Original Article

Evaluation of the Therapeutic Effect of *Achillea wilhelmsii* C. Koch Aqueous Extract in Acetic-acid-induced Ulcerative Colitis in Rat

Ali Ghobadi, Mohammad Heydarian[1], Gholamreza Bahrami[1], Leila Hosseinzadeh[1], Yalda Shokoohinia[1], Mohammad Hosein Farzaei[1], Hosna Khazaei[1], Kimia Aghaei[1]

Research Institute for Islamic and Complementary Medicine, Iran University of Medical Sciences, Tehran, [1]Pharmaceutical Sciences Research Center, Health Institute, Kermanshah University of Medical Sciences, Kermanshah, Iran

Abstract

Context: Ulcerative colitis (UC) is a chronic, idiopathic, and recurrent disease with unknown etiology. *Achillea wilhelmsii* has been introduced as a herbal remedy for gastrointestinal ulcers and UC in traditional Persian medicine. **Aims:** We examined the effectiveness of *A. wilhelmsii* aqueous extract against acetic-acid-induced UC in rats. **Settings and Design:** Fifty-six male Wister albino rats weighing 180–200 g were randomly divided into eight groups and after induction of UC were treated with five doses of aqueous extract of *A. wilhelmsii.* **Materials and Methods:** After induction of UC by acetic acid, the aerial parts of *A. wilhelmsii* (6.25, 12.5, 25, 50, and 100 mg/kg) were administered orally. On 11th day, the animals were euthanized by overdose of ether inhalation and the intestinal tissue was rapidly dissected for macroscopic, histological, and microscopic scores. **Statistical Analysis Used:** Data were analyzed by stats Directver.2.7.9 (SAS, Cary, North Carolina). One-way analysis of variance (ANOVA) followed by Newman–Keul's *post hoc* test for multiple comparisons. A value of $P < 0.05$ was considered as significant level. Results were expressed as mean ± standard error of the mean (SEM). **Results:** All doses of *A. wilhelmsii* extract significantly reduced macroscopic and microscopic scores of colitis without significant changes in bodyweight of animals. **Conclusions:** Treatment of the rats with *A. wilhelmsii* extract improved UC via its anti-inflammatory, antioxidant, and antimicrobial activities. According to the results of this study, *A. wilhelmsii* has a therapeutic effect against acetic-acid-induced UC in the animal model.

Keywords: *Achillea wilhelmsii C. Koch, antioxidant, herbal remedy, inflammation, ulcerative colitis*

Key Messages: Achillea wilhelmsii C. Koch aqueous extract can be considered as a potent treatment against UC induced by acetic acid in the rat model. This therapeutic effect was comparable with the standard drug. It has been suggested that these therapeutic effects are due to its anti-inflammatory, antioxidant, and antimicrobial actions.

Introduction

Inflammatory bowel disease (IBD) is a chronic, idiopathic, and recurrent disease[1] with two major subtypes, including Crohn's and colitis.[2] Crohn's disease (CD) can involve all parts of the gastrointestinal tract, whereas UC just affects colonic mucosa.[3] Increased inflammatory mediators due to immune defects or infectious agents are the most important factors involved in IBD pathogenesis.[4] During the progression of IBD, the immune system has not appropriate response against the infectious agents to the initial state and the intestinal tissue remains inflamed;[4] even it may lead to invasive cancer.[5] These inflammatory responses generally including overexpression of pro-inflammatory cytokines such as interleukin-1ß (IL-1ß), interferon-γ (INF-γ), tumor necrosis factor-α (TNF-α), and interleukin-23 (IL-23).[6-9] IBD first spread in high-income countries such as Northern Europe and North America with the high percentage. So that 1.4 million persons in the US and 2.2 million persons in Europe have IBD. It has spread in Asia and Southern Europe with an annual incidence of 6.3 per 100,000 persons for UC and 20.2 per 100,000 persons for CD.[10] IBD usually occurs between the ages of 15 and 30 and rarely occurs in patients with 50–60 years old.[11] The pathogenesis of IBD is not clear yet but it has been suggested that environment, geographic location, and smoking are important factors that can lead to IBD in a genetic background.[6,12,13] Anti-inflammatory drugs such as corticosteroids (prednisolone, methylprednisolone, and budesonide),[14] and amino salicylates have been used by patients suffering from IBD. 5-aminosalicylic acid (5-ASA) can be used

How to cite this article: Ghobadi A, Heydarian M, Bahrami G, Hosseinzadeh L, Shokoohinia Y, Farzaei M, *et al*. Evaluation of the therapeutic effect of *Achillea wilhelmsii* C. Koch aqueous extract in acetic-acid-induced ulcerative colitis in rat. J Rep Pharm Sci 2021;10:29-34.

