

Original Research

Prevention of Inflammation Initiation on Acetic Acid-Induced Ulcerative Colitis in Rats by *Malva nicaeensis* All.

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Abstract

Background: Aerial parts of *Malva nicaeensis* All. are preferred in the prevention and treatment of intestinal infections and hemorrhoids in Turkish traditional medicine. This study is planned to evaluate the pharmacological activity of *M. nicaeensis* extracts on rats with acetic acid-induced colitis. **Methods:** The plant material was subsequently extracted with *n*-hexane, ethanol, and water, respectively. All of these extracts were tested for efficacy in the acetic acid-induced rat colitis model. The aqueous and polysaccharide extracts regulated cytokine levels and antioxidant parameters. Furthermore, the aqueous extract in particular regulated myeloperoxidase and caspase-3 levels in this rat model. In addition, the polysaccharide-rich fraction was separated from the aqueous extract. **Results:** The polysaccharide-rich fraction and aqueous extract regulated cytokine levels and antioxidant parameters. The aqueous extract also positively affected myeloperoxidase and caspase-3 levels. The phytochemical studies revealed that the aqueous extract had the highest phenolic content. In addition, the polysaccharide fraction was found to contain total sugars, sulfated groups, uronic acids, and total proteins in 78.4%, 0.9%, 1.5%, and 14.7%, respectively, and was rich in monosaccharide-type compounds, especially galactose (36.4%). **Conclusions:** *M. nicaeensis* was discovered to be a drug lead in the future treatment of irritable bowel diseases or as a complementary therapeutic agent that aided conventional treatments.

Keywords: colitis; herbal medicine; *Malva nicaeensis*; Malvaceae; metabolic disorders; myeloperoxidase

1. Introduction

Idiopathic inflammatory bowel diseases (IBDs) include Crohn's disease and ulcerative colitis (UC), which show retention in different regions of the gastrointestinal system and affect people differently. UC mostly affects the colonic mucosa and is characterized by acute, non-infectious inflammation [1,2]. Both genetic and environmental factors are important in the onset of this damage. UC gives rise to some symptoms, including diarrhea with blood and tenesmus. UC is usually seen between the ages of 15 and 30 [3]. Side effects and inadequate treatment outcomes of the present medications used for UC encouraged scientists to examine better alternative treatments with fewer side effects [4].

In recent years, numerous plants and plant constituents have been considered potential drug leads for treating UC [5].

Members of the *Malva* L. genus (Malvaceae) are mainly distributed in widespread throughout the world and especially in the Mediterranean region. They are usually known as "Mallow (in English) and Ebegümeçi (in Turkish)" [6,7]. *Malva* sp. has been used in traditional medicines for its therapeutic properties such as mucolytic, antiseptic [8], diuretic, sedative, spasmolytic, and laxative

effects [7]. Previous studies which evaluated the effects of *Malva sylvestris* L. on UC showed that *Malva* species can be worth researching alternative sources for the medication of UC [4,5]. In traditional Lebanese medicine, whole parts of *Malva nicaeensis* All. are used to stop coughing, for wound healing [9], for renal infections, and for kidney stones [10]. The aerial parts of *M. nicaeensis* are used in Turkish folk medicine as an expectorant for coughs [11]. According to phytochemical analysis on *Malva* species, monoterpenes, diterpenes, coumarins, flavonoids, malvin, malvone A (a naphthoquinone), malvaline and many polyphenols have been identified [7,12–14].

Since there are traditional records of *Malva* sp. especially *M. nicaeensis* in infectious diseases, this study was planned to investigate the effectiveness of *Malva nicaeensis* in an experimental colitis model in rats.

2. Materials and Methods

2.1 Plant Material

Aerial parts of *M. nicaeensis* were gathered from Antalya, Turkey, in June 2019. The voucher specimen (Herbarium number: GUEF3836) has been deposited at the Herbarium of the Faculty of Pharmacy of Gazi University, Ankara, Turkey.



2.2 Extraction Procedure

The air-dried and powdered aerial parts of *M. nicaeensis* (300 g) were extracted with *n*-hexane, ethanol, and water (3 × 1 L) over 48 hours, subsequently. The pooled extracts were concentrated under low pressure at 40 °C by a rotary evaporator, and the remaining water extract was further lyophilized. The extracts were kept protected from light at –20 °C until use. The extract yields for *n*-hexane, ethanol, and aqueous extracts were 3.6%, 9.4%, and 12.9%, respectively.

