Advances in Pharmacology and Therapeutics Journal
Shahid Sadoughi University of Medical Sciences, Yazd, Iran

SHAHID SADOUGHI UNIVERSITY OF MEDICAL SCIENCES

Original Article

Impact of Eugenol on Acetic Acid-induced Colitis in Rats

Running Title: *Eugenol improves Inflammatory Bowel Diseases (IBD)*

Mohsen Zabihi[1], Faeze Aminalroayaei[1], Ehsan Motaghi-Dastnaei[2], Tahereh Dara[3*]

[1]Department of Pharmacology, School of Pharmacy, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
[2] Department of Physiology and Pharmacology, School of Medicine, Kurdistan University of Medical Sciences, Sanandaj, Iran
[3] Department of Pharmaceutics, School of Pharmacy, Shahid Sadoughi University of Medical Sciences, Yazd, Iran

A R T I C L E I N F O

Received: 10/18/2023

Accepted: 12/05/2023

***Corresponding author**
Assistant professor in Pharmaceutics, Department of Pharmaceutics, School of Pharmacy, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
Tel: +98-9131566814
Fax: 035-38203418
E-mail
taherehdara@yahoo.com

Abstract

Background: Inflammatory bowel diseases (IBD) including ulcerative colitis and Crohn's disease, are immune-mediated chronic relapsing intestinal disorders characterized by the presence of an acute or chronic inflammatory process in the bowel wall. Eugenol essential oil is used in traditional medicine to treat digestive disorders such as gastrointestinal ulcers, indigestion, and inflammation of the intestines.

Aim: To investigate the effects of Eugenol on colitis in rat models.

Methods: Eugenol was administered per rectum (5 and 10 mg/kg) and intraperitoneally (0.25 and 0.5 mg/kg) for 6 days after induction of colitis by acetic acid. The changes were examined macroscopically, histologically, and biochemically and compared with Dexamethasone.

Results: Results showed a significant decrease in the macroscopic damage score ($P < 0.05$), and reduction in the weight ratio of the colon ($P < 0.01$), the histological signs of inflammation ($P < 0.01$) such as infiltration of lymphocytes and macrophages into the mucosa, mucin depletion, crypt abscess, edema, and tissue damage, as well as leukocyte accumulation and myeloperoxidase level in compare with the colitis control group.

Conclusion: This animal trial demonstrates the beneficial anti-inflammatory and wound-healing effects of Eugenol in the treatment of acetic acid-induced colitis in rats. Eugenol can potentially be advantageous as a supplemental remedy for the treatment of IBD.

Keywords: Acetic Acid, Eugenol, Inflammatory Bowel Diseases, Traditional Medicine, Ulcerative Colitis

Citation: Zabihi M, Aminalroayaei F, Motaghi-Dastnaei E, Dara T. Impact of Eugenol on Acetic acid-induced Colitis in Rats. Adv Pharmacol Ther J. 2023;3(3): 114-122.

Introduction

Inflammatory bowel diseases (IBDs) which include ulcerative colitis and Crohn's disease are immune-mediated intestinal disorders characterized by the presence of acute, chronic, or relapsing-remitting inflammatory processes in the bowel wall. Though the pathogenesis of IBD remains unclear, current literature proposes that immune factors, genetic, microbial, and environmental are involved in its development (1). The principal physical symptoms of ulcerative colitis consist of abdominal pain, diarrhea, mucus or blood passage, weight loss, etc., while psychosocial consequences may include anxiety, lack of energy, depression, isolation, and fear (2, 3). The pathological characteristics of ulcerative colitis consist of nonspecific intestinal inflammation marked by mucosal infiltration of neutrophils, lymphocytes, and macrophages, ultimately leading to ulcer and hemorrhage (4). Furthermore, it has been suggested that the inflammatory cascade in the pathological process of colitis results from the overproduction of pro-inflammatory mediators like reactive oxygen species, eicosanoids, cytokines, and neutrophil granule contents (5). Acetic acid (AA)-induced colitis is one of the models of experimental colitis in rats and is made by intra-rectal administration of AA. Oxidative destruction is supposed to be the pathogenetic factor in this scenario, and the injury is visible in the gross morphology of the colon (6). It is believed that a similar phenomenon occurs in colitis in humans (7). Available current therapy for IBD includes immunosuppressants, corticosteroids, and 5-aminosalicylates, though they are not completely effective in most cases while both short-term and long-term adverse effects limit their use (5). Although the use of chemical drugs is very common today, their harmful effects have led to the consideration of some medicinal plants. Today, there is a serious tendency to try herbal medicines hoping their less side effects, and especially in line with the suggestion of using medicinal plants by the World Health Organization among many other reasons (8, 9).

