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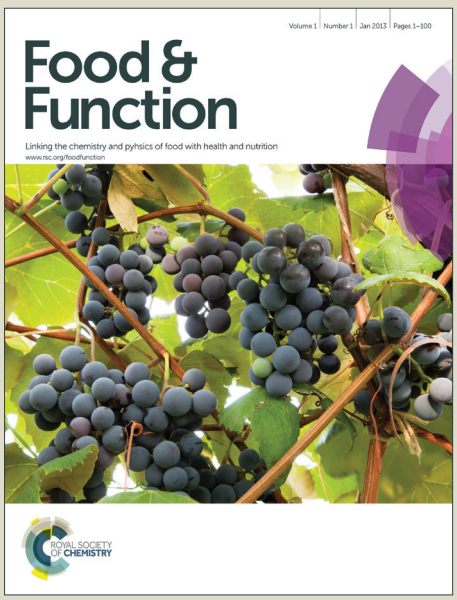

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Oral administration of geraniol ameliorates acute experimental murine colitis by inhibiting pro-inflammatory cytokines and NF-κB signaling

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Abstract

Ulcerative colitis is associated with a considerable reduction in the quality of life of the patients. The use of phyto-ingredients is becoming an increasingly attractive approach for the management of colitis. Geraniol, a monoterpene with anti-inflammatory and antioxidative properties. In this study, we investigated the therapeutic potential of geraniol as complementary and alternative medicine against dextran sulphate sodium (DSS)-induced ulcerative colitis in mice. Disease activity indices (DAI) comprising body weight loss, presence of occult blood and stool consistency were assessed for evaluation of colitis symptoms. Intestinal damage was assessed by evaluating colon length and its histology. Pre-treatment with geraniol significantly reduced DAI score, improved stool consistency (without occult blood) and increased the colon length. The amount of pro-inflammatory cytokines, specifically TNF-α, IL-1β and IL-6 and the activity of myeloperoxidase in colon tissue were significantly decreased in geraniol pre-treated mice. Western blot analyses revealed that geraniol interfered NF-κB signaling by inhibiting NF-κB (p65)-DNA binding, IκBα phosphorylation and its degradation and subsequent increase in nuclear translocation. Moreover, the expression of downstream target pro-inflammatory enzymes such as iNOS and COX-2 were significantly reduced by geraniol. Pre-treatment with geraniol also restored the DSS-induced decline in antioxidant parameters such as reduced glutathione and superoxide dismutase activity and attenuated the increase in lipid peroxidation marker, thiobarbituric acid reactive substances and nitrative stress marker, nitrites in colon tissue. Thus, our results suggest that geraniol is a potential therapeutic agent for inflammatory bowel disease.

Keywords: Geraniol, Murine colitis, Pro-inflammatory cytokines, Myeloperoxidase, Nuclear factor-kappa B, Antioxidants

Introduction

Inflammatory bowel disease (IBD) is a chronic relapsing and destructive inflammatory disorder of the gastrointestinal tract (GIT), with an annual cost of more than $1.7 billion in the United States.[1, 2] Approximately 1 in 150 people is affected with IBD in westernized nations and incidence is dramatically increasing in developing countries.[3] Ulcerative colitis and Crohn's disease are the two forms of IBD. Out of them, ulcerative colitis is the major form of IBD and mainly affecting the colonic mucosa and submucosa.[4] A recent year of evidence suggest that the incidence of occurrence of colorectal cancer is up to 30% of affected patients after 35 years of ulcerative colitis.[5] The etiology of ulcerative colitis is complex and remains unclear. Based on the literature related to IBD, the dysregulation of mucosal immune response, infiltration and activation of macrophages, activation of MAPKs and NF-κB signaling pathways and induction of proinflammatory cytokine expressions such as TNF-α, IL-1β and IL-6 in the colon tissue are thought to play an essential role in the pathogenesis of IBD.[6] In addition, the upregulation of certain proteins, i.e., cyclooxygenase (COX)-2 or inducible nitric oxide synthase (iNOS) supporting a critical role in the pathogenesis of ulcerative colitis.[4] Despite significant progress in the management of the disease and the currently available treatment options for ulcerative colitis such as corticosteroids, immunosuppressants and monoclonal antibody–based therapies against TNF-α, there is still no ideal medical therapies are available that may completely cure the disease.[7, 8] Therefore, alternative strategies are warranted for developing new therapeutics against ulcerative colitis.

The present available literature and the epidemiological evidence clearly shows that the use of herbal product as complementary medication is beneficial for the treatment of several gastrointestinal diseases including IBD.[8, 9, 10] Geraniol (3, 7-dimethyl-2, 6 octadien-1-ol) is an acyclic monoterpene alcohol approved by the United States Food and Drug

Administration (USFDA) as a food additive to flavour, beverages, candies and ice creams.[11] It is mainly found in the essential oils of lemon grass and citrus fruits, ginger, lemon, lime, orange, nutmeg, rose, palmarosa, citronella, lavender, coriander and in the oils of aromatic herbs.[11, 12] A number of reports reveal that geraniol possess multiple pharmacological activities, including anti-inflammatory, anti-microbial, anti-oxidant, antitumor, anti-lithic, neuroprotective, expectorant and gastroprotective properties.[11, 13, 14] It has also been reported that geraniol inhibits lipopolysaccharide-induced nitric oxide (NO) and prostaglandin E_2 (PGE_2) production, iNOS and COX2 expression and activation of NF-κB signaling pathway in murine macrophage RAW 264.7 cells.[15] However, the therapeutic potential of geraniol for other inflammation-associated disorders, such as ulcerative colitis has not been investigated. Thus, we hypothesized that geraniol may block NF-κB signaling pathway and iNOS and COX2 expressions and thereby attenuate experimental Dextran Sulphate Sodium (DSS)-induced ulcerative colitis in mice.

