Research Journal of Pharmacognosy (RJP) 11(2), 2024: 61–70
Received: 9 Jan 2024
Final revision: 21 Feb 2024
Accepted: 4 March 2023
Published online: 6 Mar 2024
DOI: 10.22127/RJP.2024.434773.2323

Original article

Assessing the Anti-Colitis Properties of Aqueous and Hydroalcoholic Extracts of *Pinus eldarica* in Rats with Acetic Acid-Induced Colitis

Mohsen Minaiyan[1*], Zahra Dastanian[2], Behzad Zolfaghari[3], Ardeshir Talebi[4]

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

Abstract

Background and objectives: Ulcerative colitis is a challenging inflammatory bowel disease that requires new treatments. *Pinus eldarica* can be a suitable candidate for this disease due to its anti-inflammatory, anti-ulcerative and antioxidant properties. **Methods**: *Pinus eladarica* aqueous and hydroalcoholic extracts of barks were standardized according to the total phenols, flavonoids and proanthocyanidin contents. Three doses (100, 200, and 400 mg/kg, p.o.) of both extracts were separately administered to rats with acetic acid-induced colitis for a period of five days. Reference groups received dexamethasone (1 mg/kg, i.p.) or mesalazine (150 mg/kg, p.o.) while control groups were treated with normal saline. **Results**: Both extracts reduced the macroscopic parameters of colitis (weight of colon, ulcer area, ulcer severity and ulcer index) significantly compared with control groups, especially in lower doses (100, 200 mg/kg). Similarly, the extracts improved the microscopic parameters (severity and extent of inflammation, leukocyte infiltration, crypt damage, and total colitis score) except for the dose of 400 mg, which was not effective. The decrease in myeloperoxidase activity and malondeladehyde values was also significant for both extracts at all doses. **Conclusion**: *Pinus eldarica* bark extracts are effective in treating and reducing the damage caused by colitis, although it is necessary to adjust the effective dosage. Lower doses of extracts, especially hydroalcoholic one showed better therapeutic effects. Further studies are necessary to identify effective compounds, particularly in the hydroalcoholic extract, for producing an herbal drug for the clinical setting.

Keywords: inflammation; *Pinus eldarica*; plant extract; rat; ulcerative colitis

Citation: Minaiyan M, Dastanian Z, Zolfaghari B, Talebi A. Assessing the anti-colitis properties of aqueous and hydroalcoholic extracts of *Pinus eldarica* in rats with acetic acid-induced colitis. Res J Pharmacogn. 2024; 11(2): 61–70.

Introduction

Inflammatory bowel disease (IBD) is well-known as ulcerative colitis and Crohn's disease as gastrointestinal disorder. IBD is a complex disorder and is influenced by multiple factors, which are combination of genetics, environmental, immune system, and microbial factors. Additionally, other factors which have a critical impact on the IBD pathogenesis are oxidative stress, gut microbiota, nuclear factor-kappa B (NF-κB), and nitric oxide (NO) [1]. An

*Corresponding author: minaiyan@pharm.mui.ac.ir

imbalance between pro-inflammatory cytokines such as tumor necrosis factor (TNF-α), interleukins (IL-8, IL-17A) and anti-inflammatory cytokines (IL-4, IL-13) could ultimately lead to immune system disorders like IBD [2]. One of the well-known treatment strategies for IBD is the administration of 5-aminosalicylates derivatives which inhibit cyclooxygenase and lipoxygenase in the arachidonic acid metabolic pathway as well as scavenging oxidoradicals and diminishing oxidative stress in engaged tissue [3]. Corticosteroids, immunomodulatory drugs (thiopurines and methotrexate), monoclonal antibodies (adalimumab and infliximab) and recently probiotics are other approved conventional therapies for these conditions [4,5]. Due to the rising cost of conventional drugs and concerns about their side effects, both therapists and patients are eager to use alternative or complementary medicine. The identification of different potent compounds found in medicinal herbs, including alkaloids [6], flavonoids [7], polyphenols [8], and terpenoids [9], have provided promising and safe treatment options for IBD and especially ulcerative colitis. As a result, there has been an increase in research on traditional and indigenous herbal plants, in which *Pinus* species are promising as novel alternative interventions. One of the well-known species of *Pinus* is *Pinus eldarica* Medw. (*P. eldarica*, Tehran Pine, Afghan Pine), which belongs to the Pinaceae family [10]. Different parts of Tehran pine (nuts, needles, cones and bark) have been used in traditional Asian medicine amongst the most important uses could be mentioned are asthma, allergy, dermatitis, skin wounds, high blood pressure and diabetes mellitus [11-13]. Meanwhile, the nuts, resin, and essential oil of this plant are abundantly used in the food, pharmaceutical, and cosmetic industries [14]. This plant has shown antioxidant and anti-inflammatory effects due to the various biologically active components such as α-pinene, β-pinene, β-caryophyllene, longifolene, α-humulene, δ-3-carene, junipene and high quantities of phenolic compounds including catechin, ferulic acid, caffeic acid, and taxifolin [14-16]. Since no study has been conducted on the anti-colitis effects of *P. eldarica* bark, this study was carried out to investigate this property. Therefore, the well-known model of induction with acetic acid was used and the aqueous and hydroalcoholic extracts of *P. eldarica* bark were evaluated in three increasing doses.

Material and Methods

Ethical considerations

The guideline for the care and use of laboratory animals represented by Iran national committee for ethics in animal research accessible on Ministry of Health and Medical Education website was followed. The Ethics and Research Committee of Isfahan University of Medical Sciences approved the experimental protocol with the code number of IR.MUI.RESEARCH.REC.1400.350.