Eugenol [2-Methoxy-4-(prop-2-en-1-yl) phenol] is the principal component of clove (45-90% of its essential oil) and a phenolic oil from the class of phenylpropanoids (10). It is used in the food industry as a preservative, chiefly due to its antioxidant properties (11), and as a flavoring agent for foods and cosmetics (12). Furthermore, clove is also known for its anti-inflammatory activity (13), likely attributable to Eugenol. Many reports are confirming the antibacterial, antifungal, and antiviral properties of Eugenol presumed to be due to its protective ability against free radicals (14). The present study assesses the anti-inflammatory potential of Eugenol in a rat model of acetic acid-induced colitis. In this animal trial, we checked out the inflammatory response by macroscopic and histopathological examination as well as the determination of tissue myeloperoxidase (MPO) activity as an inflammation marker.

Material and Methods

Animals: Male Wistar rats weighing about 230–270 g were purchased from the animal house in the School of Pharmaccy, Shahid Sadoughi University of Medical Sciences, Yazd, Iran. The rats were housed in groups of 6 in temperature-

DOI: https://doi.org/10.18502/aptj.v3i3.14415

and humidity-controlled rooms (20-23 °C, 50-60% humidity) with a 12-hour light/dark cycle and free access to standard food and tap water. The animals were kept and handled according to the local guidelines of care and work on laboratory animals in this university.

Chemicals: Dexamethasone was sourced from Iran Hormone Pharmaceutical Company (Tehran, Iran). All other chemicals including Eugenol, O-dianisidine dihydrochloride, formalin solution 35% w/w, and hexadecyl trimethyl-ammonium bromide (HTAB) were purchased from Merck (Darmstadt, Germany). Monobasic potassium phosphate, dibasic potassium phosphate, o-dianisidine dihydrochloride, and hydrogen peroxide (H2O2) were used for the determination of the myeloperoxidase (MPO) activity.

Body weight measurement

Rats were individually weighed by an electronic balance (model V-3000, Acculab, USA) at the beginning of the intervention and the end of the study.

Induction of experimental colitis

The animals were kept fast in stainless steel cages for 24 hours before colitis induction. Acute colitis was induced by acetic acid as described previously (15). After mild anesthesia, an 8-cm tube was entirely inserted into the large intestine through the anus for a slow infusion of acetic acid (2 mL, 3% v/v in normal saline). The rats were held in a head-down position for 30 seconds (sec) to decrease leakage of the solution from the anus.

Animal groups

The animals were randomly divided into 7 groups of 6 rats in each: the sham group received normal saline 2 mL/kg, intra-rectal followed 30 sec later by 2 mL/kg intra-rectal normal saline (without induction of colitis); the control group received normal saline 2 mL/kg, intraperitoneally (ip) followed 30 sec later by induction of colitis; the dexamethasone group had dexamethasone 1 mg/kg, ip followed 30 sec later by induction of colitis. The 2 ip test groups received Eugenol at doses of 0.25 or 0.5 mg/kg, ip followed 30 sec later by induction of colitis, while the 2 rectal test groups were given Eugenol 5 or 10 mg/kg, per rectum followed 30 sec later by induction of colitis. All of the above-mentioned treatments were repeated for a total of 6 days.

