Effects of Mesalazine and Coenzyme-Q10 on Colonic Histology in Rat Model of Ulcerative Colitis

Hannah Pirzada,[1] Samreen Hameed,[2] Ajmal Afzal,[3] Kanwar Sajid Ali,[4] Mahwash Malik,[5] Muhammad Faisal Javaid,[6]

Abstract

Objective: To observe the preventive effects of mesalazine and Coenzyme-Q10 on colonic histology in dextran sulfate sodium induced rat model of Ulcerative Colitis.

Material and Methods: It was an animal experimental study conducted in the Department of Pharmacology, King Edward Medical University and University of Veterinary & Animal Sciences, Lahore. Forty eight 48 healthy male albino rats were divided into 6 groups. Groups A and B were taken as healthy and diseased control groups. Groups B, C, D, E, and F were given 4% Dextran Sulfate Sodium in drinking water for inducing ulcerative colitis. Concomitantly, the rats were treated as per group designation, with full or half doses of mesalazine (50, 100mg/kg/day) and Coenzyme-Q10 (30mg/kg/day). Animals were euthanized after the study and colonic tissue was examined for histopathological changes (extent of inflammation, extent of crypt damage, cryptitis, crypt abscesses, and basal cell plasmacytosis).

Results: There was a marked improvement in the crypt architecture as well as the inflammatory changes in the combined treatment group as compared to the groups given Mesalazine and Coenzyme-Q10 separately.

Conclusion: Combined treatment with Mesalazine and coenzyme-Q10 has a better protective effect on colon in ulcerative colitis than either of them used alone.

Keywords: ulcerative colitis, mesalazine, coenzyme-Q10, histopathological, dextran sulfate sodium

How to cite: Pirzada H, Hameed S, Afzal A, Ali KS, Malik M, Javaid MF. Effects of Mesalazine and Coenzyme-Q10 On Colonic Histology in Rat Model of Ulcerative Colitis. Esculapio - JSIMS 2024;20(03): *360-365*

DOI: https://doi.org/10.51273/esc24.251320314

Introduction

Ulcerative colitis (UC) is a major phenotype of inflammatory bowel disease (IBD). It is a common chronic disease characterized pathologically by intestinal inflammation and epithelial injury. It is a chronic remitting-relapsing disease and manifests by abdominal pain, diarrhea, blood in stool, pallor, fever, low energy, and weight loss.[1,2] Disease is usually mild but it can be life-threatening during severe attacks. Also, there is an increased risk of colorectal cancer.[3] Studies in recent years have identified a major role of both genetic and environmental factors in the pathogenesis of UC.[4] A combination of these risk factors seems to initiate alterations in epithelial barrier function thereby allowing the translocation of luminal antigens into the bowel wall. Subsequently, aberrant and excessive cytokine responses to such environmental triggers cause subclinical or acute mucosa inflammation in a genetically susceptible host. In patients that fail to resolve acute intestinal inflammation, chronic intestinal inflammation develops that is induced by the uncontrolled activation of the mucosal immune system. In particular, mucosal immune cells such as macrophages, T cells, and subsets of innate lymphoid cells seem to respond to microbial

1. *Department of Pharmacology, Bakhtawar Amin Medical and Dental College, Multan*
2. *Department of Pathology, King Edward Medical University, Lahore*
3. *Department of Pharmacology, Gujranwala Medical College, Gujranwala*
4. *Department of Pathology, Bakhtawar Amin Medical and Dental College, Multan*
5. *Department of Pharmacology, Central Park Medical College, Lahore*
6. *Department of Biochemistry, Niazi Medical and Dental College, Sargodha*

Correspondence:
Dr. Hannah Pirzada, *Assistant professor Pharmacology, Bakhtawar Amin Medical and Dental College, Multan, Hannah.pirzada@gmail.com*

Submission Date:	25-06-2024
1st Revision Date:	15-07-2024
Acceptance Date:	15-09-2024

antigens by producing cytokines that can promote chronic inflammation of the gastrointestinal tract.[5,6] Previously, IBD was considered to be a Western disease but now, it has become a global issue.[7,8] Since 1990, the incidence has been rising in newly industrialized countries in Africa, Asia, and South America.[9] Yearly change in the incidence (specified as Annual Percentage Change) of ulcerative colitis is +14•9%.[10] From 2000 to 2010, the adjusted annual incidence rate for UC was 12.2 per 100,000 persons.[11] About 47,400 people died due to UC and Crohn's Disease together in 2015.[12] At present, no curative treatment is available that results in prolonged disease, long-term diarrhea, and recurrences that affect overall health and quality of life.[9]