Received: 07 Jan 2020
Accepted: 10 Sep 2020
Published: 31 May 2021

Address for correspondence:
Dr. Mohammad Hosein Farzaei, Pharmaceutical Sciences Research Center, Health Institute, Kermanshah University of Medical Sciences, Kermanshah, Iran.
E-mail: mh.farzaei@gmail.com

Access this article online

Website: www.jrpsjournal.com

DOI: 10.4103/jrptps.JRPTPS_4_20

Quick Response Code:

orally or topically. Mesalamine, sulfasalazine, and olsalazine are available for oral use.[15] Sulfasalazine cleaved by anaerobic bacteria in bowel to 5-ASA and sulfa pyridine (SP). Recent researches showed that therapeutic effect of sulfasalazine is due to ASA and SP that causes diarrhea, abdominal pain, nausea, and vomiting.[16,17] Because of several side effects of drugs used for IBD treatment, their long-term usage is limited.[11]

Many researches showed the beneficial effect of herbal remedies including anti-inflammatory, antioxidant, and anticancer, without above side effects that are due to treatment with chemical drugs.[18,19] *Achillea wilhelmsii* C. Koch is a perennial herb that belongs to *Asteraceae* family and includes more than 100 species worldwide,[20,21] especially central and western areas of Asia and Europe.[22,23] Several species of *Achillea* genus are known as Bumadaran in Persian.[24]

Achillea wilhelmsii contains saponins, alkaloids, volatile oils, terpenoids, and flavonoids.[25] It has been shown that antioxidant activity as well as antimicrobial properties against *Escherichia coli* and *candida*.[26] It has been shown that *A. wilhelmsii* has a stimulatory effect on humoral and cellular immunity and increases immune response due to its flavonoids and saponins content that stimulates humoral response and has immunomodulatory activity.[27] It has also been used as antispasmodic[28] immune system regulator, antiplatelet activity,[29] antitumor,[30,31] antiulcer,[32] cholera therapist,[33] and antibacterial.[34-36] This study was conducted to investigate the therapeutic effect of *A. wilhelmsii* aqueous extract in the experimental model of colitis.

Subjects and Methods

Preparation of *A. wilhelmsii* aqueous extract

Achillea wilhelmsii was gathered from Bisotun area in Kermanshah province, west of Iran, in May 2008 and authenticated by Dr. Masoumi, and voucher specimen (NO: 2729) was deposited in the central herbarium of faculty Agriculture Razi University of Kermanshah. Aerial parts of the plant were air-dried in shade and powdered by electric milling to create more surface contact with the solvent. Maceration method was used to prepare the extract. 100 g of the powder was solved in 100 mL double-distilled water and boiled for 30 min. The extract was cooled down and filtered with Whatman filter paper no.1 and extract was stored at 4°C for further use.[37]

Animal model

Fifty-six male Wister-albino rats weighing 180–200 g were accommodated under standard laboratory condition at normal temperature (23±2°C), and 12 h light/dark cycle. Ethical rules of the investigation on animals were approved by the committee of Kermanshah University of Medical Sciences (KUMS). During the adaptation, animals were fed with standard food and water for 1 week.

Induction of colitis and treatment

Acetic-acid-induced colitis, which is similar to human UC; it was first described by Macpherson according to Kojima *et al*.'s[38] method. It is a laboratory and experimental model of human IBD. According to Farzaei *et al*.'s[39] investigation, rats were fasted for 24 h and then were anesthetized with intraperitoneal (IP) injection of ketamine (10 mg/kg) with the right side position; after that, 1 mL of acetic acid (4% v/v in 0.9% saline) was instilled through rectum via rubber cannula (8 cm long). Because of the preventing of acetic acid leakage, we placed rats in a supine position. Sulfasalazine was used as the standard drug.