Evaluation of colon macroscopic damage

The rats were killed 24 hours after the last treatment (day 6). The last 8 cm of the colon was removed, opened longitudinally, washed with normal saline, and the wet weight was measured (16). Colon tissue samples were then used for macroscopic scoring and histopathological exams and to measure tissue MPO activity. The appearance of macroscopic damage was rated by an independent observer according to the following scale: 0 = no macroscopic change; 1 = mucosal erythema only; 2 = mild mucosal edema, slight bleeding or slight erosion; 3 = moderate edema, bleeding ulcers or erosions, and 4 = severe ulceration, edema, erosions, and tissue necrosis (17). Tissue samples were cut into 2 pieces, one piece for histopathological evaluation (kept in 5 mL formalin 10% as fixative) and the other for measuring MPO enzyme activity. Pieces assigned for measuring MPO activity were stored in frozen liquid nitrogen in a freezer (-70 °C). In addition, the wound area was assessed by Fiji-Win 32

image processing software (ImageJ, NIH, USA). For each sample, the ulcer index was measured by summing the wound score and wound area using the following formula:

Ulcer index = Ulcer area (cm2) + Macroscopic damage score (18).

Histopathological assessment of colon injury

The colon pieces already fixed in formalin solution were used for paraffin block preparation and then cut into slices 5 µm thick. Eventually, the hematoxylin and eosin-stained slices were scored as described previously (Rees, 1998) with some modifications. Total colitis index was measured by summing the scores of ulceration (0-1), mucin depletion (0-2), inflammation extent (0-3) and crypt damage (0-4) (19).

Determination of MPO activity

Tissue MPO activity was measured with some modifications of the method described by Bradley et al (20). A portion of fresh colon tissue (0.1 g) was homogenized in 1 mL of 50 mM potassium phosphate (pH 6) with 0.5% HTAB in an ice bath using a polytron homogenizer. More buffers were added to obtain a concentration equivalent to 5 mL per 0.1 g of colon tissue. The resultant homogenate was sonicated in an ice bath for 10 seconds, then subjected to a sequence of freezing and thawing 3 times, sonicated again for 10 seconds, and centrifuged at 4°C for 15 minutes at 15000 rpm. A total of 0.1 mL of the supernatant was mixed with 2.9 mL of 50 mM phosphate buffer (pH 6) containing 0.167 mg/mL O-dianisidine dihydrochloride and 0.0005% hydrogen peroxide. The change in absorbance at 460 nm was measured using a UV/visible photometer (model Clinic III, Tajhizat Sanjesh, Iran). MPO activity was reported as units (U) per gram (g) weight of wet colon tissue.

Statistical analysis

The statistical tests included one-way ANOVA followed by Tukey's post hoc test. A paired t-test was applied for the comparison of weight changes. Data are expressed as mean ± SD. All statistical analyses were made using SPSS software (version 22, IBM, USA).

Result

Animals' body weight changes

The difference in animal body weight among groups before induction of colitis was not significant (P> 0.05). Induction of experimental colitis was associated with a decrease in body weight in the control group compared with the sham group (P < 0.001). Eugenol (5, 10 mg/kg, rectal or 0.25, 0.5 mg/kg, i.p.) significantly prevented body weight loss at day 6. Dexamethasone (1 mg/kg, i.p.) prevented body weight loss, too (P < 0.01) (**Figure 1**).

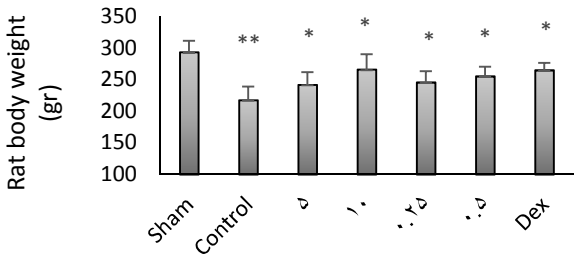

Figure 1: Effect of Eugenol on the rat body weight in grams, at day 6 after induction of colitis. Data are shown as mean and SD (n=6). **P < 0.001 in comparison with the sham group, one-way ANOVA followed by Tukey's post hoc test. *P < 0.01 in comparison with the control group, one-way ANOVA followed by Tukey's post hoc test. 5, 10: Eugenol groups receiving 5 or 10 mg/kg Eugenol, rectal. 0.25, 0.5: Eugenol groups receiving 0.25 or 0.5 mg/kg Eugenol, ip. Dex: the group receiving 1 mg/kg dexamethasone, ip.

