Taylor & Francis
Taylor & Francis Group

Journal of Investigative Surgery

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/iivs20

Improving Animal Model of Induced Colitis by Acetic Acid in Terms of Fibrosis and Inflammation Incidence in the Colon

Gholamreza Bahrami , Hossein Malekshahi , Shahram Miraghaee , Hamid Madani & Atefeh Babaei

To cite this article: Gholamreza Bahrami , Hossein Malekshahi , Shahram Miraghaee , Hamid Madani & Atefeh Babaei (2020): Improving Animal Model of Induced Colitis by Acetic Acid in Terms of Fibrosis and Inflammation Incidence in the Colon, Journal of Investigative Surgery, DOI: 10.1080/08941939.2020.1821844

To link to this article: https://doi.org/10.1080/08941939.2020.1821844

JOURNAL OF INVESTIGATIVE SURGERY
https://doi.org/10.1080/08941939.2020.1821844

Taylor & Francis
Taylor & Francis Group

ORIGINAL RESEARCH

Improving Animal Model of Induced Colitis by Acetic Acid in Terms of Fibrosis and Inflammation Incidence in the Colon

Gholamreza Bahrami[a,b], Hossein Malekshahi[b], Shahram Miraghaee[a], Hamid Madani[c], and Atefeh Babaei[a]

[a]Medical Biology Research Center, Health Technology Institute, Kermanshah University of Medical Sciences, Kermanshah, Iran; [b]School of Pharmacy, Kermanshah University of Medical Sciences, Kermanshah, Iran; [c]Clinical Research Development Center, Imam Reza Hospital, Kermanshah University of Medical Sciences, Kermanshah, Iran

ABSTRACT

Background: One of the animal models of colitis is induced by acetic acid, which mimics some features of ulcerative colitis disease. In order to increase the similarity of this model to human IBD and determine the suitable duration of acid contact to the colon and the day of induction of colitis were investigated in this study.

Methods: Fifty rats were randomly separated into five groups (n = 10) with different durations (30 s, 5, 10, and 20 min) of exposure of the colon to 4% acetic acid and colitis was investigated for 0-9 days. The extent of the mucosal ulcers, colon tissue thickening, and mucosal bleeding were scored by the Gerald classification system score. Slides of tissues were prepared for pathologic assay using the modified Wallace method.

Results: In all groups, inflammation was severe three days after the colitis induction, but no inflammation was observed in the 30 s group after five days. Acid contact with the colon surface did not result in fibrosis for the 30 s and the colon fibrosis was mild in 5 min group and severe in 10 and 20 min groups. The tissue damage was higher in groups of 20, 10, 5 min, and 30 s, respectively. Over time, the recovery rates in the 30 s and 5 min groups were higher than other groups.

Conclusion: Our results showed that the evaluation of the disease process from 3 days to nine days after a 10 min contact of acid to the colon is a suitable model that mimics the histological features of the disease.

ARTICLE HISTORY
Received 1 May 2020
Revised 30 May 2020
Accepted 4 September 2020

KEYWORDS
Ulcerative colitis; animal model; acetic acid fibrosis; inflammation

Introduction

Inflammatory bowel disease (IBD) is a chronic inflammatory bowel disease that affects the gastrointestinal mucosa [1]. Although IBD etiology has not been fully understood, it is affected by many genetic, infectious, immunological, and environmental factors [2, 3]. IBD consist of two major diseases, namely, Crohn's disease (CD) and ulcerative colitis (UC) [4]. UC is a condition where inflammatory response and morphological changes are limited to the large intestine and may include rectum [5]. Various chemicals, genetic, and bacterial models have been proposed in order to evaluate the effects of various chemical compounds on the improvement and prevention of colitis. Chemical methods include the use of Dextran Sulfate Sodium (DSS), 2, 4, 6-Trinitrobenzene Sulfonic Acid (TNBS), Oxazolone, Indomethacin, Cartagena Iodoacetamide, and Acetic Acid [6]. Injection of diluted acetic acid into the rectum is an alternative method for inducing mucosal epithelium damage, which mimics some features of UC [7]. In this model, the damage is related to epithelial necrosis and edema that penetrate the intestinal mucosal layer due to the concentration and duration of exposure to acetic acid [8]. The initial injury in this model was a relatively bland epithelial necrosis and edema that variably extended into the lamina propria, submucosa, or external muscle layers, depending of the concentrations and length of exposure of acetic acid. Transient localized ischemia may lead to acute injury, but neutrophils are not apparently involved in very early stages [9]. Mucosal inflammation is due to the initial injury and is associated with the activation of NF-κB and other inflammatory mediators [9]. Molecular changes in rodents include the release of protons from acidic protein forms into the intracellular space, leading to its acidification and epithelial neuropathy [10]. Common clinical symptoms of this model include body weight loss, diarrhea, the presence of blood in the stool, the increase of the colon weight, thickening of the intestinal wall, reduced mucus production, ulcers, and inflammation [11]. The advantages of this method are lower cost and ease of disease induction [12]. Fabia (1992) examined different doses of 4, 6, and 8% acetic acid at 10-30 seconds, and suggested that the use of 4% acid in contact with the colon within 15 seconds could be similar to the ulcerative colitis disease. Using higher concentrations of acetic acid causes frequent perforations [13].Many studies have used concentrations of 3-10% and exposure times of 15-30 seconds in an acetic acid volume of 1-2 ml [14–17].

