Evaluating the Effect of \textit{Melilotus officinalis} L. Aqueous Extracts on Healing of Acetic Acid-Induced Ulcerative Colitis in Male Rats

Nader Tanideh,\textsuperscript{1} Mehgdad Bahrani,\textsuperscript{2} Mohammad J. Khoshnood-Mansoorkhani,\textsuperscript{3} Davood Mehrabani,\textsuperscript{4} Donya Firoozi,\textsuperscript{5,\*} Omid Koohi-Hosseinabadi,\textsuperscript{6} and Aida Iraji\textsuperscript{7}

\textsuperscript{1}Colorectal Research Center, Shiraz University of Medical Sciences, Shiraz, IR Iran
\textsuperscript{2}School of Nutrition and Food Science, Shiraz University of Medical Sciences, Shiraz, IR Iran
\textsuperscript{3}Department of Pharmacology and Toxicology, School of Pharmacy, Shiraz University of Medical Sciences, Shiraz, IR Iran
\textsuperscript{4}Center of Experimental and Comparative Medicine, Shiraz University of Medical Sciences, Shiraz, IR Iran
\textsuperscript{5}Corresponding author; Donya Firoozi, School of Nutrition and Food Science, Shiraz University of Medical Sciences, Shiraz, IR Iran. Tel/Fax: +98-7137251007, E-mail: donyafiroozi@gmail.com

Received 2016 October 10; Revised 2016 November 23; Accepted 2016 November 25.

Abstract

\textbf{Background:} Ulcerative colitis (UC) is a form of inflammatory bowel disease (IBD) that is characterized by acute and chronic inflammation. The etiology and pathophysiology of IBD is unidentified, and there are many obstacles on the definite treatment of this disease. Recently, the useful effects of some herbal medicine on improving UC have been studied. \textit{Melilotus officinalis} L. (\textit{M. officinalis}) is an herb with antioxidant and anti-inflammatory effects used as food, forage and medicine.

\textbf{Objectives:} This study evaluated the antioxidant effects of \textit{M. officinalis} aqueous extracts in the acetic acid-induced ulcerative colitis in rats.

\textbf{Methods:} Fifty rats were randomly divided into five equal groups. Group I (Control healthy group) received 1 ml/kg of normal saline orally. Group II (control colitis group) received 1 ml/kg of normal saline orally. Group III (positive control) received 3 mg/kg prednisolone orally. Group IV received 1000 mg/kg \textit{M. officinalis} aqueous extracts orally. Group V received 2000 mg/kg \textit{M. officinalis} aqueous extracts orally. Ulcerative colitis was induced by intra-rectal acetic acid (3% v/v) administration. All treatments were done 24 hours after induction of colitis and continued for seven days. On the eighth day, the rats were sacrificed and colonic biopsies were taken for histopathological and biochemical studies. Data analysis was performed, using SPSS software and significance level was set at \(P \leq 0.05\).

\textbf{Results:} Treatment with \textit{M. officinalis} aqueous extract could enhance colonic antioxidant capacity and decrease inflammation and acute colonic injury induced by acetic acid, which is dose-dependent. In addition, administering the extract significantly (\(P \leq 0.05\)) reduced the colonic level of malondialdehyde and myeloperoxidase, and significantly (\(P \leq 0.05\)) increased the level of reduced glutathione (\(P \leq 0.05\)). The extract had more effects at the dose of 2000 mg/kg than 1000 mg/kg dosage and prednisolone.

\textbf{Conclusions:} This study revealed that \textit{M. officinalis} had apparent curative effects on treating UC because of its antioxidant and anti-inflammatory activities.

\textbf{Keywords:} Inflammatory Bowel Disease, Ulcerative Colitis, Antioxidant, \textit{Melilotus officinalis} L.

1. Background

Ulcerative colitis (UC) is a form of inflammatory bowel disease (IBD) that is characterized by acute and chronic inflammation of the mucosa, ulceration of the colon, bloody diarrhea, rectal bleeding, abdominal pain, cramping and weight loss (1, 2). UC is a non-transmittable inflammatory disease that is confined to the colon (3). The incidence of UC has been reported about 10 - 20 in 100,000 per year, with a prevalence of 100 - 200 cases per 100,000 in Western countries (4). The prevalence of UC is increasing in developing countries due to their lifestyle (5).