DOI: https://doi.org/10.18502/aptj.v3i3.14415

Effect of Eugenol on Colon Weight

Induction of experimental colitis induced an increase in colon weight (likely due to inflammation and edema) during the experimental period in the control group ($P < 0.001$ vs. the sham group). Treatment with Eugenol (0.25/ 0.5 mg/kg, ip. or 10 mg/kg, rectal, but not 5 mg/kg) significantly ($P < 0.01$ vs. the control group) prevented colon weight gain at day 6. Dexamethasone (1 mg/kg, ip.) halted colon weight gain, too ($P < 0.01$ vs. the control group) **(Figure 2).**

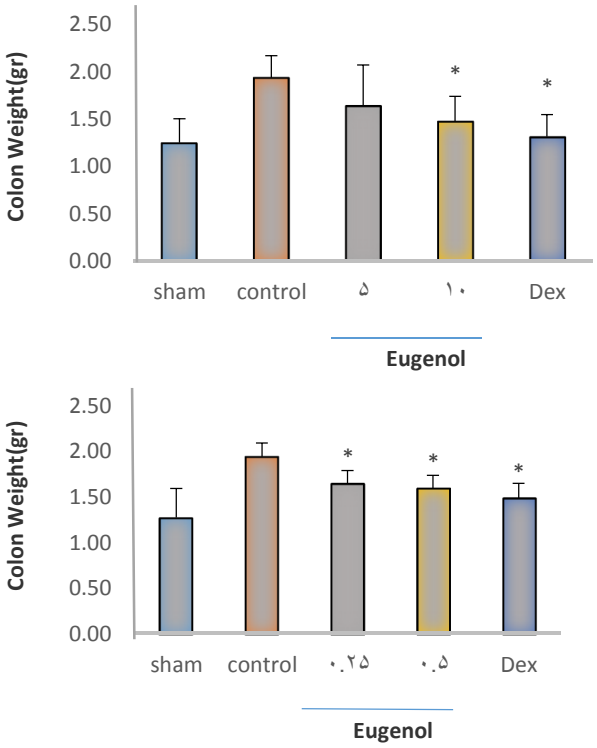

Figure 2. Effect of Eugenol on the rat colon weight in grams, at day 6 after induction of colitis. Data are shown as mean and SD (n=6). *$P < 0.01$ in comparison with the control group, one-way ANOVA followed by Tukey's post hoc test. 5, 10: Eugenol groups receiving 5 or 10 mg/kg Eugenol, rectal. 0.25, 0.5: Eugenol groups receiving 0.25 or 0.5 mg/kg Eugenol, ip. Dex: the group receiving 1 mg/kg dexamethasone, ip.

Effect of Eugenol on level of inflammation, ulcer index

Induction of experimental colitis induced an increase in the level of inflammation, ulcers, thickening of the epithelial wall of the colon, edema, and sometimes necrosis of the colon during the experimental period in the control group. Dexamethasone significantly reduced the level of inflammation, and ulcer index compared to the control group ($P<0.001$). On the other hand, Eugenol (0.25, 0.5 mg/kg, i.p. - and 5, 10 mg/kg, rectal) indicates a significant decrease in the level of inflammation, and ulcer index compared to the control group ($P < 0.001$) **(Figure 3).**

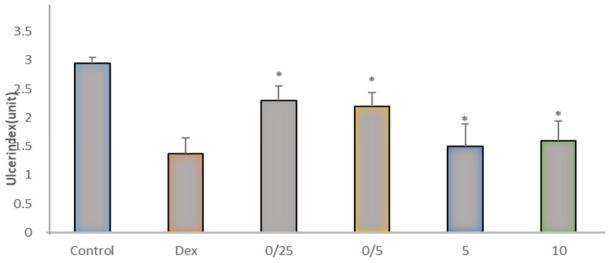

Figure 3. Effect of Eugenol on level of inflammation, after induction of ulcerative colitis. Data are analyzed as mean SD, (n=6). *$P < 0.05$, in comparison with the control group, one-way ANOVA followed by Tukey's post hoc test.

Effect of Eugenol on histopathological features

In the sham group, histological evaluation of the colon mucosa indicates no inflammation or necrosis **(Table 1, 2; Figure 4)**. On the other hand, acetic acid-induced colitis indicates demolition of transmural necrosis, edema, epithelium, areas of hemorrhage, and submucosal inflammatory cellular. Dexamethasone (1 mg/kg) and Eugenol (0.25, 0.5 mg/kg, ip. and 5, 10 mg/kg, rectal) showed a significant reduction of edema ($P< 0.01$), and extent or severity of tissue damage, as evaluated by total colitis index **(Table 1, 2 and Figure 4)**.