Pharmacologic management aims to induce remissions and prevent UC exacerbation or acute flare. Various drug groups are used for this purpose with varying control of the disease. Sometimes, surgeries are also required. However, to date, no curative plan has been devised. The drugs used in general include sulfasalazine, aminosalicylates or 5ASA (e.g, mesalazine, olsalazine, basalazide), steroids in high doses (e.g, prednisolone, methylprednisolone, hydrocortisone, beclometasone) and other immunomodulators (e.g., azathioprine, 6-mercaptopurine, methotrexate). Antibiotics are also given if required.[13] Mesalazine is considered one of the most potent drugs used in UC. Its regular use is protective against mucosal damage and permeation. It is used in most UC patients to induce and maintain remissions. The precise mechanism of action of mesalazine is unknown but evidence has shown that it acts by targeting and inhibiting the COX-1 and COX-2 enzymes. It also inhibits the activation of various inflammatory cells and biomarkers and restores the pathophysiological balance of the disease to normal.[14,15] Co-Q10 is used to treat various disorders related primarily to suboptimal cellular energy metabolism and oxidative injury. Studies supporting the efficacy of Co-Q10 appear most promising for a variety of diseases, including UC.[16] The Dextran Sulfate sodium-induced UC model is one of the widely used models as it can be easily developed owing to the wide availability and effectiveness of DSS. Some researchers have suggested that DSS mainly affects the large intestine i.e. middle and distal third of the large intestine and resembles human disease both symptomatically as well as histologically.[17] The present study is designed to observe the role of mesalazine and Co-Q10 alone and in combination on colonic histology in UC and to hypothesize that the combination therapy has a better effect than each given separately.

Material and Methods

The study design was an animal experimental study. The study was conducted in the King Edward Medical University and University of Veterinary & Animal Sciences, Lahore. After taking the approval from ethnical committee with IRB No. 789/RC/KEMU dated 13-05-2019. Sampling Technique was Simple random sampling. Sample Size was forty-eight rats were divided into six groups by lottery method. Male Sprague-Dawley rats, weighing 180g to 220g. Rats showing signs of any disease. 48 adult healthy albino rats of male gender were purchased from and kept in the animal house UVAS (University of Veterinary and Animal Sciences), Lahore. Animals were divided randomly into 6 equal groups having 8 rats in each group. The rats were exposed to natural day and night cycles at room temperature of 22±2°C with 50±5% humidity throughout the experiment. They had free access to rat chow and water ad libitum. An interval of seven days was given to them to get acclimatized before the start of the experiment. The calculated dose for an individual rat, i.e. 100mg/kg/day of Mesalazine[18] and 30mg/kg/day of Co-Q10[19] were weighed and prepared in 4% methocel solution and 0.5% carboxymethylcellulose respectively. DSS was added to the drinking water of rats of groups B, C, D, E, and F. A 4% w/v solution was made by adding DSS (molecular weight 40-50kDa) in water and rats were given this solution to drink throughout the study.[20] Forty-eight rats were divided into six groups randomly by lottery method, with 8 rats in each group. These groups were labeled as A, B, C, D, E, and F. Rats in group A (normal control group) were fed with standard rat diet throughout the study period of 7 days. Rats in group B (disease control group) were fed with a normal diet but 4% w/v solution of DSS was given instead of water. Rats in group C were given oral mesalazine prepared in 4% methocel solution at a dose of 100mg/kg/day once daily, DSS in drinking water, and placebo vehicle (0.5% carboxymethylcellulose). The rats in group D were given oral Co-Q10 prepared in 0.5% carboxymethylcellulose at a dose of 30mg/kg/day once daily, DSS in drinking water, and placebo vehicle (4% methocel). The rats in group E were given mesalazine prepared in 4% methocel solution at dose of 50mg/kg/day orally once daily, Co-Q10 prepared in 0.5% carboxymethylcellulose at dose of 30mg/kg/day orally once daily and DSS in drinking water and the rats in group F were given