The etiology and pathophysiology of IBD is unidentified and depends on a number of factors such as environmental factors, genetic, reactive oxygen species and gastrointestinal infections (1, 6). Oxidative stress is an important factor in the pathogenesis of UC disease (7). A decrease in antioxidant defense against increasing oxidative materials in colonic mucosal of the patients with UC causes tissue damage and inflammation of the colon (8). Drugs such as amino salicylates, corticosteroids, and immune modulators are normally used to treat IBD, but these drugs have more adverse effects and are only for temporary relief, not definitive treatment (9).

Therefore, many IBD patients use complementary and
alternative medicine (CAM) such as homeopathy, herbal medication and vitamin therapy for treatment (10). Experimental information acquired from animal models of colitis suggested the useful effects of the plant extracts such as *Teucrium polium* (11), *Calendula officinalis* (8), *Agave Americana* Linn (12), *Carum carvi* (13), *Naringenin* (14), and *Pterocarpus marsupium* (15) on improving UC.

*Melilotus officinalis* L. (sweet clover), belongs to the family of Leguminosae (Fabaceae). The plant is grown in Pakistan, Kashmir, India, Tibet, Russia, China, Turkey, Middle and Southern Europe (16). *M. officinalis* is an herb used as food, forage and medicine (17). The main phenolic compounds in *M. officinalis* are coumarin derivatives, rutin (flavonoid), and ferulic acid (phenol carboxylic acids) (18). It also contains vitamin C, allantoin, tannins, mineral salts, and flavonoids (19). *M. officinalis* grass has many effects like keratolytic, bio stimulant, regenerative, vasodilator, anticoagulant, expectorant, anti-inflammatory, softener and carminative (20, 21). In traditional medicine, it is used as softening, laxative, expectorant, sedative, diuretic, antibacterial, hypotensive, analgesic, and antispasmodic effects (22). As indicated in several studies, the excessive production of ROS in UC leads to oxidative damage in tissues (23). Hence, it is necessary to find antioxidant and anti-inflammatory compounds to reduce damages in UC disease. More in-depth research is suggested to find an efficient and useful treatment method.

2. Objectives

The aim of this study was to evaluate the antioxidant capacity of *M. officinalis* aqueous extracts at the doses of 1000 and 2000 mg/kg/d to reduce oxidative and tissue damages, histopathological changes, malondialdehyde (MDA), myeloperoxidase (MPO) and glutathione (GSH) level in experimental ulcerative colitis in rats.

3. Methods

3.1. Ethical Statement

This study was authorized by the animal care and apply committee of Shiraz University of Medical Sciences, Shiraz, Iran.

3.2. Preparation of the Extract

*M. officinalis* fresh plants were identified and gathered from north of Shiraz. The plants were dried in room temperature. All parts of the dried plant were powdered and cast in plenty of boiling water (a ratio of 1 to 15) and placed on the heater for 30 minutes at a temperature of 70 - 80°C. They were then wrapped in an aluminum foil container and kept in the dark room for 24 hours. First, the extract was flat with cotton and then by filter paper, and condensed in a rotary machine. The condensed extract was frozen at -20°C, and dried in a freeze dryer. Finally, the aqueous extract was prepared in different concentrations (24). The total phenols in the *M. officinalis* extract were determined by the Folin-Ciocalteua method. Results are given as gallic acid equivalent (GAE/g) of the extract (25).

3.3. Animals

Fifty male Sprague Dawley rats weighing 200 ± 20 g were purchased from laboratory animal center of Shiraz University of Medical Sciences. The rats were housed in standard condition as ambient temperature of 21 ± 2°C and 65 ± 5% relative humidity, with 12 lights and 12 dark cycles. Each animal received a balanced diet and had free access to water and chow.