Chemicals

Dexamethasone and mesalazine were received as gifts from Chemi-Darou and Darou-Pakhsh Pharmaceuticals Co., respectively. O-Dianisidine dihydrochloride (ODZ), gallic acid, cyanidine, quercetin and hexadecyl-trimethyl ammonium bromide (HTAB) were purchased from Sigma-Aldrich Company (Germany). Navand-Salamat kit (Urmia, Iran) for MDA evaluation was also prepared. All other chemicals and solvents were from Merck (Germany).

Plant material

The *Pinus eldarica* bark was gathered from the Isfahan University of Medical Sciences campus, Isfahan, Iran in the spring 2023. Experts from the Pharmacognosy Department of Isfahan School of Pharmacy confirmed its genus and species. A voucher specimen No. 3318 was deposited at the Herbarium collection of the Isfahan School of Pharmacy. The barks were dried at dark place to avoid chemical degradation and powdered using an electric mill. This powder was used for the following extraction process.

Preparation of the plant extracts

Pinus eldarica aqueous extract

The powder (70 g) was blended with 350 mL of distilled water, maintained for 12 h, shook for 2h and placed on a bain-marie until its temperature reached 80 ºC. After 20 min, the mixture was filtered and the filtrate was freeze dried. This step was repeated three times [17].

Pinus eldarica hydroalcoholic extract

The powder (100 g) and 500 mL of ethanol/water (70/30) were mixed and kept for 24h. Then, the extract was shook for 2h and filtered. This step

was repeated three times, concentrated by rotary evaporation at 50 ºC, and freeze dried [17].

Determination of polyphenolic compounds

The Folin-Ciochalteu method was employed to verify the polyphenol compounds [18]. In brief, 500 mg of aqueous and hydroalcoholic extracts of pine were separately dissolved in 96% ethanol (10 mL) and diluted to obtain 100 mL stock solution. Gallic acid (0.5 g) solution was prepared in 96% ethanol and six different concentrations were made (0, 100, 200, 300, 500 mg/mL) to depict standard curve (at 765 nm). The amount of total polyphenols were finally calculated as gallic acid equivalent (GAE)/g of each dried extract.

Determination of flavonoid compounds

The aluminum chloride colorimetric test was used to determine the total flavonoid content, using UV-Vis spectrophotometry [19]. The extracts (100 mg) were separately dissolved in acetone, mixed with aluminum chloride (20%) and glacial acetic acid (2 drops), poured off to each tube, and the volume reached to 3 mL with methanol. After 40 min, the absorbance was recorded at 415 nm. To prepare the standard samples, quercetin powder was dissolved in methanol, and concentrations of 0, 4, 20, 100, 500 mg/mL were prepared. The amount of total flavonoids were finally calculated as quercetin equivalent (QE)/g of each dried extract.

Determination of proanthocyanidin compounds

Based on the Porter et al., 1985 method, the total proanthocyanidin content was determined. The extract (10 mg) was mixed with pure methanol (10 mL). Next, the solution (5 mL) was blended with n-butanol/hydrochloric acid (30 mL, 95:5, V/V). Subsequently, ammonium sulfate ferric reagent (2N, 1 mL) was added and placed in water-bath (95 °C for 40 min). The mixture was cooled and the absorbance was recorded at 550 nm. The results were determined as cyanidine equivalent (CE/g) of each dried extract [20].

Experimental animals and induction of acute colitis

The male Wistar rats (n= 60, 10 groups), weighing 180-220 g, were obtained from the animal house of Isfahan School of Pharmacy, Iran. The rats were housed under standardized conditions, including temperature (21-23 °C) and humidity status (30-50%), a 12 h light/dark cycle, and freely accessible water and chow pellets. The rats were subjected to a 24 h fasting and then anesthetized by using desflurane. Next, a catheter (inner diameter of 2 mm and 8 cm long) was inserted into the anus of each rat to inject 3% acetic acid (2 mL). After injection, before removing the catheter, animals were put in a head-down status (60 sec) to prevent the emitting of acetic acid [21]. The groups of experimental rats and their interventions are summarized in Table 1. The suspensions of both extracts were mixed with Tween 80 (0.1% v/v) in normal saline to obtain 5 mL final volume. The prepared suspensions were administrated orally (5 mL/kg) once daily for five days. At the 6th day, the rats were sacrificed in CO_2 chamber.

Table 1. The groups of experimental rats and their interventions

Groups	Interventions started 2h prior to acetic acid injection (3%, 2mL) and continued (orally, 5mL/kg) for 5 days
Normal	Normal saline without acetic acid injection
Control	Normal saline with acetic acid colitis
PEAE100	*Pinus eldarica* aqueous extract (100 mg/kg)
PEAE200	*P. eldarica* aqueous extract (200mg/kg)
PEAE400	*P. eldarica* aqueous extract (400mg/kg)
PEHE100	*P. eldarica* hydroalcoholic extract (100 mg/kg)
PEHE200	*P. eldarica* hydroalcoholic extract (200 mg/kg)
PEHE400	*P. eldarica* hydroalcoholic extract (400 mg/kg)
Dexa.1	Dexamethasone (1mg/kg) intraperitoneally
Mesa.150	Mesalazine (150 mg/kg)

Macroscopic assessment of colon damage

Colon sections were incised longitudinally and washed with normal saline and then weighted. Colon samples were fixed on a white sheet and imaged using Sony camera. Colitis lesions were graded on a scale of 0 to 3, including 0 (no macroscopic findings), 1 (inflammation, edema, thickness, and superficial erosions), 2 (hemorrhagic spots, bleeding, and deep ulcers), and 3 (necrosis and/or perforation) [22]. Ulcer area was also measured by Fiji-win 32 software. Ulcer index (UI) was finally calculated for each sample by using UI = US (ulcer score) + UA (mean of ulcer area) formula [23].

Histological assessment of colon damage

A part of the colon tissue from each animal that was previously fixed in 10% formalin was selected and prepared through multiple steps of paraffin embedding, molding, sectioning and staining with hematoxylin and eosin (H&E). Then the prepared tissue samples were carefully examined by an expert pathologist who was

unaware of the interventions. The basis of tissue evaluation was the pathological grading provided by Cooper et al. [24].

