Physiology and Pharmacology 28 (2024) 56-65
Experimental Research Article

Evaluation of anti-ulcerative effect of *Eryngium billardieri* extracts on experimental colitis in rats

Zahra Heydari[1], Masoud Sadeghi-Dinani[2], Ardeshir Talebi[3], Mohsen Minaiyan[1,4*]

1. Department of Pharmacology and Toxicology, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, Iran
2. Department of Pharmacognosy, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, Iran
3. Department of Clinical Pathology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran
4. Pharmaceutical Sciences Research Centre, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, Iran

ABSTRACT

Introduction: *Eryngium billardieri* has been demonstrated in previous studies to possess anti-inflammatory, antioxidant, and wound-healing properties. Colitis, an inflammatory bowel disease with unknown causes, often leads to numerous side effects associated with current medications. Therefore, this study aimed to investigate the anti-ulcerative potential of *E. billardieri* extracts in experimental colitis.

Methods: The hydroalcoholic extract of *E. billardieri* and its aqueous and ethyl acetate partitions were prepared using the maceration method, and the polyphenol content was determined for each extract. Male Wistar rats with acetic acid-induced colitis were orally administered three different doses (50, 100, 200 mg/kg) of the extract and each partition for 5 consecutive days. On the sixth day, the rats' colons were removed and analyzed for macroscopic parameters (ulcer index), microscopic parameters (total colitis index), as well as inflammatory and oxidative stress markers, including myeloperoxidase and malondialdehyde, respectively.

Results: The total phenol content for the dry extract and aqueous and ethyl acetate partitions were 6.51, 4.15, and 8.59 mg gallic acid equivalent/g, respectively. The hydroalcoholic extract and ethyl acetate partition at all three examined doses were able to significantly alleviate most parameters related to colitis. However, the aqueous partition did not improve most of the colitis features except for the tissue level of malondialdehyde.

Conclusion: The study concludes that the total extract of *E. billardieri*, as well as the ethyl acetate partition, exhibited anti-colitis properties in a dose-related manner. It is suggested that the effective substances responsible for these properties are non-polar compounds that are not extracted by aqueous partitioning. Further studies are needed to identify and characterize these effective compounds.

Keywords:
Anti-inflammatory
Ulcerative colitis
E. billardieri
Plant extract
Rats

Introduction

Inflammatory bowel disease (IBD) is a common and debilitating gastrointestinal (GI) disorder that affects both men and women almost equally (Ng et al., 2017). Ulcerative colitis and Crohn's disease are two main types of IBD (Perler et al., 2019).

* Corresponding author: Mohsen Minaiyan, miniayan@pharm.mui.ac.ir
Received 25 April 2023; Revised from 6 September 2023; Accepted 29 September 2023

Citation: Heydari Z, Sadeghi-Dinani M, Talebi A, Minaiyan M. Evaluation of anti-ulcerative effect of *Eryngium billardieri* extracts on experimental colitis in rats. Physiology and Pharmacology 2024; 28: 56-65. http://dx.doi.org/10.61186/phypha.28.1.56

The main causes of this disease are unknown, but researchers believe that it involves a complex interplay of factors including inflammation, autoimmunity, genetics, and environmental influences. Several hypotheses have been proposed to explain the pathology of IBD, including immune system dysregulation triggered by environmental or genetic factors, disturbances in gastrointestinal (GI) microbiota, mucosal integrity issues, oxidative stress, and dysregulation of cytokines such as NF-κB, nitric oxide, abnormal cyclooxygenase-2 activity, and leukotriene B4 (Baumgart et al., 2007; Ishihara et al., 2009).

Today, drugs such as mesalazine, sulfasalazine, glucocorticoids, and immunosuppressive drugs are commonly employed in the treatment of IBD (Cai et al., 2021).

The efficacy, safety, and inadequate potency of drugs, as well as the side effects and complaints of patients, have created a strong incentive to adopt new and effective therapeutic measures, including new biological agents (probiotics), monoclonal antibodies (e.g. infliximab, adalimomab) (Gajendran et al., 2019; Ungaro et al., 2019), as well as herbal medicine (Akshaya et al., 2019). Herbal remedies have long attracted the attention of the public and researchers due to their abundant and diverse effective ingredients, easy access, low cost, and minimal side effects (Akshaya et al., 2019).

Among medicinal plants, the genus Eryngium (Apiaceae family) stands out as one of the largest, most complex, and diverse genera. It is rich in secondary metabolites such as flavonoids, coumarins, terpenes, essential oils, and acetylene compounds, which possess numerous biological properties (Thiviya et al., 2021; Calvino et al., 2008).

Eryngium billardieri F. Delaroche. (*E. billardieri*) (Bughenagh in local language) is one of the species of this family that is utilized globally and in Iranian traditional medicine for treating various inflammatory disorders. These include conditions like rheumatoid arthritis, otitis, sinusitis, wounds, renal infections, insect bites, and hypothyroidism (Zargari, 2015). Also, antioxidant, analgesic, antibacterial, antifungal, anti-malarial, anti-snake, and scorpion venom activities have been indicated for this species (Wang et al., 2012).

The geographical distribution of Eryngium billardieri F. Delaroche spans several provinces in Iran, including East Azarbaijan, Hamadan, Isfahan, and Kurdistan. Its root and fruit contain compounds such as tannin, sucrose, saponin, and alkaloids. The aerial parts of the plant are rich in flavonoids, coumarones, beta-carotene, chlorogenic acids, terpenoids (mono and triterpenes), caffeic acid, kaempferol, and lutein. Many of these compounds possess anti-inflammatory and antioxidant properties (Zarei et al., 2015; Khani et al., 2021; Roshanravan et al., 2018), which could potentially be beneficial for the treatment of IBD.

