCR amplification was performed in triplicate using a SYBR Green kit, following the manufacturer's guidelines [17,18]. The HPRT gene, a housekeeping gene, was employed as a reference. The forward and reverse primers for mRNA amplification can be found in Table 2. The results were presented as 2-ΔΔCt (mean fold change).

Table 2- Forward and reverse primers used in Real-Time PCR

| Cytokines | Forward primes | Reverse primer | References |
|---|---|---|---|
| TNFα | 5′-CATCTTCTCAAAATTCGAGTGACAA-3′ | 5′-TGGGAGTAGACAAGGTACAACCC-3′ | [19] |
| IFN-γ | 5′-TCAAGTGGCATAGATGTGGAAGAA-3′ | 5′-TGGCTCTGCAGGATTTTCATG-3′ | [19] |
| IL-6 | 5′- GACAACTTTGGCATTGTGG -3′ | 5′- ATGCAGGGATGATGTTCTG -3′ | [20] |
| IL-4 | 5′-CACTTAGCTGTGACACACTT | 5′-CAAGAAGTTTTCCAACGTACTCTG | [21] |

| | | | |
|---|---|---|---|
| | CTCGAGAGAC-3 | GTTGGC-3′ | |
| GAPDH | 5′- CAAAGCCAGAGTCCTTCAGA-3 | 5′- GATGGTCTTGGTCCTTAGCC -3 | [21] |

### 2.10. Statistical analysis

The Shapiro-Wilk test was employed to establish the normal distribution of the data. The Kruskal-Wallis test was utilized to analyze the disease activity index. The findings were then subjected to a one-way analysis of variance (ANOVA) and Tukey's post hoc test for further research. The results were expressed as mean ±SD, and the significance level was $p<0.05$.

## 3. Results and discussion

One of the existing tools used to measure multimorbidity is the cumulative disease score, which considers all medical problems encountered in primary care [21].

In this study, disease cumulative score (CDS) of each rat was monitored every day after the instillation of acetic acid into the colons of rats. As expected, colonic instillation of acetic acid led to a high CDS score and high mortality rate in the ulcerative colitis control group (Figure 1). Obtained data showed that both therapies could reduce the CDS index (Figure 1). However, it decreased in the group receiving prednisolone as an immuno-suppressor drug decreased more than the group receiving *D. moldavica* extract. Although it was not statistically significant ($p < 0.05$). The amount of CDS in the intact group remained close to zero until the end of day 10. It also showed the amount of CDS on the last day of treatment (day 10). As can be seen, the cumulative disease index on the previous day in the *D. moldavica* and prednisolone groups decreased significantly compared to the ulcerative colitis control group. Still, the difference between *D. moldavica* and prednisolone groups was not statistically significant.

