Current Trends in Biotechnology and Pharmacy
Vol. 19(3) 2525-2535, July 2025, ISSN 0973-8916 (Print), 2230-7303 (Online)
DOI: 10.5530/ctbp.2025.3.35

Sinapine and Sinapic acid Impart Anti-Inflammatory Effects in Dextran Sulfate Sodium-Induced Experimental Murine Ulcerative Colitis

Raneem Ismail Alkhaleel[1], Mysuru L Sumedini[2], Kurnegala Manikanta[2], Syed Baker[3], C. S. Shivakumara[4], Chakrabhavi Dhananjaya Mohan[5,6], S. Niranjan Raj[3], Kesturu S Girish[7], Siddaiah Chandra Nayaka[1,*]

[1]Department of Studies in Biotechnology, University of Mysore, Manasagangotri, Mysore 570006, Karnataka, India
[2]Department of Studies in Biochemistry, University of Mysore, Manasagangotri, Mysore 570006, Karnataka, India
[3]Department of Studies in Microbiology, Karnataka State Open University, Mukthagangotri, Mysore 570006, Karnataka, India
[4]Department of Clinical Nutrition and Dietetics, Sri Devaraj Urs Academy of Higher Education and Research, Kolar 563101, Karnataka, India
[5]Systems Toxicology Group, FEST Division, CSIR-Indian Institute of Toxicology Research, Vishvigyan Bhawan, Lucknow 226 001, Uttar Pradesh, India
[6]Academy of Scientific and Innovative Research, Ghaziabad 201002, India.
[7]Department of Studies and Research in Biochemistry, Tumkur University, Tumakuru 572103, Karnataka India

***Corresponding Author**: moonnayak@gmail.com

Abstract

Ulcerative colitis is an inflammatory bowel disease that affects a significant population and remains as one of the major public health concerns worldwide. Individuals with ulcerative colitis are at a higher risk of developing various other diseases including colon cancer. Several efforts are underway in the discovery of new small molecules for the treatment of ulcerative colitis due to adverse effects and the limited efficacy of existing therapeutic agents. In the present study, the effect of sinapine (SC) and sinapic acid (SA) (bioactive compounds found in plants of the family Brassicaceae) was examined on dextran sulfate sodium (DSS)-induced colitis in mice models. The onset of ulcerative colitis was evidenced by the reduction in the colon length, rectal bleeding, loose stool, elevated myeloperoxidase activity in colon tissue extracts, and increased pro-inflammatory cytokines, reactive oxygen species (ROS), and catalase activity in the serum of colitis mice. The *per os* administration of SA and SC demonstrated a significant reduction of disease activity index as witnessed by the restoration of colon length, reduced rectal bleeding, and consistent stool. Furthermore, SA and SC were found to decrease the myeloperoxidase activity in the colon tissue extracts. Also, there was a significant reduction in the levels of pro-inflammatory cytokines (IL-1β and IL-6), ROS, and catalase activity in the serum of colitis mice. The histochemical analysis demonstrated that SA and SC restored the architecture of the crypt and mucosa, which otherwise displayed hyperplasia with necrosis and submucosal inflammation in the colitis mice. The bioactivity of SA and SC was comparable with sulfasalazine and etacept. In conclusion, SA and SC were demonstrated as potent anti-inflammatory

agents against DSS-induced colitis in rodent models.

Keywords: Inflammatory bowel disease, ulcerative colitis, natural compounds, anti-inflammatory compounds.

Introduction

Inflammatory bowel disease (IBD) is characterized by the infection-independent chronic inflammation of the alimentary canal which encompasses ulcerative colitis, Crohn's disease, and indeterminate colitis [1-3]. Although the precise causes are unknown, environmental factors, immune system deregulation, and genetic factors are believed to contribute to the development of these diseases [4-6]. Crohn's disease can affect any part of the gastrointestinal tract whereas colitis is restricted to the colon [7, 8]. Age advancement, race (being white), family history, and consumption of some antibiotics or non-steroidal anti-inflammatory drugs are the risk factors associated with IBD [9, 10]. An increased risk of IBD was noted in individuals within one year of infections with Salmonella/ Campylobacter [11]. The transmural infiltration of immune cells including macrophages, neutrophils, lymphocytes, and mast cells results in disruption of the mucosal layer and ulceration [12, 13]. Ulcerative colitis is more prevalent than Crohn's disease. Ulcerative colitis-affected individuals are at a higher risk of developing colon cancer, osteoporosis, renal caliculi, and liver disease. Aminosalicylates (sulfasalazine, mesalamine, and olsalazine), corticosteroids, TNF-α inhibitors (etanercept), thiopurines, calcineurin inhibitors, and some immunomodulators (cyclosporine, azathioprine, and methotrexate) are some of the therapeutic agents used in the treatment of ulcerative colitis. Despite having a broad range of medications for the treatment of IBD in general, ulcerative colitis in particular, these drugs have limited efficacy and are associated with complications in long-term use which demands the discovery of new safer therapeutic agents with increased efficacy.

