

The effect of aqueous extract of *Dracocephalum polychaetum* Burnm on ulcerative colitis in adult male rats

Kohpyma H¹, Abbasnejad M^{1*}, Ahmadinejad S¹, Mostafavi A^{2,3}, Derakhshanfar A⁴

Received: 09/7/2015

Revised: 02/29/2016

Accepted: 04/23/2016

1. Dept. of Biology, Faculty of Sciences, Shahid Bahonar University of Kerman, Kerman, Iran
2. Dept. of chemistry, Faculty of Sciences, Shahid Bahonar University of Kerman, Kerman, Iran
3. Medicinal Plants Research Nucleus, Faculty of Sciences, Shahid Bahonar University of Kerman, Kerman, Iran
4. Dept. of Pathobiology, Faculty of Veterinary Medicine, Shiraz, Shiraz, Iran

Pars Journal of Medical Sciences, Vol. 14, No.1, Spring 2016

Par J Med Sci 2016; 14(1):7-14

Abstract

Introduction:

Ulcerative colitis is a chronic inflammatory disease that affects mucosa and submucosa of the colon and rectum. Its etiopathology is attributed to the imbalance of the mucosal immune response to the resident bacterial flora together with genetic and environmental factors. *Dracocephalum polychaetum* has traditionally been used to treat digestive tract diseases. Therefore, the present study aimed to evaluate the effect of this herbal aqueous extract on ulcerative colitis.

Materials and Methods:

This experimental study was conducted on 48 male Wistar rats (230-280 g). After inducing ulcerative colitis, rats were divided into the following groups: experimental groups with different doses of extract (50, 100, 200 mg/kg bw), vehicle, prednisolon and control. Ulcerative colitis was induced through acetic acid 4% enema.

Results:

The colon weight/length ratio was significantly reduced in the prednisolone group and the extract group at different doses of the extract: 50 mg/kg ($P < 0.01$), 100 mg/kg ($P < 0.001$), and 200 mg/kg ($P < 0.001$) compared with the vehicle group. The severity and extent of inflammation significantly decreased in the extract group at the dose of 200 mg/kg ($P < 0.05$) as compared with the vehicle group. The concentration of malondialdehyde increased in acetic acid-treated groups, while it decreased in the groups treated with *D. polychaetum* extract and prednisolon).

Conclusion:

It is concluded that the aqueous extract of *D. polychaetum* Burnm is effective in treating acetic acid-induced ulcerative colitis.

Keywords: Ulcerative colitis, Acetic Acid, Rat

Introduction

The etiopathology of ulcerative colitis (UC) is associated with unbalanced mucous immunity, bacterial flora in the oral mucosa as well as environmental and genetic factors (1). Despite the response of mucosal immune cells to inflammatory bowel diseases, recent studies have focused on

cytotoxic T cells (CD4 T cells), and the potential findings can be quite helpful in controlling the inflammation (2). Evidence shows that cytokines and some chemokines such as TNF- α , prostaglandins and leukotrienes mediate the inflammatory responses in all organs including the bowel

* Corresponding author, Address: Dept. of Biolog, Faculty of Sciences, Shahid Bahonar University of Kerman, Kerman, Iran

Tel: +989131409009

Email: mabbas@mail.uk.ac.ir

(3). The inflammation can be effectively controlled by inhibiting the synthesis of cyclooxygenase-2 (COX-2), which increases the number of prostaglandins and significantly contributes to inflammatory intestinal epithelial cells (IECs) (4). Non-steroidal anti-inflammatory medicines such as prednisolone are currently used to treat UC. These medicines inhibit the transcription of the synthesizing enzymes such as nitric oxide (NO), COX, thromboxane A2 and NF- κ B, and prevent the production of inflammatory cytokines (IL1, TNF) and chemokines (IL-8) (5).

Dracocephalum polychaetum bornm is a genus in the mint family Lamiaceae with fifty different species, eight of which are available only in Iran. This species is one of these eight species of the genus *Dracocephalum* that grows exclusively in Kerman Province, Iran (6). This highly branched shrub grows in high altitudes, has wooden roots of 20 cm, is specific to rocky soils and native to Kerman, and is more widespread in the surrounding mountains such as Koochpayeh, Lalezar and Hezar. This plant is covered with short white hairs and many aromatic glands and is also known as Kerman or Lalezar *Melissa officinalis* (7). This aromatic plant is known in Kerman's traditional medicine as a carminative painkiller in treating digestive disorders and also as a sedative called Mofaro. It is also used to treat kidney disorders, toothache, cold, liver disorders and congestion. Antioxidant activities are also attributed to this plant (8).