2.3 Preparation of the Polysaccharide Fraction

Aqueous extract of *M. nicaeensis* was macerated with absolute ethanol, stirred vigorously for 8 hours, and kept overnight at 4 °C. After centrifugation (20 minutes at 4 °C and 16 000 G), the supernatant was removed and the remaining part was freeze-dried. The precipitated polysaccharide-rich fraction was initially applied to an ion exchange column (equilibrated Sephadex A-25, Pharmacia, Uppsala, Sweden), eluted with gradient solutions of 0.02 to 2 M NaCl, and then applied to a size exclusion column (Sephacrose CL-6B, GE Healthcare, Uppsala, Sweden). The isolated polysaccharide fraction was lyophilized and kept at –20 °C until usage (Yield: 6.1%) [4].

2.4 Chemical Composition of Polysaccharides

Lowry and colleagues' method (1951) [15] was used to estimating the protein content of the polysaccharide fraction. Total sugars, total uronic acids, and sulphated groups were determined by the methods previously published by Dubois *et al.* (1956) [16], Bitter and Muir (1962) [17], and Lloyd *et al.* (1961) [18], respectively. Monosaccharide composition was determined after hydrolyzation of the sugars followed by high-performance anion-exchange chromatography. Concentrations of the selected nine monosaccharides (glucose, galactose, fucose, xylose, mannose, rhamnose, galacturonic acid, and glucuronic acid) were calculated by using external calibration with an equimolar mixture of the standards (Sigma Chemicals, St. Louis, MO, USA).

2.5 Determination of Total Phenolic Content

The method previously published by Singleton and Rossi (1965) was used to measure the phenolic content of the samples [19]. 2 g of dried extracts were mixed with 50 mL of *n*-hexane. In order to extract phenolic metabolites, hexane extract is partitioned with 60% methanol (3 × 100 mL). The obtained alcoholic phase was mixed with 20 mL of *n*-hexane and dried with a rotary evaporator at 40 °C. Phenolic metabolites were dissolved in a sufficient amount of distilled water and then extracted with petroleum ether (60:80). The aqueous phase was saturated with NaCl and partitioned with 4 volumes of EtOAc (3 times). Pooled ethyl acetate fractions were dried with anhydrous Na₂SO₄, and EtOAc was evaporated. 5 mL of Folin-Ciocalteu

reagent was mixed with 1 mL of various concentrations of gallic acid standard and working samples. After 5 minutes, 4 mL of a 7.9% sodium carbonate solution was added. After 2 hours of incubation at room temperature and in the dark, absorbances at 740 nm were measured.

2.6 Biological Activity Studies

2.6.1 Animals

After the permission of the Experimental Animal Ethics Committee of Kobay (Protocol number: 234) male Sprague-Dawley rats (180–200 g) were obtained from Kobay Laboratory (Ankara, Turkey) and kept in the working laboratory conditions for 3 days prior to the study. Rats were fed standard pellet feed and tap water during their adaptation period, housed in a 12-hour light/12-hour dark cycle, and kept at room temperature. Rats were divided into six groups, each with six rats. All procedures were carried out in accordance with international laws protecting the rights of animals used in research and biodiversity.

2.6.2 Preparation of Test Samples

The extracts were suspended in 0.5% carboxymethyl cellulose (CMC). The control group of rats received only 0.5% CMC, while the reference group received the drug sulfasalazine at 100 mg/kg dose that was prepared with 0.5% CMC [20]. Meanwhile, 100 mg/kg of the extracts were administered to the rats.

2.6.3 Induction of Colitis

Colitis was induced by injecting 2 mL of acetic acid solution (3% v/v) into the rectum through a polyurethane tube for enteral feeding (2 mm in diameter) that was inserted to a depth of 4.5 cm. To prevent solution leakage, the rats were held in the Trendelenburg position throughout rectal instillation and for 1 min following instillation [21].