Table 1. Effects of intraperitoneally Eugenol on the microscopic parameters of colitis induced by acetic acid in rats.

Treatment	mucin depletion 0-2	ulceration 0-1	inflammation extent 0-3	crypt abscess or damage 0-4	Total score 0-10
Sham	0	0	0	0	0
Chontrol	2	1	3	4	10***
Acetic acid-Eugenol, 0.25	2	1	2	2	7**
Acetic acid-Eugenol, 0.5	1	1	2	1	4***
Acetic acid – Dexamethasone	0	0	1	1	3***

Data are expressed as median (range). **P < 0.01, ***P < 0.001, indicates significant difference versus control

Table 2. Effects of rectal administration of Eugenol on the microscopic parameters of colitis induced by acetic acid in rats

Treatment	mucin depletion 0-2	ulceration 0-1	inflammation extent 0-3	crypt abscess or damage 0-4	Total score 0-10
Sham	0	0	0	0	0
Control	2	1	3	4	10***
Acetic acid-Eugenol, 5	1	1	2	2	6**
Acetic acid-Eugenol, 10	1	1	1	1	4***
Acetic acid-dexamethasone	0	0	0	1	3***

Data are expressed as median (range). **P< 0.01, ***P < 0.001, indicates significant difference versus control.

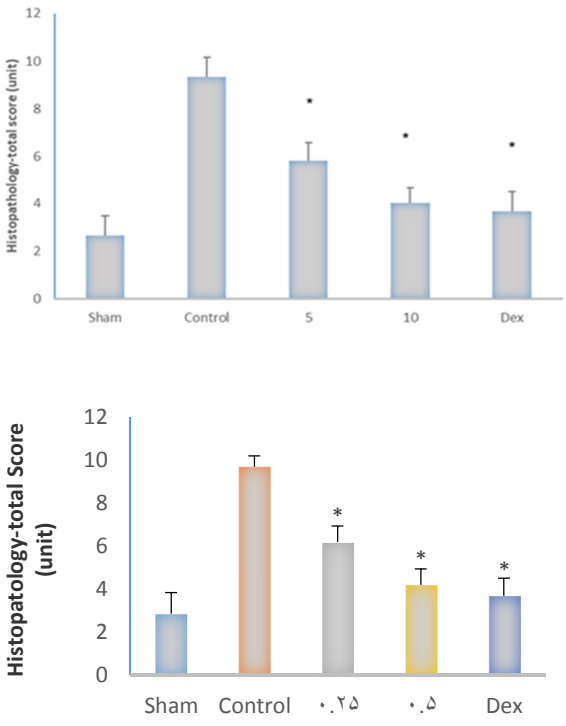

Figure 4. Effect of Eugenol on the rat histopathology total scores, at day 6 after induction of colitis. Data are shown as mean and SD (n=6). *P < 0.01 in comparison with the control group, one-way ANOVA followed by Tukey's post hoc test. 5, 10: Eugenol groups receiving 5 or 10 mg/kg Eugenol, rectal. 0.25, 0.5: Eugenol groups receiving 0.25 or 0.5 mg/kg Eugenol, ip. Dex: the group receiving 1 mg/kg dexamethasone, ip.

Effect of Eugenol on MPO activity

Intrarectal administration of acetic acid caused a significant enhancement in the level of MPO in colonic tissue when compared to the sham group (P < 0.001). In contrast, MPO activity was decreased (P < 0.001) in groups treated with Eugenol (0.25, 0.5 mg/kg, ip, and 5, 10 mg/kg,

DOI: https://doi.org/10.18502/aptj.v3i3.14415

rectal) as well as dexamethasone (1 mg/kg) (**Figure 5**).

