Original Article

Ulcer-Healing Effect of Hydroalcoholic Extract and Essential Oil of *Achillea millefolium* L. on Murine Model of Colitis

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Abstract

Bakground: Ulcerative colitis (UC) is an inflammatory bowel disease that can be treated with many medications but they have various side effects and low cure rate. So, the need for finding novel drugs with better healing characters and less toxicity would be mandatory. *Achillea millefolium* (*A. millefolium,* Yarrow) has been traditionally used to treat bleeding, ulcers, wounds, liver, and bile disorders, and recently it has been shown to have anti-ulcer, analgesic, anti-inflammatory, antioxidant, and appetizing effects that make it as a good candidate for UC. **Methods:** UC was induced with intra-rectal instillation of acetic acid. *A. millefolium* hydroalcoholic extract (AMHE, 200, 400, and 600 mg/kg/day) and essential oil (AMEO, 62.5, 125, and 250 µl/kg/day) were given to six groups of male Wistar rats for 5 days. Dexamethasone (1 mg/kg/day, intra-peritoneal) and mesalazine (100 mg/kg/day, orally) were used as reference drugs. Colon tissue specimens were separated for assessing macroscopic, pathologic, and biochemical markers. **Results:** For AMHE, 77.2 mg/g equivalent to gallic acid was obtained for total phenols. Main assessed markers, including ulcer index, total colitis index, colon weight/length ratio, rats' weight gain, and malondialdehyde levels were significantly improved in AMHE (400 and 600 mg/kg/day) and AMEO (125 and 250 µl/kg/day) groups compared to controls. Myeloperoxidase activity was only attenuated in AMHE groups significantly. **Conclusions:** Both AMHE and AMEO were effective in healing experimental colitis. It seems antioxidant, anti-inflammatory, and anti-ulcer activities of Yarrow are responsible for these beneficial effects. Further studies are warranted to elucidate the exact mechanisms involved.

Keywords: *Achillea millefolium, anti-inflammatory, colitis, rats, essential oil, plant extract*

Introduction

One of the auto-immune and inflammatory diseases with no clear-cut cure is ulcerative colitis (UC).[1] Some drugs, such as 5-aminosalicylic acid derivatives (5-ASAs), glucocorticosteroids, azathioprine, cyclosporine A, monoclonal antibodies, antibiotics, and probiotics, have been established for the treatment of colitis, but they have serious side effects, such as anemia, liver abnormalities, cataracts, osteoporosis, hypothalamic pituitary axis suppression, immunocompromised state, nephrotoxicity, hypersensitivity reactions, and infections.[2] So, the need of exploring new drugs with better safety profile, less toxicity, and reasonable efficacy is necessary.[3]

Medicinal plants with beneficial active components and shining history of usage especially in gastrointestinal ailments are good cases to study for their biological effects on UC.[4] *Punica granatum, Carum carvi, Rosmarinus officinalis*, and *Moringa oleifera* are among medicinal plants with suitable anti-colitis effects on experimental and/or clinical states of colitis.[5-8]

Achillea millefolium with common name of "Yarrow" originally comes from Europe and most often grows in grasslands, distributing from Europe to Western Asia, Australia, and Northern America from May to June commonly at 3500 mean sea level.[9]

A. millefolium is a perennial plant from the Asteracea (Compositae) family reaching up to 50 cm tall. The leaves have 5–20 cm length and the flowers (aerial parts) are mostly white with corymb-like egg-shaped heads at the top of extremities.[10]

A. millefolium is one of the oldest herbs to use as medicine for more than 3000 years ago especially by Greek soldier; Achilles in Trojan War. As in different folk and

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traditional medicines especially in Iranian traditional medicine, it has been introduced as diaphoretic, emmenagogic, diuretic, tonic, and expectorant, and it is recommended for treating bleeding, wounds, ulcers, rheumatism, pneumonia, liver and bile disorders, sweating, varicose veins, and constipation.[9,11]