2.6.4 Experimental Design

The control group was assigned as the ulcerative colitis-induced rats that were orally treated with 0.5% CMC, daily for 6 days and intra-rectally injected with acetic acid (2 mL of 3% (v/v) in 0.9% NaCl) on day 4. The extract (*n*-hexane, ethanol, aqueous extract, polysaccharide fraction) groups were assigned as the ulcerative colitis-induced rats that were orally treated with extracts, daily for 6 days and intra-rectally injected with acetic acid (2 mL of 3% (v/v) in 0.9% NaCl) on day 4. The reference group represented the ulcerative colitis-induced rats that were orally treated with sulfasalazine as a reference drug (100 mg/kg/day) for 6 days and then injected with acetic acid (2 mL of 3% (v/v) in 0.9% NaCl) intra-rectally on day 4 [21].

2.6.5 Collection of Samples

On day 6, all groups received samples one last time, and rats were sacrificed on day 7. The entire colon was

removed, opened longitudinally, and the fecal content was cleared with normal saline. The evaluation of biochemical parameters such as caspase-3, and myeloperoxidase (MPO) was conducted in the proximal part of the colon (6–7 cm). This part of the colon was stored in a physiological buffer at pH 7.4 until the homogenization of the samples. Further, a small part of the proximal colon was removed and kept in 10% formalin for histopathological analysis as well. The homogenization of tissue was performed in a cold environment at a concentration of 10% (w/v) in 11.5 g/L solutions of KCl, and the homogenized samples were centrifuged for 15 minutes at 10,000 rpm and 4 °C. The supernatant part was taken and separated for the biochemical investigations [21].

2.6.6 Assay of Colonic Myeloperoxidase (MPO) Activity

The MPO activity of colon tissue was estimated according to the previously published method by Kupeli Akkol *et al.* (2019) [21]. A total of 0.1 mL of the supernatant was mixed with 2.9 mL of 50 mM phosphate buffer (pH 6) containing 0.167 mg/mL O-dianisidine dihydrochloride and 0.0005% hydrogen peroxide. The change in absorbance at 460 nm was measured using a UV/VIS spectrophotometer (Beckman, Fullerton, CA, USA). Myeloperoxidase activity was reported as units (U) per gram (g) weight of wet colon tissue. The myeloperoxidase hyperactivity was calculated according to the following formula:

$$\text{MPO activity (mU/mg)} = 1000 \times [\text{X/weight of the colon tissue taken (mg)}]$$

where $X = 10 \times$ difference in absorption per minute/volume of tissue homogenate taken in the final reaction.

2.6.7 Nitrite Measurements

Griess reagent (100 μ L) was added to the 100 μ L of serum and colonic tissue homogenates. Sulfanilamide was used as a standard, and the calculations were performed at 540 nm. The concentrations were determined using a standard curve of sodium nitrate and the results were expressed as ng/ μ g of wet tissue [21].

2.6.8 Tumor Necrosis Factor (TNF)- α and Interleukin (IL)-6 Measurements

Enzyme-linked immunosorbent assay kits (Elabscience, Maryland, USA) were used for the measurement of TNF- α (Cat Number: E-EL-M3063) and IL-6 (Cat Number: E-MSEL-M0001) levels of the tissue and serum samples.

2.6.9 Caspase-3 Measurements

Caspase-3 activity is assessed according to the method of Jonges *et al.* (2001) [22]. The tissues were suspended in 1 mL of ice-cold PBS and washed twice. The resulting pellet was resuspended in a lysis buffer consisting of 10 mM HEPES, pH 7.0, 40 mM β -glycerophosphate, 50

mM NaCl, 2 mM MgCl₂, and 5 mM Ethylene glycol-bis(2-aminoethylether)-N,N,N',N'-tetraacetic acid (EGTA). After 10 minutes on ice, the cells were disrupted by four cycles of freezing and thawing and then stored at -80 °C. For measurement, 50 mg of protein was incubated with 40 nmol of the enzyme-substrate Ac-Asp-Glu-Val-sp-7-amino-4-methylcoumarin (DEVD-AMC) in a buffer solution comprising 100 mM HEPES, pH 7.25, 10% (w/v) sucrose, 0.1% (v/v) Nonidet P40, and 10 mM DTT. After substrate cleavage, fluorescent AMC was released, which was observed at an excitation wavelength of 360 nm and emission wavelength of 460 nm using a Perkin-Elmer plate reader. Calibration curves were constructed using free AMC. Caspase-3 activity was adjusted for the number of cells density, as microscopically determined in a sequential slide by surface measurement using an ocular grid. The amount of caspase-3 activity per milligram of tissue was measured in picomoles of AMC per minute.