Dracocephalum can be divided into volatile (essence) components and non-volatile components including flavonoids. The main essence obtained from this plant include sesquiterpenes, delta-cadinene and caryophyllene oxide. The plant extract also includes monoterpenes such as limonene, cis-limonene oxide, terpinen-4-ol, perillaldehyde, α -terpineol, trans-carveol, carvone, pergiern, limonene-10-ol, α -terpinene and a number of alkanes. The non-volatile components of this plant that are separate from its aerial twigs include

different flavonoids such as luteolin and apigenin. The main components of *Dracocephalum* include limonene (16.5%) and perillaldehyde (69.6%), as the two members of the monoterpenes family (9). The limonene in its extract is anti-acid (10), anti-tumor, sedative and anti-anxiety (8). Perillaldehyde is a vasodilator monoterpenes and a calcium channel blocker that comprises about 69.60% of the essence although it was not reported in any of the species studied (8). α -terpinene is a volatile monoterpene alcohol with anti-inflammatory properties (11). The terpinene in the extract also is an immunomodulator (12). Significant anti-inflammatory effects were also reported for luteolin in this plant (13, 14 and 15). The chemoprotective agent apigenin with antioxidant properties is also another anti-inflammatory component that exerts its effect through TNF- α , IL8, IL6 and COX2 (16, 17 and 18).

Limonene and perillaldehyde are the two main components of the essence with bactericidal properties caused by effective components such as α -terpineol, carvone and flavonoids such as apigenin and luteolin, which are certainly known to inhibit inflammatory cytokines such as COX2, IL1, IL6 and TNF- α . *Dracocephalum* is also commonly used by the natives to cure digestive disorders. The present study was therefore conducted to investigate the therapeutic effect of the plant extract on acetic acid-induced colitis.

Materials and Methods

Forty-eight adult male Wistar rats weighing 230-280 g were collected from the proliferation center of laboratory animals in Shahid Bahonar University of Kerman and randomly divided into six groups of eight. All the rats were housed in proper light conditions of 12:12 light-dark cycles at 23 \pm 1 °C. The ethical principles of laboratory animal research were also observed throughout the study.

After inducing the ulcer, the rats in group 1, 2 and 3 received 50, 100 and 200 mg/kg

doses of the extract for six days (8). The sham group received normal saline and another group oral prednisolone (19). The sixth group remained intact as the control group.

In order to induce colitis using acetic acid (16), the rats were kept in separate wire cages with mesh floors to prevent them from eating their stool and provided with only water for 36 hours while they were deprived of food. Each rat was then mildly anesthetized using CO₂ and their rectum was irrigated with 2 ml acetic acid 4% using a plastic cannula of 2.5 mm wide and 8 cm long (20). These rats were kept in these cages for another 24 hours to confirm ulcerative colitis by observing the color and consistency of stool and the presence of bloody stool. The extract was used for six days to treat the ulcers developed. The rats were then killed in compliance with the ethical principles, their colons were extracted through their abdominal cavity and some inflammatory indicators were studied. All the rats' weights were recorded at specific times of the day during the entire period of the experiment.

The ulcer was evaluated by two people who were blinded to the study. Eight cm of the colon was cut beginning 3 cm from the rectum, cut longitudinally and rinsed with cold normal saline and weighed (16). The weight to length ratio of the colon and the ulcer area were then measured and recorded as the ulcer index for each rat (16).

Myeloperoxidase (MPO) activity was calculated as follows:

MPO activity (U g⁻¹) = X/weight of the piece of tissue taken

Optical absorbance changes per minute × 10
The supernatant volume taken in the final reaction

The malondialdehyde density was also calculated as follows considering the initial absorbance:

$$A = \epsilon BC$$

where, A is the optical absorbance read at 532 nm, E is extinction coefficient in $\mu\text{mol}\cdot\text{cm}^{-1}$, B is the cuvette diameter (1 cm) and C is density in $\mu\text{mol}\cdot\text{g}^{-1}$.