CONTACT Atefeh Babaei Atefeh.babaie@gmail.com Medical Biology Research Center, P. O. Box 67155-1616, Kermanshah University of Medical Sciences, Parastar St., Daneshghah Blvd., Kermanshah, Iran.

Table 1. Exterior scoring criteria tissue damage in patients with colitis (Gerald method).

Criteria of scoring of IBD morphological damage (Gerald method)	Score
No damage	0
localized hyperemia, but no ulcers	1
Linear ulcers with no significant inflammation	2
Linear ulcers with inflammation at one site	3
Two or more sites of ulceration and / or inflammation	4
Two or more major sites of inflammation and ulceration or one major site of Inflammation and ulceration extending > 1 cm along the length of the colon	5

To investigate effects of different drugs and compounds on the induced colitis with acetic acid, it is first necessary to confirm the disease induction, determine the suitable duration of acid contact to the colon and the day of induction of colitis and the duration the body is involving with the disease.

In our survey about different effects of compounds on the common animal model of induced colitis by acetic acid in rats, it was observed that many induced rats healed without any treatment over time. Furthermore, the incidence rate of inflammation, fibrosis, and the depth of injury were very low according to our macroscopic histology studies of some induced rats.

As a result, this model was evaluated here with different doses of acid, different durations of colon exposure to the acid, and confirmation of induction on different days from the time of acid administration. The aim of the present study was to improve the induced colitis model by acetic acid in rats.

Materials and methods

Animals

In this study, 50 healthy adult Wistar female rats (aged 8-9 weeks) weighing 170 ± 15 g were kept in the animal house of the Faculty of Pharmacy, *xxx University of Medical Sciences*. The animals were maintained in a room under standard conditions of temperature (22 ± 2 °C), humidity (50% ± 20%), and 12 h day/night cycles. Before the experiment, the rats were adapted to the environment and laboratory conditions within two weeks. The rats were kept in clean Plexiglas cages with wood-shaving bedding (42 × 27 × 15 cm) with a maximum of three animals per cage. Standard laboratory pelleted formula and tap water were provided ad libitum. The whole processes of working with experimental animals were approved by the Ethics Committee of *xxx University of MedicalSciences.*

Experimental design

Fifty healthy rats were randomly divided into five groups (n = 10). Before the induction of colitis, the rats were fasted for 24 h and then anesthetized with ketamine (10% body weight, Alfasan-Holland). For induction of colitis, one ml of 4% acetic acid (Sigma-Aldrich, United States of America) was injected intra-rectum with a penetration depth of 8 cm into the anus [13]. In the control group, 1 ml of normal saline was injected intra-rectum. The duration of acid contact to the colon was accurately recorded by a stopwatch and during this time, the acid evacuation was prevented by closing the anus with fingers. After completion of the desired duration, the acid was allowed to leave completely. After acid induction of colitis, the colon was sampled randomly in all groups in order to determine the time of colitis induction in different days (1, 2 and 3), and to determine the duration of colon involvement with colitis symptoms until day 9. The experimental groups received 1 ml acetic acid 4% treatments during 30 s, 5, 10, and 20 min contact with the colon surface, with a control group administered by 1 ml of normal saline for 20 min contact with the colon surface.

Bodyweight changes

Body weights of the rats were measured and recorded from day 1 to the end of the study using a weighing scale (Ek-4152 Camry). The water and food consumption levels of rats were also recorded during day 1 to the end of the study.

Macroscopic observations

On the days set for different groups, the rats were anesthetized with ether and exsanguinated for autopsy, which was limited to removal of 7 cm from their colon tissue. The extents of the ulcer, colonic tissue density, tissue hyperemia, damage, and inflammation were studied and scored by the Gerald Classification System Score (Table 1) [18].

Microscopic observations

After being prepared in a pathology laboratory, the colon tissue was placed in 10% buffered formalin and sections with a thickness of five micrometers were prepared and stained by Hematoxylin- Eosin for histological study. Prepared slides were evaluated by an expert histologist, without normal groups and acid-treated groups' knowledge, using a Modified Wallace method (Table 2) [19].

Masson trichrome staining was conducted as following: After deparaffinized and rehydrated, the sections were stained in hematoxylin solution for 8 min. Then, ponceau acid fuchsine solution for 5 min after rinsed in running tap water for 8 min. After differentiated in phosphomolybdic–-phosphotungstic acid for 5 min, sections were transferred into aniline blue solution for 5 min. Then sections were differentiated in 0.2% acetic acid for 2 min and followed by dehydration, clearing, and mounting. Photos of sections were taken by light microscope [20].