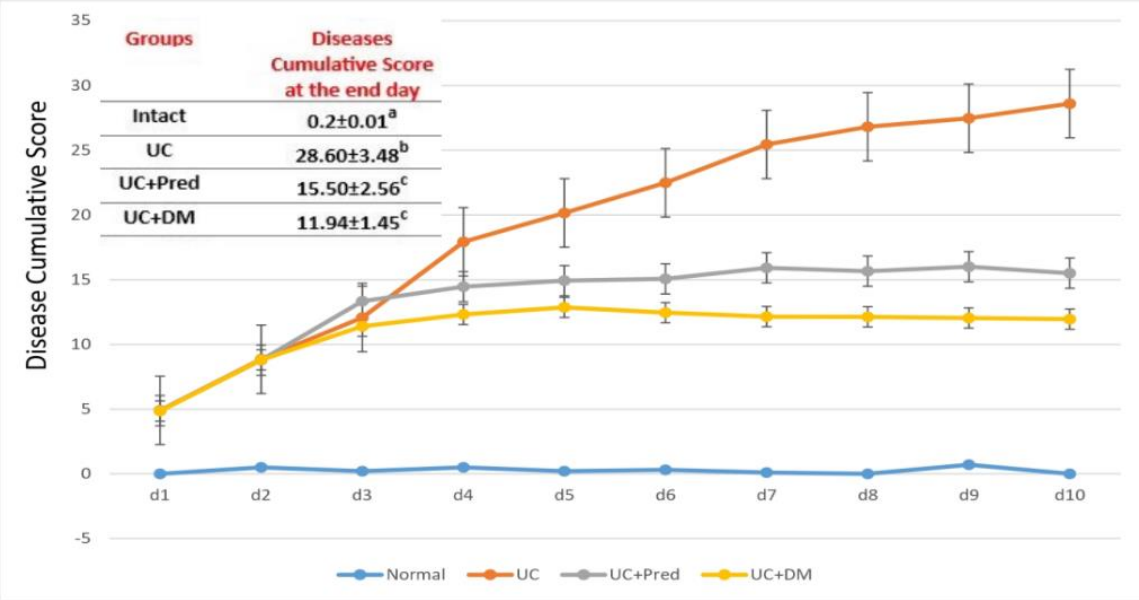


Figure 1- Evaluation of cumulative disease score; UC: ulcerative colitis; Pred: prednisolone; DM: *Dracocephalum moldavica*. Different letters show significant difference ($p < 0.05$).

Activity of myeloperoxidase and nitric oxide enzymes is shown in Figure 2 as an indicator of the level of inflammation in the body. Figure 2a shows myeloperoxidase activity. As can be seen in the figure, the activity of this enzyme decreased in the groups treated with prednisolone, and the *D. moldavica* group, and both treated groups were significantly different from each other, as well as from the control group and the group with ulcerative colitis. The group treated with *D. moldavica* responded better

to the treatment than the prednisolone group. Figure 2b which shows the level of nitric oxide enzyme activity, the situation is slightly different. There was no significant difference in activity of this enzyme between the prednisolone and *D. moldavica* groups. However, there was a considerable difference between the control and intact groups. The decrease in the activity of these enzymes indicates a reduction in the level of inflammation and a decrease in the severity of the autoimmune disease in the animal.

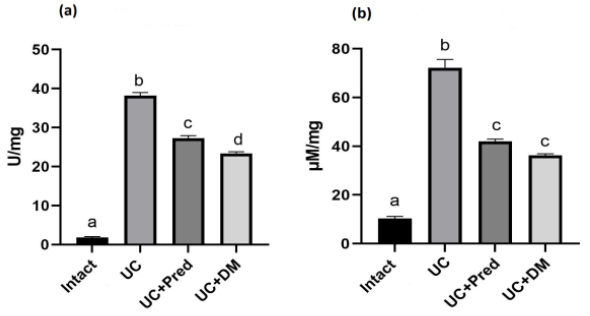


Figure 2- Myeloperoxidase activity (a), and nitric oxide activity (b), in Wistar rats induced with ulcerative colitis. Different letters show significant difference ($p < 0.05$).

The induction of colitis led to a significant decrease in the antioxidant properties of the colonic homogenate specimens in colitis rats compared to normal animals (Table 3). The data obtained showed that treatment with *D. moldavica* resulted in a more pronounced improvement in restoring the overall antioxidant capacity of the colonic homogenate specimens in colitis rats compared to treatment with prednisolone. As a result, *D. moldavica* brought the antioxidant capacity back to the level observed in normal rats in colonic samples (Table 3). Additionally, the total protein content of the colonic homogenate in colitis rats was significantly reduced compared to normal animals (Table 3). The level of complete protein in colonic specimens increased substantially in both treatment groups, with no significant difference, compared to control-positive rats (Table 3).

Table 3- Results of biochemical changes in the colonic tissues

| Groups | Malondialdehyde (μM/mg) | Total protein (mg/g tissue) |
|---|---|---|
| Intact | $1.63 \pm 0.19^{a}$ | $257.3 \pm 28.1^{a}$ |
| UC | $40.22 \pm 6.01^{b}$ | $66.3 \pm 10.8^{b}$ |
| UC+Pred | $16.22 \pm 1.92^{c}$ | $134.6 \pm 13.04^{b}$ |
| UC+DM | $9.64 \pm 1.50^{c}$ | $129.19 \pm 11.9^{a}$ |

*Different letters in the columns show significant difference ($p < 0.05$).