Natural compounds have been demonstrated to possess good therapeutic efficacy against various human ailments with a favorable safety profile. A wide range of natural compounds are in clinical practice and many are undergoing clinical trials for the treatment of various human diseases [14-16]. Many studies have examined the efficacy of natural compounds against IBD including ulcerative colitis. About 21-60% of individuals with IBD were estimated to use herbal formulations as complementary medicine with a perception of having reduced adverse effects and about 75% do not reveal this to their clinicians [17]. Curcumin, mastiha, carvacrol, thymol, eucalyptol, and numerous other natural products have been demonstrated to mitigate IBD by exerting anti-inflammatory activity in experimental animals [18]. Sinapic acid (SA) and sinapine (SC) are the bioactive compounds present in the plants of the family Brassicaceae, citrus peels, rapeseed, cereals, and oil-seed crops. Structurally, SC is the choline (a quaternary amine) ester of SA. An interesting study demonstrated that SC can enter the mitochondria to decrease the mitochondrial oxidative stress in cardiomyocytes whereas SA could not cross the mitochondrial membranes [19]. Both the compounds were able to reduce the cytosolic oxidative stress in cardiomyocytes suggesting that SA and SC are endowed with good antioxidant potential. Recent studies have demonstrated the suppressive effect of sinapic acid in acetic acid/dextran sulfate sodium (DSS)-induced rodent colitis models [20, 21]. These reports suggest that SA and SC have inherent antioxidant properties which demand their evaluation of their potency in inflammatory diseases. In the present report, the anti-inflammatory effect of SA and SC was examined in the DSS-induced murine colitis model.

Materials and Methods

Reagents

Sinapic acid (CAT# D7927), Sinapine Chloride (CAT# PHL84156), and hexadecyltrimethylammonium bromide (CAT# H5882) were purchased from Sigma-Aldrich.

Murine mini-ELISA kits for IL-1β (900-K47), IL-6 (900-K50), and IL-10 (900-K53) cytokines were purchased from PeproTech Ltd. Dextran sulfate sodium 36−50 kDa (CAT# 160110) was purchased from MP Biomedicals. Sulfasalazine gastro-resistant tablets IP 500 mg (FJMTL4) from Ipca laboratories Ltd., Etacept (CAT# 20131298) from Cipla Ltd, o-dianisidine (CAT# 119-90-4) from Loba Chemie. All other fundamental chemicals were of analytical grade.

Animals

Swiss albino male mice aged about 6-7 weeks were obtained from the Department of Zoology, University of Mysore, Mysore, India (approved by the IAEC of University of Mysore No. UOM/IAEC/01/2022-2023). Animals were acclimatized before the experiments under a 12 h light/dark cycle at 22°C and 60% humidity and fed with a standard laboratory rodent diet and RO water.

Colitis model

Dextran sulfate sodium (5%, DSS) in drinking water was given for 4 days to induce colitis as described earlier [22]. Animals were randomly divided into 10 groups (n=5) and experimental conditions were maintained as indicated below [Sulfasalazine (SZ), and Etacept (ET)]. ET alone and SZ alone served as the positive control group while group I served as a negative control.

Group I: Vehicle control group (Only drinking water)

Group II: DSS for 4 days

Group III: DSS for 4 days followed by SA (10 mg/kg/day *per os* from day 5 to 9)

Group IV: DSS for 4 days followed by SA (20 mg/kg/day *per os* from day 5 to 9)

Group V: DSS for 4 days followed by SC (5 mg/kg/day *per os* from day 5 to 9)

Group VI: DSS for 4 days followed by SC (10 mg/kg/day *per os* from day 5 to 9)

Group VII: DSS for 4 days followed by SZ (500 mg/kg/day *per os* from day 5 to 9)

Group VIII: DSS for 4 days followed by ET (5 mg/kg/day subcutaneous from day 5 to 9)

Group IX: SA alone (20 mg/kg/day *per os* from day 5 to 9)

Group X: SC alone (10 mg/kg/day *per os* from day 5 to 9)

All the animals were sacrificed on day 10 and blood was collected to analyze the pro- and anti-inflammatory cytokines levels, ROS levels, and enzyme activities in serum. Colon was collected for histopathological studies and for determining disease activity index (DAI).