Given the lack of prior research on the anti-colitis effects of *E. billardieri* and its readily available nature along with its diverse therapeutic properties, *E. billardieri* emerges as a promising candidate for the therapy and/or prevention of inflammatory bowel disease (IBD) in humans. Hence, this study aimed to explore the potential therapeutic or preventive effects of extracts and partitions of this plant in a murine model of colitis induced in rats.

Material and Methods

Plant material and preparation of extract

E. billardieri was collected from the Paveh region in Kermanshah province during the summer of 2022 at an altitude of 1500 meters above sea level. The collected plant material was dried in the shade. The aerial parts of the plant were finely chopped, and the dry powder obtained was used for extraction with a solvent consisting of ethanol/water (70/30) using the maceration method. The extraction process was repeated in three consecutive stages, each lasting for three days. Finally, the resulting extracts were combined and concentrated using a rotary evaporator (Handa et al., 2008).

To obtain partitions containing non-polar and semi-polar compounds from the plant, half of the total extract was concentrated using ethyl acetate solvent in three consecutive steps. The ethyl acetate layers collected from each step were combined. The aqueous residue from the remaining extract, containing more polar compounds, was concentrated using the rotary evaporator. Finally, the whole extract, along with the ethyl acetate and aqueous partitions, were dried using a freeze dryer (Handa et al., 2008).

The yield values for the extract and its partitions were determined, and the phenolic compounds in each were measured using the Folin-Ciocalteu reagent (Ainsworth et al., 2007). The final powdered extract was used to prepare the desired concentrations (50-200 mg/kg) for each extract.

Folin-Ciochalteu method

The standardization of both the hydroalcoholic extract and their partitions was conducted using the Folin-Ciocalteu method. According to this method, the polyphenol content of the extracts was measured relative to gallic acid. Initially, solutions with increasing concentrations of gallic acid (50, 100, 150, 250, 500 mg/kg) were prepared. Subsequently, the absorbance of these solutions was measured in the presence of Folin-Ciocalteu reagent and sodium bicarbonate at a wavelength of 765 nm. Finally, the total phenolic compounds of the extract and its partitions were determined as milligrams equivalent to gallic acid (GAE/mg) per gram of dry extract (Ainsworth et al., 2007).

Chemicals

Dexamethasone and mesalazine as pure powders were procured from Daru-Pakhsh Co. (Tehran, Iran). Navand Salamat (Urmia, Iran) Company prepared malondialdehyde (MDA) determination kit. Hexadecyl trimethyl ammonium bromide (HTAB), O-Dianisidine (ODZ), and gallic acid were prepared by Sigma-Aldrich Co. (Darmstadt, Germany).

Animals

Animal house of Isfahan School of Pharmacy prepared 78 male Wistar rats (180-220 g). The animals were housed at standard and desired humidity (40-50%), temperature (21-23 °C) and light/dark cycle (12/12 h) conditions. The rat's food and water were easily accessible. Three rats were kept in each cage. Each group of animals were kept in the animal room of the laboratory for one week in order to get acquainted with the laboratory environment.

This study received approval from Isfahan Medical Sciences University's Ethics Committee and all animal experiments performed in accordance with the general guidelines of the National Ethics Committee which emphasized the standards of care and work on animals (IR.MUI.RESEARCH.REC.1400.351).

Animal grouping

The animals were separated into thirteen groups of six rats: normal, negative control (colitis control), positive control (reference), and test groups as follows:

1: Normal group: Normal saline (5 ml/kg) was given orally (p.o.) for 5 days without colitis induction.

2: Negative control group: Normal saline (5 ml/kg, p.o.) was given for 5 days after colitis induction by acetic acid (2 mL, 3% intra-rectally).

3, 4, and 5: *E. billardieri* total (hydroalcoholic) extract (EBTE) groups: Increasing doses of extract (50, 100, and 200 mg/kg, p.o.) were given for 5 days after colitis induction (Zarei et al., 2015).

6, 7, and 8: *E. billardieri* aqueous partition (EBAP) groups: Increasing doses of aqueous partition (50, 100, and 200 mg/kg, p.o.) were given for 5 days after colitis induction.

9, 10, and 11: *E. billardieri* ethyl acetate partition (EBEAP) groups: Increasing doses of ethyl acetate partition (50, 100, and 200 mg/kg, PO) were given for 5 days after colitis induction.

12: Dexamethasone group: Dexamethasone (1 mg/kg, i.p.) was given for 5 days after colitis induction (Motavallian-Naeini et al., 2012).

13: Mesalazine group: Mesalazine (150 mg/kg, p.o.) was given for 5 days after colitis induction (Niknami et al., 2020).

Experimental protocol

Ulcerative colitis was induced in rats that had been fasted for 24 hours. Then, the rats were first anesthetized with a ketamine/midazolam mixture (75/5 mg/kg, i.p.), and 3% acetic acid (2 mL) was injected into the colon using a suitable tube. All interventions were administrated via gavage, 2 h before the induction of colitis, and then continued every 24 h for 5 days. Twenty-four hours after the last dose, the animals were euthanized in a CO2-saturated box. A length of 8 cm of colon tissue near the anus (3 cm) was removed and washed with normal saline. The Colon's wet weight was measured and subsequently used for macroscopic evaluation (Heidari et al., 2016).

Evaluation of colon macroscopic damage

The colon tissue was first spread on the working surface, and appropriate photos were taken with the help of a high-resolution camera. The ulcer surface area (cm^2) was measured using Fiji P (Image Analysis Program). Finally, the severity of injuries was graded as follows: 0 for no ulcer, 1 for inflammation, edema, thickness, and superficial ulcer, 2 for bleeding and deep ulcer, and 3 for severe ulceration, erosions, edema, and tissue necrosis. The ulcer index was calculated for each specimen by adding the ulcer score and the ulcer area, applying the

following formula:

(UI = US+UA) (Heidari et al, 2016; Niknami et al., 2020).

