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(RESEARCH ARTICLE)

Establishment of a dextran sulfate sodium-induced ulcerative disease mouse model

Yu-Hsing Lin [1, #], Yun-Xuan Chang [2, #], Tzu-Yun Chi [2, #], Tsung-Han Wu [2, #], Ya-Peng Wang [2], Yen-Jung Lu [2], Chia-Yu Lin [2], Ping-Min Huang [2], Guan-Hong Chen [2], Chien-Chao Chiu [2], Ching-Feng Chiu [3], Hsuan-Wen Chiu [4], Wei-Huang Tsai [5], Chia-Chi Chen [2, *], and Shao-Wen Hung [2, 6, *]

[1] *Department of Pet Healthcare, College of Medical Technology and Nursing, Yuanpei University of Medical Technology, Xiangshan, Hsinchu 300, Taiwan.*
[2] *Division of Animal Industry, Animal Technology Research Center, Agricultural Technology Research Institute, Xiangshan, Hsinchu 300, Taiwan.*
[3] *Graduate Institute of Metabolism and Obesity Sciences, College of Nutrition, Taipei Medical University, Taipei 110, Taiwan.*
[4] *Department of Biotechnology and Bioindustry Sciences, College of Bioscience and Biotechnology, National Cheng Kung University, Tainan 701, Taiwan.*
[5] *Department of Science and Technology, Council of Agriculture, Executive Yuan, Taipei 100, Taiwan.*
[6] *Department of Nursing, Yuanpei University of Medical Technology, Hsinchu 300, Taiwan.*
[#] *Equally distributed authors.*

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Abstract

Inflammatory bowel diseases (IBD) are multifactorial chronic intestinal disorders. Currently, mesalamine etc. and therapeutic strategies were suggested for IBD therapy. However, the etiology of IBD remains unclear which is an ongoing challenge and side effects of therapeutic drugs must be also considered. Thus, the aim of this study was to establish an optimal mouse model of IBD for the drug and therapeutic strategy investigations. Herein, 12 mice with 2% dextran sulfate sodium (DSS)-induced colitis (the negative control group) were via oral administration. Twelve mice were administered with drinking water without 2% DSS (the normal control group) via the same method as DSS-induced mice. At the end of the experiment, the body weight (BW), the stool appearance/status, the macroscopic and microscopic colonic injuries, and myeloperoxidase (MPO) activity were monitored, measured, and scored. The results showed that BALB/c mice' BW decreased on D6-D8 of 2% DSS induction and then BALB/c mice' BW continuously increased until D13 of the experiment. The stool appearance/status was seen soft stool on D2 of 2% DSS induction. The soft stool was mainly occurred on D2-D6 of 2% DSS induction. In addition, the watery stool was occurred on D4 of 2% DSS induction and was continuous until the end of the experiment (D14). The macroscopic colonic injuries were showed that colon length of the negative group (2% DSS-induced group) was significantly shorter than that of the normal control group ($p < 0.001$). The colon weight of the negative group was significantly increase than that of the normal control group ($p < 0.001$). The colon weight / length ratio in the negative group was significantly higher than that of the normal control group ($p < 0.001$). According to the histopathologic scores (evaluation of the microscopic colonic injuries), the scores of area, ulceration, inflammation, and edema in the colon tissues of the negative group was significantly higher than that of the normal control group ($p < 0.001$). The total histopathologic scores in the negative group was significantly higher than that of the normal control group ($p < 0.001$). The myeloperoxidase (MPO) activity in the inflamed colon tissue of the negative group was significantly higher than that of the normal control group ($p < 0.001$). Taken all results together, a DSS-induced ulcerative disease mouse model was successfully established. We hope that this animal model may be a useful tool for the research of the better therapeutics for IBD.

[*] Corresponding author: Chia-Chi Chen and Shao-Wen Hung
Division of Animal Industry, Animal Technology Research Center, Agricultural Technology Research Institute, Xiangshan, Hsinchu 300, Taiwan.

