Physiology and Pharmacology 28 (2024) 169-179
Experimental Research Article

The evaluation of synergistic effects of combination therapy with sulfasalazine and angiotensin-converting enzyme inhibitor in the treatment of experimental colitis in mice

Asma Mostafapour[1#], Fereshteh Asgharzadeh[2#], Seyedeh Elnaz Nazari[2], Moein Eskandari[3], Niloufar Naghibzadeh[2], Javad baharara[4], Amir Avan[5], Seyed Mahdi Hassanian[3], Majid Khazaei[2,5*]

1. Department of Biology, Mashhad Branch, Islamic Azad University, Mashhad, Iran
2. Department of Physiology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
3. Department of Medical Biochemistry, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
4. Department of Biology & Research Center for Animal Development Applied Biology, Mashhad Branch, Islamic Azad University, Mashhad, Iran
5. Metabolic Syndrome Research Center, Mashhad University of Medical Sciences, Mashhad, Iran

ABSTRACT

Introduction: Intestinal colitis, also known as ulcerative colitis, is an inflammatory bowel disease characterized by long-term inflammation and ulcers in the gastrointestinal tract. It has been suggested that the mucosal expression of angiotensin II (AT-II) is increased in colitis. This study aimed to examine the potential therapeutic effects of combination therapy with Enalapril, an angiotensin-converting enzyme inhibitor, and sulfasalazine (SSZ) in a murine colitis model.

Methods: Male C57BL/6 mice were divided into five groups: control group (distilled water), dextran sulphate sodium (DSS) group (colitis group) (1% DSS), SSZ group (positive control group) with 100 mg/kg/day, Enalapril alone group with 4 mg/kg/day, and Enalapril (4 mg/kg/day) + SSZ (100 mg/kg/day) group.

Results: There was a significant reduction in the disease activity index among the mice receiving the combination of Enalapril and SSZ compared to the colitis group. Enalapril and SSZ treatment was associated with a lower reduction in colon length, decreased colon weight, spleen weight, and spleen-to-body weight in mice with colitis. Following DSS administration, Enalapril and SSZ also significantly decreased MDA levels, an oxidant marker, and increased total thiol, SOD, and CAT levels, as antioxidants. Additionally, mucosal damage, crypt loss, pathological changes, and inflammation scores decreased after treatment with Enalapril and SSZ in comparison with the colitis group. The combination of Enalapril and SSZ reduced colon collagen content and caused a decrease in fibrosis compared to the colitis group.

Conclusion: The results of this study indicated that Enalapril alone and in combination with SSZ decreased inflammation and clinical symptoms of colitis induced by DSS.

Keywords:
Colitis
Angiotensin-converting enzyme inhibitor
Enalapril
Inflammation

* Corresponding author: Majid Khazaei, Khazaeim@mums.ac.ir
# Equally contributed as the first author

Received 2 February 2023; Revised from 8 December 2023; Accepted 18 December 2023

Citation: Mostafapour A, Asgharzadeh F, Nazari S.E., Eskandari M, Naghibzadeh N, baharara J, Avan A, Hassanian S.M., Khazaei M. The evaluation of synergistic effects of combination therapy with sulfasalazine and angiotensin-converting enzyme inhibitor in the treatment of experimental colitis in mice. Physiology and Pharmacology 2024; 28: 169-179. http://dx.doi.org/10.61186/phypha.28.2.169

Ulcerative colitis (UC), a type of inflammatory bowel disease (IBD), is a chronic condition affecting the gastrointestinal (GI) tract. UC primarily affects young individuals, significantly impacting their quality of life (Chassaing et al., 2014; Heidari et al., 2020). Although the exact cause of colitis remains unknown, environmental factors, genetic predispositions, and mucosal immunodeficiency contribute to the disease's development. Animal models of GI inflammation have provided valuable insights into the mechanisms underlying the inflammatory process (Beniwal-Patel and Shaker 2019). Various chemical compounds are used to cause UC.

The clinical symptoms and tissue damage caused by DSS closely resemble the mucosal inflammation observed in human UC (Heidari et al., 2020). UC is marked by chronic inflammation of the mucosal tissue, abdominal pain, dysentery, fatigue, nausea, and weight loss (Flynn and Eisenstein 2019). Consequently, DSS-induced colitis in laboratory models is a common method for studying and developing treatments for this disease (Asgharzadeh et al., 2021; Perše and Cerar 2012).

Drug therapy for UC aims to induce and maintain remission in patients. Amino salicylates have always been used as one of the first treatment options in UC treatment (Nikfar et al., 2009). Sulfasalazine (SSZ) is composed of an antibacterial agent (sulfapyridine) and a salicylate (5-aminosalicylic acid). In UC treatment, the therapeutic component is 5-aminosalicylic acid, while sulfapyridine acts as an inactive carrier facilitating the release of the active substance in the colon (Asgharzadeh et al., 2021). Despite partial absorption in the small intestine, approximately 90% of SSZ reaches the colon (Asgharzadeh et al., 2021; Ghafouri et al., 2020). In colitis, changes in the gut's microbial flora occur, and SSZ's antibiotic properties help modify this flora, aiding in disease improvement (Feagan and MacDonald 2012; Sutherland and MacDonald 2006; Walker et al., 1997). However, significant side effects lead to the discontinuation of SSZ in about 15% of patients. These side effects can be categorized as dose-dependent and non-dose-dependent. Dose-depended side effects include nausea, vomiting, anorexia, headache, alopecia, back pain, and lack of folate absorption. Non-dose-dependent side effects include skin rash, fever, hemolytic anemia, agranulocytosis, pancreatitis, hepatitis, reversible male infertility, colitis, alveolar fibrosis, pericarditis, and myocarditis (Amidon et al., 2015; Kumar and Mutlu 2015; Moum 2008). Due to these adverse effects, researchers are exploring alternative or adjunctive therapies to SSZ.

Among the many hypertensive medications, Enalapril is the most widely used agent that inhibits angiotensin-converting enzyme (ACE) (Lee et al., 2014). Activation of the renin-angiotensin system (RAS) in monocytes and cultures of vascular smooth muscle cells can activate the NF-κB pathway, one of the body's oldest proinflammatory systems. IBD patients have higher colonic mucosal concentrations of angiotensin I and II, in addition to angiotensin II receptors. Inflammation of the colon may be mediated by the RAS, which activates NF-κB signaling. As a therapeutic target, RAS inhibition may reduce NF-κB expression, potentially benefiting IBD situations. By inhibiting NF-κB activation, Enalapril can also exert anti-inflammatory effects (Lee et al., 2014).