Statistical analyses were conducted in SPSS 17 using One-Way ANOVA and Tukey's post-hoc test. The one-sample t-test was also used to confirm the ulcer development in the sham group compared to that in the control group. P values less than 0.05 were considered significant. The data obtained were presented as Mean \pm Standard Error of the Mean (SEM).

Results:

Wide areas of bleeding ulcers and extensive necrosis were observed in the sham group, while no types of ulcer and inflammation were observed in the control, which had not received acetic acid. The data related to factors including level, intensity and ulcer index as well as pathological observations including the degree and intensity of inflammation and also the percentage of the area affected were zero in the control group and the associated columns were therefore eliminated from the relevant diagrams.

Weight loss is an evident morphological feature in UC. As observed in diagram 1, the changes in the body weight is significantly reduced in the control group compared to that in the sham group ($P < 0.05$).

As seen in diagram 2, the weight to length ratio of the colon is significantly reduced in the groups receiving 100 and 200 mg/kg dose ($P < 0.001$) and also 50 mg/kg ($P < 0.01$) compared to that in the sham group.

A significant reduction was observed in the area of mild colon ulcers in the sham group compared to that in the prednisolone (10 mg/kg) group ($P < 0.01$) and the groups receiving 100 and 200 mg/kg dose of the extract ($P < 0.05$). Similarly, a significant reduction was observed in the area of severe colon ulcers in the sham group compared to that in the prednisolone ($P < 0.01$) and in those receiving 200 mg/kg dose of the extract ($P < 0.05$).

According to diagram 3, the ulcer index variations in the sham group are significantly different from those in the prednisolone group ($P < 0.001$), the group

receiving 200 mg/kg dose ($P<0.01$) and 100 mg/kg dose ($P<0.05$).

According to diagram 4, the sham group is significantly different from the prednisolone group ($P<0.001$), the group receiving the 200 mg/kg dose ($P<0.01$) and 100 mg/kg dose ($P<0.05$) in terms of the percentage of the area affected by the ulcer. Diagram 5 suggests that the sham group is significantly different from the control and

prednisolone group ($P<0.01$) in terms of the degree of absorption of the MPO activity.

The malondialdehyde density was also found to be significantly different in the sham group compared to that in the control group and prednisolone group ($P<0.01$) as well as in the 200 mg/kg group ($P<0.05$) (diagram 6).

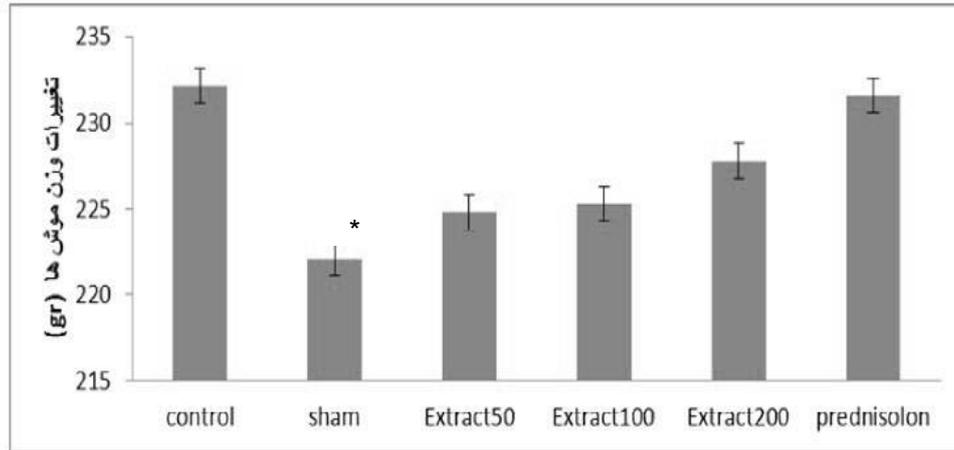


Diagram 1: Comparing the rats' weights (g) in the extract groups (50, 100 and 200 mg/kg), the prednisolone group (10 mg/kg), the sham group and the control group. Each column represents Mean \pm SEM and $n=8$. The control and prednisolone group (10 mg/kg) were found to have significant differences from the sham group ($P<0.05$)