Materials and methods

Chemicals, kits and antibodies

Geraniol (purity: ~97%) was obtained from Alfa Aesar, Heysham, Lancashire LA3 2XY, United Kingdom. Dextran Sulphate Sodium (DSS) (Molecular weight: 36,000-50,000) was purchased from MP Biomedicals, USA. Reduced glutathione, 5, 5'-dithio-bis (2-nitrobenzoic acid) (DTNB), 2-thiobarbituric acid (TBA), superoxide dismutase (SOD) assay kit, o-dianisidine were obtained from Sigma-Aldrich Co, St Louis, MO, USA. Sulphanilamide was purchased from Loba chemie Pvt Ltd. Mumbai and N-(1-naphthyl)-ethylenediamine dichloride (NED) was obtained from S.D Fine Chem Ltd. Mumbai, India. Polyvinylidine difluoride membranes (PVDF), Radioimmunoprecipitation (RIPA) buffer, Halt protease inhibitor cocktail, NE-PER nuclear and cytoplasmic extraction kit and Bicinchoninic acid (BCA) protein assay kits were obtained from Pierce Biotechnology, Rockford, IL, USA. NF-

κB (p65) transcription factor ELISA assay kit was obtained from Cayman Chemical Company, Ann Arbor, MI. Mouse TNF-α, IL-1β and IL-6 ELISA (Ready-SET-Go) kits were obtained from eBioscience, USA. Antibodies against NF-κB (p^{65}), p-IκBα, IκBα, COX2, β-actin, Lamin B and horseradish peroxidase (HRP)-conjugated secondary antibody were purchased from Cell Signaling Technology (Boston, MA). Antibody against iNOS was obtained from Sigma-Aldrich Co, St Louis, MO, USA. All other chemicals were of analytical grade.

Animals

The study protocol concerning animal usage was approved by the Institutional Animal Ethics Committee (IAEC) of the CSIR-Indian Institute of Chemical Technology (IICT) (Permit No-IICT/PHARM/SRK/281/14/31). Male Balb/C mice (weighing 25-28g, 7-8 weeks old) were obtained from National Institute of Nutrition, Hyderabad, India and were housed in BIOSAFE animal quarantine facility of the institute (Registration No: 97/1999/CPCSEA). They were maintained under standard laboratory conditions of temperature 22±2°C, relative humidity 50±15% and 12:12 h light: dark cycle throughout the study period. All the experimental animals were given free access to food and water *ad libitum* throughout the study period. Animals were allowed to acclimatise in the laboratory conditions and maintained under quarantine for a period of 7 days. All experiments were carried out in line with the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines, provided by the Government of India for the care and safe use of laboratory animals (http://cpcsea.nic.in).

Induction of ulcerative colitis and assessment of DAI

The published protocol of disease induction developed by Dou et al. [16] was followed to induce ulcerative colitis in mice. Dextran sulphate sodium (DSS) at a concentration of 4% (w/v) was dissolved in the drinking water for animals and treatment was continued for 5 days.

The DSS solution was replaced daily with fresh solution. For comparison purpose, the vehicle control group was maintained on drinking water (without DSS) throughout the duration of the experiment. Animals were examined daily from day 0 to 5 for body weight change and disease activity index (DAI) as described previously.[17] Disease Activity Index (DAI) was determined by the sum of scores given for: body weight loss (scored as: 0, none; 1, 1–5%; 2, 5–10%; 3, 10–20%; 4, over 20%), presence or absence of fecal blood (scored as: 0, negative hemoccult test; 2, positive hemoccult test; 4, gross bleeding) and stool consistency (scored as: 0, well-formed pellets; 2, loose stools; 4, diarrhoea). At the end of 5 days (i.e. on 6^{th} day of DSS administration), the animals were euthanized under CO_2 asphyxiation and the colon tissue was dissected. Colons were measured for length and weight (without stool) and were also examined for gross macroscopic appearance and the consistency of the stool found within of tissue.

Experimental design

Mice were randomized into four groups consisting 6 animals in each. Group 1, vehicle control, animals were administered with normal saline orally (10 ml/kg BW) once per day for 12 consecutive days; Group 2, DSS control, animals were administered with normal saline orally (10 ml/kg BW) once per day for 12 consecutive days and 4% (w/v) DSS (molecular weight 36 000–50 000 Da; MP Biomedicals) in drinking-water from days 8 to 12; Group 3, DSS+GE-50, animals were administered with geraniol at the dose of 50 mg/kg body weight mixed in normal saline orally (10 ml/kg BW) once per day for 12 consecutive days and 4% (w/v) DSS in drinking-water from days 8 to 12; Group 4, DSS+GE-100, animals were administered with geraniol at the dose of 100 mg/kg body weight mixed in normal saline orally (10 ml/kg BW) once per day for 12 consecutive days and 4% (w/v) DSS in drinking-water from days 8 to 12. The dose of the geraniol was selected based on the previous literature.[14, 18] In each time geraniol was mixed in normal saline and vortexed vigorously

before administration to animals through oral route. In the present study, we have not included the geraniol alone treated group (Geraniol control) as the geraniol was reported to be safe at 100mg/kg [12] and the constraint on the use of the animals to prove a known and reported phenomenon posed some restriction in our study design in the submission of ethical approval.

At the end of study (i.e on 12th day of study or 5th day of DSS administration), the presence of diarrhoea, rectal bleeding and weight loss were recorded and the animals were euthanized under CO_2 asphyxiation. The colon tissue was dissected, length and weight (without stool) of colon were recorded and also examined for gross macroscopic appearance and the consistency of the stool found within of tissue.

