LOKMAN HEKIM HEALTH SCIENCES

DOI: 10.14744/lhhs.2024.2001
Lokman Hekim Health Sci 2024;4(2):97–102

lokmanhekimhs.com

ORIGINAL ARTICLE

Effects of Dexpanthenol and Tocopherol Applied Alone and in Combination on Experimental Colitis

Dekspantenol ve Tokoferolün Ayrı Ayrı ve Birlikte Uygulanmasının Deneysel Kolit Üzerine Etkileri

Semahat Çelikten[1], Ferhat Şirinyıldız[2], Rauf Onur Ek[3]

[1]Department of Medical Biology, Ege University Institute of Health Science, İzmir, Türkiye
[2]Department of Physiology, Aydın Adnan Menderes University Faculty of Medicine, Aydın, Türkiye
[3]Department of Physiology, İstinye University Faculty of Medicine, İstanbul, Türkiye

Abstract

Introduction: The pathogenesis of inflammatory bowel diseases emphasizes oxidative stress and inflammation as significant risk factors. Dexpanthenol accelerates wound healing and also possesses anti-inflammatory and soothing properties. Tocopherol acts as a fat-soluble essential vitamin with antioxidant functions. In this study, the effects of dexpanthenol and vitamin E on a rat model of colitis induced by trinitrobenzenesulfonic acid (TNBS) are investigated.

Methods: Wistar-albino rats were used in the study and divided into five groups (n=10): sham control, TNBS, TNBS + tocopherol (T), TNBS + dexpanthenol (D), and TNBS + (TD). The sham control group received 0.9% saline solution. TNBS was administered rectally to induce colitis in the TNBS group under anesthesia. In the T group, colitis was induced by TNBS, which was followed by daily intraperitoneal administration of 30-IU/kg T. The D group received 500-mg/kg D daily, and the TD group received both 30-IU/kg T and 500-mg/kg D daily. After 4 days, all rats were sacrificed, and 10-cm segments of their colons were removed. These colon segments underwent biochemical and histopathological examinations.

Results: Tissue levels of malondialdehyde exhibited notable reductions in both the D and TD intervention cohorts, whereas myeloperoxidase levels experienced significant decreases across the T, D, and TD treatment sets. Glutathione levels exhibited marked elevations in the T, D, and TD treatment groups, whereas catalase levels displayed no discernible variances among the control, colitis, and treatment populations.

Discussion and Conclusion: These results reveal that substances with antioxidant and anti-inflammatory properties, such as dexpanthenol and vitamin E, can reduce colon tissue damage in TNBS-induced colitis.

Keywords: Colitis; Dexpanthenol; Inflammatory bowel diseases; TNBS; Tocopherol

This study was adapted from Semahat Çelikten's master's thesis.

Cite this article as: *Çelikten S, Şirinyıldız F, Ek RO. Effects of Dexpanthenol and Tocopherol Applied Alone and in Combination on Experimental Colitis. Lokman Hekim Health Sci 2024;4(2):97–102.*

Correspondence: Ferhat Şirinyıldız, M.D. Adnan Menderes Üniversitesi Tıp Fakültesi, Fizyoloji Anabilim Dalı, Aydın, Türkiye
E-mail: ferhat.sirinyildiz@adu.edu.tr **Submitted:** 23.02.2024 **Revised:** 01.08.2024 **Accepted:** 07.08.2024

Inflammatory bowel diseases (IBDs) are chronic gastrointestinal system disorders that commence with intestinal inflammation and mucosal tissue damage, progress with the disruption of immune responses, and lead to symptoms in both the gastrointestinal and extraintestinal systems, which significantly affect the patient's quality of life.[1] Symptoms can manifest in both gastrointestinal and extraintestinal forms, and depending on the location of the disease, gastrointestinal symptoms may vary. The most common symptoms include bloody-mucous diarrhea, abdominal pain, and rectal bleeding. In cases of severe bleeding, anemia may develop. Fatigue, loss of appetite, and weight loss are also common. Extraintestinal symptoms include skin lesions, eye inflammation, liver diseases, and joint pains.

