ACS OMEGA

http://pubs.acs.org/journal/acsodf

Article

α-Terpineol Mitigates Dextran Sulfate Sodium-Induced Colitis in Rats by Attenuating Inflammation and Apoptosis

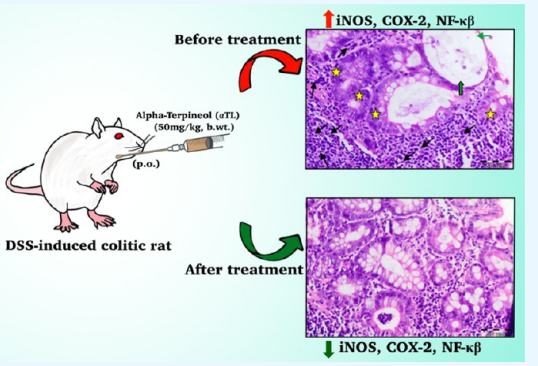
Rehan Khan,* Chandrashekhar Jori, Md. Meraj Ansari, Anas Ahmad, Ahmed Nadeem, Nahid Siddiqui, and Sarwat Sultana

Cite This: *ACS Omega* 2023, 8, 29794−29802

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: Ulcerative colitis (UC) is one of the major inflammatory disorders of the gastrointestinal tract. α-Terpineol (αTL) is naturally present in several plants, and it belongs to the monoterpenes category. αTL possesses various pharmacological properties such as antioxidant, antibacterial, antifungal, anticancer, and antiulcer activities. Importantly, αTL has been reported to possess potent anti-inflammatory effects also. In this study, we hypothesize that αTL may have protective effects against dextran sodium sulfate (DSS)-induced colitis in Wistar rats. Animals were randomly allocated to 3 groups of 6 rats each. In group III, αTL was administered at a dose of 50 mg/kg b. wt. orally from days 1 to 14, while in groups II and III, 4% DSS in drinking water was given to rats ad libitum from the 7th to 14th days. After 24 h of the last dose of αTL, all animals were euthanized. αTL administration reduced the DSS-induced colonic disease activity index, tissue damage, and goblet cell disintegration. αTL suppressed the orchestration of mast cells in the inflamed colon, enhanced the immunostaining of NF-kB-p65, COX-2, iNOS, p53, caspase-9, and cleaved caspase-3, and suppressed the immunostaining of connexin-43, survivin, and Bcl-2. The activities of caspases-9 and -3 were reduced significantly by αTL pretreatment, as also confirmed by calorimetric assays. Moreover, αTL significantly attenuated the nitric oxide level and myeloperoxidase activity. Histological results further support the fact that αTL reduced DSS-induced colonic damage and reduced inflammatory cell infiltration. Overall, our findings suggest that αTL has strong protective effects against DSS-induced colitis by mitigating inflammatory and apoptotic responses.



INTRODUCTION

Ulcerative colitis (UC) and Crohn's disease (CD) are classified under the category of inflammatory bowel disease (IBD), which are the major inflammation-related gastrointestinal diseases. Globally, UC is the most commonly diagnosed form of IBD, which is mainly identified by recurrent inflammation and colon damage, ultimately resulting in the shortening of the colon.[1] The precise mechanism of disease development is still not known, but colonic epithelial cells undergo extensive cell death, which causes tissue damage.[2,3] It has been reported that in response to an inflammatory stimulus, nuclear factor-kappaB (NF-kB) activation plays a crucial role in inflammation through its capability to regulate the expression of different inflammatory markers, viz., tumor necrosis factor-α (TNF-α), cyclooxygenase-2 (COX-2), and inducible nitric oxide synthase (iNOS). Furthermore, NF-kB also regulates the expression of apoptotic markers, viz., B-cell lymphoma-2 (Bcl-2) and survivin.[4,5] The integrity and functionality of the mucosal barrier can be compromised by aberrant apoptosis in epithelial cells or colonocytes, which mostly leads to colonic tissue damage.[3] Deregulation of connexin-43 (Cx-43), which is the foremost connexin that leads to changes in gap junctional intercellular communication (GJIC), has been shown to be involved in the pathophysiology of various intestinal epithelial cell barrier diseases such as inflammatory bowel diseases (ulcerative colitis) and cancer.[6]

Oral administration of dextran sulfate sodium (DSS) in drinking water in a rodent model has been found to induce transmural colonic inflammation, which shows histopathological alterations and clinical signs similar to human ulcerative colitis. DSS-induced colitis in a rodent model may prove to be useful for a better understanding of the development of colitis and to investigate and verify new methods of therapy for the treatment as well as prevention of colitis. Several approaches have been put to use to abrogate the extent of colitis induced by DSS, and the use of dietary agents or natural compounds with pharmacological or medicinal properties is one of them.[7]

Several epidemiological studies have shown that natural compounds with pharmacological properties are linked to

Received: June 17, 2023
Accepted: July 20, 2023
Published: August 1, 2023

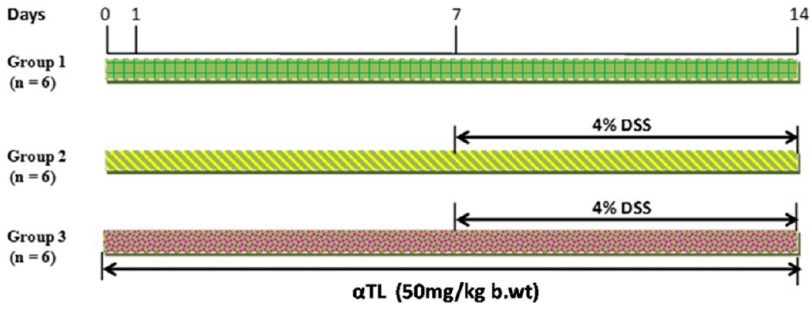


Figure 1. Schematic representation of the experimental design.

various health benefits. α-Terpineol (αTL) is naturally present in several plants, such as *Origanium vulgare*, *Antrodia cinnamomea*, *Ocimum canum*, etc.[8] It belongs to the monoterpenes category and is normally considered safe or nontoxic as the oral lethal dose (LD_{50}) in rodent models is above 5000 mg/kg.[9] αTL possesses various pharmacological properties, such as antioxidant, antibacterial, antifungal, anticancer, and antiulcer activities.[10,11] Importantly, αTL has been reported to possess potent anti-inflammatory effects.[12,13] Moreover, αTL has been reported to exert antiulcer effects or have gastroprotective activity.[14,15]

Taking into account the aforementioned biological properties of αTL, we hypothesize that it may be effective toward a colitis model induced by DSS in Wistar rats and, by targeting inflammatory and apoptotic indicators, offer certain insights into the basic mechanism that regulates αTL.

MATERIALS AND METHODS

Experimentation Protocol. Eighteen male Wistar rats were arbitrarily assigned to three distinct groups of 6 rats to evaluate the preventive application of αTL in the colitis model.

Control (group 1): Corn oil was administered orally to rats on a daily basis, dosage 5 mL/kg body weight for 14 days, and it served as the vehicle for αTL.

DSS (group 2): From the 7th to 14th days, rats were given ad libitum 4% DSS in drinking water.

