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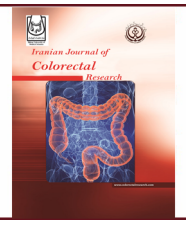

A Novel Pre-treatment Approach to Ulcerative Colitis in a Mouse Model

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Abstract

Background: Inflammatory bowel disease (IBD) is a complex multifactorial condition that includes Crohn's disease and ulcerative colitis (UC). UC is characterized by inflammation, oxidative stress, and increased intestinal epithelial cell apoptosis. The present study investigated the protective potential of five safety products, namely bromelain (Br), silibinin (SB), alpha-lipoic acid (ALA), inulin (IN), and sodium butyrate (BU), against UC.
Methods: Seventy-two male Balb/c mice were divided into nine groups and administered for 14 days with a minimum effective dose of Br, SB, ALA, IN, BU, or all five together (PAC). Mesalazine (MZ) was used to compare the therapeutic effects of the compounds. Colitis was induced by rectal injection of acetic acid (4%) on the 12th day. Blood and colon tissue were collected, and the expression of inflammatory cytokines and oxidative stress indices were examined via ELISA. SPSS v.24 was used for data analysis.
Results: All the individual therapeutic groups, including Br, IN, BU, ALA, and SB, partially improved histopathological changes due to colitis, but PAC treatment prior to colitis induction significantly ($P<0.001$) and more effectively improved colitis and alleviated the extent and intensity of the histological signs of cell damage including inflammation intensity and macroscopic and microscopic colon damage. A significant decrease in inflammatory cytokines and oxidative stress indices was also observed in the groups treated with ALA, SB, and PAC.
Conclusion: This animal study suggests that the new drug combination (PAC) is more beneficial for the prevention of UC than MZ, a usual treatment of UC.

Keywords: Ulcerative colitis, Oxidative stress, Inflammatory cytokines, Preventive drug

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Introduction

Inflammatory bowel disease (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), is a chronic inflammatory disorder affecting the gastrointestinal tract. UC, or bleeding colitis, is associated with chronic inflammation of the colon and rectum. In such a situation, the immune system is impaired, attacks the colon's internal wall, and causes various complications (1).

Generally, the incidence and prevalence of UC in Asia are generally lower than in the West (2, 3). However, recent data have described an increase in the incidence of the disease in countries of Asia with large populations such as China and India. Within the past few decades in Asia, a clear rise in the incidence of UC was observed due to the spread of the western lifestyle, particularly the increased consumption of sugars, fats, fast foods, red meat, and alcohol (4, 5). One of the long-term complications of UC is colorectal cancer (6). A wide range of complications and side effects are associated with therapies used for UC treatment, including nonsteroidal anti-inflammatory drugs (NSAIDs) (7), corticosteroids (8), antibiotics (9), immunomodulators (10), monoclonal antibody drugs (11), and probiotics (12). Inefficiency and/or serious side effects related to the administration of the drugs mentioned above, in addition to the multifactorial pathogenesis of UC, underscore the need to find novel treatment agents.

In light of these issues, we searched the scientific literature and selected some products as candidates for use in UC therapy. These products were chosen according to the different aspects of this disease and also according to the effect of each on these different aspects. Also, we worked on a pre-treatment approach to this disease and checked that these drugs had no harmful and/or counteractive effects. Finally, we were able to select the five products mentioned below.

Bromelain (Br) is an enzyme found in pineapple juice and in the pineapple stem. Therapeutic effects of Br in UC, especially in the moderate type, have been observed (13, 14). Also, Br reduces colon inflammation and cancerous lesions in the mice's large intestines (15).

Inulin (IN) is a starchy substance found in a wide variety of fruits, vegetables, and herbs, including wheat, onions, bananas, leeks, artichokes, and asparagus. This substance's effective anti-inflammatory and repairing properties have been demonstrated (12, 16, 17). In addition, IN, as a prebiotic compound, relieves UC by reducing NO synthesis (12).

Butyrate (BU), a short-chain fatty acid produced in the colon through bacterial fermentation, plays an important role in intraluminal homeostasis, inflammation modulation, cellular proliferation, differentiation, and repair of mucosal lesions (18). Legumes (beans, peas, and soybeans), fruits, nuts, cereals, and whole grains are good sources of dietary fiber. BU is also found in butter and cheese (19).

Alpha-lipoic acid (ALA) is an organosulfur compound derived from caprylic acid. Many foods contain ALA in very low amounts. They include spinach, broccoli, potatoes, yeast, tomatoes, Brussels sprouts, carrots, beets, and rice bran. ALA, a potent antioxidant, is an effective factor in UC relief and colon damage prevention (20).

Silibinin (SB) is a natural flavanone compound from medicinal plants (*Silybum marianum*), classified as a flavonoid. SB, the active ingredient of silymarin, has a key role in the treatment of colon cancer and UC in the mouse model (21).

Recent years have shown that herbal medicine and combination treatments have a preferential effect compared to the conventional single treatment of UC (22). Therefore, we hypothesized that by combining the mentioned products, we would be able to have a more effective effect against this disease. Animal models are indispensable for evaluating mechanistic details that will facilitate a better pre-clinical drug therapy design to target specific components involved in a disease. Hence, we designed this study using a mouse model to better examine our hypothesis by comparing these effects between each product as well as compared with a drug often used against this disease: mesalazine (MZ).

Therefore, the present study investigated the protective potential of five products: Br, SB, ALA, IN, and BU, or all five together (PAC), against UC.

Materials and Methods

Animal

Seventy-two male Balb/c wild-type mice (8–10 weeks of age, weighing 20-35 g) from the Razi Institute of Mashhad were transferred to our animal laboratory one week before the start of the project. The mice were kept at a temperature of 21±2 °C with a natural light period (8 am to 8 pm) for a week in the faculty animal room for adaptation to new environmental conditions. Adequate water and food were made available.

Treatment

The mice were randomly divided into nine groups of eight. The mice were weighed, and their general status was assessed daily at certain hours. The effective dose of medications was calculated and given to animals via gavage for 14 consecutive days. The doses of Br (13), IN (16), BU (23), ALA (24), SB (25), and MZ (26) were selected according to prior studies. All compounds used in this study were provided by Sigma, Merck, or MP biomedical.

The groups were as follows: Group (1), the control group (CT), lacking colitis induction and treatment; Group (2), the colitis group (CT-), with induced colitis but not treated, only received PBS; Group (3), received MZ at 100 mg/kg/day; other

groups, respectively received, (4) Br: 40 mg/kg/day, (5) IN: 800 mg/kg/day, (6) BU: 2 mg/ml, (7) ALA: 80 mg/kg/day, (8) SB: 400 mg/kg/day, and (9) "PAC": was treated with an effective dose of all (Br + IN + BU + ALA + SB). In this study, MZ was used in one group to compare its effect with others compounds. After a 12-day course of pre-treatment, UC was induced using a chemical model (27) in all treatment groups except the control group.