Ulcerative colitis (UC) is a disease that is characterized by chronic inflammation and ulceration, which usually affects the superficial part of the colon mucosa and submucosa and progresses with periods of relapse and remission. The lesions typically start in the rectum and progress proximally. Since the involvement is continuous, there are no unaffected areas between the regions where the lesion begins and ends.[2] Experimental studies have sought to clarify how stress induces pro-inflammatory effects.[3,4]

Vitamin E, or α-tocopherol, is an essential vitamin responsible for maintaining the integrity of cell membranes owing to its lipid structure.[5] Vitamin E has beneficial effects on maintaining the integrity of the intestinal epithelial barrier. The absorption rate of vitamin E in the intestine is estimated to range from 51% to 86%.[6]

Dexpanthenol is an alcohol derivative of pantothenic acid and a component of the B-vitamin complex. Numerous studies have confirmed the moisturizing and bowel barrier-strengthening properties of dexpanthenol.[7]

Malondialdehyde (MDA), one of the biomarkers in the study, is an indicator of lipid peroxidation. Glutathione peroxidase has a key role in maintaining the balance of the cell's redox function and determining cell fate. In the critical redox system of mammalian cells, glutathione peroxidase is the most prominent protein family with a versatile function that affects almost all cellular processes.[8] Myeloperoxidase (MPO) is a heme-containing peroxidase primarily expressed in neutrophils and to a lesser extent in monocytes.[9] In this study, the performance of catalase biomarker, which is an oxidative stress biomarker, is evaluated.[10]

In this study, we aimed to investigate the potential individual and combined effects of vitamin E and dexpanthenol in a TNBS (trinitrobenzenesulfonic acid)–induced experimental colitis model. Considering that the TNBS-induced colitis model reflects various aspects of IBDs, evaluating the therapeutic effects of vitamin E and dexpanthenol in this model is regarded as a potential strategy for treating these diseases. This study can contribute to a better understanding of the complexity of the pathogenesis of IBDs and the development of new treatment approaches by examining the use of these two compounds individually and in combination.

Materials and Methods

This study was initiated after obtaining ethical approval from the Aydin Adnan Menderes University Local Animal Experiments Ethics Committee on February 27, 2017, with approval number 64583101/2017/022. All animal experiments were carried out in the Animal Experimental Unit of the Aydin Adnan Menderes University Faculty of Medicine in March 2017. The study utilized a total of 40 male Wistar-albino rats, with weights ranging from 300 to 400 g. Throughout the experiment, all rats were housed in stainless steel cages measuring 425×265×180 mm, made of transparent polycarbonate material, and maintained in an environment with a relative humidity of 40%–60%, optimal temperature (22 °C), and a 12-h light/12-h dark cycle. During the experimental period, the rats were provided with access to water and pellet feed. After acclimatization to the environment, all animals were fasted for 12 h prior to the experiments commenced. Laboratory analyses were conducted at the Aydin Adnan Menderes University Central Research Laboratory. Histopathological examinations were performed in the Laboratory of Histology and Embryology, Department of Medicine, Aydin Adnan Menderes University.

In this study, a total of 40 male Wistar-albino rats (300–400 g) were utilized. Randomly, disregarding any characteristics, the rats were divided into the following groups: sham control group (n=8), TNBS group (n=8), TNBS+tocopherol (T) group (n=8), TNBS+dexpanthenol (D) group (n=8), and TNBS+tocopherol and dexpanthenol (TD) group (n=8).

The sham control group received the same procedures as the colitis group, but instead of TNBS, they were administered saline.

The TNBS group received 0.8 mL of TNBS (dissolved in 37% ethanol) intrarectally under ketamine (75 mg/kg) and xylazine (8 mg/kg) anesthesia.

The TNBS+T group was administered daily intraperitoneal injections of T at 30 IU/kg for four days after TNBS-induced colitis. Meanwhile, the TNBS+D group received daily intraperitoneal injections of D at a dosage of 500 mg/kg for 4 days following colitis induction with TNBS.