Evaluation of colon histological damage

The colon tissue was divided into two separate parts with a longitudinal section. One part was preserved in 10% formalin, sectioned, and stained with hematoxylin and eosin (H&E). Histological examination was conducted by a knowledgeable pathologist to assess the infiltration of immune cells, intensity and extent of mucosal inflammation, as well as the degree of crypt damage in the submucosal tissue (Minaiyan et al., 2008).

Measuring the myeloperoxidase (MPO) activity

The other part of the tissue was weighed and evenly chopped to maximize the extraction of MPO activity. Then, it was homogenized in 10 mM potassium phosphate buffer (pH 7) containing 0.5% HTAB. After centrifugation at 10,000 g for 30 minutes, H2O2 (0.1 mM) and ODZ (1.6 mM) were added to the supernatant solution, and the absorbance of the sample was measured at 450 nm. MPO activity (U/100 mg) was determined for the wet colon (Niknami et al, 2020).

Measuring the amount of MDA

According to the analytical kit, a standard curve was constructed within the range of 0 to 100 nM/ml, and the MDA concentration was determined using the colorimetric method at 550 nm. Briefly, 300µL of lysis buffer and 10 µL of BHT100x were added to 100 mg of colon tissue sample and homogenized. The mixture was then centrifuged at 13,000 rpm to remove insoluble materials, and the supernatant was completely separated. Subsequently, reagents were added to the supernatant and vigorously shaken. Finally, the amount of MDA (nmol/mL) was measured for the wet colon (Khoramian et al., 2020).

Statistical analysis

The results were reported as mean ± SEM and the minimal level of significance was considered at $P<0.05$. All statistical analyses were performed using the SPSS software, version 17.0.

Weight changes in the animals were analyzed by the paired Student's t-test. The differences between groups were examined using a parametric one-way analysis of variance (ANOVA) with Tukey as a post hoc test. The Kruskal-Wallis test was used to evaluate non-parametric data, followed by the Mann-Whitney U test.

Results

Yield value of the extract

The *E. billardieri* total (hydroalcoholic) extract (EBTE), aqueous partition (EBAP), and ethyl acetate partition (EBEAP) yielded 12.7% w/w, 10.1% w/w, and 2.6% w/w respectively.

TABLE 1: Weight of whole body (g) of rats in experimental groups before and after the treatments.

Groups/dose (mg/kg)	Before	After	Change %	P value
Normal	203.1 ± 13.4	212.2 ± 10.3	4.5	NS
Control	217.2 ±7.4	195.3 ± 7.2	-10.1	**
EBTE50	190.5 ± 9.7	189.2 ± 6.2	-0.7	NS
EBTE100	195.3 ± 11.5	198.3 ± 13.8	1.5	NS
EBTE200	192.8 ± 10.1	203.3 ± 11.9	5.4	NS
EBAP50	208.1 ± 8.4	195.2 ± 8.5	-6.2	*
EBAP100	191.5 ± 5.3	178.3 ± 8.8	-6.9	*
EBAP200	196.3 ± 10.3	181.1 ± 10.1	-7.7	*
EBEAP50	198.8 ± 5.8	193.5 ± 6.3	-2.7	NS
EBEAP100	207.5 ± 5.5	209.0 ± 9.3	0.7	NS
EBEAP200	197.2 ± 7.3	206.6 ± 5.3	4.7	NS
Mes.100	198.5 ± 3.4	203.5 ± 3.0	2.5	NS
Dex.1	196.8 ± 8.8	187.5 ± 11.8	-4.7	NS

Normal and control groups were treated with 5 ml/kg distilled water. EBTE: *E. billardieri* total extract, EBAP: *E. billardieri* aqueous partition, EBEAP: *E. billardieri* ethyl acetate partition. Dex: dexamethasone, Mes: mesalazine. Data are shown as mean ± SEM. The paired Student's t-test was used for analysis, (n= 6). *$P<0.05$ and **$P<0.01$ indicate significant difference. NS indicates non-significant difference

TABLE 2: Effect of *E. billardieri* total extract, aqueous, and ethyl acetate partitions on macroscopic parameters of colitis in rats.

Groups	Ulcer Severity score (0-4)	Ulcer Area (cm^2)	Ulcer Index (0-12)	Colon Weight (g/8 cm)
Norm.	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.59 ± 0.23
Control	2.8 ± 0.1$^{###}$	6.8 ± 0.4$^{###}$	9.8 ± 0.4$^{###}$	1.81 ± 0. 61$^{###}$
EBTE50	1.0 ± 0.2***	0.8 ± 0.2***	1.8 ± 0.4***	0.97 ± 0.39**
EBTE100	1.8 ± 0.4*	2.6 ± 0.6***	4.4 ± 1.0***	1.13 ± 0.51*
EBTE200	1.5 ± 0.2**	2.4 ± 0.2***	3.9 ± 0.4***	1.02 ± 0.34**
EBAP50	2.5 ± 0.2	4.8 ± 0.8	7.3 ± 0.8	1.15 ± 0.45
EBAP100	2.6 ± 0.2	5.8 ± 0.4	7.4 ± 0.6	1.34 ± 0.42
EBAP200	2.6 ±0.2	6.1 ± 0.3	8.7 ± 0.5	1.57 ± 0.58
EBEAP50	1.6 ± 0.3**	3.0 ± 0.8***	4.8 ± 1.1***	1.34 ± 0.56
EBEAP100	1.1 ± 0.1***	2.1 ± 0.2***	3.3 ± 0.2***	1.1 ± 0.21**
EBEAP200	0.8 ± 0.1***	1.7 ± 0.4***	2.5 ± 0.6***	0.88 ± 0.19***
Dex.1	0.6 ± 0.1***	1.5 ± 0.3***	2.2 ± 0.5***	0.75 ± 0.17***
Mes.100	1.0 ± 0.1***	1.6 ± 0.2***	2.6 ± 0.6***	0.83 ± 0.16***

