

Research Article

Assessment of Ameliorative Effect of *Myrica esculenta* in a DSS-Induced Murine Model against Ulcerative Colitis

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Background. Ulcerative colitis (UC) is a type of inflammatory bowel disease (IBD) delineated by inflammation or ulcers in the colon that may cause diarrhea, abdominal pain, blood in the stool, tenesmus, etc. **Objective.** To determine therapeutic effectiveness and explore the mechanism of MeEt extract against DSS (3%)-induced UC in Swiss albino mice. **Methods.** Six mice were allocated into five groups at random: group 1: vehicle control; group 2: DSS control; group 3: DSS+mesalazine (MSZ, 100 mg/kg BW); group 4: DSS+MeEt (200 mg/kg BW); and group 5: DSS+MeEt (400 mg/kg BW). Except for group 1, DSS (3%) in drinking water was given to all other groups to induce UC. %BW, average food intake, and mean stool consistency were determined for obtaining DAI scores. Mice were euthanized on the 8th day, and colonic tissues were taken to measure the colon length. Serum and tissue levels of the cytokines were also determined using ELISA kits, followed by histopathological analysis of colon tissue. In addition, hematological and serum biochemical parameters were also determined to assess the treatment effectiveness. **Result.** During the induction, DAI scores, colon length, and cytokines levels were significantly increased. MSZ at 100 mg/kg BW and MeEt at 400 mg/kg BW doses *via* oral route significantly reversed the DAI scores and colon length. Further, MeEt 400 also downregulated the expression of IL-6, IL-8, TNF- α , IFN- γ , and IL-1 and upregulated the production of IL-1 β and IL-10. Also, improvements in hematological and serum biochemical parameters were reported. Histopathological analysis revealed a decrease in inflammation and damage to colon tissue with the administration of MeEt extract. **Conclusion.** MeEt at a 400 mg/kg BW dose may be used as an alternate treatment for the management of UC, which is comparable with that of MSZ.

1. Introduction

Inflammatory bowel disease (IBD) is characterized by a protracted state of immune activation and inflammation in the gastrointestinal tract (GIT). This disease includes two most common subtypes: (i) ulcerative colitis (UC) and (ii) Crohn's disease (CD). The former is more common and affects the colonic and rectal mucosa, while the latter can infect any area of the GIT, including the mouth and anus [1]. UC prevalence is continuously growing in developing countries as a result of the adoption of western lifestyles, including dietary changes that cause crucial manifestations like rectal bleeding, bloody diarrhea, loose motions, tenesmus, and lower abdominal discomfort, all of which recur fre-

quently when the illness is exacerbated and remitted [2, 3]. Although the precise cause of the UC is not yet completely known, it is generally accepted that a confluence of the following variables contributes to its development: dysbiosis, environmental variability, and maternal inheritance. Sulfasalazine (aminosalicylates), corticosteroids, immunosuppressive drugs, and biological therapy are currently the main therapies for the treatment of UC [3]. However, these methods of treatment have few positive outcomes and are linked to several side effects, such as a higher risk of infections, loss of appetite, lymphomas, fever, and a high mortality rate. Further, only around one-third of individuals experience long-term effectiveness using these treatment approaches [4]. Side effects with current therapies are crucial

since UC necessitates longer-term treatment. Therefore, researchers are currently focused more on herb-based treatments for its control. Herbal therapy is a natural treatment approach that is considered to be safe, associated with fewer side effects, has more patient acceptability, and has a low production cost [5]. Several UC models have been beneficial in gaining knowledge of its pathophysiology and evaluating the *in vivo* effectiveness of possible treatments. Most frequently, murine models are used in which an UC-like phenotype is generated via the administration of a chemical or bacterial infection [6]. The main colitogenic agents that harm the intestinal epithelium are trinitrobenzene sulfonic acid (TNBS), acetic acid, dextran sulfate sodium (DSS), and oxazolone [7]. The DSS is the most popular since it is easy to administer (often through drinking water) and allows for precise dosage and time management. Instead of directly causing luminal inflammation, DSS breaks the epithelial barrier, exposing the lamina propria and submucosa to intestinal antigens and bacteria. Many of the inflammatory reactions are similar to those mimicked by human UC, as reported by the manifestation of defining characteristics like loss in weight, diarrhea, reduction in colon length, and fecal occult blood [8, 9]. The pathogenesis of DSS-induced UC is mediated by defective alterations in the tight junction complex and substantial increases in inflammatory cytokine production [10]. This model bears a striking resemblance to human UC and is thus used in the present study. *Myrica esculenta* Buch. and Ham (ME), which belongs to the Myricaceae family and is often called kaiphal, is a popular medicinal plant mainly found in the subtropical Himalayas. As reported by various researchers, the bark and leaves of the plant have multifaceted pharmacological and therapeutic benefits and are frequently used as traditional medicine in treating various ailments, including anemia, fever, diarrhea, ulcers, chronic bronchitis, chronic cough, and asthma [11]. However, the plant has not yet been explored for its efficacy against UC. Therefore, using the most common DSS-induced UC model, the efficacy and mechanism involved of the ethanol extract of *Myrica esculenta* (MeEt) for the management of ulcerative colitis have been evaluated in the present study.

2. Material and Methods

2.1. Chemicals and Reagents. The reagents and chemicals employed in this research were of analytical grade (for the extraction). DSS to induce ulcerative colitis was purchased from MP Biomedicals, California; mesalazine (MSZ) used as a reference standard in animal experiment was procured from HiMedia, India; diagnostic kits for all biochemical tests were purchased from Agappe Diagnostic Ltd., Kerala, India; and enzyme-linked immunosorbent assay (ELISA) kits for inflammatory cytokines were purchased from Krishgen Biosystems, Mumbai, India.

2.2. Plant Procurement and Authentication. *Myrica esculenta* Buch.-Ham stem bark, synonym for *Myrica nagi* Hook.f. also called box myrtle bark and Kaiphal/Kayphal Chaal, was procured from the local market in Yahiyaganj, Lucknow

(U.P.), India, in February 2023. The plant identification and authentication were done (NIScPR/RHMD/Consult/2022/4414-15) by the head of the Raw Material Herbarium and Museum (RHMD), CSIR-National Institute of Science Communication and Policy Research (NIScPR), Delhi, India.

2.3. Plant Extraction and Standardization

2.3.1. Extraction Procedure. The procured stem bark was washed with tap water to remove any earthy matter that might be present in it and then allowed to shade dry. After being shade dried and ground into a coarse powder, the stem bark was then put through sieve number 14. The resultant dried Me powder (200 g) was then put into a thimble-shaped Soxhlet apparatus tube and kept for 6 h on heating mental for extraction using ethanol as solvent. The resulting extract was then filtered, condensed on a rotary evaporator (R-210 BUTCHI, Switzerland), weighted, and stored in the refrigerator for further analysis [12].

2.3.2. Physicochemical Standardization and Phytochemical Screening. Various physicochemical parameters, including foreign matter, total ash, loss on drying, acid-insoluble ash, and water- and alcohol-soluble extractives, were determined on stem bark following standard protocols as previously described [13].

The presence of numerous phytochemicals such as carbohydrates, phenols, tannins, saponins, flavonoids, proteins, steroids, resin, alkaloids, and triterpenoids was also investigated using preliminary phytochemical screening [14].