Table 2. The Pathological evaluation using the modified Wallace method.

Inflamation				Depth of Lesion					Fibrosis		
Non	Mild	Medium	Severe	Non	Laminaprooria	Submucose	Muscular	Serosa	Non	Mid	Severe
–	+	++	+++	–	+	++	+++	++++	–	+	++

Stool examination

The stool consistency was examined by observation in different groups. The presence of blood in the stool was also examined using a diagnostic kit (Hema Tape, PatanTeb diagnostics, Iran). After placing stool samples on the diagnostic kit and use of solutions, the emergence of blue color indicated the presence of blood in the stool.

Statistical analysis

Statistical analyses were carried out by SPSS 16 software (SPSS/PC-16.SPSS Inc., Chicago, IL, USA). Data are expressed as mean ± standard error of means (SEM). The treated groups were compared with the control by one-way analysis of variance (ANOVA) followed by Duncan's post-hoc test at a statistical significance of $p < 0.05$. Graphical display of data was performed using the Graph Pad Prism 8 software (v8.2.1, Graph Pad Software, Inc., La Jolla, CA, USA).

Results

Weight changes

During the experiment, one death occurred in the 10 min group on day 9 and two rats died in the 20-min group on days 7 and 10. Two rats were randomly selected from each group on different days of the experiment and colon tissue was removed to confirm the day of colitis induction. At the end of the experiment, six rats were equally used for macroscopic and microscopic observations in all groups ($n = 6$). Weight loss was observed after induction of colitis in all groups, except the control group. On the first day, no significant changes were observed between all the groups ($p > 0.05$). The control group was not different significantly from the 30 s group after 3 and 5 days of colitis induction ($p < 0.05$), but it showed significant differences with groups of 5, 10, and 20 min ($p < 0.05$). The 20-min group showed a significant difference with the control group after 7 and 9 days of colitis induction ($p < 0.05$) (Figure 1).

Colon tissue inflammation, damage and fibrosis

Based on the Gerald's method (Table 1), macroscopic studies revealed the highest scores of inflammation and ulcers in groups of 20, 10, and 5 min and 30 s (4.958, 4.916, 4.125, and 3.916), respectively, 3 days of colitis induction ($p < 0.001$). Furthermore, the recoverywere differences between the groups after 9 days of induced disease. The highest improvement was observed in the 30 s and 5 min groups, and the least improvement occurred in the 20 min group (group 20 min: 4.583, group 10 min: 4.25, group 5 min 3.583, group 30 s: 0.916) ($p < 0.001$) (Figures 2 and 3).

To investigate the tissue fibrosis in chronic acetic acid -induced colitis, we observed colonic sections stained with Masson's trichrome method. Based on modified Wallace method, inflammation, depths of lesion and fibrosis were evaluated microscopically in different groups within 72 h, and 7 and 9 days after colitis induction (Figures 4 and 5 and Table 3).

In the 30 s group, severe inflammation, depths of a lesion in serosa layer, and lack of fibrous were observed after 3 days of colitis induction; Mild inflammation, depths of a lesion in the submucosal layer, and lack of fibrosis occurred after 7 days, and no inflammation, no lesion, and lack of fibrosis were detected after 9 days.

In the 5-min group, severe inflammation, depth of a lesion in serosa, and mid fibrosis were observed after 3 days of colitis induction and this condition continued until the 7th day, but severe inflammation, depth of a lesion in the serosa layer, and lack of fibrosis were observed on the 9th day.

In the 10-min group, a severe inflammation, depths of a lesion in serous layer, and mild fibrosis were observed after 72 h of colitis induction and this trend continued for 9 days.

In the 20 min group, severe inflammation, depths of a lesion in the serosa layer, and severe fibrosis were observed within 72 h to the 9th day of colitis induction (Figures 4 and 5 and Table 3).

Blood in the stool

The stool examination by a diagnostic kit in the early days confirmed the existence of blood in the stool for all groups except for the normal group, but blood in the stool was observed only in the groups of 10 and 20 min on the 9th day.

Discussion

In all the experimental groups on the 9th day, the inflammation was observed severe, except in the 30 s group and fibrosis just observed in 10 min (mid) and 20 min (severe). Over time, the recovery rate was higher in the 30 s and 5 min groups. In the 20 min group, mortality rates were higher than the other groups.