Ulcerative Colitis Induction

In this model, 24-h fasted mice were anesthetized using a mixture of ketamine and xylazine 2% (5+44 mg/kg). Then, for colitis induction, 0.1 ml of acetic acid (4%, v/v) was injected with an appropriate plastic catheter at a distance of 3 to 4 cm from the anus, and after 30 to 60 seconds of contact with the tissue, the colon washed with a physiological serum. In the normal control group, mice received 0.9% saline alone using the same technique (28). Several pilot experiments were used to find the appropriate acetic acid dosage (3% to 6%), and the best result was obtained at 4%.

Evaluation of Toxicity and Safety Dosage of the Compounds

The liver and the hematological system are the organs potentially most frequently affected during the oral administration of compounds to animals. The evaluation of the biochemical, morphological, and histopathological parameters indicated no toxicity and damage concerning the products used in this study. With oral administration in mice, Br has a very low toxicity effect with an LD50 (lethal dose) greater than 10,000 mg/kg (29); IN has no toxic effect up to 1000 mg/kg (30); BU has no toxic effect up to 1.25 g/kg (31); ALA has no toxic effect up to 500 mg/kg (32); SB has no toxic effect up to 20,000 mg/kg (33).

Assessment of Changes in Body Weight

Animal body weights were recorded daily throughout all the experiments. At the end of the experiment, after sacrificing the mice, the colons were removed aseptically from the proximal rectum. Afterward, the colon length and weight were measured. The percentage of body weight loss was calculated as the percent difference between the original body weight (day 0) and body weight on any particular day. To measure tissue edema, the colon weight/length ratio was evaluated.

Macroscopic Evaluation

The severity of colonic damage was evaluated according to a macroscopic scoring system described by Khoshakhlagh et al. in 5 levels from a normal appearance with no damage (score 0) to damage extending ≥2 cm along the length of the colon (score 4-6) (34).

Serum and Tissue Preparing

Clinical activity scores were measured, and on the 14th day, the mice were anesthetized, and blood samples (1 ml) were taken to measure the serum biomarkers. After sacrificing the mice, the colon (about 5 cm) was removed and washed with saline then its length and weight were measured. A small portion of colon tissue (50 mg) was separated and maintained at -20 °C to measure glutathione, IL-6, and TNFα. The remaining colon tissue was weighed and washed with cold normal saline and then fixed in formalin 10% for histopathologic evaluation.

Colon tissue sections were fixed and stained with hematoxylin and eosin (HE) to determine mucosal damage (35). Histological grading criteria were as follows: 0: no signs of inflammation; 1: low inflammation; 2: low levels of leukocyte infiltration; 3: high levels of leukocyte infiltration, high vascular density, thickening of the colon; and 4: transmural penetration, loss of goblet cells, high vascular density, thickening of the colon wall. Microscopic sections were examined by one pathologist in a double-blind manner, and histological reports and images were prepared. For each microscopic section, according to the Dieleman method, the crypt damage degree, the depth of damage, and the inflammation degree were scored from 0 (healthy epithelium and crypt) to 4 (complete destruction of crypt and epithelium) (36).

Cytokine Assay

Serum and colon tissue levels of TNF-α and IL-6 were determined using the ELISA (enzyme-linked immunosorbent assay) according to the manufacturer's instructions (IL-6: eBioscience Mouse IL-6 platinum ELISA kit, Affymetrix; TNF-α: eBioscience Mouse TNF alpha platinum ELISA kit, Affymetrix). To extract the proteins, 50 mg of colon tissue was completely crumbled, homogenized with RIPA lysis buffer (containing 1% protease inhibitor cocktail and 1% phosphatase inhibitor cocktail), and centrifuged for 20 minutes at 4 °C and 10,000 rpm. The supernatant was used for TNF-α and IL-6 determination.

Glutathione (GSH) Assay

Colon tissue and blood glutathione content were measured using a GSH assay kit (Bioassay Technology Laboratory E0394Mo, Mouse Glutathione peroxidase ELISA kit) according to the manufacturer's instructions. The colon tissues were suspended in 10 mM PBS solution, mixed with ice-cold 7.5% trichloroacetic acid solution, and centrifuged at 3000 ×g for 10 min. The supernatant was used for the assay. The GSH content was measured at 450 nm absorbance (37).

Statistical Analysis

In describing the data, appropriate tables and statistical indicators such as mean and standard deviation were used. In the data analysis, the normality

of the data was checked using the Shapiro-Wilk test. For normal data, parametric analysis of variance methods were used, and for pairwise comparisons, Tukey's HSD test was used. For non-normal data, non-parametric methods of the Kruskal-Wallis test were used, and the Dan-Bonferroni method was used for pairwise comparisons. The software used in this research was SPSS v. 24, and the significance level of the tests was less than 5%. In the results, P-values less than 5% are marked with a * sign, and values less than 1% are marked with a ** sign.

Results

Effects of Medicines on Body Weight Change and Tissue Edema

The weight variation curve of the mice (Figure 1) revealed that mice treated with SB, ALA, or PAC were significantly less affected by the disease (P<0.05) compared to the colitis group (CT-). On the 14th day, the average weight of mice treated by ALA and PAC showed the least weight loss compared to other groups and the colitis group. According to the weight loss index, the percentage of body weight loss was classified as unchanged, less than 5%, between 5% and 10%, and over 10%.

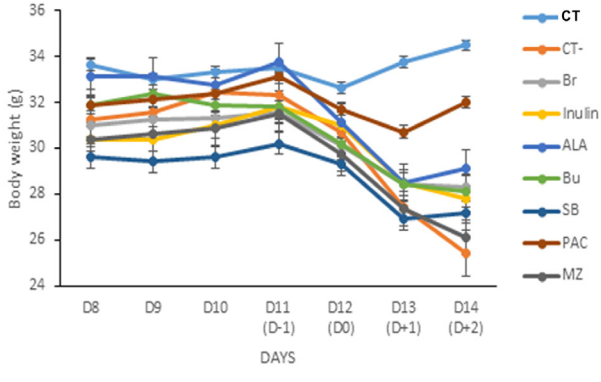

Figure 1: Body weight in different treatment groups. Body weights of mice receiving an effective dose of bromelain (Br), silibinin (SB), alpha-lipoic acid (ALA), inulin (IN), sodium butyrate (BU), the five agents together (PAC), or mesalazine (MZ), compared with the control group (CT) and colitis group (CT-) (P<0.05). Data are expressed as mean±S.E.M.