Data are expressed as mean ± SEM, n=6. $^{###}$ P<0.001 indicates significant deference versus normal group. $^{*}P$<0.05, $^{**}P$<0.01, and *** P<0.001 indicate significant difference versus control. EBTE: *E. billardieri total extract* extract, EBAP: *E. billardieri* aqueous partition, EBEAP: *E. billardieri* ethyl acetate partition. Dex: dexamethasone, Mes: mesalazine. Data are shown as mean ± SEM. One way ANOVA test was used for analysis, (n= 6).

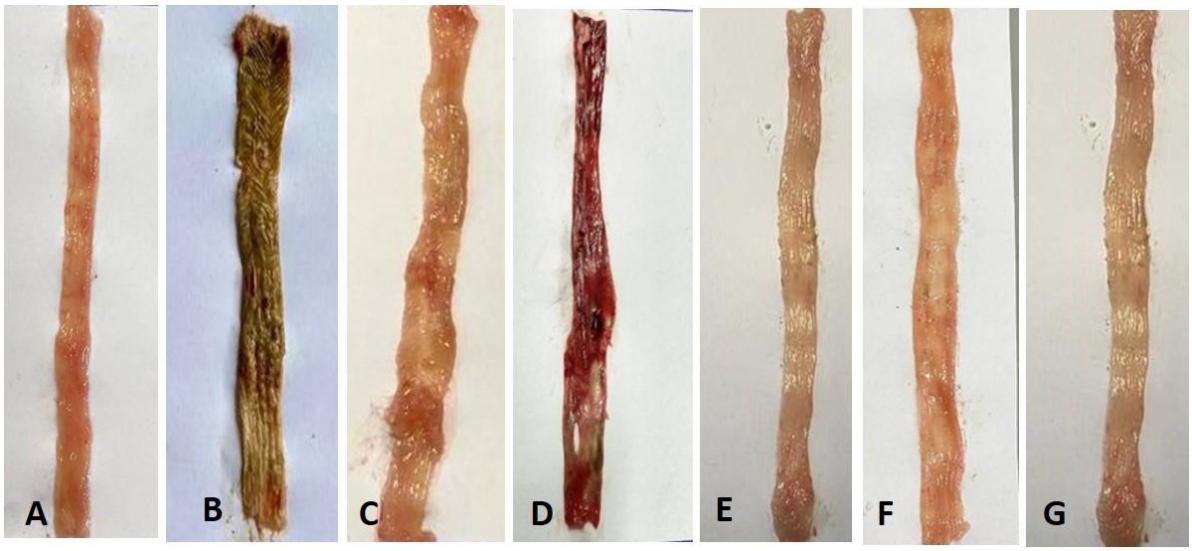

FIGURE 1. Macroscopic illustration of acetic acid-induced acute colitis in rats. (A) Normal, (B) Control (edema, erythema, thickness, ulcer, and necrosis are evident), (C) EBTE (*E. billardieri* total extract 100 mg/kg, p.o.), (D) EBAP (*E. billardieri* aqueous partition 100mg/kg, p.o.), (E) EBEAP (*E. billardieri* ethyl acetate partition 100mg/kg, p.o.), (F) Dexamethasone (1mg/kg), (G) Mesalazine (150 mg/kg).

Total content of the extract

Total phenolic content of the EBTE, EBAP, and EBEAP obtained were 6.51, 4.15, and 8.59 mg GAE/g extract respectively.

Animal weight changes

As seen in Table 1, there was a significant weight loss in the control group (P<0.01). Administration of *E. billardieri* total extract and ethyl acetate partition halted the weight loss. However, the aqueous partition did not effectively in reduce the weight loss of rats at any of the doses. The trend of weight loss was also halted with the administration of mesalazine, but dexamethasone did not prevent weight loss in the relevant group. Weight loss in the dexamethasone group was not statistically significant.

Macroscopic evaluation

Compared to the normal group, rats with colitis exhibited macroscopic damage (Table 2, Figure 1). Oral treat-

TABLE 3: Effect of *E. billardieri* total extract, aqueous, and ethyl acetate partitions on the pathologic (microscopic) parameters of colitis in rats.