Colitis is widely prevalent today. Therapies like SSZ used to improve the clinical symptoms of this disease have extensive side effects, making long-term use impractical. ACE inhibitors such as Enalapril have shown potential in improving colitis complications, with no significant side effects observed so far. Therefore, this study will investigate the effect of Enalapril in combination with SSZ in improving the clinical symptoms of colitis.

Materials and Methods

Animals

This study utilized male C57BL/6 mice weighing 22-25 g and 7-8 weeks old, sourced from the Pasteur Institute (Tehran, Iran). The animals were maintained under standard conditions (temperature of 20±22°C, 50±2% relative humidity, and a 12-hour light/12-hour dark cycle). The mice had free access to fresh food and water throughout the experiment. Animal experiments were conducted following the ARRIVE guidelines and the Guidelines for Care and Use of Laboratory Animals from the Mashhad University of Medical Sciences ethics committee. The study was approved by the ethics committee of Mashhad University of Medical Sciences (Approval ID: IR.MUMS.MEDICAL.REC.1400.131)

Experimental Drugs

The biological compounds used in this study included SSZ powder, Enalapril, hematoxylin and eosin (H&E),

TABLE 1: The DAI scoring.

DAI				
Score	Rectal bleeding	Stool consistency	Rectal prolapse	Lose weight
0	None	Normal	None	<5%
1	Red	Soft	Sign of prolapse	5-10%
2	Dark red	Very soft	Clear prolapse	10-15%
3	Gross bleeding	Diarrhea	Extensive prolapse	>15%

TABLE 2: Colonic histological scoring.

Score	0	1	2	3	4
Inflammation	None	Mild	Moderate	Severe	
Mucosal damage	None	Mucus layer	Submucosa	Muscular and serosa	
Crypt loss	None	1/3	2/3	100%+intact epithelium	100% with epithelium lose
Pathological change range	None	1-25%	26-50%	51-75%	76-100%

catalase (CAT), superoxide dismutase (SOD), malondialdehyde (MDA), and total thiol reagents. SSZ powder was prepared by the Iran Hormone Company (Tehran, Iran), while the other biological compounds were obtained from Sigma Aldrich (Saint Louis, MO).

Colitis induction

In this study, the mice were randomly divided into five groups (n=6) one week before the experiments to adapt to the environment and acclimate to the animal house conditions. The experimental period lasted ten days. The five groups included: control group (distilled water), dextran sulphate sodium (DSS) (colitis group) (1 % DSS), SSZ (positive control group) 100 mg/ kg/ day, Enalapril group 4 mg/kg/day, Enalapril (4 mg/kg/ day) + SSZ (100 mg/kg/day) (Asgharzadeh et al., 2021; Sueyoshi et al., 2013). In this study, the control group received distilled water throughout the experiment (one week). All experimental groups, except the control group, were continuously administered 1% DSS (w/v) from the first to the seventh day to induce colitis. During the experiment, distilled water was provided to the control group. Oral administration of the various interventions began on the third day and continued once daily for seven consecutive days (Fig. 1A). Bodyweight variation, visual stool consistency, and rectal bleeding were monitored daily. The DAI (Hamer et al., 2010) was calculated using the factors listed in Table 1. At the end of the experiment, the mice were sacrificed, and the entire colon was collected. After a longitudinal opening and washing with PBS solution, the length and weight of the whole colon were measured. The mice's colon was separated into three sections (the proximal, middle, and distal parts). The distal colon sections were preserved in 10% formaldehyde for histological investigations.

Histological analysis

After the distal colon sections were fixed overnight in 10% formalin, they were processed for paraffin embedding and stored as 5 mm thick tissue blocks. Subsequently, these tissue blocks were sectioned, and the sections were stained with H&E and Masson's trichrome staining. Images of the stained sections were captured using an Olympus microscope at magnifications of 40x and 100x. For the Masson's trichrome staining, the ratio of fibrotic regions was determined. The fibrotic regions were quantified using Image J software. Additionally, the colon tissue injury was scored based on the indicators listed in Table 2 (Table 2).

Oxidative stress measurement

After weighing the colon samples, they were homogenized in PBS on ice. The homogenized samples were then centrifuged at 4°C for 10 minutes at 3000 rpm. Following centrifugation, the supernatant was collected and stored at -70°C for the evaluation of oxidant/antioxidant indicators, including MDA, total thiol, SOD, and CAT activity (Bordoni et al., 2019).

MDA measurement

The amount of MDA in colon samples was measured as an indicator of lipid peroxidation. For this purpose,

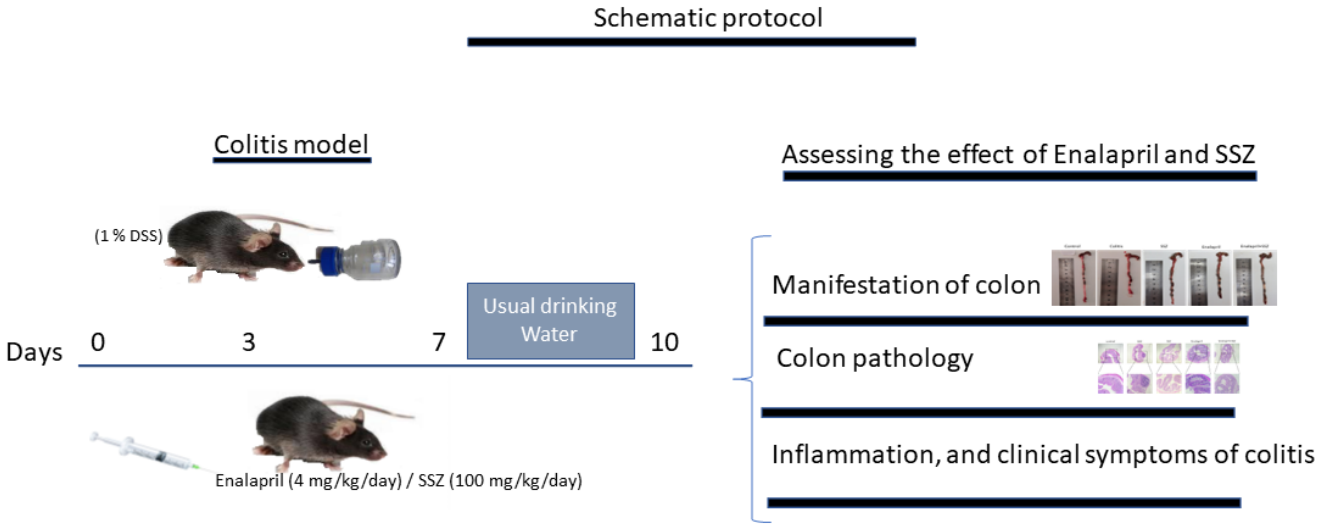

FIGURE 1. The schematic of study design

colon tissue was homogenized, and then thiobarbituric acid was added to it. The absorbance of this mixture was measured using a spectrophotometer at 535 nm against a blank. The MDA quantity in the samples was evaluated using a standard curve.