αTL + DSS (group 3): Rats received DSS as in group 2 and also received 50 mg/kg b.wt. αTL once a day for 14 days orally.

After 24 h, on day 15, all rats were administered a light anesthetic and sacrificed by cervical dislocation. Based on previously published investigations, the dosages of αTL and DSS were chosen.[8,14,16](Figure 1)

Assessment of Colitis. Clinical signs of DSS-induced colitis were assessed during the experiment to determine the disease activity index (DAI) in accordance with an earlier reported method.[3] The body weights of the animals were recorded daily. Evident occult blood or the feces containing blood, as well as the consistency of the stools (as a marker of diarrhea), were also documented daily for each rat from days 7 to 14 of DSS treatment. All results were compared to the day 7 scores, which were set at 0. The consistency of stools was scored as follows: well-formed stool pellets, 0; pasty and semi-formed stools, 2; and liquid stools, 4. Bleeding was scored as follows: no blood, 0; fecal occult blood, 2; and gross bleeding, 4. The disease activity index (DAI) is the average score of stool consistency, body weight loss, and occult or gross bleeding.[17]

Analysis of Tissue Damage. The whole colon from each group was harvested from the rats and kept between two Whatman filter papers to keep the colon flat; later, for 48 h, every colon was treated with neutral buffered formalin (10% NBF). Subsequently, staining was performed for all colons using 0.2% methylene blue in NBF solution for 3 min, then colons were gently washed with ice-cold normal saline, and colonic damage was examined using an upright light microscope (Olympus BX51).[3]

Staining for Goblet Cell Analysis. The analysis of goblet cell staining was performed according to our earlier reported method.[18]We performed Alcian blue staining to stain goblet cells as well as acidic mucins secreted by goblet cells, and counterstaining was performed using neutral red dye.

Histology. Staining with hematoxylin and eosin (H&E) was carried out to examine the histoarchitectural alterations in the colon. H&E staining was carried out according to our earlier reported method.[19]

Measurement of Caspase Activities. The activities of caspase enzymes were investigated in the cytosolic region with the help of a commercially available kit (Apotarget kit, Cat. No. KHZ 1001, Invitrogen Corporation, CA). The kit contains substrates for caspase-3 and caspase-9, and the activities of caspases were determined as per the manufacturer's instructions.

Assay for Myeloperoxidase (MPO). The technique of Bradley et al. was used to measure the activity of MPO.[20] The activity of MPO, indicated in units, is the capacity of the enzyme to break down 1 μmol of hydrogen peroxide per minute at a temperature of 25 °C. The MPO enzyme activity is given as units/min/mg protein.

Measurement of Nitric Oxide. The released NO was measured indirectly by detecting nitrite levels (an indicator of NO) in the supernatant of the tissue with the help of the Griess reagent.[21] A microplate ELISA reader (Bio RAD) was used to determine the absorbance at 540 nm.

Mast Cell Staining. The procedure outlined by Khan et al. was used to stain mast cells.[22] Toluidine blue dye was used to stain mast cells. Toluidine blue dye metachromatically stains the granules secreted by mast cells, resulting in granular cytoplasmic staining with a strong purplish-blue hue.

Immunohistochemical (IHC) Staining for the Detection of iNOS, NF-kB-p65, Caspases-3, and -9. Immunohistochemical staining was performed according to our previously reported protocol.[23] We employed the following primary antibodies: anti-rat NF-kB-p65 rabbit antibody (dilution 1:100, Biolegend), anti-rat iNOS rabbit antibody (dilution 1:100, Neomarkers), monoclonal rabbit anti-rat cleaved caspase-3 antibody (dilution 1:400, Cell Signaling), and polyclonal rabbit anti-rat caspase-9 antibody (dilution 1:300, Neomarkers).

Fluorescent Immunohistochemistry Staining to Detect p53, Connexin-43, COX-2, Survivin, and Bcl-2.