mesalazine prepared in 4% methocel solution at dose of 100mg/kg/day orally once daily, Co-Q10 prepared in 0.5% carboxymethylcellulose at dose of 30mg/kg/day orally once daily and DSS in drinking water. Twenty-four hours after the last dose administered the rats were sacrificed at the end of day 7. The colon of each rat was identified and dissected out and preserved in formalin separately. The lower colon was cut out and washed thoroughly with distilled water. A 3cm segment of the colon was separated and fixed in a 10% neutral-buffered formalin solution (pH 7.4). Processed colon tissues were fixed in paraffin wax and cut into 5um thick sections. Slides were prepared and stained with H&E stain to visualize the histological changes of inflammatory parameters (Extent of inflammation, Extent of crypt damage, Cryptitis, Crypt abscesses, Basal cell plasmacytosis). Light microscopic evaluation of DSS-induced damages to colonic mucosal and submucosal layers and response to mesalazine and Co-Q10 administration were scored as follows:

Extent of inflammation; 0 for no inflammatory changes, 1 for changes involving mucosa only, 2 for mucosal and submucosal involvement, 3 for mucosal, submucosal, and muscular layer involvement, and 4 for trans-mural changes. The extent of crypt damage was scored as; 0 for no damage, 1 for basal one-third crypt damage, 2 for basal two-third crypt damage, 3 for entire crypt damage, and 4 for crypt damage plus ulceration. Cryptitis was scored as; 0 for none, 1 for <25%, 2 for 25-50%, 3 for 50-75%, and 4 for >75%. Crypt abscesses were scored as; 0 for none, 1 for focal, and 2 for multi-focal. Basal cell plasmacytosis was scored as; 0 for none, 1 for focal, 2 for multi-focal, and 3 for diffuse.[18] Data was analyzed by using Statistical Package for Social Studies (SPSS) software for Windows (version 23.0) and Graph-Pad Prism (version 8). Histopathological changes were scored as numbers and expressed as percentages of changes in groups; the Chi-square test was used for evaluation. The significance of differences was measured through the Whitney U test. A P-value of less than 0.05 was considered significant.

Results

Inflammation was absent in all rats in Group A. In the disease group (Group B), 25% (02) rats had inflammation involving mucosa, submucosa, and muscular layer and 75% (06) rats had trans-mural extent of inflammation. In rats receiving mesalazine (Group C), 63% (05) rats had inflammation extended up to mucosa and 38% (03) rats had inflammation involving mucosa and submucosa. None of them had inflammation involving the muscular layer. In rats receiving Co-Q10 (Group D), 75% (06) rats had inflammation extended up to mucosa and submucosa and 25% (02) rats had inflammation involving mucosa, submucosa, and muscular layer. None had trans-mural involvement. In rats receiving a half dose of mesalazine along with Co-Q10 (Group E), 75% (06) rats had inflammation extended up to mucosa and submucosa and 25% (02) rats had inflammation involving mucosa, submucosa, and muscular layer. None had trans-mural involvement. While in rats receiving mesalazine in full dose along with Co-Q10 (Group F), 88% (07) rats had inflammation up to the mucosal layer and 13% (01) rats had inflammation involving mucosal and submucosal layers. None had inflammation involving the muscular layer. Comparison of the extent of inflammation among groups showed a significant difference with a p-value of .000. Crypt damage was absent in all rats in Group A. In the disease group (Group B), 25% (02) rats had damage of basal two-thirds of the crypts, and 75% (06) rats had entire crypt damage along with ulceration. In rats receiving mesalazine (Group C), 38% (03) rats had damage to basal one-third of crypts, and 63% (05) rats had damage of basal two-thirds of crypts. In rats receiving Co-Q10 (Group D), 100% (08) rats had damage of basal two-thirds of the crypts. In rats receiving a half dose of mesalazine along with Co-Q10 (Group E), 25% (02) rats had damage of basal one-third of crypts, and 75% (06) rats had damage of basal two-thirds of crypts. While in rats receiving mesalazine in full dose along with Co-Q10 (Group F), 100% (08) rats had damage of basal one-third of crypts. Comparison of the extent of crypt damage among groups showed a significant difference with a p-value of .000.