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Keywords: Establishment of Experimental Mouse Model; Inflammatory Bowel Diseases; Dextran Sulfate Sodium; Ulcerative Disease

1. Introduction

Inflammatory bowel disease (IBD) are multifactorial chronic diseases involved ulcerative colitis (UC) and Crohn's disease (CD), which was resultant from a dysfunctional/abnormal epithelial and immune responses to intestinal microorganisms, where severe disease complications often result in surgery [1-3]. At present, IBD's etiology and pathogenesis remain unclear. The environmental and genetic factors may be considered as the etiology of IBD. Until now, the cellular and molecular insights of pathogenesis of IBD were discovered as mitogen-activated protein kinases (MAPK) and nuclear transcription factor κB (NF-κB) were involved in the signalling pathway. In addition, cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS), and pro-inflammatory cytokines (TNF-α, IL-1β, and IL-6 etc.) have also been verified to participate the colonic damage [3-7].

Clinically, patients with IBD are frequently observed clinical manifestations as abdominal pain, nausea, vomiting, ileus, or diarrhea, may be attributed to deranged gastrointestinal motility that is associated with inflammation [1-3]. Currently, mesalamine (5-ASA; 5-amino salicylic acid) is a well-established drug considered and used in the first line of treatment in patients with IBD, particularly in the active mild to moderate UC [8]. 5-ASA is rapidly and extensively absorbed before reaching the colon in most clinical cases [8]. Topical treatment with 5-ASA, especially in combination with oral forms, may be more effective than either treatment alone for both distal and extensive UC. Oral or rectal corticosteroids are also commonly used but appear to be less effective than 5-ASA in inducing and maintaining remission. Moreover, 5-ASA is not free of side effects, although it is usually well tolerated [8].

The search for novel therapeutic drugs and the development of therapeutic strategies for IBD is an ongoing challenge. Therefore, there has been an urgent need for alternative medicine with high efficacy and fewer adverse effect [8-10]. The aim of this study was to establish an optimal IBD animal model for the new drug researches and novel therapeutic strategy developments.

2. Material and methods

2.1. Chemicals and Reagents

Phosphate-buffered saline (PBS; Sigma-Aldrich, Cat. No. P3813), saline (Taiwan Biotech Co., LTD, Cat. No. 100-120-1101), Zoletil 50 (Virbac, Carros, France), myeloperoxidase (MPO) activity assay kit (abcam, Cat. No. ab105136), dextran sulfate sodium salt (Spectrum Chemical, Cat. No. DE136), hexadecyltrimethylamonium bromide (HTAB; Sigma-Aldrich, Cat. No. H6269), KH_2PO_4 (Sigma-Aldrich, Cat. No. P5655), Na_2HPO_4 (Sigma-Aldrich, Cat. No. S9763), horseradish peroxidase (Sigma-Aldrich, Cat. No. 77332), and tetramethylbenzidine (TMB; Sigma-Aldrich, Cat. No. 860336) were used in this experiment.