Table 1. Biochemical findings

	Sham control	TNBS	TNBS+T	TNBS+D	TNBS+TD
MDA (nmol/g)	304.00 (±18.84)**	353.87 (±17.02)	334.37 (±24.18)	326.25 (±19.17)**	318.87 (±17.9)**
MPO (U/g)	15.60 (12.2–16.5)**	20.80 (19.8–22.6)	19.70 (18.6–1.10)*	19.7 (16.9–20.6)*	18.60 (16.5–21.1)*
GSH (nmol/g)	20.50 (18.8–22.6)**	10.15 (9.2–12.5)	11.90 (9.2–13.1)*	13.05 (10.10–5.4)**	13.20 (10.9–14.9)**
CAT (mU/ml)	5.12 (±0.63)	4.72 (±0.50)	4.63 (±0.76)	4.85 (±0.71)	4.69 (±0.78)

*: P<0.05; **: P<0.01 in comparison with the TNBS group. TNBS: Trinitrobenzenesulfonic acid; MDA: Malondialdehyde; MPO: Myeloperoxidase; GSH: Glutathione; CAT: Catalase.

The TNBS+TD group received daily intraperitoneal injections of both T at a dose of 30 IU/kg and D at a dose of 500 mg/kg for 4 days after inducing colitis with TNBS.

Throughout the experimental period, all rats were subjected to manipulations specific to their respective groups. After 4 days, all rats were sacrificed, and their colons were removed.

The bowel tissues of both the control and experimental groups of rats were removed. The tissues were weighed, and for the biochemical analysis protocols, they were homogenized in phosphate buffer. The tissue homogenates were then vortexed and centrifuged (15000 rpm;15 min; 4 °C), and the supernatants were stored at -80 °C for analysis.

Measurement of MPO activity in tissues, measurement of tissue MDA, measurement of tissue glutathione (GSH) activity, and measurement of tissue catalase (CAT) activity were performed following the biochemical analysis kit protocols. Moreover, after standard histological processing, the samples were embedded in paraffin blocks. Using a microtome, sections of 5-µm thickness were randomly cut from these blocks. These sections were stained with hematoxylin–eosin and sealed with Entellan. Using an Olympus DP20 digital camera mounted on an Olympus BX51 microscope, photographs were captured. The microscopic scoring criteria for colon mucosa are as follows: 0=no effect, 1=mild effect, 2=moderate effect, and 3=severe effect.[11]

Induction of Colitis

Rats designated for experimental colitis induction with TNBS were fasted for approximately 24 h before the procedure, but they had free access to water. On the day of colitis induction, the rats' bowels were first emptied. Following this process, anesthesia was administered. Anesthesia was achieved by intraperitoneal injection of 90-mg ketamine and 10-mg xylazine. Anesthesia took effect within approximately 5 min in the rats. The depth of anesthesia was checked by assessing the limb response. Subsequently, while the rats were under anesthesia, a 0.8-mL TNBS solution, prepared by dissolving 25 mg of 2, 4, 6-TNBS in 37% ethanol, was administered. This was done by inserting a 1-mL polyethylene cannula with a 6 French diameter of 8 cm into the anal orifice. The TNBS solution was injected slowly with a syringe to ensure the full volume was administered, and the rats were kept in a Trendelenburg position for 30s. After the procedure, the rats were placed back in their cages and monitored under appropriate temperature conditions.

Statistical Analysis

The statistical analysis of the data collected in the study was conducted using IBM® SPSS® Statistics version 15.0 (Chicago, USA). The appropriateness of the variables for normal distribution was assessed by examining the coefficient of variation, skewness–kurtosis, and the Kolmogorov–Smirnov/Shapiro–Wilk tests. Descriptive analyses were presented using the mean (±standard deviation) for normally distributed variables, and comparisons were conducted using the t-test. For variables that were not normally distributed, descriptive statistics were presented as median (minimum–maximum), and comparisons were carried out using the Mann–Whitney U test. Within the scope of the study, treatment groups and control groups were compared with the TNBS group. A significance level of $p<0.05$ was considered statistically significant. ChatGPT support was utilized for language translations.

Results

Biochemical Analyses

In this study, the effects of treatment with dexpanthenol and tocopherol on rat intestinal tissue in the TNBS-induced experimental colitis model were investigated.

During the biochemical examination, tissue levels of MDA revealed a substantial rise in the TNBS group when juxtaposed with the sham control group ($p<0.01$). In contrast to the TNBS group, tissue MDA levels showed a notable decrease in both the TNBS+D and TNBS+TD groups ($p<0.01$) (Table 1).