2.6.10 Histopathological Investigation

Rat colonic tissues were removed and deposited in a 10% formaldehyde solution. Tissue processing was carried out using Thermo Scientific Excelsior (London, UK). Histocentre 2 was used to make paraffin blocks from the processed tissues. The marine glass was utilized to cut 3.5-m slices using the Leica RM2255 microtome (Texas, USA). All tissue samples were examined under a light microscope after being stained with hematoxylin-eosin (HE) in the Shandon Varistan apparatus (Ohio, USA) [21].

2.6.11 Statistical Evaluation

Statistical analyses and comparisons were performed using GraphPad Prism (version 6.0) software (DMCA Compliance Agent, Boston, MA, USA). p values of <0.05 were considered significant * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Statistical mean \pm standard error of the mean (SEM) for all outcomes is presented.

3. Results and Discussion

Normal colon homeostasis requires the equilibrium between pro-inflammatory and anti-inflammatory cytokines in the colon mucosa. Cytokine profile disruption causes some negative conditions, such as inflammatory bowel diseases. Further, an increment of pro-inflammatory cytokines such as TNF- α and IL-1- α , IL-1 β , IL-6, IL-8, and IL-23 during UC is developed in the organism [23]. TNF- α , interleukins, nuclear factor kappa beta (NF- κ B), and chemokines are overexpressed in UC patients [24]. In the current study, the aqueous extract obtained from *M. nicaeensis* decreased serum TNF- α and IL-6 levels from 16.7 to 7.3 and from 49.3 to 19.6 pg/mL, respectively. Meanwhile, the aqueous extract decreased TNF- α and IL-6 levels in colonic tissue from 307.5 to 114.8 and from 218.9 to 146.1 pg/mg, respectively. In addition to the aqueous extract, the polysaccharide fraction also showed remarkable activity against cytokine levels. This extract dimin-

Table 1. Serum and colonic tissue TNF- α and IL-6 levels in treated groups.

Groups	Serum		Colonic tissue	
	TNF- α (pg/mL)	IL-6 (pg/mL)	TNF- α (pg/mg)	IL-6 (pg/mg)
Control	16.7 \pm 5.8	49.3 \pm 18.4	307.5 \pm 41.7	218.9 \pm 38.6
<i>n</i> -Hexane extract	20.1 \pm 9.3	47.6 \pm 11.3	298.4 \pm 46.8	210.6 \pm 41.9
Ethanol extract	14.6 \pm 4.2	40.1 \pm 10.0	267.5 \pm 40.4	181.8 \pm 25.4
Aqueous extract	7.3 \pm 1.4*	19.6 \pm 4.5**	114.8 \pm 26.2**	146.1 \pm 34.3**
Polysaccharide fraction	8.0 \pm 2.6*	22.7 \pm 6.1*	136.9 \pm 24.3*	153.2 \pm 38.1**
Sulfasalazine	5.8 \pm 1.1***	16.3 \pm 2.5***	87.4 \pm 13.9***	100.8 \pm 14.5***

*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$; The values are presented as Mean \pm S.E.M., Standard Error of Mean.

Table 2. Serum and colonic tissue NO and MDA levels in treated groups.

Groups	Serum		Colonic tissue	
	Nitrite (μ g)	MDA (nmol/mL)	Nitrite (ng/ μ g)	MDA (nmol/g)
Control	0.5 \pm 0.2	10.8 \pm 3.4	2.5 \pm 1.6	385.1 \pm 57.6
<i>n</i> -Hexane extract	0.3 \pm 0.1	11.6 \pm 3.3	2.7 \pm 1.5	403.3 \pm 49.3
Ethanol extract	0.7 \pm 0.3	9.7 \pm 2.6	2.3 \pm 1.4	344.2 \pm 48.4
Aqueous extract	1.9 \pm 0.6*	4.1 \pm 1.5**	1.0 \pm 0.4*	196.4 \pm 34.9**
Polysaccharide fraction	1.1 \pm 0.9*	4.9 \pm 2.0*	1.6 \pm 0.9	217.6 \pm 33.8*
Sulfasalazine	2.1 \pm 0.3**	2.4 \pm 0.6***	0.4 \pm 0.1***	147.9 \pm 26.4***

*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$; The values are presented as Mean \pm S.E.M., Standard Error of Mean. NO, nitric oxide; MDA, malondialdehyde.

ished serum TNF- α and IL-6 levels from 16.7 to 8.0 pg/mL and from 49.3 to 22.7 pg/mL, respectively. Similarly, the same extract diminished TNF- α and IL-6 levels in colonic tissue from 307.5 to 136.9 and from 218.9 to 153.2 pg/mg, respectively (Table 1).