2.2. Experimental Animals and Experimental Design

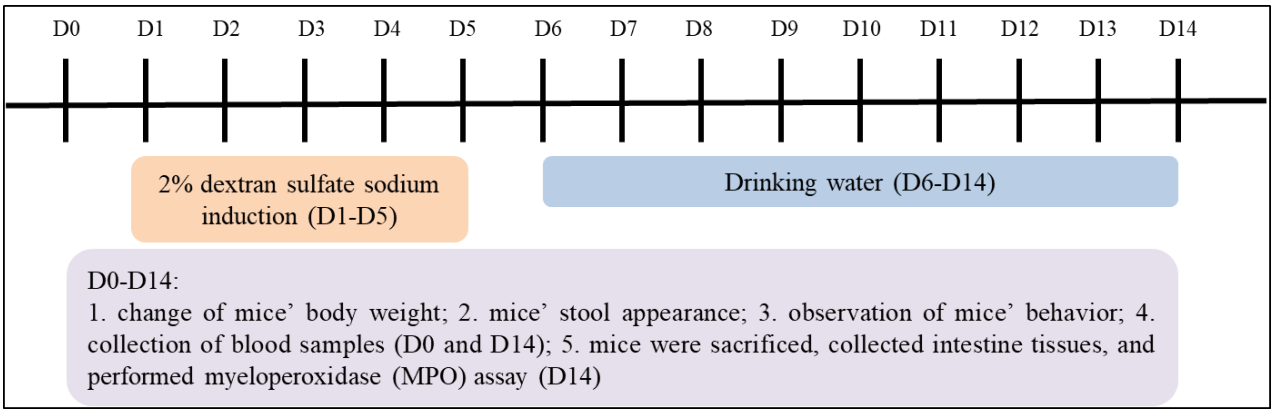

Figure 1 Experimental groups and designs

Adult male 24 BALB/c mice [8 weeks old; body weight (BW) between 24-25 g] with specific pathogen-free conditions were used for this study, were purchased from BioLASCO Taiwan Co., Ltd. (Yilan, Taiwan). The environment was

maintained room temperature (24-27°C) and 60%-70% humidity with a photoperiod of 12-hr light/12-hr dark cycle. The study will begin after a week acclimation. The Institutional Animal Care and Use Committee (IACUC) of Agricultural Technology Research Institute inspected all animal experiments and this study comply with the guidelines of protocol IACUC 990401 approved by the IACUC ethics committee. The male 24 BALB/c mice were divided respectively the normal control (n = 12) and the negative control (n = 12). All BALB/c mice were fed with standard laboratory diet (No. 5053, LabDiet®; PMI Nutrition International, St. Louis, MO, USA) ad libitum during the experimental period. The change of BLAB/c mice' BW, BALB/c mice' stool appearance/status, the observation of BALB/c mice' behaviors, and MPO expression of the collected BALB/c mice' intestine tissues were monitored, scored, and detected during the experiment (Fig. 1).

2.3. Induction of Colitis and Determination of Scores of Stool Consistency

Briefly, BALB/c mice were administrated with 2% DSS involved drinking water for 5 days. Later, the normal drinking water was provide to BALB/c mice until the end of the experiment. Finally, BALB/c mice were sacrificed after overdose anaesthetized. During the study, BALB/c mice' BW and stool consistency were recorded daily. Scores of stool consistency was defined as followed score 1, normal stool; score 2, soft stool; score 3, watery stool.

2.4. Assessment of the Severity of Colitis

The colon was removed, opened with longitudinal incision, cleaned, and rinsed with PBS to remove fecal material. Gross damage lesion of the colonic mucosa was assessed by a senior veterinary pathologist. The length, weight, and weight/length ratio of BALB/c mice' colons were measured. The individual damage features of colitis were graded according the method described [8, 10]. Colonic tissue samples were taken from macroscopic damage area and processed for subsequent MPO activity measurement.

2.5. Histological Assessment of Colitis

Colonic tissue samples of BALB/c mice were fixed in 10% buffered formalin and embedded in paraffin blocks. Five μm sections were stained with hematoxylin and eosin. The slides were then evaluated by a senior veterinary pathologist. The scores of colon injury were defined according to the method described [8, 10] as score 0, normal; score 1, one area of inflammation or no ulcer; score 2, one area of ulcer; score 3, one area of inflammation or one or two one area of ulcers; score 4, one area of inflammation, ulcers > 2 area; score 5, two area of inflammation, ulcers > 2 area; score 6, ulceration > 2 cm.

2.6. Assessment of MPO Activity

MPO activity was assessed as a marker of neutrophil infiltration slightly modified to the method described [8, 10]. Briefly, storage colonic tissue samples were removed from -80°C and allowed to thaw on ice. When the samples thawed, 1 mL of 0.5% HTAB containing 50 mM KH_2PO_4 and 0.1 M Na_2HPO_4 was added per 100 mg tissue for homogenization. Homogenates was centrifuged at 12,000 *g* for 10 minutes at 4°C after freeze/thaw for four times. The supernatant was collected for MPO activity assay. The stock solution of horseradish peroxidase was 0.5 mg/mL and used as a standard. The substrate of MPO activity assay was TMB to show up the reaction. In the reaction, 10 μL of standard and sample were added to appropriately labeled tubes. TMB was added at a volume of 100 μL to initiate the reaction, and 100 μL 0.1 M H_2SO_4 was added after 10 minutes of initiation to terminate MPO reaction. The absorbance changes were measured by a spectrophotometer at 450 nm and MPO activity was expressed as nanograms per milligram of tissue.