Total thiol groups (SH) measurement

The dithiol nitro benzoic acid (DTNB) method was applied to investigate the total thiol concentration in the samples. Reduced glutathione (GSH) was used as a standard for constructing the curve. The supernatant was incubated with DTNB (pH 8.6) in 0.1 M Tris-EDTA buffer. The mixture was then incubated for 10 minutes at 25 °C, and its absorbance was read at 412 nm using a spectrophotometer. A standard curve was used to quantify the GSH concentration.

SOD measurement

The superoxide dismutase (SOD) activity was assessed using the pyrogallol auto-inhibition method and the inhibition of MTT conversion to formazan. Following this, dimethyl sulfoxide (DMSO) was utilized to dissolve the formazan and produce stable colors. The absorbance was then measured at 570 nm (Hashemzehi et al., 2020).

CAT measurement

The catalase activity was measured by the rate of hydrogen peroxide decomposition. The amount of catalase enzyme was then read using a spectrophotometer at 240 nm.

Statistical analysis

Prism 6.0 (GraphPad Software Inc., La Jolla, CA, USA) was used for statistical analysis. The data were expressed as mean ± SEM. One-way ANOVA test was used, followed by a post-hoc LSD test. P-values <0.05 were considered statistically significant.

Results

The effect of Enalapril on reducing the symptoms of chronic colitis in mice

This study demonstrated that administration of DSS for the first three days induced colitis in mice. The results showed that mice treated with DSS experienced significant weight loss compared to the control group (P<0.001), confirming the induction of colitis by DSS. From days 5 to 10 after treatment, the weight of mice in the SSZ (P<0.01), Enalapril (P<0.001), and SSZ + Enalapril (P<0.001) groups significantly improved (Figure 2A). These results indicate that Enalapril, in combination with SSZ, effectively alleviates weight loss due to colitis from days 5 to 10. To further confirm the development of colitis, DAI was assessed in the animals. The results showed that DAI was significantly increased in the colitis group (P<0.001), confirming DSS-induced colitis (Figure 2B). Treatment with SSZ (P<0.001), Enalapril (P<0.01), and SSZ + Enalapril (P<0.001) sig-

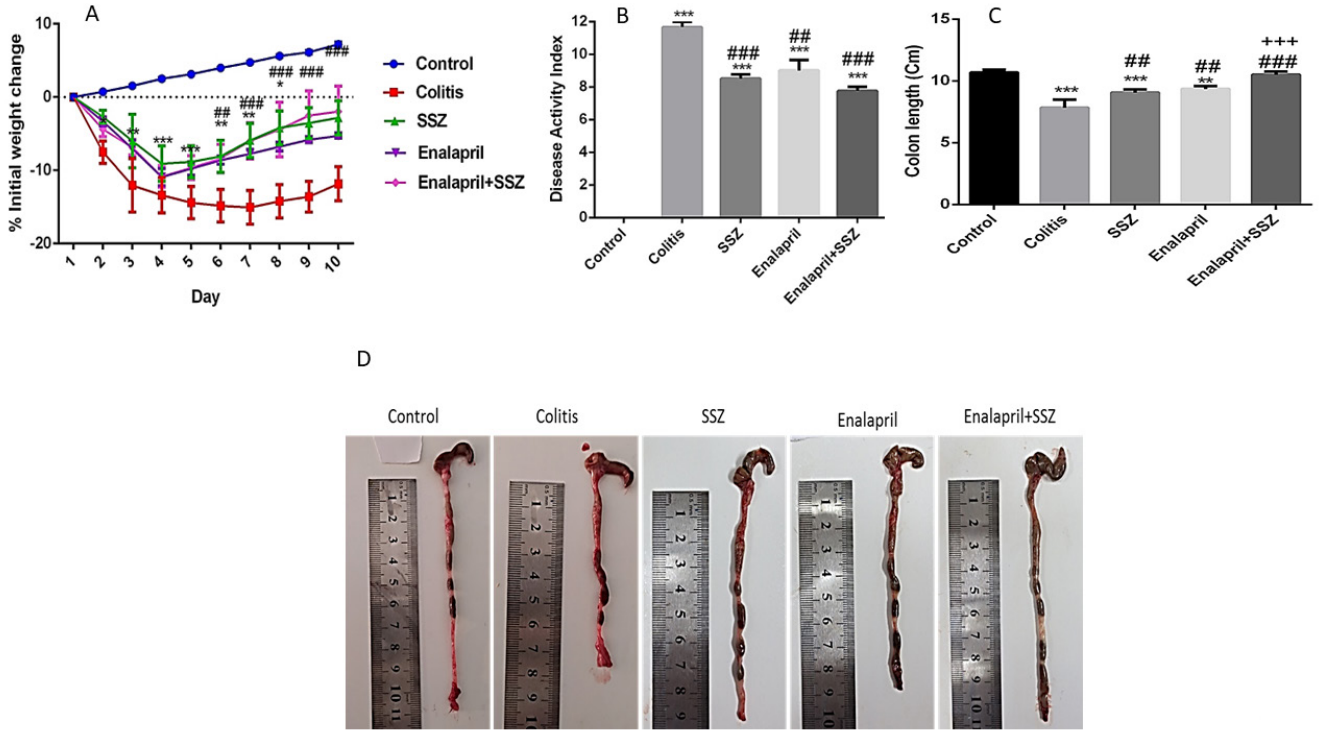

FIGURE 2. The effect of Enalapril on body weight in DSS-induced colitis (A). The effect of Enalapril in DAI-induced colitis (B). The effect of Enalapril on colon length in DSS-induced colitis (C). Macroscopic image of the colon length (D). Data are presented as Mean±SEM. $^{***}P<0.001$, in comparison to the control group. $^{###}P<0.001$ and $^{##}P<0.01$ in comparison to the colitis group. $^{+++}P<0.001$, in comparison to the SSZ group.

nificantly improved DAI compared to the colitis group (Figure 2B).

Enalapril improves DSS-induced colon damage in mice.

Colon length was significantly reduced in the colitis group compared to the control group ($P<0.001$), indicative of DSS-induced colitis (Figure 2C-D). Treatment with SSZ ($P<0.01$), Enalapril ($P<0.01$), and SSZ + Enalapril ($P<0.001$) improved colon length compared to the colitis group (Figure 2C-D). Among the treatment groups, Enalapril + SSZ resulted in a significant increase in colon length compared to the SSZ group alone ($P<0.001$) (Figure 2C-D). The measurements of colon length in the control (11 cm), colitis (7.5 cm), SSZ (9 cm), Enalapril (9 cm), and SSZ + Enalapril groups (11 cm) are illustrated in Figure 1D.