3.3.1. Induction of the Experimental Colitis in Rats

All animals were fasted for 36 hours before the induction of colitis. A polyurethane cannula (diameter of 2 mm) was applied for rectal entrance of acetic acid 3%, and the tip was inserted up to 8 cm proximal to the anus. Two milliliters of acetic acid (AA) were administered transrectally into the colon by the cannula for 15 seconds, and to induce UC, the rats underwent anesthesia with ketamine 10% (100 mg/kg) and xylazine 2% (10 mg/kg) (26).

3.3.2. Experimental Design

The rats were randomly divided into five equal groups as follows:

- Group I (control healthy group) received 1 mL/kg of normal saline orally
- Group II (control colitis group) received 1 mL/kg of normal saline orally
- Group III (positive control) received 3 mg/kg prednisolone orally
- Group IV received 1000 mg/kg *M. officinalis* aqueous extracts orally
- Group V received 2000 mg/kg *M. officinalis* aqueous extracts orally

All treatments were provided 24 hours after induction of colitis and continued for seven days. On the eighth day, the rats were sacrificed, and colonic biopsies were taken for histopathological and biochemical studies. The doses were selected based on previous studies. The doses of 1000 and 2000 mg/kg/d *M. officinalis* aqueous extracts, and the dose of prednisolone were selected based on previous studies.
3.4. Histopathological Study

For histological examination, the colon tissues were sectioned with 5 µm thickness, and stained with haematoxylin and eosin (H & E). This was done by a pathologist who was unaware of the histological injury treatment records.

3.5. Biochemical Study

Colonic samples were stored immediately at -80°C till analysis. Tissue samples were homogenized in 1 mL of 10 mmol/L Tris-HCl buffer pH 7.1, and homogenation was used to measure malondialdehyde (MDA), myeloperoxidase (MPO), and glutathione (GSH) activities. In colonic tissue homogenate, MDA, MPO and GSH contents were expressed per gram wet tissue weight. MDA production was determined in the colonic tissue by thiobarbituric acid reaction (27). MPO activity was assessed in colonic tissue based on the reaction hydrogen peroxide and o-dianisidine dihydrochloride as substrates (28), and GSH was determined in colonic tissue, described by Owens and Belcher based on the reaction of 5,5-dithiobis-(2-nitrobenzoic acid) (29).

3.6. Statistical Analysis

For statistical analysis, the data were checked for normal distribution, using 1-sample K-S test. Differences between the groups, by normal distribution, were done using one-way ANOVA and Tukey HSD post hoc test, and the data not normally distributed were analyzed by Kruskall-Wallis test, followed by Dunn’s test. All the statistical analyses were determined by SPSS software (Version 18, Chicago, IL, USA). Differences were considered significant at P < 0.05.

4. Results

4.1. Analysis of the Extract of M. officinalis

The aqueous extract yielded 18.21% w/w. The total phenols determination showed 26.51 mg GAE/g extract of the plant.

4.2. Histopathological Study

In acetic acid control group, the colonic mucosa showed epithelial necrosis, granular atrophy mucosal tissue destruction with ulcer and migration of inflammatory cells (a). In prednisolone (3 mg/kg) treated rats, the relative improvement could be seen in the wound and mucosal tissue of the colon, but inflammatory cells were still present (b). The wound improved and intestinal mucosal tissue was repaired in M. officinalis treated rats that received a dose of 1000 mg/kg orally, but inflammatory cell infiltration was still present (c). Administration of M. officinalis at a dose of 2000 mg/kg orally, protected colonic mucosa, improved the wound and caused inflammatory cells to disappear (d) (Figure 1).

4.3. Biochemical Study

4.3.1. Effects of M. officinalis on MDA Activity

Figure 2 demonstrates the mucosal MDA concentrations in colonic mucosal biopsies of the rats. MDA increased in acetic acid control when compared to normal controls. Treatment with M. officinalis with 1000 and 2000 mg/kg orally and prednisolone for a period of seven days resulted in a significant decrease in MDA levels when compared to acetic acid induced control.

4.3.2. Effects of M. officinalis on MPO Activity

Figure 3 demonstrates the mucosal MPO concentrations in colonic mucosa of the rats. Tissue MPO levels significantly increased (P < 0.05), following the intrarectal administration of acetic acid. Treating rats with M. officinalis (1000 and 2000 mg/kg) such as prednisolone caused a significant reduction (P < 0.05) in the mean MPO activity when compared to acetic acid induced controls (Group 2).