Furthermore, there are lots of studies on *A. millefolium* showing its various biological activities, such as anti-ulcer, anti-inflammatory, antioxidant, immunosuppressive, appetizing, analgesic, antispasmodic, hepatoprotective, antiproliferative, anticancer, and antitumor that are attributed to its plentiful active components.[12] Flavones, flavanones, catechins, and anthocyanins are found in total extract, and artemisia ketone, monoterpenoides, such as camphor, linalool, and linalyl acetate, and 1,8 cineole present in the essential oil fraction are among the most active ingredients of *A. millefolium* for which anti-inflammatory and anti-ulcerative properties have been documented in several studies.[9,13,14]

Concerning the above-mentioned properties, for the first time, anti-colitis effects of hydroalcoholic and essential oil fractions of *A. millefolium* in a murine model of colitis induced by acetic acid were explored.

Methods

Plant preparation

A. millefolium was collected from Tashal in Oshtorankooh area (Lorestan Province) with 2400-m height from high seas located between Aligoodarz, Azna, and Dorood cities. It was compared and checked by herbarium sample No. 1398 at Isfahan School of Pharmacy by Dr. Afasaneh Yegdaneh, a Pharmacognosist from Isfahan University of Medical Sciences. The aerial parts of *A. millefolium* were collected, dried, and powdered to become suitable for later extraction process.

Preparation of herb's aerial parts extract

For preparing AMHE by maceration method, 300 g of herb powder was put in a big container, ethanol was added (3 L, 80%), and wet for 24 h. It was stirred for 2 h, filtered, and its ethanol residue let to be evaporated in rotary evaporator. The phenolic constituent of extract was standardized as gallic acid equivalent (GALeq/g) value by Folin–Ciocalteu method. Briefly, absorption of solubilized gallic acid (50, 100, 150, 250, and 500 mg/kg) was read in the presence of Folin–Ciocalteu indicator and sodium bicarbonate at 765-nm wavelength. Then, standard curve was depicted and total phenols of extracts were measured in terms of milligram equal to gallic acid content as gram.[15]

Preparation of herb's aerial parts essential oil

To prepare AMEO, hydro-distillation was done by using 100 g of *A. millefoliums* powder in 500 ml of distilled water that was boiled in Clevenger. The obtained essential oil was separated and analyzed by GC-MS apparatus according to the method previously reported by Sajjadi *et al*. (2015).[16]

Drugs and solutions

The powders of mesalazine (MES) and dexamethasone (Dex) were procured from Iran-Hormone Pharmaceutical Co. (Tehran, Iran). Orto-dianizidin dihydrocholoride (ODD), hexa-decyl trimethyl ammonium bromide (HTAB), and Folin–Ciocalteu reagent were bought from Sigma Co. (St. Louis, MO, USA). Formaldehyde, glacial acetic acid, ethanol (96%), and diethyl ether oxide were purchased from Merck Co. (Darmstadt, Germany).

Animals

Male Wistar inbred rats weighting 180–220 g were used in this study. They were purchased from the animal house belonged to Isfahan School of Pharmacy and Pharmaceutical Sciences. All of handling and working affairs were done according to Isfahan University of Medical Sciences Ethics committee guidelines approved for utilizing laboratory animals and specified by the National Ethics Code: IR.MUI.Research.Rec. 1398.037.

Grouping of animals

In this study, 60 healthy male rats were allocated in 10 groups of six rats in each group. Normal group received normal saline (5 ml/kg, p.o.) without ulcer induction. Control group received normal saline (5 ml/kg, p.o.) 2 h before ulcer induction and repeated once daily for next 5 days. AMHE groups received *A. millefolium* hydroalcoholic extract (200, 400, and 600 mg/kg, p.o.),[17] AMEO groups received *A. millefolium* essential oils (62.5, 125, and 250 µl/kg, p.o.),[18] Dex. 1 group received Dex (1 mg/kg, i.p.) and Mes. 100 group received MES (100 mg/kg. p.o.). All treatments were made 2 h before ulcer induction and repeated daily for next 5 days.[15]

UC induction

Initially, rats were fasted for 24 h with free access to tap water. After weighting, they were lightly anesthetized and 2 ml of acetic acid 3% instilled in their colon by a suitable feeding tube (8-cm length and 2-mm diameter). Then, the rats were kept headed down for 1 min to minimize exiting acetic acid residue out of the rectum. Two hours after UC induction, the animals were allowed to eat rat's chow.