https://doi.org/10.1021/acsomega.3c04317
ACS Omega 2023, 8, 29794−29802

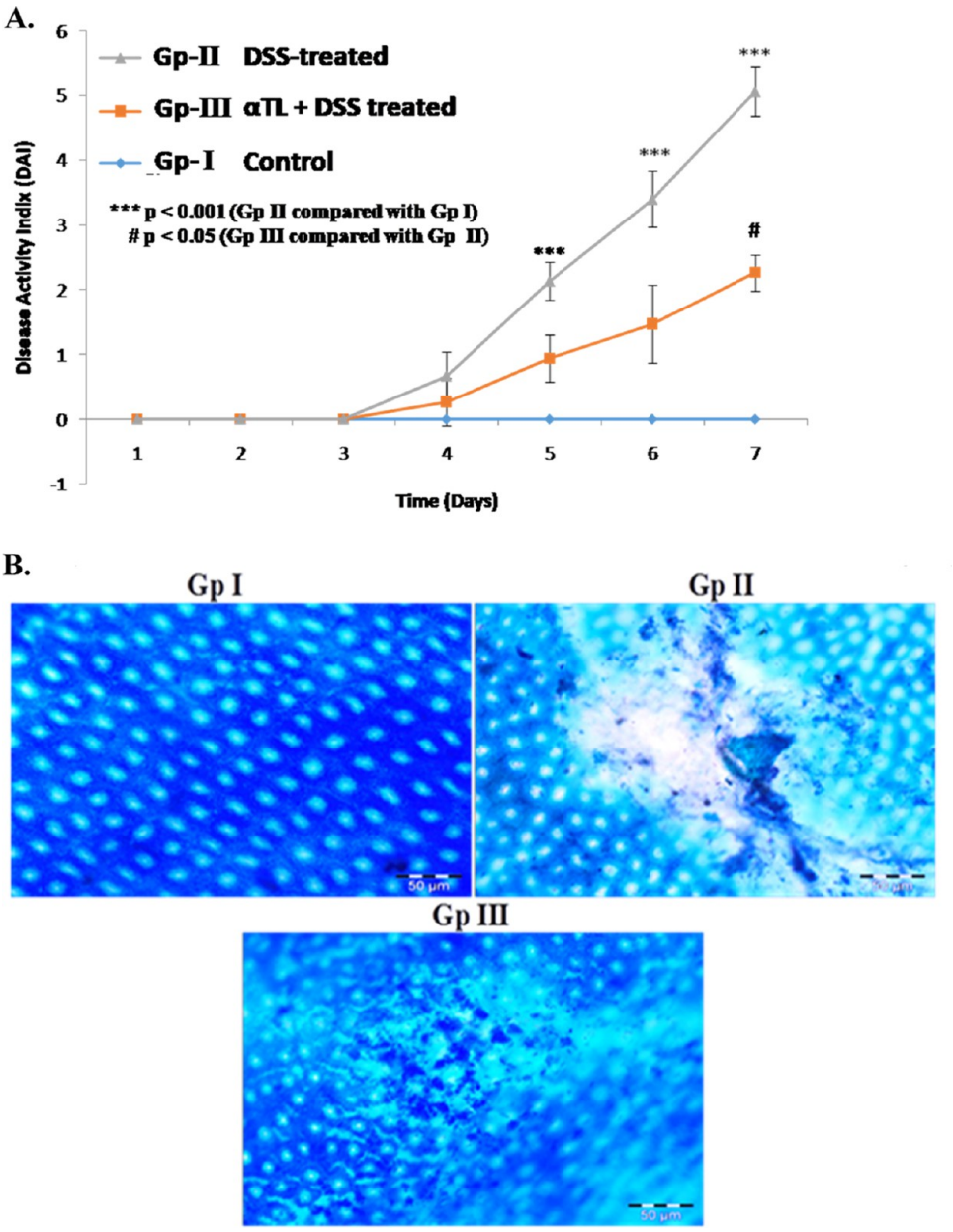


Figure 2. (A) Effects of α-terpineol (TL) and DSS on the disease activity index (DAI). Data (n = 6) are presented as mean ± SD. Significant differences between the control (Gp-I) and DSS (Gp-II) groups are shown by ***p < 0.001 and those between Gp-II and Gp-III are shown by the symbol #p < 0.05. Gp-I is the vehicle-treated healthy control group, Gp-II is the DSS-treated group, and Gp-III is the DSS + αTL-treated group. (B) Topographical view of colonic damage by methylene blue. (Gp-I) Vehicle-treated healthy control showing the normal structure of crypts. (Gp-II) DSS-treated group showing colonic damage after staining with methylene blue solution. (Gp-III) DSS + αTL-treated group showing marked reduction in colonic damage. Original magnification: 10×.

Fluorescent immunohistochemical staining was performed according to our previously reported protocol.[22] We employed the following primary antibodies: anti-rat p53 rabbit polyclonal antibody (Santa Cruz, dilution 1:100); anti-rat connexin-43 mouse monoclonal antibody (Santa Cruz, dilution 1:400); anti-rat COX-2 polyclonal antibody (Santa Cruz Biotechnology Inc., dilution 1:300); anti-rat survivin polyclonal antibody (Cell Signaling, dilution 1:300), and anti-rat Bcl-2 polyclonal antibody (Santa Cruz Biotechnology Inc., dilution 1:200). The secondary antibody was FITC-conjugated goat anti-rat antibody (Jackson ImmunoResearch Lab Inc., dilution 1:50).

Protein Estimation. Using bovine serum albumin (BSA) as a reference, the method was used to calculate the amount of protein in each sample.[24]

Statistical Assessment. The mean ± SD for all of the data from various groups is displayed. The analysis of variance (ANOVA) method was employed to examine the distinctions between various groups. In addition, the minimum threshold for statistical significance for all tests was established at p < 0.05 using the Tukey–Kramer multiple comparisons test.

RESULTS

Effects of αTL on the Disease Activity Index (DAI) and Tissue Damage. The DAI data showed that rats treated with

https://doi.org/10.1021/acsomega.3c04317
ACS Omega 2023, 8, 29794−29802

only DSS (group 2) developed significant signs of colitis as compared to rats treated with αTL + DSS (group 3) and vehicle-treated control rats (Group 1). The disease development became more florid and confluent with time, and the DAI demonstrated that signs of colitis were evident by day 7. Treatment with αTL significantly ($p < 0.05$) attenuated the DAI response on day 7 in the rats in the αTL + DSS-treated group (Group 3) compared with the only-DSS-treated group (group 2). The presence of loose stools was found on day 4 in groups 2 and 3, and watery stools were observed on day 6 in groups 2 and 3. However, the presence of occult blood was observed on day 4 in groups 2 and 3 and gross bleeding was examined on day 6 in groups 2 and 3 (Figure 2A). Treatment with DSS led to colonic damage, as visualized after staining with methylene blue solution, while pretreatment with αTL reduced DSS-induced colonic damage in Wistar rats (Figure 2B).

Colon Histoarchitecture Alterations. Colonic sections of rats in the control group displayed a normal histoarchitecture and a minor infiltration of inflammatory cells, while there was enormous infiltration of inflammatory cells, which mainly accounts for neutrophils and the abnormal glandular architecture along with crypt dilatation and ablation noted in the DSS-treated group (group 2). Mucosal edema, disruption of muscularis mucosae, and focal erosion of the colonic epithelium were also found in the DSS-treated group. Pretreatment with αTL exhibited protection against DSS-induced mucosal damage, probably by reducing inflammatory cell infiltration, which may lead to a reduction in mucosal edema, crypt ablation, and erosion of the colonic epithelium (Figure 3).

Effect of αTL against DSS-Induced Activities of Caspases. In the αTL + DSS-treated group (group 3), treatment with αTL significantly ameliorated caspase-9 ($p < 0.05$) and caspase-3 activities ($p < 0.05$) compared with the DSS-treated group (group 2) (Figure 4). The activities of these caspases were significantly increased ($p < 0.001$ for caspase-9 and $p < 0.01$ for caspase-3) in the DSS-treated group (Group 2) compared to the control group (group 1).

Effects of αTL on the DSS-Induced Myeloperoxidase (MPO) Activity and Nitric Oxide Level. The activity of myeloperoxidase enzyme was increased considerably ($p < 0.001$) in group 2 (DSS-treated group) compared to group 1 (control group); supplementing with αTL greatly ($p < 0.05$) attenuated the MPO activity in the αTL + DSS-treated group (Group 3) as compared to group 2. (Figure 5A). In the DSS-treated group (group 2), NO production was greatly ($p < 0.001$) enhanced compared to the control group (group 1), while pretreatment with αTL greatly ($p < 0.05$) attenuated the NO production in the αTL + DSS-treated group (group 3) compared to group 2, as shown in Figure 5B.

Effect of αTL against DSS-Induced Mast Cell Infiltration. In group 2, an increased number of mast cells was observed beneath the lamina propria layer of the colon. αTL treatment decreased the infiltrated mast cell number in group 3 (treatment group) compared to group 2, as shown in Figure 6.

Effects of αTL and DSS on Goblet Cells. Goblet cells were heavily reduced and disrupted in the disease group, while the control group showed intact goblet cells with normal abundance. In group 3, αTL supplementation alleviated the disruption of goblet cells compared to the disease group, as depicted in Figure 7.

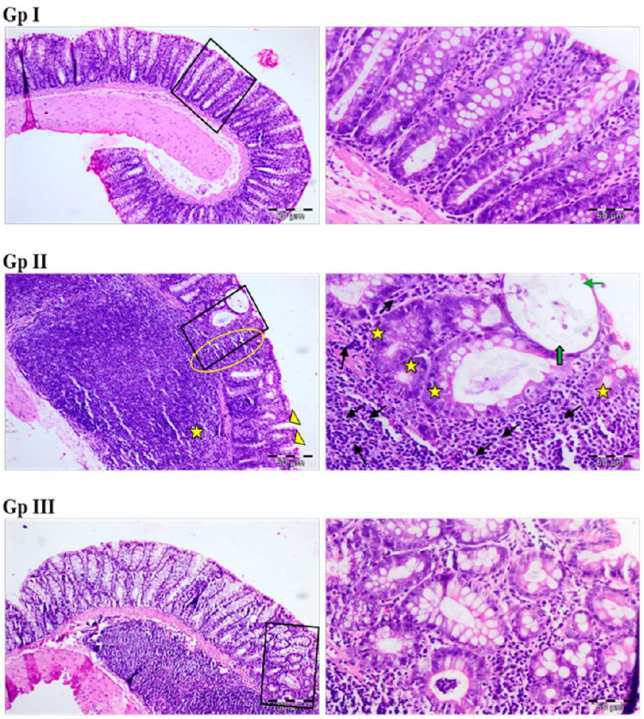


Figure 3. Effects of α-terpineol (αTL) and DSS on histopathological alterations in the colon. Microscopic images of colon sections depicting the histology of different treatment groups. The insets in the left panel with 10× magnification are shown enlarged in the right panel with 40× magnification. Yellow arrowheads indicate epithelial lining damage; black arrows and stars show inflammatory cell infiltration.