4.3.3. Effects of M. officinalis on GSH Level

Reduced glutathione content significantly decreased in acetic acid control group. Treatment with M. officinalis in a dose of 1000 and 2000 mg/kg and prednisolone (3 mg/kg) orally resulted in a significant increase in GSH content (Figure 4).

5. Discussion

This study evaluated the healing effects of M. officinalis aqueous extract in 1000 and 2000 mg/kg dietaries against acetic acid-induced UC by measuring tissue histopathology and MDA, GSH and MPO level in rats. This study found that treatment with M. officinalis aqueous extract could lead to enhancement in colonic antioxidant capacity and a decrease in inflammation and acute colonic injury induced by acetic acid, which is dose-dependent. The results were confirmed by histopathological examinations. Moreover, the groups that received 2000 mg/kg dosage of oral M. officinalis extract administration for a period of seven consecutive days produced a better response when compared with the dose of 1000 mg/kg of M. officinalis extract. We used oral prednisolone as a reference drug, and found that the 2000 mg/kg dosage of the extract was more effective when compared with prednisolone.

Induced ulcerative colitis with AA causes neutrophils and macrophages infiltration in animals, which creates epithelial lesions and necrosis, colonic damage and inflammatory conditions (30).
Tanideh N et al.

Figure 1. Histopathological Changes Associated with Experimental Ulcerative Colitis Induction and Treatment with *M. officinalis* Extract

A, acute inflammation and granular atrophy in response to acetic acid 3%; B, 3 mg/kg prednisolone, orally; C, 1000 mg/kg of *M. officinalis* extract, orally; D, 2000 mg/kg of *M. officinalis* extract, orally (H and E, ×100 and 250).

It has been well demonstrated that oxidative stress has an important role in IBD initiation and continuance (31). Inconsistency between oxidant and antioxidant material in colitis caused oxidative damage, which is a characteristic feature of colitis (32).

Antioxidant effects have been found in several compounds and can reduce the harmful effects of reactive oxygen species, created in biological systems by disabling these reactive. Natural flavonoids are important compounds as an effective source of exogenous antioxidants (33). *M. officinalis* contains phenolic acid, flavonoids and coumarin and these components have notable antioxidant (34). Phenolic compounds of *M. officinalis* extracts have an inhibitory effect on arachidonic acid metabolism through the lipoxygenase pathway; also, *M. officinalis* has anti-inflammatory effects by reducing circulating phagocytes and lowering citrulline production (35). Moreover, coumarin (the most effective component of *M. officinalis*) has antioxidant properties that affect the organization and scavenging of ROS. In addition, it can suppress superoxide production in leukocytes via its antioxidant ability, which can influence phagocyte activity (36, 37). In this study, we found that *M. officinalis* has anti-inflammatory properties on acute inflammation in ulcerative colitis models. Similarly, Plesca-Manea found that *M. officinalis* had anti-inflammatory effects against acute inflammation induced...
Figure 2. Effects of Treatments on Tissue Levels of MDA Experimental Rats

| Group Treatment | MDA (µmol/g Wet Tissue) |
|-----------------|-------------------------|
| NS              | 3                       |
| Acetic Acid     | 2                       |
| Prednis Olone   | 1                       |
| EXT 1000        | 0                       |
| EXT 2000        | 0                       |

Results are presented as the mean ± SD of 10 rats in each group. *A significant difference in the level of P < 0.05 between treatment groups and the colitis control group (group receiving acetic acid); #a significant difference in the level of P < 0.001 between treatment groups and the colitis control group (group receiving acetic acid).

Figure 3. Effects of the Treatments on Tissue Levels of MPO Experimental Rats

| Group Treatment | MPO (Unit/g Wet Tissue) |
|-----------------|-------------------------|
| NS              | 1.5                     |
| Acetic Acid     | 1.0                     |
| Prednis Olone   | 0.5                     |
| EXT 1000        | 0.0                     |
| EXT 2000        | 0.0                     |

Results are presented as the mean ± SD of 10 rats in each group. *A significant difference in the level of P < 0.05 between treatment groups and the colitis control group (group receiving acetic acid); #a significant difference in the level of P < 0.001 between treatment groups and the colitis control group (group receiving acetic acid).

by turpentine oil in male rabbits (19).

