Pharmacological Reports 67 (2015) 616–623

Contents lists available at ScienceDirect

Pharmacological Reports

journal homepage: www.elsevier.com/locate/pharep

Original research article

Effect of chenodeoxycholic acid and sodium hydrogen sulfide in dinitro benzene sulfonic acid (DNBS) – Induced ulcerative colitis in rats

Nidhi Goyal, Ajay Rana, Krishna Reddy V. Bijjem, Puneet Kumar *

Department of Pharmacology, I.S.F College of Pharmacy, Moga, India

ARTICLE INFO

Article history:
Received 12 October 2014
Received in revised form 25 December 2014
Accepted 30 December 2014
Available online 15 January 2015

Keywords:
2,4-Dinitrobenzene sulfonic acid
Farnesoid X receptor
Hydrogen sulfide
Oxidative stress
Ulcerative colitis

ABSTRACT

Background: Ulcerative colitis is a chronic inflammatory condition in which the inflammatory response confined to the colon. There is a need to explore the new targets for UC such as Farnesoid X receptor and hydrogen sulfide pathway.

Methods: Wistar rats of either sex (200–250 g) were used. 2,4-Dinitrobenzene sulfonic acid (DNBS) (25 mg/rat) given by rectal route into the colon to induced symptoms of ulcerative colitis. Chenodeoxycholic acid (CDCA) (10 and 20 mg/kg) and sodium hydrogen sulfide (NaHS) (10 and 30 μmol/kg) and a inhibitor of cystathionine-γ-lyase enzyme (CSE) i.e. DL-propargylglycine (10 mg/kg) treatment given along with 2,4-dinitrobenzene sulfonic acid. The disease activity index was assessed by daily change in body weight and rectal bleed score and change in length of colon. Oxidative stress markers (reduced glutathione, malondialdehyde (MDA), nitrite, and catalase and myeloperoxidase enzyme activity), serum glutamic-oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) levels in blood serum, and cardiac hemodynamic were performed on last day.

Results: The administration of DNBS intra-rectally in rats produced loss of body weight and bloody diarrhea with significant increase in oxidative stress markers in the colon. CDCA (10 and 20 mg/kg) and NaHS (10 and 30 μmol/kg) significantly attenuated DNBS-induced UC in rats. The combination of CDCA (10 mg/kg) and NaHS (10 μmol/kg) showed synergetic effect whereas; DL-propargylglycine reversed the protective effect of CDCA.

Conclusion: The observed beneficial effects following CDCA may be due to its action through activation of CSE enzyme which leads to hydrogen sulfide generation.

Introduction

Ulcerative colitis (UC) is a chronic inflammatory condition in which the inflammatory response and morphologic changes remain confined to the colon [1]. The most consistent feature of UC is the presence of blood and mucus mixed with stool, accompanied by lower abdominal cramping which is most intense during the passage of bowel movements [2]. The previous reports have shown that till date no appropriate treatment is available to treat UC without side effects [3]. Thus, there is a need to explore the further pathways or targets involved in the pathogenesis of UC such as Farnesoid X receptor (FXR) and hydrogen sulfide (H_2S) pathway, so that the respective treatments can be proven beneficial for the patients with lesser side effects.

In the present study we used DNBS to induce UC. DNBS is a hapten administered in combination with ethanol (40–50%), to break the mucosal barrier and allow its penetration into the bowel wall [4]. The DNBS model produces acute and chronic

Abbreviations: 6E-CDCA, 6-ethyl chenodeoxycholic acid; ALT, alanine aminotransferase; ANOVA, analysis of variance; AST, aspartate aminotransferase; CA, cholic acid; CDCA, chenodeoxycholic acid; CSE, cystathionine-γ-lyase; $CaCl_2$, calcium chloride; CO_2, carbon dioxide; DNBS, 2,4-dinitrobenzene sulfonic acid; FXR, Farnesoid X receptor; GI, gastrointestinal; GSH, glutathione; H_2O_2, hydrogen peroxide; H_2S, hydrogen sulfide; HOCl, hypochlorous acid; IBD, inflammatory bowel disease; IFN-γ, interferon-γ; IL, interleukin; iNOS, inducible nitric oxide synthase; KCl, potassium chloride; KH_2PO_4, potassium hydrogen phosphate; LPO, lipid peroxidation; LVEDP, left ventricular end diastolic pressure; MDA, malondialdehyde; MPO, myeloperoxidase enzyme; $MgSO_4$, magnesium sulfate; NaCl, sodium chloride; $NaHCO_3$, sodium bicarbonate; NaHS, sodium hydrogen sulfide; NO, nitric oxide; NSAID, non steroidal anti-inflammatory drug ;O_2oxygen; PAG, DL-propargylglycine; SGOT, serum glutamate oxaloacetate transaminase; SGPT, serum glutamate pyruvate transaminase; TNBS, 2,4,6-trinitrobenzene sulfonic acid; TNF-αtumor, necrosis factor-α; UC, ulcerative colitis.

* Corresponding author.
E-mail address: punnubansal79@gmail.com (P. Kumar).

http://dx.doi.org/10.1016/j.pharep.2014.12.018
1734-1140/

inflammation and ulceration in the colon characterized by transmural inflammation, ulceration, and fibrosis length shortening and weight increase of the colon [5].

FXR is a member of the ligand-activated nuclear hormone receptor superfamily. FXR primarily act as a bile acid sensor. Recent evidence suggests that the bile acid-FXR interaction is involved in the pathophysiology of a wide range of diseases of the liver, biliary and gastrointestinal tract, such as IBD [6]. FXR receptor is reported to play an important role in the maintenance of innate immune and inflammatory reactions and regulates barrier function and prevention of bacterial translocation in the gastrointestinal tissue [6]. Further, administration of 6-ethyl chenodeoxycholic acid (6E-CDCA), a synthetic FXR agonist has been shown to attenuate TNBS-induced colitis in mice [7]. Furthermore, FXR (−/−) mice are prone to develop severe gastric and intestinal injury, possibly *via* reduced (50%) gastrointestinal expression of CSE, an enzyme required for generation of H_2S. Interestingly, pharmacological treatment with CDCA or GW4064, selective FXR agonists, produced significant gastro protection *via* selective activation of FXR and subsequent activation of CSE-dependent mechanism [8].

Therefore, on the basis of these theories, hypothesis has been generated that CDCA (a selective FXR agonist) exerts its positive effect on the UC by increasing CSE expression and subsequently H_2S release, evaluated by using NaHS (a H_2S donor) and DL-propargylglycine (selective CSE inhibitor). In addition to this, cardiac parameters are also assessed to evaluate the effect of CDCA on an isolated rat heart.

Materials and methods

Animals

The experimental protocol used in the present study was approved by Institutional Animal Ethics Committee (Approval No. IAEC/CPCSEA/M9/P161, dated: 08/03/2014). All the experiments were carried out according to guidelines of Indian National Science Academy for care and use of animals in scientific research. Forty-eight Wistar rats (180–250 g) of either sex, divided into 9 groups ($n = 6$), were employed in the present study. All the animals were fed standard chow diet and water *ad libitum*. They were acclimatized in animal house and were exposed to normal cycle of light and darkness.