Determination of pro-inflammatory cytokines (TNF-α, IL-1β and IL-6)

Colon tissue of around 60 mg from each animal was weighed, minced and a 10% tissue homogenate was prepared in ice-cold phosphate buffer saline (PBS, pH 7.4) containing 1% Halt protease inhibitor cocktail. The homogenate was centrifuged at 4000rpm for 15min at 4°C and the supernatant obtained was used for the estimation of TNF-α, IL-1β and IL-6 using mouse specific TNF-α, IL-1β and IL-6 ELISA kits (eBioscience, USA) respectively, as per manufacturer instructions. The total protein content in the supernatant was estimated using BCA protein assay kit (Pierce Biotechnology, Rockford, IL, USA) against bovine serum albumin (BSA) as standard. The concentrations of all cytokines were expressed as pg/mg protein.

Determination of myeloperoxidase (MPO) activity

Myeloperoxidase activity, an enzymatic marker generally used to quantify the extent of inflammatory cell infiltration in whole-tissue colon, was determined as previously described.[19] Briefly, the frozen colon tissue samples were homogenized in ice-cold 50mM

potassium phosphate buffer (pH 6) with 0.5% hexadecyltrimethylammonium bromide (HTAB) and 10mM EDTA. The tissue homogenates were then subjected to freeze and thaw at least two cycles. The samples were then centrifuged for 20 min at 13,100g at 4°C. The supernatant obtained was separated and an aliquot of the supernatant (0.1 ml) was mixed with 2.9 ml of 50 mM phosphate buffer (pH 6.0) containing 0.167 mg of o-dianisidine and 0.0005% hydrogen peroxide. The change in absorbance was measured at 460 nm. The activity of MPO was expressed as U/g of tissue.

Measurement of nitrite levels

The accumulation of nitrite in the colon tissue, as an indicator of the production of nitric oxide was determined by a colorimetric assay with Greiss reagent (0.1% N-(1-napththyl) ethylene diamine dihydrochloride, 1% sulphanilamide and 5% phosphoric acid).[20] Sodium nitrite was used for the preparation of standard curve and the concentration of nitrite in the colon tissue homogenate was determined from sodium nitrite standard curve.

Measurement of reduced glutathione content

The reduced glutathione (GSH) content in colon tissue was estimated by using Ellman's reagent ((5, 5'-dithio-bis (2-nitrobenzoic acid)) according to the method described by Ellman[21] and was expressed as µg/g tissue.

Measurement of superoxide dismutase activity

Colonic SOD activity was determined using superoxide dismutase assay kit (Sigma–Aldrich Co., St. Louis, MO, USA) according to the manufacturer specifications.

Measurement of lipid peroxidation

Thiobarbituric acid reactive substances (TBARS), as a measure of lipid peroxidation were determined spectrophotometrically at 532nm by heating the colon tissue homogenate

containing 10% trichloroacetic acid (TCA) with 0.67% (w/v) 2-thiobarbituric acid (Sigma-Aldrich) as described previously.[22]

Histopathology

For light microscopic examination of colon tissues, immediately after the sacrifice of animals, colon tissue was dissected and fixed in 10% buffered formalin for 48h. Then, the colon tissue samples were processed and embedded in paraffin. Sections were cut at 5 μm thicknesses on a rotary microtome, mounted and stained with hematoxylin and eosin (H and E). These sections were evaluated for histological changes under light microscopy (Zeiss microscope, Axioplan 2 Imaging, Axiovision software).

Western blot analyses

Frozen colon tissue (distal part) from each experimental group was minced and homogenized in ice-cold radioimmunoprecipitation (RIPA) buffer (Pierce Biotechnology, Rockford, IL, USA) containing 1% Halt protease inhibitor to prepare whole protein extract as per manufacturer instruction. Similarly, NE-PER nuclear and cytoplasmic extraction kits (Pierce Biotechnology, Rockford, IL, USA) was used to prepare nuclear fraction. The protein concentration in homogenates was determined using Bicinchoninic acid (BCA) protein assay kits (Pierce Biotechnology, Rockford, IL, USA). Western blot analysis was performed as previously described method.[23] In nuclear and whole protein extracts, the amount of NF-κB (p65) (rabbit, monoclonal, Cell Signaling Technology; diluted to 1:500) and in whole protein extracts, the amount of p-IκBα (rabbit, monoclonal, Cell Signaling Technology; diluted to 1:1000), IκBα (mouse, monoclonal, Cell Signaling Technology; diluted to 1:1000), COX-2 (rabbit, monoclonal, Cell Signaling Technology; diluted to 1:1000), iNOS (rabbit, monoclonal, Sigma-Aldrich; diluted to 1:500) were determined. β-actin and Lamin B (rabbit, monoclonal, Cell Signaling Technology; diluted to 1:1000) were used for internal control for equal loading of whole and nuclear protein extracts respectively. The membranes were

incubated with horseradish peroxidase (HRP)-conjugated secondary antibody (diluted to 1:4000) for 1 h and then visualized by enhanced chemiluminescence reagent (Supersignal West Pico, Pierce Biotechnology, Rockford, IL, USA) and Vilber-Fusion-Western blot-Chemiluminescence Imaging system. The densitometry analysis of each blot was performed by employing Image J software, NIH, USA.

NF-κB (p65) transcription assay

A 96-well enzyme-linked immunosorbent assay (ELISA) based NF-κB (p65) transcription assay was carried out to detect DNA binding activity of NF-κB (p65) in nuclear extracts of colon tissue as per manufacturer instructions (Cayman Chemical Company, Ann Arbor, MI).

Statistical analysis

Data are expressed as the means± S.E.M. Statistical significance was analysed using a one-way ANOVA analysis (Graph Pad Prism software (version 5.0)) followed by Dunnett's multiple comparison procedure. Statistical significance was considered when the p value was less than 0.05.