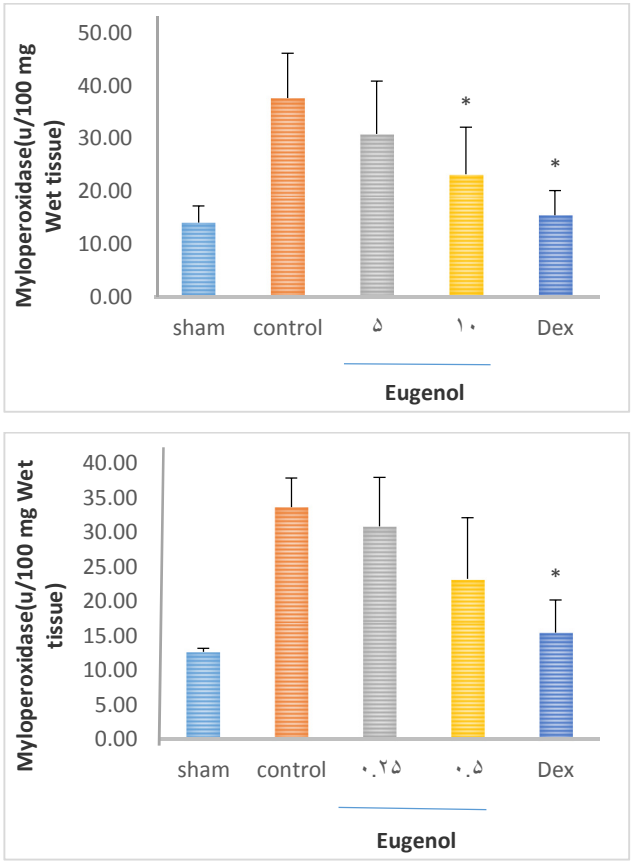

Figure 5. Effect of Eugenol on MPO, after induction of ulcerative colitis. Data are analyzed as mean S.D, (n=6). *P < 0.05, **P < 0.01 in comparison with the control group, one-way ANOVA followed by Tukey's post hoc test. (Sham: Normal saline group, Control: Acetic acid group, Eugenol group: (0.25, 0.5 mg/kg, ip, and 5, 10 mg/kg, rectal), Dex group: receiving dexamethasone).

Discussion

The present study shows that Eugenol decreases tissue damage in an experimental model of acetic acid-induced colitis as established macroscopic, histological, and biochemical changes. Intra-rectal administration of acetic acid is one of the reputable methods for induction of an experimental model of IBD. This method is widely approved to screen potential drugs because of its similarity to human IBD. Demolition of the colon structure and mucosal barrier by chemical stimulation increased vasopermeability, and enhanced inflammatory mediators are major contributing factors in the induction of this animal model (21, 22). An increase in the weight of inflamed colon tissue can be directly associated with the severity and extent of inflammation (23). Our finding showed that treatment with Eugenol could decrease the weight ratio of colon and macroscopic damage score compared with the colitis control. Furthermore, our findings showed that treatment with Eugenol essential oil decreased the histological signs of inflammation such as infiltration of lymphocytes and macrophages into the mucosa, edema, and tissue damage. MPO is a proteolytic enzyme found in neutrophil granulocytes that fights bacteria. These enzymes are released from activated neutrophils and catalyze the formation of hypochlorous acid from hydrogen peroxide and chloride ions, leading to oxidative damage associated with colitis induction (24). Hence, the reduction in the activity of MPO can be described as a manifestation of the anti-inflammatory effect of a given drug. Our research displays that the administration of Eugenol reduced the MPO level during acetic acid-induced colitis indicating an excellent anti-inflammatory activity on the experimental colitis model.

Eugenol is a compound derived from the clove plant. Clove represents an important plant source of phenolic compounds such as flavonoids, hydroxybenzoic acids, hydroxycinnamic acid, and propene hydroxyphenyl. Eugenol has very high antioxidant properties (25) Additionally, a recent study reported that Eugenol also has anti-

inflammatory activity in acute lung injury caused by lipopolysaccharide (LPS). Pretreatment with Eugenol inhibited the inflammatory response and leukocyte uptake into lung tissue by reducing the regulation of pro-inflammatory cytokine expression (IL-6 and TNF-a) and NFKB signaling. Furthermore, Eugenol also increased the catalase (CAT), glutathione peroxidase (GPx), superoxide dismutase (SOD), and Glutathione-S-transferase (GST), which are substantial anti-oxidative enzymes (26). Eugenol prevents the liberation of inflammatory mediators from macrophages. Macrophages are cells of the immune system that help produce mediators (e.g., pro-inflammatory cytokines and nitric oxide), which are important for cellular and vascular events during the installation and progression of an inflammatory process (27). Furthermore, a recent study reported that Eugenol advances cytotoxicity against breast cancer cells (TNBC) and animal models and synergistic chemotherapeutic effects with cisplatin. A key point in this effect was the inhibition of the NF-kB signaling pathway, which resulted in the inhibition of phosphorylation of p50 and p65 subunits and the resulting migration to the cell nucleus, reducing IL6 and IL-8 levels (28).