Enalapril improves DSS-induced inflammation in mice

This study demonstrated that spleen weight was significantly increased in the colitis group compared to the control group ($P<0.001$). Treatment with SSZ ($P<0.001$), Enalapril ($P<0.001$), and SSZ + Enalapril ($P<0.001$) led to a significant reduction in spleen weight compared to the colitis group (Figure 3A). There was also a significant increase in the ratio of spleen weight to body weight in the colitis group compared to the control group ($P<0.001$). Treatment with SSZ ($P<0.001$), Enalapril ($P<0.001$), and SSZ + Enalapril ($P<0.001$) resulted in a significant decrease in the spleen weight to body weight ratio compared to the colitis group (Figure 3B-C). These findings indicate that Enalapril significantly reduces inflammation when combined with SSZ.

Enalapril improves DSS-induced oxidative stress in mice.

The results showed that treatment with SSZ ($P<0.001$), Enalapril ($P<0.001$), and SSZ + Enalapril ($P<0.001$) significantly reduced the level of MDA compared to the colitis group. Additionally, in the SSZ + Enalapril group, the level of MDA was significantly reduced compared to the SSZ group ($P<0.01$) (Figure 4A). Total thiol levels were significantly decreased in the colitis group compared to the control group ($P<0.001$). Treatment with SSZ ($P<0.001$), Enalapril ($P<0.001$), and SSZ +

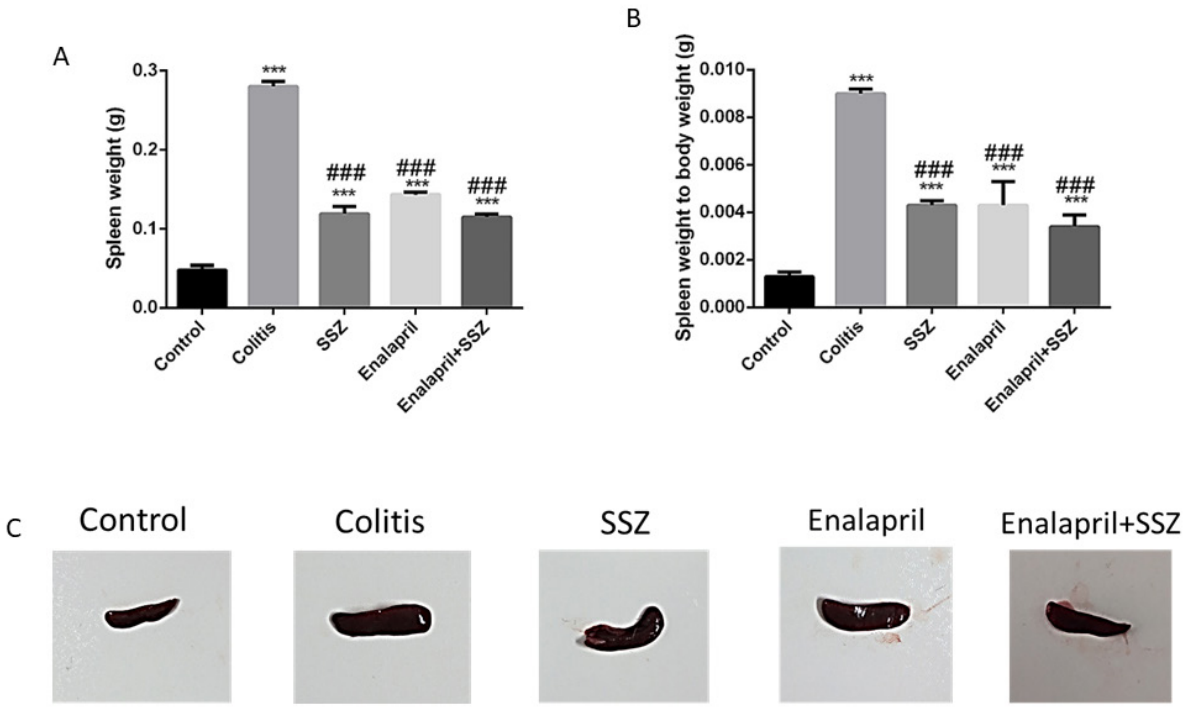

FIGURE 3. The effect of Enalapril on spleen weight in DSS-induced colitis (A). The effect of Enalapril on spleen weight to body weight ratio (B). Macroscopic image of the spleen (C). Data are presented as Mean±SEM. $^{***}P<0.001$, in comparison to the control group. $^{###}$ $P<0.001$ in comparison to the colitis group.