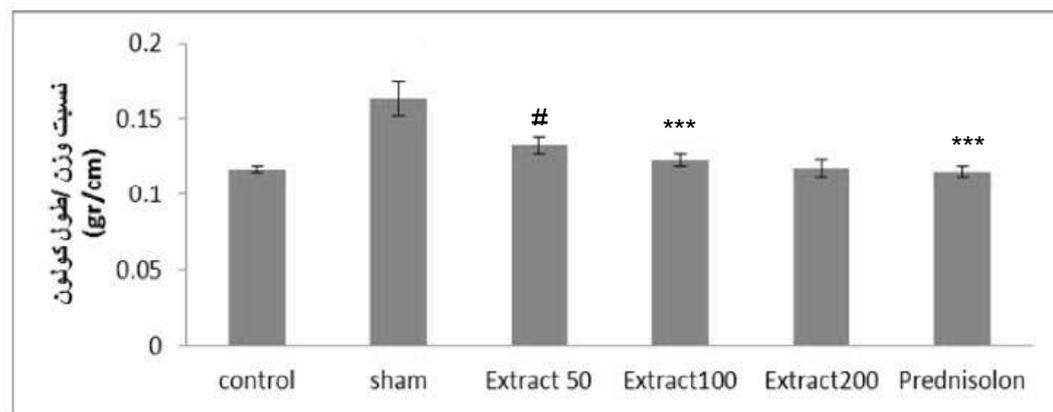


Diagram 2: The changes in the weight to length ratio of the isolated colon tissue (g/cm) in the extract groups (50, 100 and 200 mg/kg) as well as the prednisolone, sham and control groups. Each column represents Mean \pm SEM and $n=8$. The sham group was found to be significantly different from the control group, the prednisolone (10 mg/kg) group and also those receiving 100 and 200 mg/kg dose of the extract ($P<0.001$). A significant difference was also observed between the sham group and the 50 mg/kg dose group ($P<0.01$).

Significantly different from the 50 mg/kg dose group
 *** Significantly different from the control group, the prednisolone group as well as the 100 and 200 mg/kg dose group ($P<0.001$)

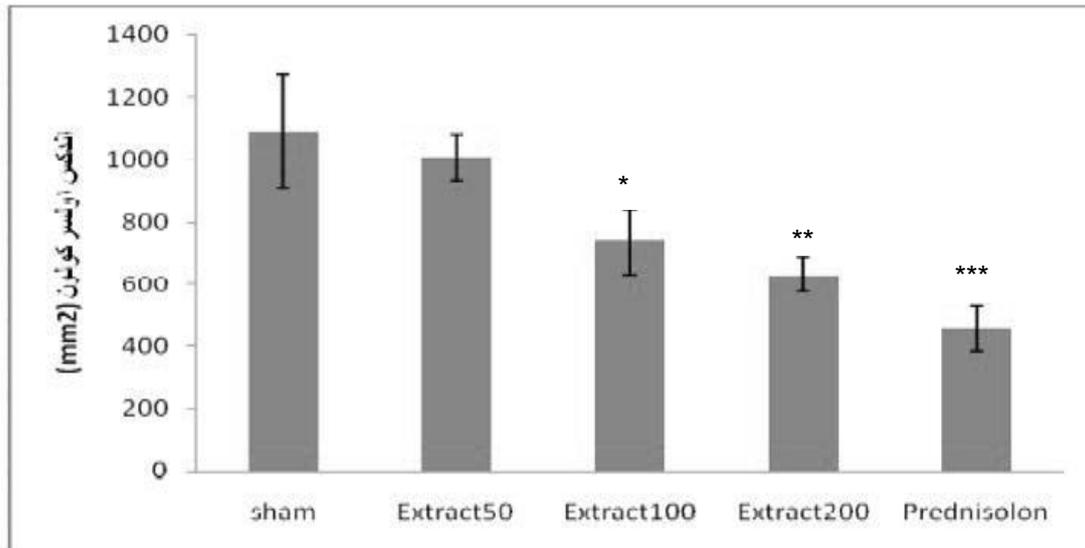


Diagram 3: The ulcer index variations in the extract groups (50, 100 and 200 mg/kg), the prednisolone (10 mg/kg) group and the sham group. Each column represents Mean \pm SEM and n=8. The difference between the sham group and the prednisolone group was found to be significant ($P<0.001$). The sham group was also found to be significantly different from the group receiving 200 mg/kg dose ($P<0.01$) and 100 mg/kg dose ($P<0.05$).