2.3.3. High-Performance Liquid Chromatography (HPLC). The presence of gallic acid (GA), myricetin (My), and quercetin (Q) in MeEt extract was quantified using HPLC, following an earlier published protocol [15] with slight modifications. Briefly, quantification was done on a HPLC platform (Shimadzu, Japan) model: Prominence (pump: LC-20AD, autosampler: SIL-20AC, and oven: CTO-10ASvp) using a photodiode array detector (PDA: SPD-M20A), software (LabSolutions 6.72 SP1), and a C18 column (ShimadzuRP-C-18, 250 × 4.6 mm, 5 μm pore size). The method involved solvent A (1% acetic acid) in water and solvent B (acetonitrile) in isocratic elution mode with a 0.6 mL/min flow rate. The volume of injection was 20 μL, and the detector wavelength was set at 254 nm. For the identification and quantification of chemical constituents, the standards were used to compare the UV absorbance and retention time.

2.4. Animal Experiments. Animal experiments in the present research were carried out by following the standard procedures given by the Organisation for Economic Cooperation and Development (OECD) (1987) to care for and utilize experimental animals. A plethora of variables can affect the outcome of the studies; thus, to minimize individual differences, mice of nearby weight, age group, and similar gender were used. Thirty adult (6-7 weeks old) male and female Swiss albino mice (20-25 g) were used in this research. All the animals were acclimatized for a period of 1 week prior to the experiment and kept under standard laboratory conditions (25 ± 1°C, 55 ± 5% relative humidity, and

an automatic 12 h light/12 h dark cycle) [16]. All the mice were thus kept in controlled environmental conditions, and careful handling was carried out during the experiments. Animals were given a commercial-standard food and unlimited access to water. The experimental study was carried out under the protocol CCSEA/IAEC/SLSRPL/18/07/2023-02, which was duly authorized by the Institutional Animal Ethics Committee (IAEC) of Systemic Life Sciences & Research Pvt Ltd., constituted under the Committee for Control and Supervision of Experiments on Animals (CCSEA), Govt. of India.

2.4.1. Animal Grouping and Induction of UC. Animals were given the earlier reported treatment to induce UC [17]. Briefly, for 7 days, mice were administered with 3% (*w/v*) DSS in drinking water [18]. 30 Swiss albino mice were randomly separated into 5 groups ($n = 6$). Group 1, vehicle control, receives distilled water (no DSS administration). Group 2 is DSS control, which receives DSS (3%). Group 3 receives DSS (3%) and the standard drug mesalazine (MSZ) at 100 mg/kg BW orally. Groups 4 and 5 are the treatment groups that receive DSS (3%) and MeEt extract at 200 and 400 mg/kg BW orally. Administration of MSZ and MeEt was started at the same time as the DSS induction was initiated. The mice were administrated orally with MSZ and MeEt extract once a day for seven days in groups 3, 4, and 5. Daily monitoring of body weight and disease activity was done throughout the seven days. On the eighth day, all the animals were slaughtered after fasting for 12 hours. On the day of the sacrifice, measurements were made on the colon's weight and length, blood collection, and serum separation [19].

2.4.2. Estimation of Disease Activity Index. Disease activity scores were determined considering the following parameters: visible stool consistency, blood in stool, and loss in body weight. Consistency of stool is evaluated on a 4-point scale: 0 for formed and hard stool, 1 for formed but soft feces, 2 for loose feces, and 3 for runny and watery stool. The percent weight loss was calculated relative to the percent body weight of the first day. Further, traces of the occult blood in the stool were examined using Hemocult SENSAs single slides based on the following criteria: 0, Hemocult negative (-); 1, Hemocult positive (+); 2, Hemocult positive (++); and 3, bleeding on the anus site (+++). The animals were then sacrificed, and the colon was removed for measuring the length and histopathological analysis [20].

2.4.3. Colon Weight-to-Length Ratio. Mice had their abdomens dissected, and the colons were removed by tracing backward. Wet weights of 10 cm colon lengths were measured after they had been removed, longitudinally opened, and washed in saline. The wet weights were then divided by the colon length to calculate the colon weight-to-length ratio [21].

2.4.4. Measurement of Inflammatory Cytokines in Serum and Colon Tissues. ELISA kits (Krishgen Biosystems, Mumbai, India) were used to measure the levels of several cytokines, including IL-6, IL-8, IL-10, IL-1, TNF- α , and IFN- γ , in serum and colon tissue samples. The tests were conducted in accordance with the manufacturer's instructions.

2.4.5. Hematological Analysis. To perform a complete blood count (CBC) and serum analysis, blood was taken from the retro orbital plexus of each mouse's eye. Cell counts of collected whole blood (without adding anticoagulant) samples were performed on a hematology analyzer (Mindray BC 3000 Plus, Agappe, India).

2.4.6. Effect of MeEt on Biochemical Parameters. Serum samples were analyzed to determine the level of various biochemical parameters such as urea, serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvate transaminase (SGPT), creatinine, alkaline phosphatase (ALP), uric acid, LDL (low-density lipoprotein), HDL (high-density lipoprotein), and bilirubin using diagnostic kits of Agappe, India. The biochemical tests were performed as per the manufacturer's instructions.

2.4.7. Tissue Histopathology and Histopathological Score. Colon tissues were embedded in paraffin wax after being preserved in 10% neutral-buffered formalin. The sections of 5 μ m were cut and stained with hematoxylin and eosin for histological evaluation. Under the microscope (Leica, DM 750, Germany), sections were observed under at 400x to determine the degree of colonic inflammation caused due to DSS induction [22], which was scored on a subjective scale, i.e., 0, normal colonic mucosa; 1, loss of one-third of the crypts; 2, the loss of two-thirds of the crypts; 3, a single epithelial layer covering the lamina propria; and 4, erosions and significant inflammatory cell infiltration [22].

2.5. Statistical Analysis. The mean and SEM of the number of separate experiments are used to illustrate the results. The one-way analysis of variance (ANOVA) and Tukey's post hoc test were used to evaluate the statistical significance between the experimental groups. Data with a *p* value of 0.05 or below were deemed significant. GraphPad Prism version 5.01 was used to analyze the data.

3. Results

3.1. Physicochemical Standardization. Total ash, water-soluble ash, acid-insoluble ash, extractive values, moisture content, loss on drying (LOD), and pH values of 1% and 10% solutions were all used to indicate the purity of any material in the presence of foreign matter in physicochemical research. The Me bark estimate of $0.6 \pm 0.04\%$ indicates that the powdered crude material was thoroughly dried and kept in an airtight container. The computed LOD for the Me bark was $8.2 \pm 0.37\%$. The ash value was found to be within limits (Supplementary Table 1). Further, the preliminary phytochemical screening of Me extract revealed the presence of major photochemicals in it, such as alkaloids, flavonoids, saponins, tannins, steroids, resins, phenolics, triterpenoids, and carbohydrates. Furthermore, proteins and saponins were not detected in our analysis (Supplementary Table 2).