Results

Effect of geraniol on DSS-induced DAI, body weight and colon length change

Administration of 4% (w/v) DSS for five consecutive days produced a reproducible colitis, which is indicated by a significant decrease in body weight, loose stool and rectal bleeding when compared with vehicle control mice (Fig. 1A). In DSS-induced mice, the DAI increased rapidly, reaching 3.27 ± 0.13 after 5 days of DSS administration (i.e. on 12^{th} day of the study). Treatment with geraniol at both the doses (50 and 100 mg/kg body weight) markedly (DAI score, 2.66 ± 0.12 for 50mg/kg geraniol and 1.6 ± 0.17 for 100mg/kg geraniol on 12^{th} day of study) reduced the DAI score when compared with DSS alone treated mice. Similarly, the body weight (Fig. 1B) and the colon length (Fig. 1C) were significantly ($p<$

0.001 for body weight and $p< 0.01$ for colon length) decreased in DSS-induced mice when compared with vehicle control mice. Treatment with geraniol along with DSS at the dose of 100 mg/kg body weight significantly ($p< 0.001$) prevented the decline in body weight and the colon length shortening when compared with the DSS only treated mice. Though geraniol at 50 mg/kg significantly ($p< 0.001$) attenuated the colon length decrease, treatment with geraniol at 50 mg/kg did not produce any significant ($p> 0.05$) change in the body weight when compared with the DSS only induced mice.

Effect of geraniol and/or DSS on total protein content in colon

The group of animals treated with DSS alone showed a significant ($p< 0.01$) decrease in total protein content in colonic tissue when compared with the vehicle control mice. Treatment with geraniol at both of the doses (50 and 100 mg/kg body weight) significantly ($p< 0.01$ at 50 mg/kg and $p< 0.05$ at 100 mg/kg) attenuated the decline in total protein content when compared with the DSS alone treated mice (Fig. 1D).

Effect of geraniol on DSS-induced changes in colon histology

Fig. 2 depicts the morphology and size of colons from control and experimental animals. Hematoxylin and eosin (H and E) staining and light microscopic examination (Fig. 3) of colon tissue sections from vehicle control mice (3A) revealed intact colon structure. Tissue sections from DSS-induced mice (3B) revealed disruption of epithelial lining, marked infiltration of inflammatory cells and a decrease in the number of crypts in comparison of the vehicle control mice (without DSS). Tissue sections from geraniol at 50 mg/kg (3C) and 100 mg/kg (3D) pre-treated DSS-induced mice markedly attenuated the DSS-induced alterations of the epithelial structural integrity and preserved the mucosal lining in colon tissue.

Effect of geraniol on DSS-induced pro-inflammatory cytokines and MPO activity

As shown in Fig. 4, the group of animals treated with DSS alone showed a significant ($p < 0.001$) increase in the concentrations of TNF-α (Fig. 4A), IL-1β (Fig. 4B) and IL-6 (Fig. 4C) and the MPO activity (Fig. 4D) in colon tissue when compared with the vehicle control mice. Treatment with geraniol at both the doses (50 and 100 mg/kg body weight) significantly ($p < 0.001$) prevented the increase in pro-inflammatory cytokines and the MPO activity when compared with the DSS alone treated mice.

Effect of geraniol on DSS-induced NF-κB (p65) nuclear translocation, IκBα phosphorylation and NF-κB (p65)-DNA binding

Western blot analysis (Fig. 5) revealed a significant increase in the amount of nuclear and total NF-κB (p65) ($p < 0.001$ for nuclear and $p < 0.05$ for total NF-κB) and the ratio of p-IκBα/ IκBα expression ($p < 0.05$) in the colon tissue of DSS-induced mice when compared with the vehicle control mice (Fig. 5A). Pretreatment with geraniol at both the doses (50 and 100 mg/kg body weight) significantly decreased the nuclear and total amount of NF-κB (p65) and prevented the IκBα phosphorylation when compared with the DSS alone treated mice. Similarly, the NF-κB (p65)-DNA binding activity of the nuclear extracts from DSS-induced animals was significantly ($p < 0.001$) increased compared with the vehicle control group (Fig. 5B). Pretreatment with geraniol at both the doses significantly ($p < 0.05$ at 50 mg/kg and $p < 0.01$ at 100 mg/kg) prevented the NF-κB (p65)-DNA binding activity when compared with the DSS-induced animals.

Effect of geraniol on DSS-induced iNOS and COX-2 expression

As shown in Fig. 6, the group of animals treated with DSS alone showed a significant ($p < 0.05$) increase in the amount of inflammatory proteins namely, iNOS and COX-2 when compared with the vehicle control mice. Treatment with geraniol along with DSS at 100 mg/kg significantly ($p < 0.01$) decreased the iNOS and COX-2 expressions when compared

with the DSS alone treated mice. Though geraniol at low dose (50 mg/kg) did not produce any significant ($p > 0.05$) change in COX-2 expression, the amount of iNOS protein was significantly ($p < 0.05$) decreased compared with the DSS alone treated mice.

Effect of geraniol on DSS-induced changes in nitrites, TBARS, GSH and SOD levels in colon tissues

The levels of nitrites and thiobarbituric acid reactive substances (TBARS) were significantly ($p < 0.01$) increased and the levels of reduced glutathione and superoxide dismutase (SOD) activity were significantly ($p < 0.01$) decreased in colon tissues of DSS-induced mice when compared with the vehicle control mice (Fig. 7). Pre-treatment with geraniol at both the doss (50 and 100 mg/kg) along with DSS significantly ($p < 0.01$) prevented the increase in tissue nitrites and TBARS levels and significantly ($p < 0.01$) restored the SOD activity when compared with the only DSS-induced mice. Similarly, though geraniol at low dose (50 mg/kg) did not produce any significant ($p > 0.05$) change in GSH levels, the concentrations of GSH was significantly ($p < 0.01$) restored when compared with the DSS alone treated mice.