Drugs and chemicals

2,4-Dinitrobenzene sulfonic acid, sodium hydrogen sulfide, DL-propargylglycine (PAG) and o-dianisidine hydrochloride were obtained from Sigma–Aldrich, St. Louis, USA. Chenodeoxycholic acid was obtained from Genetix Biotech Asia Pvt. Ltd., New Delhi, India. SGOT and SGPT kits were procured from Erba Mannheim, Germany.

Induction of colitis

Animals were kept for overnight fasting and next day fasted rats were lightly anesthetized with ether. In anesthetized rats, a catheter was inserted into the colon via the anus until approximately the splenic flexure (8 cm from the anus) containing DNBS (25 mg/rat) dissolved in 50% ethanol. Thereafter, the animals were kept for 15 minutes in a Trendelenburg position to avoid reflux. The body temperature of the animals was kept at 37 °C and the animals were allowed to recover from the anesthesia. They were then given unlimited normal diet, water and drug treatment for the next seven days [9].

- Group 1 – Normal Control;
- Group 2 – Disease Control (DNBS, 25 mg/rat intra-rectal) single injection;
- Group 3 and 4 – DNBS (25 mg/rat intra-rectal) + CDCA (10 and 20 mg/kg, *po* for 10 days);
- Group 5 and 6 – DNBS (25 mg/rat intra-rectal) + NaHS (10 and 30 μmol/kg, intra-rectal for 7 days);
- Group 7 – DNBS (25 mg/rat intra-rectal) + CDCA (10 mg/kg, *po*) + NaHS (10 μmol/kg, intra-rectal);
- Group 8 – DNBS (25 mg/rat intra-rectal) + CDCA (20 mg/kg, *po*) + PAG (10 mg/kg, *ip* for 7 days);
- Group 9 – DNBS (25 mg/rat intra-rectal) + sulfasalazine (300 mg/kg, *po* for 7 days).

All the drugs were given for 7 days after DNBS injection except CDCA. CDCA was given for 10 days starting 3 days before and continues for 7 days after DNBS injection. The doses and schedule were selected on the basis of previous studies.

Assessment of physical appearances

Body weight were observed on 1st and 7th day of the study whereas rectal bleeding was observed as part of index to determine disease activity daily from 1st to 7th day and average of bleeding score was shown in the graph. The rectal bleed scoring was given from 0 to 4 depending on the stool consistency and presence of blood. The scores were allotted as follows, 0 – normal stool, 1 – solid stool with water, 2 – liquid stool with no bleeding, 3 – liquid stool with little bleeding, 4 – gross bleeding (blood with stool). The animals were observed for seven days and on day eight, the animals were sacrificed and abdomen was opened by a midline incision. The colon was removed, freed from surrounding tissues, opened along the anti-mesenteric border, rinsed, weighed and the length of the colon was measured. Further, the colon was processed for histology and other biochemical parameters [10].

Biochemical parameters

Measurement of lipid peroxidation (LPO)

The extent of LPO in the colon homogenate was determined quantitatively by performing the method as described by Wills [19]. The values were calculated using the molar extinction co-efficient of chromophore [$1.56 \times 105\,(\text{mol/l})^{-1}\,\text{cm}^{-1}$].

Estimation of nitrite

The accumulation of nitrite in the supernatant, an indicator of the production of nitric oxide was determined by a colorimetric assay with Greiss reagent [11].

Estimation of reduced glutathione levels

Reduced glutathione was estimated according to the method described by Ellman [12]. Results were calculated using molar extinction co-efficient of the chromophore ($1.36 \times 10^4\,(\text{mol/l})^{-1}\,\text{cm}^{-1}$).

Catalase activity

Catalase activity was assessed by the method of Luck wherein the breakdown of H_2O_2 is measured [13]. The results were expressed as micromoles of hydrogen peroxide decomposed per min per mg of protein.

Estimation of TNF-α level

The quantification of TNF-α was done by rat TNF-α immunoassay kit (RayBiotech, Inc. Norcross, GA). Concentrations of TNF-α were calculated from their respective standard curves.

Measurement of myeloperoxidase (MPO) activity

Neutrophil infiltration was monitored by measuring MPO activity in colon segments according to the protocol described by Patel et al. One unit of MPO activity was defined as that degrading one micromole of peroxidise per minute at 25 °C.

Hemodynamic parameters

When the rats were sacrificed by spinal dislocation, thorax was opened and heart was excised after intraperitonial heparinization (500 U/kg body weight) and placed into chilled, heparinized perfusate to arrest the beating of the heart. The heart was immediately mounted on digital Langendorff's apparatus (RADNOTI, Monrovia, CA, USA) and perfused with Kreb's Hensleit solution (NaCl: 118 mM; KCl: 4.7 mM; $CaCl_2$: 2.5 mM; $MgSO_4 \cdot 7H_2O$: 1.2 mM; $NaHCO_3$: 25 mM; KH_2PO_4: 1.2 mM; glucose: 11 mM), gassed with 95% O_2, 5% CO_2, pH 7.4, maintained at 37 °C for the measurement of cardiac functions, a double distilled water filled latex balloon was inserted through the mitral valve into the left ventricle, and left ventricular end diastolic pressure (LVEDP) and rate of pressure development (dp/dt_{max}) were measured using pressure transducer (BIOPAC MP 100 System, CA, USA) [14].

Estimation of Liver enzyme level

Serum was separated by centrifuging blood at 2500 rpm for 10 min and the levels of SGOT and SGPT were analyzed by using a commercially available enzymatic kit (IFCC method, Erba, Germany).

Histopathological studies

The microscopic evaluation of proximal and distal parts of colon samples were fixed in 10% formaldehyde and processed routinely for embedding in paraffin. Paraffin sections were stained with hematoxylin and eosin to indicate histological features.

Statistical analysis

The results are expressed as mean ± SD The colon length, rectal bleeding and biochemical parameters were analyzed by one-way analysis of variance (ANOVA) followed by Bonferroni's *post hoc* test for multiple comparisons. The body weight and hemodynamic parameters were analyzed by two-way ANOVA followed by Bonferroni's multiple comparison *post hoc* test. $p < 0.05$ was considered as statistically significant.

Results

Effect of CDCA and NaHS alone and/or in combination on body weight and bleeding score in DNBS treated rats

Body weight were observed on 1st and 7th day of the study whereas the bleeding score was given daily from 1st to 7th day and average of bleeding score was shown in Fig. 2 DNBS treated rats showed significant decrease in body weight on day 7th day whereas the bleeding score was significantly increased as the disease progress as compared to control group. Treatment with NaHS (10 and 30 μmol/kg, *ip*), CDCA (10 and 20 mg/kg, *po*) and sulfasalazine (300 mg/kg, *po*) significantly increased body weight and decreased bleeding in DNBS treated rats. Further, the combination of low dose of NaHS (10 μmol/kg, *ip*) and CDCA (10 mg/kg, *po*) showed synergistic effect as compared to their effects alone in DNBS treated rats. However, when PAG (10 mg/kg, *ip*) was given along with CDCA (20 mg/kg, *po*), it significantly reversed the protective effect of CDCA as compared with its effect alone (Figs. 1 and 2).