Figure 3 displays the results of the Real-time PCR test. The image reveals a decrease in TNFα gene expression in the groups treated with *D. moldavica* and prednisolone, with the most significant reduction observed in the *D. moldavica* group. TNFα is a crucial inflammatory cytokine released during the initial phase of inflammatory diseases. Furthermore, the gene expression of the inflammatory cytokines IFNɣ and IL-6 decreased more in the *D. moldavica* treated groups compared to the prednisolone-treated groups. This decrease in the expression of inflammatory genes indicates a favorable response to treatment, considering prednisolone's role as a vital immunosuppressive drug prescribed for autoimmune diseases. In contrast, the face of the IL-4 gene, an anti-inflammatory cytokine, decreased in the untreated group with the disease (ulcerative colitis control). However, it increased in the groups treated with prednisolone and *D. moldavica*, with the highest increase observed in the *D. moldavica* group.

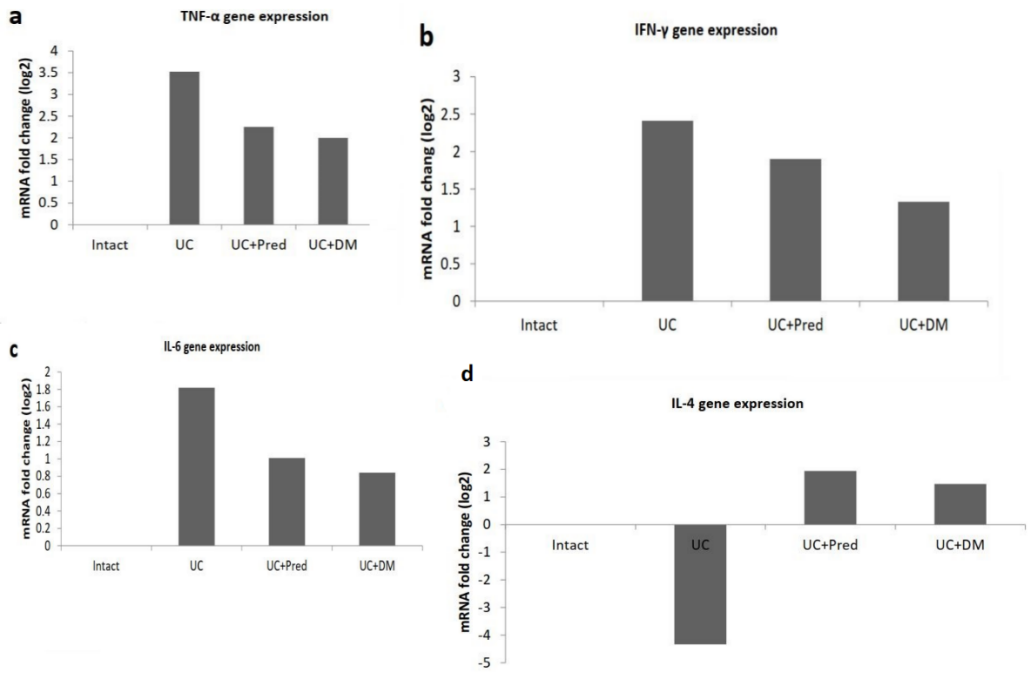


Figure 3- Real-time PCR analysis of cytokines in Wistar rats induced with ulcerative colitis. TNFα (a), IFNɣ (b), and IL-6 (c) are pro-inflammatory cytokines, and IL-4 (d) is anti-inflammatory cytokine.

Multiple research studies have demonstrated that an equilibrium within the intestinal microbiota plays a role in developing intestinal inflammation linked to ulcerative colitis. Consequently, maintaining the balance of gut microbiota is of utmost importance in treating ulcerative colitis [22]. The acetic acid-induced destruction of the intestinal mucosa results in acute local inflammation in both the rectum and the colon, causing a pathological pattern resembling ulcerative colitis [23]. The pathology is driven primarily by a significant infiltration of neutrophils and oxidative and nitrative damages. [24]. Despite the ability of corticosteroids such as prednisolone to effectively manage inflammation, they do not have any impact on tissue oxidative or nitrative injuries. Moreover, the immunosuppressive characteristics of corticosteroids, along with numerous other side effects, have prompted the researchers to seek an alternative therapy [12]. *D. moldavica* L. extract, a typical traditional Chinese medicine, has been demonstrated to possess numerous pharmacological effects, such as antioxidative, anti-inflammatory, and antibacterial properties. The efficacy of *D. moldavica* extract in ameliorating ulcerative colitis has been established through various measures such as improvement in disease activity index, weight loss, colon length, and histological scoring. Additionally, the administration of *D. moldavica* extract has been found to enhance the count of Lactobacillus, a beneficial probiotic. Moreover, network pharmacology analysis has revealed that the active ingredients present in the *D. moldavica* extract, including luteolin, rosmarinic acid, oleanolic acid, ursolic acid, apigenin, acacetin, kaempferol, and isorhamnetin, are closely associated with anti-inflammatory activity. These active ingredients exert their effects through multiple signaling pathways, including the NF κB, IL 17, TNF–α, and Toll-like receptor (TLR) signaling pathways. [22].