Myeloperoxidase (MPO) activity in colon tissue

Another part of tissues (0.1 g) were thawed and mixed with phosphate buffer (5 mL) containing HTAB (0.5% w/v) and sent to homogenizer (3 cycles for 45 sec with 60 sec intervals). The obtained solution was sonicated (10 sec) and centrifuged (4000 rpm for 15 min). Next, phosphate buffer (2.9 mL) containing H_2O_2 (0.005%) and ODZ (0.167 mg/mL) was mixed with supernatant (100µL). The absorption at 450 nm wavelength was recorded to determine the activity of MPO at 0 and 3 min [25]. The activity of MPO was expressed as U/g of wet colon weight.

Malondialdehyde (MDA) content in colon tissue

The last part of frozen tissue (0.1g) was mixed with 1 mL of potassium chloride (1.15% v/w) and homogenized. The supernatant was collected after centrifuge (1200 rpm for 10 min) to determine MDA content using a commercial assay kit. Next centrifuge was done again at 3000 rpm (15 min), and the absorption at 532 nm was recorded and its levels were determined according to the formula: MDA level (nmol/mL) = (MDA OD – 0.0433) / 0.1982 [21].

Statistical analysis

The data are presented as either mean ± SD or median (range). Statistical significance level was $p<0.05$. Differences between experimental groups were reported after one-way analysis of variance (ANOVA) test with Tukey post hoc analysis for parametric data. The Mann-Whitney U test analyzed the non-parametric data using the SPSS statistical version 16.0.

Results and Discussion

In our study based on the Folin-Ciochalteu assay, polyphenols were about 40.8 and 72.8 mg in aqueous and hydroalcoholic extracts GAE/g, respectively (Table 2). In the study conducted by Vieito et al., the amount of polyphenols in bark extract of *P. pinaster* was equivalent to 63.8 mg GAE/g of extract which is near to our results [26]. If polyphenolic substances are considered as one of the main components of the effective substances of pine's bark, it can be expected that *P. eldarica* and *P. pinaster* have similar biological and therapeutic properties [16]. Total flavonoids and proanthocyanidins obtained from aqueous and hydroalcoholic extracts were 0.27 and 0.44 GE/g and 74.1 and 94.8 AE/g of dried extracts, respectively (Table 2). These results are consistent with the study conducted by Kim et al., who represented that pine flavonoids and proanthocyanidins were better extracted by ethanol than water, and therefore had a higher yield value [17].

Table 2. *Pinus eldarica* bark extracts yield value and main components

Extract	Yield value (w/w)	Dry weight (mg)	Polyphenols (GAE/g)	Flavonoids (QE/g)	Proanthocyanidins (CE/g)
PEAE	18.3 ± 2.8	520 mg ± 46.7	40.8 ± 3.8	0.27 ± 0.07	74.1 ± 4.0
PEHE	9.2 ± 1.2	720 mg ± 53.6	72.8 ± 6.7	0.44 ± 0.07	94.8 ± 7.1

PEAE: *Pinus eldarica* aqueous extract; PEHA: *P. eldarica* hydroalcoholic extract; GAE/g: gallic acid equivalent/g of dried extract; QE/g: quercetin equivalent/g of dried extract; CE/g: cyanidine equivalent /g of dried extract; data are mean± SD (n=3)

Table 3. The effects of *Pinus eldarica* bark extracts on macroscopic parameters of experimental colitis

Groups	Ulcer area (cm^2)	Ulcer score (0-3)	Ulcer index (0-8)	Weight of colon (g/8cm)
Normal	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.52 ± 0.07
Control	5.52 ± 0.77###	2.66 (2-3)###	8.60 ± 0.96###	1.45 ± 0.15###
PEHE100	2.02 ± 0.92*	1.33 (0-3) *	3.78 ± 1.50*	1.17 ± 0.12*
PEHE200	2.53 ± 1.51	1.66(0-3)	3.50 ± 1.40*	1.26 ± 0.10
PEHE400	3.60 ± 0.40	1.83 (1-3)	4.30 ± 1.08*	1.29 ± 0.09
PEAE100	1.73 ± 0.83*	1.16 (0-2)*	2.92 ± 1.30*	1.10 ± 0.05*
PEAE200	0.96 ± 0.47***	0.66 (0-2)**	1.60 ± 0.79***	0.98 ± 0.09***
PEAE400	3.71 ± 0.70	1.83 (0-3)	4.71 ± 1.30*	1.34 ± 0.19
Dexa.1	0.84 ± 0.40***	0.5 (0-2)***	1.41 ± 0.95***	0.95 ± 0.07***
Mesa.150	0.93 ± 0.61***	0.66 (0-2)***	1.22 ± 0.66**	0.72 ± 0.05***

PEAE: *Pinus eldarica* aqueous extract; PEHE: *P. eldarica* hydroalcoholic extract; Dexa.1: dexamethasone (1mg/kg); Mesa.150: mesalazine (150 mg/kg); * $p<0.05$, ** $p<0.01$, *** $p<0.001$ significant difference compared with control group; ### $p<0.001$ compared with normal group; data are mean± SD (n=6) or median (range) for scores

We investigated the anti-inflammatory potential of *P. eldarica* two extracts via evaluation of different macroscopic and microscopic parameters and MPO, MDA levels in rats. The induction of acute colitis via acetic acid was confirmed according to the significant difference between all macroscopic, microscopic and biochemical results versus normal rats (Tables 3, 4 and Figures 1-4).