Experimental design

Rats were randomly divided into eight groups ($n = 8$). Induction of colitis was performed by instillation of acetic acid. Animals were grouped as follows: (1) animals without UC and any treatment, (2) animals with UC and received 6.25 mg/kg/d of *A. wilhelmsii* extract, (3) animals with UC and received 12.5 mg/kg/d of *A. wilhelmsii* extract, (4) animals with UC and received 25 mg/kg/d of *A. wilhelmsii* extract, (5) animals with UC and received 50 mg/kg/d of *A. wilhelmsii* extract, (6) animals with UC and received 100 mg/kg/d of *A. wilhelmsii* extract, (7) animals with UC that received distilled water, and (8) animals with UC and treated by 100 mg/kg/day sulfasalazine. All treated groups received *A. wilhelmsii* extract dissolved in water and administered orally by gavage. Treatment was administered to the animals for 10 days, 1 week before induction of colitis up to 3 days after induction of colitis. On the 11th day, the animals were euthanized by an overdose of ether inhalation and intestinal tissue was rapidly dissected.

Microscopic and macroscopic assessment of colonic damage

Both macroscopic and histopathologic assessments were performed in this research. According to Farzaei *et al*.'s[39] method, the samples were sliced in two pieces: one piece for histopathology assessment and the other for measuring biomarkers that were weighed and maintained at –20ºC for 24 h.

Macroscopic scoring

For macroscopic evaluation, on the 11th day, pieces were separated from colon at 5 cm in length and fixed in 10 mL formalin 10%. Macroscopic scoring was performed by using a method based on Mustafa *et al*.'s[40] study, according to scale ranging from 0 to 4 as shown in Table 1.

Microscopic scoring

Very thin cut of colonic tissue should be made for the light to pass the through and fixed in 10% formalin in phosphate-

Table 1: Histological assessment due to acetic acid inoculation in rats

Damage of inflammation	Scores
No macroscopic changes	0
Mucosal erythema only	1
Mild mucosal edema, slight bleeding, or small erosions	2
Moderate edema, bleeding ulcers, or erosions	3
Severe ulceration, erosions, edema, and tissue necrosis	4

Table 2: Histological assessment of biopsy specimen thickness in animals with UC induced by acetic acid inoculation

Neutrophil infiltrate			
None 0	**Slight increase 1**	**Marked increase 2**	
Epithelium			(0–2)
Lamina propria			(0–2)
Muscularis mucosa			(0–2)
Submucosa			(0–2)
Muscularis propria			(0–2)
Serosa			(0–2)
Fibrin deposition			
Absent 0	Present 1		
Mucosa			(0–1)
Submucosa			(0–1)
Submucosal neutrophil margination			
Absent 0	Present 1		(0–1)
Submucosal edema			
Nil 0	Patchy 1	Confluent 2	(0–2)
Epithelial necrosis			
Nil 0	Localized 1	Extensive 2	(0–2)
Epithelial ulceration			
Absent 0	Present 1		(0–1)
Maximum score			20

buffered saline and then stained with hematoxylin and eosin.[40] This thin cut was scored by histopathologist according to scoring system shown in Table 2.[40,41]

Statistical analysis

Data were analyzed by stats Directver.2.7.9 (SAS, Cary, North Carolina) One-way analysis of variance (ANOVA) followed by Newman–Keul's *post hoc* test for multiple comparisons. A value of $P < 0.05$ was considered as significant level. Results were expressed as mean ± standard error of the mean (SEM).

Results

Macroscopic and microscopic assessment of colonic damage

Results of macroscopic score are shown in Figure 1. According to the chart, higher macroscopic scores are seen in control group. Intra-rectal administration of acetic acid to the control group exhibited ulceration, adhesion, wall thickening, and severe inflammation in comparison with the normal group. The best healing activity among treated groups belonged to 12.5 mg/kg of *A. wilhelmsii* extract. Higher and lower doses reduce the therapeutic effect of *A. wilhelmsiias*. As well as administration of sulfasalazine (100 mg/kg) improves ulcerating, adhesion, wall thickening, and inflammation than other groups and have lowest macroscopic score. Macroscopic results show that the score of treatment group with sulfasalazine was very similar to the treatment group with 12.5 mg/kg of *A. wilhelmsii* extract.