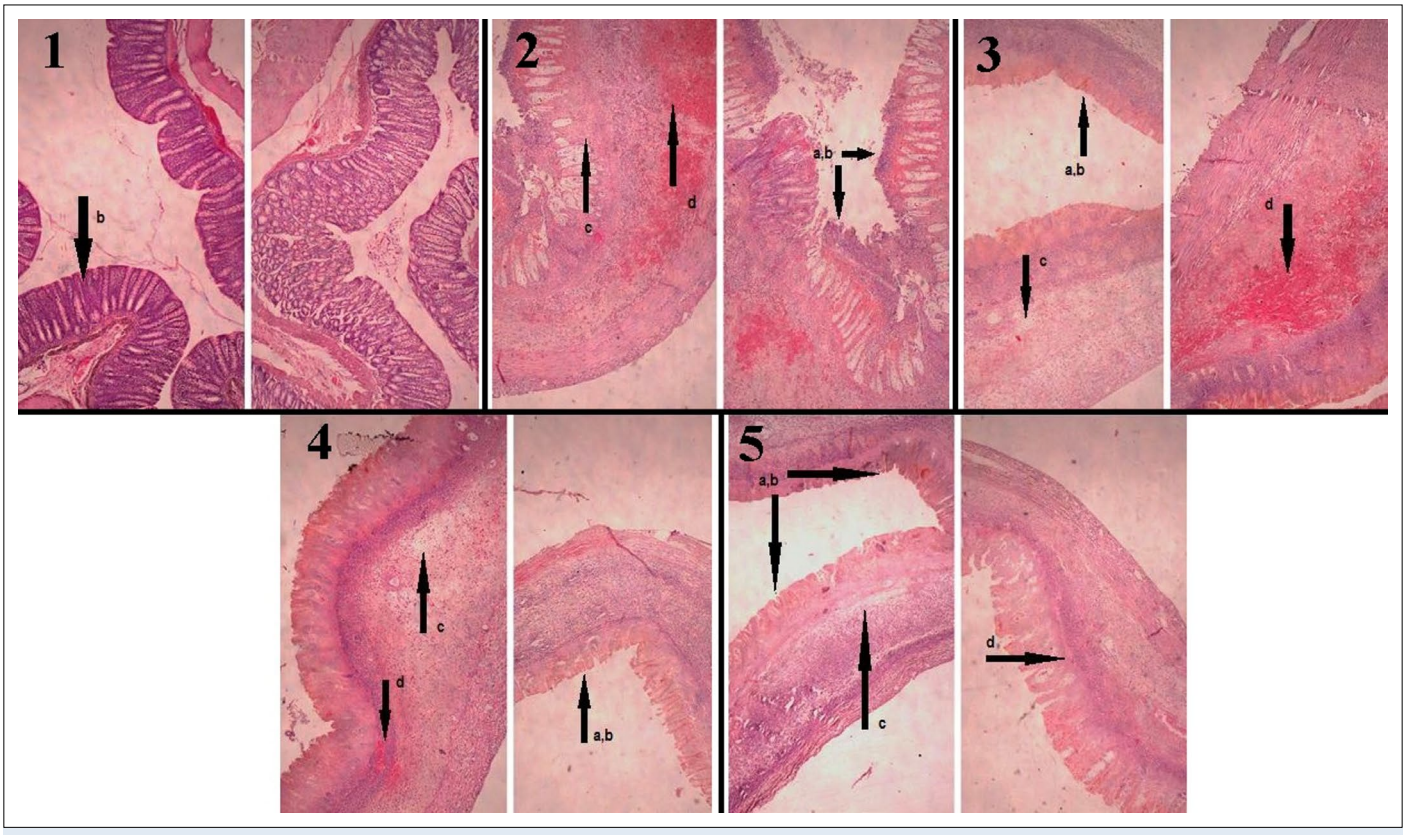

Figure 1. Hematoxylin–eosin staining—×40 magnification; a; mucosal damage/necrosis b; inflammation c; edema d; hemorrhage 1. Sham control group 2. TNBS group; 3. TNBS+T group; 4. TNBS+D group 5. TNBS+TD group.

