Chicoric acid enhances the antioxidative defense system and protects against inflammation and apoptosis associated with the colitis model induced by dextran sulfate sodium in rats

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Research Article

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Abstract

Although several anticolitic drugs are available, their application is associated with numerous side effects. Here, the potential anticolitic efficiency of chicoric acid (CA; a hydroxycinnamic acid) against dextran sulfate sodium-induced colitis in rats was examined in rats. Animals were randomly assigned to the following five groups: control, CA (100 mg/kg body weight), DSS [(DSS); 4% w/v], CA + DSS (100 mg/kg), and the 5-aminosalicylic acid (100 mg/kg) + DSS group. The obtained data revealed that CA significantly prevented the shortening of colon length in addition to alleviating colon histological changes in colitis rats. Meanwhile, the oxidative stress-related enzymes, in addition to malondialdehyde and nitric oxide, were markedly modulated by CA. The results also indicated that CA exerted an anti-apoptotic effect in the colitis rats by inhibiting pro-apoptogenic indices (Bax and caspase-3) and enhancing Bcl-2, the anti-apoptogenic protein. Moreover, DSS caused an elevation of pro-inflammatory mediators, including interleukin-1β, tumor necrosis factor-α, myeloperoxidase, cyclooxygenase II, prostaglandin E2, and peroxisome proliferator-activated receptor gamma. Interestingly, these changes were significantly attenuated following the CA administration. At the molecular level, CA supplementation has increased the expression level of nuclear factor erythroid 2-related factor-2 (Nrf2) and decreased the expressions of nitric oxide synthase and mitogen-activated protein kinase 14. CA has been determined to lessen DSS-induced colitis by activating Nrf2 and its derived antioxidant molecules and suppressing inflammation and apoptosis cascades associated with the development of colitis; suggesting that CA could be used as an alternative naturally-derived anticolitic agent.

Introduction

Ulcerative colitis (UC), which is defined as a chronic inflammatory disorder of the colon, is often marked by inflammation of the mucous membranes and the formation of ulcers. With sluggish recovery, it is overwhelmingly recurrent and is regarded as a refractory digestive tract disorder (Bewtra et al. 2015, Li et al. 2017). The prevalence of UC is high in Western countries, and it has been rapidly increasing in the East, where prevalence was previously low (Ng et al. 2018). The main symptoms of UC are abdominal pain, bleeding of the rectum, bloody diarrhea, weight loss, fatigue, and fever, which can start gradually or begin all at once (Rodriguez-Canales et al. 2016). 5-Aminosalicylic acid (5-ASA), monoclonal antibodies (infliximab), immunosuppressive agents, and glucocorticoids are drugs currently used for UC therapy. Nevertheless, the long-term use of these therapeutic drugs can lead to allergy, toxicity, bleeding of the upper gastrointestinal part, and other side effects (Gao et al. 2019). Therefore, it is urgent to explore new strategies for UC treatment that are safer, cheaper, more effective, and have fewer side effects. In this context, natural products, in addition to their chemical compounds, have been proposed as candidates for new drug development owing to their wide therapeutic range as well as low toxicity, side effects, and cost (Park &Park 2013).

It is thus important to control inflammation to prevent conditions such as allergies, autoimmune diseases, metabolic syndrome, and cancer (Elinav et al. 2013). NF-E2-related factor-2 (Nrf2) is one of the main transcription factors responsible for cellular protection from oxidative stress, and it has been

identified to mitigate inflammation (Kobayashi et al. 2013). In the normal physiological state in the absence of stress, Nrf2 is degraded following attachment to cytoplasmic protein Kelch-like ECH-associated protein-1 (Keap1). On the other hand, Nrf2 degradation is inhibited in the presence of oxidative stress, which then leads to Nrf2 accumulation. Nrf2 then moves into the nucleus and binds with one of the small Maf proteins to form a heterodimer (Gerstgrasser et al. 2017), which binds to the regulatory regions of Nrf2 target genes, promoting their increased transcription. It was reported that Nrf2 deficiency could increase the severity of inflammation in animal models, such as models of sepsis and emphysema (Thimmulappa et al. 2006).

Natural products are a great source of drugs, and they have the potential for drug screening. Chicoric acid is extracted from *Cichorium intybus* L and other plants. Chicoric acid has been found to ameliorate inflammation induced by lipopolysaccharides in both cell culture and mice. Reduced inflammation was associated with the downregulation of nuclear factor kappa B (NF-κB) and tumor necrosis factor-alpha (TNF-α), which are two major regulators of inflammation responses (Liu et al. 2017a, Liu et al. 2017b). Several other proinflammatory factors, including nitric oxide synthase, cyclooxygenase-2, prostaglandin E2, interleukin1b (IL-1b), IL-12, and IL-18, have also been reported to be downregulated by chicoric acid (Kour et al. 2016, Liu et al. 2017a, Liu et al. 2017b, Matthias et al. 2007). However, two relevant studies showed results inconsistent with the above. Matthias et al. (2008) reported that LPS inhibited NF-κB expression, which was reversed by chicoric acid treatments in Jurkat E6.1 leukemia T cell lymphoblasts. The other reported that echinacea extracts (containing chicoric acid) upregulated LPS-induced TNF-α in rat alveolar macrophages (Goel et al. 2002).

Chicoric acid has been found to have a high oxygen radical scavenging capacity, reducing the ROS level and protecting cells from free radical-induced cytotoxicity (Dalar &Konczak 2014, Wang et al. 2017). Moreover, chicoric acid increases the generation of antioxidative enzymes that reduce ROS levels, that is, glutathione, glutathione peroxidase, superoxide dismutase, chloramphenicol acetyltransferase, heme oxygenase, and NAD(P)H dehydrogenase, in various cells (Chen et al. 2017, Wang et al. 2017). The underlying mechanism of the antioxidative effects of chicoric acid is attributed to its ability to enhance nuclear translocation of nuclear factor erythroid 2-related factor 2 (Nrf-2) and the level of peroxisome proliferator-activated receptor-c coactivator a (PGC-1a) (Schlernitzauer et al. 2013, Wang et al. 2017). Since oxidative stress is closely related to the development of certain cancers and chronic diseases, all these findings suggest the potential future application of chicoric acid (CA) for oxidative stress-associated disorders (Peng et al. 2019). Therefore, this present study was designed to investigate other possible mechanisms responsible for the protective effects of CA against colitis induced by dextran sulfate sodium (DSS) in rats that could lead to the plausible use of CA in the treatment of UC.