Groups (doses)	Inflam. Severity (0-3)	Inflam. Extent (0-3)	Leuk. Infilt. (0-3)	Crypt damage (0-3)	Total colitis index (0-12)
Normal	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Control	3.0 (3-3)$^{###}$	3.0 (3-3)$^{###}$	3.0 (2-3)$^{###}$	3.0 (3-3)$^{###}$	12.0 (11-12)$^{###}$
EBTE50	1.5 (1-3)***	1.5 (1-3)***	1.0 (1-3)***	2.0 (1-3)*	6.0 (4-12)**
EBTE100	2.0 (1-3)*	2.0 (1-3)*	1.5 (0-2)**	2.5 (1-3)	8.0 (3-11)*
EBTE200	2.5 (1-3)	2.0 (1-3)*	2.0 (1-2)	2.5 (1-2)	9.0 (4-10)
EBAP50	2.5 (2-3)	2.5 (1-3)	2.0 (2-3)	2.5 (2-3)	9.5 (7-12)
EBAP100	2.5 (2-3)	2.5 (2-3)	2.5 (2-3)	3.0 (2-3)	10.0 (8-12)
EBAP200	2.5 (2-3)	2.5 (2-3)	2.0 (2-3)	2.5 (3-3)	9.5 (9-12)
EBEAP50	1.5 (1-3)***	1.5 (1-3)***	1.0 (1-2)***	1.0 (1-2)***	5.0 (4-10)***
EBEAP100	1.5 (1-2) ***	1.5 (1-2) ***	1.0 (1-2) ***	1.0 (1-2) ***	5.0 (4-8) ***
EBEAP200	1.0 (1-2) ***	1.0 (0-2) ***	1.0 (1-2) ***	1.0 (1-2) ***	4.0 (3-8) ***
Dex.1	0.5 (0-1) ***	0.5 (0-2) ***	0.5 (0-1) ***	1.0 (1-2) ***	2.5 (2-6) ***
Mes.100	1.0 (1-2) ***	1.0 (1-2) ***	0.5 (1-2) ***	1.5 (1-2) ***	3.0 (4-8) ***

Data are expressed as median (range). EBTE: *E. billardieri* total extract, EBAP: *E. billardieri* aqueous partition, EBEAP: *E. billardieri* ethyl acetate partition. Dex: dexamethasone, Mes: mesalasin. $P<0.05$ was considered as significant. * $P<0.05$, ** $P<0.01$, *** and ### $P<0.001$ indicate significant difference versus control (Mann-Whitney U test), (n=6).

ment with EBTE and EBEAP in all doses diminished the macroscopic parameters of colitis (at least $P<0.05$). However, the EBAP, on the contrary, was not significantly effective on any of the macroscopic parameters of colitis ($P>0.05$) (Table 2, Figure 1). Treatment with dexamethasone and mesalazine alleviated all the macroscopic features of colitis significantly ($P<0.001$) (Table 2, Figure 1).

Pathological evaluation

All pathological parameters of the negative control group, including inflammation extent and severity, leucocyte infiltration, and crypt damage, showed significant changes ($P<0.001$) compared to the normal group (Table 3, Figure 2).

Administration of EBTE and EBEAP at different doses (50, 100, and 200 mg/kg) reduced histopathological variables such as inflammation severity and extent, *infiltration of immune cells,* crypt destruction, and total colitis index (at least $P<0.05$) (Table3).

Consistent with the results obtained in the macroscopic evaluation, the aqueous partition of the EBAP was not effective on any of the microscopic parameters of colitis ($P>0.05$) (table 3). While, as expected, the reference drugs dexamethasone (1mg/kg) and mesalazine (100 mg/kg) effectively reduced all pathological parameters of colitis ($P<0.001$) (Table 3, Figure 2).

MPO activity and MDA values

The results in Table 4 show that the induction of colitis with acetic acid in rats (control group) was associated with a significant increase in MDA and MPO levels ($P<0.001$). Treatment with EBTE and its ethyl acetate partition (EBEAP) resulted in a significant decrease in MPO and MDA values (at least $P<0.05$). However, the aqueous partition at all three applied doses failed to significantly reduce MPO activity ($P>0.05$), although it did decrease MDA levels significantly. Also, the results showed that EBEAP at the doses of 100 and 200 mg/kg were significantly more effective than EBAP in this regard (at least $P<0.05$). As expected, the reference drugs dexamethasone and mesalazine effectively reduced MPO and MDA levels significantly ($P<0.001$) (Table 4).

Discussion

The acetic acid model of experimental colitis has been described as both quick and reproducible, with many pathological and clinical similarities to human colitis (MacPherson et al., 1978). Acetic acid induces inflammation by penetrating the mucous and sub-mucosal layers, initiating activity in the arachidonic pathway, and stimulating lipid peroxidase, lipoxygenase, and cyclooxygenase pathways (Kumar et al., 2014). Concurrently, immune cells such as neutrophils, which are the main source of inflammatory cytokine production, increase in