Effects of αTL and DSS on Colonic Tissue Levels of iNOS, NF-kB-p65, Cleaved Caspase-3, and Caspase-9. Immunopositive staining was carried out for the colon sections of group 2, and iNOS, NF-kB-p65, cleaved caspase-3, and caspase-9 (arrows) showed more brown color than that in group 1 (control group). Pretreatment with αTL decreased cells immunopositive for iNOS, NF-kB-p65, cleaved caspase-3, and caspase-9 in group 3 compared to group 2. In immunohistochemical analyses, iNOS-, NF-kB-p65-, cleaved caspase-3-, and caspase-9-specific immunostaining are indicated by a brown color, while hematoxylin staining is indicated by a light-blue color. The original magnification was 40× [Figure 8 (A–D)].

Effects of αTL and DSS on the Expression of Different Biomarkers. The expression of different biomarkers, such as COX-2 and p53, was increased, while levels of Bcl-2, Cx-43, and survivin expression were decreased in the disease group (group 2). In group 2, colonic sections showed enhanced COX-2 and p53 staining of immunopositive cells but reduced connexin-43, survivin, and Bcl-2 immunopositive staining as compared to the control group (group 1). In group 3, pretreatment with αTL ameliorated staining due to reduced immunopositive cells for COX-2 and p53 and increased connexin-43, survivin, and Bcl-2 compared to group 2. The particular immunostaining of COX-2, p53, connexin-43, survivin, and Bcl-2 for fluorescence immunohistochemical analyses is shown by a greenish-yellow color, and the original magnification was 40× [Figure 9 (A–E)].

https://doi.org/10.1021/acsomega.3c04317
ACS Omega 2023, 8, 29794–29802

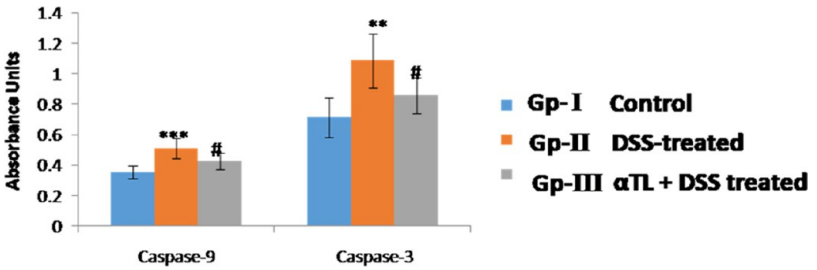


Figure 4. Effect of α-terpineol (αTL) and DSS on the caspase-3 and -9 activities. Data ($n = 6$) are presented as mean ± SD. Significant differences between Gp-I (control) and Gp-II (DSS) are shown by ***$p < 0.001$ and those between Gp-II and Gp-III are shown by the symbol #$p < 0.05$.

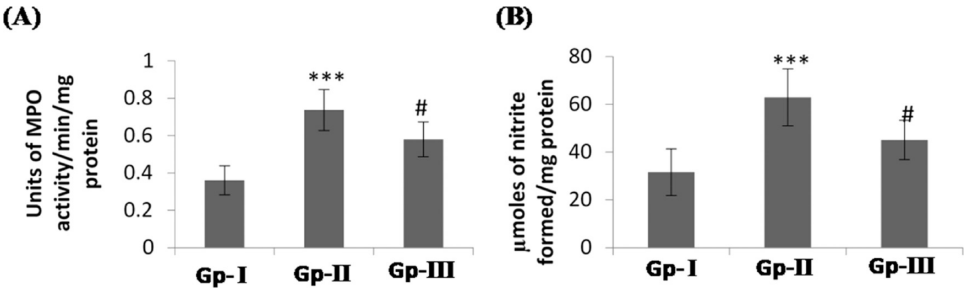


Figure 5. Graphs showing (A) MPO activity and (B) NO level. Data ($n = 6$) are presented as mean ± SD. Significant differences between Gp-I (control) and Gp-II (DSS) are shown by ***$p < 0.001$ and those between Gp-II and Gp-III are shown by the symbol #$p < 0.05$.

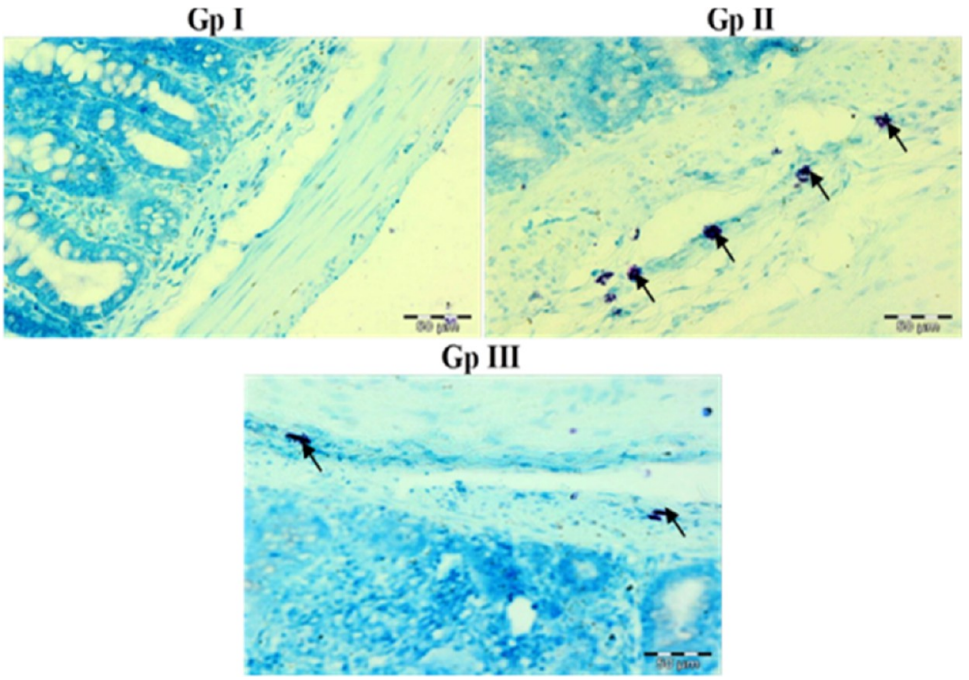


Figure 6. Effects of α-terpineol (αTL) and DSS on colon mast cell infiltration. Microscopic images of colonic sections depict mast cell granules by toluidine blue staining. Original magnification: 40×. Black arrows show mast cell infiltration.