Results of microscopic score are shown in Figure 2. According to Table 2, microscopic evaluation of control group (Score 5) showed severe edema, hemorrhage, necrosis, ulceration, and mucosal and submucosal polymorphonuclear. In the positive control group (sulfasalazine), minor lesions and mild

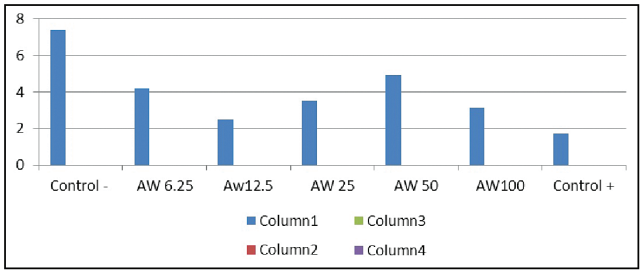

Figure 1: Ulcer score in different groups with UC receiving *Achillea wilhelmsii* extract

mucosal inflammation was observed. In the treatment group with 100 mg/kg of *A. wilhelmsii* extract, crypt destruction, submucosal inflammation, and polymorphonuclear was observed. In treatment groups with 25 and 50 mg/kg of *A. wilhelmsii* extract, mild inflammation, diffuse destruction of crypts, and edema in some parts were observed. As well as, colon features were normal in normal groups. Finally, according to microscopic evidence as only mucosal inflammation was seen in the sulfasalazine group, so it is expected to have the least microscopic score (Score 1) like the treatment group with 12.5 mg/kg of *A. wilhelmsii* extract (Score 1).

Discussion

This study investigates the therapeutic effects and possible mechanism and chemical constituents of *A. wilhelmsii* aqueous extract. Our research showed the antimicrobial effects of *A. wilhelmsii* on UC by laboratory evidence, microscopic and macroscopic tissue studie,s and its effects were comparable with sulfasalazine. The reduction in microscopic and macroscopic score is due to administration of different doses of *A. wilhelmsii* (6.25, 12.5, 25, 50, and 100 mg/kg) that dramatically improvement of UC was observed in treatment