Twenty-four hours after the last treatment, animals were weighted and euthanized with CO_2 gas inhalation, afterward their abdominal cavity was opened for the following evaluations.[8]

Macroscopic evaluations

Proximal to the anus (3 cm), 8 cm of colons were separated and cut. Then, the colons were opened longitudinally and irrigated by normal saline. Then, they were weighted, fixed on working sheet, and appropriate photos were taken by a mobile camera.

Ulcer score (U_S) was estimated using qualitative evaluation, 0: No ulcer or erosion, 1: inflammation, edema, thickness, and superficial erosions, 2: hemorrhage and evident erosion, and 3: sever ulceration, tissue necrosis, and/or perforation. Ulcer area (U_A) was measured by Fiji win 32 program followed by calculating ulcer index using following equation: Ulcer Index = $U_{A+} U_S$.[8]

Microscopic evaluations

Initially, fixed colonic tissue specimens were embedded in paraffin, processed, and sectioned in 4-mm thick layers. Afterward, they were deparaffinized with xylene and hydrated by ethanol. At last, they were colored with hematoxylin and eosin (H and E). Inflammation severity (0: none, 1: slight, 2: moderate, and 3: severe), inflammation extent (0: none, 1: mucosal, 2: mucosal, and sub-mucosal, and 3: transmural invasion), crypt damage (0: none, 1: basal 1/3 damaged, 2: basal 2/3 damaged, 3: surface epithelium was intact only, and 4: crypts and surface epithelium were intact), and leukocyte infiltration (0: trace, 1: mild, 2: moderated, and 3: sever) were evaluated in H and E-stained and encoded samples following modification of a validated scoring scheme described by Cooper *et al*.[19] Total colitis index (TCI) was measured by following equation:

TCI = Inflammation severity + inflammation extent + crypt damage + leukocyte infiltration.

Histopathological assessment was conducted with the use of a Zeiss microscope that was equipped with a Sony color video camera (Sony, Japan) to provide digital imaging.

Evaluation of myeloperoxidase (MPO) activity

Evaluation of MPO activity as an index of immune cells migration and penetration in the tissue was measured according to the method described by Motavallian-Naeini *et al*.[20] One hundred milligram of colon specimens was homogenized in potassium buffer (5 ml, pH, 6) containing 0.5% HTAB. Then, the mixture was sonicated for 10 s in an ice bath and the homogenate was centrifuged (4000 rpm). The supernatant in the volume of 0.1 ml and 2.9 ml of 50 mM phosphate buffer (pH, 6) was mixed containing 0.167 mg/ml ODD and 0.0005% hydrogen peroxide. After incubation, the absorbance was measured at 450 nm using UV-Vis spectrophotometer (LSI Model Alfa-1502) at 0 and 3 min time interval. MPO activity was reported as U/100 mg for the weight of wet colon tissue.

Evaluation of malondialdehyde (MDA) level

Evaluation of MDA as an index of lipid peroxidation was carried out by adding 1 ml of potassium chloride 1.15% w/v to 0.1 g of colonic tissue, and then samples were homogenized and centrifuged (1200 rpm for 10 min). The supernatant was separated, centrifuged (3000 rpm for 15 min), and the absorbance was measured at 532 nm. All the experiments for MDA measurement was done with Navand assay kit (Navand-Salamat, Urmia, Iran) according to the company's brochure instruction.[15]

Statistical analysis

All data were presented as mean ± standard error of the mean (SEM) and analyzed with SPSS (Statistical Package for the Social Science) version 16. One-way ANOVA was used for parametric data, with Tukey's post hoc test. Mann–Whitney U test for the non-parametric data and Student's *t*-pairs test for paired groups were also utilized. Scoring data were presented as median (range) in related tables. $P < 0.05$ was considered as significant for all comparisons.

Results

Yield value and total phenolic contents

The yield value of 22.4% (W/W) and 0.18% (V/W) for AMHE and AMEO, respectively was obtained. According to Folin–Ciocalteu method, each gram of extract had 77.2 mg total phenolic compounds equivalent to gallic acid. Top components isolated with more than 3% of total essential oils were respectively 1, 8-cineole, Alpha-copaene, copaene, and L-calamenene.