Materials And Methods

Animal care and use statement

Adult male Wistar rats (200–225 g) were purchased from EgyVac (Helwan, Cairo, Egypt) for use in this current study. Animals were given free access to a standard rodent diet and water under a 12 h light/dark cycle and were housed at a constant 24°C. Experiments were performed in accordance with the Animal and Research Ethics Committee for Laboratory Animal Care, Faculty of Science, Helwan University (Cairo, Egypt), which approved the protocol (number HU2020/Z/AEG0120-02).

Colitis And Ca Treatment

Colitis was induced by supplementing drinking water with 4% (w/v) DSS)40 kDa; Sigma-Aldrich Chemical Company, St. Louis, MO, United States) for 7 d (Qin et al. 2019). Rats were randomly assigned to the following six groups ($n = 7$): the control group, which received normal drinking water without DSS; the CA group, which received oral administration of CA (Weikeqi Biological Technology Co., Ltd, Sichuan, China) at 100 mg/kg body weight to test the safety of CA when administered in rats, the dose selected as per Wang et al. (2017); the DSS group, which received 4% DSS in drinking water; the CA + DSS group, which received 4% DSS in drinking water and an oral administration of CA (100 mg/kg); and the 5-ASA + DSS group, which received 4% DSS in drinking water and was orally administered 5-ASA (Minapharm-Egypt, Cairo, Egypt) at a dose of 100 mg/kg, as per Almeer et al. (2018). All groups received treatment for 7 d and were sacrificed on the eighth day *via* cervical dislocation. Colons were then harvested for macroscopic, biochemical, molecular, and histological examinations.

Assessment Of Macroscopic Colonic Damage

Macroscopic colonic damage was estimated following the scoring system used by Almeer et al. (2018) as follows: no macroscopic changes (0 points), no ulceration + hyperemia (1 point), slight mucosal edema (2 points), moderate edema at one site (3 points), severe ulcers at more than one site < 5 mm (4 points), and extreme ulcers at more than one site > 5 mm (5 points).

Histopathological Examination

Colon tissue samples were collected and fixed in 10% neutral formalin; these were then embedded in paraffin and stained with hematoxylin and eosin for histological assessment. The histological injury was estimated as the collective score of the infiltration of inflammatory cells (score 0–3) along with the damage of mucosa (score 0–3) as described by Zhang et al. (2014) and Yue et al. (2018).

Assessment Of Oxidative Stress Markers

Colon samples were mixed and homogenized using 50 mmol Tris–HCl buffer (pH: 7.4), and the homogenate was centrifuged at 3000 x g at 4°C for 10 min. Malondialdehyde (MDA), nitric oxide (NO), and reduced glutathione levels were detected in the supernatants based on the methods reported by

Ohkawa et al. (1979), Green et al. (1982), and Sedlak and Lindsay (1968), respectively. The antioxidant enzymatic activities of superoxide dismutase (SOD) and catalase (CAT) were assessed based on the methods previously described by Nishikimi et al. (1972) and Aebi (1984), respectively. The activities of glutathione peroxidase (GPx) and glutathione reductase (GR) were also evaluated based on the methods described by Paglia and Valentine (1967) and Carlberg and Mannervik (1985), respectively.

Assessment Of Apoptotic Mediators

The level of Bax protein in the supernatant was determined using an ELISA kit according to the manufacturer's protocol (Abcam, cat no. ab32503). Additionally, caspase-3 activity was assessed using an ELISA kit obtained from Cusabio (cat no. CSB-E08857r). The level of Bcl-2 was estimated using an ELISA kit (MyBioSource; cat no: MBS2881713) according to the manufacturer's instructions.

Assessment Of Inflammatory Mediators

The levels of IL-1β (cat no. RLB00), IL-6 (cat no. R6000B), and tumor necrosis factor-α (TNF-α) (cat no. RTA00) were detected in the supernatant using ELISA kits following the manufacturer's instructions (R and D systems, Minneapolis, MN, United States). Colon myeloperoxidase (MPO) activity was also estimated as a marker of neutrophil infiltration using the method described by Bradley et al. (1982), with minor modifications. Homogenates were then frozen and thawed for three cycles, followed by centrifugation for 10 min at 10000 x g at 4°C. Subsequently, 0.1 mL of the supernatant was mixed with 2.9 mL of 0.05 mol/L phosphate buffer (pH 6.0) and 1 mL of 1.6 mmol/L o-dianisidine hydrochloride containing 0.0005% H_2O_2 (v/v). The absorbance change was reported at 460 nm. Additionally, prostaglandin E2 (PGE2; cat no. CSB-E07967r) levels, cyclooxygenase II (cat no. CSB-E13399r) activity, and peroxisome proliferator-activated receptor gamma (PPARγ; cat no. CSB-E08624r) levels were measured using the corresponding ELISA kits (Cusabio, Hubei, China) following the manufacturer's instructions.

Expression of colonic Nos2, Mapk14, and Nfe2l2

Total RNA was extracted from the colon, from which complementary deoxyribonucleic acid was synthesized using the protocol provided by Abdel Moneim (2016). A reverse transcriptase-polymerase chain reaction was used to assess the expression of *Nos2, Mapk14*, and *Nfe2l2* using an Applied Biosystems 7500 device and SYBR Green Master Mix (Life Technologies, CA, United States). Transcription of the target genes was normalized to messenger ribonucleic acid (mRNA) levels of *Gapdh*, and data analysis was performed using the $2^{-\Delta\Delta CT}$ method. The primer sequences of the target genes used are shown in Table 2.