2.7. Statistical Analysis

SPSS (Statistical package for the social sciences) statistical software (version 28.0) were used for statistical analysis. Measurement data were expressed as mean ± standard error of mean (SEM). All comparisons were made by one-way ANOVA (Analysis of Variance). All significant differences are reported at $^{*}p < 0.05$.

3. Results

3.1. Change of BW of BALB/c Mice

Change of BW of BALB/c mice was monitored every day. Data showed that change of BW of BALB/c mice found on D6-D8 of 2% DSS induction and then continuously increased BALB/c mice' BW until D13 of the experiment (Fig. 2).

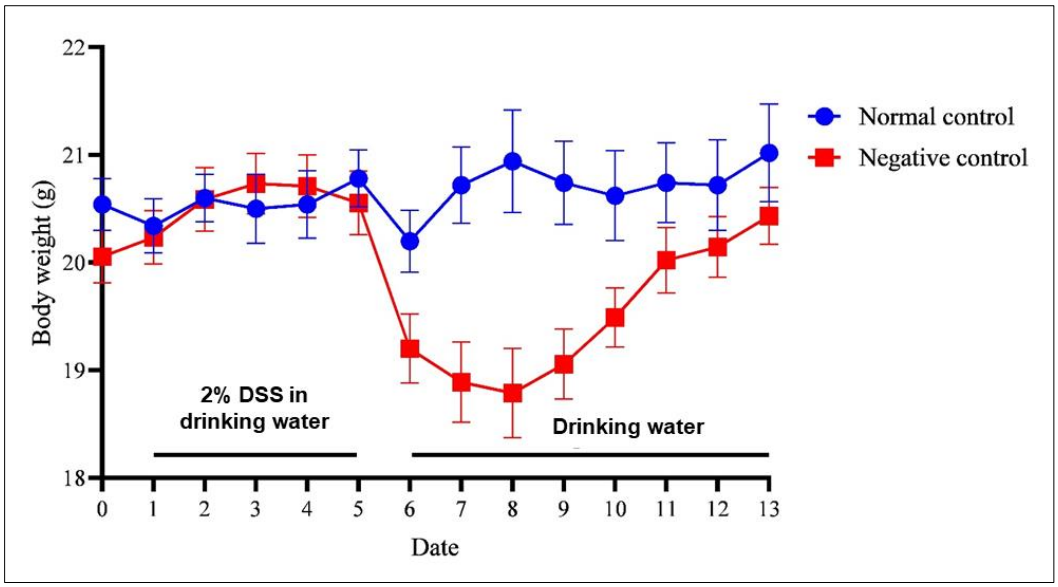

Figure 2 The change of BW of BALB/c mice during the experiment. The male BALB/c mice were divided respectively as the normal control (n = 12) and the negative control (n = 12). BALB/c mice were administrated with 2% DSS involved drinking water for 5 days. Later, the normal drinking water was provide to BALB/c mice until the end of the experiment

3.2. The Stool Appearance/Status of BALB/c Mice

The stool appearance/status of BALB/c mice was monitored and scored every day. Data showed that stool appearance/status was presented that soft stool was occurred on D2 of 2% DSS induction. The soft stool was mainly occurred on D2-D6 of 2% DSS induction. In addition, the watery stool was occurred on D4 of 2% DSS induction and was continuous until the end of the experiment (Fig. 3).