The percentage of body weight loss in the control group (CT) did not show any changes. In the colitis group, mice showed a body weight loss of over 10% (P<0.05). The remaining groups showed the same results explained above, compatible with the histology results below (P<0.05). Interestingly, the PAC group showed the least body weight loss.

The colon weight/length ratio, as a measure of tissue edema, was significantly increased in the colitis group (P<0.05) compared with the control group (Figure 2). Using SB and PAC caused a significant reduction in this parameter compared with the colitis group (P<0.05). In other groups, tissue edema decreased but did not reach statistical significance.

Effects of Medicines on Histopathological Changes

Figure 3 shows that in the colitis group (CT-), inflammation extended to the mucosa, submucosa, and epithelial cells (area 1). Also, extensive tissue granulation with infiltration of leukocytes and

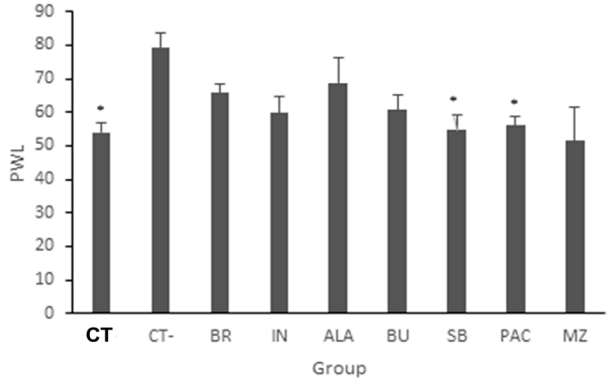

Figure 2: Effects of drugs on tissue edema. The chart of colon weight/length. Data are expressed as the means±S.E.M. *P<0.05, **P<0.01 and ***P<0.001 vs. colitis group (CT-); #P<0.05 VS. mesalazine.

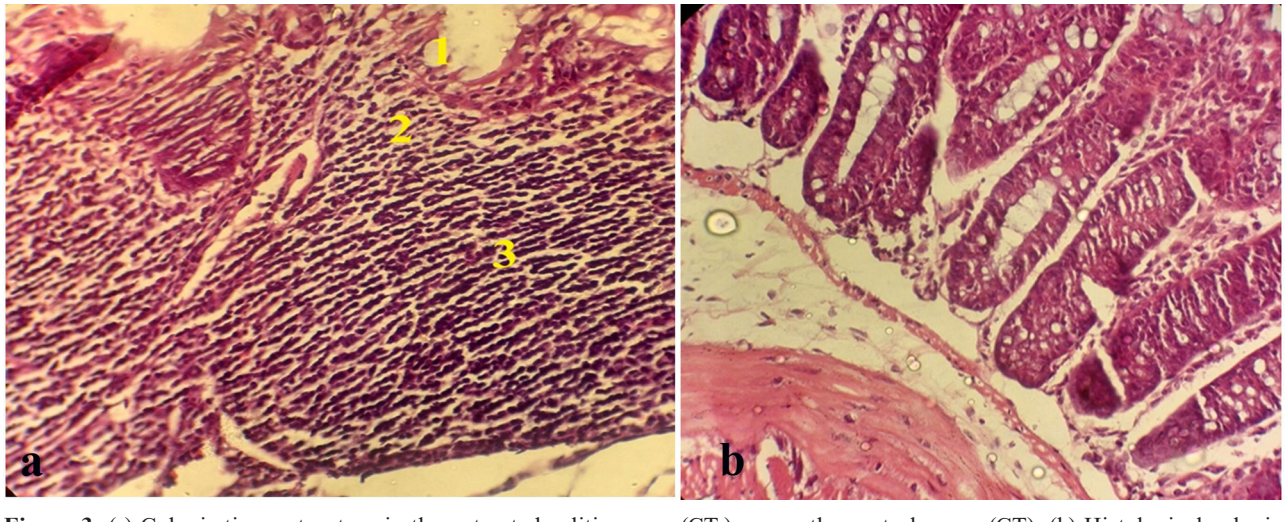

Figure 3: (a) Colonic tissue structure in the untreated colitis group (CT-) versus the control group (CT); (b) Histological colonic mucosal sections of colitis group (a) showing severe destruction of epithelium with inflammatory cell infiltration (1), loss of cryptic structure and decrease in the number of goblet cells and leukocyte infiltration (2 and 3), and normal mice (b) showing normal mucosa with intact epithelia. Hematoxylin-eosin stain; original magnification (400x).

Goblet cells was observed. In some sections of ulcerated areas, necrotic tissue adjacent to surface cells with extensive crypt destruction could be observed (areas 2, 3). Treatment of mice with PAC significantly attenuated the extent and severity of the histological signs of cell damage and colitis. In addition, all the other therapeutic groups partially improved colitis (Figure 4).

Histopathological Scores of Colitis

Microscopic pathological scores significantly increased in the colitis group (CT-) compared with that of the control group (CT). PAC significantly decreased the pathological scores in acetic acid-induced colitis mice as compared with the colitis group ($P<0.001$), showing a much better effect than the MZ group ($P<0.05$) (Figure 5).

Serum Cytokine Assay

Serum levels of IL-6 were significantly lower in all treatment groups compared with the colitis group (CT-). Also, in the PAC group, the IL-6 level was significantly lower ($P<0.05$) in comparison with the MZ group (Figure 6A).

Serum level of TNF-α decreased significantly in the Br and BU groups ($P<0.01$, each) and in the ALA, SB, and PAC groups ($P<0.001$, each) compared with the colitis group (CT-). However, the decrease in the MZ and IN groups compared with the colitis group (CT-) did not reach statistical significance (Figure 6B).

Colon Cytokine Assay

Administration of acetic acid caused a significant elevation in colonic levels of IL-6 and TNF-α in the colitis group (CT-), as compared with those in the control group (CT) (Figure 7). Treatment of mice with ALA ($P<0.01$) or PAC ($P<0.05$) significantly reduced the production of IL-6 compared with the colitis group (CT-), as evident in Figure 7A. Administration of ALA and BU significantly ($P<0.05$) reduced TNF-α levels compared with the colitis group. In the PAC group, the colonic TNF-α level decreased significantly ($P<0.01$) compared with the colitis and MZ groups (Figure 7B).