Effect of CDCA and NaHS alone and/or in combination on colon length in DNBS treated rats

DNBS treated rats showed significant decrease in colon length on last day as compared to control group. Treatment with NaHS (10 and 30 μmol/kg, *ip*), CDCA (10 and 20 mg/kg, *po*) and sulfasalazine (300 mg/kg, *po*) significantly attenuated DNBS induced changes in colon as compared to disease control group. Further, the combination of NaHS (10 μmol/kg, *ip*) and CDCA (10 mg/kg, *po*) showed synergistic effect as compared to their effects alone in DNBS treated rats. However, when PAG (10 mg/kg, *ip*) was given along with CDCA (20 mg/kg, *po*), it significantly reversed the protective effect of CDCA as compared with its effect alone (Fig. 3).

Effect of CDCA and NaHS alone and/or in combination on biochemical parameters (LPO, GSH, catalase and nitrite) in DNBS treated rats

DNBS treated rats showed significant increases MDA and nitrite levels (Figs. 4 and 7), whereas decreases reduced GSH and catalase levels (Figs. 5 and 6) as compared to control group. Treatment with NaHS (10 and 30 μmol/kg, *ip*) and CDCA (10 and 20 mg/kg, *po*) significantly decreased MDA and nitrite level and increased reduced GSH and catalase level in DNBS treated rats as compared to disease control group. The sulfasalazine (300 mg/kg, *po*) also showed significant effects as compared to DNBS treated rats. Further, the combination of low dose of NaHS (10 μmol/kg, *ip*) and CDCA (10 mg/kg, *po*) showed synergistic decrease in MDA and nitrite level and increase in reduced GSH and catalase level as compared to their effects alone in DNBS treated rats. However, when PAG (10 mg/kg, *ip*) was given along with CDCA (20 mg/kg, *po*),

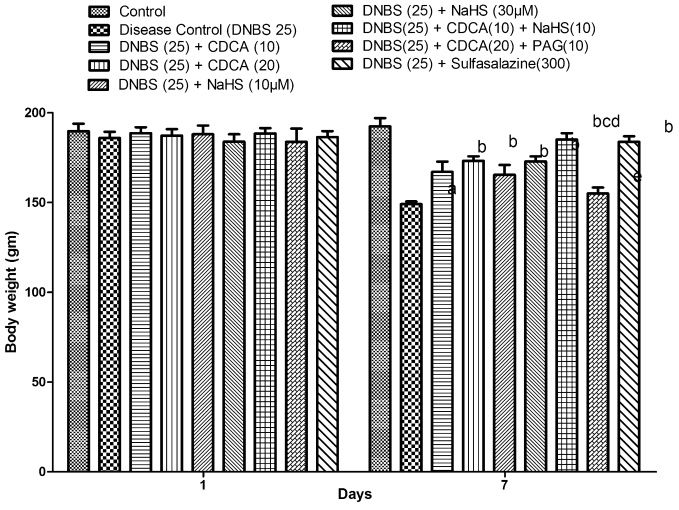

Fig. 1. Effect of CDCA and NaHS alone and/or in combination on body weight in DNBS treated rats. Data expressed as mean ± SD. [a]$p < 0.05$ *vs* normal control, [b]$p < 0.05$ *vs* DNBS treated group, [c]$p < 0.05$ *vs* CDCA (10 mg/kg), [d]$p < 0.05$ *vs* NaHS (10 μM/kg), [e]$p < 0.05$ *vs* CDCA (20 mg/kg). CDCA – chenodeoxycholic acid, NaHS – sodium hydrogen sulfide, PAG – DL-propargylglycine, DNBS – 2,4-dintrobenzene sulfonic acid.

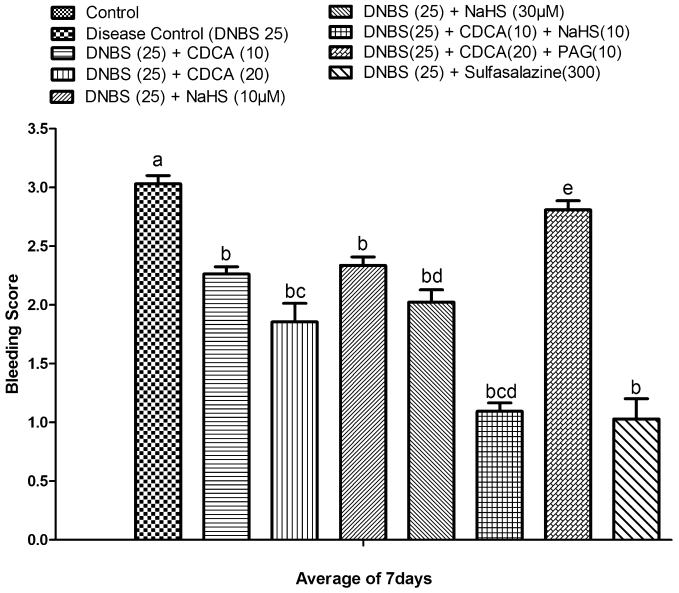

Fig. 2. Effect of CDCA and NaHS alone and/or in combination on colon length in DNBS treated rats. Data expressed as mean ± SD. $^{a}p < 0.05$ *vs* normal control, $^{b}p < 0.05$ *vs* DNBS treated group, $^{c}p < 0.05$ *vs* CDCA (10 mg/kg), $^{d}p < 0.05$ *vs* NaHS (10 μM/kg), $^{e}p < 0.05$ *vs* CDCA (20 mg/kg).

it significantly reversed the protective effect of CDCA as compared with its effect alone.

Effect of CDCA and NaHS alone and/or in combination on MPO and TNF-α in DNBS treated rats

DNBS treated rats showed significant increase in the level of MPO and TNF-α as compared to control group. Treatment with NaHS (10 and 30 μmol/kg, *ip*) and CDCA (10 and 20 mg/kg, *po*, for 10 days) significantly decreased MPO and TNF-α level in DNBS treated rats as compared to disease control group. The sulfasalazine (300 mg/kg, *po*) also showed significant decrease in MPO

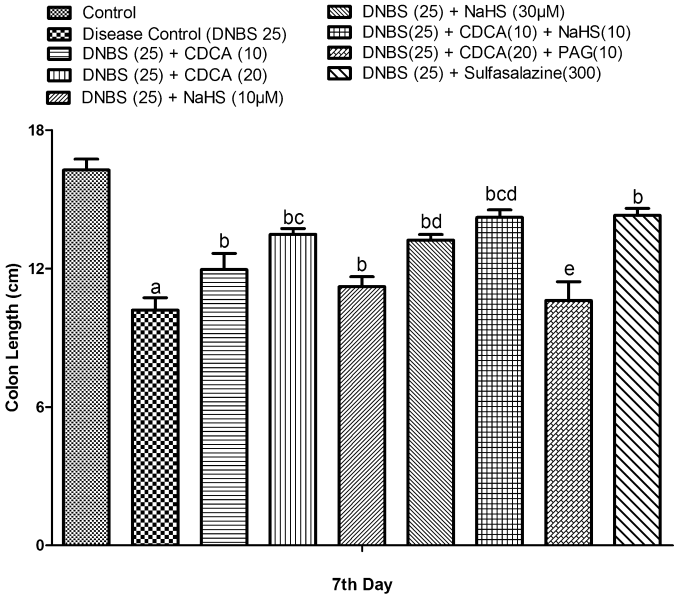

Fig. 3. Effect of CDCA and NaHS alone and/or in combination on bleeding score in DNBS treated rats. Data expressed as mean ± SD. $^{a}p < 0.05$ *vs* normal control, $^{b}p < 0.05$ *vs* DNBS treated group, $^{c}p < 0.05$ *vs* CDCA (10 mg/kg), $^{d}p < 0.05$ *vs* NaHS (10 μM/kg), $^{e}p < 0.05$ *vs* CDCA (20 mg/kg).