DISCUSSION

As far as we know, this is the first report to show how α-terpineol (TL) exhibited protective effects against DSS-induced colitis in an animal model. We evaluated several parameters to observe the efficacy of αTL. In this research, we found that αTL reduced DSS-induced colonic injury as indicated by the tissue damage analysis by methylene blue staining and by histological evaluation of the colon. Histological findings revealed that the DSS-treated group showed colonic damage, which is evident by the irregular glandular structure, mucosal edema, disruption of muscularis mucosae, and focal erosion of the colonic epithelium along with crypt ablation or crypt dilatation. However, prophylactic treatment with αTL markedly alleviated DSS-induced colonic injury. Furthermore, DAI data also showed that pretreatment of αTL reduced clinical signs of colitis.

Goblet cells are special types of cells that secrete predominantly acidic mucins. These mucins are high-molecular-weight proteins. These mucins possess heavy glycosylation, forming a thick gel-like layer in the form of

https://doi.org/10.1021/acsomega.3c04317
ACS Omega 2023, 8, 29794−29802

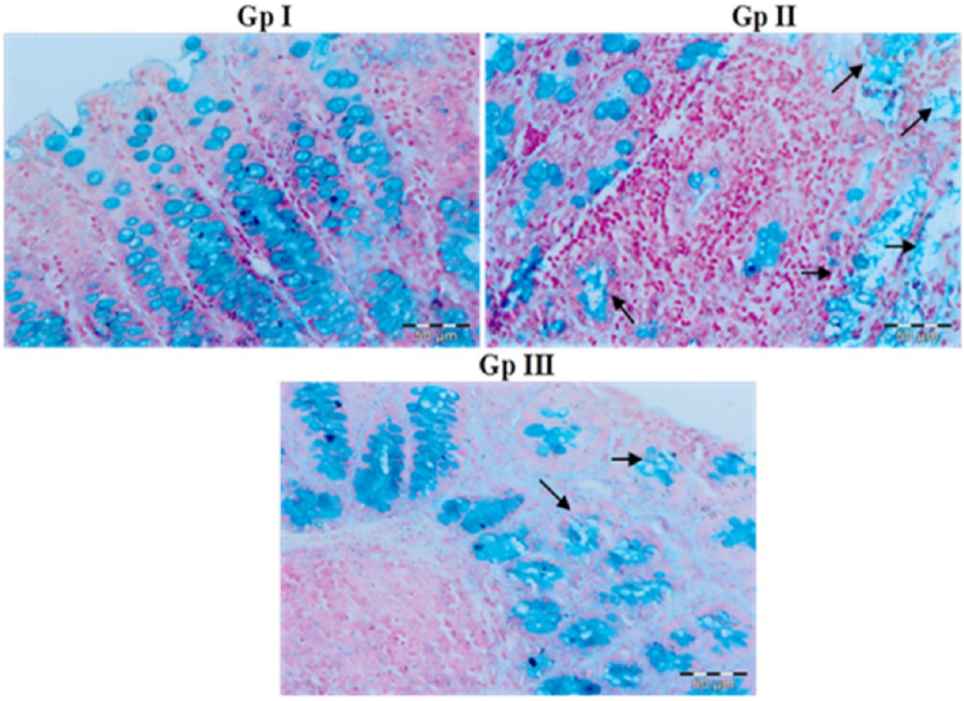


Figure 7. Effects of α-terpineol (αTL) and DSS on goblet cells. Photomicrographs of colonic sections depict goblet cells stained blue by Alcian blue and counterstained by nuclear red. Original magnification, 40×. Black arrows show goblet cell staining.

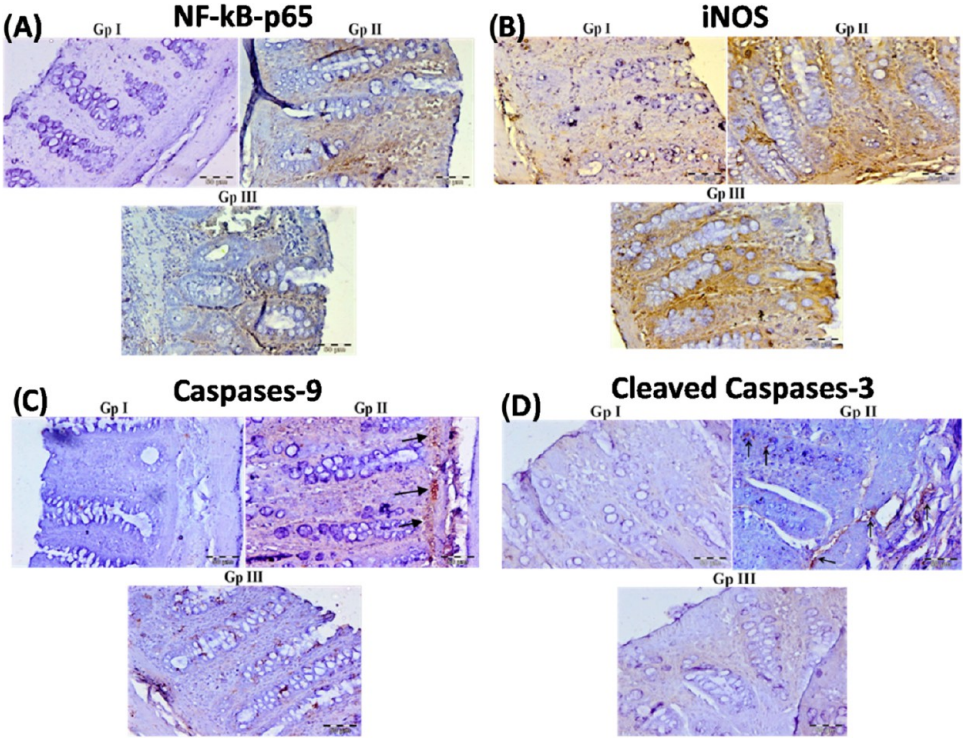


Figure 8. Immunohistochemical staining of (A) NF-kB-p65, (B) iNOS, (C) caspase-9, and (D) cleaved caspase-3. Photomicrographs show specific immunostaining for NF-kB-p65, iNOS, caspase-9, and cleaved caspase-3, as indicated by the brown color, while hematoxylin staining is indicated by a light-blue color. Black arrows indicate the immunopositive cells. Original magnification, 40×.

mucus, which acts as a protective barrier.[25,26] It has already been well-documented in various reports that DSS treatment leads to the typical loss of goblet cells, which is one of the hallmarks of DSS-induced colonic injury.[27−29] In our study, DSS administration also results in the loss or disintegration of goblet cells, and pretreatment with αTL markedly reduced goblet cell disintegration.