A lot of information about the various mechanisms involved in colitis has been obtained from animal models and new drugs have been discovered for the treatment of this disease [9]. One ofthe common animal model of colitis induction includes intra-rectum injection of 4% acetic acid, exposure durations of 30-15 s, and investigating the effects of chemical compounds after 24 h of induction with

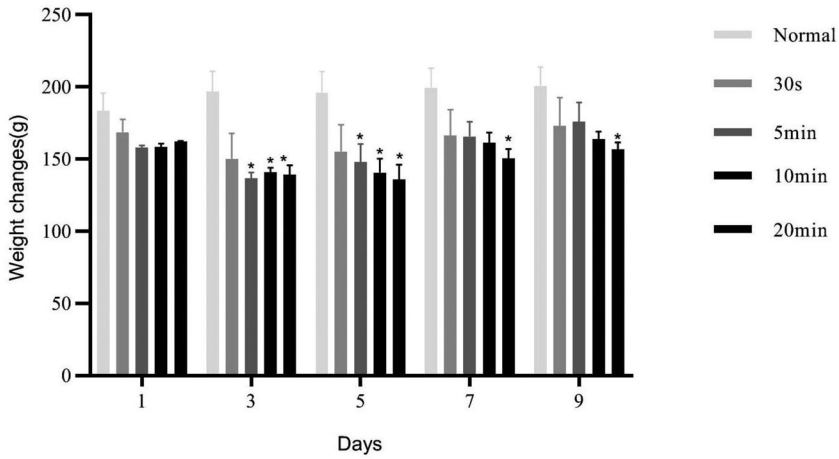

Figure 1. The results of evaluating body weight changes (g) in different groups. Mean ± SEM., *Significantly different compared with control ($p < 0.05$) ($n = 6$).

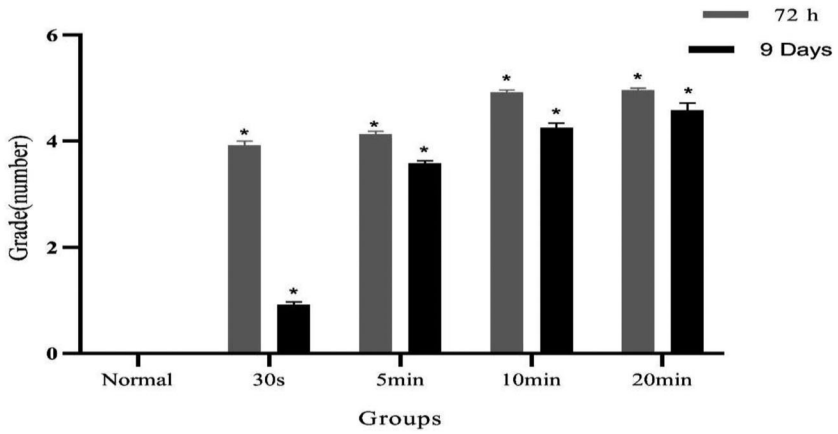

Figure 2. Scoring tissue damage based on Gerald Method, *72 hours and 9 days after induction of Colitis*. *Significantly different compared with control ($p < 0.001$), ($n = 6$).