Cryptitis was absent in all rats in Group A. In disease group (Group B), 25% (02) rats had cyptitis that involved 25-50% of the crypt area, 38% (03) rats had cryptitis involving 50-75% of the crypt area, and 38% (03) rats had cryptitis involving >75% of crypt area. In rats receiving mesalazine (Group C), 100% (08) of rats had cyptitis that involved <25% of the crypt area. In rats receiving Co-Q10 (Group D), 75% (06) rats had cyptitis that involved <25% of the crypt area, and 25% (02) rats had cyptitis that involved 25-50% of the crypt area. In rats receiving a half dose of mesalazine along with Co-Q10 (Group E), 25% (02) rats had cyptitis that involved <25% of the crypt area, and 75% (06) rats had cryptitis involving 50% of the crypt area. While in rats receiving mesalazine in full dose along with Co-Q10 (Group F),

100% (08) rats had cyptitis that involved <25% of the crypt area. Comparison of cryptitis among groups showed a significant difference with a p-value of .000.

Absent in all rats in Group A. In the disease group (Group B), 100% (08) of rats had multi-focal crypt abscesses. In rats receiving mesalazine (Group C), 100% (08) of rats had focal crypt abscesses. In rats receiving Co-Q10 (Group D), 75% (06) rats had focal crypt abscesses and 25% (02) rats had multi-focal crypt abscesses. In rats receiving a half dose of mesalazine along with Co-Q10 (Group E), 25% (02) rats had focal crypt abscesses and 75% (06) rats had multi-focal crypt abscesses. While in rats receiving mesalazine in full dose along with Co-Q10 (Group F), 100% (08) rats had focal crypt abscesses. Comparison of crypt abscesses among groups showed a significant difference with a p-value of .000.

seen in any rat in Group A. In the disease group (Group B), 100% (08) of rats had diffuse basal cell plasmacytosis. In rats receiving mesalazine (Group C), 63% (05) rats had focal basal cell plasmacytosis and 38% (03) rats had multifocal basal cell plasmacytosis. In rats receiving Co-Q10 (Group D), 75% (06) rats had multifocal basal cell plasmacytosis and 25% (02) rats had diffuse basal cell plasmacytosis. In rats receiving a half dose of mesalazine along with Co-Q10 (Group E), 88% (07) rats had multifocal basal cell plasmacytosis and 13% (01) rats had diffuse basal cell plasmacytosis. While in rats receiving mesalazine in full dose along with Co-Q10 (Group F), 100% (08) rats had focal basal cell plasmacytosis. Comparison of basal cell plasmacytosis among groups showed a significant difference with a p-value of .000.

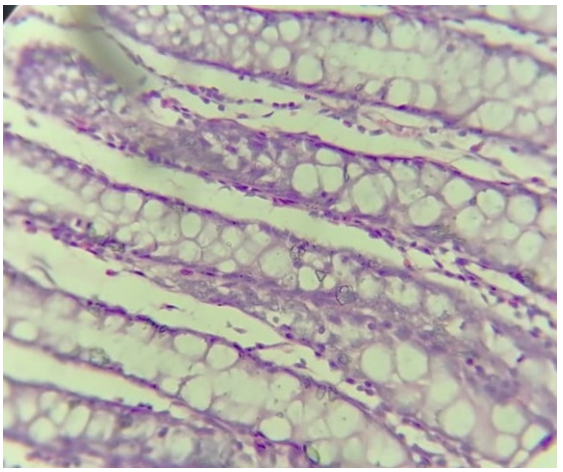

Figure 1: *Picture showing normal histology of the colon. Normal crypt architecture with no inflammation seen in group A (H&E 40X)*

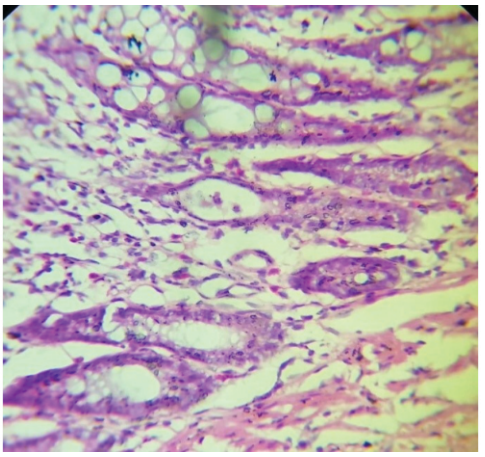

Figure 2: *Figure shows significant crypt abscesses and total loss of crypt architecture in group B. Significant inflammation can be seen with cryptitis (H&E 40X)*