Massive infiltration of neutrophils is the foremost response to intestinal inflammation, which undoubtedly accounts for the increased MPO activity. MPO is an enzyme present within neutrophils, and therefore, it is an indirect marker of

https://doi.org/10.1021/acsomega.3c04317
ACS Omega 2023, 8, 29794−29802

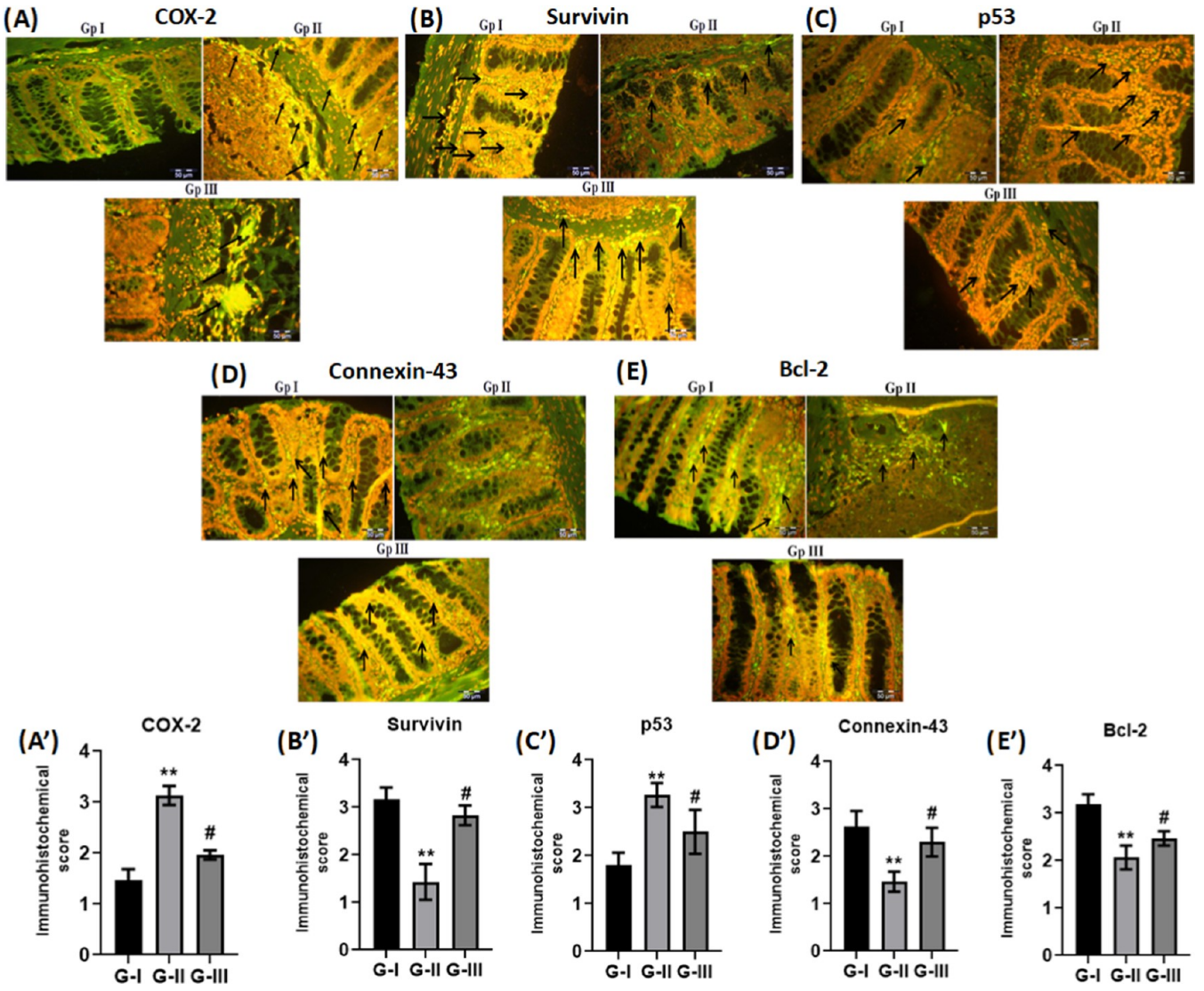


Figure 9. (A–E). Fluorescent immunohistochemical staining of (A) COX-2, (B) survivin, (C) p53, (D) connexin-43, and (E) Bcl-2. Photomicrographs of particular immunostaining of COX-2, p53, connexin-43, survivin, and Bcl-2 for fluorescence immunohistochemistry analyses shown by a greenish-yellow color and nuclear propidium iodide staining shown by a red color. Original magnification, 40×. Black arrows indicate immunopositive cells. Significant differences between Gp-I (control) and Gp-II (DSS) are shown by ***$p < 0.001$ and those between Gp-II and Gp-III are shown by the symbol #$p < 0.05$.

neutrophil infiltration.[30,31] MPO may also contribute to colonic damage through its capacity to oxidize, nitrosylate, and chlorinate proteins when oxidants and nitric oxide are produced by neutrophils.[1] Our results are in corroboration with previous findings, which exhibited increased MPO activity in DSS-induced colitis.[3,32,33] αTL supplementation alleviated DSS-induced MPO activity, which is also evident by the histological examinations showing that pretreatment with αTL reduced infiltration of inflammatory cells (∼70% population among inflammatory cells is of neutrophils). Thus, these markers support the anti-inflammatory potential of αTL.

Inflammation is caused in part by mast cells by releasing preformed TNF-α from the granules present with the mast cells.[34] In spite of the mediating inflammatory responses, TNF-α and NO also act as key signaling molecules, which lead to the activation of NF-κB, a redox-sensitive transcription factor.[35] NF-κB is reported to be upregulated in IBD, and the activation of NF-κB mediates the unchecked transcriptional activation of its downstream targets, including COX-2 and iNOS.[5,36−38] In our study, we observed that pretreatment with αTL ameliorated DSS-induced orchestration of intracolonic mast cells and also the level of TNF-α and NO. αTL administration inhibited the stimulation of NF-κB and, therefore, reduced the levels of COX-2 and iNOS. Several other studies also show that natural compounds having antioxidant potential inhibit NF-κB activation by blocking phosphorylation and thus the proteosomal degradation of IκB.[38]

Connexin-43 (Cx-43) is a type of transmembrane protein in the cells that helps to form channels between closely adhered cells. These channels are called gap junctions.[39] Cx-43 is a key connexin that plays a role in intercellular communication through gap junctions. Altered expression/levels of Cx-43 reportedly play a role in the pathogenesis of several illnesses, including IBDs.[6] p53 is a transcription factor that is responsible for the regulation of the gene transcription involved in the cell cycle and DNA damage repair.[40] Both Cx-43 and p53 regulate the process of apoptosis.[40,41] Specifically, p53 controls Bcl-2

https://doi.org/10.1021/acsomega.3c04317
ACS Omega 2023, 8, 29794−29802

function and further leads to apoptosis by stimulating the mitochondrial release of cytochrome c, which results in the activation of apoptotic protease activating factor-1 (Apaf-1) and caspase-9, subsequently activating caspases-9 and -3.[42] Survivin is a protein that inhibits apoptosis, and thus, it supports the survival of cells.[43] Survivin is generally not expressed in mature differentiated tissues, and diminished expression of survivin is a hallmark of cell death.[44] In this study, it was found that αTL treatment attenuated the DSS-induced increased expression of p53, caspase-9, and cleaved caspase-3 and decreased the expression of Cx-43, Bcl-2, and survivin. αTL treatment increased the anti-apoptotic Bcl-2 expression to alleviate colitis.