The findings represented that the reduction of macroscopic parameters including ulcer severity, ulcer area, and ulcer index overall as well as the weight of colon-separated (8 cm) were diminished compared to the control animals. Unexpectedly, with increasing the dose of the extracts, especially hydroalcoholic one, the effectiveness decreased (Tables 3, 4), in a way that the dose of 400 mg/kg of this extract was ineffective on most macroscopic and microscopic parameters. Also, the lowest dose (100 mg/kg) of aqueous extract showed better results (Tables 3, 4). These results might indicate that some non-beneficial substances in the extracts, which are present in small amounts, might become more prominent with the increase of the dose and counteract the beneficial effects of the whole extract [27]. Toxicology studies on Tehran pine extract have shown that it has been safe in both single acute (2000 mg/kg) and multiple sub-acute (125, 250 and 500 mg/kg/day for 28 days) tests. The only adverse effect with the highest dose examined was inflammation in the liver and kidney, which has been claimed to have no effect on the functional and biochemical parameters of these organs [28]. Following the administration of aqueous and hydroalcoholic extracts, the levels of MPO and MDA declined significantly ($p<0.05$) compared to the control group (Figures 3, 4). Even by increasing the dose of extracts to 400 mg/kg, this reduction was meaningful, indicating the presence of antioxidants and anti-inflammatory ingredients in both extracts [15,16].

Table 4. The effects of *Pinus eldarica* bark extracts on pathological parameters of experimental colitis

Groups	Inflam. score (0-3)	Inflam. extent (0-3)	Leucocyte infiltratin (0-3)	Crypt damage (0-4)	Total colitis index (0-13)
Normal	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Control	3.0 (2-3) ###	3.0 (2-3)###	3.0 (3-3)###	4.0 (2-3) ###	13.0 (9-13) ###
PEAE100	2.0 (1-3)*	1.5 (1-3)**	1.5 (1-2) ***	1.5 (2-3)**	6.0 (6-11)*
PEAE200	1.5 (2-3)**	2.0 (1-3)*	1.5 (1-3) **	2.0 (1-3) *	7.5 (6-11)*
PEAE400	2.5 (1-3)	2.5 (2-3)	2.0 (2-3)	3.0 (2-3)	10.0 (8-12)
PEHE100	1.5 (1-3)**	1.0 (1-2)***	1.0 (0-2)***	1.5 (2-3)**	5.0 (5-10)**
PEHE200	1.5 (0-2)***	1.0 (1-2)***	1.0 (1-3)***	0.5 (0-2) ***	4.5 (3-7)***
PEHE400	3.0 (2-3)	2.5 (2-3)	3.0 (3-3)	3.0 (2-3)	11.5 (9-12)
Dexa. 1	1.0 (1 -2)***	0.5 (0-2)***	1.0 (0-2)***	0.5 (0-2)***	3.0 (2-7)***
Mesa.150	1.5 (1-2)***	0.5 (1-2)***	1.0 (1-3)***	1.5 (1-2)***	4.0 (3-8)***

PEAE and PEHE: *Pinus eldarica* aqueous and hydroalcoholic extract 100, 200, and 400 mg/kg, respectively; Dexa.1: dexamethasone (1 mg/kg); Mesa.150: mesalazine (150 mg/kg); Inflam: inflammation * $p<0.05$, ** $p<0.01$, *** $p<0.001$ significant difference compared to control group; ### $p<0.001$ compared to normal group; Data are median (range) for scores.

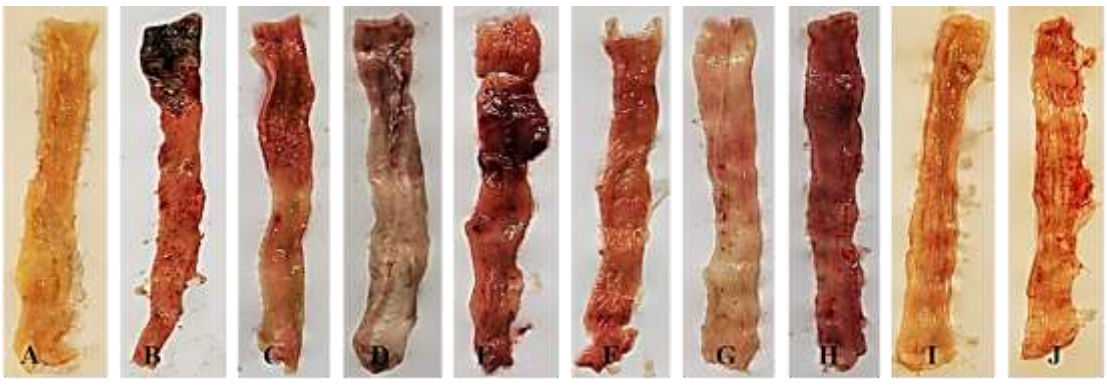

Figure 1. Macroscopic view of colitis in rats; (A) normal colon receiving normal saline; (B) control colitis receiving normal saline; (C, D, E) colitis treated with three doses of aqueous bark extract of *Pinus eldarica* (100, 200, 400 mg/kg, respectively); (F, G, H) colitis treated with three doses of hydroalcoholic bark extract of *P. eldarica* (100, 200, 400 mg/kg, respectively); (I) colitis treated with dexamethasone (1 mg/kg); (J) colitis treated with mesalazine (150 mg/kg)