Additionally, oxidative stress can be one of the initial factors in UC development. Changes in the levels of oxidative parameters such as Glutathione (GSH), Myeloperoxidase (MPO), and MDA have been seen during the inflammatory response [25]. In the current study, the control group exerted an important increase in lipid peroxides due to oxidative stress. Furthermore, the aqueous extract and polysaccharide fraction significantly increased serum nitrite levels from 0.5 to 1.9 and 1.1 μ g, respectively. On the contrary, the aqueous extract and polysaccharide fraction decreased nitrite levels in colonic tissue from 2.5 to 1.0 and 1.6 ng/ μ g, respectively. Additionally, MDA levels were significantly diminished in both serum and colonic tissues (Table 2).

Under various pathological conditions or by the mechanism initiated by apoptotic tissue damage, apoptosis is a process that involves the normal turnover of damaged cells. Caspases are classified as inactive procaspases that are present in the majority of cells. When activated, they can cause the activation of other procaspases, which is what leads to the breakdown of intracellular proteins and programmed rapid cell death of the cells [26]. Similar to starter caspases, executioner caspases, such as caspase-3, is in charge of causing cell death during apoptosis. In the cur-

rent investigation, a significant increase in colonic caspase-3 activity was seen in the rats in the control group, but a significant decrease in the mean value of caspase-3 in the colon of the experimental group was discovered (Table 3).

Acute inflammation is characterized by an increase in neutrophil MPO activity that is observed after the infiltration of polymorphonuclear leukocytes. One of the most crucial components of an organism's immune system is MPO [27]. In the current study, an increment in MPO activity was detected in the inflamed colon, while a decrease in MPO levels was detected in the aqueous extract and polysaccharide fraction along with the sulfasalazine-treated group animals when compared to the control group. Thus, it was established that the rise in MPO levels and acetic acid-induced colitis were closely connected. All of these dangerous biochemical characteristics created by acetic acid treatment were reversed by treatment with aqueous extract and polysaccharide fraction (Table 3).

The colitis model triggered with acetic acid is one of the most utilized methods for evaluating the efficacy of drug leads against IBD. After administering acetic acid intrarectally, massive necrosis diffuses inflammatory leukocyte infiltration and ulcerated mucosa. Linear ulcers, inflammation, localized hyperemia, significant loss of mucosal structure, cellular infiltration, cryptic abscess, and goblet cell decline are observed in the colon tissues treated by acetic acid in the intrarectal route [28]. In the control group, there were several mucosal erosions, including the invasion of certain inflammatory cells in the lamina pro-

Table 3. Effects of test samples on Caspase-3 and MPO in tissue homogenate.

Groups	Caspase-3 level	MPO concentration
	(pmolAMC/min/mg/protein)	(U/mg of tissue)
Control	46.2 ± 8.4	25.53 ± 2.21
<i>n</i> -Hexane extract	48.4 ± 6.3	20.33 ± 2.07
Ethanol extract	43.6 ± 5.1	19.26 ± 1.45
Aqueous extract	27.9 ± 3.0**	8.47 ± 0.93**
Polysaccharide fraction	34.2 ± 4.4**	10.96 ± 1.38*
Sulfasalazine	11.6 ± 3.5***	4.94 ± 0.43***

*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$; The values are presented as Mean ± S.E.M., Standard Error of Mean; MPO, Myeloperoxidase.

Table 4. Chemical compositions of polysaccharide fraction of *M. nicaeensis*.