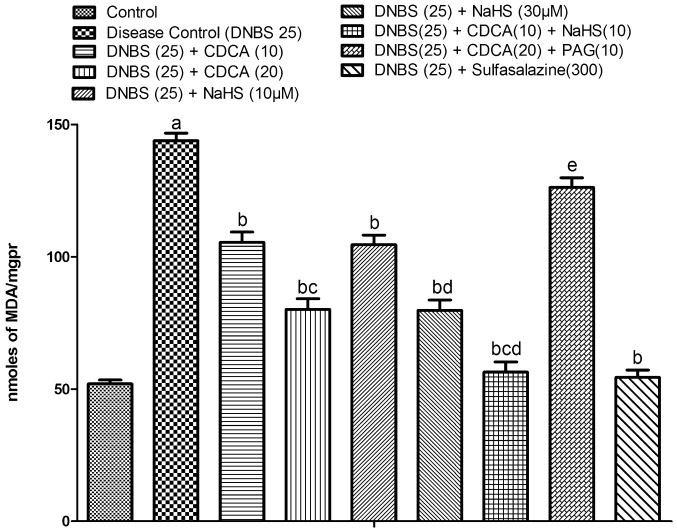

Fig. 4. Effect of CDCA and NaHS alone and/or in combination on lipid peroxidation in DNBS treated rats. Data expressed as mean ± SD. $^{a}p < 0.05$ *vs* normal control, $^{b}p < 0.05$ *vs* DNBS treated group, $^{c}p < 0.05$ *vs* CDCA (10 mg/kg), $^{d}p < 0.05$ *vs* NaHS (10 μM/kg), $^{e}p < 0.05$ *vs* CDCA (20 mg/kg).

and TNF-α in DNBS treated rats. Further, the combination of low dose of NaHS (10 μmol/kg, *ip*) and CDCA (10 mg/kg, *po*) showed significant decrease in MPO and TNF-α level as compared to their effects alone in DNBS treated rats. However, when PAG (10 mg/kg, *ip*) was given along with CDCA (20 mg/kg, *po*), it significantly reversed the protective effect of CDCA as compared with its effect alone (Figs. 8 and 9).

Effect of CDCA and NaHS alone and/or in combination on LVEDP, +dp/dt, ALT and AST in DNBS treated rats

The DNBS and pharmacological intervention with various drugs (i.e. CDCA and NaHS) treated rats did not show any significant effect on LVEDP, +dp/dt, ALT and AST level as compared to control group (Figs. 10–13).

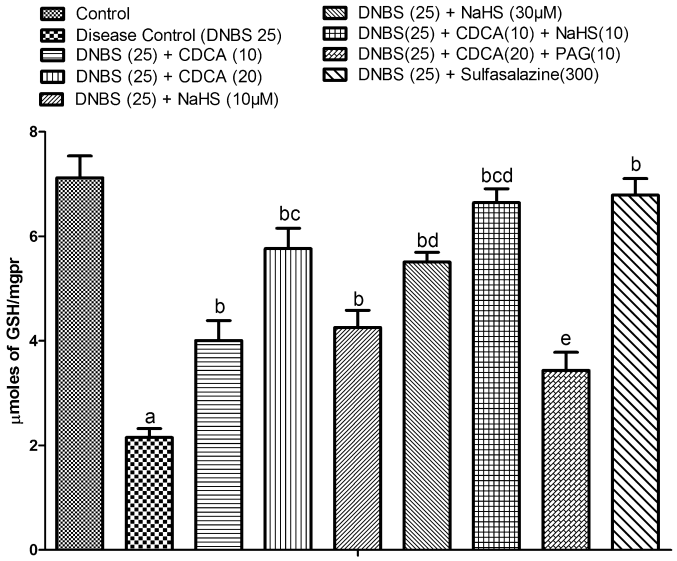

Fig. 5. Effect of CDCA and NaHS alone and/or in combination on reduced glutathione in DNBS treated rats. Data expressed as mean ± SD. $^{a}p < 0.05$ *vs* normal control, $^{b}p < 0.05$ *vs* DNBS treated group, $^{c}p < 0.05$ *vs* CDCA (10 mg/kg), $^{d}p < 0.05$ *vs* NaHS (10 μM/kg), $^{e}p < 0.05$ *vs* CDCA (20 mg/kg).

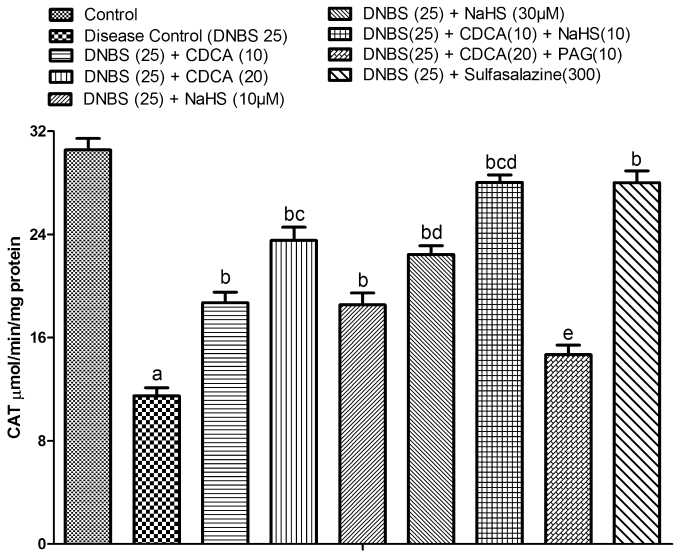

Fig. 6. Effect of CDCA and NaHS alone and/or in combination on catalase in DNBS treated rats. Data expressed as mean ± SD. $^{a}p < 0.05$ *vs* normal control, $^{b}p < 0.05$ *vs* DNBS treated group, $^{c}p < 0.05$ *vs* CDCA (10 mg/kg), $^{d}p < 0.05$ *vs* NaHS (10 μM/kg), $^{e}p < 0.05$ *vs* CDCA (20 mg/kg).