Table 1
Criteria for disease activity index

Score	Weight loss (%)	Stool consistency	Bloodstain or gross bleeding
1	None	Normal	Negative
2	1–5	Loose stool	Negative
3	5–10	Loose stool	Positive
4	10–15	Diarrhea	Positive
5	>15	Diarrhea	Gross bleeding

Table 2
Primer sequences of genes analyzed in real time polymerase chain reaction

Name	Forward primer (5'-3')	Reverse primer (5'-3')
Gapdh	AGTGCCAGCCTCGTCTCATA	GATGGTGATGGGTTTCCCGT
Nfe2l2	TTGTAGATGACCATGAGTCGC	ACTTCCAGGGGCACTGTCTA
Nos2	GTTCCTCAGGCTTGGGTCTT	TGGGGGAACACAGTAATGGC
Mapk14	AGAGTCTCTGTCGACCTGCT	GGGTCGTGGTACTGAGCAAA

Gapdh: Glyceraldehyde-3-phosphate dehydrogenase; Nfe2l2: Nuclear factor erythroid 2-related factor 2; Nos2: Inducible nitric oxide synthase; Mapk14: Mitogen-activated protein kinases.

Statistical analysis

All quantitative data were represented as means ± SD. Statistical comparisons between groups were determined using ANOVA, followed by Duncan's multiple comparison test (Statistic Package for Social Science, version 17.0). For the macroscopic and histological scores, Kruskal–Wallis test was used followed by Dunn multiple comparison test. Statistical differences were deemed significant when $P < 0.05$.

Results

Effects of CA on macroscopic colonic damage and histopathological examination in colitis rats

Macroscopic images of experimental colitis revealed transmural inflammation of the colon, accompanied by signs of hyperemia, mucosal edema, corrosion, necrosis, and ulcerations (Fig. 1A). However, co-administration of CA (100 mg/kg) doses led to less severe hyperemia, mucosal edema, necrosis, corrosion, and ulceration, with a significant reduction in the grade of colonic damage by about 32% and

49%, respectively, in comparison with untreated rats (Fig. 1B). The administration of 5-ASA (a reference drug used for colitis treatment) showed a better reduction than CA.

Furthermore, healthy and CA-treated rats showed a normal mucosal epithelium layer (Figs. 2A and B). However, histopathological examination of the colitis rats revealed erosion of the mucosal layer with subsequent desquamation and detachment of the epithelial layer, necrosis in the intestinal crypts, bleeding, and focal infiltration of neutrophils (Fig. 2C). However, CA-treated rats manifested a decrease in the degree of ulcerative lesions and crypt damage (Fig. 2D). 5-ASA showed a reduction in the degree of ulcerative lesions and crypt damage (Fig. 2E). Consistent with the pathological observations, a significant reduction of the histopathological score was observed in the CA-treated colitis rats compared to the colitis group, particularly at the high dose of CA. Similarly, the histopathological scores in the CA-treated and 5-ASA-treated groups were decreased compared to the colitis group (Fig. 1C).

Effect Of Ca On Oxidative Stress In Colitis Rats

The glutathione level and SOD, CAT, GPx, and GR activities in colitis rats have been observed to have significantly diminished as compared to the control group. However, these reduced levels and activities were significantly alleviated with CA or 5-ASA administration. Compared with 5-ASA, CA exhibited a greater ameliorative effect. On the other hand, MDA levels were significantly elevated in colitis rats, while CA administration exerted a remarkable decrease in this elevation. Similarly, DSS-induced colitis markedly increased the levels of NO and *Nos2* gene expression compared to the control group. Nevertheless, CA or 5-ASA administration notably reduced these elevated levels (Fig. 3). The abovementioned results support that CA treatment increased antioxidant ability in the colon in colitis rats.

Effect of CA on the mRNA expression of Nfe2l2 in colitis rats

DSS treatment caused the downregulation of *Nfe2l2* mRNA levels (Fig. 4). CA or 5-ASA administration led to the attenuation of this altered mRNA expression. Moreover, the attenuating effect of CA (100 mg/kg) was greater than that of 5-ASA.

Effect Of Ca On Inflammatory Status In Colitis Rats

The levels of the pro-inflammatory cytokines (IL-1β, IL-6, and TNF-α) were notably elevated in colitis rats compared to the control group. Even so, these elevated levels were significantly diminished upon CA or 5-ASA administration. Also, the data clarified that the COX-II activity, PGE2 levels, and MPO activity, in addition to *Mapk14* mRNA expression, were markedly increased in the DSS group, and CA administration has dramatically decreased these levels. The PPAR-γ levels were also decreased in the DSS group, the loss of which was attenuated with CA administration. Interestingly, CA (100 mg/kg) showed higher improvements in the measured inflammatory markers compared to 5-ASA (100 mg/kg). These data illustrated the anti-inflammatory properties of CA, as well as its ability to attenuate neutrophil infiltration,

as manifested by the repression of MPO activity, an enzyme released from neutrophils, in colitis rats (Fig. 5).

Effect Of Ca On Apoptotic Markers In Colitis Rats

Apoptosis was enhanced in the colitis group, as indicated by increased levels of Bax and caspase-3 activity, in addition to the reduced levels of Bcl-2 (Fig. 6). These levels were significantly mitigated upon CA or 5-ASA administration. These results suggest that CA exerted an anti-apoptotic effect in colitis rats.

Discussion

Ulcerative colitis is often characterized by intensive injury and inflammation of the colon, along with a series of clinical symptoms, such as visceral pain, diarrhea, bloody stool, and body weight loss (Fumery et al. 2018). DSS administration for 6–10 d is the most common model of induced colitis in rats, resulting in inflammation mostly in the intestinal wall mucosal lining, with disease characteristics similar to human UC (Neurath &Travis 2012). In this present study, UC was established in rats *via* administration of 4% DSS in the drinking water for 7 successive days. Histopathological examination (represented as histopathological score) found intense damage on the colon mucosa, recognized by goblet cell loss and infiltration of immune cells, in DSS-treated rats. However, CA significantly ameliorated this colon damage induced by DSS administration. The obtained results are consistent with those of Mateus et al. (2022), who reported that phenolic compounds protect from colitis, as it reduced the expansion of inflammation.