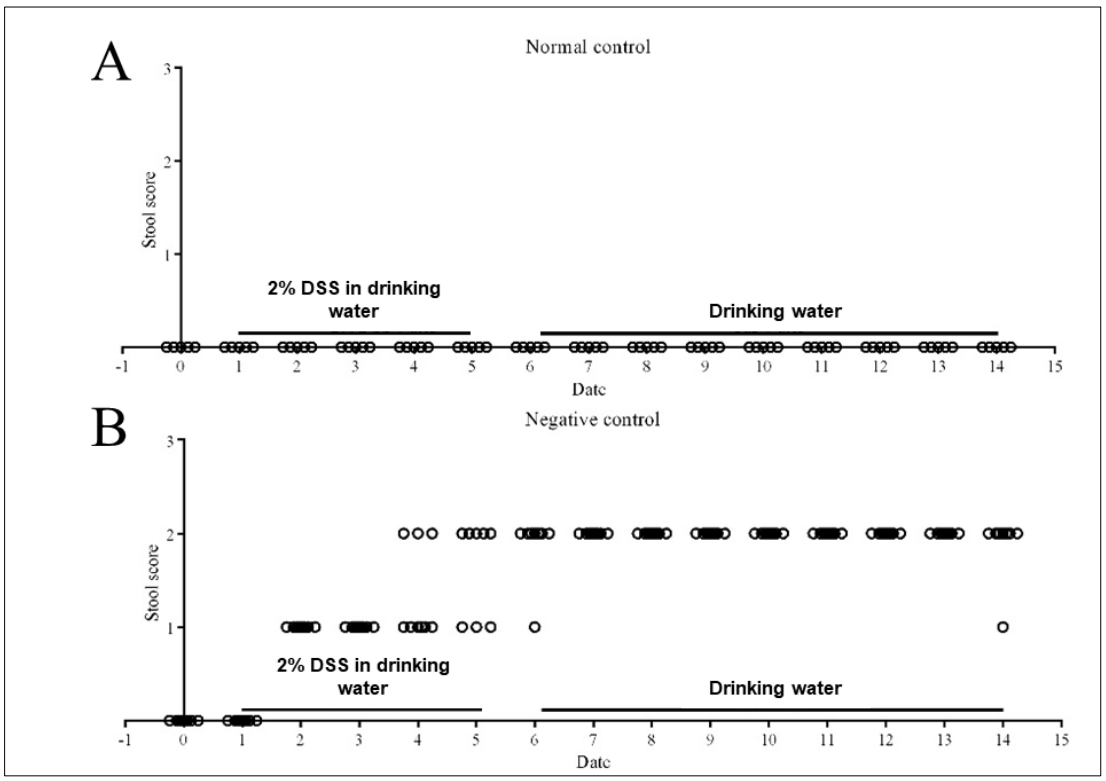

Figure 3 The stool appearance/status and scoring of BALB/c mice during the experiment. The male BALB/c mice were divided respectively as the normal control (n = 12) and the negative control (n = 12). BALB/c mice were administrated with 2% DSS involved drinking water for 5 days. Later, the normal drinking water was provide to BALB/c mice until the

end of the experiment. Scores of stool consistency was defined as followed score 1, normal stool; score 2, soft stool; score 3, watery stool

3.3. The Macroscopic Colonic Injuries of BALB/c Mice

The macroscopic colons of BALB/c mice was performed at the end of the experiment. Data showed that macroscopic colonic injuries were presented that colon length of the 2% DSS-induced group was significantly short than that of the normal control group ($p < 0.001$). The colon weight of the 2% DSS-induced group was significantly increase than that of the normal control group ($p < 0.001$). The colon weight / length ratio in the 2% DSS-induced group was significantly higher than that of the normal control group ($p < 0.001$) (Fig. 4).