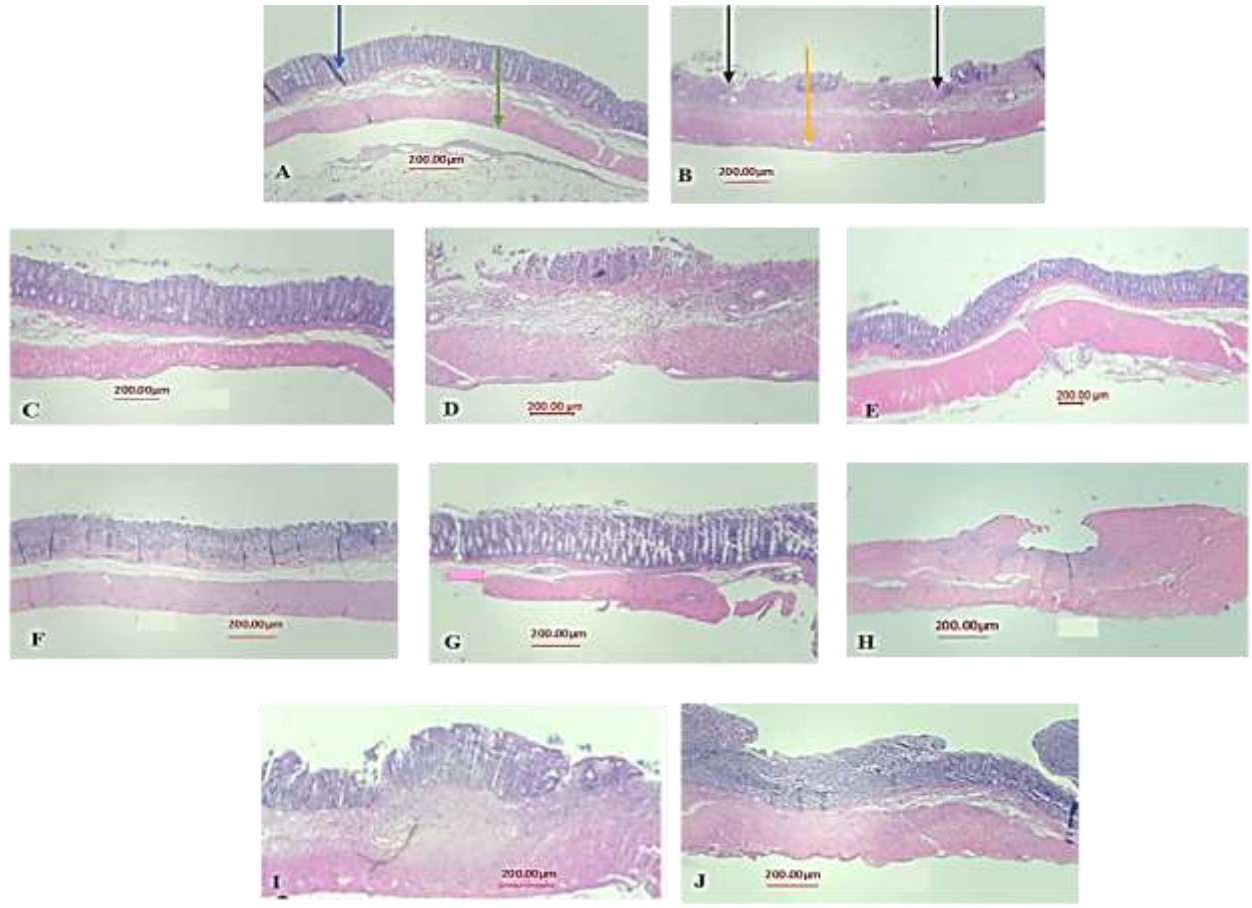

Figure 2. Microscopic view of rat's colon tissue stained with H&E (10 x magnification); (A) normal colon receiving normal saline; (B) control colitis receiving normal saline; (C, D, E) colitis treated with three doses of aqueous bark extract of *Pinus eldarica* (100, 200, 400 mg/kg, respectively); (F, G, H) colitis treated with 3 doses of hydroalcoholic bark extract of *P. eldarica* (100, 200, 400 mg/kg, respectively); (I) colitis treated with dexamethasone (1 mg/kg); (G) colitis treated with mesalazine (150 mg/kg); the features were marked with blue arrow (normal tissue containing normal mucus), green arrow (normal sub-mucosal layers), black arrow (ulcers and crypt damage), and yellow arrow (thickness of the sub-mucosal layer and leukocyte infiltration).

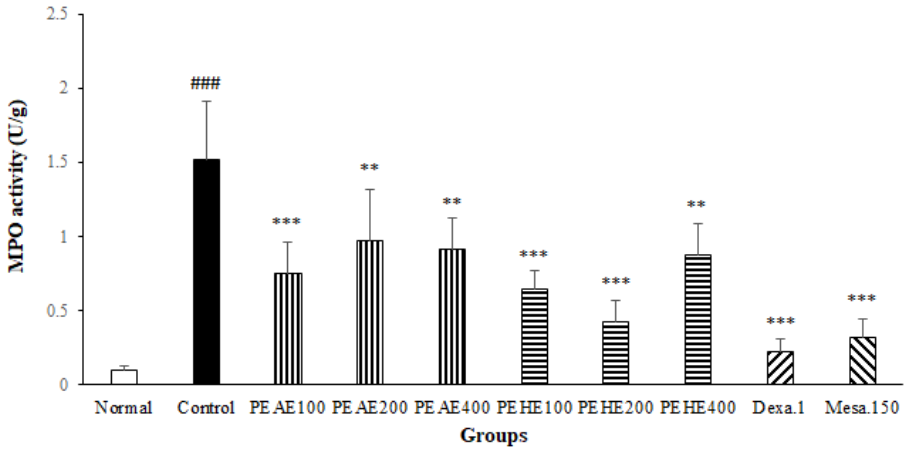

Figure 3. Myeloperoxidase (MPO) activity obtained from the colon tissue in experimental rats; PEAE and PEHE: *Pinus eldarica* aqueous and hydroalcoholic extract 100, 200, and 400 mg/kg, respectively; Dexa.1: dexamethasone (1 mg/kg); Mesa.150: mesalazine (150 mg/kg); data are mean ± SD (n=6); *p<0.05, ** p<0.01, and *** p<0.001 compared with control group; ### p<0.001 compared with normal group