Macroscopic evaluations

Animal weight change and colonic weight/length ratio

In normal group with no colitis induction, there was no ulcer or inflammation, so its relevant data including body weight change and colon weight to length ratio had minimum value with zero ulcer index [Table 1, Figures 1, 2].

Control group with colitis induction exhibited maximum amount of body weight change, colon weight to length ratio, and ulcer index showed acetic acid was powerful enough to develop ulcerative colitis [Table 1, Figure 1, 2].

Groups treated with AMHE600, AMEO250, and Mes. 100 exhibited an improvement in weight gain (at least

Table 1: Changes in body weight of rats treated with *A. millefolium*

Groups/Dose (mg or µg/kg)	Before	After	*P*
Normal	211.8±2.0	222.9±3.3	<0.001*
Control	217.2±2.2	197.3±2.0	-
AMHE200	179.2±3.6	177.0±3.8	>0.05
AMHE400	200.2±7.3	204.0±8.4	>0.05
AMHE600	205.1±3.8	217.3±4.8	<0.05*
AMEO62.5	195.7±4.1	189.3±3.4	>0.05
AMEO125	183.2±4.4	191.3±5.6	>0.05
AMEO250	171.8±3.2	186.0±4.0	<0.01*
Dex. 1	188.8±4.6	189.1±3.3	>0.05
Mes. 100	202.2±3.6	215.0±4.5	<0.05*

Normal=Normal rats treated with normal saline (5 ml/kg), Control=Rats with colitis treated with normal saline (5 ml/kg), AMHE=*Achillea millefolium* hydroalcoholic extract, AMEO=*Achillea millefolium* essential oil, Dex=dexamethasone, MES=mesalazine. *Significant difference versus control group

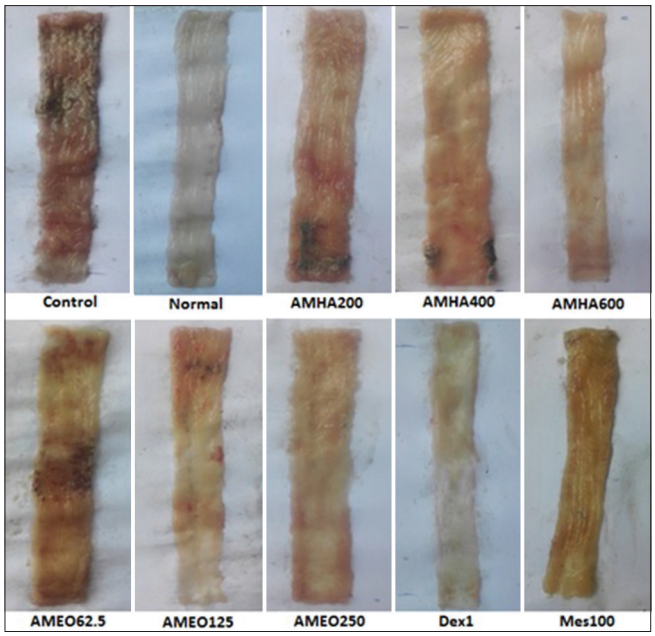

Figure 1: Effect of *A. millefolium* on macroscopic features of colitis. Normal = normal rats treated with normal saline (5 ml/kg), Control = rats with colitis treated with normal saline (5 ml/kg), AMHE = *A. millefolium* hydroalcoholic extract (200, 400, 600 mg/kg), AMEO = *A. millefolium* essential oil (62.5, 125, 250 µl/kg), Dex = dexamethasone, Mes = mesalazine