Tissue MPO levels exhibited a significant increase in the TNBS group in comparison to the sham control group ($p<0.01$). Conversely, tissue MPO levels experienced a significant decrease in the TNBS+T, TNBS+D, and TNBS+TD groups what these inconsistencies preserve the TNBS group ($p<0.05$) (Table 1).

In the TNBS group, tissue levels of GSH exhibited a significant reduction when compared with the sham control group ($p<0.01$). Nonetheless, within the TNBS group, there was a notable increase in tissue GSH levels in the TNBS+T group ($p<0.05$), as well as in the TNBS+D and TNBS+TD groups ($p<0.01$) (Table 1).

Histopathological Analyses

Damage/necrosis, inflammation, edema, and hemorrhage conditions in the colonic tissues were assessed. For histological scoring, the following grading scale was employed: 0=no damage, 1=mild damage, 2=moderate damage, and 3=severe damage.

In the sham control group, a mild increase in inflammation was observed. In the TNBS group, intense mucosal damage/necrosis, inflammation, hemorrhage, and mild edema were detected. Likewise, in the TNBS+T group, intense mucosal damage/necrosis, inflammation, hemorrhage, and moderate edema were observed. In the TNBS+D and TNBS+TD groups, intense mucosal damage and hemorrhage, moderate inflammation, and edema were observed. The histopathological findings are shown in Figure 1.

Discussion

In this study, it was determined that prophylactic intraperitoneal administration of D and TD significantly decreased MDA activity ($p<0.01$), and this difference reached statistical significance. Prophylactic intraperitoneal administration of T did not result in statistically significant changes in MDA activity. The decrease in tissue MDA levels in the D and TD groups suggested that they effectively inhibited lipid peroxidation in TNBS-induced colitis. In a study investigating the effect of phlorizin on colon tissues in TNBS-induced colitis, it was concluded that phlorizin at doses of 20, 40, and 80 mg/kg reduced MDA levels in colon tissues.[12] Gnanaraj et al.[13] examined the potential effects of karanjin on TNBS-induced colitis. Karanjin led to a decrease in MPO and MDA levels. The results obtained

at the end of the study indicated that karanjin has potential in the treatment of colitis. Various studies have shown that iloprost has protective effects owing to its antioxidant and anti-inflammatory activity. In light of this information, a study that evaluates the effects of iloprost on oxidant/antioxidant status and colon histopathology in experimental colitis showed a significant decrease in elevated MDA levels induced by colitis.[14] In a study that investigates the effects of a plant-derived flavonoid, epigallocatechin-3-gallate (green tea extract), on dextran sulfate sodium (DSS)–induced colitis, it was also observed that the elevated MDA levels associated with induced colitis were reduced.[15]

In this study, it was observed that prophylactic intraperitoneal administration of T, D, and TD significantly decreased MPO activity ($p<0.05$). The decrease in tissue MPO levels observed in all treatment groups in TNBS-induced colitis led to the conclusion that it reduced inflammation and, consequently, neutrophil activity. An experimental colitis model showed that administration of N-acetylcysteine to mice resulted in significantly lower MPO levels in intestinal tissues, consistent with histological findings.[16]

Rosmarinic acid has anti-inflammatory and antioxidant effects. In mice with acute and chronic UC induced by DSS, rosmarinic acid treatment reduced elevated colon MPO levels.[17] In experimental colitis induced by DSS, MPO activity increased, and the treatment group with Cyclocarya paliurus, a medicinal plant with antidiabetic, antitumor, and immune-boosting properties, demonstrated a statistically significant decrease.[18]

In this study, it was determined that prophylactic intraperitoneal administration of D and TD significantly increased GSH activity ($p<0.01$), and prophylactic intraperitoneal administration of T significantly increased GSH activity ($p<0.05$), and the difference reached statistical significance (Table 1). The increase in tissue GSH levels observed in all treatment groups in TNBS-induced colitis suggested that these treatment groups activated antioxidant defense mechanisms that protect cells from oxidative damage. In a 2023 study, the effect of 1000-mg/kg Acacia arabica and Ocimum basilicum (basil) on UC was investigated in an acetic acid–induced colitis model. It was found that GSH activity decreased and showed significant antioxidant activity by increasing SOD and GSH levels after treatment.[19]