CONCLUSIONS

Overall, αTL has histoprotective effects against DSS-induced colonic damage. αTL has been shown to alleviate inflammation, as demonstrated through various inflammatory indicators. Extensive apoptosis of colon epithelial cells resulted in tissue deterioration. We observed that pretreatment with αTL also significantly counters the apoptotic response exerted by DSS. Anti-apoptotic biomarkers such as Bcl-2 could play a major role in the pathophysiology of ulcerative colitis and could be important markers to target the treatment for ulcerative colitis. The exact mechanism of the histoprotective effects of αTL against DSS-induced colonic injury is still unclear, but the most likely mechanistic action might be a reduced inflammatory and apoptotic response. To further understand the protective mechanism of αTL, more research is required.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c04317.

Details of chemicals used and animal maintenance and ethical statement (PDF)

AUTHOR INFORMATION

Corresponding Author

Rehan Khan − *Chemical Biology Unit, Institute of Nano Science and Technology, Mohali 140306 Punjab, India;* orcid.org/0000-0002-2406-1129; Phone: +91-172-2210075; Email: rehankhan@inst.ac.in

Authors

Chandrashekhar Jori − *Chemical Biology Unit, Institute of Nano Science and Technology, Mohali 140306 Punjab, India*

Md. Meraj Ansari − *Centre for Pharmaceutical Nanotechnology, Department of Pharmaceutics, National Institute of Pharmaceutical Education and Research, Mohali 160062 Punjab, India;* orcid.org/0000-0001-5562-2892

Anas Ahmad − *Julia McFarlane Diabetes Research Centre (JMDRC) and Department of Microbiology, Immunology and Infectious Diseases, Snyder Institute for Chronic Diseases and Hotchkiss Brain Institute, Cumming School of Medicine, University of Calgary, Calgary, Alberta T2N 4N1, Canada;* orcid.org/0000-0002-1105-7526

Ahmed Nadeem − *Department of Pharmacology and Toxicology, College of Pharmacy, King Saud University, Riyadh 11451, Saudi Arabia*

Nahid Siddiqui − *Amity Institute of Biotechnology, Amity University, Noida 201303, India*

Sarwat Sultana − *Department of Medical Elementology and Toxicology, Jamia Hamdard, New Delhi 110062, India*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.3c04317

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The authors acknowledge and extend their appreciation to the Researchers Supporting Project Number (RSP2023R124), King Saud University, Riyadh, Saudi Arabia.

REFERENCES

(1) Stucchi, A. F.; Shofer, S.; Leeman, S.; et al. NK-1 antagonist reduces colonic inflammation and oxidative stress in dextran sulfate-induced colitis in rats. *Am. J. Physiol.: Gastrointest. Liver Physiol.* **2000**, *279*, G1298−G1306.

(2) Li, J. H.; Yu, J. P.; Yu, H. G.; et al. Expression and significance of nuclear factor κB p65 in colon tissues of rats with TNBS-induced colitis. *World J. Gastroenterol.* **2005**, *11*, 1759−1763.

(3) Qiu, W.; Wu, B.; Wang, X.; et al. PUMA-mediated intestinal epithelial apoptosis contributes to ulcerative colitis in humans and mice. *J. Clin. Invest.* **2011**, *121*, 1722−1732.

(4) Orlowski, R. Z.; Baldwin, A. S., Jr. NF-kappaB as a therapeutic target in cancer. *Trends Mol. Med.* **2002**, *8*, 385−389.

(5) Mantovani, A.; Allavena, P.; Sica, A.; Balkwill, F. Cancer-related inflammation. *Nature* **2008**, *454*, 436−444.

(6) Ey, B.; Eyking, A.; Gerken, G.; et al. TLR2 mediates gap junctional intercellular communication through connexin-43 in intestinal epithelial barrier injury. *J. Biol. Chem.* **2009**, *284*, 22332−22343.

(7) Bak, Y. K.; Lampe, J. W.; Sung, M. K. Dietary supplementation of glucosamine sulfate attenuates intestinal inflammation in a mouse model of experimental colitis. *J. Gastroenterol. Hepatol.* **2007**, *22*, 1859−1865.

(8) Sabino, C. K.; Fereira-Filho, E. S.; Mendes, M.; da Silva-Filho, J. C.; Ponte, M.; Moura, L.; Oliveira, E.; Quintans-Junior, L. J.; dos Santos, M.; Oliveira, R.; et al. Cardiovascular effects induced by α-terpineol in hypertensive rats. *Flavour Fragrance J.* **2013**, *28*, 333−339.

(9) Choi, Y. J.; Sim, C.; Choi, H. K.; Lee, S. H.; Lee, B. H. α-Terpineol induces fatty liver in mice mediated by the AMP-activated kinase and sterol response element binding protein pathway. *Food Chem. Toxicol.* **2013**, *55*, 129−136.

(10) Ishaque, A.; Salim, A.; Simjee, S. U.; Khan, I.; Adli, D.S.H. Alpha terpineol directs bone marrow mesenchymal stem cells toward neuronal lineage through regulation of wnt signaling pathway. *Cell Biochem. Funct.* **2023**, *41*, 223−233.

(11) Hassan, S. B.; Gali-Muhtasib, H.; Göransson, H.; Larsson, R. Alpha terpineol: a potential anticancer agent which acts through suppressing NF-kappaB signalling. *Anticancer Res.* **2010**, *30*, 1911−1919.

(12) Held, S.; Schieberle, P.; Somoza, V. Characterization of alphaTerpineol as an anti-inflammatory component of orange juice by in vitro studies using oral buccal cells. *J. Agric. Food Chem.* **2007**, *55*, 8040−8046.

(13) de Oliveira, M. G.; Marques, R. B.; de Santana, M. F.; Santos, A. B.; Brito, F. A.; Barreto, E. O.; De Sousa, D. P.; Almeida, F. R.; Badauê-Passos, D., Jr; Antoniolli, A. R.; Quintans-Júnior, L. J. α-terpineol reduces mechanical hypernociception and inflammatory response. *Basic Clin. Pharmacol. Toxicol.* **2012**, *111*, 120−125.

(14) Souza, R. H.; Cardoso, M. S.; Menezes, C. T.; Silva, J. P.; De Sousa, D. P.; Batista, J. S. Gastroprotective activity of α-terpineol in two experimental models of gastric ulcer in rats, DARU. *J. Pharm. Sci.* **2011**, *19*, 277−281.