Conclusion

The present study displays that Eugenol could inhibit acetic acid-induced colitis in rats by decreasing leukocyte accumulation and inhibiting MPO production. The results of this study display that Eugenol could potentially be beneficial as a supplemental remedy for the treatment of IBD. However, further investigations are needed to elucidate the entire mechanism of action of Eugenol essential oil and its isolated compounds on the cell receptors and signaling pathways associated with colitis.

Declarations

Acknowledgments: None.

Conflict of interest: The authors declare no conflict of interest in conducting this study.

Funding: This study was funded by Shahid Sadoughi University of Medical Sciences, Yazd, Iran. (Grant No. 6526).

Ethical considerations: This study was reviewed and approved by the Ethics Committee of the Shahid Sadoughi University of Medical Sciences, Yazd, Iran. (Registration Code: IR.SSU.MEDICINE.REC.1398.053).

Code of Ethics: IR.SSU.MEDICINE.REC.1398.053

Authors' contribution: Conceptualization, Supervision, and Methodology: M.Z and E.M; Investigation: All of the authors; Writing – original draft: F.A; Review & editing: M.Z, T.D and E.M; Data collection: F.A; Data analysis: F.A; Funding Administration: M.Z.

Consent for publication: All of the authors give consent for the publication of identifiable details, which can include photographs, tables, and details within the text to be published in this Journal and Article.

Availability of data and materials: The source of data and materials are available.

References

1. Alireza Taghavi S, Reza Safarpour A, Hosseini SV, Noroozi H, Safarpour M, Rahimikazerooni S. Epidemiology of inflammatory bowel diseases (IBD) in Iran: a review of 740 patients in Fars Province, Southern Iran. Annals of Colorectal Research. 2013;1(1):1-2.