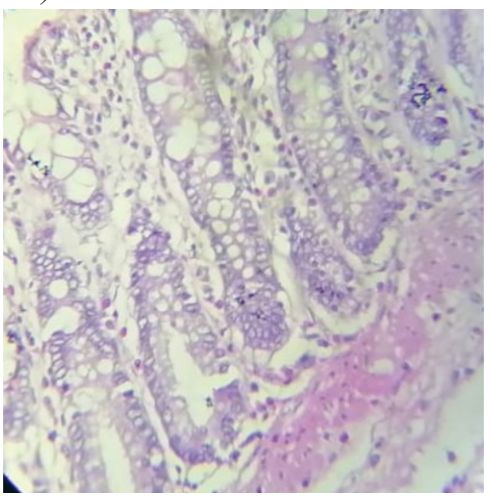

Figure 3: *Figure showing basal cell plasmacytosis in group B (H&E 40X)*

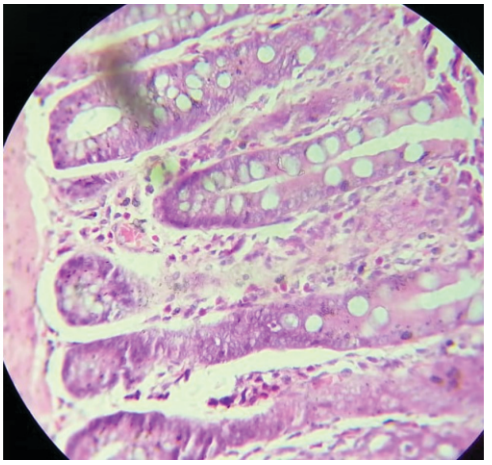

Figure 4: *Figure shows normal crypt architecture, and mild inflammation in the group treated with mesalazine (Group C). No cryptitis or crypt abscess was*

seen. (H&E 20X)

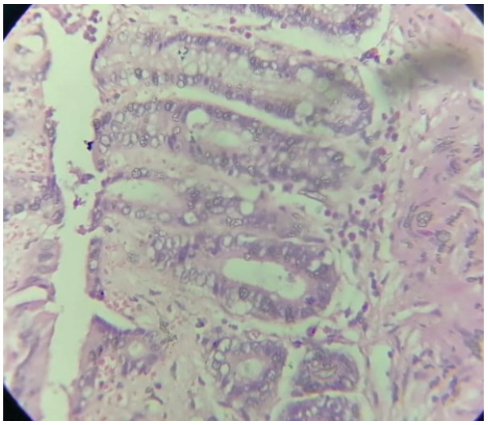

Figure 5: *Figure shows mild cryptitis and inflammation in the group treated with Co-Q10 (Group D). No crypt abscesses were seen. (H&E 20X)*

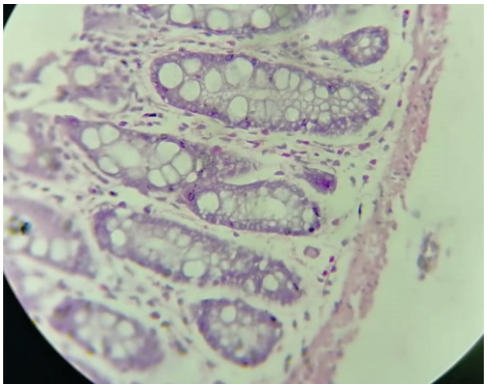

Figure 6: *Figure shows mild cryptitis, inflammation, and focal crypt abscesses in group E. (H&E 10X)*