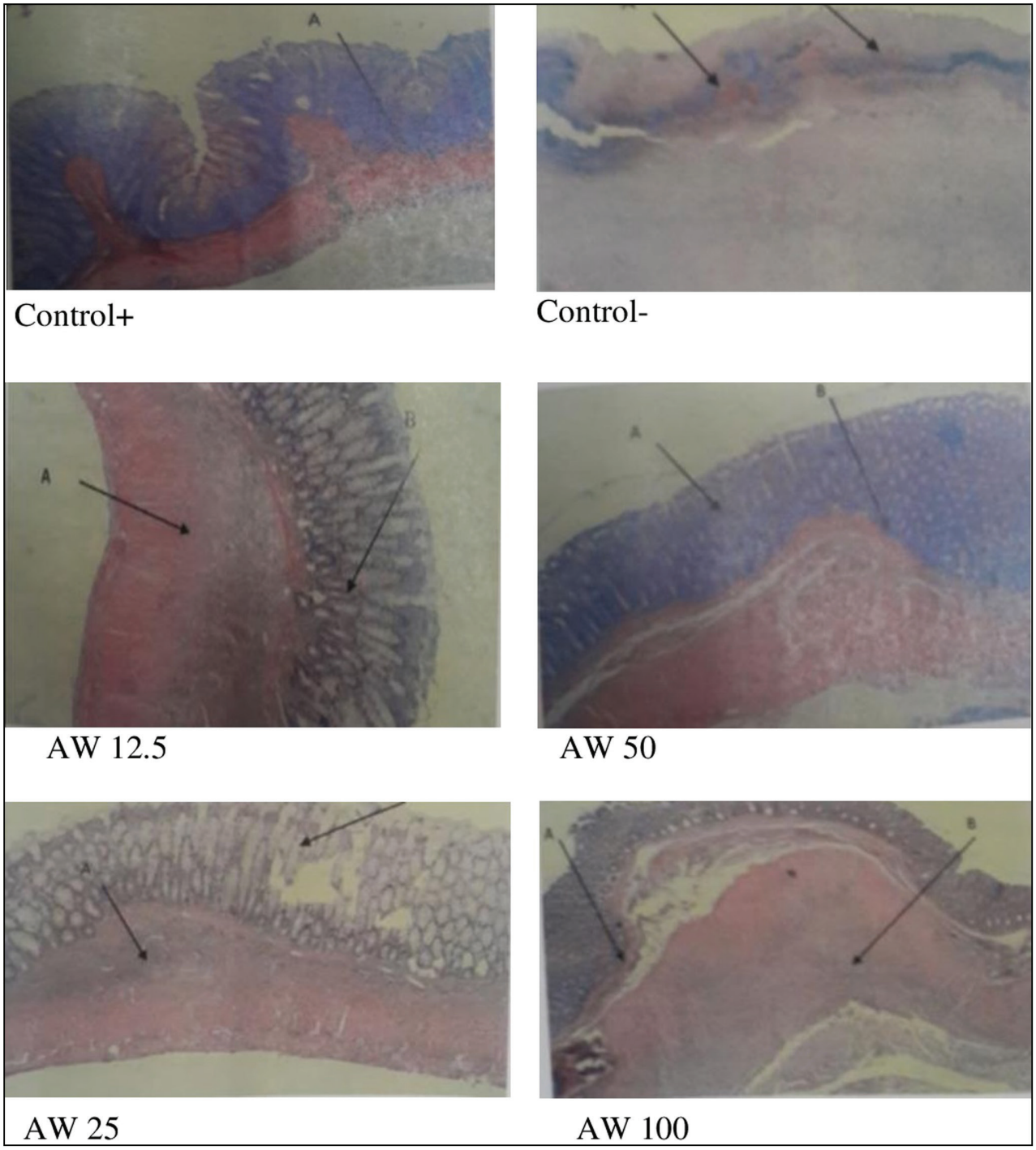

Figure 2: Histological assessment of colon tissue in different groups. Microscopic evaluation of control group (Score 5) showed intense transdermal inflammation and/or diffuse necrosis (A) and sever crypt destruction (B). Control+ group (Score 1) showed minimal mucosal inflammation (A), in AW-50 (Score 2) polymorphonuclear (PMN) accumulation (A) and destruction of crypts (B), in AW-25 group (Score 2) creation of crypts and submucosal inflammation and mild PMN accumulation (B), in AW-100 (Score 3) PMN accumulation (A) and destruction of crypts (B) were observed

group with 12.5 mg/kg of *A. Wilhelmsii* extract. Several studies showed that in proinflammatory situation, some of the cytokines such as TNF-α and IL-6 produced in large quantities leading to activation of mesenchymal cells[42] and cause exacerbate in the inflammatory response and severe tissue damage which induce the production of other cytokines by cells that eventually lead to intestinal necrosis, edema and neutrophil infiltration of the tissue.[43,44] Researches showed that blocking inflammatory mediators reduces acute and chronic disorders caused by them. Some of the cellular and animal studies have shown the anti-inflammatory and inhibitory effects of *A. wilhelmsii* extract on inflammatory cytokines.[45] Studies show that *A. wilhelmsii* extract does its effect by increasing macrophages and B-lymphocytes levels.[46] Flavonols as

one of the active compounds of *A. wilhelmsii* extract can stimulate proliferation of human peripheral blood leukocyte and raises the activity of helper T cells, cytokines, interleukin 2, g-interferon, and macrophages so it can be considered as a potent immunomodulatory herb.[47] IBD is the result of improper immune response (the phenomenon of the autoimmunity and non-auto immunity).[48] Important characteristic of UC is bloody diarrhea and in more severe cases form bloody stool with dyspepsia before extraction, but Crohn's is heterogeneous disease and its manifestations depending on where it is involved and symptoms usually include: non-bloody diarrhea, fever, dyspepsia, and lose weight.[49,50] Among the animal models used to UC studies, mice are the most appropriate model. UC induced by acetic acid inoculation because it induces colitis similar to human colitis in animal models.[11,51] Acetic acid does not directly cause intestinal inflammation[11] but it induces IBD by overexpression of pro-inflammatory cytokines such as TNF-α, IL-6, and IL-23;[1] so acetic acid is used to investigate the therapeutic effect of medications. For this purpose, these medications should be taken 24h after the administration of acetic acid.[11] *Achillea wilhelmsii* has beneficial effects such as hypertensive,[52] hepatoprotective,[53] and gastric acid output.[54] HPLC chromatogram of the *A. wilhelmsii* aqueous extract shows this extract contains isoschaftoside, schaftoside, vicenin 2, vicenin 3, caffeic acid, isovitexin, leucodin; *A. wilhelmsii* aqueous extract shows a high content of phenols and flavonoids.[55] Mahmoudabady *et al*.[24] showed that flavonoids are one of the most common antioxidant and have a beneficial effect on reducing oxidative stress. As well as, the antioxidative activity of *A. wilhelmsii* extract (75 mg/kg) causes significant changes in hematologic parameters such as mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular hemoglobin (MCH) reduction. Non-toxicity is confirmed by histopathologic and microscopic investigation. Niazmand *et al*.[54] showed that *A. wilhelmsii* inhibits extracellular calcium entry into cells by blocking voltage-dependent channels and cause vasodilation in the aorta.