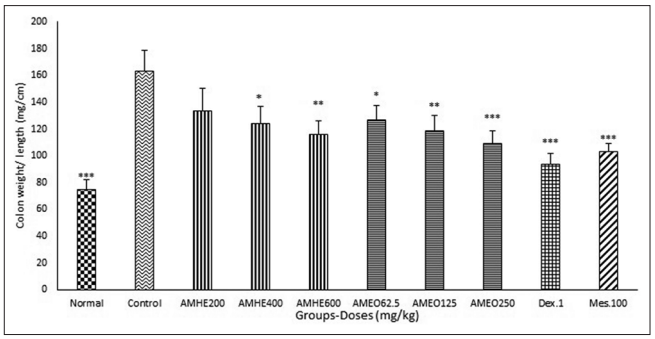

Figure 2: Effect of *A. millefolium* on colonic weight to length ratio (mg/cm). Normal = normal rats treated with normal saline (5 ml/kg), Control = Rats with colitis treated with normal saline (5 ml/kg), AMHE = *A. millefolium* hydroalcoholic extract, AMEO = *A. millefolium* essential oil, Dex = dexamethasone, Mes = mesalazine. Data are presented as mean ± SEM, $n = 6$. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ show significant difference versus control group

$P < 0.05$). Other treatments and even Dex. 1 showed no significant change in body weight through the experimental period ($P > 0.05$) [Table 1].

All groups received AMHE and AMEO as well as reference drugs showed significant decrease in weight to length ratio of colons in comparison to control group (at least $P < 0.05$). AMHE200 was the exception and could not ameliorate this parameter ($P > 0.05$).

Ulcer index

All groups received AMHE and AMEO as well as reference drugs showed an attenuation in ulcer index of colons in comparison to control group; however, this effect was only significant (at least $P < 0.05$) for greater doses of AMHE (400 and 600 mg/kg) and AMEO (125 and 250 µl/kg) [Figure 3].

Pathologic evaluations

In normal group, it was obvious that the mucosal and sub-mucosal layers and crypts were at normal status with no leucocyte infiltration and inflammation. Therefore, TCI was scored zero.

In control group with colitis inflammation of mucosal and sub-mucosal layers, crypt damage and leukocyte infiltration were at maximum level, therefore, TCI was top scored [Table 2 and Figure 4].

TCI

Histopathologic signs and eventually TCI revealed significant improvement in groups treated with AMHE (400 and 600 mg/kg) and AMEO (125 and 250 µl/kg) compared to control group (at least $P < 0.05$) [Table 2]. Treatment with Dex. 1 and Mes. 100 decreased all microscopic features of colitis versus control group ($P < 0.01$) [Table 2].

Biochemical evaluation

MPO activity

MPO activity in groups treated with AMHE at all doses as well as Dex and MES declared significant decline in comparison to control group (at least $P < 0.05$), whereas AMEO at different doses had no significant effect ($P > 0.05$) [Figure 5].

MDA value

All groups received AMHE and AMEO as well as reference drugs showed significant decrease in MDA value of colons in comparison to control group (at least $P < 0.05$). AMHE200 was the exception and could not decrease this parameter [Figure 6].

Discussion

In this study, ulcer-healing effects of hydroalcoholic extract and essential oil of *A. millefolium* were studied on an experimental model of acute colitis in rats. The findings revealed that both extract and essential oil fractions of plant in applied doses (AMHE: 200 mg/kg and AMEO: 62.5 µl/kg are not included) improved macroscopic, microscopic, and biochemical indices of colitis in animals. Although more obvious improvements were found after using greater doses of AMHE and AMEO, the dose–effect relationship was not attained for the majority of results. The animals had also better weight gain after treating with AMHE (600 mg/kg) and AMEO (250 µl/kg) suggesting improvement in colitis and/or enhancing in appetite of rats because of appetizer property of Yarrow had demonstrated by Nematy *et al*.[21] They reported that *A. millefolium* at doses of 50 and 100 mg/kg increased appetite in Wistar rats. This could be helpful in chronic