3.2. HPLC. The result pertaining to the HPLC profile while comparing the retention time of mixed standards with MeEt extract revealed the presence of quercetin, myricetin, and gallic acid (Figure 1). In all, 57 peaks with various retention

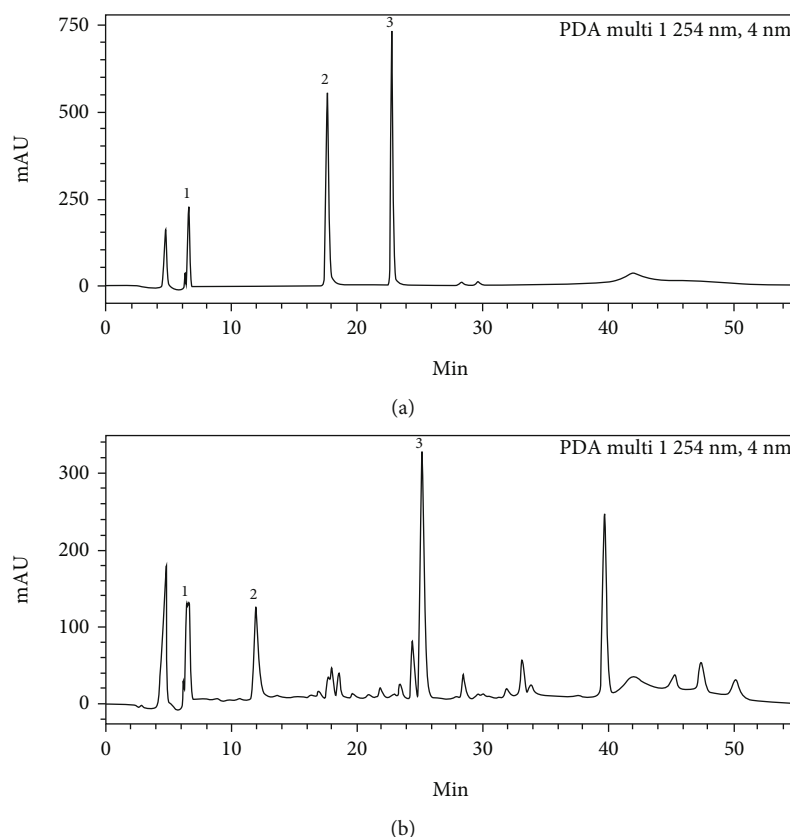


FIGURE 1: HPLC analysis of ethanolic extract of *M. esculenta* (MeEt): (a) Representative chromatograms of standard mixture of (1) gallic acid, (2) myricetin, and (3) quercetin; (b) chromatograms of MeEt extract indicating the presence of (1) gallic acid, (2) myricetin, and (3) quercetin at 254 nm.

times and peak areas were found in the HPLC chromatogram. The standard content by HPLC was found to be 0.0147% for gallic acid, 0.0014% for myricetin, and 0.0008% for quercetin, respectively.

3.3. Effect of MeEt on DSS-Induced UC. DSS (3%) administration caused mice to develop acute UC, which was shown by a decrease in average food intake, body weight, a marked change in consistency of stool, and presence of bloody stool in the DSS group (Figure 2). A significant difference was observed after the administration of the standard drug MSZ at 100 mg/kg BW and MeEt at 400 mg/kg BW in terms of % weight loss, average food intake, mean stool consistency, and mean stool blood score on days 5 and 7 in contrast to the DSS control group, whereas no significant difference was observed after the administration of MeEt at a 200 mg/kg BW dose as compared to the DSS control group.

3.4. Effect of MeEt on Colon and Spleen. The length of the colon is an important parameter to determine the extent of inflammation that occurred due to DSS administration. As observed in Figures 3(a) and 3(b), in the DSS control group, a reduction in colon length was observed in mice (6.3 ± 0.3 vs. 10.1 ± 0.3) when compared to the vehicle control group. A significant enlargement in colon length was recorded in groups treated with MSZ (8.6 ± 0.3) and MeEt 400 (8.2 ± 0.2), while groups treated with MeEt 200 (7.8 ± 0.4)

did not protect the colon tissue from damage as compared to the DSS control group. Further, the colon weight-to-length ratio (mg/cm^{-1}) was determined for each group of mice. The weight-to-length ratio was 43.1 ± 2.8 in the vehicle control group, while in the DSS control group, it was significantly increased to 87.8 ± 3.0 . Groups treated with MSZ and MeEt 400 showed a significant weight-to-length ratio of 57.7 ± 8.5 and 60.6 ± 3.8 , respectively, as compared to the DSS control group. Group treated with MeEt 200 showed a less significant difference (67.1 ± 3.3) as compared to the DSS control group.

The weight of the spleen was also determined, as it has been reported to be positively associated with the UC (Figure 3). In this study, the spleen weight was also significantly increased after the induction of DSS as compared to the vehicle control group (0.32 ± 0.02 vs. 0.15 ± 0.01). A significant reduction in spleen weight was obtained in the group treated with the standard drug MSZ (0.20 ± 0.01) in comparison to the DSS control group. Group treated with MeEt at 400 and 200 mg/kg BW showed a less significant difference of 0.23 ± 0.02 and 0.24 ± 0.02 when compared to the DSS control group.

3.5. Effect of MeEt on Serum and Tissue Cytokine Levels. The UC inflammatory response begins with an increased level of inflammatory cytokines, and the effect of MeEt extract on serum and tissue cytokine levels was examined using ELISA