Chemical group	% Composition
Total sugars	78.4 ± 3.9
Uronic acids	1.5 ± 0.7
Sulfated groups	0.9 ± 0.1
Total proteins	14.7 ± 3.8

pria, villus flattening, and some damage to the crypts and cylindrical epithelium in the mucosa in the duodenum and jejunum and it was determined that mucosal inflammation developed on the duodenum (duodenitis) and jejunum (jejunitis) (Fig. 1A). The *n*-hexane extract-treated group of animals exerted histopathological modifications, such as new villus formation for intestinal absorption, little collagen fiber regeneration in connective tissue, and tight regular lamina propria (Fig. 1B). The polysaccharide fraction treated group showed histopathological changes, like villus formation, and some lipid vacuole formation in connective tissue (Fig. 1E). Finally, the ethanol and aqueous extract treated groups also had some histopathological modifications, such as regeneration in the mucosa and villus degeneration as well as collagen fiber regeneration in the submucosa (Fig. 1C,D). In the positive control group namely the sulfasalazine-treated group, villus and collagen fiber regeneration in mucosa and submucosa were examined, respectively (Fig. 1F).

In the current study, not only the pharmacological activities of the extracts and fraction were evaluated, but their phytochemical compositions were also determined. According to our results, the highest phenolic content was found in the aqueous extract, which is mainly important in terms of antioxidant activity during inflammation. The phenolic content value was 1.2 ± 0.9 mg/g for the *n*-hexane extract, 2.5 ± 0.4 mg/g for the ethanol extract, and 4.8 ± 0.7 mg/g for the aqueous extract of the dried extract.

The amounts of total sugars, sulfated groups, uronic acids, and total proteins in the polysaccharide fraction were calculated as 78.4%, 0.9%, 1.5%, and 14.7%, respectively (Table 4).

Table 5. Monosaccharide compositions (%) of the polysaccharide fraction obtained from *M. nicaeensis*.

Monosaccharide	% Composition
Galactose	36.4 ± 3.5
Mannose	8.7 ± 1.9
Glucose	19.7 ± 2.6
Arabinose	0.5 ± 0.1
Rhamnose	4.9 ± 1.3
Glucuronic acid	32.3 ± 5.8

The findings demonstrated that the polysaccharide fraction is rich in monosaccharides, with galactose (36.4%) being the main monosaccharide and arabinose (0.5%) being the least prevalent (Table 5).

UC is a multifactorial disease that originates from a dysfunctional epithelial, innate, and adaptive immune response to intestinal microorganisms. Currently, the management of IBD is a challenging process for gastroenterologists; 5-aminosalicylates, immunomodulators, antibiotics, steroids, and surgery have been used to alleviate the symptoms of the disease and its remission. However, long-term use of these conventional treatments is associated with severe toxicities for patients [29]. Therefore, due to those causes, scientists are looking for new and safer treatments for UC.

In the present study, the effects of various extracts obtained from *M. nicaeensis* were appraised in acetic acid-induced UC in rats. Macroscopically, no defect was seen in the colon of the rats treated with the aqueous extract and polysaccharide fraction of *M. nicaeensis*. The effects of the tested extracts against UC seem to be related to their polysaccharide and polyphenol content. Polyphenols such as anthocyanins, flavonoids, and tannins are secondary metabolites with different biological activities such as anticarcinogenic, antimicrobial, vasodilatory, wound healing [30–32], antioxidant, and anti-inflammatory properties [33–36]. These data were in parallel with the results of previous publications on *M. sylvestris* polysaccharides [37–39].

Previous reports showed that the aqueous extract prepared from different parts of *M. sylvestris* was known to be