Table 2: Microscopic parameters of colitis in rats treated with *A. millefolium*

Groups/Doses (mg or µg/kg)	Inflammation Severity (0-3)	Inflammation Extent (0-3)	Leukocyte Infiltration (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 (3-3)	2.5 (2-3)	3.0 (2-3)	3.5 (3-4)	12.0 (11-13)
AMHE200	2.0 (1-3)	2.0 (1-3)	2.0 (1-3)*	3.0 (1-4)	9.0 (4-13)*
AMHE400	1.0 (0-3)**	1.5 (0-1)**	1.0 (0-2)**	1.0 (1-3)**	4.5 (1-9)**
AMHE600	2.0 (2-3)**	1.0 (1-3)	2.5 (1-3)	2.0 (2-3)*	7.5 (6-11)**
AMEO62.5	2.0 (1-3)*	2.0 (1-3)	2.5 (2-3)	3.0 (3-4)	9.5 (7-13)
AMEO125	1.5 (0-1)**	1.5 (0-2)*	1.5 (1-2)*	1.5 (1-2)**	6.0 (3-7)**
AMEO250	1.0 (0-2)**	1.0 (0-2)**	1.5 (1-2)**	1.0 (1-2)**	4.5 (2-8)**
Dex. 1	1.0 (0-1)**	0.5 (0-1)***	1.0 (0-1)**	0.0 (0-0)***	2.5 (0-3)***
Mes. 100	0.5 (0-1)***	0.5 (0-1)***	1.0 (0-1)**	1.0 (0-2)**	3.0 (0-5)**

Normal=Normal rats treated with normal saline (5 ml/kg), Control=Rats with colitis treated with normal saline/tween (5 ml/kg), AMHE=*A. millefolium* hydroalcoholic extract, AMEO=*A. millefolium* essential oil, Dex=dexamethasone, Mes=measalamine. Data are presented as median (range), n=6. *P<0.05, **P<0.01, and ***P<0.001 show significant difference versus control group

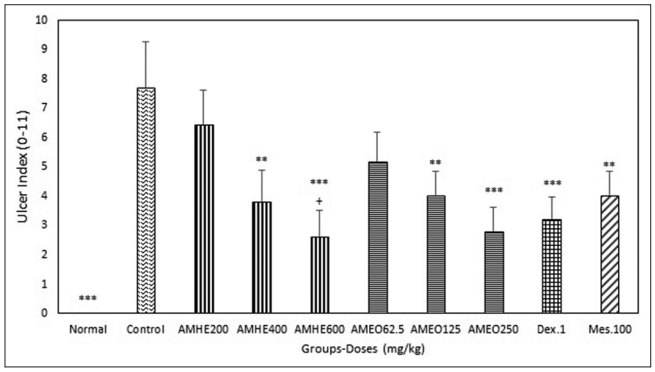

Figure 3: Effect of *A. millefolium* on ulcer index of colitis. Normal = normal rats treated with normal saline (5 ml/kg), Control = Rats with colitis treated with normal saline (5 ml/kg), AMHE = *A. millefolium* hydroalcoholic extract, AMEO = *A. millefolium* essential oil, Dex = dexamethasone, Mes = mesalazine. Data are presented as mean ± SEM, n = 6. *P < 0.05, **P < 0.01, and ***P < 0.001 show significant difference versus control group, +P < 0.05 versus AMHE200 group

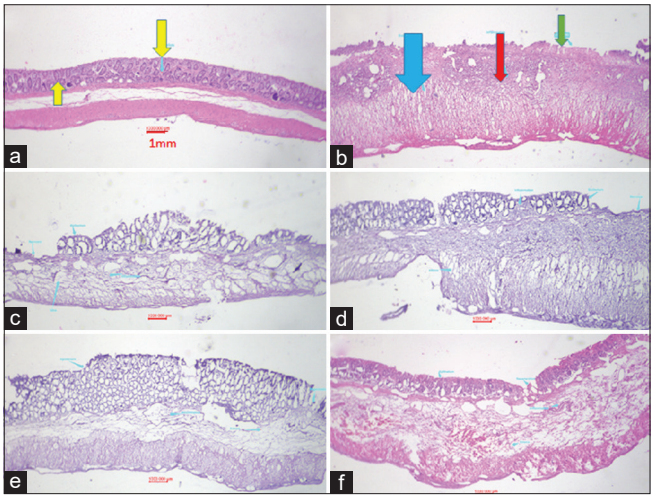

Figure 4: Effect of *A. millefolium* on microscopic features of colitis (a) Normal colon shows normal tissue architecture including intact epithelium and normal crypts (yellow arrow) (b) Control colitis tissue shows highest tissue damage including disrupted epithelium (green arrow), crypt damage (red arrow), leucocyte infiltration, inflammation, and edema (blue arrow) (c) Colitis treated with ACHE600 mg/kg (d) colitis treated with AMEO125 µl/kg (e) Colitis treated with dexamethasone 1 mg/kg and (f) Colitis treated with mesalazine 100 mg/kg. In these last four images, the indices of colitis have improved in some degrees. H and E staining and ×10 magnification was made