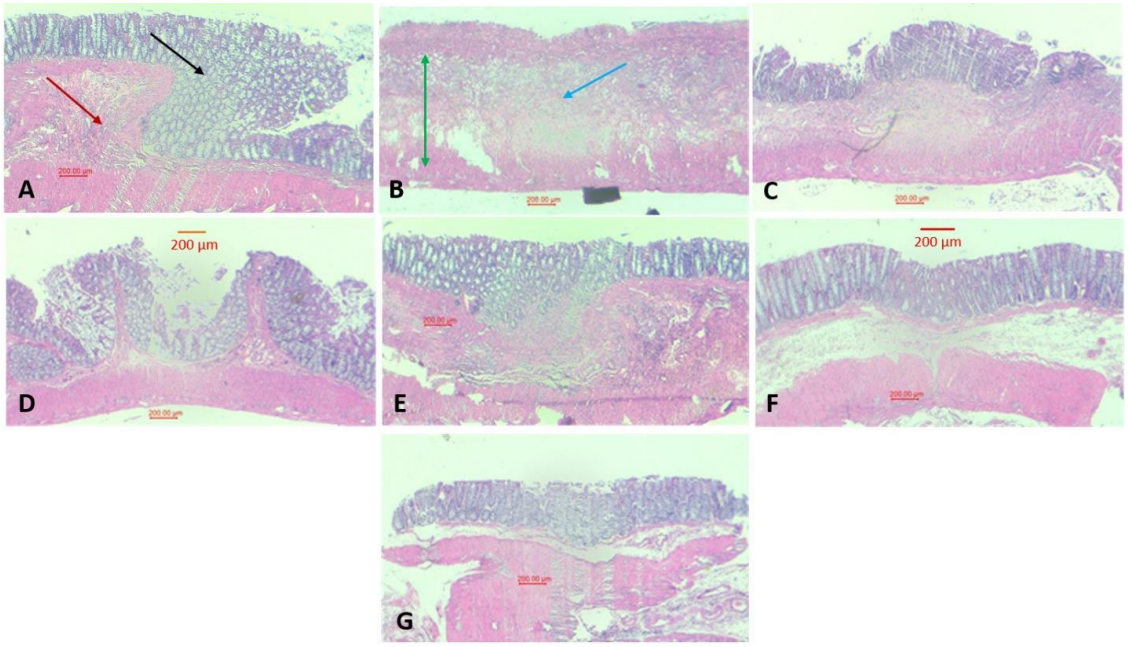

FIGURE 2. Microscopic illustration of acetic acid-induced acute colitis in rats. (A) Normal colon, mucosal, sub-mucosal layer (red arrow), and crypts (black arrow) are normal and intact, (B) Control colitis, mucosal layer, and crypts have destroyed while inflammation of sub-mucosal layer (green arrow) and leucocyte infiltration (blue arrow) are evident. (C) Colitis treated with EBTE (*E. billardieri* total extract 100 mg/kg, p.o.), (D) Colitis treated with EBAP (*E. billardieri* aqueous partition 100mg/kg, p.o., (E) colitis treated with EBEAP (*E. billardieri* ethyl acetate partition 100mg/kg, p.o.), (F) Colitis treated with dexamethasone (1 mg/kg), (G) Mesalazine (150 mg/kg). The treatment groups show varying degrees of improvement in the histological aspects of colitis.

TABLE 4: Effect of *E. billardieri* total extract, aqueous, and ethyl acetate partitions on the myeloperoxidase (MPO) activity and malondialdehyde (MDA) level of colon tissue.

Groups/dose (mg/kg)	MPO (U/100 mg)	MDA (nmol/ml)
Normal	0.53±0.3	68.8±11.2
Control$^{###}$	2.75±0.34$^{###}$	471.5±79.3$^{###}$
EBTE50	1.01±0.5**	180.8±43.3***
EBTE100	1.31±0.5**	206.4±48.5***
EBTE200	1.58±0.3**	257.3±51.3**
EBAP50	1.94±0.4	269.3±46.1**
EBAP100	1.98±0.4	283.2±52.3**
EBAP200	2.07±0.3	301.3±55.9*
EBEAP50	0.97±0.3***	154.4±33.7***
EBEAP100	0.65±0.3***+	111.6±26.1***
EBEAP200	0.54±0.2***+	97.1±24.9***+
Dex.1	0.41±0.2***	86.6±26.3***
Mes.100	0.63±0.2***	113.3±33.3***

Data are expressed as mean ± SEM. $^{###}$ $P < 0.001$ significant deference versus normal group. $^{*}P<0.05$, $^{**}P<0.01$, and $^{***}P<0.001$ indicate significant difference versus control group, $+P<0.05$ indicates significant difference versus EBAP with similar dose (one way ANOVA), (n=6). EBTE: *E. billardieri* total extract, EBAP: *E. billardieri* aqueous partition, EBEAP: *E. billardieri* ethyl acetate partition. Dex: dexamethasone (1mg/kg), Mes: mesalazine (150 mg/kg).

the control groups receiving acetic acid, leading to elevated MPO and MDA values. Thus, all colitis features are evident in the control colitis group and are reflected in macroscopic and pathologic variables (Gholap et al, 2012).

Our findings showed that experimental colitis significantly resulted in weight loss in rats, consistent with previous studies (Niknami et al, 2020; Khoramian et al., 2020). Loss of appetite, increased bowel movement, and diarrhea likely contribute to this weight loss. Treatment with the total extract and ethyl acetate partition could prevent this weight loss, while the aqueous partition was ineffective. The tannins and alkaloids in *E. billardieri* extracts may play an important role in this effect due to their astringent and antispasmodic properties (Khani et al, 2021).

Both macroscopic findings (ulcer index) and the histology results (total colitis index) demonstrated that the total extract and its ethyl acetate partition of the *E. billardieri* showed a dose-related anti-ulcerogenic activity. Eryngium species are effective against various inflammatory conditions and a wide range of GI ailments which have been mentioned in the Iranian traditional medicines and worldwide (Zargari, 2015). The results of other investigations have indicated that this plant possesses various biological effects, including anti-inflammatory, antioxidant, wound healing, anti-apoptotic, and antibacterial properties. Each of these effects may contribute to the anti-colitis effect observed with the use of this plant (Zarei et al., 2015, Yesilada et al., 1989).

The aqueous partition demonstrated a significant reduction in the level of MDA across all three examined doses, while showing no significant effect on other variables such as ulcer index, total colitis index and MPO activity). Since MDA serves as an indirect measure of the antioxidant effect of the extracts, it is likely that the antioxidant effect alone may not suffice to protect the colon tissue against the ulcer-causing agent. However, considering that natural antioxidants and phytochemicals typically exhibit multifunctional properties, a more comprehensive antioxidant assay would involve the measurement of multiple properties of this system for greater reliability (Tahan et al., 2011; Frankel at al., 2000).