The high rate of inflammation in colon tissue can lead to the overproduction of ROS and induction of oxidative stress, which can then cause intestinal cell injury and further increase the degree of inflammation (Jena et al. 2012). SOD, CAT, GPx, and GR are enzymatic antioxidants responsible for the suppression of ROS generation. It has been reported that the enzymatic activities of SOD, CAT, and GPx are reduced during colitis (Ighodaro &Akinloye 2018). Consistent with this, SOD, CAT, GPx, and GR activities were reduced in the DSS treatment group, while CA administration rescued these enzymatic activities as compared to the DSS group. Moreover, markedly elevated levels of MDA, NO, and *Nos2* mRNA expressions were observed in the DSS-challenged rats, whereas CA attenuated these abnormal elevations. The antioxidant activity of CA has been previously reported (Zhu et al. 2018), in addition to its ability to reduce MDA levels responses (Liu et al. 2017a).

In this current study, a marked increase in the levels of the apoptotic proteins Bax and caspase-3 and a significant decrease in the level of the anti-apoptotic protein Bcl-2 were detected in the DSS group. This finding is compatible with a study suggesting that gut inflammation can lead to apoptotic activity enhancement in the mucosa of the colon (Andaloussi et al. 2013). The obtained results indicated that CA exerted an anti-apoptotic effect in colitis-induced rats. This result is compatible with the findings of Tsai et al. (2017), who recorded that CA inhibited the apoptotic pathway in oxidized low-density lipoprotein-induced human endothelial dysfunction *via* downregulating caspase-3 and Bax expression. The anti-

apoptotic property of CA is related to its ability to extinguish ROS and elicit mitochondrial apoptotic reactions (Tsai et al. 2017).

Various pro-inflammatory cytokines released from immune cells have important roles in colitis initiation and development. For instance, IL-1β, IL-6, and TNF-α can evoke signal transduction related to inflammation after binding with specific receptors, thus intensifying the inflammatory response and leading to tissue injury in several inflammatory disorders (Almeer et al. 2019). Hence, one of the main targets in colitis therapy is these pro-inflammatory cytokines (Yamamoto-Furusho 2018). In the present study, high levels of these pro-inflammatory cytokines (1β, IL-6, and TNF-α) in the colon tissues of colitis-induced rats were observed, whereas, upon CA administration, these elevated levels were observed to decrease. MPO, an enzyme released from neutrophils, induces the production of ROS. The release of MPO from cells can exacerbate the damage to tissues through inflammation of the extracellular matrix. MPO, which is a marker for the infiltration of neutrophils, can be used to assess the severity of ulcerative colitis (Masoodi et al. 2012). Inducible nitric oxide synthase (iNOS) and COX-II are enzymes responsible for the synthesis of NO and PGE2, respectively, which are found in damaged intestinal mucosa and submucosa (Oh et al. 2014). Therefore, iNOS and COX-II can be regarded as inflammatory damage indicators. In this present work, CA treatment significantly decreased the elevation of MPO, iNOS, NO, COX-II, and PGE2 in the DSS group. These results are consistent with the previous study that reported that CA can alleviate the inflammation in acute liver injury induced by lipopolysaccharide (LPS) and d-galactosamine through downregulation of the expression of NF-κB and MAPKs in addition to MPO activity (Li et al. 2020). Furthermore, CA showed an anti-inflammatory effect in LPS-induced inflammation on yak peripheral blood mononuclear cells by downregulating inflammation-related factors and cytokines (Xue et al. 2021). It was also declared that CA diminished the expression of iNOS and NO production because of its capability to scavenge NO (Park et al. 2011).

Inflammatory stimuli can also activate the MAPK signaling pathway. MAPKs have been determined to be involved in the regulation of the release of pro-inflammatory cytokines TNF-α, IL-1β, and IL-6, as well as anti-inflammatory cytokine IL-10 (Jia et al. 2020, Qian et al. 2015). It was reported that MAPK activity was markedly elevated upon intestinal epithelial damage in IBD; moreover, tissue immunohistochemistry showed elevated p38 MAPK expression levels in neutrophils and macrophages of the intestinal lamina propria (Waetzig et al. 2002). Phosphorylation of p38 MAPK was drastically increased in patients with UC compared to normal individuals, and a positive correlation was observed with the degree of UC (Zhao et al. 2011). In this current study, the expression level of the *Mapk14* gene (which encodes Mapk14, one of the four p38 MAPKs) was elevated in colitis rats, and CA was able to alleviate this elevation.

PPAR-γ, a negative regulator of inflammation, is reduced in several inflammation-related disorders, such as UC (Byrav et al. 2013). PPAR-γ can suppress the activation and nuclear import of NF-κB through the IκBα pathway, in which NF-κB plays a major role in the regulation of the inflammatory response and pathogenesis of IBD (Su et al. 1999). A broad spectrum of PPAR-γ ligands, such as thiazolidinediones, oleanolic acids, prostaglandins, and eicosanoids, has been revealed to possess powerful anti-inflammatory activities, which are mediated *via* the binding and suppression of NF-κB by activated PPAR-

γ (Choo et al. 2015). In the present study, CA significantly increased the PPAR-γ level compared to the DSS group. Upregulation of PPAR-γ expression in colon epithelial cells can cause the suppression of the NF-κB and MAPK signaling pathways and repress inflammatory gene transcription (Abdel-Ghany et al. 2015, Moon 2014). Therefore, the anti-inflammatory effects of CA may be due in part to its PPAR-γ agonistic activity.