Previous studies reported the useful effects of the plant extracts such as *Calendula officinalis* (8), *Carum carvi* (13) and *Naringenin* (14) on improving UC. In addition, Safarpour et al. reported (38) some beneficial effects of *M. officinalis* in treating UC. In this study, we evaluated the healing effects of *M. officinalis* at the higher doses to investigate the probable side effects. We found that *M. officinalis* aqueous extract at the dose of 1000 and 2000 mg/kg did not have any toxic effects, but we noticed that UC treatment improved at the dose of 2000 mg/kg.

Several reports have shown that the contents of MDA increase in UC. MDA have acceptable relations with the degree of lipid peroxidation; hence, it is frequently used in measuring lipid peroxide levels (39, 40). Furthermore, studies have clearly demonstrated that depletion of GSH leads to the cellular damage as well as colonic injuries. GSH is one of the first lines of oxidative defense mechanisms against free radicals (41), and can prevent ROS oxidative injuries (42, 43).

In our study, the levels of GSH decreased in colonic tissue of the colitis in the control group, while MDA contents increased significantly. To date, no study has examined the effects of *M. officinalis* on the level of GSH in animal’s model of UC. In this study, treatment with *M. officinalis* caused a remarkable increase in GSH and a decrease in MDA in colonic tissue, which was dose-dependent. *M. officinalis* provided protective effects in UC, perhaps through the scavenging radicals and its antioxidant properties. This can be one of the important and underlying mechanisms of *M. officinalis* protection against UC. Similar to findings of this study, Safarpour and et al. revealed that treatment by *M. officinalis* in colitis rats decreased the level of MDA and improved UC symptoms (38).

MPO is an enzyme that exists in neutrophils. The level of MPO activity is directly related to the neutrophil concentration in the inflamed tissue. Therefore, measuring MPO activity is considered as a quantitative and sensitive assay for acute intestinal inflammation (44). Many studies showed that herbs and their main components such as phenols and polyphenols decreased MPO level and improved inflammation in the colonic tissue of ulcerative colitis animals (45-47). In this study, a significant reduction was detected in neutrophil infiltration in colonic mucus and MPO activity of animal subjects with colitis treated with *M. officinalis*. The beneficial effects of this plant on MPO and inflammatory condition may be due to its phenolic components like coumarin. Previously, no study examined the effects of *M. officinalis* on the level of MPO in animal’s...
model of UC.

In conclusion, this study demonstrated that daily oral administration of *M. officinalis* extract could relieve the ulcerative colitis induced by acetic acid in the rats’ colon. Results of tissue MDA, MPO and GSH and histopathological evaluations indicated a reduction in the inflammation. The healing, anti-inflammatory and antioxidant properties of *M. officinalis* can make it an appropriate choice of supplement for treating ulcerative colitis. However, further studies are required to confirm its clinical effect on humans.

**Acknowledgments**

The authors would like to thank the research consultation center (RCC) of Shiraz University of Medical Sciences for their invaluable assistance in editing this article.

**References**

1. Jagtap AG, Shirke SS, Phadke AS. Effect of polyherbal formulation on experimental models of inflammatory bowel diseases. J Ethnopharmacol. 2004;90(2-3):195-204. doi: 10.1016/j.jep.2003.09.042. [PubMed: 15031818].

2. Papadalis KA, Targar SR. Role of cytokines in the pathogenesis of inflammatory bowel disease. Annu Rev Med. 2000;51:289-98. doi: 10.1146/annurev.med.51.2.289. [PubMed: 10774465].

3. Baumgart DC, Sandborn WJ. Inflammatory bowel disease: clinical aspects and established and evolving therapies. Lancet. 2007;369(9573):1641-57. doi: 10.1016/S0140-6736(07)60751-X. [PubMed: 17499606].

4. Loftus EJ. Clinical epidemiology of inflammatory bowel disease: Incidence, prevalence, and environmental influences. Gastroenterology. 2004;126(6):2004-17. [PubMed: 15688613].