The effectiveness of ethyl acetate and total extracts, contrasted with the ineffectiveness of the aqueous extract at similar doses, suggests that the active compounds with anti-colitis and anti-ulcer properties are probably non-polar in nature. these compounds likely accumulated in the ethyl acetate fraction, exerting a significant impact on the colitis indices (Sefidkon et al., 2004; Wang et al., 2012). This observation aligns with the total phenolic content of the extracts. Specifically, the ethyl acetate partition (8.59mg GAE/g), which exhibited the most promising results, contained approximately twice the polyphenol content of the aqueous partition (4.15 mg GAE/g) and 50% more than the total extract (6.51mg GAE/g). Tannins, lignins, flavonoids, and terpenoids are among the most important and abundant phenolic components of *E. billardieri*, while some of the alkaloids, saponins, coumarone, chlorogenic acid, caffeic acid, and beta carotene are among the active ingredients of *E. billardieri* are predominantly non-polar or semi-polar in nature and tend to be accumulated in hydroalcoholic and ethyl acetate partition (Sefidkon et al., 2004; Wang et al., 2012).

In this study, the therapies were administered orally over a period of 6 days. Therefore, it is plausible that the effective substances in treating colitis were able to be sufficiently absorbed or reach the colon through the digestive lumen. Additionally, this timeframe allowed for the contribution of delayed mechanisms. These mechanisms likely include the scavenging of oxidoreductases and the repair of mucosal layers (Minaiyan et al., 2014; Siddhuraju et al., 2003).

Both the roots and the aerial sections of the plant contain high concentrations of polyphenolic chemicals, which are abundant in *E. billardieri*. Secondary plant metabolites, known as phenolic compounds, play a vital role in the defensive systems of plants against pathogens and free radicals (Maisuthisaul et al., 2007; Khani et al., 2021; Zarei et al., 2015).

E. billardieri contains antioxidant chemicals, which indicate the scavenging action caused by hydrogen proton donation (Daneshzadeh et al., 2020). In this regard, flavonoids represent a subgroup of phenolic chemicals within a broader category, and the majority of the positive benefits of *E. billardieri* may be attributed to their antioxidant and anti-inflammatory capabilities. Inhibition of cytokine-stimulated nitric oxide synthesis, an anti-inflammatory mechanism related to hydroalcoholic extract, has also been reported by Wang et al., 2012. Due to limitations in laboratory equipments and budget, it is recommended to isolate at least one group of effective

ingredients (*e.g.* flavonoids) from each extract or partition and conduct further studies on them. Furthermore, measuring inflammatory cytokines most related to colitis, such as interleukins, TNF-alpha, and NF-kB, would be valuable for further mechanistic studies.

Conclusion

This study showed that the total (hydroalcoholic) extract of *E. billardieri*, along with its ethyl acetate partition, effectively reduced macroscopic, microscopic, MDA, and MPO activity parameters, leading to an improvement in experimental colitis. These effects are likely attributed to the presence of compounds with antioxidant, anti-inflammatory, and wound healing properties. However, to develop new drugs or supplements based on this plant, further analytical, mechanistic, and toxicological studies are required in both preclinical and clinical settings.

Acknowledgement

The authors might intend to express gratitude to their University's Vice Chancellor of Research and Technology for financial support of this project approved upon 3400616 codes.

Conflict of interest

The authors declare that they have no conflict of interest regarding to this work.

References

Akshaya K, Chitra V. A review on pathological state and herbal remedies on ulcerative colitis. Res J Pharm Technol. 2019; 12(3): 1409-17. https://doi.org/10.5958/0974-360X.2019.00235.X

Baumgart D C, Carding S R. Inflammatory bowel disease: cause and immunobiology. The Lancet. 2007; 12;369: 1627-40. https://doi.org/10.1016/S0140-6736(07)60750-8

Cai Z, Wang S, Li J. Treatment of inflammatory bowel disease: a comprehensive review. Front Med. 2021; 20(8): 765474. https://doi.org/10.3389/fmed.2021.765474

Calvino C I, Martínez S G, Downie S R. The evolutionary history of Eryngium (Apiaceae, Saniculoideae): rapid radiations, long distance dispersals, and hybridizations. Mol Phylogenet Evol. 2008; 46(3): 1129-50. https://doi.org/10.1016/j.ympev.2007.10.021

Cosnes J, Gower-Rousseau C, Seksik P, Cortot A. Epidemiology and natural history of inflammatory bowel diseases. Gastroenterology. 2011; 140(6): 1785-94. https://doi.org/10.1053/j.gastro.2011.01.055

Daneshzadeh M S, Abbaspour H, Amjad L, Nafchi A M. An investigation on phytochemical, antioxidant and antibacterial properties of extract from Eryngium billardieri F. Delaroche. J Food Meas Chara. 2020; 14(2): 708-15. https://doi.org/10.1007/s11694-019-00317-y

Frankel E N, Meyer A S. The problems of using one-dimensional methods to evaluate multifunctional food and biological antioxidants. J Sci Food Agr. 2000; 80(13): 1925-41. https://doi.org/10.1002/1097-0010(200010)80:13<1925::AID-JSFA714>3.0.CO;2-4

Gajendran M, Loganathan P, Jimenez G, Catinella A P, Ng N, Umapathy C, et al. A comprehensive review and update on ulcerative colitis. Dis Mon. 2019; 65(12): 100851. https://doi.org/10.1016/j.disamonth.2019.02.004

Gholap P A, Nirmal S A, Pattan S R, Pal S C, Mandal S C. Potential of Moringa oleifera root and Citrus sinensis fruit rind extracts in the treatment of ulcerative colitis in mice. Pharm Biol. 2012; 50(10): 1297-02. https://doi.org/10.3109/13880209.2012.674142

Handa S S, Khanuja S P, Longo G, Rakesh D U. editors. Extraction technologies for medicinal and aromatic plants. 1st ed. Trieste: ISC-UNDO publication; 2008.

Heidari B, Sajjadi S E, Minaiyan M. Effect of Coriandrum sativum hydroalcoholic extract and its essential oil on acetic acid- induced acute colitis in rats. Avicenna J Phytomed 2016; 6(2): 205-14.