Disease activity index

All the experimental animals were carefully monitored throughout the study duration for disease as well as prognostic symptoms and DAI scores were assigned. Animal weight, stool consistency (presence of diarrhea), and rectal bleeding were considered to assign DAI scores as given below.

Table 1: DAI scoring system

Score	Weight loss	Stool consistency	Blood in feces
0	None	Normal	None
1	1 – 5 %	Loose	Slight bleeding
2	6 – 10 %	Loose	Mild bleeding
3	11 - 20%	Diarrhea	Moderate bleeding
4	>20 %	Diarrhea	Gross bleeding

Colon collection

The colons were dissected from the cecum to the anus, rinsed with ice-cold phosphate-buffered saline (10 mM, pH 7.4), weighed, and their length was measured to determine the length/weight ratio, and preserved for further analysis. The efficacy of SA and SC in treating colitis was compared with that of etacept (a standard TNF-α inhibitor) and sulfasalazine (a standard treatment for colitis).

Preparation of colons for histological analysis

The Swiss roll of colons was prepared for histological analysis by dissecting them from the caecum to the anus after ensuring they remained intact. They were flushed with ice-cold phosphate-buffered saline (10 mM, pH 7.4) to remove any remaining fecal matter and to preserve tissue integrity. Colon lengths were measured, and photographs of the harvested colon were taken for the presentation. Next, the colons were cut open and gently rolled keeping the segment flat open with the luminal side up from the anus to the caecum and pierced with the 26-gauge needle to hold it in place. The rolled colons were then fixed in 10% phosphate-buffered formalin to preserve tissue structure. After fixation, the Swiss rolls were processed through dehydration, clearing, and embedding in paraffin wax followed by slicing (5µm) mounting on slides, and staining with hematoxylin & eosin dye (H&E) for microscopic examination to assess the damage markers such as crypt and goblet cell morphology, epithelial erosion, and ulceration. The histological scoring was given as follows.

Table 2: Histologic Scoring System for DSS-Induced Colitis

Score	Description		
	Severity of Inflammation	Extent of Inflammation	Crypt Damage
0	None	None	None
1	Mild	Mucosa	1/3 Damaged
2	Moderate	Mucosa and submucosa	2/3 Damaged
3	Severe	Transmural	Crypts lost, Surface Epithelium present

Serum cytokine analysis

The blood sample collected from the experimental animals was centrifuged at 3000 rpm for 10 min to separate the serum. The supernatant (serum) was collected and the levels of various cytokines including IL-1β, IL-6, and IL-10 were measured using ELISA kits as per the manufacturer's protocol (PeproTech).

Myeloperoxidase (MPO) activity

Briefly, 0.2 g of the excised colonic tissue from all the groups was homogenized in 50 mM potassium phosphate buffer (pH 6.0) containing 0.5% hexadecyltrimethylammonium bromide. Tissue debris was removed by centrifugation at 12,000 rpm for 20 min at 4 °C. Supernatant was collected in a 96-well plate in triplicates, and 200 µl of ODA-H_2O_2 reagent (0.167 mg/ml o-dianisidine dihydrochloride and 0.05% H_2O_2) was added to each well including the well containing 10 µl of buffer alone, which served as blank. Absorbance was measured at 450 nm using a multimode plate reader (Thermo Scientific) at 0, 30, and 60 S. The difference between two-time points was taken, and the MPO activity was calculated using the formula (MPO constant: 1.13×10^{-2}) *(ε* is the extinction coefficient of oxidized *o*-dianisidine, which has been determined to be $1.13 \times 10^4\ M^{-1}cm^{-1}$ for a wavelength of 460 nm at room temperature. *b* is the optical path length, which is 1 cm for the cuvettes recommended in this protocol)

$$Act = \frac{\Delta c}{Vrea \times Csn}$$

$$\Delta C = \frac{\Delta Abs}{\varepsilon b} = \frac{\Delta Abs}{1.13 \times 10^4 \times 1.0}$$

Determination of reactive oxygen species (ROS) in serum

To determine the oxidative stress, the total ROS level in the serum was measured using the DCFDA method. Briefly, the aliquot of serum samples from all groups was taken in a 96-well plate with PBS. DCFDA (10 µM) was added, and ROS levels were analyzed using a Varioskan multimode plate reader (Thermo Scientific, USA) by their specific excitation/ emission wavelengths (488/530)

Estimation of catalase activity in serum

Catalase activity was determined by measuring the rate of hydrolysis of H_2O_2 at 240 nm. To the reaction mixture (1 ml) containing H_2O_2 (8.8 mM) in sodium phosphate buffer (0.1 M, pH 7.0), an aliquot of serum samples (0.05 mg protein) was added independently. Readings were taken at 0, 60, 120, and 180 s at 240 nm wavelength in the spectrophotometer. The absorbance was monitored for 3 min and the activity was expressed as µmol H_2O_2 decomposed/min/mg protein.