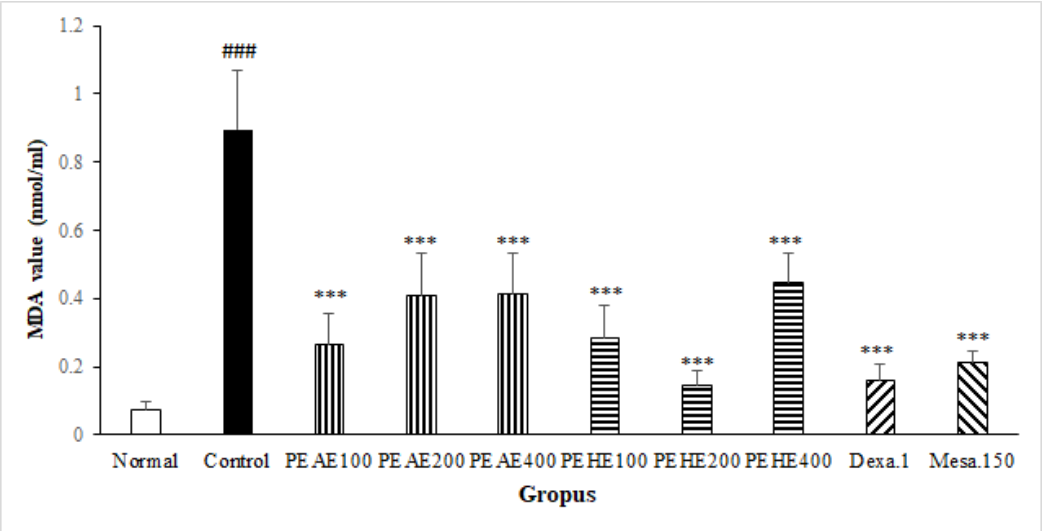

Figure 4. Malondialdehyde **(**MDA) values obtained in the colon tissue in experimental rats; PEAE and PEHE: *Pinus eldarica* aqueous and hydroalcoholic extract 100, 200, and 400 mg/kg, respectively; Dexa.1: dexamethasone (1 mg/kg); Mesa.150: mesalazine (150mg/kg); data are mean ± SD (n=6); *p<0.05, ** p<0.01, and *** p<0.001 compared with control group; ### p<0.001 compared with normal group

MDA, an organic compound, is produced during the peroxidation of unsaturated fatty acids via free radicals and it is considered a valuable oxidative stress marker [29]. MPO, as a peroxidase enzyme, is naturally produced in the granules of neutrophils and is released into the extracellular space after neutrophil degranulation. This enzyme is a marker to assay the level of leukocyte infiltration, inflammation and oxidative stress indirectly in tissues [30]. Based on this fact, successful induction of colitis in colon tissue was verified in the control group due to the high level of MDA (oxidative stress marker), MPO (an inflammatory marker), and leukocytes infiltration (Table 4, Figures 3, 4). Since higher doses of the extracts could not significantly reduce colitis indices in the treatment groups, it can be concluded that other mechanisms plus antioxidant effects could improve colitis complications [31]. Overall, the effect of *Pinus eldarica* hydroalcoholic extract was better compared to aqueous one which might be associated with higher levels of effective beneficial compounds like polyphenols, flavonoids, and proanthocyanidins in the hydroalcoholic extract. It seems that the hydroalcoholic solvent is a more suitable medium for chemicals extraction with anti-colitis properties [26]. In the groups that received the reference drugs, dexamethasone and mesalazine, all macroscopic and microscopic indices besides the MPO and MDA level were considerably lower than the control group ($p<0.01$). This effective reduction of indices via reference drugs indicated that anti-inflammatory mechanisms were probably mediated by both corticosteroid and non-corticosteroid receptors [32].

The bark extract of Tehran pine is rich in phenolic compounds that present antioxidant and anti-inflammatory effects. Catechin and taxifolin are major components of these compounds [15]. Huseini et al., identified eight polyphenols in the extract of *P. eldarica* nuts among which catechin, epicatechin, and tyrosol showed the highest amounts [33]. The presence of high amounts of catechin and procatechin in the bark extract of French pine (pycnogenol) has also been proven, and many biological effects, including antioxidant, anti-hyperglycemic and trapping free radicals, are attributed to these substances [34]. Considering the presence of significant amounts of gallic acid and tannins in pine bark extracts and the fact that tannins play a protective and healing role in colon ulcers, the effectiveness of the extracts can also be attributed to these substance [35].

In addition, flavonoids such as proanthocyanidins and flavonols were extracted from the leaf extract of Tehran pine in Kaundun et al., study [36]. Flavonols such as quercetin and kaempferol were also frequently identified. Flavonoids prevent the prostaglandins biosynthesis and neutrophil

degranulation, and reduce arachidonic acid releasing, which is a precursor to prostaglandins [37]. In addition, flavonoids inhibit inflammatory cytokines such as IL-6, IL-7 and TNF-α. Also some flavonoids inhibit the NF-κB pathway, which leads to a decrease in the production of TNF-α and IL-1 as the key elements in the activation of inflammatory cells. The reduction of IL-1 synthesis by this group of compounds in turn leads to the reduction of IL-2 and TNF-α production [38]. Quercetin as a common flavonoid present in pine extract, is one of the most prominent dietary antioxidants that exhibits a wide range of biological activities. Its beneficial health effects were approved in cancer, diabetes, hypertension, and aging. In IBD, quercetin exerts its anti-inflammatory effects by improving the intestinal mucus barrier, modulating local immune homeostasis, and suppressing oxidative stress. Although it's oral bioavailability is low, its optimal plasma levels can be obtained with repeated administration [39]. The authors attributed part of the anti-inflammatory and anti-ulcerative effects of Tehran pine to the presence of flavonols and flavonoids [36]. However, the antioxidant effect alone is not enough for anticolitis property and it is reflected in many parameters which cannot be fully evaluated by measuring MDA value [40].