Glutathione (GSH) Assay in Colon and Blood

Considering the role of oxidative stress in the development of colitis, a GSH test was performed. Colon tissue and blood GSH content in various groups were measured. Higher serum levels of GSH were found in the control group compared with the colitis group ($P<0.001$). However, serum levels of GSH were significantly higher in the ALA, SB, Br, and PAC groups ($P<0.01$), while no significant differences were observed between the IN and BU groups compared with the colitis group (CT-) (Figure 8A).

The colon level of GSH increased in all groups compared with the colitis group, but in the PAC, ALA, and SB groups ($P<0.05$, each), this increase was significantly higher (Figure 8B).

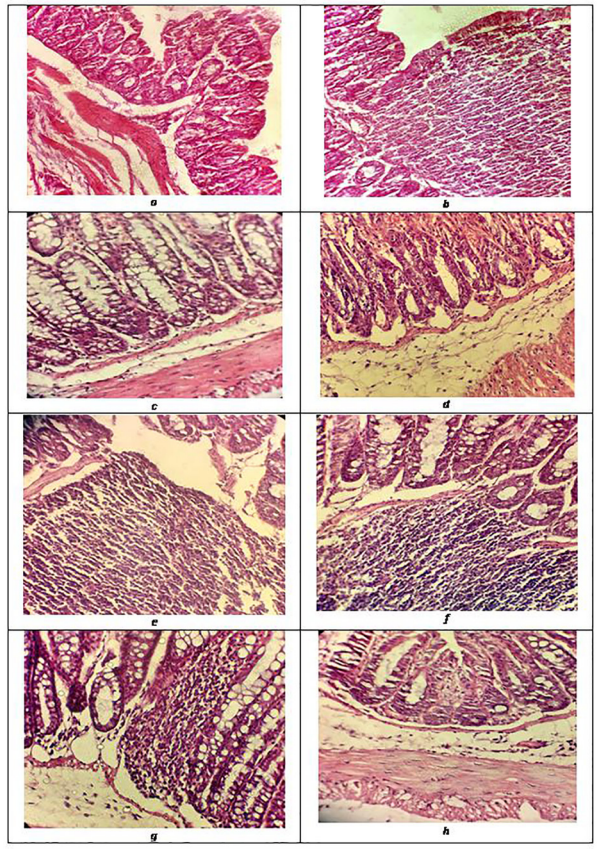

Figure 4: Effects of medicines on the microscopic appearances of the mice colon with 14 consecutive days of pre-treatment with induction of colitis by acetic acid on the 12th day. Treatment with (a) alpha lipoic acid 80 mg/kg/day (healthy area, no colitis) and (b) moderate colitis; (c) Silibinin 400 mg/kg/Day (healthy area, no colitis); (d) Butyrate 2 mg/ml (healthy area, no colitis); (e) Mesalazine 100 mg/kg/Day (moderate colitis); (f) Inulin 800 mg/kg/day (moderate colitis); (g) Bromelain 40 mg/kg/day (mild colitis); (h) effective dose of all five agents (PAC group) revealing healthy mucosa (no colitis). Hematoxylin-eosin stain; original magnification (400x).

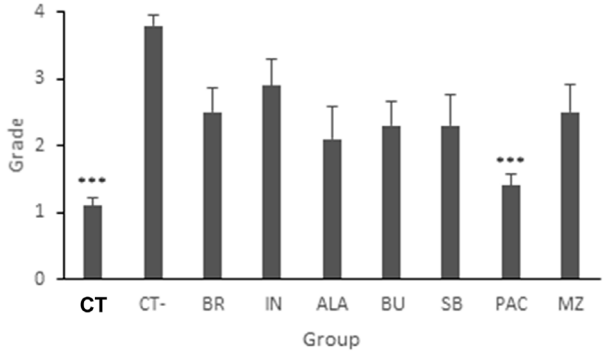

Figure 5: Microscopic histopathological scores of colonic damage from acetic acid in mice. Data are expressed as the mean±S.E.M. *$P<0.05$, **$P<0.01$ and ***$P<0.001$ vs. colitis group (CT-); # $P<0.05$ vs. mesalazine

Discussion

Ulcerative colitis (UC) is characterized by inflammation, oxidative stress, and increased intestinal epithelial cell apoptosis. UC is a multifactorial disease for which current therapeutic options do not suffice, each leading to different

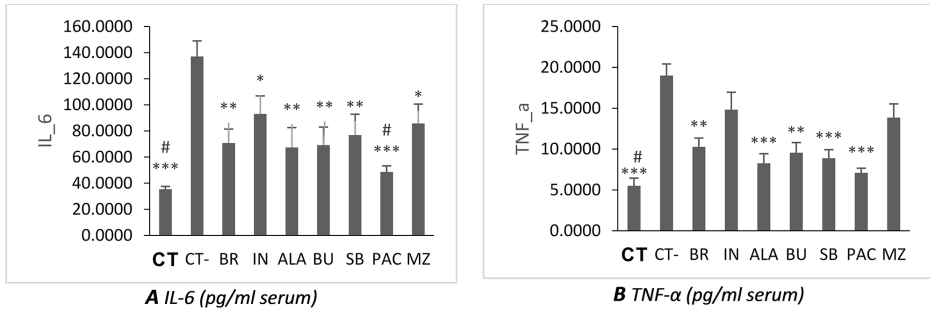

Figure 6: (A) Effects of medicines on the levels of IL-6 in serum; (B) Effects of medicines on the levels of TNF-α in serum. Data are expressed as the mean±S.E.M. *P<0.05, **P<0.01 and ***P<0.001 vs. colitis group (CT-); #P<0.05 vs. mesalazine (MZ)

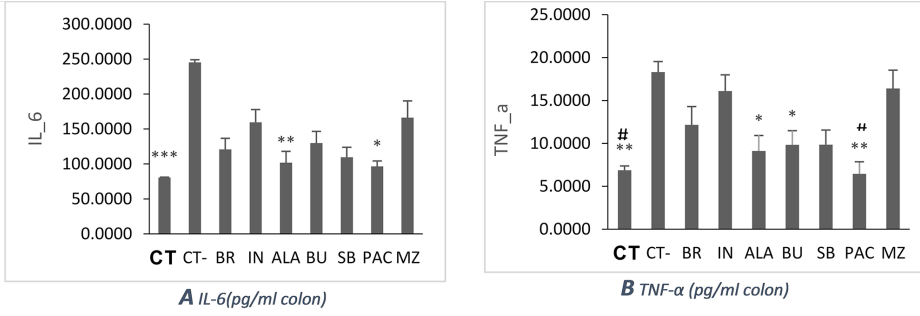

Figure 7: (A) Effects of medicines on the levels of IL-6 in the colon; (B) Effects of medicines on the levels of TNF-α in the colon. Data are expressed as the mean±S.E.M. * P<0.05, **P<0.01 and ***P<0.001 vs. colitis group (CT-); #P<0.05 vs. mesalazine