Discussion

In this study, we demonstrated that the treatment with geraniol, a monoterpene with anti-inflammatory and antioxidative properties can confer protection against DSS-induced colitis in a mice model. Dextran sulphate sodium (DSS)-induced colitis in mice is a widely used preclinical model of the human ulcerative colitis.[14, 15, 16] DSS administration at a concentration of 2 to 6% (w/v) in drinking water for 5 to 10 consecutive days in mice produces clinical features such as production of pro-inflammatory mediators and leukocyte infiltration, superficial ulceration and mucosal damage, very similar to those observed in ulcerative colitis patients.[26] Administration of DSS in drinking water directly affects the gut epithelial cells of the basal crypts and alters the integrity of the mucosal barrier resulting in

bloody diarrhoea, weight loss and shortening of colon length.[27] In our study, the mice treated with DSS (4% (w/v)) for 5 consecutive days showed a pronounced disease activity index (DAI) with significant loss in body weight, bloody diarrhoea and shortening of colon length when compared with the vehicle control (no DSS) mice. Pretreatment with geraniol (50 and 100 mg/kg body weight) revealed an overall lower impact of DSS-induced colonic damage, which was evidenced by a reduced DAI, improved stool consistency without occult blood and increase in colon length in comparison with DSS-induced colitis mice. In addition, the light microscopic examination of colon tissue revealed that pretreatment with geraniol attenuated the DSS-induced alterations of the epithelial structural integrity and preserved mucosal lining in colon tissue. Since no report about the protective effect of geraniol on ulcerative colitis is available, our findings are unique in establishing the role of geraniol in ameliorating the colitis-like features in murine models of inflammatory bowel disease.

It is well recognized that the infiltration of inflammatory cells and the expression of pro-inflammatory cytokines such as TNF-α, IL-1β and IL-6 in colon tissue is a hallmark of ulcerative colitis.[4, 17, 24] Tumor necrosis factor-α (TNF-α) is an important pro-inflammatory cytokine produced chiefly by resident macrophages in response to harmful stimuli such as DSS, eventually, leading to amplified inflammatory responses and further development of the clinical signs of inflammation.[28] The activity of myeloperoxidase, which is linearly associated with the infiltration of inflammatory cells such as neutrophils, is used as surrogate marker for extent of inflammatory infiltration.[29] In our study, the levels of pro-inflammatory cytokines, TNF-α, IL-1β and IL-6 and the activity of myeloperoxidase in colon tissue were significantly increased in DSS-induced colitic mice. Pretreatment with geraniol significantly decreased the elevation of both pro-inflammatory cytokine levels and the myeloperoxidase activity when compared with the DSS alone induced mice. Thus, our findings suggest that the anti-inflammatory effect of geraniol is likely to be associated with its ability to inhibit the

inflammatory cell influx, as well as, release of proinflammatory cytokines in the colon tissue after DSS administration.

Several lines of evidence suggest that transcription factor, nuclear factor-kappa B (NF-κB) plays a critical role in the development and progression of inflammatory response in IBD.[4, 9, 17] The NF-κB is normally sequestered in the cytoplasm by binding to the inhibitory protein IκBα. Following activation and phosphorylation of IκBα by diverse stimuli including oxidative stress, pro-inflammatory cytokines, microbes and microbial products, the freed NF-κB translocate to the nucleus to drives the expression of target genes.[24] The activation of NF-κB leads to the induction of varieties of genes encoding proinflammatory cytokines and downstream inducible proinflammatory enzymes such as iNOS and COX-2 and exacerbate the inflammatory condition of intestine by affecting colon mucosa integrity.[9, 17] Moreover, it has also been recognized that activation of IκBα, accumulation of pro-inflammatory cytokines and upregulation of iNOS and COX-2 proteins in intestinal epithelial cells of IBD patients.[4, 9] In the present study, we also observed a significant amount of NF-kB (p65) nuclear translocation and its DNA binding, which is a basic step for NF-κB transactivation of its downstream proinflammatory genes in DSS-induced colitic animals when compared with the vehicle control animals. Of note, earlier studies have shown that geraniol can down regulate the production of NO and PGE_2, the expression of iNOS and COX-2 and the activation of NF-κB in lipopolysaccharide-induced macrophage RAW264.7 cells.[15] Corroborating with this anti-inflammatory effect of geraniol, in this study, pretreatment with geraniol attenuated the DSS-induced NF-κB nuclear translocation and its activation and prevented the IκBα degradation and phosphorylation in the colon tissue. In addition, geraniol decreased COX-2 and iNOS expression in colon tissue. Thus, these findings explain the anti-inflammatory effect of geraniol in the DSS-induced colitis experimental model.

Oxidative stress and profound lipid peroxidation in colon plays an important role in pathophysiology of ulcerative colitis.[30, 31] Leukocyte infiltration is an important source of reactive oxygen and nitrogen species generation, which attack the cellular macromolecules and disrupt epithelial cell integrity.[30, 32] Interestingly, it has also been reported that patients with ulcerative colitis have very low concentration of serum antioxidants compared to healthy individuals.[33] Supporting the importance of antioxidant pathways in intestinal homeostasis, it has been reported that the mice deficient in both Gpx1 and Gpx2 develop spontaneous colitis.[34] Moreover, the production of NO by iNOS has a detrimental effect on the development of colitis.[32] As expected, in the present study, we also observed a significant elevation of TBARS levels, as a marker for lipid peroxidation and the nitrites and iNOS levels, as an index of nitrosative stress and a significant decrease in GSH and SOD levels in the colon tissue of DSS-induced mice compared with the vehicle control (no DSS) mice. Such changes were significantly attenuated by geraniol treatment (100 mg/kg) and substantiating its antioxidant property.

In conclusion, the results of the present study showed that geraniol mitigates the severity of colitis by inhibiting the infiltration of inflammatory cells and the expressions of pro-inflammatory cytokines in colon tissue. In addition, geraniol attenuated the DSS-induced NF-κB activation and subsequent iNOS and COX-2 expression in colon tissue. These findings suggest that geraniol has potential therapeutic value for the treatment of ulcerative colitis.

Notes

The authors declare that there are no conflicts of interest.