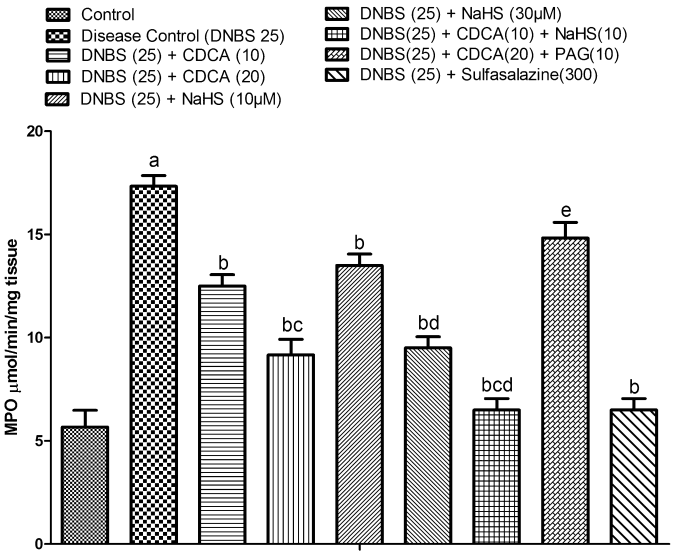

Fig. 8. Effect of CDCA and NaHS alone and/or in combination on MPO in DNBS treated rats. Data expressed as mean ± SD. $^{a}p < 0.05$ *vs* normal control, $^{b}p < 0.05$ *vs* DNBS treated group, $^{c}p < 0.05$ *vs* CDCA (10 mg/kg), $^{d}p < 0.05$ *vs* NaHS (10 μM/kg), $^{e}p < 0.05$ *vs* CDCA (20 mg/kg).

Effect of CDCA and NaHS alone and/or in combination on histopathological changes in DNBS treated rats

Rat's colon cross sections revealed normal looking mucosal epithelium with no necrosis or inflammation in the control group (Fig. 14A). In DNBS-treated rats, a diffused active UC was seen along with severe necrosis and extensive morphological disorientation, edema and diffuse inflammatory cells infiltrate in the submucosa of colon section (Fig. 14B). Treatment with CDCA (10 and 20 mg/kg, *po*) and NaHS (10 and 30 μmol/kg, *ip*) reversed histological damage, as represented by healing of the mucosal epithelium with less eroded surface surrounded by fewer inflammatory edema and less necrosis (Fig. 14C–F). Pretreatment with the combination of low dose of CDCA (10 mg/kg, *po*) and NaHS (10 μmol/kg, *ip*) resulted in a total healing of the superficial eroded mucosa with no hemorrhage, edema and necrosis and a few inflammatory cells infiltrate (Fig. 14G). Co-administration of PAG (10 mg/kg, *ip*) with CDCA (20 mg/kg, *po*) increased sub-mucosal edema and cellular infiltrates and reversed the protective effect of CDCA (Fig. 14H). Pretreatment with sulfasalazine (300 mg/kg, *po*) showed markedly healed and improved intestinal mucosa as compared to disease control group (Fig. 14I).

Discussion

IBD is a multifactorial perturbation of intestinal immune homeostasis involving alterations of intestinal barrier permeability and resulting in an aberrant immune response to luminal microbiota [15]. UC is an idiopathic, chronic inflammatory disorder of the colonic mucosa, which starts in the rectum and generally extends proximally in a continuous manner through part of, or the entire colon. The most consistent feature of UC is the presence of

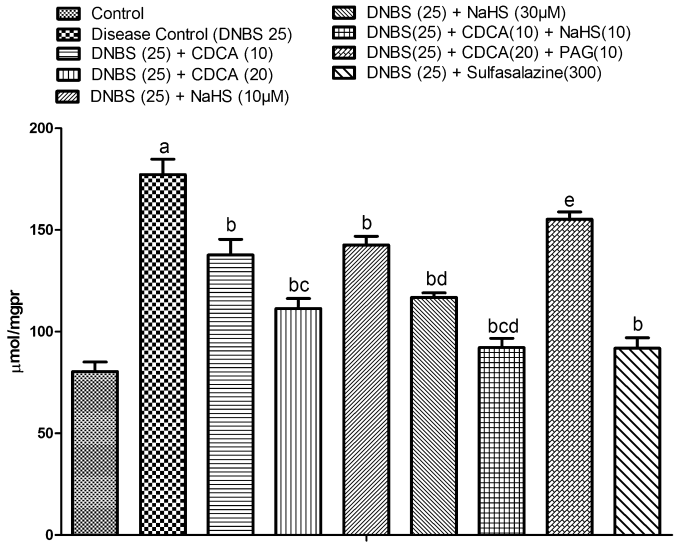

Fig. 7. Effect of CDCA and NaHS alone and/or in combination on nitrite in DNBS treated rats. Data expressed as mean ± SD. $^{a}p < 0.05$ *vs* normal control, $^{b}p < 0.05$ *vs* DNBS treated group, $^{c}p < 0.05$ *vs* CDCA (10 mg/kg), $^{d}p < 0.05$ *vs* NaHS (10 μM/kg), $^{e}p < 0.05$ *vs* CDCA (20 mg/kg).

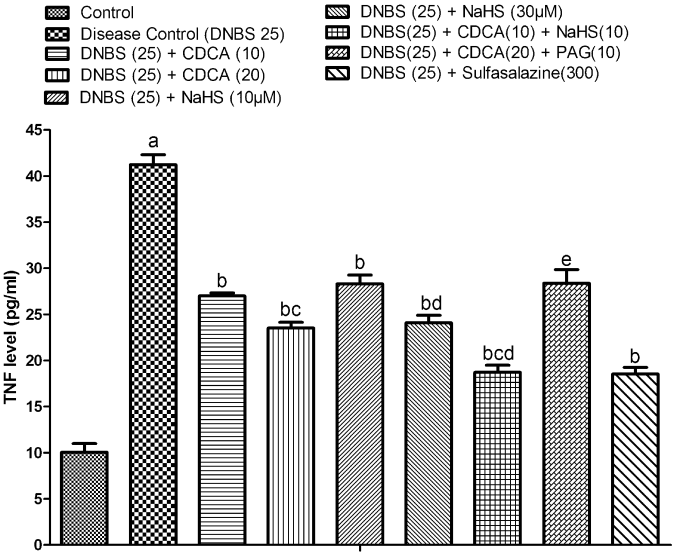

Fig. 9. Effect of CDCA and NaHS alone and/or in combination on TNF-α in DNBS treated rats. Data expressed as mean ± SD. $^{a}p < 0.05$ *vs* normal control, $^{b}p < 0.05$ *vs* DNBS treated group, $^{c}p < 0.05$ *vs* CDCA (10 mg/kg), $^{d}p < 0.05$ *vs* NaHS (10 μM/kg), $^{e}p < 0.05$ *vs* CDCA (20 mg/kg).