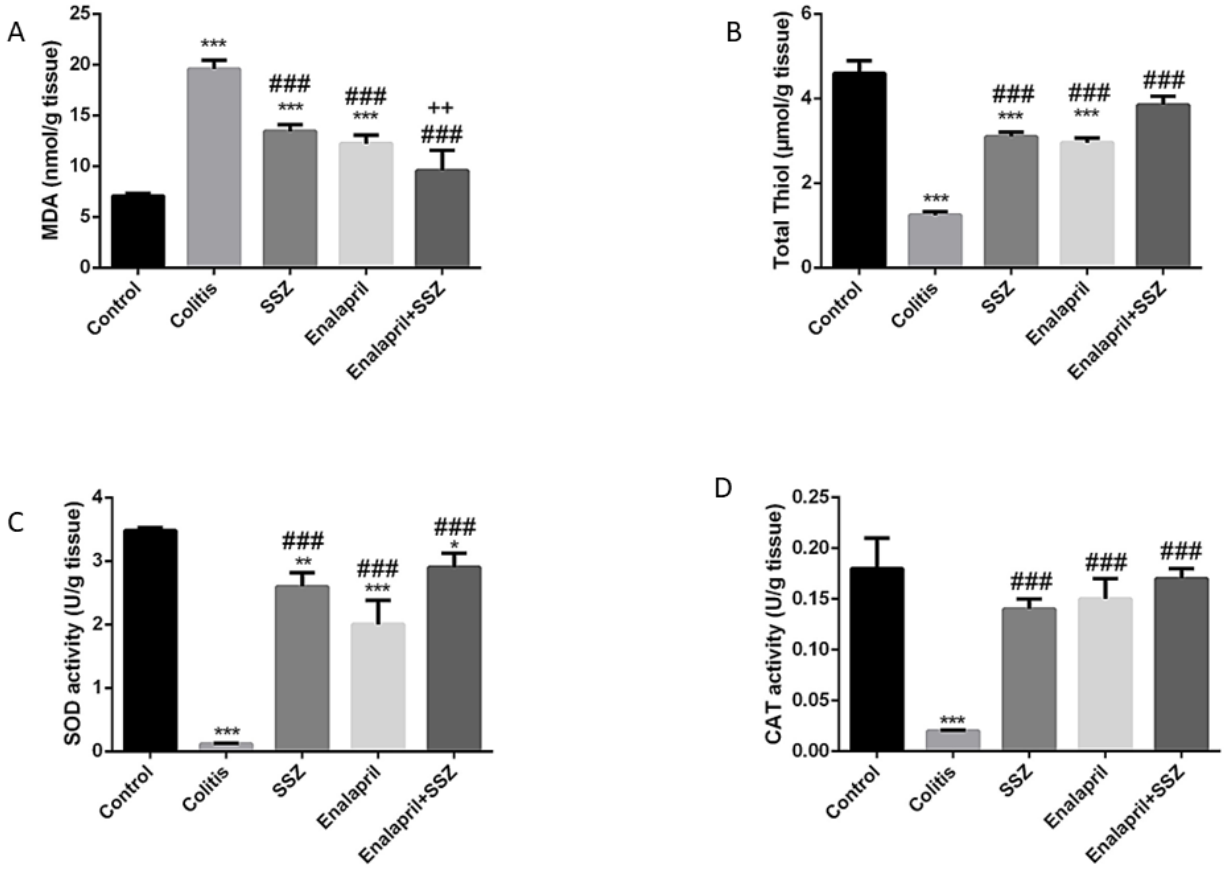

FIGURE 4. The effect of Enalapril on oxidative stress in DSS-induced colitis mice.
The effect of Enalapril on MDA (A), total thiol (B), SOD activity (C), and CAT activity (D). Data are presented as Mean±SEM. $^{**}P<0.01$, and $^{***}P<0.001$ compared to the control group. $^{###}$ $P<0.001$ in comparison to the colitis group. $^{++}$ $P<0.01$ compared to the SSZ group.

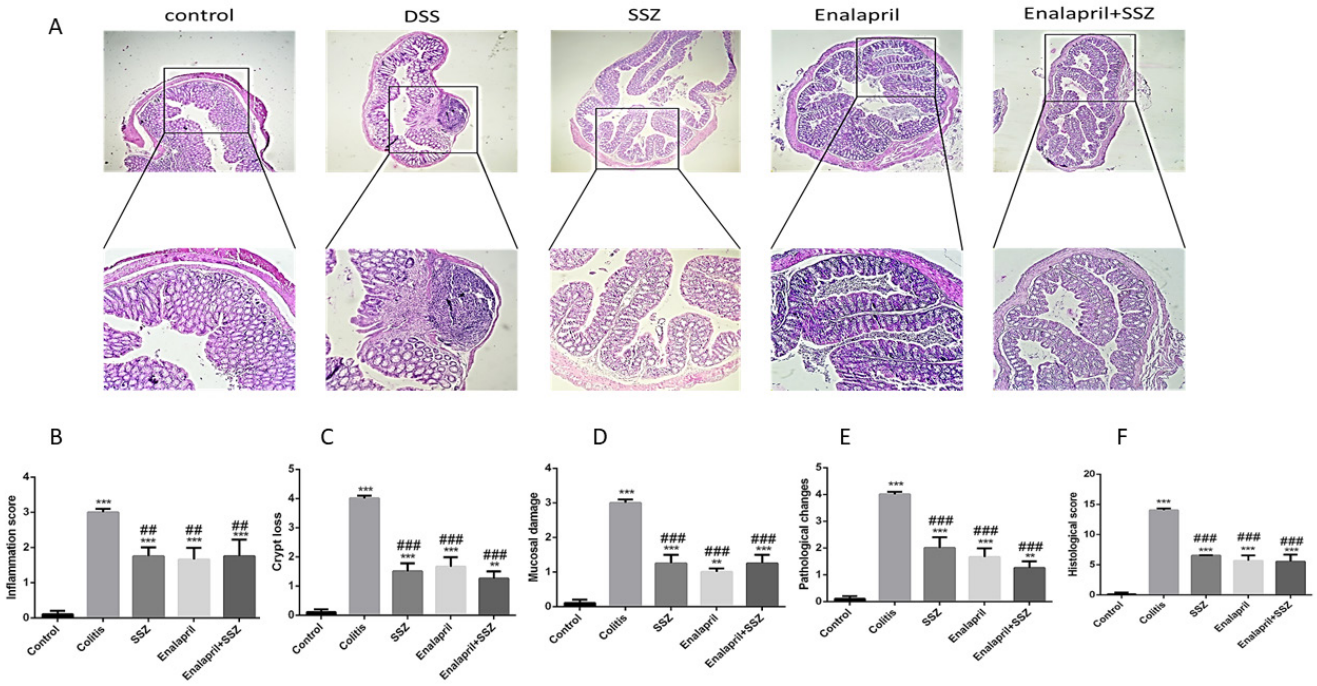

FIGURE 5. Histopathological image of the colon via H&E staining (A). The effect of Enalapril on inflammation score in DSS-induced colitis (B). The effect of Enalapril on crypt loss in DSS-induced colitis (C). The effect of Enalapril on mucosal damage in DSS-induced colitis (D). The effect of Enalapril on pathological changes in DSS-induced colitis (E). The effect of Enalapril in histological score in DSS-induced colitis (F). Data are presented as Mean±SEM. $^{**}P<0.01$, and $^{***}P<0.001$ compared to the control group. $^{\#\#}P<0.01$, and $^{\#\#\#}P<0.001$ in comparison to the colitis group.

Enalapril ($P<0.001$) increased total thiol levels compared to the colitis group (Figure 4B). SOD activity was decreased in the colitis group compared to the control group ($P<0.001$). In the SSZ ($P<0.001$), Enalapril ($P<0.001$), and SSZ + Enalapril ($P<0.001$) groups, SOD activity significantly increased compared to the colitis group (Figure 4C). CAT activity was also decreased in the colitis group compared to the control group ($P<0.001$). Treatment with SSZ ($P<0.001$), Enalapril ($P<0.001$), and SSZ + Enalapril ($P<0.001$) significantly increased CAT activity compared to the colitis group (Figure 4D).

Enalapril improves DSS-induced histopathological changes in mice.

Histopathological changes in colon tissue from DSS-induced colitis in mice were assessed using H&E staining (Figure 5A). Inflammation scores in the colitis group significantly increased compared to the control group ($P<0.001$). The inflammation scores in the SSZ ($P<0.001$), Enalapril ($P<0.001$), and SSZ + Enalapril ($P<0.001$) groups decreased compared to the colitis group. These results suggest that Enalapril combined with SSZ reduces inflammation (Figure 5B). The level of crypt loss in the colitis group increased significantly compared to the control group ($P<0.001$). The level of crypt loss in the SSZ ($P<0.001$), Enalapril ($P<0.001$), and SSZ +Enalapril ($P<0.001$) significantly decreased compared to the colitis group (Figure 5C). Thus, Enalapril in combination with SSZ helps prevent the reduction of colon crypts. In the DSS-induced colitis group, mucosal damage increased significantly compared to the control group ($P<0.001$). Mucosal damage in the SSZ ($P<0.001$), Enalapril ($P<0.001$), and SSZ + Enalapril ($P<0.001$) groups significantly decreased compared to the colitis group (Figure 5D). Therefore, Enalapril combined with SSZ improves mucosal damage.