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Figure legends

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$^{\dagger}p< 0.05$ and $^{\#}p< 0.001$ vs Vehicle control

$^{*}p< 0.05$, $^{**}p< 0.01$ and $^{***}p< 0.001$ vs DSS control

Fig. 2: Representative photographs showing colon tissues from (A) vehicle control, (B) Dextran sulphate sodium (DSS) alone treated mice, (C) Geraniol at 50 mg/kg body weight pre-treated DSS-induced mice and (D) Geraniol at 100 mg/kg body weight pre-treated DSS-induced mice.

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weight treated mice showing predominant intact colon histology with reduced extent of epithelial injury, intact crypt structure and mild inflammatory cell infiltrate.

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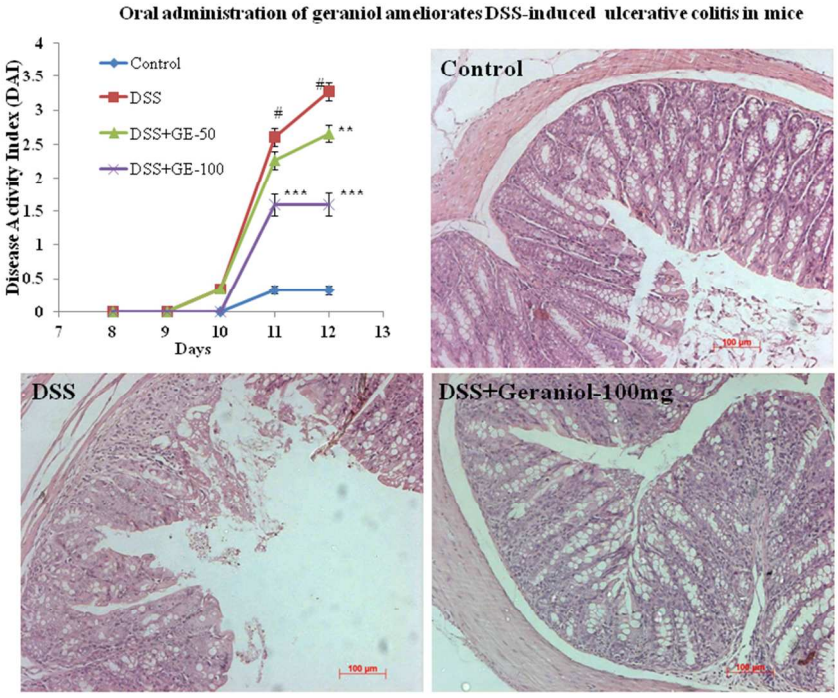

Graphical Abstract
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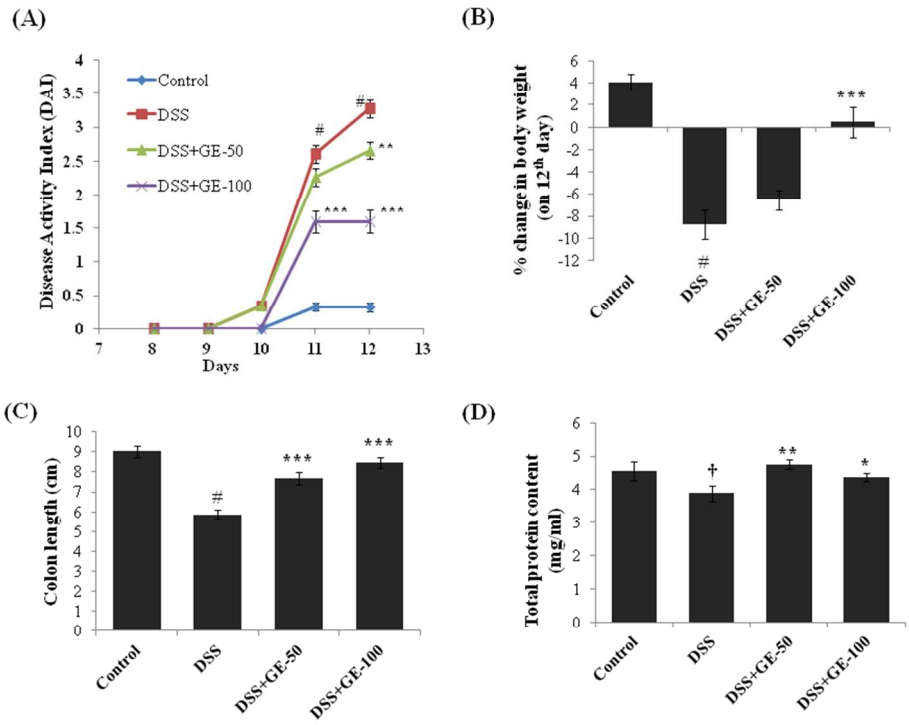

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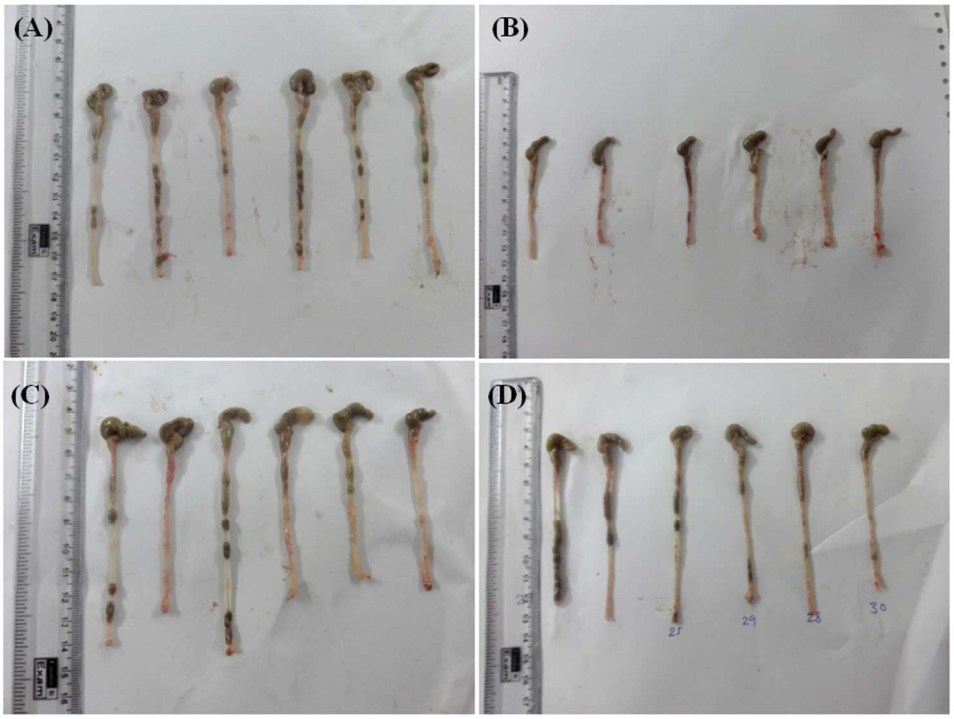