Protein estimation

The protein estimation was done according to the method of Lowry et al., using BSA as standard.

Statistical analysis

Results were expressed as mean ± SEM of three independent experiments. Statistical significance among groups was determined by one-way/ two-way analysis of variance (ANOVA) followed by Tukey's test for comparison of means ($^{*/\#} < 0.05$, $p^{**/\#\#} < 0.02$, $p^{***/\#\#\#} < 0.01$, $p^{****/\#\#\#\#}$). All analyses were done using GraphPad Prism software (Version 8.0.2).

Results and Discussion

SA and SC reverted the DSS-induced shortening of the colon length

The chemical structure of sinapine and sinapic acid is given in Figure 1A. To examine the anti-colitis efficacy of SA and SC, a colitis murine model was established by feeding the experimental animals with DSS through drinking water till day 4 and subsequently administering test compounds till day 9 as detailed in methods (Figure 1B). The results demonstrated a significant decline in the colon length in DSS-treated animals compared to untreated animals. The administration with either SA or SC significantly reverted the DSS-induced shortening of colon length compared to the DSS alone group (Figure 2A-B). SZ and ET were used as positive controls. The restoration activity of SA and SC was comparable with SZ and ET suggesting that SA and SC may mitigate the DSS-induced colitis.

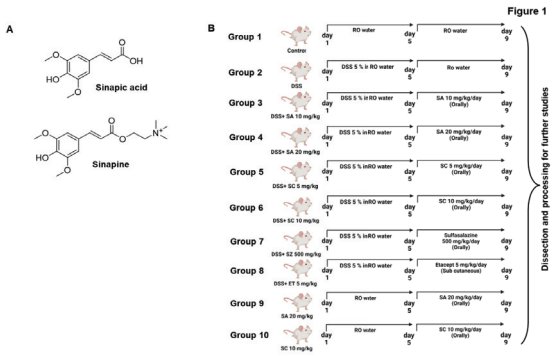

Figure 1. (A) Chemical structure of SA and SC. (B) Flowchart depicting the experimental strategy used in establishing colitis model and examination of compounds of interest.

SA and SC reduced the DAI in colitis mice

The DAI score was assigned by examining the stool consistency and rectal bleeding in animals administered with DSS alone or with test agents. Loose stool and mild rectal bleeding were observed in animals administered with DSS whereas SA- and SC-administered animals displayed good stool consistency and reduced rectal bleeding (Figure 2C). SA-administered animals displayed low DAI compared to SC-administered animals.

The overall DAI score was significantly downregulated in SA- and SC-administered animals from day 6 till day 9 compared to DSS-administered animals (Figure 2D-E). SZ- and ET-administered animals also displayed a reduction in DAI compared to the diseased group.

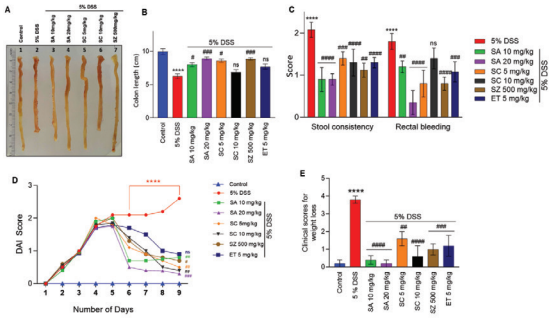

Figure 2. Effect of SA and SC on colon length and DAI in colitis mice. (A) Images of colon of experimental mice of different groups. (B) Colon length (centimetre), each bar represents by the Mean + SEM shows the significant difference in the colon length between each treated group and the control group (**** p<0.001, significant compared to control group; #p<0.05, ###p<0.01 significantly different from the DSS group). (C) Stool consistency and rectal bleeding scores to each group (D) The DAI score (E) Weight loss score. Data are presented as mean ± SEM. (2C-E: **** p<0.001 significant compared to control group; ##p< 0.02, ###p<0.01, ####p<0.001 significantly different from the DSS group).

SA and SC decreased the MPO activity in the colon tissue of colitis mice

The catalytic activity of MPO was measured in the colonic tissue extracts of experimental animals. It was found that the MPO activity was significantly elevated in DSS-administered animals whereas it was significantly reduced in SA- and SC-administered animals (Figure 3A). The MPO activity in SA- and SC-administered groups was comparable to the untreated and SZ-treated groups.