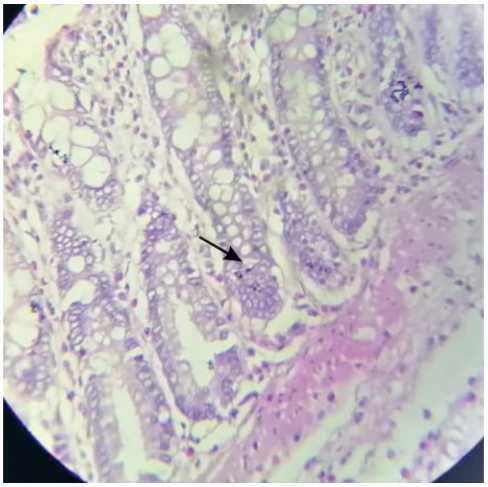

Figure 7: *The figure shows normal crypt architecture, no inflammation, no cryptitis, and no crypt abscesses seen in the group treated with a combination of mesalazine and Co-Q10 (Group F) (H&E 20X)*

Discussion

Histopathological study with light and deca-head microscopy revealed some major changes in inflammatory changes and architecture of crypts. DSS led to a complete distortion of the crypt architecture. It caused severe inflammatory changes, cryptitis, and basal cell plasmacytosis which led to degeneration of the crypts and produced focal and dense crypt abscesses. Similar findings were reported in the research works using the rat model of DSS-induced UC.[18,21] After the administration of Mesalazine for 7 days, there was some reversal of these histopathological changes in the colonic sections of the rats of group C. The extent of crypt abscesses, cryptitis, and inflammation was decreased. A significant restoration of crypt architecture was seen. Such results were seen in a study where Mesalazine was used to treat DSS-induced UC in rats.[18] Administration of Co-Q10 for 7 days in rats of group D, inflammation was reduced and crypt architecture restoration was seen to some extent. Crypt abscesses were settled to a significant extent. Similar results were seen in a study where Co-Q10 was given for UC.[16] Administration of half dose of Mesalazine and full dose of Co-Q10 for 7 days, in rats of group E, reduced inflammation and restored crypt architecture to some extent. Full doses of both Mesalazine and Co-Q10 when administered together completely settled down the inflammation, cryptitis, crypt abscesses, and basal cell plasmacytosis and completely restored the crypt architecture. No study to date has suggested the potentiation of therapeutic effects of Mesalazine with administration of Co-Q10.

Conclusion

This study has demonstrated that the combined administration of Mesalazine and Co-Q10 has exerted a stronger effect on restoring colonic histology to normal in the UC rat model as compared to both of these drugs given alone.

Conflict of interest: *None*

Funding Source: *None*

References

1. Tesija Kuna A. Serological markers of inflammatory bowel disease. Biochemia Medica. 2013;23(1):28-42. doi: 10.11613/bm.2013.006