Conclusion

The result of this study showed that the aqueous extract of *A. wilhelmsii* treated acetic-acid-induced UC in rats. As well as, macroscopic and microscopic scores in all groups that received *A. wilhelmsii* were less than control group, but the most effective dose of *A. wilhelmsii* extract was 12.5 mg/kg. In this study the necessity of herbal remedies and its superiority compared with chemical treatments was emphasized. As well as therapeutic effects of *A. wilhelmsii* were discussed in detail and showed its best therapeutic effect at 12.5 mg/kg. Hope to be considered as a complementary treatment of colitis in the near future.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Rahimi R, Nikfar S, Abdollahi M. Induction of clinical response and remission of inflammatory bowel disease by use of herbal medicines: A meta-analysis. World J Gastroenterol 2013;19:5738-49.
2. Fiocchi C. Inflammatory bowel disease: Etiology and pathogenesis. Gastroenterology 1998;115:182-205.
3. Bamias G, Nyce MR, De La Rue SA, Cominelli F; American College of Physicians; American Physiological Society. New concepts in the pathophysiology of inflammatory bowel disease. Ann Intern Med 2005;143:895-904.
4. Hanauer SB. Inflammatory bowel disease: Epidemiology, pathogenesis, and therapeutic opportunities. Inflamm Bowel Dis 2006;12:S3-9.
5. Ullman T, Croog V, Harpaz N, Sachar D, Itzkowitz S. Progression of flat low-grade dysplasia to advanced neoplasia in patients with ulcerative colitis. Gastroenterology 2003;125:1311-9.
6. Baumgart DC, Carding SR. Inflammatory bowel disease: Cause and immunobiology. Lancet 2007;369:1627-40.
7. Baumgart DC, Sandborn WJ. Inflammatory bowel disease: Clinical aspects and established and evolving therapies. Lancet 2007;369:1641-57.
8. Cho JH, Abraham C. Inflammatory bowel disease genetics: Nod2. Annu Rev Med 2007;58:401-16.
9. Lucas A, Cobelens PM, Kavelaars A, Heijnen CJ, Holtmann G, Haag S, *et al*. Disturbed in vitro adrenergic modulation of cytokine production in inflammatory bowel diseases in remission. J Neuroimmunol 2007;182:195-203.
10. Molodecky NA, Soon IS, Rabi DM, Ghali WA, Ferris M, Chernoff G, *et al*. Increasing incidence and prevalence of the inflammatory bowel diseases with time, based on systematic review. Gastroenterology 2012;142:46-54.e42; quiz e30.
11. Low D, Nguyen DD, Mizoguchi E. Animal models of ulcerative colitis and their application in drug research. Drug Des Devel Ther 2013;7:1341-57.
12. Pithadia AB, Jain S. Treatment of inflammatory bowel disease (IBD). Pharmacol Rep 2011;63:629-42.
13. Podolsky DK. Inflammatory bowel disease (1). N Engl J Med 1991;325:928-37.
14. Baron JH, Connell AM, Kanaghinis TG, Lennard-Jones JE, Jones AF. Out-patient treatment of ulcerative colitis. Comparison between three doses of oral prednisone. Br Med J 1962;2:441-3.
15. Bernstein CN, Fried M, Krabshuis JH, Cohen H, Eliakim R, Fedail S, *et al*. World gastroenterology organization practice guidelines for the diagnosis and management of IBD in 2010. Inflamm Bowel Dis 2010;16:112-24.
16. Brown JP, McGarraugh GV, Parkinson TM, Wingard RE Jr, Onderdonk AB. A polymeric drug for treatment of inflammatory bowel disease. J Med Chem 1983;26:1300-7.
17. Gisbert JP, González-Lama Y, Maté J. 5-aminosalicylates and renal function in inflammatory bowel disease: A systematic review. Inflamm Bowel Dis 2007;13:629-38.
18. Dattner AM. From medical herbalism to phytotherapy in dermatology: Back to the future. Dermatol Ther 2003;16:106-13.
19. Huffman MA. Animal self-medication and ethno-medicine: Exploration and exploitation of the medicinal properties of plants. Proc Nutr Soc 2003;62:371-81.
20. Dokhani S, Cottrell T, Khajeddin J, Mazza G. Analysis of aroma and phenolic components of selected *Achillea* species. Plant Foods Hum Nutr 2005;60:55-62.
21. Majnooni MB, Mohammadi-Farani A, Gholivand MB, Nikbakht MR, Bahrami GR. Chemical composition and anxiolytic evaluation of