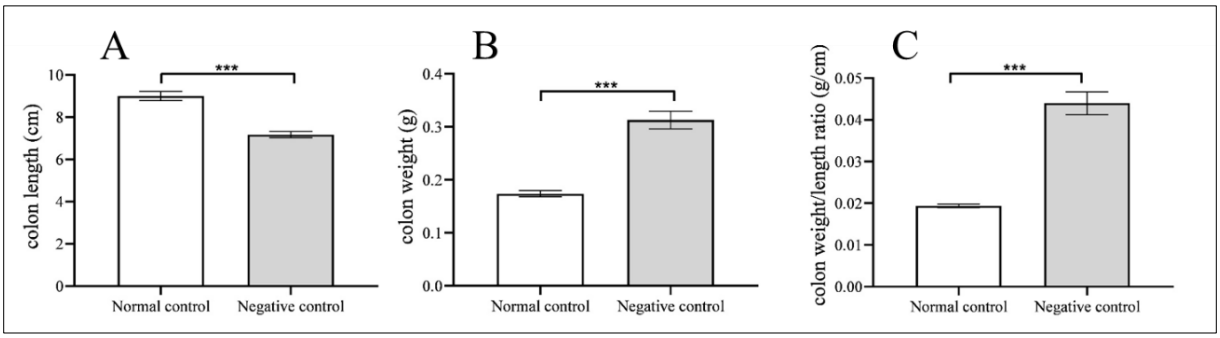

Figure 4 The macroscopic inner appearance of colon tissues of BALB/c mice. The male BALB/c mice were divided respectively as the normal control (n = 12) and the negative control (n = 12). (A) The colon length. (B) The colon weight. (C) The colon weight/length ratio. The length (cm), weight (g), and weight/length ratio (g/cm) of colon were measured. Data presented mean ± SEM and $^{***}p < 0.001$

3.4. The Microscopic Colonic Tissue Injuries of BALB/c Mice

The microscopic colons of BALB/c mice was performed at the end of the experiment (Fig. 5). Data showed that microscopic colonic injuries (area, ulceration, inflammation, and edema) of 2% DSS-induced group was significantly increase than that of the normal control group ($p < 0.001$). Total histopathologic scores in the 2% DSS-induced group was also significantly higher than that of the normal control group ($p < 0.001$) (Fig. 6).

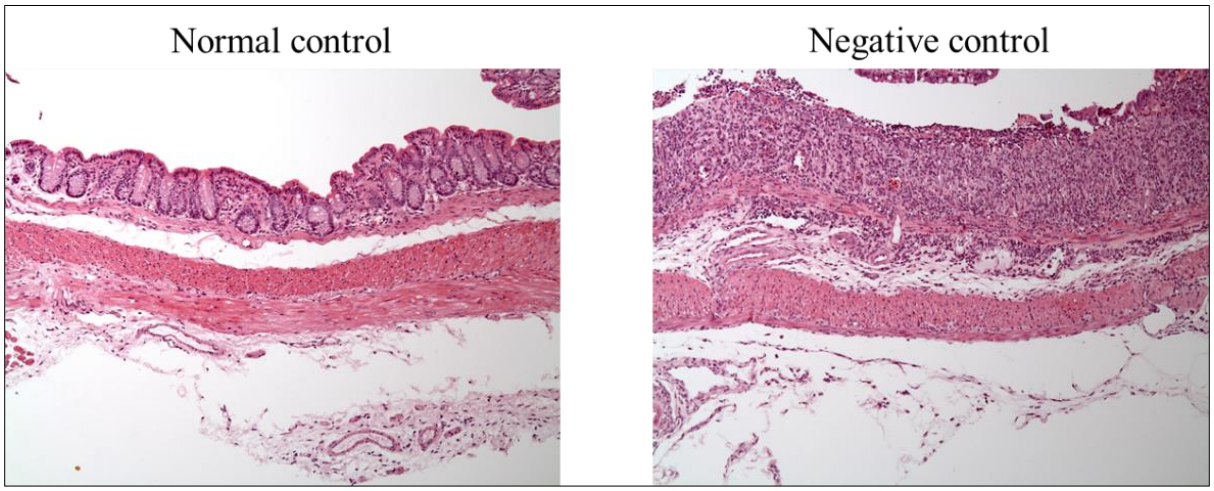

Figure 5 The microscopic colonic tissue injuries of BALB/c mice.