  DOI: https://doi.org/10.18502/aptj.v3i3.14415

2. Monteleone G, Pallone F, MacDonald TT. Emerging immunological targets in inflammatory bowel disease. Current opinion in pharmacology. 2011;11(6):640-5.
3. Casati J, Toner BB, De Rooy EC, Drossman DA, Maunder RG. Concerns of patients with inflammatory bowel disease. Digestive diseases and sciences. 2000;45(1):26-31.
4. Fiocchi C. Inflammatory bowel disease: etiology and pathogenesis. Gastroenterology. 1998;115(1):182-205.
5. Yao J, Wang J-Y, Liu L, Li Y-X, Xun A-Y, Zeng W-S, et al. Anti-oxidant effects of resveratrol on mice with DSS-induced ulcerative colitis. Archives of medical research. 2010;41(4):288-94.
6. Pereira C, Grácio D, Teixeira JP, Magro F. Oxidative stress and DNA damage: implications in inflammatory bowel disease. Inflammatory bowel diseases. 2015;21(10):2403-17.
7. Vishwakarma N, Ganeshpurkar A, Pandey V, Dubey N, Bansal D. Mesalazine–probiotics beads for acetic acid experimental colitis: formulation and characterization of a promising new therapeutic strategy for ulcerative colitis. Drug Delivery. 2015;22(1):94-9.
8. Boisnic S, Branchet M, Ermosilla V. Healing effect of a spray containing Rhealba oat colloidal extract in an in vitro reconstitution model of skin. International journal of tissue reactions. 2005;27(3):83-9.
9. Chevallier A. Encyclopedia of Herbal Medicine: The Definitive Home Reference Guide to 550 Key Herbs with all their Uses as Remedies for Common Ailments: Dorling Kindersley; 2000.
10. Zhang P, Zhang E, Xiao M, Chen C, Xu W. Study of anti-inflammatory activities of α-d-glucosylated eugenol. Archives of pharmacal research. 2013;36(1):109-15.
11. Zhang H, Chen X, He J. Pharmacological action of clove oil and its application in oral care products. Oral Care Industry. 2009;19:23-4.
12. Chatterjee D, Bhattacharjee P. Use of eugenol-lean clove extract as a flavoring agent and natural antioxidant in mayonnaise: product characterization and storage study. Journal of food science and technology. 2015;52(8):4945-54.
13. Han X, Parker TL. Anti-inflammatory activity of clove (Eugenia caryophyllata) essential oil in human dermal fibroblasts. Pharmaceutical Biology. 2017;55(1):1619-22.
14. Lee S-J, Lee K-G. Inhibitory effects of volatile antioxidants found in various beans on malonaldehyde formation in horse blood plasma. Food and chemical toxicology. 2005;43(4):515-20.
15. MacPherson B, Pfeiffer C. Experimental production of diffuse colitis in rats. Digestion. 1978;17(2):135-50.
16. Rashidian A, Mehrzadi S, Ghannadi AR, Mahzooni P, Sadr S, Minaiyan M. Protective effect of ginger volatile oil against acetic acid-induced colitis in rats: a light microscopic evaluation. Journal of Integrative Medicine. 2014;12(2):115-20.
17. Deshmukh C, Veeresh B, Pawar A. Protective effect of Emblica officinalis fruit extract on acetic acid induced colitis in rats. Journal of Herbal Medicine and Toxicology. 2010;4(2):83-7.
18. Minaiyan M, Asghari G, Taheri D, Saeidi M, Nasr-Esfahani S. Anti-inflammatory effect of Moringa oleifera Lam. seeds on acetic acid-induced acute colitis in rats. Avicenna journal of phytomedicine. 2014;4(2):127.
19. Minaiyan M, Hajhashemi V, Rabbani M, Fattahian E, Mahzouni P. Evaluation of anti-colitic effect of fluvoxamine against acetic acid-induced colitis in normal and reserpinized depressed rats. European journal of pharmacology. 2015;746:293-300.
20. Bradley P, Da P. Christensen RD, Rothstein G: Measurement of cutaneous inflammation: Estimation of neutrophil content with an enzyme marker. J invest Dermatol. 1982;78:206-9.
21. NOA M, MÁS R, CARBAJAL D. Effect of D-002 on acetic acid-induced colitis in rats at single and repeated doses. Pharmacological research. 2000;41(4):391-5.
22. Zabihi M, Hajhashemi V, Talebi A, Minaiyan M. Evaluation of central and peripheral effects of doxepin on acetic acid-induced colitis in rat and the involved mechanisms. EXCLI journal. 2017;16:414.
23. Torres J, Billioud V, Sachar DB, Peyrin-Biroulet L, Colombel J-F. Ulcerative colitis as a progressive disease: the forgotten evidence. Inflammatory bowel diseases. 2012;18(7):1356-63.
24. Bachiega TF, de Sousa JPB, Bastos JK, Sforcin JM. Clove and eugenol in noncytotoxic concentrations exert immunomodulatory/anti□inflammatory action on cytokine production by murine macrophages. Journal of Pharmacy and Pharmacology. 2012;64(4):610-6.
25. de Araújo Lopes A, da Fonseca FN, Rocha TM, de Freitas LB, Araújo EVO, Wong DVT, et al. Eugenol as a promising molecule for the treatment of dermatitis: antioxidant and anti-inflammatory activities and its nanoformulation. Oxidative Medicine and Cellular Longevity. 2018;2018.
26. Huang X, Liu Y, Lu Y, Ma C. Anti-inflammatory effects of eugenol on lipopolysaccharide-induced inflammatory reaction in acute lung injury via regulating inflammation and redox status. International immunopharmacology. 2015;26(1):265-71.
27. Arango Duque G, Descoteaux A. Macrophage cytokines: involvement in immunity and infectious diseases. Frontiers in immunology. 2014;5:491.
28. Islam SS, Al□Sharif I, Sultan A, Al□Mazrou A, Remmal A, Aboussekhra A. Eugenol potentiates cisplatin anti□cancer activity through inhibition of ALDH□positive breast cancer stem cells and the NF□κB signaling pathway. Molecular carcinogenesis. 2018;57(3):333-46.