Achillea wilhelmsii C. Koch essential oil in rat. Res Pharm Sci 2013;8:269-75.
22. Saeidnia S, Gohari A, Mokhber-Dezfuli N, Kiuchi F. A review on phytochemistry and medicinal properties of the genus *Achillea*. Daru 2011;19:173-86.
23. Asgary S, Naderi GH, Sarrafzadegan N, Mohammadifard N, Mostafavi S, Vakili R. Antihypertensive and antihyperlipidemic effects of *Achillea wilhelmsii*. Drugs Exp Clin Res 2000;26:89-93.
24. Mahmoudabady M, Lashkari M, Niazmand S, Soukhtanloo M. Cardioprotective effects of *Achillea wilhelmsii* on the isolated rat heart in ischemia-reperfusion. J Tradit Complement Med 2017;7:501-7.
25. Etman M, Hassan A, Atta A, Fahmy G, Youssef A, Nadia A, *et al*. Phytochemical and pharmacological studies on *Achillea santolina* L. plant [Egypt]. Egypt J Vet Sci 1987.
26. Kazemi M, Rostami H. Chemical composition and biological activities of Iranian *Achillea wilhelmsii* L. essential oil: A high effectiveness against Candida spp. and Escherichia strains. Nat Prod Res 2015;29:286-8.
27. Sharififar F, Pournourmohammadi S, Arabnejad M. Immunomodulatory activity of aqueous extract of *Achillea wilhelmsii* C. Koch in mice. Indian J Exp Biol 2009;47:668-71.
28. Ghazanfari G, Minaie B, Yasa N, Nakhai LA, Mohammadirad A, Nikfar S, *et al*. Biochemical and histopathological evidences for beneficial effects of satureja khuzestanica jamzad essential oil on the mouse model of inflammatory bowel diseases. Toxicol Mech Methods 2006;16:365-72.
29. Rahimi R, Mozaffari S, Abdollahi M. On the use of herbal medicines in management of inflammatory bowel diseases: A systematic review of animal and human studies. Dig Dis Sci 2009;54:471-80.
30. Csupor-Löffler B, Hajdú Z, Zupkó I, Réthy B, Falkay G, Forgo P, *et al*. Antiproliferative effect of flavonoids and sesquiterpenoids from *Achillea millefolium* sl on cultured human tumour cell lines. Phytother Res 2009;23:672-6.
31. Tozyo T, Yoshimura Y, Sakurai K, Uchida N, Takeda Y, Nakai H, *et al*. Novel antitumor sesquiterpenoids in *Achillea millefolium*. Chem Pharm Bull (Tokyo) 1994;42:1096-100.
32. Cavalcanti AM, Baggio CH, Freitas CS, Rieck L, de Sousa RS, Da Silva-Santos JE, *et al*. Safety and antiulcer efficacy studies of *Achillea millefolium* L. After chronic treatment in wistar rats. J Ethnopharmacol 2006;107:277-84.
33. Benedek B, Geisz N, Jäger W, Thalhammer T, Kopp B. Choleretic effects of yarrow (*Achillea millefolium* s.l.) In the isolated perfused rat liver. Phytomedicine 2006;13:702-6.
34. Candan F, Unlu M, Tepe B, Daferera D, Polissiou M, Sökmen A, *et al*. Antioxidant and antimicrobial activity of the essential oil and methanol extracts of *Achillea millefolium* subsp. millefolium Afan. (Asteraceae). J Ethnopharmacol 2003;87:215-20.
35. Mahady GB, Pendland SL, Stoia A, Hamill FA, Fabricant D, Dietz BM, *et al*. In vitro susceptibility of helicobacter pylori to botanical extracts used traditionally for the treatment of gastrointestinal disorders. Phytother Res 2005;19:988-91.
36. Stojanović G, Radulović N, Hashimoto T, Palić R. In vitro antimicrobial activity of extracts of four *Achillea* species: The composition of *Achillea clavennae* L. (Asteraceae) extract. J Ethnopharmacol 2005;101:185-90.
37. Ahmed S, Saifullah, Ahmad M, Swami BL, Ikram S. Green synthesis of silver nanoparticles using Azadirachta indica aqueous leaf extract. J Radiat Res Appl Sci 2016;9:1-7.