- * Significantly different from the 100 mg/kg dose group ($P<0.05$)
- ** Significantly different from the 200 mg/kg dose group ($P<0.01$)
- *** Significantly different from the prednisolone group ($P<0.001$)

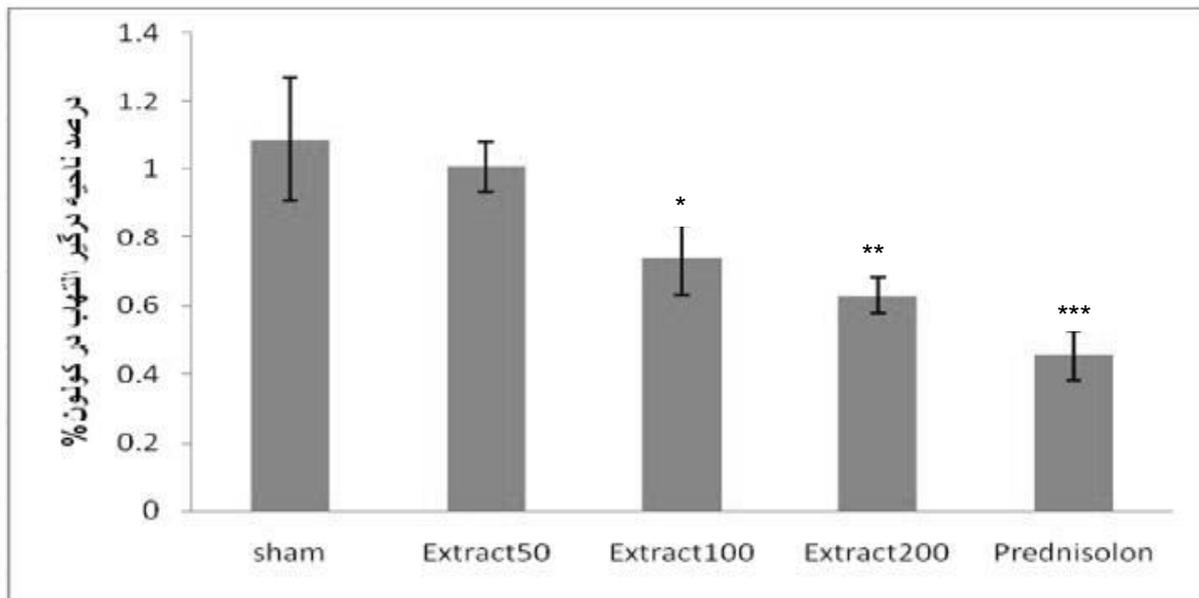


Diagram 4: Variations in the percentage of the area affected in the extract groups (50, 100 and 200 mg/kg), the prednisolone group and the sham group. Each column represents Mean \pm SEM and n=8. The sham group was found to be significantly different from the prednisolone group ($P<0.001$), the group receiving the 200 mg/kg dose ($P<0.01$) and 100 mg/kg dose ($P<0.05$).

- * Significantly different from the 100 mg/kg dose group ($P<0.05$)
- ** Significantly different from the 200 mg/kg dose group ($P<0.01$)
- *** Significantly different from the prednisolone group ($P<0.001$)