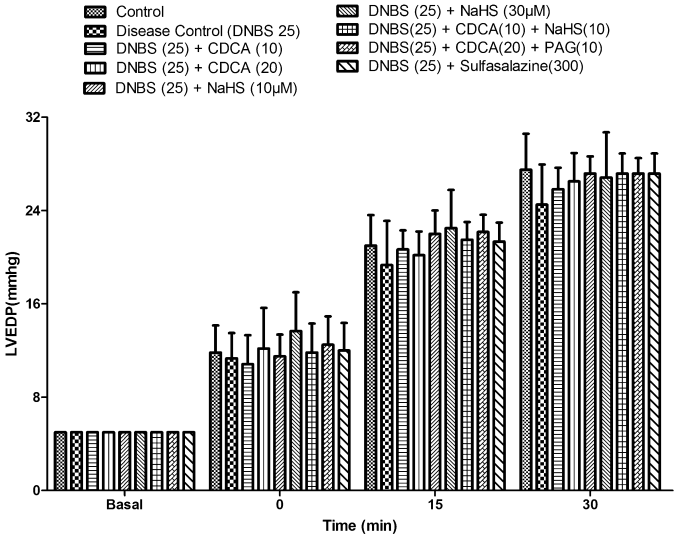

Fig. 10. Effect of CDCA and NaHS alone and/or in combination on LVEDP in DNBS treated rats. CDCA – chenodeoxycholic acid, NaHS – sodium hydrogen sulfide, PAG – DL-propargylglycine, DNBS – 2,4-dintrobenzene sulfonic acid.

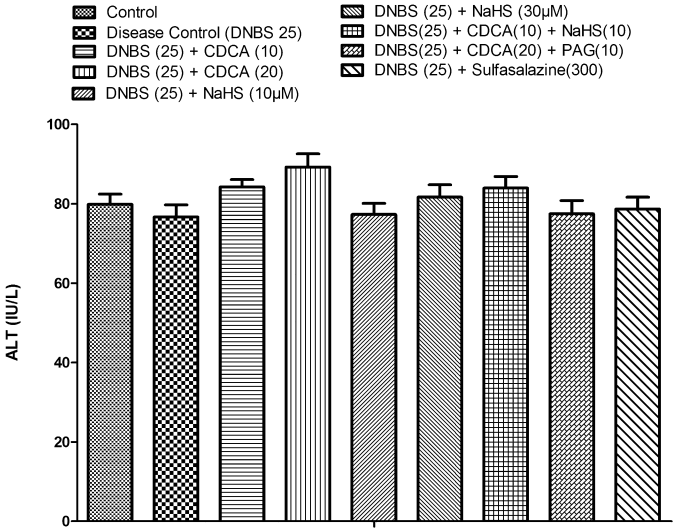

Fig. 12. Effect of CDCA and NaHS alone and/or in combination on ALT in DNBS treated rats. CDCA – chenodeoxycholic acid, NaHS – sodium hydrogen sulfide, PAG – DL-propargylglycine, DNBS – 2,4-dintrobenzene sulfonic acid.

blood and mucus mixed with stool, weight loss, fever, accompanied by lower abdominal cramping, which is most intense during the passage of bowel movements [16]. The clinical course of UC is unpredictable, marked by alternating periods of exacerbation and remission [17]. So the study needs to be done for therapeutic treatment of exacerbation and which can decrease the consequences of remission.

In this study, DNBS induced UC model is used because the symptoms produced by DNBS are clinically relevant to human UC [18]. DNBS is a hapten administered as an enema in rats or mice in combination with ethanol (40–50%), to break the mucosal barrier and allow DNBS penetration into the bowel wall. DNBS reacts with autologous proteins and stimulates delayed type of hypersensitivity which provokes severe ulcerations of the mucosal and epithelial barrier characterized by transmural infiltration of mononuclear cells [19]. DNBS leads to erosion and hemorrhagic ulceration in epithelial mucosa which may lead to rectal bleeding along with stool [9].

DNBS treated rats has been found to be associated with an overproduction of nitric oxide (NO) because of the expression of the inducible isoform of NO synthase (iNOS) which results in oxidative tissue damage [20]. In this study, DNBS treated rats has shown the elevated level of MDA because DNBS causes cell damage and hence increasing the lipid peroxidation. Since, DNBS treated rats have shown to increase oxidative stress by increasing the oxidant levels in rats, the anti-oxidant activity of catalase and reduced GSH has been found to decrease.

Increased oxidative stress is also associated with increased levels of MPO which catalyses the conversion of proportionally more stable hydrogen peroxide to unstable hypochlorous acid (HOCl) which is known to be cytotoxic in nature. In the present study, DNBS induces a strong inflammatory response and a significant increase in MPO activity compared to controls [21]. Moreover, activated macrophages produce cytokines such

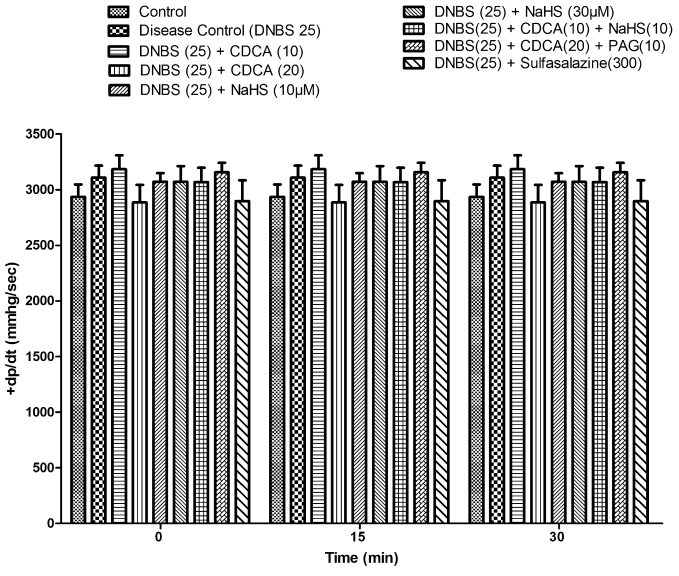

Fig. 11. Effect of CDCA and NaHS alone and/or in combination on +dp/dt in DNBS treated rats. CDCA – chenodeoxycholic acid, NaHS – sodium hydrogen sulfide, PAG – DL-propargylglycine, DNBS – 2,4-dintrobenzene sulfonic acid.

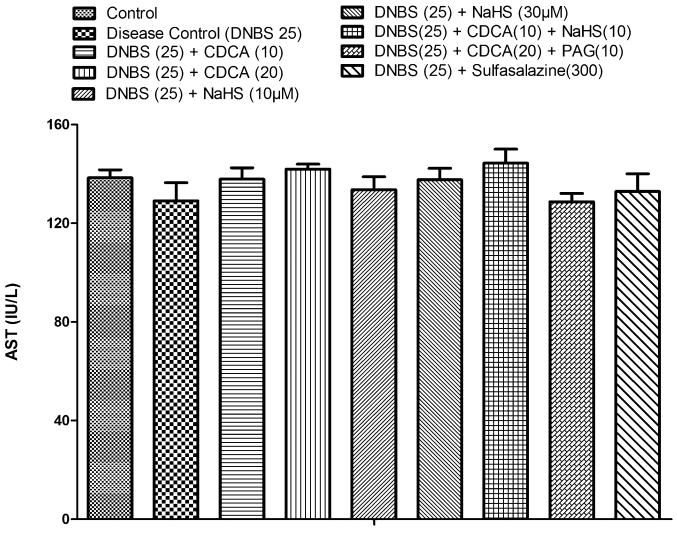

Fig. 13. Effect of CDCA and NaHS alone and/or in combination on AST in DNBS treated rats. CDCA – chenodeoxycholic acid, NaHS – sodium hydrogen sulfide, PAG – DL-propargylglycine, DNBS – 2,4-dintrobenzene sulfonic acid.