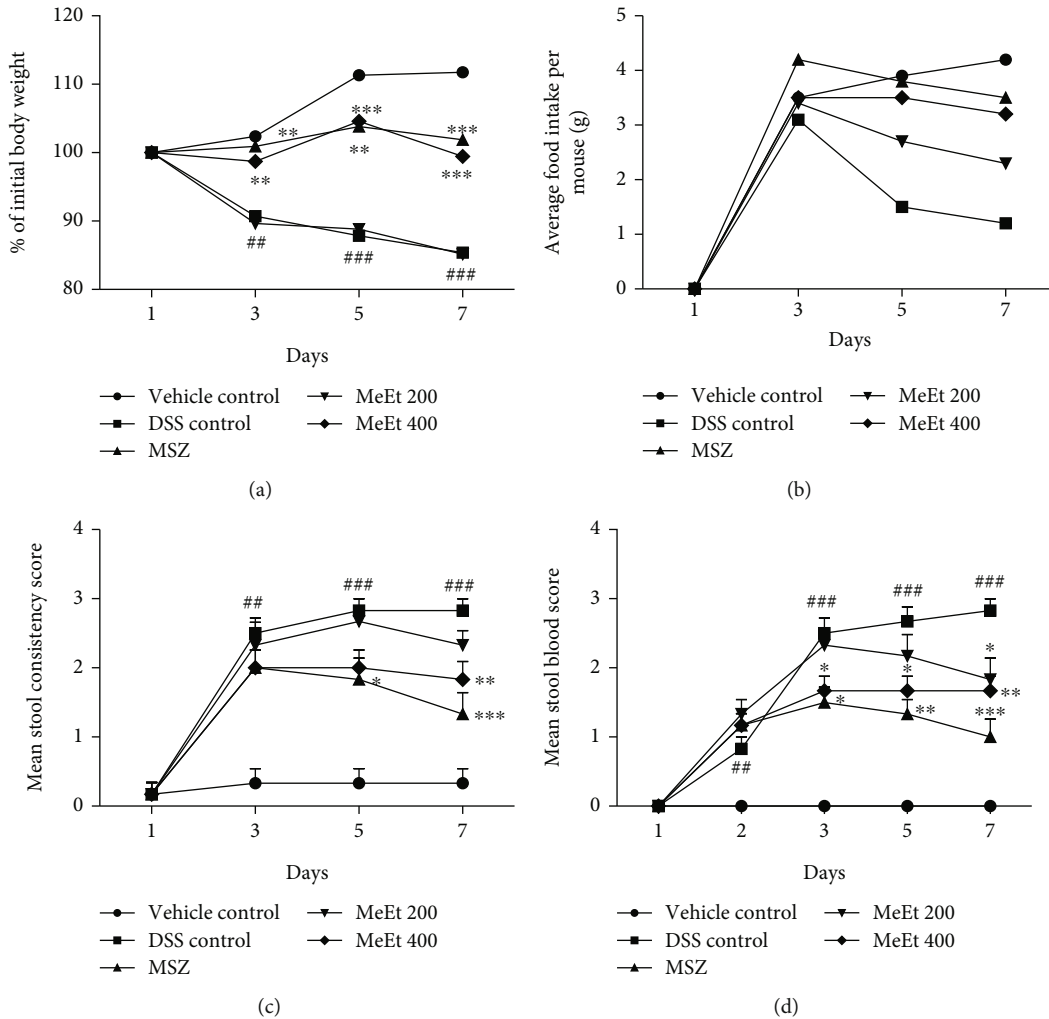


FIGURE 2: Effect of MeEt extract on body weight and disease activity index (DAI) against DSS-induced UC in mice. UC was induced in all the groups except vehicle control group by treating animals with the DSS (3%) in drinking water for 7 days. Mesalazine/MSZ (100 mg/kg BW) was administered orally as a standard drug. MeEt extract was administered orally at the doses of 200 and 400 mg/kg BW once a day for 7 days: (a) % of initial body weight; (b) average food intake per mouse (g); (c) mean stool consistency score; (d) mean stool blood score. All data are presented as mean + SEM ($n = 6$). Tukey's multiple comparison test along with a one-way ANOVA was utilized to identify the significant differences among the groups. $###p < 0.001$, $##p < 0.01$, and $*p < 0.05$, DSS control group vs. vehicle control group; $***p < 0.001$, $**p < 0.01$, and $*p < 0.05$, treatment groups vs. DSS control group.

kits (Figure 4). It was observed that in the DSS control group, the level of inflammatory cytokines such as IL-6, TNF- α , IL-1 β , IL-8, and IFN- γ was significantly increased, while due to the administration of DSS, the level of anti-inflammatory cytokines like IL-10 was significantly reduced as compared to the vehicle control group. Group treated with MeEt 400 showed a significant reduction in serum levels of IL-6 (74.2 ± 3.0), TNF- α (600.3 ± 30.1), IL-1 β (388.3 ± 1.8), IL-10 (102.4 ± 2.4), IL-8 (383.2 ± 16.4), and IFN- γ (664.8 ± 26.0) as compared to the DSS control group (88.5 ± 2.0 , 849.3 ± 68.8 , 487.9 ± 1.6 , 54.3 ± 6.4 , 520.8 ± 48.3 , and 900.3 ± 63.6 , respectively). This decreased level of serum cytokines was also observed in the group treated with the standard drug MSZ as compared to the DSS control group. In MeEt 200 treatment group, a significant decrease was only observed for IL-6 and IL-1 β (78.5 ± 2.5 and 478.5 ± 2.0 , respectively).

Further, oral administration of MeEt 400 also resulted in a decreased level of inflammatory cytokines in colon tissue. The statistical differences for MeEt 400 vs. DSS control group for IL-6 (65.4 ± 7.4 vs. 86.7 ± 8.2), TNF- α (580.0 ± 30.6 vs. 771.3 ± 44.9), IL-1 β (418.9 ± 40.5 vs. 574.5 ± 21.8), IL-10 (97.5 ± 9.0 vs. 3.7), IL-8 (408.5 ± 32.2 vs. 545.5 ± 26.6), and IFN- γ (527.5 ± 30.8 vs. 706.1 ± 40.3) were obtained significant. Treatment with the standard drug MSZ also showed a significant reduction in the above-mentioned cytokine level, while the group treated with MeEt 200 showed no significant reduction in the cytokine level of colon tissue.

3.6. *Effect of MeEt on Hematological Parameters.* Various hematological parameters were estimated to evaluate the efficacy of MeEt extract against UC. The findings obtained showed that in the DSS control group, the levels of white

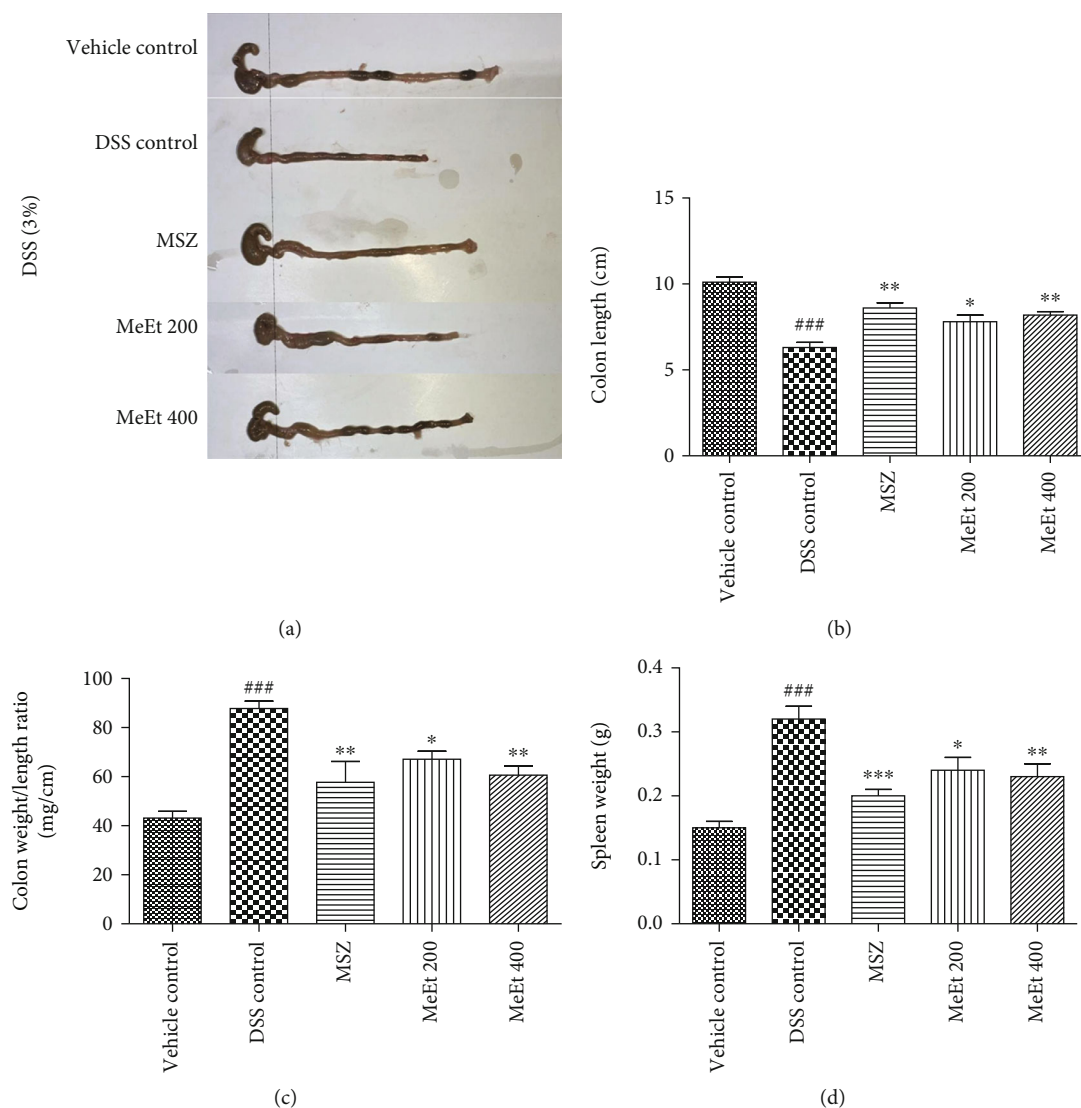


FIGURE 3: Effect of MeEt extract on colon and spleen tissues against DSS-induced UC in mice. UC was induced in all the groups except vehicle control group by treating animals with the DSS (3%) in drinking water for 7 days. Mesalazine/MSZ (100 mg/kg BW) was administered orally as a standard drug. MeEt extract was administered orally at the doses of 200 and 400 mg/kg BW once a day for 7 days. Animals from all the groups were sacrificed after 24 h of the last dose, and colons were harvested: (a) group-wise images of colon, harvested on day 7; (b) colon length; (c) colon weight/length ratio (mg/cm); (d) spleen weight (g). All data are presented as mean + SEM ($n = 6$). Tukey's multiple comparison test along with a one-way ANOVA was utilized to identify the significant differences among the groups. ^{###} $p < 0.001$, ^{**} $p < 0.01$, and ^{*} $p < 0.05$, DSS control group vs. vehicle control group; ^{***} $p < 0.001$, ^{**} $p < 0.01$, and ^{*} $p < 0.05$, treatment groups vs. DSS control group.