According to the obtained results, the *Nfe2l2* expression level was markedly elevated in CA-treated groups compared to the DSS group, while the expression level of Keap1 was decreased in the CA-treated groups compared to the DSS group. Hence, the inhibition of Keap1 may lead to activation of Nrf2, which can further enhance downstream antioxidant enzyme expression. Therefore, CA is assumed to restore the redox balance through the downregulation of Keap1 and upregulation of Nrf2, thereby repressing oxidative stress and inflammatory response and reducing the severity of colitis injury. Consistent with the present results, various agents have been reported to ameliorate colitis induced by DSS and colitis-associated colon cancer through Nrf2 activation (Lu et al. 2016, Wang et al. 2016).

Furthermore, the obtained results demonstrated that the anticolitic effect of CA at the higher dose is similar to the 5-ASA. *In vitro*, 5-ASA has many of the pharmacological actions of NSAIDs (non-steroidal anti-inflammatory drugs) such as the anti-inflammatory and anti-neoplastic actions. The anti-inflammatory action of 5-ASA included the suppression of NF-κB translocation, prevention of epithelial cell damage and death induced by oxidative stress, activation of heat shock protein response, inhibition of colonic biosynthesis of chemoattractant leukotrienes, and modulation of prostaglandin action. Moreover, 5-ASA produces effects similar to PPAR-γ (Desreumaux &Ghosh 2006).

Conclusion

This study concluded that the possible protective mechanisms of CA in a colitis animal model may involve the attenuation of oxidative stress, inflammatory cell accumulation in the colon, and inflammation through the downregulation of Keap1 and activation of Nrf2, in addition to its PPAR-γ agonistic activity. Further, the obtained results may warrant extending the action of the mechanism that cooperates with the prevention of colitis. Therefore, CA might be a useful anti-inflammatory agent and may be used as a nutraceutical to prevent colitis. But, further study is needed to explore deeply the exact anticolitic action of CA.

Declarations

Ethical approval

All the experimental protocols were approved by the Committee of Research Ethics for Laboratory Animal Care, Taif University (approval no. 44-031).

Consent to participate

Not applicable.

Consent for publication

Consented.

Authors contributions

Fahad Alharthi has performed all the research items

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Conflict of interest

The author declares no conflict of interest.

Data Availability

The collected data in this study are available from the author.

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Figures

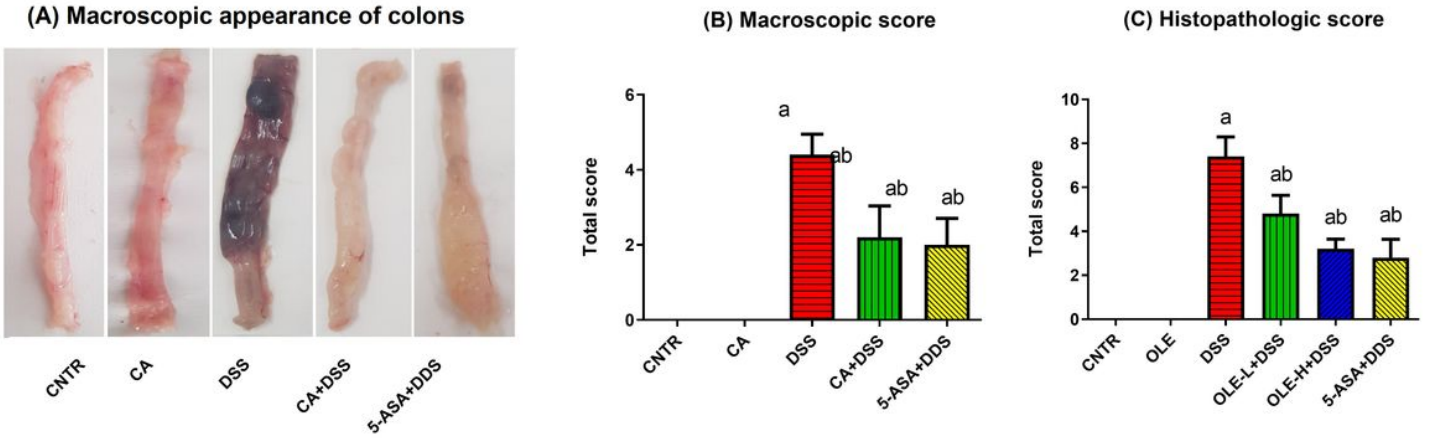

Figure 1

Effect of chicoric acid on dextran sulfate sodium-induced colitis in rats. A: Macroscopic appearance of rat colons in different experimental groups; B: Macroscopic score; and C: histological score. The results are expressed as means ± SD (n = 7). [a]P < 0.05 compared to the control group (healthy group); [b]P < 0.05 compared to the dextran sulfate sodium-treated group. CNTR: Control group; CA: Colon from the chicoric acid; DSS: Dextran sulfate sodium; 5-ASA: 5-Aminosalicylic acid; DAI: Disease activity index.