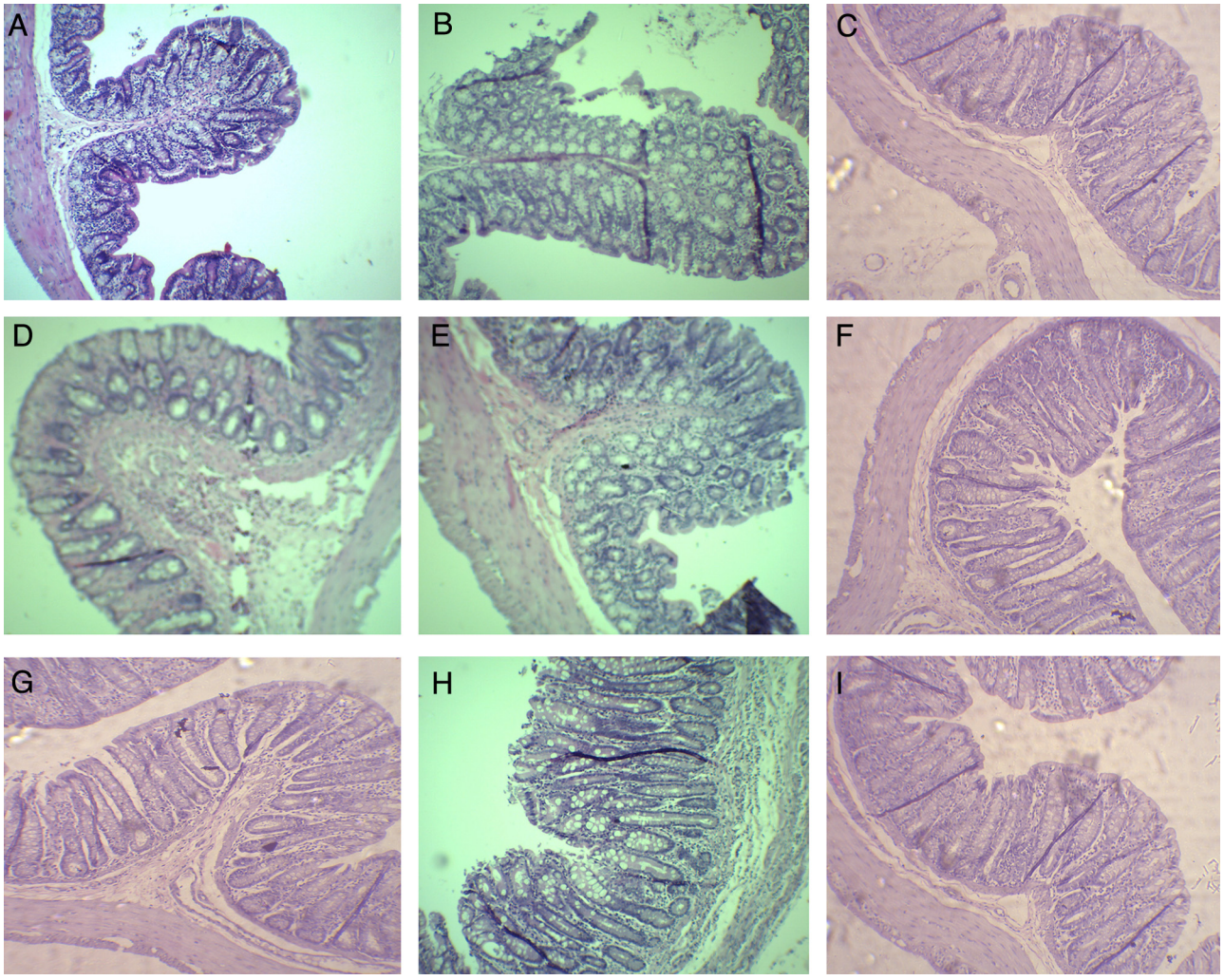

Fig. 14. Effect of CDCA and NaHS alone and/or in combination on histopathological changes in DNBS treated rats. (A) Normal rat colon with intact mucosal layer and epithelial; (B) DNBS treated rat colon with diffused active colitis, chronic inflammatory cells infiltrate with extensive disrupted intestinal layer and necrosis; (C and D) CDCA (10 and 20 mg/kg) treated rat colon partially repaired epithelial changes and restoration of intestinal layer; (E and F) NaHS (10 and 30 μmol/kg) treated rat colon showed only mild superficial inflammatory cell infiltration with no ulceration. (G) Combination of CDCA (10 mg/kg) and NaHS (10 μmol/kg) administered rats attenuated cell damage with complete healing of intestinal layer and prevented cellular inflammatory infiltrate; (H) CDCA (20 mg/kg) with PAG (10 mg/kg) increased mucosal damage and cellular infiltrate as compared to test drug treated group; (I) sulfasalazine (300 mg/kg) attenuated cell damage with complete ulcer healing in DNBS treated group.

as TNF-α, interleukin (IL)-6, IL-8, *etc*. which further stimulates the inflammatory cascade [22]. Patients with UC suffering from chronic inflammation have also increased levels of TNF-α and several pro-inflammatory cytokines both involved in innate and adaptive immune responses [23]. DNBS (25 mg/rat) treated rats in the present study caused severe necrosis and extensive morphological disorientation, edema and diffuse inflammatory cells infiltrate in the submucosa of colon section during histopathological studies, as in accordance with previous data [9].

In the present study, it has been observed that the administration of CDCA (10 and 20 mg/kg) and NaHS (10 and 30 μmol/kg) at both low and high doses significantly showed preventive effect in comparison to DNBS treated rats. They decreased the colon length shortening and rectal bleeding score significantly as compared to the disease control group and hence significantly increased the body weight. Moreover, they decreased the levels of oxidants i.e. MDA and nitrite whereas the anti-oxidant levels are comparatively increased i.e. catalase and GSH. CDCA and NaHS also significantly decreased the MPO and TNF-α level in comparison to DNBS treated rats, proving the anti-inflammatory effect.

Also, when low dose NaHS (10 μmol/kg) and CDCA (10 mg/kg) were given in combination, they showed synergistic effect in body weight, rectal bleeding, colon length shortening and oxidative stress and inflammatory markers in comparison to single administration of both low doses of CDCA and NaHS and also showed significant improvement as compared to DNBS treated rats. However, when high dose of CDCA (30 mg/kg) was given with PAG (10 mg/kg), the protective effect of CDCA was abrogated. There results showed that CDCA protective effect might be mediated through increase secretion of H_2S. This also hypothesizes that activation of FXR receptors directly or indirectly affects the secretion of H_2S. Furthermore, in our study treatment with CDCA and NaHS resulted in a total healing of the superficial eroded mucosa with no hemorrhage, edema and necrosis and a few inflammatory cells infiltrate.