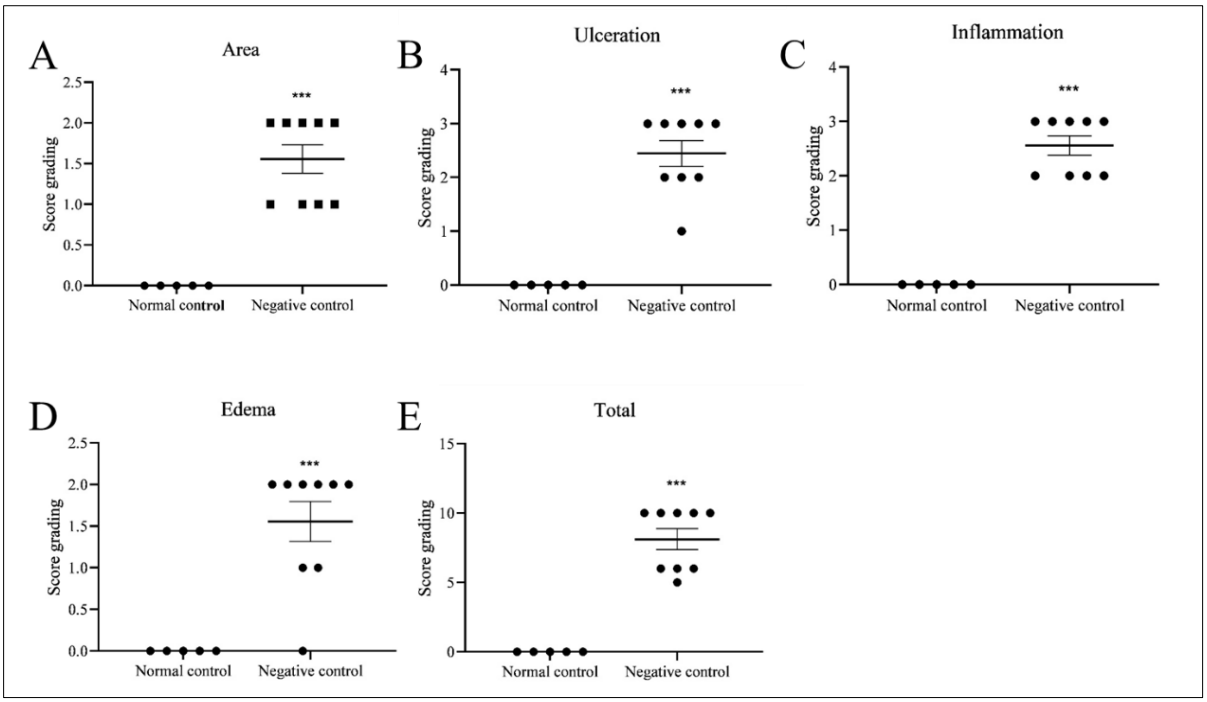

Figure 6 The microscopic inner appearance of colon tissues of BALB/c mice. The male BALB/c mice were divided respectively as the normal control (n = 12) and the negative control (n = 12). (A) Area. (B) Ulceration. (C) Inflammation. (D) Edema. (E) Total scores. The scores of colon injury were performed as score 0, normal; score 1, one area of inflammation or no ulcer; score 2, one area of ulcer; score 3, one area of inflammation or one or two one area of ulcers; score 4, one area of inflammation, ulcers > 2 area; score 5, two area of inflammation, ulcers > 2 area; score 6, ulceration > 2 cm. Data presented mean ± SEM and $^{***}p < 0.001$.

3.5. MPO Activity in the Colon Tissues of BALB/c Mice

Evaluating MPO activity was performed by using MPO activity assay kit. Data showed that MPO activity in colonic tissue of the 2% DSS-induced group was significantly higher the normal control ($p < 0.001$) (Fig. 7).