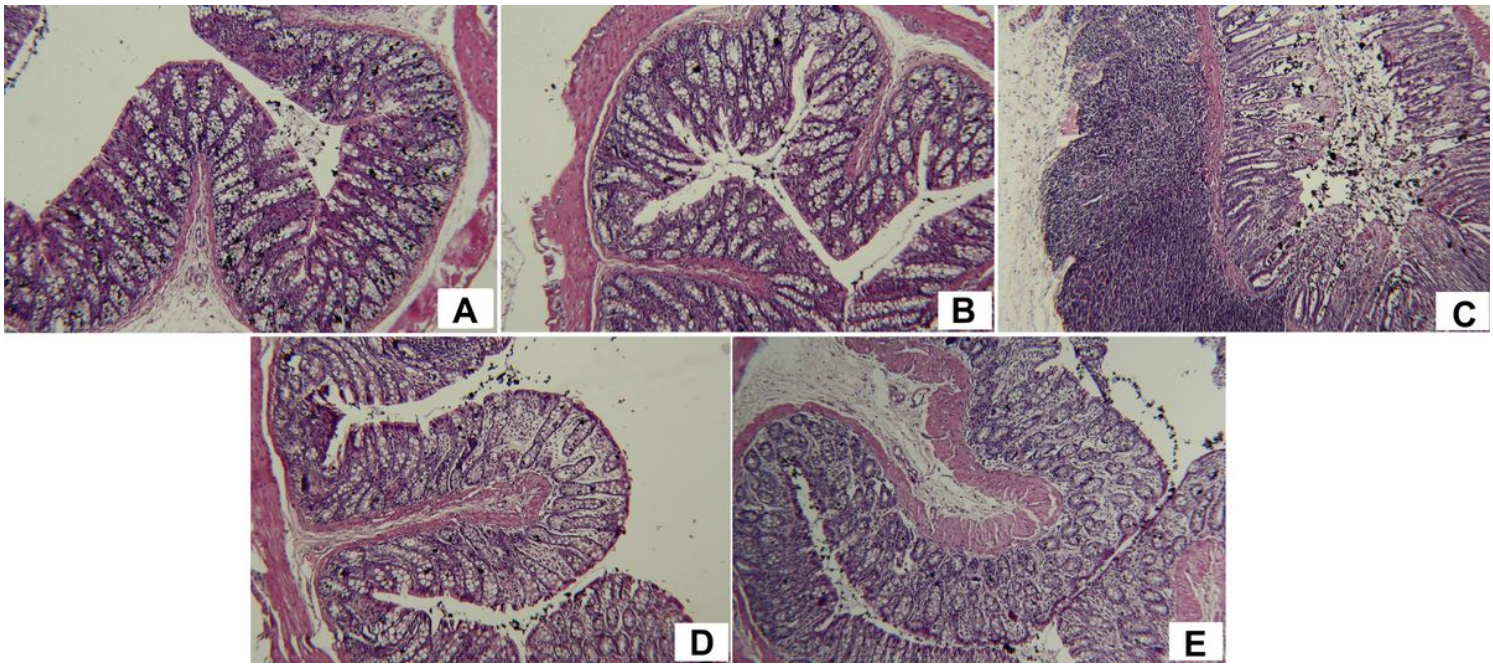

Figure 2

Effect of chicoric acid on dextran sulfate sodium-induced colitis in rats. A: Colon from the control group (healthy group); B: Colon from the chicoric acid (CA) group (without dextran sulfate sodium); C: Colon from dextran sulfate sodium experimental colitis group; D: Colon from CA (100 mg/kg) and experimental

colitis-treated group; E: Colon from 5-aminosalicylic acid (100 mg/kg) and experimental colitis-treated group. All images were taken at 200 × magnification.

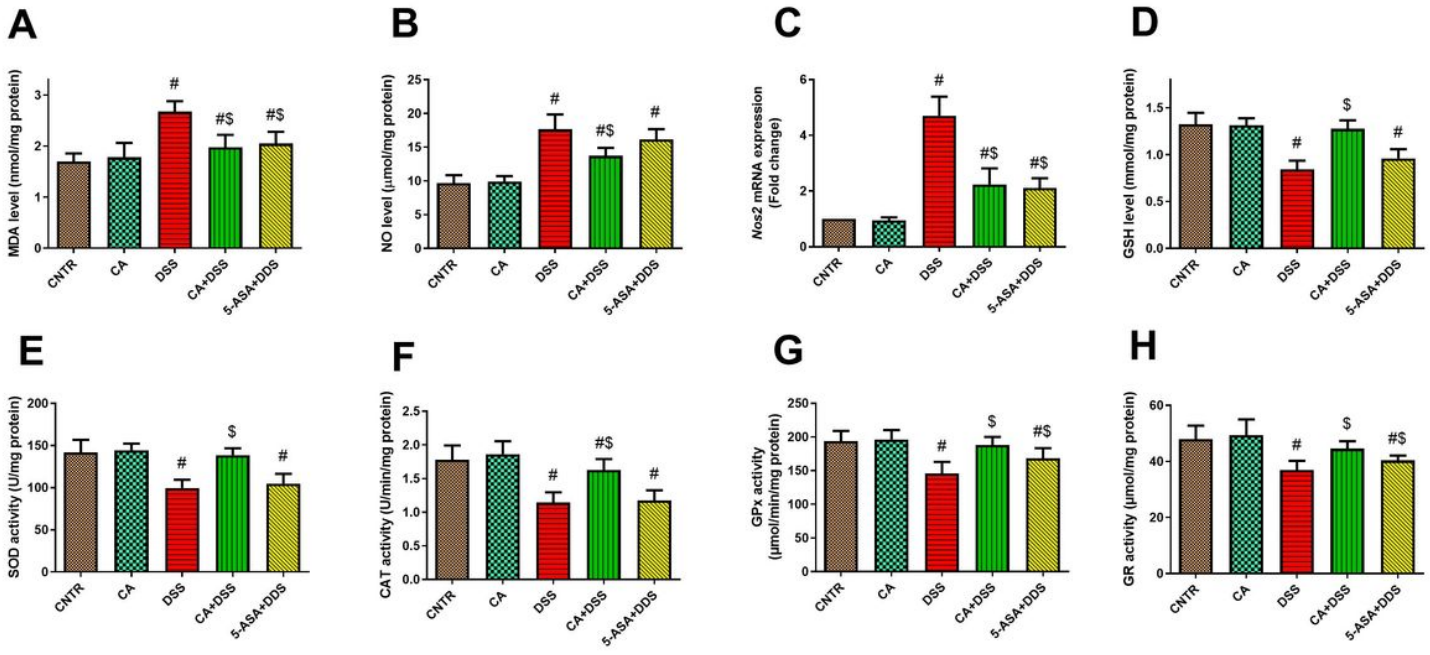

Figure 3

Antioxidant effects of chicoric acid on dextran sulfate sodium-induced colitis in rats. A: Malondialdehyde; B: Nitric oxide; C: Nos2; D: Glutathione; E: Glutathione peroxidase; F: Glutathione reductase; G: Superoxide dismutase; and H: Catalase. The biochemical results are expressed as means ± SD (n = 7). Reverse transcriptase-polymerase chain reaction results of Nos2 were normalized with Gapdh messenger ribonucleic acid levels and were represented as fold change compared to messenger ribonucleic acid levels in control group rats. [a]P < 0.05 compared to the control group (healthy group); [b]P < 0.05 compared to the dextran sulfate sodium group. CNTR: Control group; CA: Colon from the chicoric acid; DSS: Dextran sulfate sodium; 5-ASA: 5-Aminosalicylic acid; DAI: Disease activity index; GSH: Glutathione; MDA: Malondialdehyde; mRNA: Messenger ribonucleic acid; GPx: Glutathione peroxidase; GR: Glutathione reductase; SOD: Superoxide dismutase; CAT: Catalase.