Ishihara S, Aziz M M, Yuki T, Kazumori H, Kinoshita Y. Inflammatory bowel disease: review from the aspect of genetics. J Gastroenterol. 2009; 44: 1097-08. https://doi.org/10.1007/s00535-009-0141-8

Khani S, Abdollahi M, Asadi Z, Nazeri M, Nasiri M A, Yusefi H, et al. Hypoglycemic, hepatoprotective, and hypolipidemic effects of hydroalcoholic extract of Erynium billardieri root on nicotinamide/streptozotocin-induced type II diabetic rats. Res Pharm Sci. 2021; 16(2): 193. https://doi.org/10.4103/1735-5362.310526

Khoramian L, Sajjadi SE, Miniaiyan M. Anti-inflammatory effect of Adiantum capillus-veneris hydroalcoholic and aqueous extracts on acetic acid-induced colitis in rats. Avicenn J Phytomed 2020; 10(5): 492-03.

Kumar V S, Rajmane A R, Adil M, Kandhare A D, Ghosh P, Bodhankar S L. Naringin ameliorates acetic acid induced colitis through modulation of endogenous oxido-nitrosative balance and DNA damage in rats. J Biomed Res. 2014; 28(2): 132. https://doi.org/10.7555/JBR.27.20120082

MacPherson B, Pfeiffer C. Experimental production of diffuse colitis in rats. Digestion. 1978; 17(2): 135-50. https://doi.org/10.1159/000198104

Maisuthisakul P, Suttajit M, Pongsawatmanit R. Assessment of phenolic content and free radical-scavenging capacity of some Thai indigenous plants. Food Chem. 2007; 100(4): 1409-18. https://doi.org/10.1016/j.foodchem.2005.11.032

Minaiyan M, Ghannadi A, Mahzouni P, Nabi-Meibodi M. Anti-ulcerogenic effect of ginger (rhizome of Zingiber officinale Roscoe) hydroalcoholic extract on acetic acid-induced acute colitis in rats. Res Pharm Sci. 2008;3(2):79-86.

Minaiyan M. Sajjadi SE, Naderi N, Taheri D. Anti-inflammatory effect of Kelussia odoratissima Mozaff. hydroalcoholic extract on acetic acid- induced acute colitis in rats. J Reports Pharm Sci 2014; 3(1): 28-35. https://doi.org/10.4103/2322-1232.222545

Motavallian-Naeini A, Minaiyan M, Rabbani M, Mahzuni P. Anti-inflammatory effect of ondansetron through 5-HT3 receptors on TNBS-induced colitis in rat. EXCLI J. 2012; 11:30.

Ng S C, Shi H Y, Hamidi N, Underwood F E, Tang W, Benchimol E I, et al. Worldwide incidence and prevalence of inflammatory bowel disease in the 21st century: a systematic review of population-based studies. Lancet. 2017; 390: 2769-78. https://doi.org/10.1016/S0140-6736(17)32448-0

Niknami E, Sajjadi S E, Talebi A, Minaiyan M. Protective effect of Vitis vinifera (Black Grape) seed extract and oil on acetic acid-induced colitis in rats. Int J Prev Med 2020; 362: 19.

Perler B K, Ungaro R, Baird G, Mallette M, Bright R, Shah S, et al. Presenting symptoms in inflammatory bowel disease: descriptive analysis of a community-based inception cohort. BMC Gastroenterol. 2019;19(1), 1-8. https://doi.org/10.1186/s12876-019-0963-7

Roshanravan N, Asgharian P, Dariushnejad H, Alamdari N M, Mansoori B, Mohammadi A, et al. Eryngium billardieri induces apoptosis via bax gene expression in pancreatic cancer cells. Adv Pharm Bull. 2018; 8(4): 667. https://doi.org/10.15171/apb.2018.075

Sefidkon F, Dabiri M, Alamshahi A. Chemical composition of the essential oil of eryngium billardieri F. delaroche from Iran. J Essen Oil Res. 2004; 16(1): 42-3. https://doi.org/10.1080/10412905.2004.9698648

Siddhuraju P, Becker K. Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (Moringa oleifera Lam.) leaves. J Agr Food Chem. 2003; 51(8): 2144-55. https://doi.org/10.1021/jf020444+

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

Thiviya P, Gamage A, Piumali D, Merah O, Madhujith T. Apiaceae as an important source of antioxidants and their applications. Cosmetics. 2021; 8(4): 111. https://doi.org/10.3390/cosmetics8040111

Ungaro R, Colombel J F, Lissoos T, Peyrin-Biroulet L. A treat-to-target update in ulcerative colitis: a systematic review. Am J Gastroenterol. 2019; 114(6): 874. https://doi.org/10.14309/ajg.0000000000000183

Wang P, Su Z, Yuan W, Deng G, Li S. Phytochemical constituents and pharmacological activities of Eryngium billardieri (Apiaceae). 2012; 3(1): 99-120. https://doi.org/10.2174/2210290601203010099

Yesilada E, Tanaka S h, Tabata M, Sezik E. The anti-inflammatory activity of the fractions from Eryngium billardieri in mice. Phytother Res. 1989; 3(1): 38-40. https://doi.org/10.1002/ptr.2650030111

Zarei A, Ashtiyani S C, Hamidizadeh S, Rezaei A, Ramezani M, Aalizadeh A. The study of the effects hydro-alcoholic extract of Eryngium billardieri on lipid profiles levels and liver and renal functions tests in hypercholesterolemic rats. J Chem Pharm Res. 2015; 7(2): 200-6.

Zargari A. Pharmaceutical plants (persian). 8th ed. Tehran: Tehran University Press; 2015. 212-4.