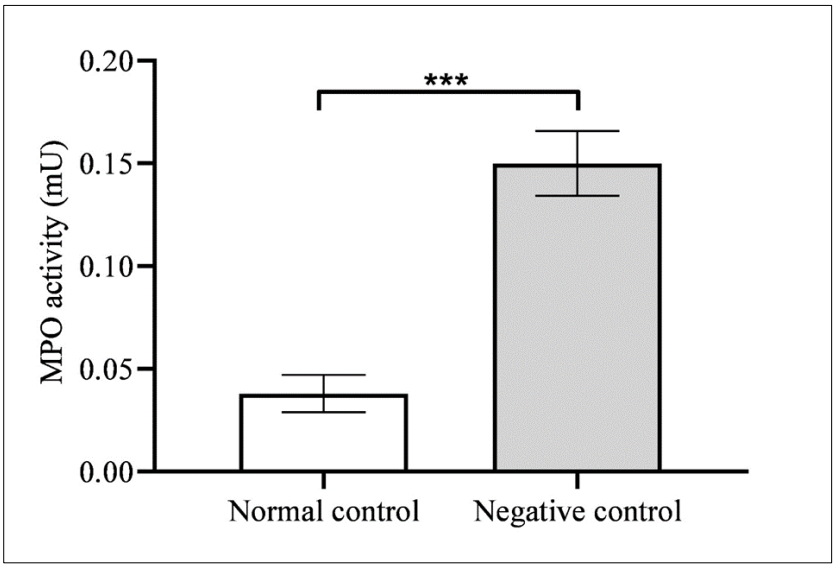

Figure 7 Expression of MPO activity in the colon tissues of BALB/c mice. The male BALB/c mice were divided respectively as the normal control (n = 12) and the negative control (n = 12). Data presented mean ± SEM and $^{***}p < 0.001$

4. Discussion

IBD (mainly covers CD and UC) is a group of idiopathic, chronic, and relapsing inflammatory conditions mainly affecting colon and small intestine and characterized by severe abdominal pain and diarrhea. CD and UC are chronic and progressive inflammatory diseases that may affect GI tract and might be associated with an increased risk for colon cancer [9, 11-13]. Currently, several animal models of IBD have been developed. These animal models of IBD were provided to help out in identification of novel drug targets and therapeutic strategies. Although these animal models have lot of shortcomings but still some promising new drugs have been developed by utilization of these preclinical animal models [14-19].

Since the exact etiology of IBD is not known yet, a number of animal models have been developed over past decades to study the possible mechanism involved in pathogenesis of disease and new therapeutic targets [9, 20-32]. The different types of animal models are developed for the study of IBD. Currently, animal models of IBD were classified as chemical inductions, Immunological inductions, spontaneous inductions, gene knock-out inductions, and transgenic inductions. Chemicals-induced animal models included as dinitrobenzene sulfonic acid (DNBS)-induced animal models, DSS-induced animal models, 2,4,6-Trinitrobenzene sulfonic acid (TNBS)-induced animal models, recurrent TNBS-induced models, acetic acid-induced animal models, carrageenan-induced animal model, iodoacetamide-induced animal models, indomethacin-induced animal models, and oxazolone-induced animal models. In DSS-induced animal models can present BW loss, diarrhea, bloody stools, piloerection, anemia, and death as same as our DSS-induced mouse model in this study. Moreover, other classified IBD as immunologic animal model of colitis: dinitrochlorobenzene (DNCB)-induced animal models and bacterial induction of colitis (*Salmonella*-induced colitis); Spontaneous mouse model species as C3H/HejBir mice model and SAMP1/Yit mice model. Conventional gene knock-out animal models as IL-10, TGF-β, IL-2, NOD2, A20, MDR1A, Gai2, T-cell receptor a (TCRa), IL-23, XBP1, and XBP1 gene knock-out etc. Transgenic mouse animal models of colitis as IL-7 Tg, STAT4 HLA-B27, SOCS1 Tg, DNN-cadherin transgenic mice, and Keratin 8 knock-out mice [20-32]. These animal models have provided translational knowledge and a framework to think about the impact of hormones, genes, and various environmental factors like sound, light, and so forth, on IBD pathophysiology.

Although various animal models for studying IBD mechanisms have been developed and exploited efficiently, leading to better understanding of the pathophysiology of the disorder, which are discussed here, emphasizing on their clinical and histological features showing relevance to human IBD. In this study, an optimal DSS-induced mouse model of IBD has been successfully established. We hope that this animal model may be a useful tool for the research of the better therapeutics for IBD.

5. Conclusion

IBD are multifactorial chronic intestinal disorders. Currently, mesalamine etc. and therapeutic strategies were suggested for IBD therapy. However, the etiology of IBD remains unclear and side effects of therapeutic drugs must be considered thus, the aim of this study was to establish an optimal IBD-induced mouse model for the new drugs and the novel therapeutic strategies investigations. Taken all results together, a DSS-induced ulcerative disease mouse model was successfully established. This model may be a useful tool for the research of the better therapeutics for IBD.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare no conflict of interest.

Statement of ethical approval

The Institutional Animal Care and Use Committee (IACUC) of Agricultural Technology Research Institute inspected all animal experiments and this study comply with the guidelines of protocol IACUC 990401 approved by the IACUC ethics committee.

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