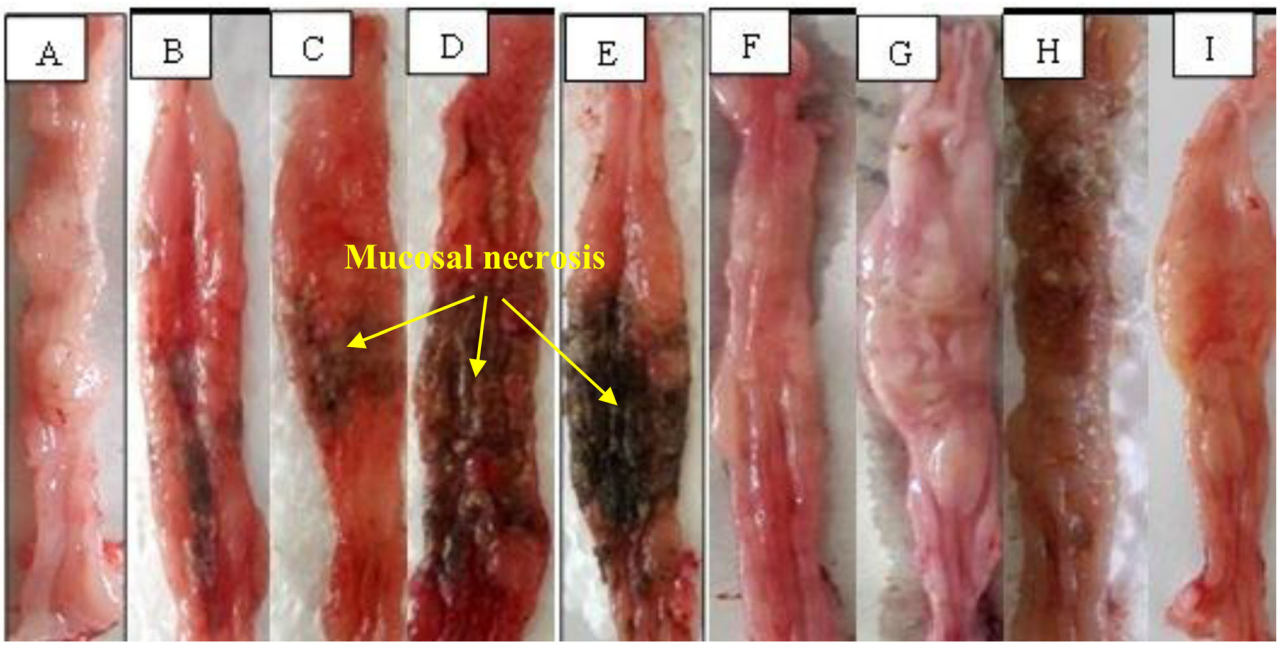

Figure 3. Rat colonic mucosa (macroscopic): A: Normal rat. B, C, D, E (exposure duration of acetic acid with the colon: 30 seconds, 51,020 minutes respectively and confirmation of induction of colitis, 3 days after administration of acid. F, G, H, I (exposure duration of acetic acid with the colon: 30 seconds, 51,020 minutes respectively and the length of induced colitis recovery 9 days after administration of acid)($n = 6$).

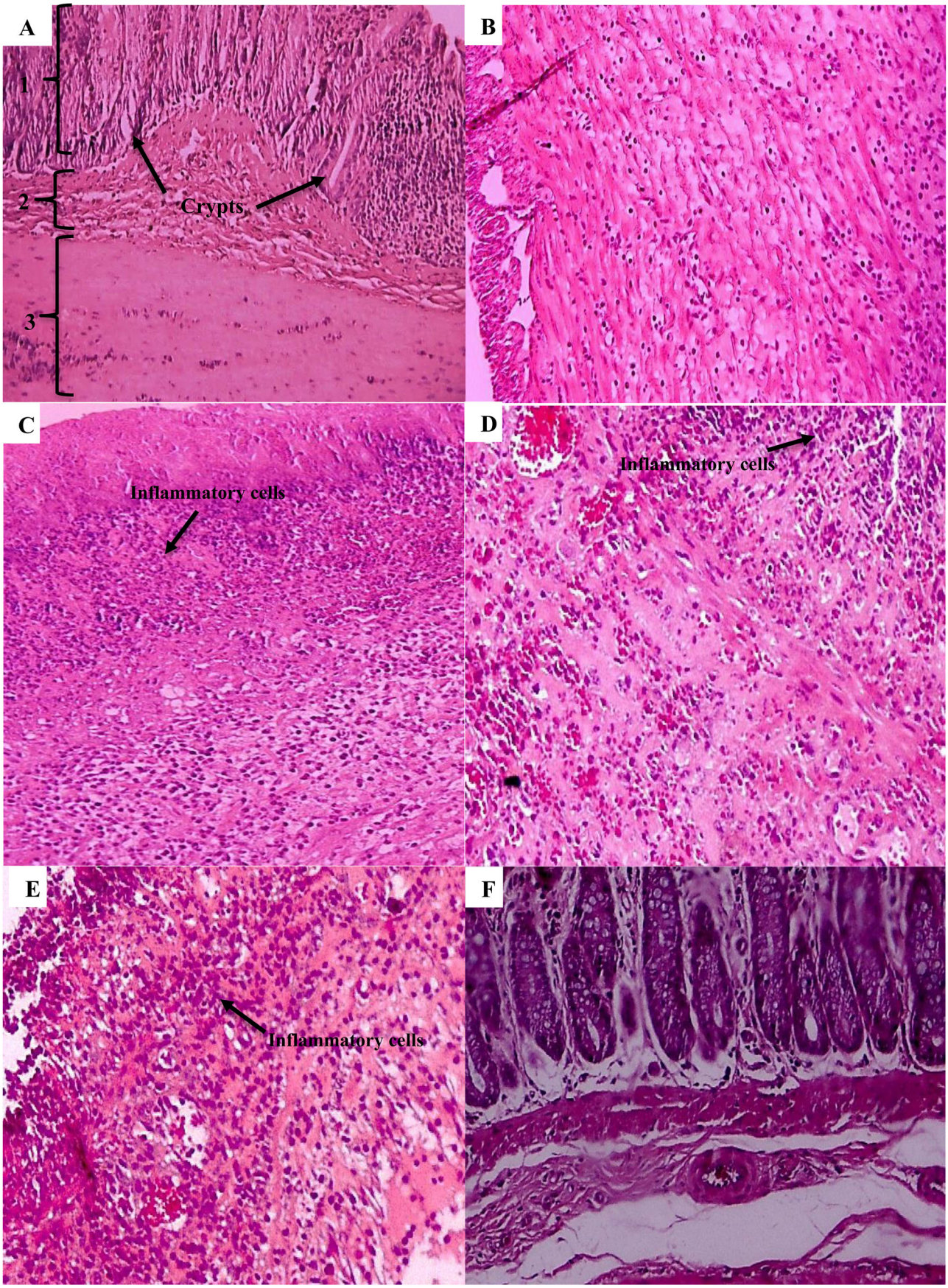

Figure 4. Photomicrographs of the rat colon stained with H&E stain (x 200). A: photomicrographs of normal rat colon (1; Mucosa, 2: Submucosa, 3: Muscularis). B-E: exposure duration of acetic acid with the colon 30 seconds, 5, 10 and 20 minutes respectively and confirmation of induction of colitis 3 days after administration of acid. F-I : exposure duration of acetic acid with the colon 30 seconds, 51,020 minutes respectively and the length of induced colitis recovery 9 days after administration of acid.