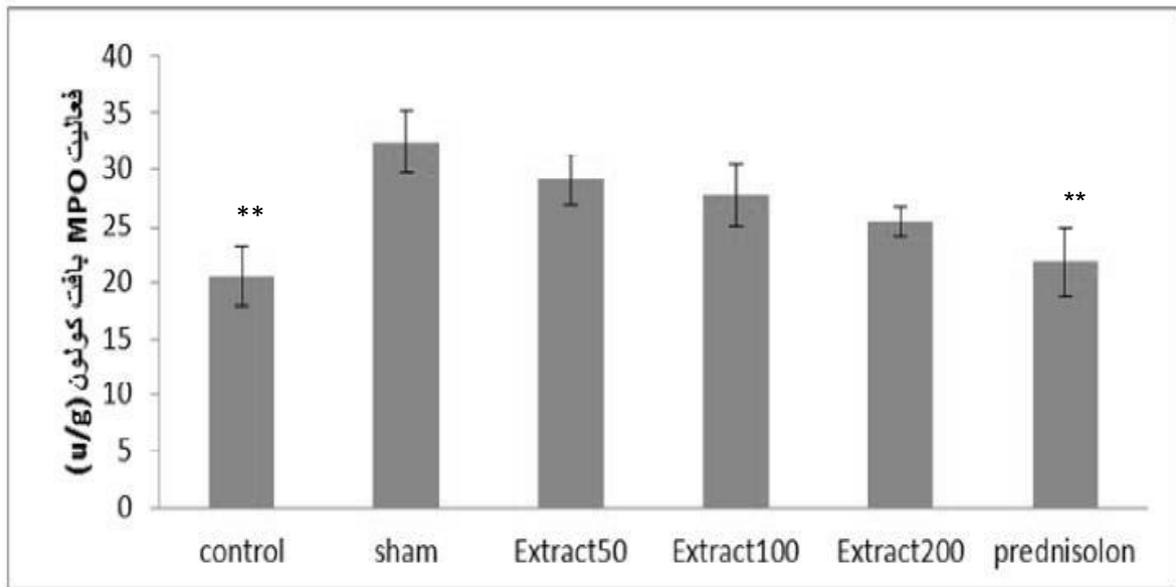


Diagram 5: Variations in the degree of absorption of the MPO activity in the extract groups (50, 100 and 200 mg/kg), the prednisolone group (10 mg/kg), the sham and the control group. Each column represents Mean \pm SEM and n=8. The sham group was found to be significantly different from both the control group and the prednisolone group ($P<0.01$).

** Significantly different from the control group and the prednisolone group ($P<0.01$)

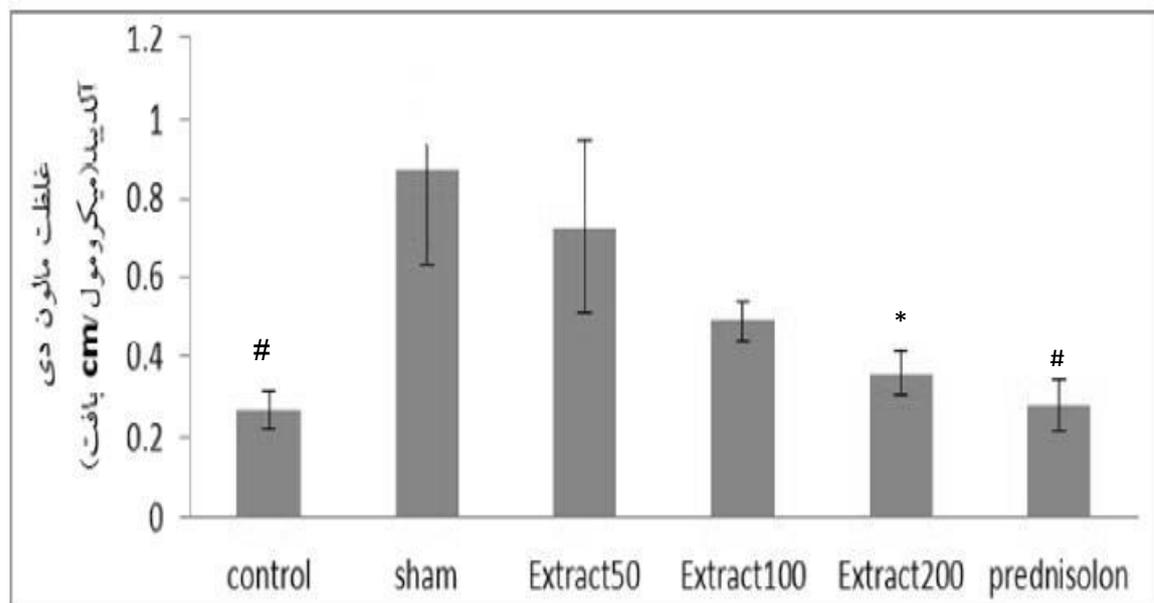


Diagram 6: The malondialdehyde density in the extract groups (50, 100 and 200 mg/kg), the prednisolone (10 mg/kg) group, the sham and control group. Each column represents Mean \pm SEM and n=8. The sham group was found to be significantly different from the control group and the prednisolone group ($P<0.01$) as well as the group receiving 200 mg/kg dose ($P<0.05$).