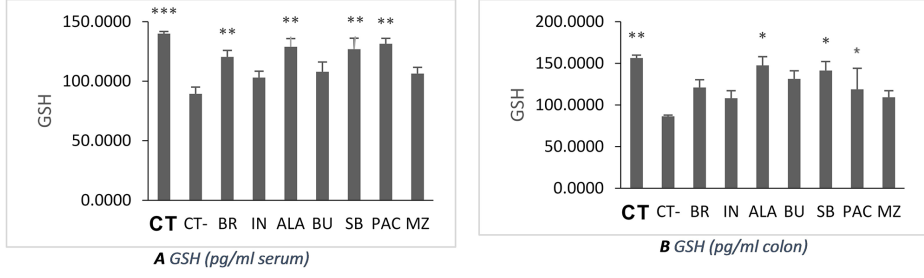

Figure 8: (A) Effects of medicines on the levels of glutathione (GSH) in serum; (B) Effects of medicines on the levels of GSH in the colon. Data are expressed as the mean±S.E.M. *P<0.05, **P<0.01 and ***P<0.001 vs. colitis group (CT-)

complications. This study aimed to investigate the protective potential of five agents (Br, SB, ALA, IN, and BU) against UC in a mouse model, with MZ as a usual treatment used to compare the therapeutic effects.

The results on body weight change and tissue edema showed that the group treated with PAC (the combination of all five agents) had the least weight loss and colon weight/length ratio, while the colitis group (CT-) showed the most body weight (over 10%) loss and an increased colon weight/length ratio. Previous studies have claimed that components of PAC including Br, IN, BU, ALA, and SB act as anti-edematous and anti-inflammatory agents (38-42). Colon weight/length ratio increment and body weight loss due to dehydration are recognized as consequences of UC (43). It is likely that in terms of controlling weight loss and tissue edema in UC, the administration of the studied compounds in combination acts more effectively than each alone. It should be noted that on the 11th day, food was withheld for 24 hours from all groups, which caused a brief reduction in weight on the 12th day. As there was no significant difference between the groups before the treatment, the weight gain in each group should be compared to themselves.

Acetic acid injection into the colon of experimental mice causes acute and severe inflammatory responses characterized by macroscopic changes, microscopic lesions of the colon wall, ulcers, submucosal edema, and a reduced number of goblet cells. Based on our results, although all the individual agents partially improved histopathological changes due to colitis, PAC significantly and more effectively alleviated the extent and intensity of the histological signs of cell damage including inflammation intensity and macroscopic and microscopic colon damage, improving the colitis. Previous reports have demonstrated that Br (13), IN (44), BU (45), ALA (46), and SB (21) improved the histopathological changes due to colitis, which is in accordance with the results of our study.

TNF-α is an inflammatory cytokine that plays an important role in IBD, including UC (47). The IL-6 cytokine level is a representative parameter of IBD that significantly correlates with the severity and extent of CD and UC (48). Our results showed a significant decrease in IL-6 and TNF-α in the serum

of all groups (only a non-significant decrease in TNF-α for the IN and MZ groups); however, in the colon, the decrease in IL-6 was significant only in the PAC and ALA groups, and the decrease in TNFα was significant in the PAC, ALA, and BU groups in comparison with colitis group. In addition, the level of colon TNFα and serum IL-6 was significantly lower in the PAC group than in the MZ group, which had a similar level to the control group. Acetic acid injection and the development of colitis increased the IL-6 and TNFα levels in the colon and blood compared to the control group. This is while the PAC group had significantly lower levels of these cytokines in the serum and colon tissues compared with the colitis group.

Silibinin (SB) inhibits transcription factor NF-κB and stops its induction of tumor necrosis factor-alpha (TNF-α) in a dose-dependent method (49). Also, it inhibits the formation of prostaglandins and cyclooxygenase 2, which are involved in neutrophil accumulation in the mucosa, production of reactive oxygen species (ROS), and oxidation in inflamed tissues (50). Probably, SB exerts beneficial effects due to its potent antioxidant properties. Antioxidant and protective properties of SB have been proven in laboratory animals with acute or chronic lesions (51). This study's results agree with previous studies that showed that oral intake of SB in 50 and 100 mg/kg concentrations would improve inflammation and tissue damage of the colon (52).

In an in vitro study on colon tissue obtained from patients with IBD, bromelain (Br) treatment reduced the secretion of pro-inflammatory cytokines and chemokines (53). In another in vitro research, the effects of purified fruit bromelain (PFB) on Tumor Necrosis Factor Receptors (TNFRs) indicated that epithelial TNFR1 and TNFR2 expression significantly increased in a rat colitis model but was neutralized by PFB. PFB also ameliorated colitis symptoms including infiltration of inflammatory cells, cytokine profiles, and epithelial cell apoptosis (54). The reduction of IL-6 and TNFα in the Br group of our research is consistent with the results of the mentioned studies.

An inulin (IN)-based diet reduces the risk of colon cancer (55) and increases the absorption of calcium, magnesium, zinc, and iron in the colon (56). In one study, the potential preventive effects of IN (1% (w/v) orally for 7 days) on dextran sulfate sodium (DSS)-induced colitis were studied. The prebiotic IN improved colitis symptoms by decreasing NO production and mucus secretion induction, suggesting an anti-inflammatory effect in preventing colitis (12). In our study, IN also improved colitis, but less than the other studied agents. Furthermore, IN reduced the level of IL-6 but not that of TNFα. Few studies have illustrated the effect of IN on increasing electrolyte permeability in intestines and promoting wound healing (12). Since the mice were sacrificed 48 hours after wound development in our study, there was possibly not enough time to heal the intestinal ulcer.

Sodium butyrate (BU) has been used to repair intestinal mucous membranes and has an anti-inflammatory role. By inhibiting TNFα, IL-13, and IFNγ, BU can improve IBD, especially UC (57-59). In another study, simultaneous administration of BU and MZ in UC treatment showed a better improvement in UC (60). In another report, mice received BU (0.5% via oral) for 14 days in DSS-induced UC. Results revealed that BU improves mucosal lesions and the inflammatory profile of the intestinal mucosa (61). In this study, BU significantly reduced the pro-inflammatory and inflammatory cytokines in the serum and tissue, which is consistent with other studies (61). BU derivatives play an important role in keeping the colon healthy through the inhibition of the histone deacetylase enzyme, which plays a critical role in gene regulation, immune system modulation, cancer suppression, cell differentiation, reducing oxidative stress, controlling diarrhea, visceral sensitivity, and modulating intestinal motion (61, 62).