In this study, likewise, no significant change in CAT activity was observed. Reportedly, there was no change in CAT activity in DSS-induced colitis models with the plant extract Cyrtocapra procera (sumac). Researchers suggested that the lack of change in CAT levels may be due to the elimination of H202 by other cellular antioxidant mechanisms.[20]

In this study, the histology of colon sections obtained from the treatment groups showed only mild inflammation improvement. In a study carried out in 2022, the effect of 20, 40, and 80 mg/kg of naringenin on a TNBS-induced colitis model was investigated. It was found that naringenin reduced inflammation.[21] When evaluated clinically, it was found that colon inflammation decreased in people who received various treatments such as Infliximab and Vedolizumab.[22]

Conclusion

Severe mucosal damage/necrosis, inflammation, hemorrhage, and mild edema were observed microscopically in colitis-associated groups. These differences support the efficacy of TNBS as a chemical agent to create a chronic colitis model and are consistent with the literature. In the group treated with dexpanthenol, MPO and MDA levels were low, and GSH levels were high. This shows the antioxidant and anti-inflammatory activity of dexpanthenol. In the vitamin E–treated group, MPO levels were low, and GSH levels were high, supporting the antioxidant and anti-inflammatory activity of vitamin E. In the group treated with both dexpanthenol and vitamin E, MPO and MDA levels were the lowest, and GSH levels were the highest. This suggests the synergistic antioxidant and anti-inflammatory effects of dexpanthenol and vitamin E. Given the potential positive role of dexpanthenol and vitamin E in both prophylactic and therapeutic use in IBD prognosis, further animal and human studies may provide valuable guidance.

Ethics Committee Approval: The Aydın Adnan Menderes University Locak Animal Experiments Ethics Committee granted approval for this study (date: 27.02.2017, number: 64583101/2017/022).

Authorship Contributions: Concept: SÇ, ROE; Design: SÇ, ROE, FŞ; Supervision: ROE; Fundings: ROE; Materials: SÇ, FŞ; Data Collection or Processing: SÇ, FŞ, ROE; Analysis or Interpretation: FŞ, SÇ; Literature Search: FŞ; Writing: FŞ, SÇ; Critical Review: ROE.

Conflict of Interest: None declared.

Use of AI for Writing Assistance: Not declared.

Financial Disclosure: The authors declared that this study received no financial support.

Peer-review: Externally peer-reviewed.