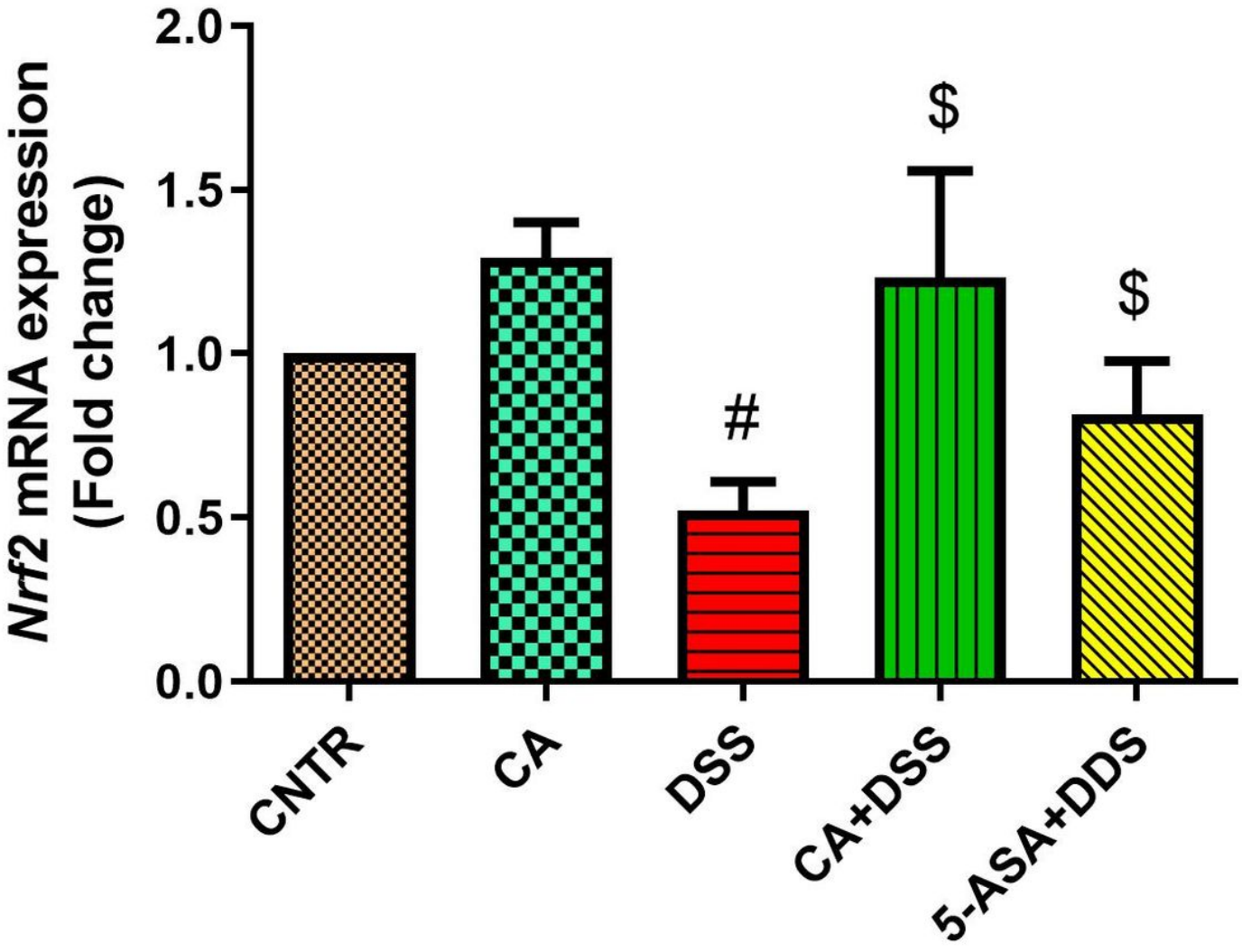

Figure 4

Effect of chicoric acid on Nfe2L2 messenger ribonucleic acid (mRNA) expression in colonic tissue of experimental colitis in rats. The results are expressed as means ± SD (n = 7). Reverse transcriptase-polymerase chain reaction results were normalized to Gapdh mRNA levels and represented as fold change compared to mRNA levels in control group rats. [a]P < 0.05 compared to the control group (healthy group); [b]P < 0.05 compared to dextran sulfate sodium group. CNTR: Control group; CA: Colon from the chicoric acid; DSS: Dextran sulfate sodium; 5-ASA: 5-Aminosalicylic acid; Nrf2: NF-E2-related factor-2.