The extracted compounds from *P. eldarica* from Iran include α-pinene, caryophyllene oxide, camphor, β-caryophyllene, and myrtenal besides a significant amount of polyphenolic compounds. Basholli-Salihu et al., demonstrated that α-pinene may possess an anti-inflammatory activity due to the decline in secretion of cytokines (IL-1β, IL-6, and TNF-α) which is induced by lipopolysaccharide [41]. Alpha-pinene is a key compound found in all pine species. In this study, due to the lack of adequate funds, we failed to measure inflammatory mediators and cytokines such as IL-1β, IL-6, TNF-α and NF-κB in the colon tissue so, these evaluations are recommended for future studies. Also, since the colitis induction model with acetic acid is an acute inflammatory and ulcerative model, it is recommended to investigate the therapeutic effect of Tehran pine's extracts in a chronic model such as dextran sulfate sodium- induced model.

Considering the positive effects of Tehran pine extracts and their effective mechanisms in reducing colitis ulceration and inflammation, it is needed to formulate the active compounds of this plant and utilize them as adjunct or alternative therapies to conventional drugs after well-conducted clinical trials. It seems that these novel formulations could improve IBD and diminish the adverse effects of commonly used medications.

Conclusion

Treatment with aqueous and hydroalcoholic extracts of *P. eldarica* showed the potential to reduce various features of experimental ulcerative colitis. In fact, specific active ingredients of *P. eldarica* responsible for anti-inflammatory, anti-oxidant, and wound healing properties have been isolated; however, their absolute role in colitis prevention and/or treatment should be investigated.

Acknowledgments

The authors would like to thank Vice Chancellor for Research, Isfahan University of Medical Sciences for supporting this project No. 3400682.

Author contributions

Mohsen Minaiyan presented the idea of research, designed and supervised all parts related to grouping of animals, determining the doses of drugs, arrangement of interventions, induction of colitis and statistical analysis of data; Zahra Dastanian executed the experiments and interventions under supervision of professors; Behzad Zolfaghari designed and supervised the experiments related to identification, preparation and evaluation of herbal materials and extracts; Ardeshir Talebi designed and supervised the experiments related to the sampling, preparation and evaluation of tissues for histopathologic analysis. All authors contributed in writing, reviewing and preparation of the manuscript.

Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the accuracy and integrity of the paper content.

References

[1] Guan Q. A comprehensive review and update on the pathogenesis of inflammatory bowel disease. *J Immunol Res*. 2019; Article ID 7247238.

[2] Friedrich M, Pohin M, Powrie F. Cytokine networks in the pathophysiology of inflammatory bowel disease. *Immunity*. 2019; 50(4): 992-1006.

[3] Terdiman JP, Steinbuch M, Blumentals WA,

Ullman TA, Rubin DT. 5-Aminosalicylic acid therapy and the risk of colorectal cancer among patients with inflammatory bowel disease. *Inflamm Bowel Dis*. 2007; 13(4): 367-371.

[4] Summers RW. Novel and future medical management of inflammatory bowel disease. *Surg Clin North Am*. 2007; 87(3): 727-741.

[5] Selvamani S, Mehta V, El Enshasy HA, Thevarajoo S, El Adawi H, Zeini I, Pham K, Varzakas T, Abomoelak B. Efficacy of probiotics-based interventions as therapy for inflammatory bowel disease: a recent update. *Saudi J Biol Sci*. 2022; 29(5): 3546-3567.

[6] Li C, Wang J, Ma R, Li L, Wu W, Cai D, Lu Q. Natural-derived alkaloids exhibit great potential in the treatment of ulcerative colitis. *Pharmacol Res*. 2022; Articl ID 105972.

[7] Ribeiro D, Proenca C, Rocha S, Lima JL, Carvalho F, Fernandes E, Freitas M. Immunomodulatory effects of flavonoids in the prophylaxis and treatment of inflammatory bowel diseases: a comprehensive review. *Curr Med Chem*. 2018; 25(28): 3374-3412.

[8] Caban M, Lewandowska U. Polyphenols and the potential mechanisms of their therapeutic benefits against inflammatory bowel diseases. *J Funct Foods*. 2022; Article ID 105181.

[9] Ge J, Liu Z, Zhong Z, Wang L, Zhuo X, Li J, Xiaoying J, Xiang-Yang Y, Tian X, Renren B. Natural terpenoids with anti-inflammatory activities: Potential leads for anti-inflammatory drug discovery. *Bioorg Chem*. 2022; Article ID 105817.

[10] Zargary A. Medicinal plants. 5th ed. Tehran: Tehran University Press, 1996.

[11] Mamedov N, Craker LE. Medicinal plants used for the treatment of bronchial asthma in Russia and Central Asia. *J Herbs Spices Med Plants*. 2001; 8(2-3): 91-117.

[12] Mamedov N, Gardner Z, Craker LE. Medicinal plants used in Russia and Central Asia for the treatment of selected skin conditions. *J Herbs Spices Med Plants*. 2005; 11(1-2): 191-222.

[13] Gulati OP. The nutraceutical pycnogenol: its role in cardiovascular health and blood glucose control. *Biomed Rev*. 2005; 16: 49-57.

[14] Afsharypour S, Sanaty F. Essential oil constituents of leaves and fruits of *Pinus eldarica* Medw. *J Essent oil Res*. 2005; 17(3): 327-328.

[15] Iravani S, Zolfaghari B. Phytochemical analysis of *Pinus eldarica* bark. *Res Pharm Sci*. 2014; 9(4): 243-250.

[16] Yesil-Celiktas O, Ganzera M, Akgun I, Sevimli C, Korkmaz KS, Bedir E. Determination of polyphenolic constituents and biological activities of bark extracts from different *Pinus* species. *J Sci Food Agric.* 2009; 89(8): 1339-1345.

[17] Kim NY, Jang MK, Lee DG, Yu KH, Jang H, Kim M, Kim SG, Yoo BH, Lee SH. Comparison of methods for proanthocyanidin extraction from pine (*Pinus densiflora*) needles and biological activities of the extracts. *Nutr Res Pract*. 2010; 4(1): 16-22.