Pathological changes increased significantly in the colitis group ($P<0.001$). Enalapril ($P<0.001$), SSZ ($P<0.001$), and SSZ + Enalapril ($P<0.001$) decreased pathological changes induced by DSS (Figure 5E).

Similarly, Enalapril combined with SSZ decreased histological changes induced by DSS ($P<0.001$). Both SSZ ($P<0.001$) and SSZ + Enalapril ($P<0.001$) reduced histological changes compared to the colitis group (Figure 5F).

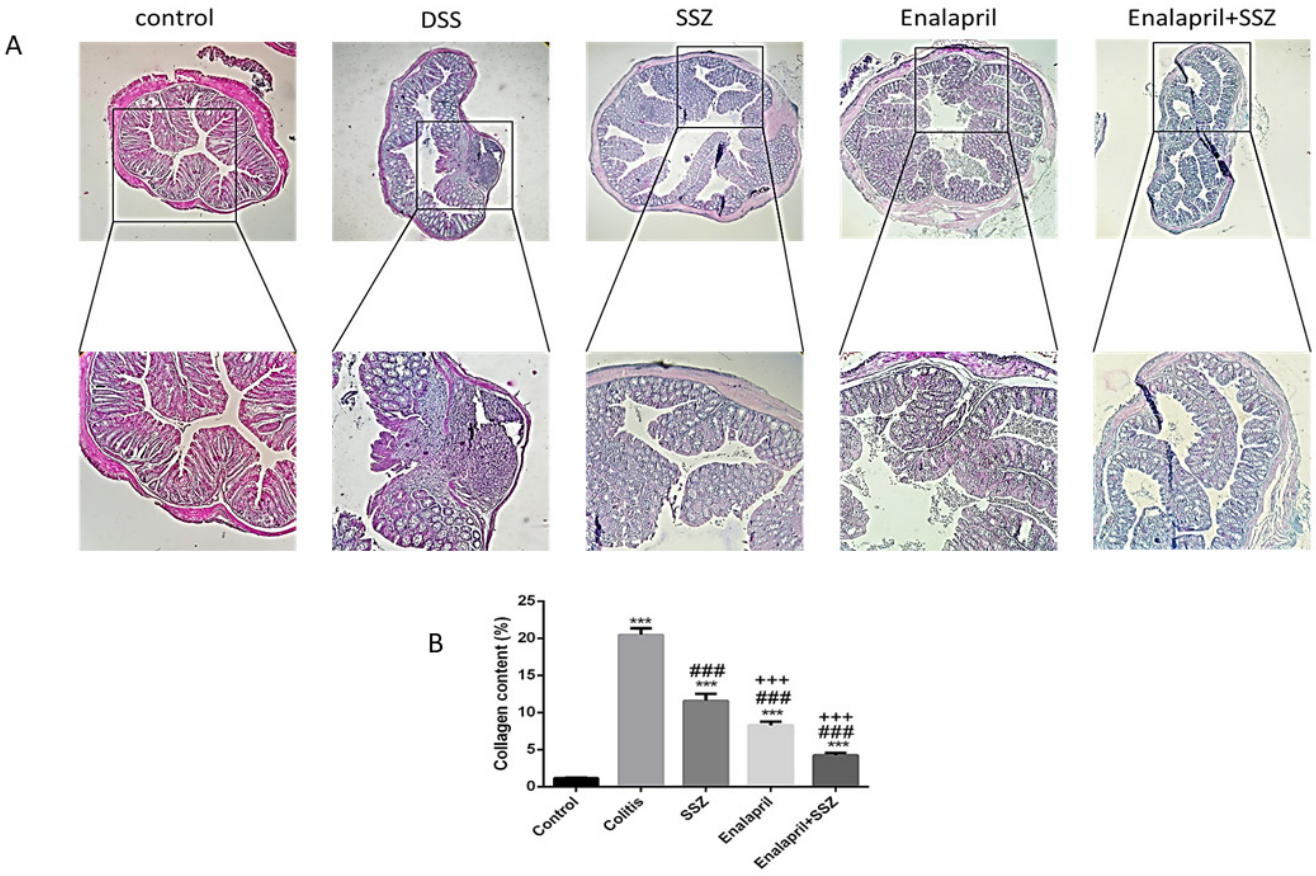

FIGURE 6. Histopathological image of the colon via trichrome staining (A). The effect of Enalapril on collagen content of the colon in DSS-induced colitis (B). Data are presented as Mean±SEM. $^{***}P<0.001$, in comparison to the control group. $^{###}P<0.001$ in comparison to the colitis group. $^{+++}P<0.001$compared to the SSZ group.

Overall, these results indicate that the combination of SSZ and Enalapril significantly reduces inflammation and histological changes induced by DSS in a mouse model of colitis.

Enalapril improves DSS-induced colon fibrosis in mice

One of the complications of colitis is excessive collagen deposition in the colon tissue, leading to fibrosis. The results of histological examination with Masson's trichrome staining show that fibrosis in the colon tissue increased sharply due to colitis. However, fibrosis significantly decreased in the treatment groups, especially in the SSZ + Enalapril group (Figure 6A). Additionally, the amount of collagen in the treatment groups, SSZ ($P<0.001$), Enalapril ($P<0.001$), and SSZ + Enalapril ($P<0.001$), significantly decreased compared to the colitis group. The most significant reduction in collagen deposition was observed in the SSZ + Enalapril group ($P<0.001$) (Figure 6B). These results indicate that Enalapril in combination with SSZ effectively decreases colon fibrosis.

Discussion

The absence of a definitive treatment for colitis underscores the need to explore alternative therapeutic approaches. One potential strategy involves combining existing drugs to reduce dosages, which can decrease adverse effects and improve effectiveness.

In the present study, the effects of Enalapril alone and in combination with SSZ on the clinical symptoms of DSS-induced colitis were investigated. This study showed that Enalapril improved the clinical and histological symptoms of colitis. Colitis was induced using DSS to mimic clinical symptoms in humans, and the results align with other studies (Chassaing et al., 2014; Perše and Cerar 2012).