In a rat model of UC, alpha-lipoic acid (ALA) at a dose of 25 mg/kg for three days decreased inflammation of the intestine with the loss of neutrophil accumulation and the inhibition of oxidative reactions, as well as a change in the intestinal morphology (20, 24). In a recently published study on a mouse model of UC, ALA carried out its protective role by controlling nuclear factor kappa B, cyclooxygenase 2, and IL-17 (24). The results of this study are in accordance with previous studies, as ALA (80 mg/kg) improved inflammation and tissue damage due to UC (20, 24). Our results of the immunohistochemistry study showed that ALA significantly reduced acetic acid-induced levels of pro-inflammatory and anti-inflammatory cytokines in the colon and serum, in accordance with previous studies (24). We found that ALA could improve colitis and was more effective than the other compounds used (even MZ), but less effective than PAC.

Therapeutic effects of medicines on histopathological changes showed that treatment with SB, BU, and PAC could relieve colitis, while ALA, IN, and Br relatively relieved colitis. Our research results indicate that PAC is able to reduce the expression of IL-6 in the colon tissue. The reduction of inflammatory cytokines (TNFα) and histology results indicate an effective improvement in disease in mice. Figure 9 summarizes the mechanism of action of the various compounds used in this study against UC.

Glutathione (GSH) is the most abundant intracellular antioxidant component with a wide range of antioxidant capacities. A significant reduction in GSH levels was shown in the colitis group due to colonic tissue damage. An increase in oxidative stress and consequent redox adjustment by antioxidants, such as reduced GSH, are involved in the pathophysiology of UC in both animals and clinical trial studies (63, 64). Any reduction in the

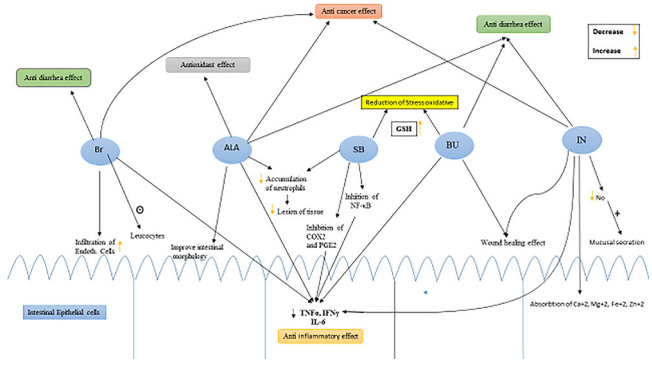

Figure 9: Mechanism of action of different compounds used against ulcerative colitis: bromelain (Br), silibinin (SB), alpha-lipoic acid (ALA), inulin (IN), and sodium butyrate (BU).

level of reduced GSH has often been positively correlated with an increased risk of oxidative stress (65). Accordingly, the reduction of GSH content showed in the acetic acid-positive control group may be due to increased oxidative stress in this group. The results of the present study are similar to those reported earlier (66, 67).

Conclusion

Ulcerative colitis (UC) is an inflammatory disorder of the gastrointestinal tract, with an increasing prevalence in developed countries. Each of the drugs used in this study was relatively useful in improving the UC induced in the mouse model. However, the results showed that the synergistic effects of medicines in the PAC group led to more potency and efficacy in improving UC induced in a mouse model. Although more clinical studies are required, our study demonstrated that PAC might be beneficial for UC prevention and treatment.

Ethics Approval

All the experiments were conducted at the Medical Research and Innovation Center under the supervision of the research assistant of Medical Sciences of Mashhad Islamic Azad University. All experimental procedures were performed following approval by the Ethical Committee for Animal Care and Use of the Islamic Azad University of Mashhad (Ethical approval number: IR.IAU.MSHD.REC.1396.113). Also, all animal experiments were performed in accordance with National Institutes of Health (NIH) animal care guidelines.

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Authors' Contribution

VP and AHM contributed to the study conception and design. Material preparation, data collection, and analysis were performed by VP, VA. The first draft of the manuscript was written by AP, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Conflict of interest: None declared.

References

1. Feuerstein JD, Cheifetz AS, editors. Ulcerative colitis: epidemiology, diagnosis, and management. Mayo Clinic Proceedings; 2014: Elsevier.
2. Ling K-L, Ooi C-J, Luman W, Cheong W-K, Choen FS, Ng H-S. Clinical Characteristics of Ulcerative Colitis in Singapore, a Multiracial City–State. Journal of clinical gastroenterology. 2002;35(2):144-8.
3. Lok KH, Hung HG, Ng CH, Kwong KC, Yip WM, Lau SF, et al. Epidemiology and clinical characteristics of ulcerative colitis in Chinese population: experience from a single center in Hong Kong. Journal of gastroenterology and hepatology. 2008;23(3):406-10.
4. Jantchou P, Morois S, Clavel-Chapelon F, Boutron-Ruault M-C, Carbonnel F. Animal protein intake and risk of inflammatory bowel disease: The E3N prospective study. The American journal of gastroenterology. 2010;105(10):2195.
5. Jowett S, Seal C, Pearce M, Phillips E, Gregory W, Barton J, et al. Influence of dietary factors on the clinical course of ulcerative colitis: a prospective cohort study. Gut. 2004;53(10):1479-84.
6. Travis S, Stange E, Lémann M, Øresland T, Bemelman W, Chowers Y, et al. European evidence-based consensus on the management of ulcerative colitis: current management. Journal of Crohn's and Colitis. 2008;2(1):24-62.
7. Wolfe MM, Lichtenstein DR, Singh G. Gastrointestinal toxicity of nonsteroidal antiinflammatory drugs. New England Journal of Medicine. 1999;340(24):1888-99.
8. Mowat C, Cole A, Windsor A, Ahmad T, Arnott I, Driscoll R, et al. Guidelines for the management of inflammatory bowel disease in adults. Gut. 2011;60(5):571-607.