5. Aghazadeh R, Zali MR, Bahari A, Amin K, Ghaighaie F, Firouzi F. Inflammatory bowel disease in Iran: a review of 457 cases. J Gastroenterol Hepatol. 2005;20(1):1691-5. doi: 10.1111/j.1440-1746.2005.03905.x. [PubMed: 16248877].

6. Chen CY, Lee KT, Charles Tzu-Chi I, Lai WT, Huang YB. Epidemiology and Disease Burden of Ulcerative Colitis in Taiwan: A Nationwide Population-Based Study. Value Health Region Issues. 2013;2(1):127-34. doi: 10.1016/j.valh.2013.02.006.

7. Pavlik KP, Laroux FS, Fuseler J, Wolf RE, Gray L, Hoffman J, et al. Role of reactive metabolites of oxygen and nitrogen in inflammatory bowel disease. Free Radic Biol Med. 2002;33(3):311-22. [PubMed: 12126753].

8. Mehrabani D, Ziael M, Hosseini SV, Ghahramani L, Banazadeh AM, Ashraf MJ, et al. The effect of calendula officinalis in therapy of acetic Acid induced ulcerative colitis in dog as an animal model. Iran Red Crescent Med J. 2011;13(12):884-90. [PubMed: 22757434].

9. Hashmohseny S, Hoang BV, Wu M, Lynn CA, Feinle-Bisetti C, Howarth GS. Clinical and structural effects of traditional Chinese medicine and the herbal preparation, Iberogast, in a rat model of ulcerative colitis. J Ethnopharmacol. 2008;119(1):10-9. doi: 10.1016/j.jep.2007.11.009. [PubMed: 18222658].

10. Mehrabani D, Bahrami F, Hosseini SV, Ashraf MJ, Tanideh N, Rezazadeh A, et al. The healing effect of Turmeric polium in Acetic Acid Induced Ulcerative Colitis in the Dog as an Animal Model. Middle East J Dig Dis. 2012;4(1):40-7. [PubMed: 24829634].

11. Mannaasheh BA, Kulkarni PV, Sangsrekkop M, Savant C, Mohan A. Protective effect of Agave americana Linn. leaf extract in acetic acid-induced ulcerative colitis in rats. Ayu. 2015;36(1):100-6. doi: 10.4019/0034-8520-2015-034-018. [PubMed: 26730148].

12. Keshavarz A, Minaykan M, Ghanndi A, Mahzouni P. Effects of Carum carvi L. (Caraway) extract and essential oil on TNBS-induced colitis in rats. Exp Toxicol Pathol. 2015;67(4):195-201. doi: 10.1016/j.etp.2015.02.005. [PubMed: 25688613].

13. Al-Rejaie SS, Abouhashish RM, Al-Enazi MM, Al-Assaf AH, Parmar MY, Ahmed MM. Protective effect of naringenin on acetic acid-induced ulcerative colitis in rats. World J Gastroenterol. 2013;19(34):5633-44. doi: 10.3748/wjg.v19.i34.5633. [PubMed: 24039355].

14. Mathew MM, Han NV, Murugesan A, Raj EA, Prasanth KG. Evaluation of the protective effect of Pterocarpus marsupium on acetic acid-induced ulcerative colitis in rats. Inflammopharmacol. 2015;23(2):195-201. doi: 10.1007/s10787-015-0234-3. [PubMed: 25991550].

15. Anwer MS, Mohtasheem M, Ashraf M, Ahmed SW, Banoo H. Chemical constituents from Melilotus officinalis. J Basic Appl Sci. 2008;4(2):89-94. [PubMed: 19153923].

16. Hiraoka T, Okawa M, Kinjo J, Nohara T. A new oleane glucoside obtained from the aerial parts of Melilotus officinalis. Chem Pharm Bull (Tokyo). 2000;48(2):286-7. [PubMed: 10705521].

17. Houth JR, Paya M. Pharmacological and biochemical actions of simple coumarins: natural products with therapeutic potential. Gen Pharmacol. 1996;27(4):703-22. [PubMed: 8553100].