blood cells (WBC), lymphocytes, monocytes, and granulocytes increased significantly when compared to the vehicle control group, while due to DSS induction, the levels of red blood cells (RBC), HGB (hemoglobin), and platelet counts decreased significantly (Figure 5). In group treated with MeEt at 400 mg/kg BW, a significant decrease in WBC (7.9 ± 1.4 from 14.6 ± 1.7), lymphocytes (6.9 ± 0.8 from 12.5 ± 1.2), monocytes (22.9 ± 2.1 from 33.9 ± 2.9), and granulocytes (1.1 ± 0.3 from 5.3 ± 0.9) was recorded, while a significant increase in RBC (8.9 ± 1.7 from 4.2 ± 0.3), HGB (12.9 ± 1.2 from 6.3 ± 0.5), and platelets (522.3 ± 38.1 from 314.5 ± 36.0) was recorded. Treatment with MSZ at 100 mg/kg BW also showed significant decrease in WBC, lymphocytes, monocytes, and granulocyte levels

and also resulted in a significant increase in RBC, HGB, and platelet count. After the administration of MeEt at 200 mg/kg BW as compared to the DSS control group, the changes were insignificant.

3.7. Effect of MeEt on Serum Biochemical Parameters. UC is reported to be associated with liver and kidney disorders; thus, to evaluate it, different biochemical parameters were assessed. After the induction of DSS-induced UC, there was an increased level of serum biochemical enzymes such as SGPT, SGOT, uric acid, urea, creatinine, LDL, and bilirubin following the decreased levels of HDL and ALP. Treatment with MSZ at 100 mg/kg BW and MeEt at 400 mg/kg BW showed a significant reduction in the level of these

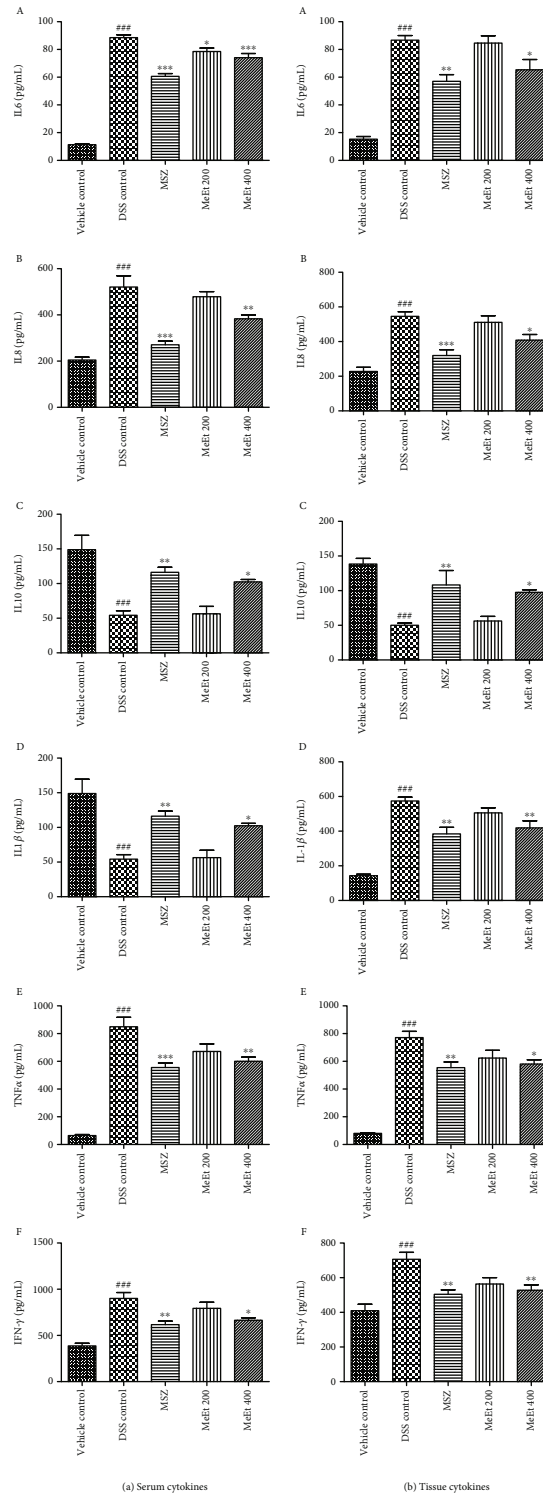


FIGURE 4: Effect of MeEt extract on the production of inflammatory cytokines in (a) serum and (b) colon tissues against DSS-induced UC in mice. Determination of cytokine production was done by ELISA. UC was induced in all the groups except vehicle control group by treating animals with the DSS (3%) in drinking water for 7 days. Mesalazine/MSZ (100 mg/kg BW) was administered orally as a standard drug. MeEt extract was administered orally at the doses of 200 and 400 mg/kg BW once a day for 7 days. (A) IL6 (pg/mL); (B) IL8 (pg/mL); (C) IL10 (pg/mL); (D) IL1β (pg/mL); (E) TNFα (pg/mL); (F) IFN-γ (pg/mL) production in colon tissue on day 7. All data are presented as mean + SEM (n = 6). Tukey's multiple comparison test along with a one-way ANOVA was utilized to identify the significant differences among the groups. ###p < 0.001, ##p < 0.01, and #p < 0.05, DSS control group vs. vehicle control group; ***p < 0.001, **p < 0.01, and *p < 0.05, treatment groups vs. DSS control group.