SA and SC downregulated the oxidative stress in the serum of DSS-administered mice

Subsequently, levels of ROS in the serum of all experimental animals were quantified using the DCFDA method. There was a significant elevation in the levels of ROS in DSS-administered animals and a significant decline was observed in SA- and SC (only in 10 mg/kg)-administered animals (Figure 3B). In parallel, the catalytic activity of catalase was measured in serum samples to understand the level of oxidative stress in different groups of animals. It was noticed that a significant decline in the catalase activity in DSS-administered animals and its significant restoration in all other groups (Figure 3C) suggesting that both SA and SC have good antioxidant potential and thereby are reducing the DSS-induced oxidative stress in the colitis-affected mice.

SA and SC treatment altered the levels of inflammation-related cytokines

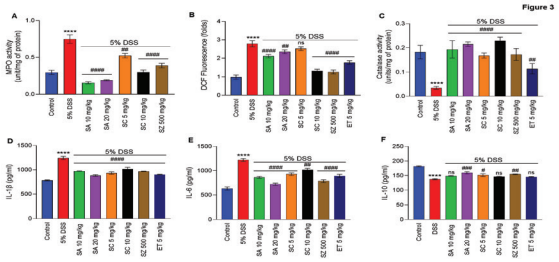

Figure 3. Effect of SA and SC on MPO activity, total ROS, catalase activity, and cytokine levels. A) Severity of colonic inflammation was assessed by MPO activity in colon extracts from all the groups. B) The severity of oxidative stress was measured by measuring the total ROS level in serum using DCFDA method. C) Catalase activity was measured in the serum. Serum levels of (D) IL-1β, (E) IL-6, (F) IL-10 were measured using ELISA kit method and expressed in concentration pg/ml. Data are presented as mean ± SEM, n=5, **** p<0.001, significant compared to control group;#p<0.05, ##p<0.02, ###p<0.01, ####p<0.001 significantly different from the DSS group.

Current Trends in Biotechnology and Pharmacy
Vol. 19(3) 2525-2535, July 2025, ISSN 0973-8916 (Print), 2230-7303 (Online)
DOI: 10.5530/ctbp.2025.3.35

The levels of pro-inflammatory and anti-inflammatory cytokines were measured in the serum samples to understand the extent of inflammation in experimental animals. There was a significant upregulation in the levels of pro-inflammatory cytokines (IL-1β and IL-6) and downregulation in the levels of anti-inflammatory cytokine (IL-10) in the serum of DSS-administered animals (Figure 3D-F). In contrast, the levels of pro- and anti-inflammatory cytokines were restored to normal levels in SA- and SC-administered animals indicating that SA and SC impart anti-inflammatory activity in colitis-affected mice.

SA and SC treatment restored the mucosa and crypt architecture of the colon

Histological analysis was performed to visualize the effect of DSS and compounds of interest on the mucosal layer and crypt architecture. Crypt hyperplasia with necrosis, congestion, and submucosal inflammation was observed in DSS-administered animals whereas the administration of either SA or SC significantly restored the mucosal epithelial morphology which is comparable with the group treated with SZ and ET (Figure 4A-B). All these results suggest that both SA and SC can revert the DSS-induced colitis in experimental mice. SA has shown anti-colitis activity in

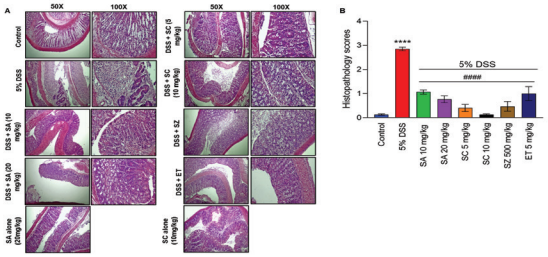

Figure 4: Effects of SA and SC on the architecture of the colon. (A) Histological analysis of colon of all the groups by H&E staining method. (B) Bar graphs represent the histopathology scores for the colon histology sections. Data are presented as mean ± SEM, n=5, **** p<0.001, significant compared to control group; ####p<0.001 significant compared to DSS group.