The colonic mucosa of patients with active ulcerative colitis exhibits elevated levels of proinflammatory cytokines such as IL-1 beta, IL-6, IL-8, and TNF–α when cultured in an organ setting [25]. In 2019, Dianbo Yao and colleagues showcased the impact of disrupting the intestinal mucosal barrier and bacterial invasion on the development of intestinal inflam-

mation. This disruption led to the stimulation of TLR4/NF-κB in intestinal epithelial cells, resulting in inflammatory cell infiltration and the release of inflammatory cytokines. These processes provided survival advantages to susceptible cells and promoted abnormal proliferation. Additionally, the study highlighted the specific roles of TLR4/NF-κB, TNF–α, and IL-6 in both intestinal epithelial cells and inflammatory cells [26]. In the present investigation, the ulcerative colitis group exhibited the highest levels of inflammatory cytokines, whereas these levels decreased in the *D. moldavica* treated group. The comparison between the decreasing values of the *D. moldavica* group and the prednisolone treated group, along with the increase in the secretion of the anti-inflammatory cytokine IL-4, signifies the restoration of the body's immunomodulatory state and its approach toward normalcy. Undoubtedly, this indicates the success of the treatment. Furthermore, when considering the reduction in disease cumulative score, which serves as a cumulative index of the disease, it becomes evident that *D. moldavica* treatment has been effective.

On the other hand, the activity of the myeloperoxidase enzyme serves as a widely recognized indicator for assessing the functionality of neutrophils and leukocytes, as well as determining the extent of inflammation within the body [27]. It is believed that during sterile inflammation, the presence of myeloperoxidase and the resulting oxidants contribute to the escalation of inflammation and tissue harm. The levels of myeloperoxidase have been found to rise in various autoimmune diseases and during the inflammatory phase of these conditions. Myeloperoxidase is reputed to enhance vascular permeability and trigger inflammatory immune reactions. [28]. It seems that nitric oxide activity is also used to measure the level of inflammation [29]. Moreover, the lipid peroxidation process in an organism is initiated by free radicals. Polyunsaturated fatty acid peroxidation within cells produces malondialdehyde as one of its end products. The excessive presence of free radicals results in an elevated production of malondialdehyde. Consequently, the level of Malondialdehyde serves as an indicator of oxidative stress and the antioxidant status in individuals diagnosed with cancer. [30].

In our investigation, we observed a decline in the activity of myeloperoxidase and nitric oxide enzymes in the *D. moldavica* treated group. Additionally, a reduction in malondialdehyde and total protein levels indicated a decrease in inflammation in the same group. An analysis of the collected data reveals that the *D. moldavica* treated group exhibited positive responses to the treatment, or at the very least, disease control. These findings will be further supported by comparing the results to those of the prednisolone group. Considering the numerous challenges that CU poses for patients and the drawbacks of immunosuppressive medications, we propose using *D. moldavica* plants to treat this condition. However, it is strongly advised that further research be conducted on immunological findings, cell counting, and marker assays of these cells.

## 4. Conclusion

To summarize, the findings indicate that utilizing natural antioxidants and immunomodulatory plants can be a hopeful approach to reducing inflammation in a rat model of ulcerative colitis. Acknowledging the positive impacts of *D. moldavica* hydro-alcoholic extract, such as its direct antioxidant and immunomodulatory properties and direct anti-inflammatory effects, is reasonable. Nevertheless, there may be additional mechanisms at play that require further investigation.

## 5. Conflict of Interest

There are no competing interests to be declared.