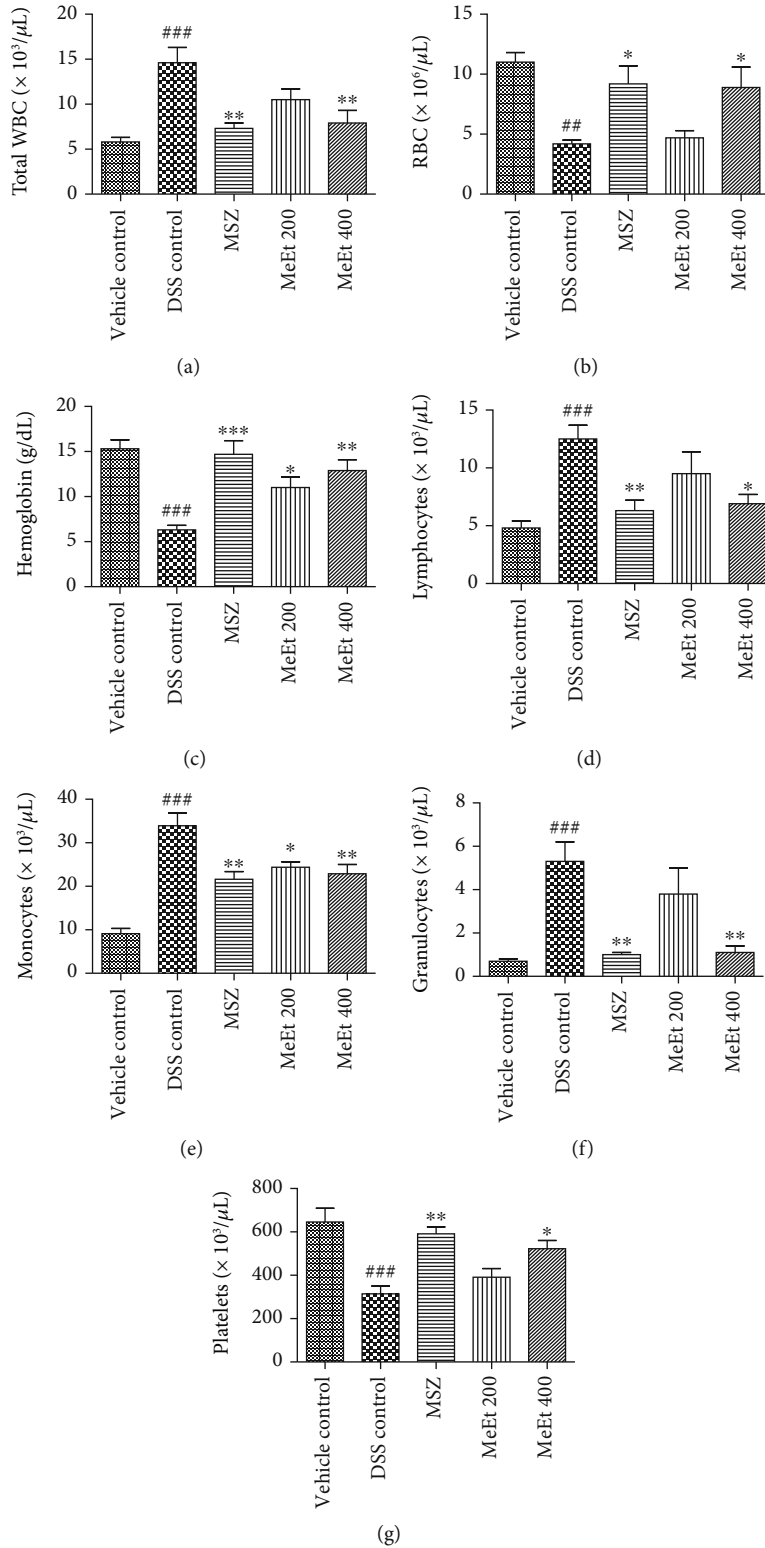


FIGURE 5: Effect of MeEt extract on hematological parameters against DSS-induced UC in mice. UC was induced in all the groups except vehicle control group by treating animals with the DSS (3%) in drinking water for 7 days. Mesalazine/MSZ (100 mg/kg BW) was administered orally as a standard drug. MeEt extract was administered orally at the doses of 200 and 400 mg/kg BW once a day for 7 days: (a) total WBC ($\times 10^3/\mu\text{L}$); (b) RBC ($\times 10^6/\mu\text{L}$); (c) hemoglobin (g/dL); (d) lymphocytes ($\times 10^3/\mu\text{L}$); (e) monocytes ($\times 10^3/\mu\text{L}$); (f) granulocytes ($\times 10^3/\mu\text{L}$); (g) platelets ($\times 10^3/\mu\text{L}$). All data are presented as mean + SEM ($n = 6$). Tukey's multiple comparison test along with a one-way ANOVA was utilized to identify the significant differences among the groups. ### $p < 0.001$, ## $p < 0.01$, and * $p < 0.05$, DSS control group vs. vehicle control group; *** $p < 0.001$, ** $p < 0.01$, and * $p < 0.05$, treatment groups vs. DSS control group.

TABLE 1: Effect of MeEt extract at 200 and 400 mg/kg BW on serum biochemical parameters against DSS-induced UC in mice.

Biochemical tests	Vehicle control	DSS control	MSZ	MeEt 200	MeEt 400
SGOT (U/L)	35.8 ± 3.7	89.7 ± 7.6 ^{###}	50.7 ± 5.9 ^{**}	63.1 ± 7.1 [*]	55.6 ± 6.5 ^{**}
SGPT (U/L)	39.7 ± 5.3	73.7 ± 6.8 ^{###}	43.7 ± 3.2 ^{**}	55.8 ± 2.9	46.4 ± 4.5 ^{**}
ALP (U/L)	364.9 ± 21.9	216.4 ± 23.4 ^{###}	326.8 ± 23.2 ^{**}	298.7 ± 19.6	319.2 ± 18.0 [*]
Creatinine (mg/dL)	13.4 ± 2.0	29.5 ± 3.8 ^{##}	16.7 ± 2.8 [*]	19.5 ± 3.6	17.8 ± 1.5
Urea (mg/dL)	4.7 ± 0.6	15.4 ± 3.1 ^{##}	7.2 ± 1.1 [*]	9.1 ± 1.6	8.1 ± 1.1 [*]
Uric acid (mg/dL)	2.0 ± 0.5	7.4 ± 0.7 ^{###}	3.2 ± 0.6 ^{***}	4.5 ± 0.9 [*]	4.11 ± 0.4 [*]
HDL (mg/dL)	96.3 ± 5.9	46.6 ± 5.7 ^{###}	85.6 ± 6.4 ^{**}	70.9 ± 8.2	82.6 ± 8.2 ^{**}
LDL (mg/dL)	13.8 ± 1.5	35.6 ± 5.2 ^{##}	16.5 ± 3.0 ^{**}	29.3 ± 2.9	18.1 ± 3.4 [*]
Bilirubin (mg/dL)	0.9 ± 0.1	3.8 ± 0.7 ^{##}	1.6 ± 0.2 [*]	3.1 ± 0.6	1.9 ± 0.3

All data are presented as mean ± SEM ($n=6$). Tukey's multiple comparison test along with a one-way ANOVA was utilized to identify the significant differences among the groups. ^{###} $p < 0.001$, ^{##} $p < 0.01$, and ^{*} $p < 0.05$, DSS control group vs. vehicle control group; ^{***} $p < 0.001$, ^{**} $p < 0.01$, and ^{*} $p < 0.05$, treatment groups vs. DSS control group.