IBD in general, and ulcerative colitis in particular is one of the major health concerns worldwide. Since the precise causes of ulcerative colitis are unknown, emphasis is given to the development of therapeutic agents that are relatively safe and efficacious. Natural compounds that are endowed with anti-inflammatory activity have demonstrated good suppression of colitis in preclinical disease models. The anti-inflammatory activity of SA is well-studied in the preclinical colitis model. The earlier studies have demonstrated that colitis that is induced using either 2,4,6-trinitrobenzene sulfonic acid, DSS, or acetic acid can be reverted by SA [19-21]. SA was found to decrease the activation of NLRP3 inflammasome in the colitis mice [23]. SA was also reported to restore in antioxidant system by stimulating the Nrf2/keap1 pathway and enhancing autophagy in colitis mice [24]. Although SA and SC are closely related structures (except esterified choline in SC), SC was found to be capable of crossing the mitochondrial membrane in cardiomyocytes while SA could not. SA displayed antioxidant potential in cytoplasm while SC was found to be distributed in mitochondria as well as cytoplasm to impart antioxidant activity in both the compartments of cardiomyocytes [19]. Considering the antioxidant potential of sinapine from the above study, we examined its anti-colitis potential in a DSS-induced mice model. It was found that SC displayed consistently good anti-colitis activity as evidenced by the restoration of colon length, reduced DAI, reduction of MPO activity, decreased levels of pro-inflammatory cytokines, and re-establishment of crypt architecture in DSS-induced experimental mice.

During the study period, experimental animals were administered with DSS to induce colitis. DSS-induced mice excreted loose stool and displayed mild rectal bleeding which confirmed the onset of disease. SA displayed more potency than SC in improving stool consistency and rectal bleeding. The DAI score was found to be significantly low in groups treated with SA (20 mg/kg) and SC (10 mg/kg)

demonstrating the potential anti-colitis effect of SA and SC. MPO is a prominent pro-inflammatory enzyme that is present in the azurophilic granules of neutrophils and monocytes. MPO drives the formation of hypochlorite from hydrogen peroxide and chloride to eliminate pathogenic microorganisms. MPO can serve as a classical biomarker of colonic inflammation in colitis. There was a significant elevation in the MPO activity in the DSS-induced mice indicating induction of colitis whereas the SA and SC significantly reduced the MPO activity in DSS-induced rodents. Overproduction of ROS is a hallmark feature of IBD that regulates inflammatory pathways [25]. ROS activates macrophages and thereby promotes the release of pro-inflammatory cytokines to further elevate inflammation [26]. There was a significant increase in ROS in the serum of DSS-administered animals whereas SA and SC substantially reduced levels of ROS. The results of the catalase assay were also in agreement with serum ROS levels. Elevation in the pro-inflammatory cytokines and decline in the anti-inflammatory cytokines in the serum of DSS-administered animals further confirmed the occurrence of disease whereas administration of SA and SC reversed the levels of these cytokines. Histochemical staining of the colon also endorsed the observations made in all the previous assays. Additionally, the effect of SA and SC was examined on the normal mice and no significant alteration in body weight and morphology of liver and spleen was observed. The images of liver and spleen are provided as supplementary information.

From all these results, it can be concluded that colitis is a complicated disease that may alter the functions of other organs due to elevation in circulating ROS as well as pro-inflammatory cytokines. Therefore, it is highly essential to administer compounds with good anti-inflammatory properties. This study does not present the precise cellular target of SA and SC which is the lacunae of the study and attempts will be made to identify the target of these compounds in future studies. The present study advises the incorporation of commodities that are rich in sinapine and sinapic acid in a diet of patients with inflammatory diseases in general, and colitis in particular to enhance the effectiveness of therapeutic agents. Based on our results as well as previous reports, SA and SC have been demonstrated to be endowed with good anti-inflammatory potential in the colitis model and therefore, they may be considered for clinical evaluation in inflammatory diseases either for monotherapy or adjunct therapy. In conclusion, the present report demonstrates SA and SC as potent suppressors of colitis in rodent preclinical models.

Conclusion

Sinapic acid (SA) and sinapine (SC), bioactive compounds found in plants of the Brassicaceae family, were examined for their anti-inflammatory effects in a dextran sulfate sodium (DSS)-induced murine model of ulcerative colitis. DSS administration led to reduced colon length, rectal bleeding, loose stool, elevated myeloperoxidase activity in colon tissue, and increased pro-inflammatory cytokines, reactive oxygen species, and catalase activity in serum. Treatment with SA and SC significantly reduced the disease activity index, restored colon length, decreased myeloperoxidase activity, pro-inflammatory cytokines, reactive oxygen species, and catalase activity. Histological analysis showed that SA and SC restored the crypt and mucosal architecture in the colon. The efficacy of SA and SC was comparable to sulfasalazine and etacept. The study demonstrates the potent anti-inflammatory effects of SA and SC in DSS-induced experimental colitis.

Acknowledgements

The authors thank the University of Mysore for providing infrastructure to carryout this research work.