18. Plesca-Manea L, Parvu AE, Parvu M, Taamas M, Brua R, Puia M. Effects of Melilotus officinalis on acute inflammation. Phytother Res. 2002;16(4):316-9. doi: 10.1002/pr.875. [PubMed: 1212285].

19. Eder M, Mehnert W. The importance of concomitant compounds in plant extracts. Pharmazie. 1998;53(5):285-9. [PubMed: 9631497].

Ann Colorectal Res. 2016; 4(4):e42856.
21. Born SL, Rodriguez PA, Eddy CI, Lehman-McKeeman LD. Synthesis and reactivity of coumarin 3,4-epoxide. Drug Metab Dispos. 1997;25(11):1388-24. [PubMed: 9359900].
22. Weber US, Steffen B, Siegers CP. Antitumor-activities of coumarin, 7-hydroxy-coumarin and its glucuronide in several human tumor cell lines. Res Commun Mol Pathol Pharmacol. 1998;99(2):193-206. [PubMed: 9583093].
23. Head KA, Jurenka JS. Inflammatory bowel disease part I: ulcerative colitis-pathophysiology and conventional and alternative treatment options. Altern Med Rev. 2001;6(1):247-83.
24. Sharafati-Chaleshtori R, Rafieian-Kopaei M. Screening of antibacterial effect of the Scrophularia striata against E. coli in vitro. J Herb Med Pharm. 2014;3(1):31-4.
25. Minaiany M, Ghamandi A, Etemad M, Mahzouni P. A study of the effects of Cydonia oblonga Miller (Quince) on TNBS-induced ulcerative colitis in rats. Res Pharm Sci. 2012;7(2):303-10. [PubMed: 2281087].
26. Tanideh N, Nematollahi SL, Hosseini SV, Hosseinzadeh M, Mehrabani D, Safarpour A, et al. The healing effect of Hypericum perforatum extract on acetic acid-induced ulcerative colitis in rat. Ann Colorect Res. 2014;4(2):4.
27. Subbarao KV, Richardson JS, Ang LC. Autopsy samples of Alzheimer’s cortex show increased peroxidation in vitro. J Neurochem. 1990;53(1):342-5. [PubMed: 2355227].
28. Mullane KM, Kraemer R, Smith B. Myeloperoxidase activity as a quantitative assessment of neutrophil infiltration into ischemic myocardium. J Pharmacol Methods. 1985;14(3):315-7. [PubMed: 2997548].
29. Beutler E. Red cell metabolism: a manual of biochemical methods. Grune & Stratton; 1975.
30. Hartmann RM, Morgan Martins MI, Tieppo J, Fillmann HS, Maroni NP. Effect of Boswellia serrata on antioxidant status in an experimental model of colitis rats induced by acetic acid. Dig Dis Sci. 2012;57(8):2038-44. doi: 10.1007/s10620-012-2134-3. [PubMed: 2245198].
31. Kraidenier L, Verspaget HW. Review article: oxidative stress as a pathogenic factor in inflammatory bowel disease-radiicals or ridiculous?. Aliment Pharmacol Ther. 2002;16(12):1997-2015. [PubMed: 1245293].
32. Droge W. Free radicals in the physiological control of cell function. Physiol Rev. 2002;82(1):47-95. doi: 10.1152/physrev.00018.2001. [PubMed: 11773609].
33. Borges Burbos G, da Rocha Vianna D, Medina-Remon A, von Poser G, Maria Lamuela-Raventos R, Lucia Eifler-Lima V, et al. The Antioxidant Activity of Coumarins and Flavonoids. Mini Rev Med Chem. 2013;13(1):318-34. doi: 10.2174/13895575138000200.
34. Trouillas P, Calliste C, Aliass D, Simon A, Marfak A, Delage C, et al. Antioxidant, anti-inflammatory and antiproliferative properties of sixteen water plant extracts used in the Limousin countryside as herbal teas. Food Chemistry. 2003;80(3):399-407. doi: 10.1016/s0308-8146(02)00282-0.