## References

1. Tomar N, De RK. A brief outline of the immune system. Methods in Molecular Biology. 2014; 1184: 3-12. https://doi.org/10.1007/978-1-4939-1115-8_1

2. Shaddel M, Shamsi H, Tavakoli P, Abtahi Foroushani SM, Shamsi R. Comparing the effects of aloe vera leaf extract on K562 tumor cell and peripheral blood

mononuclear cells. Internal Medicine Today. 2019; 25(3): 216-229.
http://dx.doi.org/10.32598/hms.25.3.216

3. Mousavi SM, Hashemi SA, Behbudi G, Mazraedoost S, Omidifar N, Gholami A, et al. A review on health benefits of *Malva sylvestris* L. nutritional compounds for metabolites, antioxidants, and anti-inflammatory, anticancer, and antimicrobial applications. Evidence-Based Complementary and Alternative Medicine. 2021: 5548404.
https://doi.org/10.1155/2021/5548404

4. Xing J, Peng K, Cao W, Lian X, Wang Q, Wang X. Effects of total flavonoids from *Dracocephalum moldavica* on the proliferation, migration, and adhesion molecule expression of rat vascular smooth muscle cells induced by TNF-α. Informa Healthcare. 2013; 51(1): 74–83.
https://doi.org/10.3109/13880209.2012.711839

5. Najafi, M., et al., Effects of total extract of *Dracocephalum moldavica* on ischemia/reperfusion induced arrhythmias and infarct size in the isolated rat heart. Iranian Journal of Basic Medical Sciences. 2009; 11(4): 229-235.
https://doi.org/10.22038/ijbms.2009.5186

6. Song R, Roberts BL, Lee EO, Lam P, Bae SC. A randomized study of the effects of t'ai chi on muscle strength, bone mineral density, and fear of falling in women with osteoarthritis. The Journal of Alternative and Complementary Medicine. 2010; 16(3) :227-33.
https://doi.org/10.1089/acm.2009.0165

7. Grassi D, Desideri G, Croce G, Tiberti S, Aggio A, Ferri C. Flavonoids, vascular function and cardiovascular protection. Current Pharmaceutical Design. 2009; 15(10): 1072-1084.
https://doi.org/10.2174/138161209787846982

8. Rajesh M, Mukhopadhyay P, Haskó G, Huffman JW, Mackie K, Pacher P. CB2 cannabinoid receptor agonists attenuate TNF-α-induced human vascular smooth muscle cell proliferation and migration. British Journal of Pharmacology. 2008; 153(2): 347-357.
https://doi.org/10.1038%2Fsj.bjp.0707569

9. Osterud B, Bjorklid E. Role of monocytes in atherogenesis. Physiological Reviews. 2003; 83(4): 1069-1112.
https://doi.org/10.1152/physrev.00005.2003

10. Bernstein CN, Fried M, Krabshuis JH, Cohen H, Eliakim R, Fedail S, et al. World gastroenterology organization practice guidelines for the diagnosis and management of IBD in 2010. Inflammatory Bowel Disease. 2010; 16(1): 112-124.
https://doi.org/10.1002/ibd.21048

11. Ng SC, Shi HY, Hamidi N, Underwood FE, Tang W, Benchimol EI, et al. Worldwide incidence and prevalence of inflammatory bowel disease in the 21st century: a systematic review of population-based studies. Lancet. 2017; 390(10114): 2769-2778.
https://doi.org/10.1016/s0140-6736(17)32448-0

12. Salamatian M, Mohammadi V, Froushani SMA. Ameliorative effects of aqueous cinnamon extract on ulcerative colitis in rats. Physiology and Pharmacology. 2019; 23(2): 140-149.

13. Low D, Nguyen DD, Mizoguchi E. Animal models of ulcerative colitis and their application in drug research. Drug Design, Development and Therapy. 2013; 7: 1341-1357.
https://doi.org/10.2147%2FDDDT.S40107

14. Pulli B, Ali M, Forghani R, Schob S, Hsieh KLC, Wojtkiewicz G, et al. Measuring myeloperoxidase activity in biological samples. PLoS One. 2013; 8(7): e67976.
https://doi.org/10.1371/journal.pone.0067976

15. Al-Rejaie SS, Abuohashish HM, Al-Enazi MM, Al-Assaf AH, Parmar MY, Ahmed MM. Protective effect of naringenin on acetic acid-induced ulcerative colitis in rats. World Journal of Gastroenterology. 2013; 19(34): 5633–5644.
https://doi.org/10.3748%2Fwjg.v19.i34.5633