[18] Ainsworth EA, Gillespie KM. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin Ciocalteu reagent. *Nat Protoc*. 2007; 2(4): 875-877.

[19] Karapandzova M, Stefkov G, Cvetkovikj I, Stanoeva JP, Stefova M, Kulevanova S. Flavonoids and other phenolic compounds in needles of *Pinus peuce* and other pine species from the *Macedonian flora*. *Nat Prod Commun*. 2015; 10(6): 987-990.

[20] Porter LJ, Hrstich LN, Chan BG. The conversion of procyanidins and prodelphinidins to cyanidin and delphinidin. *Phytochemistry*. 1985; 25(1): 223-230.

[21] Kayvanara AH, Yegdaneh A, Talebi A, Minaiyan M. Evaluating anti-Inflammatory effect of hydroalcoholic extracts of *Citrus medica* L. pulp and peel on rat model of acute colitis. *Res J Pharmacogn.* 2023; 10(2): 29-38.

[22] Jalalipour M, Yegdaneh A, Talebi A, Minaiyan M. *Salvia officinalis* leaf extracts protect against acute colitis in rats. *Res Pharm Sci*. 2022; 17(4): 350-359.

[23] 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*. 2009; 3(2): 15-22.

[24] Cooper HS, Murthy SN, Shah RS, Sedergran DJ. Clinicopathologic study of dextran sulfate sodium experimental murine colitis. *Lab Invest*. 1993; 69(2): 238-249.

[25] 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: 30-44.

[26] Vieito C, Fernandes É, Velho MV, Pires P. The effect of different solvents on extraction yield, total phenolic content and antioxidant activity of extracts from pine bark (*Pinus pinaster* subsp. *atlantica*). *Chem Eng Trans*. 2018; 64: 127-132.

[27] 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 Rep Pharm Sci*. 2014; 3(1): 28-35.

[28] Ghadirkhomi A, Safaeian L, Zolfaghari B, Agha Ghazvini MR, Rezaei P. Evaluation of acute and sub-acute toxicity of *Pinus eldarica* bark extract in Wistar rats. *Avicenna J Phytomed*. 2016; 6(5): 558-566.

[29] Draper HH, Hadley M. Malondialdehyde

determination as index of lipid peroxidation. *Meth Enzymol*. 1990; 186: 421-431.
[30] Tian Y, Wang K, Wang Z, Li N, Ji G. Chemopreventive effect of dietary glutamine on colitis-associated colon tumorigenesis in mice. *J Carcinog*. 2013; 34(7): 1593-1600.
[31] Villablanca EJ, Selin K, Hedin CRH. Mechanisms of mucosal healing: treating inflammatory bowel disease without immunosuppression. *Nat Rev Gastroenterol Hepatol*. 2022; 19(8): 493-507.
[32] McQuaid KR. Drugs used in the treatment of gastrointestinal disease. In: Katzung BG, editor. Basic and clinical pharmacology. 11th ed. New York: McGraw Hill Companies, 2007.
[33] Huseini HF, Anvari MS, Khoob YT, Rabbani S, Sharifi F, Arzaghi SM, Fakhrzadeh H. Anti-hyperlipidemic and anti-atherosclerotic effects of *Pinus eldarica* Medw. nut in hypercholesterolemic rabbits. *DARU J Pharm Sci*. 2015; 23(1): 32-37.
[34] Maritim A, Dene B, Sanders R, Watkins III J. Effects of pycnogenol treatment on oxidative stress in streptozotocin-induced diabetic rats. *J Biochem Mol Toxicol*. 2003; 17(3): 193-199.
[35] Piazza S, Fumagalli M, Martinelli G, Pozzoli C, Maranta N, Angarano M. Hydrolyzable tannins in the management of Th1, Th2 and Th17 inflammatory-related diseases. *Molecules.* 2022; Article ID 27217593.
[36] Kaundun SS, Fady B, Lebreton P. Genetic differences between *Pinus halepensis*, *Pinus brutia* and *Pinus eldarica* based on needle flavonoids. *Biochem Syst Ecol*. 1997; 25(6): 553-562.
[37] O'Leary KA, de Pascual-Tereasa S, Needs PW, Bao YP, O'Brien NM, Williamson G. Effect of flavonoids and vitamin E on cyclooxygenase-2 (COX-2) transcription. *Mutat Res*. 2004; 551(1-2): 245-254.
[38] Gupta M, Mishra V, Gulati M, Kapoor B, Kaur A, Gupta R, Tambuwala MM. Natural compounds as safe therapeutic options for ulcerative colitis. *Inflammopharmacol*. 2022; 30(2): 397-434.
[39] Lyu YL, Zhou HF, Yang J, Wang FX, Sun F, Li JY. Biological activities underlying the therapeutic effect of quercetin on inflammatory bowel disease. *Mediators Inflamm*. 2022; Article ID 5665778.
[40] Frankel EN, Meyer AS. The problems of using one-dimensional methods to evaluate multifunctional food and biological antioxidants. *J Sci Food Agric.* 2000; 80(13): 1925-1941.
[41] Basholli-Salihu M, Schuster R, Hajdari A, Mulla D, Viernstein H, Mustafa B, Mueller M. Phytochemical composition, anti-inflammatory activity and cytotoxic effects of essential oils from three *Pinus* spp. *Pharm Biol*. 2017; 55(1): 1553-1560.

Abbreviations

IBD: inflammatory bowel disease; MPO: myeloperoxidase; MDA: malondealdehyde; NF-κB: nuclear factor-kappa B; TNF-α: tumor necrosis factor alpha; NO: nitric oxide; IL: Interleukin; ODZ: orthodianizidine; HTAB: hexadecy-trimethyl ammonium bromide