(15) Matsunaga, T.; Hasegawa, C.; Kawasuji, T.; Suzuki, H.; Saito, H.; et al. Isolation of the antiulcer compound in essential oil from the leaves of Cryptomeria japonica. *Biol. Pharm. Bull.* **2000**, *23*, 595−598.
(16) Diaz-Granados, N.; Howe, K.; Lu, J.; McKay, D. M. Dextran sulfate sodiuminduced colonic histopathology, but not altered epithelial ion transport, is reduced by inhibition of phosphodiesterase activity. *Am. J. Pathol.* **2000**, *156*, 2169−2177.
(17) Cooper, H. S.; Murthy, S. N.; Shah, R. S.; Sedergran, D. J. Clinicopathologic study of dextran sulfate sodium experimental murine colitis. *Lab. Invest.* **1993**, *69*, 238−249.
(18) Khan, R.; Khan, A. Q.; Qamar, W.; et al. Chrysin abrogates cisplatin-induced oxidative stress, p53 expression, goblet cell disintegration and apoptotic responses in the jejunum of Wistar rats. *Br. J. Nutr.* **2012**, *108*, 1574−1585.
(19) Khan, R.; Sultana, S. Farnesol attenuates 1,2-dimethylhydrazine induced oxidative stress, inflammation and apoptotic responses in the colon of Wistar rats. *Chem. Biol. Interact.* **2011**, *192*, 193−200.
(20) Bradley, P. P.; Priebat, D. A.; Christensen, R. D.; Rothstein, G. Measurement of cutaneous inflammation: estimation of neutrophil content with an enzyme marker. *J. Invest. Dermatol.* **1982**, *78*, 206−209.
(21) Green, L. C.; Wagner, D. A.; Glogowski, J.; et al. Analysis of nitrate, nitrite, and [15N] nitrate in biological fluids. *Anal. Biochem.* **1982**, *126*, 131−138.
(22) Khan, R.; Khan, A. Q.; Lateef, A.; et al. Glycyrrhizic Acid Suppresses the Development of Precancerous Lesions via Regulating the Hyperproliferation, Inflammation, Angiogenesis and Apoptosis in the Colon of Wistar Rats. *PLoS One* **2013**, *8*, No. e56020.
(23) Khan, R.; Khan, A. Q.; Qamar, W.; et al. Chrysin protects against cisplatin-induced colon toxicity via amelioration of oxidative stress and apoptosis: probable role of p38MAPK and p53. *Toxicol. Appl. Pharmacol.* **2012**, *258*, 315−329.
(24) Lowry, O. H.; Rosebrough, N. J.; Farr, A.; Randall, R. J. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **1951**, *193*, 265−275.
(25) Robbe, C.; Capon, C.; Coddeville, B.; Michalski, J. C. Structural diversity and specific distribution of O-glycans in normal human mucins along the intestinal tract. *Biochem. J.* **2004**, *384*, 307−316.
(26) Specian, R. D.; Oliver, M. G. Functional biology of intestinal goblet cells. *Am. J. Physiol.* **1991**, *260*, C183−C193.
(27) Camacho-Barquero, L.; Villegas, I.; Sánchez-Calvo, J. M.; et al. Curcumin, a Curcuma longa constituent, acts on MAPK p38 pathway modulating COX-2 and iNOS expression in chronic experimental colitis. *Int. Immunopharmacol.* **2007**, *7*, 333−342.
(28) Jin, X.; Malykhina, A. P.; Lupu, F.; Akbarali, H. I. Altered gene expression and increased bursting activity of colonic smooth muscle ATP-sensitive K+ channels inexperimental colitis. *Am. J. Physiol.: Gastrointest. Liver Physiol.* **2004**, *287*, G274−G285.
(29) Gaudio, E.; Taddei, G.; Vetuschi, A.; et al. Dextran sulfate sodium (DSS) colitis in rats: clinical, structural, and ultrastructural aspects. *Dig. Dis. Sci.* **1999**, *44*, 1458−1475.
(30) Barbara, G.; Xing, Z.; Hogaboam, C. M.; et al. Interleukin 10 gene transfer prevents experimental colitis in rats. *Gut* **2000**, *46*, 344−349.
(31) Massa, F.; Marsicano, G.; Hermann, H.; et al. The endogenous cannabinoid system protects against colonic inflammation. *J. Clin. Invest.* **2004**, *113*, 1202−1209.
(32) Blackburn, A. C.; Doe, W. F.; Buffinton, G. D. Colonic antioxidant status in dextran sulfate-induced colitis in mice. *Inflammatory Bowel Dis.* **1997**, *3*, 198−203.
(33) Camuesco, D.; Ivez, J. G.; Nieto, A.; et al. Dietary olive oil supplemented with fish oil, rich in EPA and DHA (n-3) polyunsaturated fatty acids, attenuates colonic inflammation in rats with DSS-induced colitis. *J. Nutr.* **2005**, *135*, 687−694.
(34) Galli, S. J.; Nakae, S.; Tsai, M. (2005) Mast cells in the development of adaptiveimmune responses. *Nat Immunol* **2005**, *6*, 135−142.
(35) Kundu, J. K.; Surh, Y. J. Inflammation: gearing the journey to cancer. *Mutat. Res., Rev. Mutat. Res.* **2008**, *659*, 15−30.
(36) Rogler, G.; Brand, K.; Vogl, D.; et al. Nuclear factor kB is activated in macrophages and epithelial cells of inflamed intestinal mucosa. *Gastroenterology* **1998**, *115*, 357−369.
(37) Karin, M.; Greten, F. R. NF-kappaB: linking inflammation and immunity to cancer development and progression. *Nat. Rev. Immunol.* **2005**, *5*, 749−759.
(38) Surh, Y. J.; Chun, K. S.; Cha, H. H.; et al. Molecular mechanisms underlying chemopreventive activities of anti-inflammatory phytochemicals: down-regulation of COX-2 and iNOS through suppression of NF-κB activation. *Mutat. Res., Fundam. Mol. Mech. Mutagen.* **2001**, *480*, 243−268.
(39) Sáez, J. C.; Berthoud, V. M.; Branes, M. C.; et al. Plasma membrane channels formed by connexins: their regulation and functions. *Physiol. Rev.* **2003**, *83*, 1359−1400.
(40) Riley, T.; Sontag, E.; Chen, P.; Levine, A. Transcriptional control of human p53- regulated genes. *Nat. Rev. Mol. Cell Biol.* **2008**, *9*, 402−412.
(41) Sirnes, S.; Bruun, J.; Kolberg, M.; et al. Connexin43 acts as a colorectal cancer tumor suppressor and predicts disease outcome. *Int. J. Cancer* **2012**, *131*, 570−581.
(42) Miyashita, T.; Krajewski, S.; Krajewska, M.; et al. Tumor suppressor p53 is a regulator of bcl-2 and bax gene expression in vitro and in vivo. *Oncogene* **1994**, *9*, 1799−1805.
(43) Tamm, I.; Wang, Y.; Sausville, E.; et al. IAP-family protein survivin inhibits caspase activity and apoptosis induced by Fas (CD95), Bax, caspases and anticancer drugs. *Cancer Res.* **1998**, *58*, 5315−5320.
(44) Ambrosini, G.; Adida, C.; Altieri, D. C. A novel anti-apoptosis gene, survivin, expressed in cancer and lymphoma. *Nat. Med.* **1997**, *3*, 917−921.

https://doi.org/10.1021/acsomega.3c04317
ACS Omega 2023, 8, 29794−29802