16. Orsonneau JL, Douet P, Massoubre C, Lustenberger P, Bernard S. An improved pyrogallol red-molybdate method for determining total urinary protein. Clinical Chemistry. 1989; 35(11): 2233-2236.
https://doi.org/10.1093/clinchem/35.11.2233

17. Mahmouzadeh L, Abtahi Froushani SM, Hobbenaghi R, Mahmoudian A, Mohammadi V. Altered immune responses in mice after receiving nicotine-pulsed mesenchymal stem cell-conditioned medium. Archives of Advances in Biosciences. 2021; 12: 1-11.
https://doi.org/10.22037/aab.v12i4.35358

18. Giulietti A, Overbergh L, Valckx D, Decallonne B, Bouillon R, Mathieu C. An overview of real-time quantitative PCR: applications to quantify cytokine gene expression. Methods. 2001; 25(4): 386-401.
https://doi.org/10.1006/meth.2001.1261

19. Patil C, Zhu X, Rossa CJ, Kim YJ, Kirkwood KL. p38 MAPK regulates IL-1beta induced IL-6 expression through mRNA stability in osteoblasts. Immunological Investigations. 2004; 33(2): 213-233.
https://doi.org/10.1081%2Fimm-120034231

20. Rogan DF, Cousins DJ, Santangelo S, Ioannou PA, Antoniou M, Lee TH. Analysis of intergenic transcription in the human IL-4/IL-13 gene cluster. Proceedings of the National Academy of Sciences of the United States of America. 2004; 101(8): 2446-2451. https://doi.org/10.1073%2Fpnas.0308327100

21. Hudon C, Fortin M, Vanasse A. Cumulative illness rating scale was a reliable and valid index in a family practice context. Journal of Clinical Epidemiology. 2005; 58(6): 603–608. https://doi.org/10.1016/j.jclinepi.2004.10.017

22. Gang S, Bai W, Yu H, Gula A, Wang Z. *Dracocephalum moldavica* L. extract alleviates experimental colitis in rats by modulating gut microbiome and inflammatory pathways. Molecular Medicine Reports. 2023; 28(6). https://doi.org/10.3892/mmr.2023.13115

23. Rezaie A, Parker RD, Abdollahi M. Oxidative stress and pathogenesis of inflammatory bowel disease: an epiphenomenon or the cause? Digestive Diseases and Sciences. 2007; 52(9): 2015-2021. https://doi.org/10.1007/s10620-006-9622-2

24. Wallace JL, McKnight W, Asfaha S, Liu DY. Reduction of acute and reactivated colitis in rats by an inhibitor of neutrophil activation. American Physiological Society Journal. 1998; 274(5): 802-808. https://doi.org/10.1152/ajpgi.1998.274.5.g802

25. Murata Y, Ishiguro Y, Itoh J, Munakata A, Yoshida Y. The role of proinflammatory and immunoregulatory cytokines in the pathogenesis of ulcerative colitis. Journal of Gastroenterology. 1995: 56-60.

26. Yao D, Dong M, Dai C, Wu S. Inflammation and inflammatory cytokine contribute to the initiation and development of ulcerative colitis and its associated cancer. Inflammatory Bowel Diseases. 2019; 25(10): 1595–1602. http://dx.doi.org/10.1093/ibd/izz149

27. El-Agamy DS, Shaaban AA, Almaramhy HH, Elkablawy S, Elkablawy MA. Pristimerin as a novel hepatoprotective agent against experimental autoimmune hepatitis. Frontiers in Pharmacology. 2018; 37(9). https://doi.org/10.3389/fphar.2018.00292

28. Strzepa A, Pritchard KA, Dittel BN. Myeloperoxidase: a new player in autoimmunity. Cellular Immunology. 2017; 317:1-8. https://doi.org/10.1016%2Fj.cellimm.2017.05.002

29. Liy PM, Puzi NNA, Jose S, Vidyadaran S. Nitric oxide modulation in neuroinflammation and the role of mesenchymal stem cells. Experimental Biology and Medicine. 2021; 246(22): 2399-2406. https://doi.org/10.1177%2F1535370221997052

30. Gawel S, Wardas M, Niedworok E, Wardas P. Malondialdehyde (MDA) as a lipid peroxidation marker. Wiadomosci Lekarskie. 2004; 57(9-10): 453-455.