Although CDCA is a FXR ligand and is reported to be a cardiotoxic, our study revealed no significant changes in the LVEDP and +dp/dt levels in the normal as well as DNBS treated rats. Furthermore, when tested for ALT and AST levels in the blood serum, CDCA does not show any significant effect on their levels in comparison to both normal and DNBS treated rats.

Pharmacological treatment with CDCA has shown to provide significant gastroprotection against NSAIDs-induced gastric damage *via* selective activation of FXR and subsequent activation of CSE dependent mechanism [8].

CSE (cystathionine gamma-lyase) is an enzyme responsible for the generation of H_2S in the body which is an endogenous gaseous mediator that exerts several anti-inflammatory effects and increases the resistance of stomach to the injury [24]. H_2S can also attenuate some elements of inflammation (leukocyte adherence, edema formation, *etc.*) and accelerate the healing of injured GI tissue by reducing NO, TNF-α, IL-1β, IFN-γ levels and appears to act as a tonic down regulator of granulocyte infiltration into intestinal tissue [25]. Since, CSE is responsible for the production of H_2S, no study has been done to verify the mechanistic pathway involved in the ulceroprotective role of FXR agonist against the colitis.

Hence, in the present study we have evaluated the downstream pathway involved in the beneficial effect of FXR which is secondary to the increased generation of H_2S as supported by the above results. CDCA (FXR agonist) in combination with NaHS (H_2S donor) synergistically attenuated the UC-like symptoms in DNBS treated animals. But this protective effect was abolished when CDCA was given with the CSE antagonist i.e. PAG, which suggests that FXR ligand exhibits ulceroprotective effects by directly or indirectly increasing the generation of H_2S gas in the body through regulation of the expression of CSE enzyme and thus H_2S further shows its anti-inflammatory activity leading to amelioration of the disease.

Conflict of interest statement

The authors have no conflict of interest.

Funding

Authors would like to thank Punjab State Council of Science & Technology, Chandigarh, India for partial funding for this research work.

Acknowledgement

We express our word of thanks to the Chairman of the college Mr. Parveen Garg for providing us excellent research facilities.

References

[1] Head KA, Jurenka JS. Inflammatory bowel disease Part 1: Ulcerative colitis – pathophysiology and conventional and alternative treatment options. Altern Med Rev 2003;8(3):247.
[2] Rutgeerts P, Sandborn WJ, Feagan BG, Reinisch W, Olson A, Johanns J, et al. Infliximab for induction and maintenance therapy for ulcerative colitis. New Engl J Med 2005;353(23):2462–76.
[3] Burisch J. Crohn's disease and ulcerative colitis. Occurrence, course and prognosis during the first year of disease in a European population-based inception cohort. Dan Med J 2014;61(1):B4778.
[4] Wallace JL, Le T, Carter L, Appleyard CB, Beck PL. Hapten-induced chronic colitis in the rat: alternatives to trinitrobenzene sulfonic acid. J Pharmacol Toxicol Methods 1995;33:237–9.
[5] Goyal N, Rana A, Ahlawat A, Bijjem KRV, Kumar P. Animal models of inflammatory bowel disease: a review. Inflammopharmacology 2014;1–15.
[6] Gadaleta RM, van Mil SW, Oldenburg B, Siersema PD, Klomp LW, van Erpecum KJ. Bile acids and their nuclear receptor FXR: relevance for hepatobiliary and gastrointestinal disease. BBA – Mol Cell Biol Lipids 2010;1801(7):683–92.
[7] Vavassori P, Mencarelli A, Renga B, Distrutti E, Fiorucci S. The bile acid receptor FXR is a modulator of intestinal innate immunity. J Immunol 2009;183:6251–61.
[8] Fiorucci S, Mencarelli A, Cipriani S, Renga B, Palladino G, Santucci L, et al. Activation of the Farnesoid-X receptor protects against gastrointestinal injury caused by non-steroidal anti-inflammatory drugs in mice. Br J Pharmacol 2011;164(8):1929–38.
[9] Joshi SV, Vyas BA, Shah PD, Shah DR, Shah SA, Gandhi TR. Protective effect of aqueous extract of *Oroxylum indicum* Linn (root bark) against DNBS-induced colitis in rats. Indian J Pharmacol 2011;43(6):656.
[10] Shifrin H, Nadler-Milbauer M, Shoham S, Weinstock M. Rivastigmine alleviates experimentally induced colitis in mice and rats by acting at central and peripheral sites to modulate immune responses. PLOS ONE 2013;8(2):e57668.
[11] Wills ED. Mechanisms of lipid peroxide formation in animal tissues. Biochem J 1966;99:667–76.
[12] Sastry KVH, Moudgal RP, Mohan J, Tyagi JS, Rao G. Spectrophotometric determination of serum nitrite and nitrate by copper–cadmium alloy. Anal Biochem 2002;306(1):79–82.
[13] Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophys 1959;82(1):70–7.
[14] Luck H. Catalase. In: Bergmeyer HU, editor. Methods of enzymatic analysis. New York: Academic Press; 1971. p. 885–93.
[15] Langendorff O. Untersuchungen am iiberlebenden Saugetierherzen. Pflugers Archiv 1898;61:291–332.
[16] Schirbel A, Fiocchi C. Inflammatory bowel disease: established and evolving considerations on its etiopathogenesis and therapy. J Dig Dis 2010;11:266–76.
[17] Ordás I, Eckmann L, Talamini M, Baumgart DC, Sandborn WJ. Ulcerative colitis. Lancet 2012;380:1606–19.
[18] Tran CD, Katsikeros R, Abimosleh SM. Current and Novel Treatments for Ulcerative Colitis. In: Shennak M, editor. Ulcerative Colitis from Genetics to Complications. InTech; ISBN: 2012978-953-307-853-3. http://dx.doi.org/10.5772/25592.
[19] Dothel G, Vasina V, Barbara G, Ponti FD. Animal models of chemically induced intestinal inflammation: predictivity and ethical issues. Pharmacol Ther 2013;139(1):71–86.
[20] McCafferty DM. Peroxynitrite and inflammatory bowel disease. Gut 2000;46:436–9.
[21] Wallace JL. Release of platelet-activating factor (PAF) and accelerated healing induced by a PAF antagonist in an animal model of chronic colitis. Can J Physiol Pharmacol 1988;66(4):422–5.
[22] Cuzzocrea S, Mazzon E, Di Paola R, Patel NS, Genovese T, Muià C, et al. Erythropoietin reduces the development of experimental inflammatory bowel disease. J Pharmacol Exp Ther 2004;311(3):1272–80.
[23] Xavier RJ, Podolsky DK. Unravelling the pathogenesis of inflammatory bowel disease. Nature 2007;448:427–34.
[24] Gadaleta RM, van Erpecum KJ, Oldenburg B, Willemsen EC, Renooij W, Murzilli S, et al. Farnesoid X receptor activation inhibits inflammation and preserves the intestinal barrier in inflammatory bowel disease. Gut 2011. http://dx.doi.org/10.1136/gut.2010.212159.
[25] Wallace JL. Hydrogen sulfide-releasing anti-inflammatory drugs. Trends Pharmacol Sci 2007;28:501–5.