38. Kojima R, Hamamoto S, Moriwaki M, Iwadate K, Ohwaki T. The new experimental ulcerative colitis model in rats induced by subserosal injection of acetic acid. Nihon Yakurigaku Zasshi Folia Pharmacol Japon 2001;118:123-30.
39. Farzaei MH, Ghasemi-Niri SF, Abdolghafari AH, Baeeri M, Khanavi M, Navaei-Nigjeh M, *et al*. Biochemical and histopathological evidence on the beneficial effects of tragopogon graminifolius in TNBS-induced colitis. Pharm Biol 2015;53:429-36.
40. Mustafa A, El-Medany A, Hagar HH, El-Medany G. Ginkgo biloba attenuates mucosal damage in a rat model of ulcerative colitis. Pharmacol Res 2006;53:324-30.
41. Millar AD, Rampton DS, Chander CL, Claxson AW, Blades S, Coumbe A, *et al*. Evaluating the antioxidant potential of new treatments for inflammatory bowel disease using a rat model of colitis. Gut 1996;39:407-15.
42. Fausel R, Afzali A. Biologics in the management of ulcerative colitis–comparative safety and efficacy of TNF-α antagonists. Ther Clin Risk Manag 2015;11:63.
43. Catanzaro R, Occhipinti S, Calabrese F, Anzalone MG, Milazzo M, Italia A, *et al*. Irritable bowel syndrome: New findings in pathophysiological and therapeutic field. Minerva Gastroenterol Dietol 2014;60:151-63.
44. Pedersen J, Coskun M, Soendergaard C, Salem M, Nielsen OH. Inflammatory pathways of importance for management of inflammatory bowel disease. World J Gastroenterol 2014;20:64-77.
45. Bashi DS, Fazly Bazzaz BS, Sahebkar A, Karimkhani MM, Ahmadi A. Investigation of optimal extraction, antioxidant, and antimicrobial activities of *Achillea* biebersteinii and A. Wilhelmsii. Pharm Biol 2012;50:1168-76.
46. Mitra S, Gupta M, Sarma D. Immunomodulatory effect of IM-133. Phytother Res 1999;13:341-3.
47. Kawakita S, Giedlin H, Nomoto K. Immunomodulators from higher plants. J Nat Med 2005;46:34-8.
48. Wells BG, DiPiro JT, Schwinghammer TL, DiPiro CV. Pharmacotherapy Handbook, 9/E. Virginia, United states: McGraw Hill Professional; 2014.
49. Longo DL, Fauci AS, Kasper DL, Hauser SL, Jameson JL, Loscalzo J. Harrison's principles of internal medicine. New York: Mcgraw-Hill; 2012.
50. Michener WM, Whelan G, Greenstreet RL, Farmer RG. Comparison of the clinical features of Crohn's disease and ulcerative colitis with onset in childhood or adolescence. Cleve Clin Q 1982;49:13-6.
51. Dodda D, Chhajed R, Mishra J. Protective effect of quercetin against acetic acid induced inflammatory bowel disease (IBD) like symptoms in rats: Possible morphological and biochemical alterations. Pharmacol Rep 2014;66:169-73.
52. Niazmand S, Esparham M, Rezaee S, Harandizadeh F. Hypotensive effect of *Achillea wilhelmsii* aqueous-ethanolic extract in rabbit. Avicenna J Phytomed 2011;1:51-6.
53. Yaeesh S, Jamal Q, Khan Au, Gilani AH. Studies on hepatoprotective, antispasmodic and calcium antagonist activities of the aqueous-methanol extract of *Achillea millefolium*. Phytother Res 2006;20:546-51.
54. Niazmand S, Khooshnood E, Derakhshan M. Effects of *Achillea wilhelmsii* on rat's gastric acid output at basal, vagotomized, and vagal-stimulated conditions. Pharmacogn Mag 2010; 6:282.
55. Khazneh E, Hřibová P, Hošek J, Suchý P, Kollár P, Pražanová G, *et al*. The chemical composition of *Achillea wilhelmsii* C. Koch and its desirable effects on hyperglycemia, inflammatory mediators and hypercholesterolemia as risk factors for cardiometabolic disease. Molecules 2016;21:404.