Angiotensin II is one of the most critical factors in

causing cellular apoptosis in the inflammatory process, activated by ACE. Angiotensin II also induces the expression of tumor necrosis factor-α (TNF-α), Bcl-2, and Bax. In DSS-induced colitis, the expression of these factors increases, playing an essential role in cellular apoptosis in the inflammatory process, especially in colon epithelial cells (Haendeler et al., 2000; Shi et al., 2016; WANG et al., 2001). It has been reported that ACE inhibitors such as Enalapril reduce levels of angiotensin II, TNF-α, Bcl-2, and Bax, thereby decreasing apoptosis in epithelial cells and reducing clinical symptoms of colitis, including body weight loss and histological changes (Spencer et al., 2007). The results of this study support and confirm these findings.

Enalapril treatment of DSS-induced colitis improves symptoms, colon damage, and pathological changes by inhibiting the renin-angiotensin system (RAS) and NF-κB signaling pathway (Lee et al., 2014; Takahashi et al., 2008). Enalapril blocks RAS and inhibits NF-κB activation, thereby reducing inflammation. This leads to an improvement in colitis symptoms (weight loss and DAI), a reduction in colon damage (weight and length of the colon), and a decrease in pathological changes (pathological score). Therefore, Enalapril effectively reduces the inflammatory process in colitis (Lee et al., 2014).

The administration of ACE inhibitors has been shown to reduce colon fibrosis, symptoms, and damage in colitis (Alican et al., 2005). Colitis increased the activity of the RAS system. In this system, angiotensin II in the colon tissue induces collagen proliferation and secretion in colonic myofibroblasts, leading to collagen deposition and fibrosis in the colon tissue (Rieder et al., 2018). By inhibiting the function of the RAS system, ACE inhibitors prevent the conversion of angiotensinogen to angiotensin II (Wengrower et al., 2004). This inhibition prevents the stimulation of myofibroblasts to secrete collagen, thereby reducing collagen deposition and fibrosis in colonic tissue (Garg et al., 2020). These findings align with the present study results and confirm them.

The activation of the RAS system leads to the production of angiotensin II, which disrupts the oxidant/antioxidant balance and increases the production of ROS. ROS cause the peroxidation of membrane lipids, leading to inflammation and the secretion of inflammatory mediators such as TNF-α (Hamza and Dyck 2014; Lee and Hur 2019). ACE inhibitors, by suppressing the RAS and inhibiting angiotensin II activation, decrease oxidative stress and inflammation (Husain et al., 2015). The results of this study support these findings.

Regarding the efficacy of combined therapy, we observed a reduction in the DAI in the SSZ+Enalapril group compared to the SSZ group. The DAI is a composite score reflecting the severity of UC symptoms, including stool consistency, rectal bleeding, and mucosal appearance. A lower DAI score indicates better disease control and improved quality of life for patients. Therefore, our findings suggest that the addition of Enalapril to SSZ treatment may provide clinical benefits for UC patients.

While it is true that combination therapy shows a significant difference in collagen content, this finding could still have clinical implications. Fibrosis is a crucial factor in evaluating the progression and severity of colitis. Therefore, even if the combination therapy does not show significant differences in other criteria, the observed reduction in collagen content could still be considered a positive outcome.

Also, there was a significant reduction in colon length and MDA in this study. These findings are important factors in reducing the progression of colitis and further support the potential benefits of combining SSZ and Enalapril in UC treatment.

MDA is a well-known marker of oxidative stress produced during inflammation and linked to tissue damage in UC. In our study, we observed a significant decrease in MDA levels in the SSZ+Enalapril group compared to the SSZ group, indicating a reduction in oxidative stress and tissue damage. Furthermore, we found a significant decrease in colon length in the SSZ+Enalapril group, which is a hallmark of colitis progression.

These findings suggest that the addition of Enalapril to SSZ treatment may have a protective effect on the colon and reduce the progression of colitis. While further studies are needed to fully understand the mechanism behind this effect, our results provide initial evidence for the potential benefits of combining SSZ and Enalapril in UC treatment. While our study did not show significant differences between the SSZ and SSZ+Enalapril groups in some criteria, the reduction in DAI is clinically relevant.

Conclusion

The absence of a definitive colitis treatment necessi-

tates exploring alternative therapeutic approaches. In this regard, a potential strategy involves reducing drug dosages used in treating colitis, which often entail numerous adverse effects. This can be achieved by incorporating FDA-approved drugs and other beneficial medications to manage colitis more effectively.

The current study showed that combination therapy using SSZ and Enalapril can prevent inflammation in colonic tissue by inhibiting the production of angiotensin II. Our data indicated that Enalapril increased the efficacy of SSZ while decreasing its side effects. However, confirming the results of this study requires further investigation by examining cellular and molecular mechanisms.

Acknowledgement

The Mashhad University of Medical Sciences supported this research.

Conflict of interest

The authors have no conflict of interest.

References

Alican I, Şener G, Yüksel M, Gedik N, Ercan F, Jahovic N. The effect of angiotensin-converting enzyme inhibitors on experimental colitis in rats. Regulatory Peptides 2005; 130:67-74. https://doi.org/10.1016/j.regpep.2005.03.009

Amidon S, Brown J E, Dave V S. Colon-targeted oral drug delivery systems: design trends and approaches. Aaps Pharmscitech 2015; 16: 731-741. https://doi.org/10.1208/s12249-015-0350-9

Asgharzadeh F, Yaghoubi A, Nazari S E, Hashemzadeh A, Hasanian S M, Avan A, et al. The beneficial effect of combination therapy with sulfasalazine and valsartan in the treatment of ulcerative colitis. EXCLI Journal 2021; 20: 236.

Beniwal-Patel P, Shaker R. Gastrointestinal and liver disorders in women's health: A point of care clinical guide: Springer, 2019. https://doi.org/10.1007/978-3-030-25626-5

Bordoni L, Fedeli D, Nasuti C, Maggi F, Papa F, Wabitsch M, et al. Antioxidant and anti-inflammatory properties of Nigella sativa oil in human pre-adipocytes. Antioxidants 2019; 8: 51. https://doi.org/10.3390/antiox8020051

Chassaing B, Aitken J D, Malleshappa M, Vijay-Kumar M. Dextran sulfate sodium (DSS)-induced colitis in mice. Current Protocols in Immunology 2014; 104: 15.25. 1-15.25. 14. https://doi.org/10.1002/0471142735.im1525s104

Feagan B G, MacDonald J K. Oral 5-aminosalicylic acid for induction of remission in ulcerative colitis. Cochrane Database of Systematic Reviews 2012. https://doi.org/10.1002/14651858.CD000543.pub3

Flynn S, Eisenstein S. Inflammatory bowel disease presentation and diagnosis. Surgical Clinics of North America 2019; 99: 1051-62. https://doi.org/10.1016/j.suc.2019.08.001