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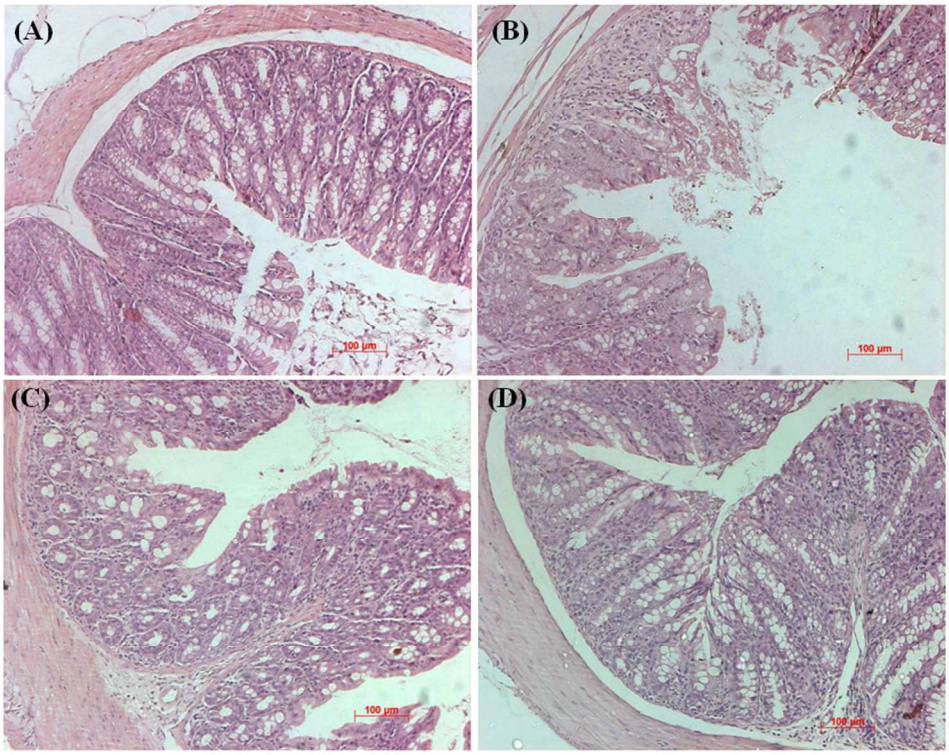

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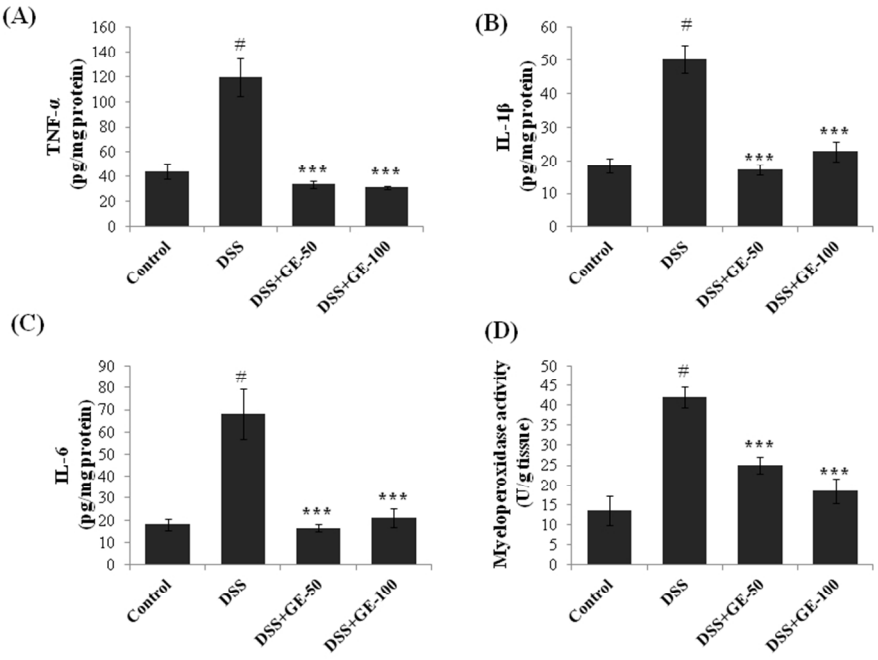