* Significantly different from the 200 mg/kg dose group ($P<0.05$)

** Significantly different from the control group and the prednisolone group ($P<0.01$)

Discussion

The aqueous extract of *Dracocephalum polychaetum* was found to fairly reduce some changes in the colon microscopic factors involved in UC such as the wet weight of the colon, the weight to length ratio of the colon, the ulcer area and the ulcer index as well as in enzymatic agents, i.e. the MPO activity and malondialdehyde level. This reduction is most significant in the group receiving a 200 mg/kg dose of the aqueous extract of *Dracocephalum polychaetum* although it is comparable with that in the group treated with prednisolone. The prevalence of inflammatory digestive diseases including UC is on the rise today owing to inappropriate lifestyle and improper nutrition. Non-steroidal anti-inflammatory medicines such as prednisolone are used to control UC due to the lack of specific treatments for this disease. As suggested in both literature and the present study, glucocorticoids such as prednisolone alleviate the inflammation by inhibiting inflammatory cytokines such as NO, TNF- α , IL1 and IL8, which is manifested as reduced inflammation area and reduced MPO activity (5).

As a key stage of development of inflammation in the gastrointestinal tract, the level of prostaglandin I₂, thromboxane A₂, prostaglandin E₂ (21) and COX2 expression significantly increase in chronic inflammatory diseases such as acetic acid-induced UC (8). Although the factor or factors affecting the inflammation were impossible to be isolated from the total extract in the present study owing to the equipment and financial constraints, components such as limonene, perillaldehyde, apigenin, luteolin (22 and 23) and α -Terpineol (20) are reported to have significant anti-inflammatory effects. The study plant is however characterized by having a significantly higher level of perillaldehyde compared to other plant species and even other genera (8). As mentioned in the Introduction, perillaldehyde has anti-inflammatory and

antispasmodic activities, which are crucial in alleviating the lesions and pain of UC.

Given that the analgesic effects of the extract of *Dracocephalum polychaetum* manifest only in the second phase of the formalin test (pain test), i.e. the pain or inflammatory phase, this plant seems to express its analgesic properties as anti-inflammatory effects (8). Research suggests that the whole extract of the study plant has sedative and anti-anxiety activities (8). Furthermore, stress and anxiety are well understood to play a key role in colitis and inflammatory bowel diseases (16). The whole extract of the plant might therefore exert part of its anti-inflammatory effect through relaxing the rats.

The measurement of MPO and TBARS as the indicators of oxidative stress in UC showed that the oral administration of the *Dracocephalum* extract, particularly the 200 mg/kg dose, can alleviate the intracellular oxidative stress. Given the results obtained and owing to the effective anti-inflammatory and anti-oxidative stress components in this extract including limonene, perillaldehyde, α -terpineol and flavonoids such as apigenin and luteolin, whose inhibitory effects on inflammatory cytokines such as IL1, COX2 and TNF- α were previously confirmed in literature, the anti-inflammatory properties of this plant can be used to treat UC. The effective components of the plant were impossible to be extracted owing to the limitations of the present study. To accurately understand the therapeutic mechanism of the total extract effect on UC, perillaldehyde as the main component of the total extract is recommended to be evaluated in the first step and other constituents in further studies.

Conclusion

The effective anti-inflammatory and anti-oxidative stress components in *Dracocephalum polychaetum* can be used to alleviate some inflammatory factors and therefore treat the inflammation of UC.

Further histological and pathological studies are certainly needed to obtain more rigorous results.

Acknowledgement

The authors would like to express their gratitude to the faculty members, advisors and officials in Shahid Bahonar University

of Kerman who supported the present study.

Conflict of interests

The Authors declare that there is no conflict of interest regarding the authorship or publication of this paper.