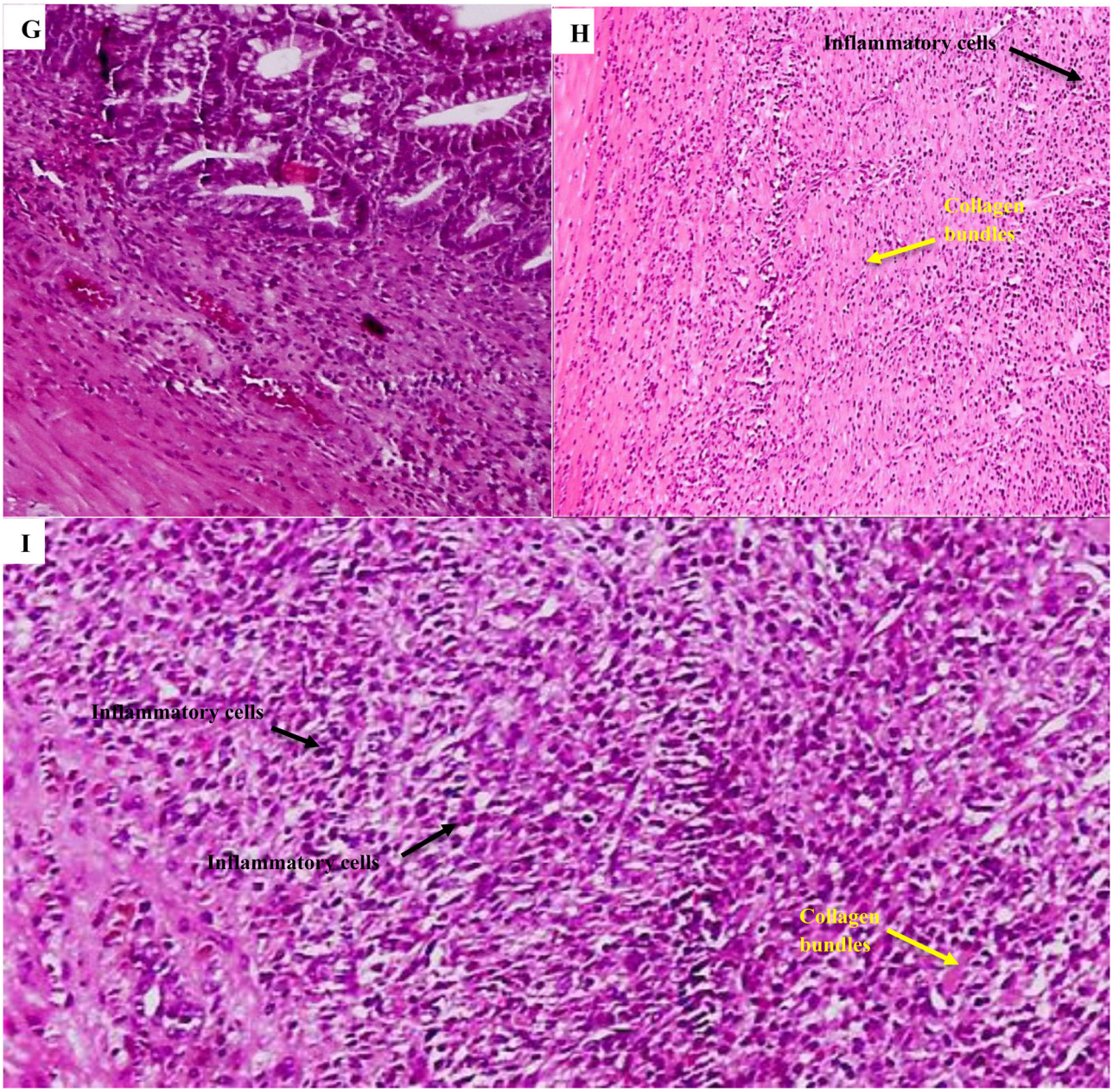

Figure 4. Continued.

different treatment periods [14–17]. Besides, the duration of acid contact to the colon, the day of disease induction, and the period of body involve with the disease should be determined in this model. The aim of our study was to modify the colitis induction by acetic acid in rat.