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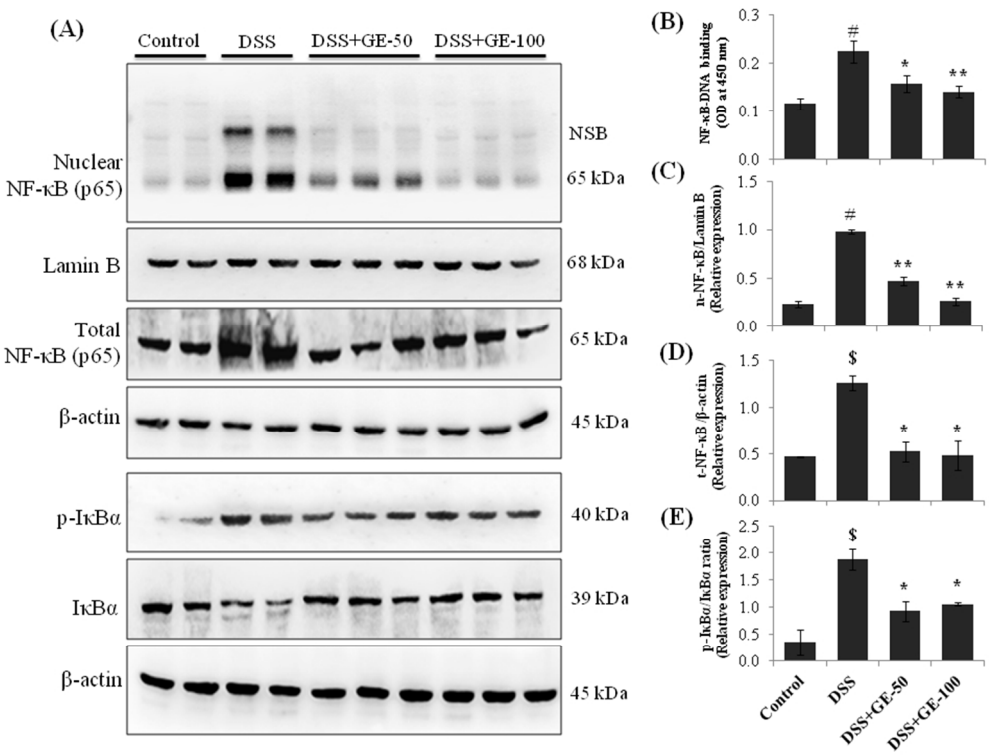

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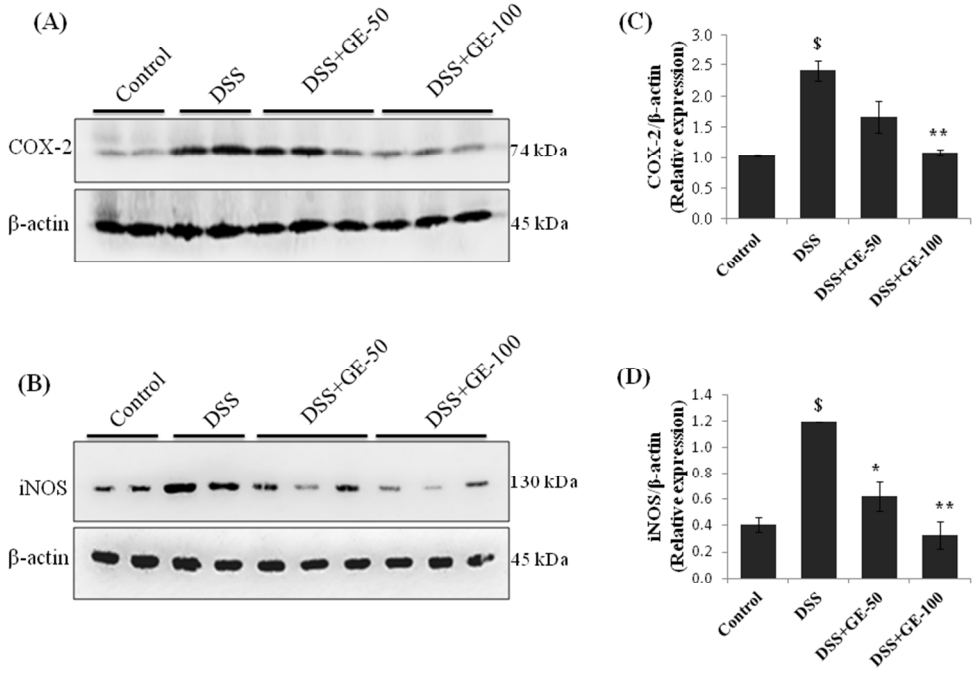

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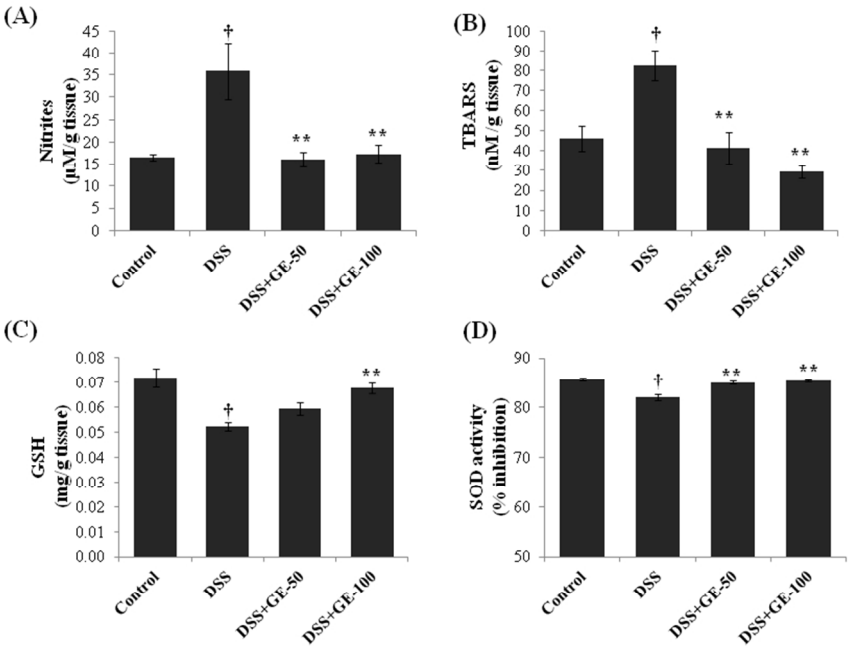

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