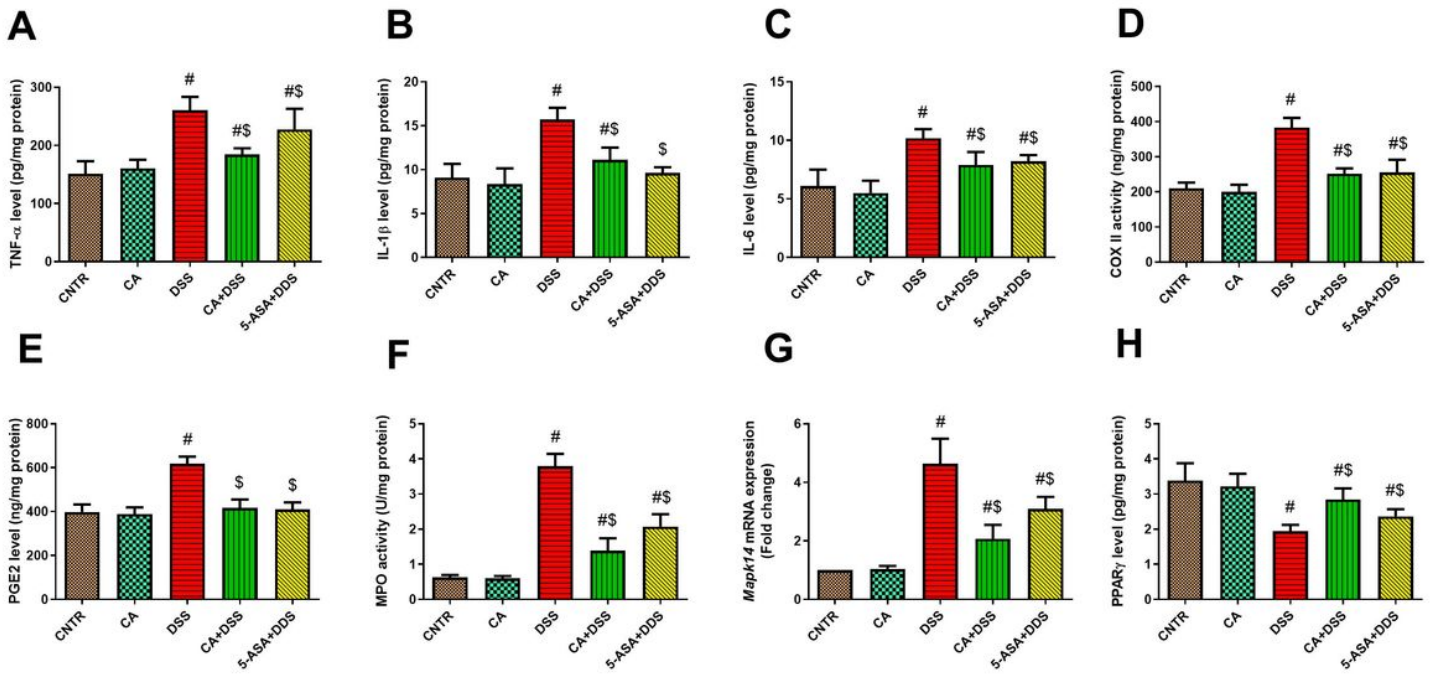

Figure 5

Effect of chicoric acid on inflammatory mediators and cytokines. A: Tumor necrosis factor-α; B: Interleukin-1β; C: Interleukin-6; D: Cyclooxygenase II; E: Prostaglandin E2; F: Myeloperoxidase; G: Mitogen-activated protein kinases (Mapk14); and H: Peroxisome proliferator-activated receptor gamma. ELISA and biochemical results are expressed as means ± SD (n = 7). Reverse transcriptase-polymerase chain reaction results of Mapk14 were normalized with Gapdh mRNA levels, and these were represented as fold change compared to mRNA levels in control group rats. [a]P < 0.05 compared to the control group (healthy group); [b]P < 0.05 compared to dextran sulfate sodium-untreated group. CNTR: Control group; CA: Colon from the chicoric acid; DSS: Dextran sulfate sodium; 5-ASA: 5-Aminosalicylic acid; IL: Interleukin; TNF-α: Tumor necrosis factor-α; PGE2: Prostaglandin E2; MPO: Myeloperoxidase; Mapk14: Mitogen-activated protein kinases; mRNA: Messenger ribonucleic acid; PPAR-γ: Peroxisome proliferator-activated receptor gamma.

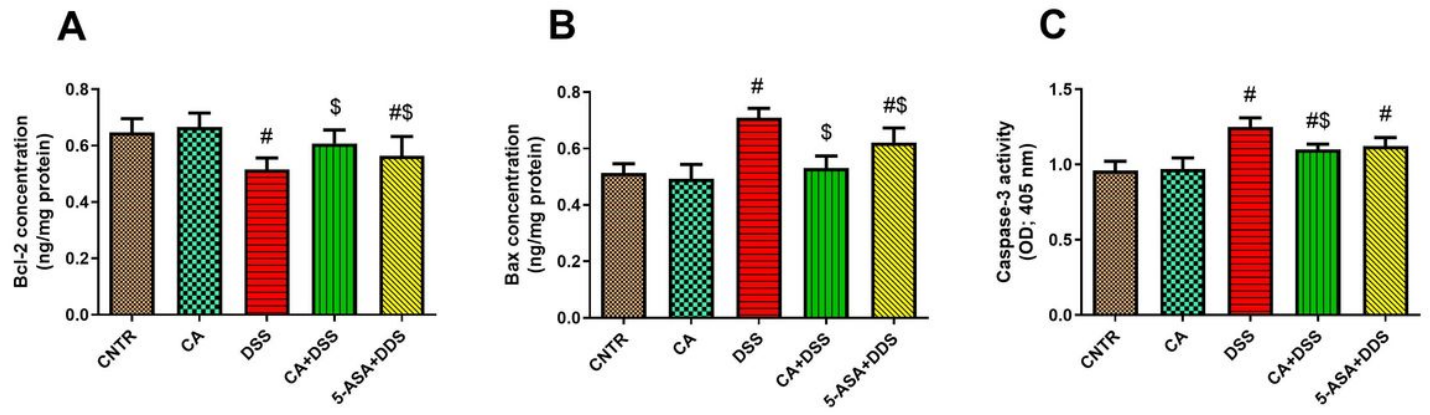

Figure 6

Effect of chicoric acid on apoptosis-related proteins. A: Bcl-2; B: Bax, and C: caspase-3 in colonic tissue in experimental colitis rats. The results are expressed as means ± standard deviation (n = 7). [a]P < 0.05 compared to the control group (healthy group); [b]P < 0.05 compared to dextran sulfate sodium group. CNTR: Control group; CA: Colon from the chicoric acid; DSS: Dextran sulfate sodium; 5-ASA: 5-Aminosalicylic acid.