9. Nitzan O, Elias M, Peretz A, Saliba W. Role of antibiotics for treatment of inflammatory bowel disease. World journal of gastroenterology. 2016;22(3):1078.
10. Connell W, Kamm M, Ritchie J, Lennard-Jones J. Bone marrow toxicity caused by azathioprine in inflammatory bowel disease: 27 years of experience. Gut. 1993;34(8):1081-5.
11. Hayes CE, Cerezo CS. Monoclonal Antibody Use in Inflammatory Bowel disease. Medicine and Health Rhode Island. 2011;94(11):337.
12. Abdelouhab K, Rafa H, Toumi R, Bouaziz S, Medjeber O, Touil-Boukoffa C. Mucosal intestinal alteration in experimental colitis correlates with nitric oxide production by peritoneal macrophages: effect of probiotics and prebiotics. Immunopharmacology and immunotoxicology. 2012;34(4):590-7.
13. Hale LP, Greer PK, Trinh CT, Gottfried MR. Treatment with oral bromelain decreases colonic inflammation in the IL-10-deficient murine model of inflammatory bowel disease. Clinical Immunology. 2005;116(2):135-42.
14. Kane S, Goldberg MJ. Use of bromelain for mild ulcerative colitis. Annals of internal medicine. 2000;132(8):680.
15. Chobotova K, Vernallis AB, Majid FAA. Bromelain's activity and potential as an anti-cancer agent: current evidence and perspectives. Cancer letters. 2010;290(2):148-56.
16. Leenen CH, Dieleman LA. Inulin and oligofructose in chronic inflammatory bowel disease. The Journal of Nutrition. 2007;137(11):2572S-5S.
17. Guarner F. Inulin and oligofructose: impact on intestinal diseases and disorders. British Journal of Nutrition. 2005;93(S1):S61-S5.
18. Vernia P. Butyrate in the treatment of ulcerative colitis. Digestive and Liver Disease Supplements. 2007;1(1):27-30.
19. Schnekenburger M, Diederich M. Nutritional epigenetic regulators in the field of cancer: new avenues for chemopreventive approaches. Epigenetic cancer therapy: Elsevier; 2015. p. 393-425.
20. Kolgazi M, Jahovic N, Yüksel M, Ercan F, Alican I. α-Lipoic acid modulates gut inflammation induced by trinitrobenzene sulfonic acid in rats. Journal of gastroenterology and hepatology. 2007;22(11):1859-65.
21. Zheng R, Ma J, Wang D, Dong W, Wang S, Liu T, et al. Chemopreventive Effects of Silibinin on Colitis-Associated Tumorigenesis by Inhibiting IL-6/STAT3 Signaling Pathway. Mediators of inflammation. 2018;2018.
22. Wan P, Chen H, Guo Y, Bai A-P. Advances in treatment of ulcerative colitis with herbs: from bench to bedside. World Journal of Gastroenterology: WJG. 2014;20(39):14099.
23. Vieira EL, Leonel AJ, Sad AP, Beltrão NR, Costa TF, Ferreira TM, et al. Oral administration of sodium butyrate attenuates inflammation and mucosal lesion in experimental acute ulcerative colitis. The Journal of nutritional biochemistry. 2012;23(5):430-6.
24. Trivedi P, Jena G. Role of α-lipoic acid in dextran sulfate sodium-induced ulcerative colitis in mice: studies on inflammation, oxidative stress, DNA damage and fibrosis. Food and chemical toxicology. 2013;59:339-55.
25. Kauntz H, Bousserouel S, Gosse F, Marescaux J, Raul F. Silibinin, a natural flavonoid, modulates the early expression of chemoprevention biomarkers in a preclinical model of colon carcinogenesis. International journal of oncology. 2012;41(3):849-54.
26. Atarbashe RK, Abu-Raghif A. Comparative treatment of induced ulcerative colitis in male rat model by using cinnarizine and sulfasalazine. Iraqi Journal of Veterinary Sciences. 2020;34(2):465-72.
27. Mousavizadeh K, Rahimian R, Fakhfouri G, Aslani F, Ghafourifar P. Anti-inflammatory effects of 5-HT3 receptor antagonist, tropisetron on experimental colitis in rats. European journal of clinical investigation. 2009;39(5):375-83.
28. Rachmilewitz D, Karmeli F, Takabayashi K, Hayashi T, Leider-Trejo L, Lee J, et al. Immunostimulatory DNA ameliorates experimental and spontaneous murine colitis. Gastroenterology. 2002;122(5):1428-41.
29. Pavan R, Jain S, Kumar A. Properties and therapeutic application of bromelain: a review. Biotechnology research international. 2012;2012.
30. Johannsen F. Toxicological profile of carboxymethyl inulin. Food and chemical toxicology. 2003;41(1):49-59.
31. Egorin MJ, Yuan Z-M, Sentz DL, Plaisance K, Eiseman JL. Plasma pharmacokinetics of butyrate after intravenous administration of sodium butyrate or oral administration of tributyrin or sodium butyrate to mice and rats. Cancer chemotherapy and pharmacology. 1999;43(6):445-53.
32. Cremer D, Rabeler R, Roberts A, Lynch B. Safety evaluation of α-lipoic acid (ALA). Regulatory Toxicology and Pharmacology. 2006;46(1):29-41.
33. Abenavoli L, Capasso R, Milic N, Capasso F. Milk thistle in liver diseases: past, present, future. Phytotherapy Research. 2010;24(10):1423-32.
34. Khoshakhlagh P B-SM, Mohammadirad A, Ashtaral-Nakhai L, Minaie B, Abdollahi M. . Beneficial effect of phosphodiesterase-5 inhibitor in experimental inflammatory bowel disease; molecular evidence for involvement of oxidative stress. . Toxicology mechanisms and methods. 2007;17(5):281-8.
35. Araki Y, Sugihara H, Hattori T. The free radical scavengers edaravone and tempol suppress experimental dextran sulfate sodium-induced colitis in mice. International journal of molecular medicine. 2006;17(2):331-4.
36. Rees V. Chronic experimental colitis induced by dextran sulphate sodium (DSS) is characterized by Th1 and Th2 cytokines. Clinical & Experimental Immunology. 1998;114(3):385-91.
37. Nishiyama Y, Kataoka T, Yamato K, Taguchi T, Yamaoka K. Suppression of dextran sulfate sodium-induced colitis in mice by radon inhalation. Mediators of inflammation. 2012;2012.
38. Rathnavelu V, Alitheen NB, Sohila S, Kanagesan S, Ramesh R. Potential role of bromelain in clinical and therapeutic applications. Biomedical reports. 2016;5(3):283-8.
39. Nicola WG, Girgiss MW, El-Arab AME, Habib DF, Elnemr ME, Ahmed NM. Role of Inulin in the Protection and Management of Metabolic Inflammation in Humans by. Biomedical and Pharmacology Journal. 2018;11(2):1083-90.
40. Bach Knudsen K, Lærke H, Hedemann M, Nielsen T, Ingerslev A, Gundelund Nielsen D, et al. Impact of diet-modulated butyrate production on intestinal barrier function and inflammation. Nutrients. 2018;10(10):1499.
41. Kiemer AK, Müller C, Vollmar AM. Inhibition of LPS-induced nitric oxide and TNF-α production by α-lipoic acid in rat Kupffer cells and in RAW 264.7 murine macrophages. Immunology and cell biology. 2002;80(6):550-7.
42. Tong W, Zhang C, Hong T, Liu D, Wang C, Li J, et al. Silibinin alleviates inflammation and induces apoptosis in human rheumatoid arthritis fibroblast-like synoviocytes and has a therapeutic effect on arthritis in rats. Scientific reports. 2018;8(1):3241.
43. Elsherif Y, Alexakis C, Mendall M. Determinants of weight loss prior to diagnosis in inflammatory bowel disease: a retrospective observational