Garg M, Royce S G, Tikellis C, Shallue C, Batu D, Velkoska E, et al. Imbalance of the renin-angiotensin system may contribute to inflammation and fibrosis in IBD: a novel therapeutic target? Gut 2020; 69: 841-851. https://doi.org/10.1136/gutjnl-2019-318512

Ghafouri Z, Seyyedian S, Nikbakht J, Kouhsari E, Bayat S, Zargar H, et al. Effect of Sodium Cromoglycate on acetic acid-induced ulcerative colitis in mice. Korean Journal of Gastroenterology 2020; 75: 39-45. https://doi.org/10.4166/kjg.2020.75.1.39

Haendeler J, Ishida M, Hunyady L, Berk B C. The third cytoplasmic loop of the angiotensin ii type 1 receptor exerts differential effects on extracellular signal-regulated kinase (ERK1/ERK2) and apoptosis via Ras-and Rap1-dependent pathways. Circulation Research 2000; 86: 729-736. https://doi.org/10.1161/01.RES.86.7.729

Hamer H M, Jonkers D M, Vanhoutvin S A, Troost F J, Rijkers G, de Bruïne A, et al. Effect of butyrate enemas on inflammation and antioxidant status in the colonic mucosa of patients with ulcerative colitis in remission. Clinical Nutrition 2010; 29: 738-744. https://doi.org/10.1016/j.clnu.2010.04.002

Hamza S M, Dyck J R. Systemic and renal oxidative stress in the pathogenesis of hypertension: modulation of long-term control of arterial blood pressure by resveratrol. Frontiers in Physiology 2014; 5: 292. https://doi.org/10.3389/fphys.2014.00292

Hashemzehi M, Naghibzadeh N, Asgharzadeh F, Mostafapour A, Hassanian S M, Ferns G A, et al. The therapeutic potential of losartan in lung metastasis of colorectal cancer. EXCLI Journal 2020; 19: 927.

Heidari M, Hashemi S M, Baghaei K, Zali M R Z M. Comparative effects of different doses of dextran sodium sulfate on the induction of chronic colitis in C57BL/6 mice. Research in Medicine 2020; 44: 346-351.

Husain K, Edu Suarez A I, Hernandez W, Ferder L. Effect of paricalcitol and enalapril on renal inflammation/oxidative stress in atherosclerosis. World Journal of Biological Chemistry 2015; 6: 240. https://doi.org/10.4331/wjbc.v6.i3.240

Kumar S D, Mutlu E A. What do I do with my medications if I become Pregnant? Safety of IBD medications during

pregnancy. Inflammatory Bowel Disease: Springer, 2015: 171-187. https://doi.org/10.1007/978-3-319-14072-8_23

Lee C, Chun J, Hwang S W, Kang S J, Im J P, Kim J S. Enalapril inhibits nuclear factor-κB signaling in intestinal epithelial cells and peritoneal macrophages and attenuates experimental colitis in mice. Life Sciences 2014; 95: 29-39. https://doi.org/10.1016/j.lfs.2013.11.005

Lee S Y, Hur S J. Effect of treatment with peptide extract from beef myofibrillar protein on oxidative stress in the brains of spontaneously hypertensive rats. Foods 2019; 8: 455. https://doi.org/10.3390/foods8100455

Moum B. Which are the 5-ASA compound side effects and how is it possible to avoid them? Inflammatory bowel diseases 2008; 14: S212-S213. https://doi.org/10.1002/ibd.20712

Nikfar S, Rahimi R, Rezaie A, Abdollahi M. A meta-analysis of the efficacy of sulfasalazine in comparison with 5-aminosalicylates in the induction of improvement and maintenance of remission in patients with ulcerative colitis. Digestive Diseases and Sciences 2009; 54: 1157-1170. https://doi.org/10.1007/s10620-008-0481-x

Perše M, Cerar A. Dextran sodium sulphate colitis mouse model: traps and tricks. Journal of Biomedicine and Biotechnology 2012; 2012. https://doi.org/10.1155/2012/718617

Rieder F, Bettenworth D, Ma C, Parker C E, Williamson L A, Nelson S A, et al. An expert consensus to standardise definitions, diagnosis and treatment targets for anti-fibrotic stricture therapies in Crohn's disease. Alimentary pharmacology & therapeutics 2018; 48: 347-357. https://doi.org/10.1111/apt.14853

Shi Y, Liu T, He L, Dougherty U, Chen L, Adhikari S, et al. Activation of the renin-angiotensin system promotes colitis development. Scientific Reports 2016; 6: 1-11. https://doi.org/10.1038/srep27552

Spencer A U, Yang H, Haxhija E Q, Wildhaber B E, Greenson J K, Teitelbaum D H. Reduced severity of a mouse colitis model with angiotensin converting enzyme inhibition. Digestive Diseases and Sciences 2007; 52: 1060-1070. https://doi.org/10.1007/s10620-006-9124-2

Sueyoshi R, Ignatoski K M W, Daignault S, Okawada M, Teitelbaum D H. Angiotensin converting enzyme-inhibitor reduces colitis severity in an IL-10 knockout model. Digestive Diseases and Sciences 2013; 58: 3165-3177. https://doi.org/10.1007/s10620-013-2825-4

Sutherland L R, MacDonald J K. Oral 5-aminosalicylic acid for maintenance of remission in ulcerative colitis. Cochrane Database of Systematic Reviews 2006. https://doi.org/10.1002/14651858.CD000544.pub2

Takahashi M, Suzuki E, Takeda R, Oba S, Nishimatsu H, Kimura K, et al. Angiotensin II and tumor necrosis factor-α synergistically promote monocyte chemoattractant protein-1 expression: roles of NF-κB, p38, and reactive oxygen species. American Journal of Physiology-Heart and Circulatory Physiology 2008; 294: H2879-H2888. https://doi.org/10.1152/ajpheart.91406.2007

Walker A M, Szneke P, Bianchi L A, Field L G, Sutherland L R, Dreyer N A. 5-Aminosalicylates, sulfasalazine, steroid use, and complications in patients with ulcerative colitis. American Journal of Gastroenterology (Springer Nature) 1997; 92.

Wang L-X, Ideishi M, Yahiro E, Urata H, Arakawa K, Saku K. Mechanism of the cardioprotective effect of inhibition of the renin-angiotensin system on ischemia/reperfusion-induced myocardial injury. Hypertension Research 2001; 24: 179-187. https://doi.org/10.1291/hypres.24.179

Wengrower D, Zannineli G, Pappo O, Latella G, Sestieri M, Villanova A, et al. Prevention of fibrosis in experimental colitis by captopril: The role of TGF-β1. Inflammatory Bowel Diseases 2004; 10: 536-545. https://doi.org/10.1097/00054725-200409000-00007