UC when weight loss, anemia, and/or anorexia are troublesome symptoms.[22] In reference groups, rats treated with MES showed weight gain; however, dexamethasone treated ones represented no weight gain. This is probably because of catabolic properties of glucocorticoids that likely counteract with disease improving associated weight gain.[23] Considering MPO activity, it is demonstrated that AMHE at different doses was effective and diminished this parameter as the effect was comparable with reference drugs (Dex and MES). This is consistent with the results of a study by Potrich *et al*.[17] in which a dose of 100 mg/kg of Yarrow hydroalcoholic extract was able to reduce MPO levels in significant amount. AMEO, on the other hand, was not effective suggesting that essential oil composition of *A. millefolium* could not likely inhibit lipid peroxidation and leucocyte infiltration in target colonic tissue presumably because of lack or paucity of flavonoid contents and/or weak availability or low adequacy of doses after oral use. Although this is the first time that the effect of yarrow essential oil on MPO levels has been studied, other studies have shown that the essential oil of this plant can affect the function of immune cells and leukocytes.[18] Furthermore, the groups treated with AMHE and AMEO at two greater doses exhibited a significant decline in MDA value compared to control groups confirming both fractions of *A. millefolium* had antioxidant and anti-inflammatory properties on colonic tissue.[24]

As it is previously mentioned, *A. millefolium* (Yarrow) is a herbaceous plant from Acteracea family that its areal parts contain flavonoids, tannins, alkaloids, mono, and sesqui-terpenes with anti-inflammatory, anti-ulcer, antioxidant, and immune-modulatory effects for which anti-colitis activity could be anticipated.[12]

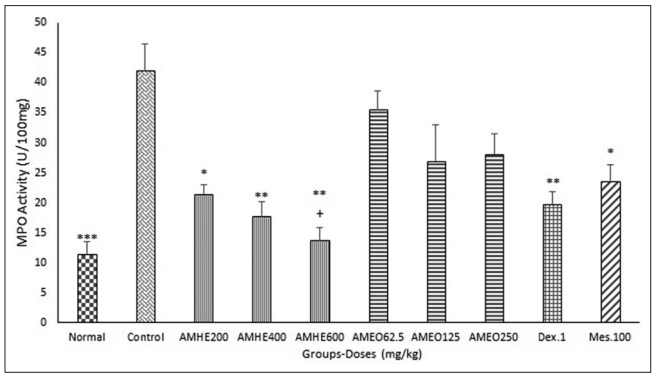

Figure 5: Effect of *A. millefolium* on MPO activity in colonic tissue. Normal = normal rats treated with normal saline (5 ml/kg), Control = Rats with colitis treated with normal saline (5 ml/kg), MPO = myeloperoxidase, AMHE = *A. millefolium* hydroalcoholic extract, AMEO = *A. millefolium* essential oil, Dex = dexamethasone, Mes = mesalazine. Data are presented as mean ± SEM, *n* = 6. **P* < 0.05, ***P* < 0.01, and ****P* < 0.001 show significant difference versus control group, ⁺*P* < 0.05 versus AMHE200 group