study. Gastroenterology research and practice. 2014;2014.
44. Videla S, Vilaseca J, Antolín M, García-Lafuente A, Guarner F, Crespo E, et al. Dietary inulin improves distal colitis induced by dextran sodium sulfate in the rat. The American journal of gastroenterology. 2001;96(5):1486.
45. Malago JJ, Sangu CL. Intraperitoneal administration of butyrate prevents the severity of acetic acid colitis in rats. Journal of Zhejiang University-SCIENCE B. 2015;16(3):224-34.
46. Sun J, Zhang H, Guan L, Zhou H, Sun M. Alpha-lipoic acid attenuates trinitrobenzene sulfonic acid-induced ulcerative colitis in mice. International journal of clinical and experimental medicine. 2015;8(1):358.
47. Kuethe F, Figulla HR, Herzau M, Voth M, Fritzenwanger M, Opfermann T, et al. Treatment with granulocyte colony–stimulating factor for mobilization of bone marrow cells in patients with acute myocardial infarction. American heart journal. 2005;150(1):115.
48. Takač B, Mihaljević S, Štefanić M, Glavaš-Obrovac L, Kibel A, Samardžija M. Importance of interleukin 6 in pathogenesis of inflammatory bowel disease. Collegium antropologicum. 2014;38(2):659-64.
49. Manna SK, Mukhopadhyay A, Van NT, Aggarwal BB. Silymarin suppresses TNF-induced activation of NF-κB, c-Jun N-terminal kinase, and apoptosis. The Journal of Immunology. 1999;163(12):6800-9.
50. Zhao J, Sharma Y, Agarwal R. Significant inhibition by the flavonoid antioxidant silymarin against 12-O-tetradecanoylphorbol 13-acetate–caused modulation of antioxidant and inflammatory enzymes, and cyclooxygenase 2 and interleukin-1α expression in SENCAR mouse epidermis: Implications in the prevention of stage I tumor promotion. Molecular carcinogenesis. 1999;26(4):321-33.
51. Osuchowski M, Johnson V, He Q, Sharma R. Alterations in regional brain neurotransmitters by silymarin, a natural antioxidant flavonoid mixture, in BALB/c mice. Pharmaceutical biology. 2004;42(4-5):384-9.
52. Esmaily H, Hosseini-Tabatabaei A, Rahimian R, Khorasani R, Baeeri M, Barazesh-Morgani A, et al. On the benefits of silymarin in murine colitis by improving balance of destructive cytokines and reduction of toxic stress in the bowel cells. Central European Journal of Biology. 2009;4(2):204.
53. Onken JE, Greer PK, Calingaert B, Hale LP. Bromelain treatment decreases secretion of pro-inflammatory cytokines and chemokines by colon biopsies in vitro. Clinical Immunology. 2008;126(3):345-52.
54. Zhou Z, Wang L, Feng P, Yin L, Wang C, Zhi S, et al. Inhibition of epithelial TNF-α receptors by purified fruit bromelain ameliorates intestinal inflammation and barrier dysfunction in colitis. Frontiers in immunology. 2017;8:1468.
55. Pool-Zobel BL. Inulin-type fructans and reduction in colon cancer risk: review of experimental and human data. British Journal of Nutrition. 2005;93(S1):S73-S90.
56. Shahzad Z, Rouached H, Rakha A. Combating mineral malnutrition through iron and zinc biofortification of cereals. Comprehensive Reviews in Food Science and Food Safety. 2014;13(3):329-46.
57. Plöger S, Stumpff F, Penner GB, Schulzke JD, Gäbel G, Martens H, et al. Microbial butyrate and its role for barrier function in the gastrointestinal tract. Annals of the New York Academy of Sciences. 2012;1258(1):52-9.
58. Kovarik JJ, Tillinger W, Hofer J, Hölzl MA, Heinzl H, Saemann MD, et al. Impaired anti-inflammatory efficacy of n-butyrate in patients with IBD. European journal of clinical investigation. 2011;41(3):291-8.
59. Diakos C, Prieschl EE, Säemann MD, Böhmig GA, Csonga R, Sobanov Y, et al. n-Butyrate inhibits Jun NH (2)-terminal kinase activation and cytokine transcription in mast cells. Biochemical and biophysical research communications. 2006;349(2):863-8.
60. Assisi R. Combined butyric acid/mesalazine treatment in ulcerative colitis with mild-moderate activity. Results of a multicentre pilot study. Minerva gastroenterologica e dietologica. 2008;54(3):231-8.
61. Chen G, Ran X, Li B, Li Y, He D, Huang B, et al. Sodium Butyrate Inhibits Inflammation and Maintains Epithelium Barrier Integrity in a TNBS-induced Inflammatory Bowel Disease Mice Model. EBioMedicine. 2018;30:317-25.
62. Hamer HM, Jonkers D, Venema K, Vanhoutvin S, Troost F, Brummer RJ. The role of butyrate on colonic function. Alimentary pharmacology & therapeutics. 2008;27(2):104-19.
63. Pravda J. Radical induction theory of ulcerative colitis. World journal of gastroenterology: WJG. 2005;11(16):2371.
64. Karp SM, Koch TR. Oxidative stress and antioxidants in inflammatory bowel disease. Disease-a-Month. 2006;5(52):199-207.
65. McLennan SV, Heffernan S, Wright L, Rae C, Fisher E, Yue DK, et al. Changes in hepatic glutathione metabolism in diabetes. Diabetes. 1991;40(3):344-8.
66. Rezaei N, Eftekhari MH, Tanideh N, Mokhtari M, Bagheri Z. Protective Effects of Honey and Spirulina Platensis on Acetic Acid-Induced Ulcerative Colitis in Rats. Iranian Red Crescent Medical Journal. 2018(In Press).
67. Nieto N, Torres M, Fernandez M, Giron M, Rios A, Suarez M, et al. Experimental ulcerative colitis impairs antioxidant defense system in rat intestine. Digestive diseases and sciences. 2000;45(9):1820-7.