35. Oetari S, Sudibyo M, Commandeur JN, Samhoedi R, Vermeulen NP. Effects of curcumin on cytochrome P450 and glutathione-S-transferase activities in rat liver. Biochem Pharm. 1995;50(3):39-45. [PubMed: 8534266].
36. Varanda EA, Raddi MS, Dias Fde L, Araujo MC, Gibran SC, Takahashi CS, et al. Mutagenic and cytotoxic activity of an isocoumarin (Paepalan- tide) isolated from Paepanthus vellosioides. Teratog Carcinog Mutagen. 1997;17(2):85-95. [PubMed: 9260222].
37. Gu J, Walker VE, Lipinskas TW, Walker DM, Ding X. Intrapertitoneal administration of coumarin causes tissue-selective depletion of cytochromes P450 and cytotoxicity in the olfactory mucosa. Toxicol Appl Pharmacol. 1997;146(1):134-43. doi: 10.1006/taap.1997.8238. [PubMed: 9299605].
38. Safaforour AR, Kaviyani F, Sephehrmanesh M, Ahmad M, Hosseinabadi OK, Tanideh N, et al. Antioxidant and Anti-Inflammatory Effects of Gel and Aqueous Extract of Mellitox officinalis L. in Induced Ulcerative Colitis: A Rattus norvegicus Model. Ann Colorect Res. 2015;3(2).
39. Cetinkaya A, Bulbuloglu E, Kurutas EB, Ciralik H, Kantarceken B, Buyukbese MA. Beneficial effects of N-acetylcysteine on acetic acid-induced colitis in rats. Tohoku J Exp Med. 2005;206(2):119-9. [PubMed: 15888969].
40. Cetinkaya A, Bulbuloglu E, Kantarceken B, Ciralik H, Kurutas EB, Buyukbese MA, et al. Effects of L-carnitine on oxidant/antioxidant status in acetic acid-induced colitis. Dig Dis Sci. 2006;51(3):488-94. doi: 10.1007/s00394-006-1609-9. [PubMed: 16614957].
41. Hagar HH, El-Medany A, El-Eter E, Arafa M. Ameliorative effect of pyrrolidine-thioicarbonate on acetic acid-induced colitis in rats. Eur J Pharmacol. 2007;574(1):69-77. doi: 10.1016/j.ejphar.2006.09.066. [PubMed: 1711250].
42. Sivaraprasad R, Nagaraj M, Varalakshmi P. Combined efficacies of lipoic acid and 2,3-dimercaptopropanesulfonic acid against lead-induced lipid peroxidation in rat liver. J Nutr Biochem. 2004;15(1):28-33. doi: 14704565.
43. Sivaraprasad R, Nagaraj M, Varalakshmi P. Lipoic acid in combination with a chelator ameliorates lead-induced peroxidative damages in rat kidney. Arch Toxicol. 2002;76(2):137-41. doi: 10.1007/s00204-002-0350-0. [PubMed: 12815470].
44. Gonzalez R, Rodriguez S, Romay C, Gonzalez A, Armento J, Remirez D, et al. Anti-Inflammatory Activity of Phycocyanin Extract in Acetic Acid-Induced Colitis in Rats. Pharmacol Res. 1999;39(3):2055-5. doi: 10.1016/s1040-6644(99)00049-3. [PubMed: 10079148].
45. Ueki A, Maity S, Karmakar S, Datta N, Vedasirimoni JR, Das PK. Curcumin, the major component of food flavour turmeric, reduces mucosal injury in trinitrobenzene sulphonic acid-induced colitis. Br J Pharmacol. 2003;139(2):209-18. doi: 10.1038/sj.bjp.0705241. [PubMed: 12770926].
46. Sanchez-Fidalgo S, Sanchez de Ibarra L, Cardeno A, Alarcon de la Lastra C. Influence of extra virgin olive oil diet enriched with hydroxytyrosol in a chronic DSS colitis model. Eur J Nutr. 2012;51(4):497-506. doi: 10.1007/s00394-012-0353-y. [PubMed: 21874330].
47. Li XL, Cai YQ, Qin H, Wu YJ. Therapeutic effect and mechanism of proanthocyanidins from grape seeds in rats with TNBS-induced ulcerative colitis. Can J Physiol Pharmacol. 2008;86(12):341-9. doi: 10.1139/Y08-089. [PubMed: 19088805].