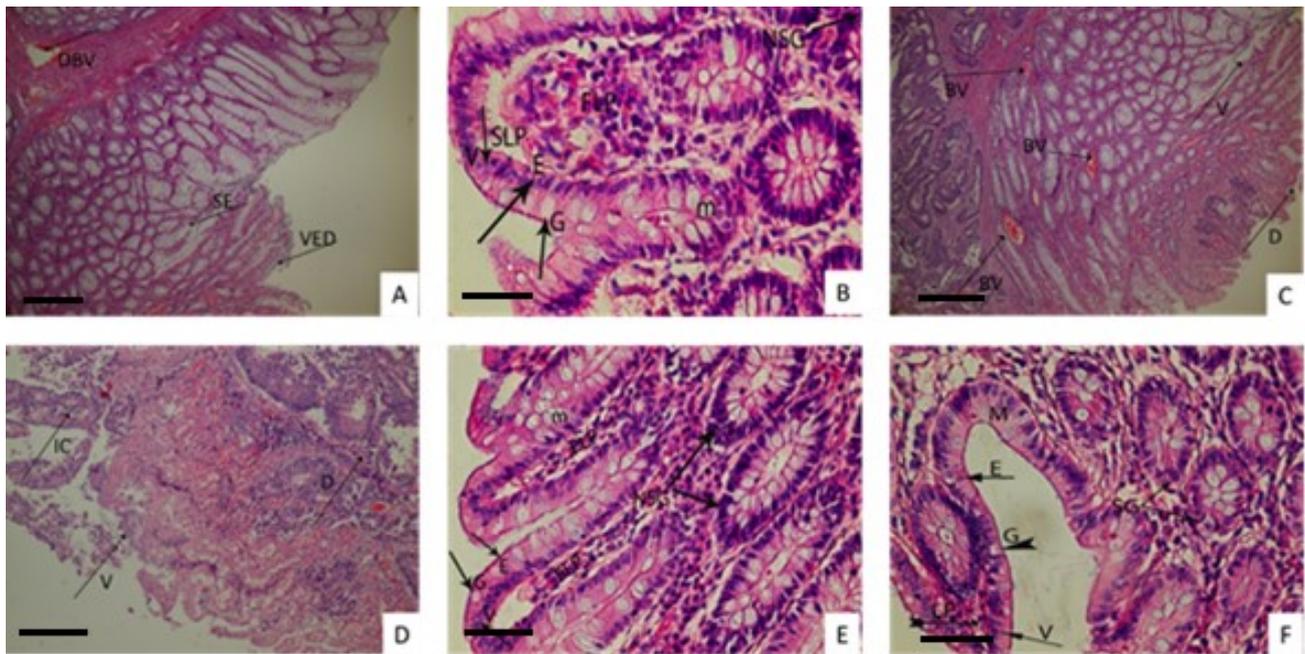


Fig. 1. Histopathological views of treated groups. The original magnification was $\times 100$ and the scale bars represent $120\ \mu\text{m}$ for figures. Data are representative of 6 animal per group. [(A) Control group. (B) *n*-Hexane extract. (C) Ethanol extract. (D) Aqueous extract. (E) Polysaccharide fraction. (F) Sulfasalazine]. DBV, Dilated blood vessel in the submucosa; SE, Separation and degeneration of the epithelium in the mucosa; VED, Villus epithelium degeneration; BV, Blood vessels; V, Villus degeneration; D, Crypts degeneration; IC, Inflammatory cells; M, Mucosa layer; SG, Secret glands in the mucosa; G, Goblet cell; E, Normal epithelium cell; LP, Lamina propria; M. Mucosa; NSG, New developing secret glands; SLP, Small gaps in the newly formed in lamina propria structure; FLP, Formation of new lamina propria structure.

rich in mucilaginous polysaccharides with rhamnose, trehalose, galacturonic acid, glucuronic acid, galactose, glucose, fructose, and sucrose [38–41]. The polysaccharides of *M. sylvestris* were stated to have anti-complementary activity, which may be one of the possible mechanisms for the experimental results. Furthermore, in the study conducted by Classen and Blaschek, high amounts of arabinogalactans were extracted from cultures of *M. sylvestris* [42]. According to the study, these polysaccharides could be the main effective compounds in the aqueous fraction of the plant. These polysaccharides were found to have preventive effects against acid-induced UC. Liu *et al.* [43] exhibited that the application of the polysaccharides significantly decreased ulcers, mortality, and colon mass. Wong *et al.* [44] reported that *Angelica sinensis* (Oliv.) Diels and *Rheum tanguticum* Maxim. ex Balf. polysaccharides could be due to the prevention of the pathological process of UC. Polysaccharides from *Vaccinium oxycoccos* reduced neutrophil infiltration and were also reported to have significant antioxidant properties [45]. Polysaccharides from *M. sylvestris* were reported to have potential antioxidant activity [46].

Magro *et al.* [47] reported that the aqueous extract of *M. sylvestris* leaves displayed a remarkable antimicrobial effect against fungi, and it was reported that *M. sylvestris*

leaves inhibited prostaglandin synthesis by the cyclooxygenase pathway. Saldanha *et al.* [29] reported that some polyphenols, including resveratrol, quercetin, kaempferol, ellagic acid, rutin, green tea polyphenols, and silymarin showed remarkable effects against UC in addition to the polysaccharides.