Multiple factors are considered important for the diagnosis of colitis in patients, including the presence of blood in the stool, frequent bloody diarrhea, and weight loss [21]. During the experiment, the highest amount of consumed water and food was observed in the 30 s group, which indicates a faster recovery process of the digestive system and a higher absorption performance in this group than the other groups. The results showed a significant reduction in weight, especially in the10 min group, until the 7th day, and in the 20-min group until the end of the experiment. During the early days of colitis induction, the presence of blood in the stool was observed in all groups, but it was confirmed only in groups of 10 and 20 min until the 9th day. In ulcerative colitis, edema, fat accumulation, and muscle layer hypertrophy may create a thick wall in the large intestine [6, 22]

The researches demonstrated that in colitis the severity of inflammation shows the infiltration of inflammatory cells, including neutrophils, and the extent of inflammation indicates the involvement of different sections of layers including the mucosa, the submucosa, and the entire width of the colon wall [2]. The mechanism through which acetic acid seems to begin the process of inflammation involves the entrance of the protonated form of acid into the epithelium where it breaks down and the resultant proton causes epithelial damage by acidifying the cellular environment [23]. Degrading the mucosa by acetic acid, the production of a series of leukotrienes, prostaglandins, and thromboxanes increases in UC [23]. The physiological roles of these materials in the intestine include the control of the movements,

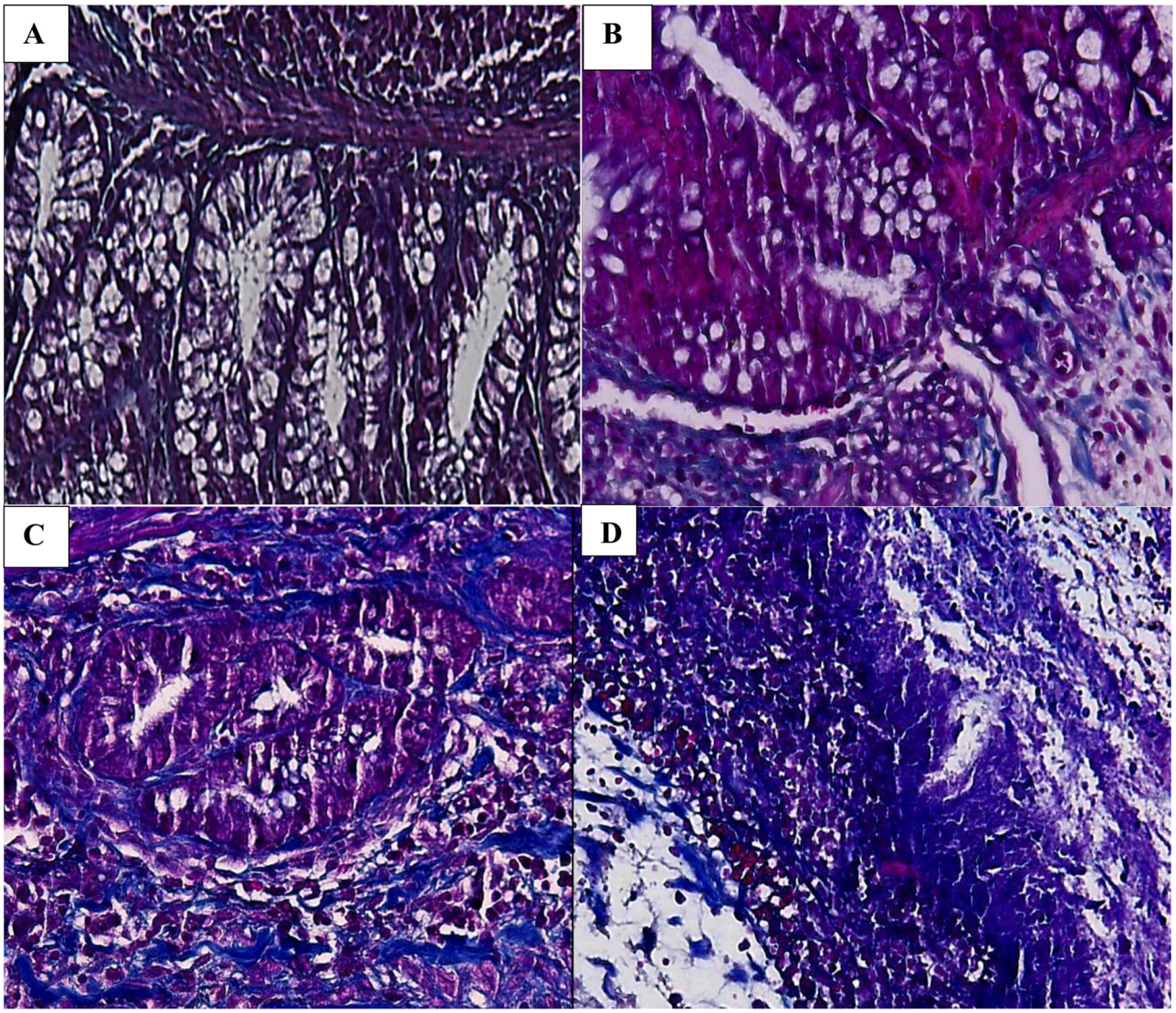

Figure 5. Photomicrographs of the rat colon stained Masson's Trichrome Stain (x 200). The exposure duration of acetic acid with the colon 30 s (A), 5 min (B), 10 min (C) and 20 min (D) in 9 days after administration of acid. The collagen deposition, shown by blue color staining area.

increase of the intestinal secretions, and create a variety of digestive diseases, including colitis [24].