References

1. Ungaro R, Mehandru S, Allen PB, Peyrin-Biroulet L, Colombel JF. Ulcerative colitis. Lancet 2017;389(10080):1756–70. [CrossRef]
2. Baran B, Mert Ozupek N, Yerli Tetik N, Acar E, Bekcioglu O, Baskin Y. Difference between left-sided and right-sided colorectal cancer: A focused review of literature. Gastroenterology Res 2018;11(4):264–73. [CrossRef]
3. Nemirovsky A, Ilan K, Lerner L, Cohen-Lavi L, Schwartz D, Goren G, et al; Israeli IBD Research Nucleus (IIRN). Brain-immune axis regulation is responsive to cognitive behavioral therapy and mindfulness intervention: Observations from a randomized controlled trial in patients with Crohn's disease. Brain Behav Immun Health 2021;19:100407. [CrossRef]
4. Pellissier S, Dantzer C, Mondillon L, Trocme C, Gauchez AS, Ducros V, et al. Relationship between vagal tone, cortisol, TNF-alpha, epinephrine and negative affects in Crohn's disease and irritable bowel syndrome. PLoS One 2014;9(9):e105328.
5. De Falco B, Amato M, Lanzotti V. Chia seeds products: An overview. Phytochem Rev 2017;16:745–60. [CrossRef]
6. Karaağaç RM, Pınarlı Ç. Fat-soluble vitamins and their effects on the gut microbiota. Eurasian J Health Sci 2023;6(3):116–22. [in Turkish]. [CrossRef]
7. Ebner F, Heller A, Rippke F, Tausch I. Topical use of dexpanthenol in skin disorders. Am J Clin Dermatol 2002;3(6):427–33. [CrossRef]
8. Pei J, Pan X, Wei G, Hua Y. Research progress of glutathione peroxidase family (GPX) in redoxidation. Front Pharmacol 2023;14:1147414. [CrossRef]
9. Lin W, Chen H, Chen X, Guo C. The roles of Neutrophil-Derived Myeloperoxidase (MPO) in diseases: The new progress. Antioxidants (Basel) 2024;13(1):132. [CrossRef]
10. Öner E, Belge Kurutas E, Demirhan İ, Güngör M. Evaluation of method performance and biological variations for oxidative stress biomarkers in plant extracts: A method validation study. Kahramanmaraş Sütçü İmam Univ Agric Nat 2022;25(5):965–73. Turkish. [CrossRef]
11. González R, Rodríguez S, Romay C, Ancheta O, González A, Armesto J, et al. Anti-inflammatory activity of phycocyanin extract in acetic acid-induced colitis in rats. Pharmacol Res 1999;39(1):55–9. [CrossRef]
12. Cheng J, Liu D, Huang Y, Chen L, Li Y, Yang Z, et al. Phlorizin mitigates dextran sulfate sodium-induced colitis in mice by modulating gut microbiota and inhibiting ferroptosis. J Agric Food Chem 2023;71(43):16043–56. [CrossRef]
13. Gnanaraj C, Govendan M, Loo CY, Yong YS, Sekar M, Mat Taib CN, et al. A Potential furanoflavonoid for neuroprotection. Available at: https://link-springer-com-443.webvpn.synu.edu.cn/article/10.1007/s11101-024-09925-z. Accessed Aug 28, 2024.
14. Şimşek O. Preventitative effect of montelukast in the radiocontrast induced nephropathy in rats via NF-KB, NOX4 and P22 phox expression Master's Thesis, İzmir. Dokuz Eylul University; 2021.
15. Wu Z, Huang S, Li T, Li N, Han D, Zhang B, et al. Gut microbiota from green tea polyphenol-dosed mice improves intestinal epithelial homeostasis and ameliorates experimental colitis. Microbiome 2021;9(1):184. [CrossRef]
16. İspiroğlu M, Kantarçeken B, Çıralık H, Bulbuloglu E. Effects of N-Acetyl cystein and β-glucan in experimental colitis model in rats. Kahramanmaraş Sütçü İmam Univ Med J 2020;16:357–64. [in Turkish]. [CrossRef]
17. Zhou B, Liu J, Wang Y, Wu F, Wang C, Wang C, et al. Protective effect of ethyl rosmarinate against ulcerative colitis in mice based on untargeted metabolomics. Int J Mol Sci 2022;23(3):1256. [CrossRef]
18. Lu H, Shen M, Chen Y, Yu Q, Chen T, Xie J. Alleviative effects of natural plant polysaccharides against DSS-induced ulcerative colitis via inhibiting inflammation and modulating gut microbiota. Food Res Int 2023;167:112630. [CrossRef]
19. Bejeshk MA, Aminizadeh AH, Rajizadeh MA, Rostamabadi F, Bagheri F, Khaksari M, et al. Ameliorating effects of Acacia arabica and Ocimum basilicum on acetic acid-induced ulcerative colitis model through mitigation of inflammation and oxidative stress. Heliyon 2023;9(11):e22355. [CrossRef]
20. Rodriguez-Canales M, Martinez-Galero E, Nava-Torres AD, Sanchez-Torres LE, Garduño-Siciliano L, Canales-Martinez MM, et al. Anti-inflammatory and antioxidant activities of the methanolic extract of cyrtocarpa procera bark reduces the severity of ulcerative colitis in a chemically induced colitis model. Mediators Inflamm 2020:5062506. [CrossRef]
21. Hambardikar VR, Mandlik DS. Protective effect of naringin ameliorates TNBS-induced colitis in rats via improving antioxidant status and pro-inflammatory cytokines. Immunopharmacol Immunotoxicol 2022;44(3):373–86.
22. Peyrin-Biroulet L, Arkkila P, Armuzzi A, Danese S, Guardiola J, Jahnsen J, et al. Comparative efficacy and safety of infliximab and vedolizumab therapy in patients with inflammatory bowel disease: A systematic review and meta-analysis. BMC Gastroenterol 2022;22(1):291. [CrossRef]