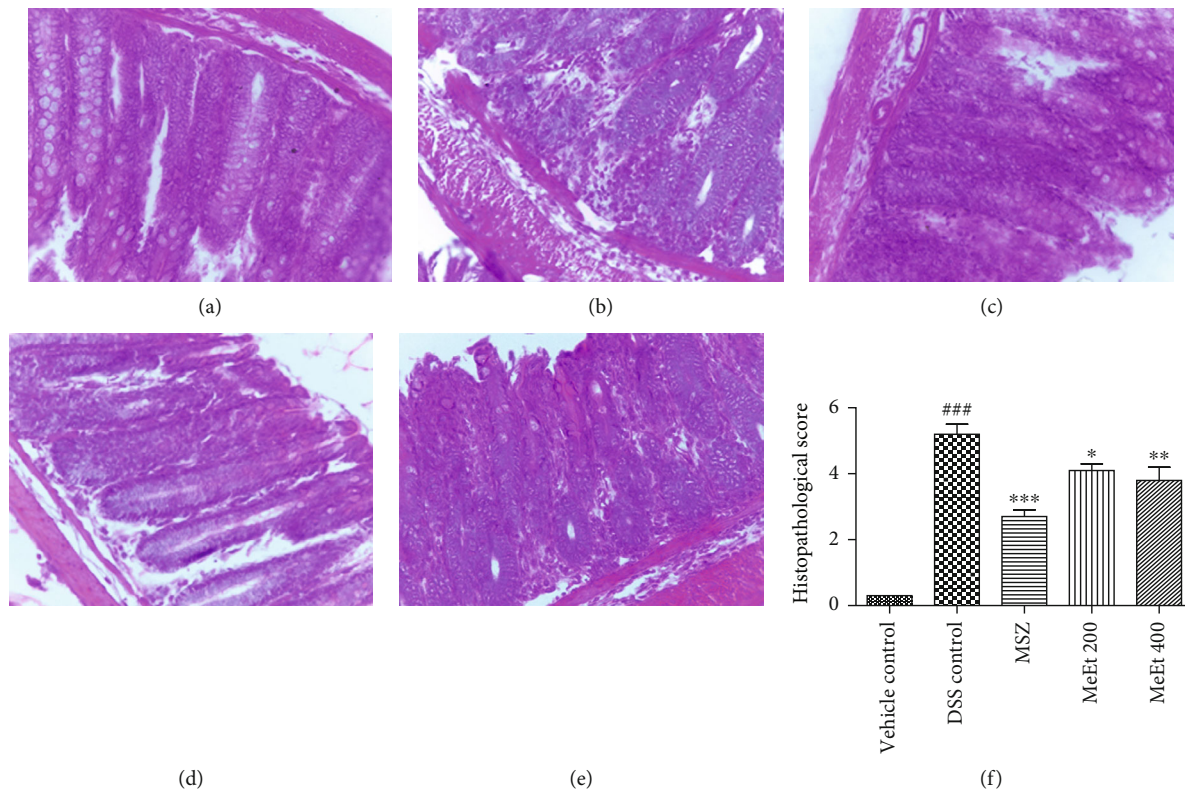


FIGURE 6: Microscopic analysis of colon tissue sections stained with H&E: (a) vehicle control group showing the normal architecture of colon with rare or no observation of inflammatory cells; (b) DSS control group showing degenerated and abnormal epithelium; (c) MSZ at 100 mg/kg BW treated group showing significant amelioration of inflammatory cell accumulation and protection as compared to DSS control group; (d) MeEt at 200 mg/kg BW showing less significant protection of epithelium as compared to DSS control group; (e) MeEt at 400 mg/kg BW showing moderate protection of epithelium as well as reduced inflammatory cell accumulation as compared to DSS control group. (f) Data of the histological analysis has been reported as colonic histopathological score, whereby the degree of colonic inflammation was scored semiquantitatively. All data are presented as mean ± SEM ($n=6$). Tukey's multiple comparison test along with a one-way ANOVA was utilized to identify the significant differences among the groups. ^{###} $p < 0.001$, ^{##} $p < 0.01$, and ^{*} $p < 0.05$, DSS control group vs. vehicle control group; ^{***} $p < 0.001$, ^{**} $p < 0.01$, and ^{*} $p < 0.05$, treatment groups vs. DSS control group.

enzymes (Table 1), while treatment with MeEt at 200 mg/kg BW resulted in a significant reduction only in the level of SGOT and uric acid, while all other parameters were nonsignificantly attenuated.

3.8. Histopathology of Colon Tissue. Colon tissues were stained with H&E (hematoxylin and eosin staining) and examined under an optical microscope at magnifications of 40x for histological analysis (Figure 6). The intestinal

TABLE 2: Histopathological scoring system*.

Histopathological score	Description
0	No sign of inflammation, intact crypt, and no ulceration foci.
1	Mild mucosal inflammation and damage to crypt and 1-2 ulceration foci.
2	Moderate inflammation that extends up to the submucosal layer, increased damage to crypt, and 3-4 ulceration foci.
3	Severe inflammation that extends to the <i>tunica muscularis</i> , entire damage to crypt, and immense ulceration.
4	Severely chronic inflammation and erosion that leads to change in epithelial surface.
5	Concurrent erosion of colonic epithelium.

*Histopathological scoring system is followed from Kim et al. [18].

epithelium is made up of a lamina propria layer and an epithelial cell layer. DSS induction caused damage to the epithelium. Findings of H&E staining of colon tissues demonstrated that in the control group, the epithelial cells were intact or normal; there was no sign of inflammation. Nevertheless, in the DSS control group, epithelial cells and crypt structures were severely injured. However, treatment with MSZ at a 100 mg/kg BW dose and MeEt at a 400 mg/kg BW dose attenuated the damage to the colon tissue. Further, histopathological scores were also determined based on the histopathological scoring system (Table 2), as described previously [19]. The lowest score was reported for the MSZ-treated group at 5.2 ± 0.3 and MeEt 400 at 3.8 ± 0.4 as compared to the DSS control group at 5.2 ± 0.3 . The scores were also found to be less significant in the MeEt 200 group in comparison to the DSS control group (Figure 6(f)).

4. Discussion

Numerous studies have shown and established the potential use of medicinal herbs in the management of inflammatory bowel diseases like UC. A seed, roots, stems, bark, leaves, and flowers are just a few of the components of numerous medicinal plants that are utilized in the treatment of this illness, all of which contain active chemical components that may possess anti-inflammatory properties. Such potent chemical substances affect different chemical mediators, enzymes, genes, or cellular processes by acting at several sites of the inflammatory pathways to ameliorate the cascade of inflammation in the body [23]. Standardization is the primary and vital step in ensuring the quality of the herbal drugs, and this ensures that the plant material is free from impurities. Our initial phytochemical analysis of MeEt extract found that flavonoids, phenolic compounds, triterpenoids, and alkaloids were present (Supplementary Table 2). Flavonoids and alkaloids from natural products are already well documented in the literature to have a prominent role in the treatment of UC [24, 25]. *Myrica esculenta* is reported to possess numerous pharmacological properties [11]. The stem bark is reported in Ayurvedic literature to be used for the treatment of various ailments like *aruchi* (anorexia), *arsa* (piles), *gulma* (abdominal tumors), *jvara* (fever), *grahani* (irregular bowel function), *pandu roga* (anemia), *hrillasa* (nausea), *mukha roga* (oral disorders),

kantha roga (ears, nose, and throat disorders), *kasa* (cough), *svasa* (dyspnea), and *agnimandya* (indigestion) [26]. Thus, the reverse pharmacological approach was conducted with the aim of evaluating the efficacy and mechanism of action of the MeEt extract of stem bark to alleviate the symptoms associated with UC. In this study, the protective effect of MeEt extract has been evaluated. MeEt extract during HPLC analysis showed the presence of myricetin [27], gallic acid [28], and quercetin (Figure 1). Various other chemical constituents have also been identified in MeEt extract, such as castalagin, epigallocatechin 3-O-gallate, chlorogenic acid, catechin, 3-hydroxybenzaldehyde, isovanillin, ferulic acid, myricitrin, myricanol, myricanone, myricanone, trihydroxytaraxaranoic acid, and dihydroxytaraxerane [12]. The administration of DSS (3%) has resulted in weight loss, diarrhea, anorexia, and blood in stool, which is the principal characteristic of the disease that may occur as a result of mucosal injury and reduced epithelial barrier function [29, 30]. MeEt at a dose of 400 mg/kg BW can relieve the symptoms of UC, which are comparable with those of the standard drug MSZ at 100 mg/kg BW. However, MeEt extract, when administered orally at a daily dose of 200 mg/kg BW, did not produce significant differences in the mice (Figure 2). After the oral administration of MeEt 400, when the colon examined, there was a prominent decrease in the weight-to-length ratio. The edema and increase in cell infiltration are mostly correlated with increased weight of the colon in proportion to tissue length. The decreased weight-to-length ratio (Figure 3) could indicate less active inflammation as a result of successful anti-inflammatory treatment [31]. Inhibiting the release of prostaglandins and histamine mediators, which produce mucus and cause mucosal edema, may be one of the effects of *M. esculenta* plant extracts on inflammatory mediators [32]. In UC animal models, splenomegaly is a reliable sign of intestinal inflammation, and reduction of spleen growth has frequently been used to confirm the moderating effects of medicines. Mice that received MeEt 400 and MSZ displayed a suppressive effect on spleen weight gain [33, 34]. In UC, different facets of the inflammatory response are under the control of cytokines. Proinflammatory and anti-inflammatory cytokine imbalances affect gut homeostasis and further the disease's progression. The DSS control group showed raised level of TNF- α , IL-6, IL-1 β , and IFN- γ (Figure 4). When an inflammatory response begins,