2. Kuna AT. Serological markers of inflammatory bowel disease. Biochem Med (Zagreb). 2013;23(1):28-42. doi: 10.11613/bm.2013.006.
3. Alvi MY, Alvi MA, Abbas M, Khan MA. Colonoscopic Evaluation of Bleeding Per Rectum in Children. Esculapio Journal of SIMS. 2015;11(4):37-9. DOI:https://doi.org/10.51273/esc15.71149
4. Mohsin A, Farhan S. Upper gastrointestinal bleeding. Esculapio Journal of SIMS. 2009;5(3):2-11. DOI: http://doi.org/10.51273/esc2513.
5. Ekbom A HC, Zack M, Adami HO, Zack M, Adami H-O. Ulcerative colitis and colorectal cancer: A population-based study. N Engl J Med. 1990;323(1):1228–33. doi: 10.1056/NEJM 199011013231802.
6. SB H. Inflammatory bowel disease: Epidemiology, pathogenesis, and therapeutic opportunities. Inflamm Bowel Dis. 2006;12(1):3-9. doi: 10.1097/01.mib.0000195385.19268.68
7. Alzoghaibi MA. Concepts of oxidative stress and antioxidant defense in Crohn's disease. World J Gastroenterol. 2013;19(39):6540-7. doi: 10.3748 /wjg. v19.i39.6540
8. Moldoveanu AC DM, Braticevici CF, Cytokines in inflammatory bowel disease. Romanian Journal Of Internal Medicine. 2015;53(2):118-27. doi: 10.1515/rjim-2015-0016
9. M'Koma AE. Inflammatory bowel disease: an expanding global health problem. Clin Med Insights Gastroenterol. 2013;6:33-47. doi: 10.4137/CGast.S12731
10. Ghosh S. Ulcerative colitis – what can we learn from the Asia-Pacific region? Canadian Journal of Gastroenterology and Hepatology. 2014;28(3):122. doi: 10.1155/2014/834909.
11. Dave M, Papadakis KA, Faubion WA, Jr. Immunology of inflammatory bowel disease and molecular targets for biologics. Gastroenterol Clin North Am. 2014;43(3):405-24. doi: 10.1016/j.gtc.2014.05.003.
12. Ng SC, Shi HY, Hamidi N, Underwood FE, Tang W, Benchimol EI, et al. The Worldwide Incidence and Prevalence of Inflammatory Bowel Disease in the 21st Century: A Systematic Review of Population-Based Studies. Gastroenterology. 2017;152(5):S970-S1. doi: 10.1016/S0140-6736(17)32448-0.
13. Shivashankar R, Tremaine WJ, Harmsen WS, Loftus EV, Jr. Incidence and Prevalence of Crohn's Disease and Ulcerative Colitis in Olmsted County, Minnesota From 1970 Through 2010. Clin Gastroenterol Hepatol. 2017;15(6):857-63. doi: 10.1016/j.cgh. 2016.10.039
14. Mortality GBD, Causes of Death C. Global, regional, and national life expectancy, all-cause mortality, and cause-specific mortality for 249 causes of death, 1980-2015: a systematic analysis for the Global Burden of Disease Study 2015. Lancet. 2016;388(10053):1459-544. doi: 10.1016/S0140-6736(16)31012-1.
15. Mortality GBD, Causes of Death C. Global, regional, and national life expectancy, all-cause mortality, and cause-specific mortality for 249 causes of death, 1980-2015: a systematic analysis for the Global Burden of Disease Study 2015. Lancet. 2016;388(10053):1459-544. doi: 10.1016/S0140-6736(16)31012-1.
16. Heath JL, Heath RD, Tamboli C, Johnson L, Wilson AS, Chervinskiy S, et al. Mesalamine desensitization in a patient with treatment-refractory ulcerative colitis and aspirin and nonsteroidal anti-inflammatory drug hypersensitivity. Annals of Allergy, asthma & immunology: official publication of the American College of Allergy, Asthma, & Immunology. 2017;118(4):518-20. doi: 10.1016/j.anai. 2017.01.026.
17. Ham M, Moss AC. Mesalamine in the treatment and maintenance of remission of ulcerative colitis. Expert Review of Clinical Pharmacology. 2012;5(2):113-23. doi: 10.1586/ecp.12.2
18. El Morsy EM, Kamel R, Ahmed MA. Attenuating effects of coenzyme Q10 and amlodipine in ulcerative colitis model in rats. Immunopharmacology and immunotoxicology. 2015;37(3):244-51. doi: 10.3109/08923973.2015.1021357.
19. Randhawa PK, Singh K, Singh N, Jaggi AS. A review on chemical-induced inflammatory bowel disease models in rodents. The Korean Journal of Physiology & pharmacology: official journal of the Korean Physiological Society and the Korean Society of Pharmacology. 2014;18(4):279-88. doi: 10.4196/kjpp.2014.18.4.279
20. Hayashi Y, Aoyagi K, Morita I, Yamamoto C, Sakisaka S. Oral administration of mesalazine protects against mucosal injury and permeation in dextran sulfate sodium-induced colitis in rats. Scandinavian journal of gastroenterology. 2009;44(11):1323-31. doi: 10.3109/00365520 903262414.
21. Khodir AE, Atef H, Said E, ElKashef HA, Salem HA. Implication of Nrf2/HO-1 pathway in the coloprotective effect of coenzyme Q10 against experimentally induced ulcerative colitis. Inflammopharmacology. 2017;25(1):119-35. doi: 10.1007/s10787-016-0305-0
22. Chassaing B, Aitken JD, 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. doi: 10.1002/0471142735.im1525s104
23. Diaz-Granados N, Kathryn Howe, Jun Lu, McKay DM. Dextran Sulfate Sodium-Induced Colonic Histopathology, but not Altered Epithelial Ion Transport, Is Reduced by Inhibition of Phosphodiesterase Activity. American Journal of Pathology. 2000;156(6):2169-77. doi: 10.1016 /S0002-9440(10)65087-0

Authors Contribution

HP: Conceptualization of Project

SH: Data Collection

AF: Literature Search

KSA: Statistical Analysis

MM: Drafting, Revision

MFJ: Writing of Manuscript