In the literature, there is no previous report on the effects of *M. nicaensis* polysaccharides. The previous studies reported that the polysaccharides of *V. oxycoccos*, *R. tanguticum*, and *A. sinensis* showed remarkable activity against UC. Similarly, these results are parallel with our results [43–45]. The aqueous extract of *M. nicaensis* displayed higher efficacy against UC than the polysaccharide fraction, suggesting that other constituents in the aqueous fraction showed synergistic effects due to the polysaccharides. Magro *et al.* [47] demonstrated that the aqueous extract of *M. sylvestris* was the only extract with significant antimicrobial effects against fungi, which was reported to inhibit prostaglandin synthesis via the cyclooxygenase pathway. The aqueous extract prepared from the leaves of *M. sylvestris* was reported to contain various terpenoids such as blumenol A, dehydrovomifoliol, linalool-1-oic acid, linalool, malvone A, and different megastigmene derivatives [13,48]. There are also some glycosides, including ascorbic acid, fructose, glucose,

and trehalose, along with several glycosidic phenolic compounds [33,49]. Additionally, Geerling *et al.* [50] exhibited that the consumption of vitamin C, fructose, magnesium, and fruit reduced the development of UC. Moreover, these kinds of metabolites were reported for significant anti-inflammatory and antioxidant potentials, which play a principal role in the observed efficacy of this fraction. As far as we know, this is the first published report about the beneficial effects of *M. sylvestris* in the treatment or prophylaxis of UC, though Sleiman and Daher previously reported on the anti-ulcerogenic and anti-inflammatory properties of the aqueous extract of *M. sylvestris* against gastric ulcers when taken orally. Through the inhibition of elastase and trypsin by the aqueous extract of *M. sylvestris*, enhanced mucous production in mucosal tissue and improved elastin promoter activity improved the elasticity of mucosal tissues [36].

Other than phenolic and terpenic metabolites, previous studies also demonstrated that the *n*-hexane extract of *M. sylvestris* was reported to include carotenoids, tocopherols, and various fatty acids, with a large amount (50% to 80%) of polyunsaturated fatty acids. Although carotenoids and tocopherols have the potential for antioxidant properties and could be responsible for the effects of those fractions. In the studies of Geerling *et al.* [50], it was demonstrated that consuming a lot of mono- and polyunsaturated fatty acids may increase the risk of developing UC, and this finding may help to explain the lower beneficial outcomes (compared to aqueous and alcoholic fractions) observed in the present study.

4. Conclusions

In conclusion, this study suggests that *M. nicaeensis* might be an encouraging therapeutic option for UC through its antioxidant and anti-inflammatory properties. Findings revealed that *M. nicaeensis* can be used in the cell, tissue, or person-specific treatments that will be developed in the future for the treatment of IBD, or as a complementary therapeutic agent that supports conventional treatments. The activity of *M. nicaeensis* could be due to its polysaccharides and phenolic contents. Furthermore, this plant can be recommended to be used as a prophylactic supplement for patients with UC to improve their health complaints or reduce the risks of adverse effects from their conventional treatment. Nonetheless, especially clinical studies are required to confirm its effects on humans.

Availability of Data and Materials

Datasets used and/or analyzed for this study are available from the corresponding author upon appropriate request.

Author Contributions

Conceptualization—EKA; Design—EKA; Supervision—EKA; Resources—EKA, HT; Materials—

EKA, GT; Data Collection and/or Processing—EKA and ES-S; Analysis and/or Interpretation—EKA, GT, HT and ES-S; Writing—EKA; Critical Reviews—EKA, and ES-S. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work to take public responsibility for appropriate portions of the content and agreed to be accountable for all aspects of the work in ensuring that questions related to its accuracy or integrity.

Ethics Approval and Consent to Participate

All experiments on Sprague-Dawley rats were performed with permission of the Experimental Animal Ethics Committee of Kobay (Protocol number: 234) (Ankara, Turkey).

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Conflict of Interest

The authors declare no conflict of interest. EKA served as one of the Guest editors of this journal. We declare that EKA had no involvement in the peer review of this article and has no access to information regarding its peer review. Full responsibility for the editorial process for this article was delegated to Luigi De Masi.

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