Funding information

No funding received to carry out this

research work.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. S. Alatab, S.G. Sepanlou, K. Ikuta, H. Vahedi, C. Bisignano, S. Safiri, A. Sadeghi, M.R. Nixon, A. Abdoli, H. Abolhassani, V. Alipour, M.A.H. Almadi, A. Almasi-Hashiani, A. Anushiravani, J. Arabloo, S. Atique, A. Awasthi, A. Badawi, A.A.A. Baig, N. Bhala, A. Bijani, A. Biondi, A.M. Borzì, K.E. Burke, F. Carvalho, A. Daryani, M. Dubey, A. Eftekhari, E. Fernandes, J.C. Fernandes, F. Fischer, A. Haj-Mirzaian, A. Haj-Mirzaian, A. Hasanzadeh, M. Hashemian, S.I. Hay, C.L. Hoang, M. Househ, O.S. Ilesanmi, N. Jafari Balalami, S.L. James, A.P. Kengne, M.M. Malekzadeh, S. Merat, T.J. Meretoja, T. Mestrovic, E.M. Mirrakhimov, H. Mirzaei, K.A. Mohammad, A.H. Mokdad, L. Monasta, I. Negoi, T.H. Nguyen, C.T. Nguyen, A. Pourshams, H. Poustchi, M. Rabiee, N. Rabiee, K. Ramezanzadeh, D.L. Rawaf, S. Rawaf, N. Rezaei, S.R. Robinson, L. Ronfani, S. Saxena, M. Sepehrimanesh, M.A. Shaikh, Z. Sharafi, M. Sharif, S. Siabani, A.R. Sima, J.A. Singh, A. Soheili, R. Sotoudehmanesh, H.A.R. Suleria, B.E. Tesfay, B. Tran, D. Tsoi, M. Vacante, A.B. Wondmieneh, A. Zarghi, Z.-J. Zhang, M. Dirac, R. Malekzadeh, M. Naghavi, The global, regional, and national burden of inflammatory bowel disease in 195 countries and territories, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017, The Lancet Gastroenterology & Hepatology, 5 (2020) 17-30.

2. G.P. Ramos, K.A. Papadakis, Mechanisms of Disease: Inflammatory Bowel Diseases, Mayo Clinic proceedings, 94 (2019) 155-165.

3. W. Qiu, Z. Wang, Q. Liu, Q. Du, X. Zeng, Z. Wu, D. Pan, X. Zhang, M. Tu, Structure and regulatory mechanisms of food-derived peptides in inflammatory bowel disease: A review, Food science & nutrition, 12 (2024) 6055-6069.

4. R. Liu, Y. Luo, J. Ma, Q. Zhang, Y. Sheng, J. Li, H. Li, T. Zhao, Traditional Chinese medicine for functional gastrointestinal disorders and inflammatory bowel disease: narrative review of the evidence and potential mechanisms involving the brain-gut axis, Frontiers in pharmacology, 15 (2024) 1444922.

5. S.S. Seyedian, F. Nokhostin, M.D. Malamir, A review of the diagnosis, prevention, and treatment methods of inflammatory bowel disease, Journal of medicine and life, 12 (2019) 113-122.

6. M. Bai, H. Guo, X.Y. Zheng, Inflammatory bowel disease and Clostridium difficile infection: clinical presentation, diagnosis, and management, Therapeutic advances in gastroenterology, 16 (2023) 17562848231207280.

7. G. Roda, S. Chien Ng, P.G. Kotze, M. Argollo, R. Panaccione, A. Spinelli, A. Kaser, L. Peyrin-Biroulet, S. Danese, Crohn's disease, Nature reviews. Disease primers, 6 (2020) 22.

8. M. Gajendran, P. Loganathan, A.P. Catinella, J.G. Hashash, A comprehensive review and update on Crohn's disease, Disease-a-month : DM, 64 (2018) 20-57.

9. Y. Ye, Z. Pang, W. Chen, S. Ju, C. Zhou, The epidemiology and risk factors of inflammatory bowel disease, International journal of clinical and experimental medicine, 8 (2015) 22529-22542.

10. A.T. Abegunde, B.H. Muhammad, O. Bhatti, T. Ali, Environmental risk factors for inflammatory bowel diseases: Evidence based literature review, World journal of gastroenterology, 22 (2016) 6296-6317.

11. J.D. Feuerstein, A.S. Cheifetz, Ulcerative colitis: epidemiology, diagnosis, and management, Mayo Clinic proceedings, 89 (2014) 1553-1563.

12. M.J. Uddin, S.O. Jeong, M. Zheng, Y. Chen, G.J. Cho, H.T. Chung, Y. Joe, Carbon monoxide attenuates dextran sulfate sodium-induced colitis via inhibition of GSK-3β signaling, Oxidative medicine and cellular longevity, 2013 (2013) 210563.