macrophages and lymphocytes both release TNF- α [35]. The proinflammatory cytokines TNF- α and IFN- γ play a significant role in the pathophysiology of UC by altering the tight junction and causing intestinal epithelial cells to undergo apoptosis [36]. TNF- α exerts its pleiotropic effects by causing the synthesis of adhesion molecules, fibroblast proliferation, and procoagulant factors, as well as the onset of cytotoxic, apoptotic, and acute phase responses. Additionally, it also has the ability to increase the production of cytokines like IL-1 and IL-6. When compared to normal colonic tissue, the tissue level of IL-8 was higher in active UC, and the serum concentration of IL-8 was also related to the endoscopic and histological severity of UC. IL-10, in contrast to all of these cytokines, inhibits antigen presentation and the production of proinflammatory cytokines, which in turn lessens inflammation in the mucosa. The major sources of IL-10 are macrophages and dendritic cells [37]. In the current study, MeEt extract suppressed the levels of IL-6, TNF- α , IL-8, IL-1 β , and IFN- γ , along with the possible restoration of IL-10 that may provide beneficial results in UC management. Apart from this influence, other biomarkers in UC are also studied. Hb and RBC levels are decreased in UC; this may be due to iron deficiency, which is the most prominent cause of anemia in UC [38]. Further WBC, monocytes, and lymphocytes provide initial line defense for the innate immune system [39] and are thus raised in UC. Therapy with MeEt 400 has suppressed their level, indicating its potent role against inflammation during UC. The most frequent extraintestinal symptoms of irritable bowel disease (IBD) are hepatobiliary changes and renal impairment. Moreover, atherosclerosis and coronary artery disease (CAD) are also associated with the development of UC. 5% of people will have chronic liver disease, and up to 30% of people will have abnormal liver tests [40–42]. Thereby, these alterations can be correlated with the disturbance in the levels of SGOT, SGPT, uric acid, HDL, and ALP. This study suggests that MeEt extract can reduce the occurrence of these extraintestinal symptoms. When the intestinal epithelial monolayer is disrupted, luminal bacteria and their associated antigens can enter the mucosa and spread proinflammatory intestinal contents into underlying tissue, causing intestinal inflammation in the DSS control group (Figure 6) [43]. MeEt 400 and MSZ have reduced the inflammation by preventing damage to the epithelial monolayer lining. Through our HPLC analysis, we can state that the protective effects of MeEt extract are observed due to the presence of myricetin, gallic acid, quercetin, and other chemical compounds in it that may have contributed to its potency in treating UC. This study thus suggests that MeEt extract at an oral dose of 400 mg/kg can serve as an alternative therapy for the management of UC and can also offer comparable treatment benefits as that of MSZ with fewer side effects. Moreover, the present study also showed that these attenuative effects are partly mediated by a decrease in number of inflammatory indicators, such as cytokines. These findings open the door to a more long-term strategy for treating UC, as current treatments that focus on a specific cytokine are likely to be constrained by the emergence of compensatory pathogenic pathways. This

study has confirmed the protective role of the ethanolic extract of *Myrica esculenta* extract against DSS-induced ulcerative colitis in mice.

5. Conclusion

Idiopathic UC is the most common type of IBD, with a prevalence of around 156 to 291 cases per one lakh people per year. Inflammation in the colon, diarrhea, and blood in the stool are the main characteristics of the disease. If the disease remains untreated, it may result in a life-threatening toxic megacolon. Current available therapies are associated with several side effects, and this disease requires treatment for a longer period of time, so herbal therapy can be considered for its management. In the current study, MeEt extract at doses of 200 and 400 mg/kg BW orally is evaluated in mice against DSS-induced UC and compared with the standard drug MSZ at 100 mg/kg BW. The consecutive seven-day treatment with MSZ and MeEt at a dose of 400 mg/kg showed suppressive effects on colonic inflammation by downregulating inflammatory cytokines such as IL-6, IL-8, IL-1 β , TNF- α , and IFN- γ . However, MeEt at 200 mg/kg BW did not show significant effects. Thus, it can be concluded that MeEt extract at 400 mg/kg BW may have the potential to serve as a therapeutic agent for the management of UC. Further, the future scope relies on developing a suitable herbal formulation and conducting clinical trials to further confirm the safety and efficacy of the plant.

Abbreviations

ALP:	Alkaline phosphatase
CD:	Crohn's disease
CBC:	Complete blood count
CCSEA:	Committee for Control and Supervision of Experiments on Animals
CAD:	Coronary artery disease
DAI:	Disease activity index
DSS:	Dextran sodium sulfate
ELISA:	Enzyme-linked immunosorbent assay
GIT:	Gastrointestinal tract
GA:	Gallic acid
HDL:	High-density lipoprotein
H&E:	Hematoxylin and eosin staining
HGB:	Hemoglobin
HPLC:	High-performance liquid chromatography
IBD:	Inflammatory bowel disease
IL-1 β :	Interleukin-1 beta
IL:	Interleukin (6, 8, 10)
IAEC:	Institutional Animal Ethic Committee
INF- γ :	Interferon gamma
LDL:	Low-density lipoprotein
LOD:	Loss on drying
Me:	<i>Myrica esculenta</i>
MeEt:	<i>Myrica esculenta</i> ethanolic extract
MSZ:	Mesalazine
My:	Myricetin
OECD:	Organisation for Economic Co-operation and Development

Q: Quercetin
 RBC: Red blood cell
 TNF- α : Tumor necrosis factor alpha
 TNBS: Trinitrobenzene sulfonic acid
 SGPT: Serum glutamic pyruvic transaminase
 SGOT: Serum glutamic oxaloacetic transaminase
 UC: Ulcerative colitis
 WBC: White blood cell.

Data Availability

All data were based on experimental studies.

Conflicts of Interest

The authors reflect no conflict of interest.

Authors' Contributions

All authors contributed equally to the concept, experimental study, and data analysis. MJ has planned the experimental study and written the manuscript. Further data analysis and manuscript review were done by MP. The final manuscript was reviewed, evaluated, and edited by AV.

Supplementary Materials

Supplementary 1. Supplementary Table 1 includes the preliminary photochemical studies carried out for the ethanolic extract of the plant *Myrica esculenta* as per the WHO guidelines and pharmacopeia. The data reveals the presence of structurally diverse classes of primary and secondary metabolites like alkaloids, carbohydrates, glycosides, flavonoids, saponin, tannins, resins, and phenolic compounds.

Supplementary 2. Supplementary Table 2 includes the data for the evaluation of purity of MeEt, standardized by physicochemical studies comprising moisture content, ash value, LOD, and extractive value.

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