References:

1. Fei K, Praveen K, Liu Z. Herbal Medicine in the Treatment of Ulcerative Colitis. *Gastroenterol* 2012; 18: 3-10.
2. Motoi U, Hiroki I, Hiroki M, et al. surgical procedure for sporadic colorectal cancer in patients with mild ulcerative colitis. *Gastroenterol* 2012; 6: 635-642.
3. Ardizzone S, Bianchi Porro G. Biologic therapy for inflammatory bowel disease. *Drugs* 2005; 65: 2253–2286.
4. Singer II, Kawka DW, Schloemann S, et al. Cyclooxygenase-2 is induced in colonic epithelial cells in inflammatory bowel disease. *Gastroenterol* 1998; 115: 297-306.
5. Amir K, Rami E. Non-Steroidal Anti-Inflammatory Drugs and Inflammatory Bowel Disease. *Pharm* 2010; 3: 1084-1092
6. Mehrabani M, Rouholahi S, Foroumadi AR. Phytochemical studies of *Dracocephalum Polychaetum* Bornm. *Med Plants* 2005; 4(16): 42-36(Persian).
7. Rechinger KH. *Flora Iranica: flora des Iranischen Hochlandes und der umrahmenden gebirge*. Graz, Austria: Akademische Druck; 1963.
8. Khodami M, Abbasnejad M, Sheibani V, et al. Evaluation of the analgesic and anxiolytic effects of *Dracocephalum polychaetum*. *Physiol Pharmacol* 2011; 15(2): 444-454(Persian).
9. Loftus JR. Clinical epidemiology of inflammatory bowel disease: incidence, prevalence and environmental influence. *Gastroenterol* 2004; 126:1504-1517.
10. Jidong S. D-limonene: safety and clinical applications. *Altern Med* 2007; 12: 3.
11. Hassan SB, Gali-Muhtasib H, Goransson H, et al. Alpha terpineol: a potential anticancer agent which acts through suppressing NF-kappaB signalling. *Anticancer Res* 2010; 30(6): 1911-9.
12. Friedrich K, Delgado IF, Santos LM, et al. Assessment of sensitization potential of monoterpenes using the popliteal lymph node assay. *Food Chem Toxicol* 2007; 45: 1516-1522.
13. Hyong J. Major in Biomodulation, Department of Agricultural Biotechnology. Seoul: Seoul National University.
14. Yi-Ching L, Chung-Hsin Y, Ming-Ling Y, et al. Luteolin Suppresses Inflammatory Mediator Expression by Blocking the Akt/NFκB Pathway in Acute Lung Injury Induced by Lipopolysaccharide in Mice. *Hindawi Publishing Corporation* 2011; 8.
15. Kim SH, Shin KJ, Kim D, et al. Luteolin inhibits the nuclear factor-κB transcriptional activity in Rat-1 fibroblasts. *Biochem Pharmacol* 2003; 66: 955–63.
16. Masoumi-ardakani Y, Abbasnejad M, Derakhshanfar A, et al. Effect of *Matricaria recutita* L. aqueous extract on acetic acid-induced ulcerative colitis in adult male rats. *Physiol Pharmacol* 2010; 14(3): 268-280.
17. Dong-Yeul K, Dae-Ki J, Young-Hwa K, et al. Antibacterial and synergistic effects of *Kochia scoparia* extracts against methicillin-resistant *Staphylococcus aureus*. *African J Microbiol Res* 2012; 6(10): 2455-2461.
18. Sanjeev S, Sanjay G. Apigenin: A Promising Molecule for Cancer Prevention. *Pharm Res* 2010; 27(6): 962-978.
19. Sylvie Hugbers. Prednisolon-induced Ca^{2+} malabsorption is caused by diminished expression of the epithelial Ca^{2+} channel TRPV6. *Physiol Gastrointest liver* 2007; 292: 99-97
20. Liao M, Lin R, Chen F, et al. Luteolin attenuates the pulmonary inflammatory response involves abilities of antioxidation and inhibition of MAPK and NFκB pathways in mice with endotoxin-induced acute lung injury. *Food Chemical Toxicol* 2011; 49: 2660–2666.
21. Kubes P, McCafferty DM. Nitric oxide and intestinal inflammation. *Am J Med* 2000; 109: 150–158
22. Gehrman J, Bonnekoh P, Miyazawa T, et al. Immunocytochemical study of an early microglial activation in ischemia. *Blood Flow Metab* 1992; 12: 257–269.
23. Noa M, Mas R, Carbajal D, et al. Effect of D-002 on acetic acid induced colitis in rats at single and repeated doses. *Pharmacol Res* 2000; 41:391-395.