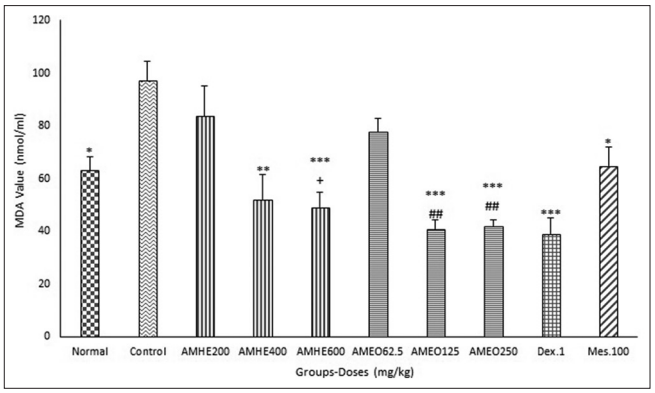

Figure 6: Effect of *A. millefolium* on MDA value in colonic tissue. Normal = normal rats treated with normal saline (5 ml/kg), Control = rats with colitis treated with normal saline (5 ml/kg), MDA = malondialdehyde, AMHE = *A. millefolium* hydroalcoholic extract, AMEO = *A. millefolium* essential oil, Dex = dexamethasone, Mes = mesalazine. Data are presented as mean ± SEM, *n* = 6. **P* < 0.05, ***P* < 0.01, and ****P* < 0.001 show significant difference versus control group, ⁺*P* < 0.05 versus AMHE200 and ##*P* < 0 versus AMEO62.5 group

Apigenin, luteolin, and their 7-O-glycoside derivatives, quercetine, cynaroside, salvigenin, and coffeoylquinic acid on the other hand are the main flavonoids of *A. millefolium* having strong antioxidant activity that could likely protect fibroblasts from active destroying radicals and leading to wound-healing effects.[25,26] Centaureidin and Achillicin III, respectively are among flavonoids and terpenoids ingredients of Yarrow have antiproliferative effect on Hela, MCF-7, and A431 cell lines suggesting an important effect on colon cancers is sometimes associated with chronic and relapsing colitis.[27,28] *A. millefolium* aerial parts have also been reported to be rich in apigenin and luteolin and their 7-O and 7-malonyl glycosides for which anti-colitis properties have been previously demonstrated.[29,30] Borneol, camphor, eucalyptol, alpha-pinene, and beta-terpineol are other ingredients in essential oil component of *A. millefolium* with radical scavenging and antioxidant properties might contribute in ameliorative effects of *A. millefolium* essence partition.[12,14,31]

A number of recent studies have also proven anti-ulcer effect of *A. millefolium* on gastric ulcer. The researchers demonstrated a healing character of *A. millefolium* by increasing mucosal cell proliferation, inhibiting acid and pepsin secretions, and rising protective factors on gastric wall.[32,33] It has been shown that *A. millefolium* can also resolve pain and cramps within gastrointestinal tract because it can decrease ileum and colon movements and spasms at the same time on stomach, it exerted prokinetic effects.[34,35] Whether these peptic ulcer healing and antispasmodic mechanisms are likely involved in alleviation of UC need more detailed experiments.

It has been shown that cytokines, eicosanoids, and local hormones, such as interleukin-6 (IL6), IL-1β, prostaglandin E2, granulocyte macrophage-colony stimulating factor, nitric oxide (NO), and tumor necrosis factor-alpha (TNF-α) have important roles in colitis pathogenesis.[28] Many bioactivity studies done with *A. millefolium* have revealed that this plant can alter the synthesis and/or activity of these factors in favor of colitis amelioration. For example, aqueous extract of *A. millefolium* (25–300 μg/ml) suppressed lipopolysaccharides-induced NO synthesis in a dose-dependent manner.[36] Additionally, the extract inhibited cyclooxygenase-2, IL-6, and TNF-α production, whereas synthesis and secretion of IL-10 (as an anti-inflammatory cytokine) was significantly increased.[12] Furthermore, *A. millefolium* methanolic extract (100 and 200 mg/kg) interestingly exhibited hepatoprotective activity in animal model of liver toxicity probably because of anti-inflammatory and anti-oxidative properties; therefore, Yarrow as one of the Live-52® ingredients can improve liver cirrhosis when using in patients with UC that suffer from extra-intestinal hepato-biliary manifestations.[37,38]

Conclusions

This study at the first time reported the healing properties of hydroalcoholic extract and essential oil of *A. millefolium* on colitis induced by acetic acid. This could be because several different active components exist within both extract and essential oil fractions and subsequently various different mechanisms of action some of which were accounted here. Therefore, more detailed studies are required to confirm suitability of this medicinal plant as complementary remedy for human colitis.

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Conflicts of interest

There are no conflicts of interest.

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