13. A.C. Nirvanappa, C.D. Mohan, S. Rangappa, H. Ananda, A.Y. Sukhorukov, M.K. Shanmugam, M.S. Sundaram, S.C. Nayaka, K.S. Girish, A. Chinnathambi, M.E. Zayed, S.A. Alharbi, G. Sethi, Basappa, K.S. Rangappa, Novel Synthetic Oxazines Target NF-κB in Colon Cancer In Vitro and Inflammatory Bowel Disease In Vivo, PLOS ONE, 11 (2016) e0163209.

14. R. Jadimurthy, S. Jagadish, S.C. Nayak, S. Kumar, C.D. Mohan, K.S. Rangappa, Phytochemicals as Invaluable Sources of Potent Antimicrobial Agents to Combat Antibiotic Resistance, Life, 13 (2023) 948.

15. C.D. Mohan, S. Rangappa, S.C. Nayak, R. Jadimurthy, L. Wang, G. Sethi, M. Garg, K.S. Rangappa, Bacteria as a treasure house of secondary metabolites with anticancer potential, Seminars in cancer biology, 86 (2022) 998-1013.

16. C.D. Mohan, S. Rangappa, H.D. Preetham, S. Chandra Nayaka, V.K. Gupta, S. Basappa, G. Sethi, K.S. Rangappa, Targeting STAT3 signaling pathway in cancer by agents derived from Mother Nature, Seminars in cancer biology, 80 (2022) 157-182.

17. M.M. Davila, E. Papada, The Role of Plant-Derived Natural Products in the Management of Inflammatory Bowel Disease—What Is the Clinical Evidence So Far?, Life, 13 (2023) 1703.

18. R.N. Subudhi, N. Poonia, D. Singh, V. Arora, Natural approaches for the management of ulcerative colitis: evidence of preclinical and clinical investigations, Natural products and bioprospecting, 14 (2024) 42.

19. D. Boulghobra, P.E. Grillet, M. Laguerre, M. Tenon, J. Fauconnier, P. Fança-Berthon, C. Reboul, O. Cazorla, Sinapine, but not sinapic acid, counteracts mitochondrial oxidative stress in cardiomyocytes, Redox biology, 34 (2020) 101554.

20. S. Jang, S. Kim, B.R. So, Y. Kim, C.K. Kim, J.J. Lee, S.K. Jung, Sinapic acid alleviates inflammatory bowel disease (IBD) through localization of tight junction proteins by direct binding to TAK1 and improves intestinal microbiota, Frontiers in pharmacology, 14 (2023) 1217111.

21. M. Shahid, M. Raish, A. Ahmad, Y.A. Bin Jardan, M.A. Ansari, A. Ahad, K.M. Alkharfy, A.L. Alaofi, F.I. Al-Jenoobi, Sinapic Acid Ameliorates Acetic Acid-Induced Ulcerative Colitis in Rats by Suppressing Inflammation, Oxidative Stress, and Apoptosis, Molecules, 27 (2022) 4139.

22. H.K. Keerthy, C.D. Mohan, K.S. Siveen, J.E. Fuchs, S. Rangappa, M.S. Sundaram, F. Li, K.S. Girish, G. Sethi, Basappa, A. Bender, K.S. Rangappa, Novel Synthetic Biscoumarins Target Tumor Necrosis Factor-α in Hepatocellular Carcinoma in Vitro and in Vivo*, Journal of Biological Chemistry, 289 (2014) 31879-31890.

23. B. Qian, C. Wang, Z. Zeng, Y. Ren, D. Li, J.-L. Song, Ameliorative Effect of Sinapic Acid on Dextran Sodium Sulfate- (DSS-) Induced Ulcerative Colitis in Kunming (KM) Mice, Oxidative medicine and cellular longevity, 2020 (2020) 8393504.

24. W.-Y. Li, J.-Y. Liu, Z.-X. Wang, K.-Y. Wang, C.-X. Huang, W. He, J.-L. Song, Sinapic Acid Attenuates Chronic DSS-Induced Intestinal Fibrosis in C57BL/6J Mice

by Modulating NLRP3 Inflammasome Activation and the Autophagy Pathway, ACS omega, 9 (2024) 1230-1241.

25. P. Muro, L. Zhang, S. Li, Z. Zhao, T. Jin, F. Mao, Z. Mao, The emerging role of oxidative stress in inflammatory bowel disease, Frontiers in endocrinology, 15 (2024) 1390351.
26. M. Herb, M. Schramm, Functions of ROS in Macrophages and Antimicrobial Immunity, Antioxidants, 10 (2021) 313.