Here, the extent of inflammation was examined through macroscopic and microscopic studies. The inflammation was observed in varying degrees until day 9 in all groups except in the 30 s group. As shown in Figure 4, B-E photomicrographs confirm an increase in the infiltration of inflammatory cells that is one of the important characteristics of UC and plays a major role in active colitis [25, 26]. Photomicrograph F in Figure 4 shows a decrease in the infiltration of inflammatory cells in the 30 s group on day 7. According to G-I photomicrographs, however, the infiltration of inflammatory cells is observed in 5, 10, and 20-min groups on day 9.

So far, the physiological pathways involved in the improvement of intestinal ulceration have been specified to some extent. During acute and chronic inflammation of the intestine, macrophages and neutrophils cause local tissue damage by secretion of reactive oxygen species and tissue degrading enzymes [27]. One of the important intestinal tissue damage mechanisms is oxidative stress through an excessive production of reactive oxygen metabolites (ROM), including superoxide anion, hydrogen peroxide, hypochlorous acid and hydroxyl radical [28]. Colitis induced by acetic acid is also known to produce excess reactive oxygen metabolites. The infiltrated and activated neutrophils represent an important source of reactive oxygen and nitrogen species [29]. The cross-linking proteins, lipids, and nucleic acids, cause cellular dysfunction and damage [30].This event happens due to the release of pre-inflammatory cytokines, as well as chemotaxis and cell activating peptides that have previously been linked to the matrix. If tissue damage is severe, myofibroblasts migrate to the site of injury. The migratory function, the ability to contract the wound area, and the production of an extracellular matrix (ECM) by intestinal myofibroblast cells play important roles in the physiological state that changes with chronic inflammation [27]. A chronic or repeated inflammation is a necessary prerequisite for the onset of gut fibrosis [27].

Fabia et al indicated that using higher concentrations of acetic acid causes frequent perforations and the 4% acid in contact with the colon within 15 seconds was similar to the

Table 3. Results of pathological evaluation using the modified Wallace method in groups (n = 6).

	Inflamation				Depth of Lesion					Fibrosis		
	Non	Mid	Medium	Severe	Non	Laminaprooria	Submucose	Muscular	Serosa	Non	Mid	Severe
72 h												
normal	–				–					–		
30s				+++					++++	–		
5min				+++					++++		+	
10min				+++					++++		+	
20min				+++					++++			++
7Day												
normal	–				–					–		
30s		+					++			–		
5min				+++					++++		+	
10min				+++					++++		+	
20min				+++					++++			++
9 day												
normal	–				–					–		
30s	–				–					–		
5min				+++					++++	–		
10min				+++					++++		+	
20min				+++					++++			++

human model [13]. In others studies have used 1-2 ml of 3-10% acid and exposure times of 15-30 seconds to colon [14–17].In the present study, 30 seconds of acetic acid contact with the colon did not result in fibrosis. During 5 min of acid contact with the colon, mild fibrosis was observed until the 7th day. In the 10 min group, fibrosis was observed until the 9th day, and finally in the 20 min group, severe fibrosis was observed until the 9th day due to the longer contact time of the colon with acid.

Other important factors in the diagnosis of colitis are tissue hyperemia, mucosal ulceration, and its extent. Hyperemia and ulcers were observed in all the groups up to 3 days after the induction of colitis. In the 30 s and 5 min groups, significant improvements were observed in hyperemia and ulceration until the 9th day.

However, the recoveries of rates were very low in the groups of 10 and 20 min. The different sections of the colon wall are involved in UC that depend on the disease severity. In the3thdays, inner -layer involvement was observed in all the groups.The damage to the inner layers continued until the 9th day in 5, 10 and 20 min groups but the 30 s group, just the outer layers were involved on the 7th day and there was no tissue damage on the 9th day.

In the present study, the use of 4% acetic acid with 30 s and 5 min of acid contact with the colon showed a recovery, which is unsuitable for assessing the effects of different chemicals according to our observations. Symptoms during 20 min of colon exposure to acid were severe and even fatal in some rats. The colon exposure to acid during 10 min is the appropriate time to induce colitis.

In other studies, treatment was started 24 h after the induction of colitis [14–17]. In this study, 72 h after colitis induction is suggested as the best time to start the treatment because macroscopic and microscopic parameters did not confirm the induction of colitis at the other times examined here. Depending on the course of the induced colitis on different days, the best course of treatment is 9 days.

Strength and limitations

The strengths of this study include considering all the factors involved in acid acetic-induced colitis to remove the effect of other variables in animal models, confirming the disease and the duration of induced colitis by acetic acid to investigate the pharmacological effects, and determining exact and sufficient time to induce colitis based on macroscopic and microscopic observations. Although, there are some limitations in our study that should be discussed. Firstly, Due to lack of funding, biochemical factors were not measured. Secondly, despite that ethical rules were followed at the whole process of working with experimental animals, there was no measure of pain threshold in the anesthetized rats after prolonged exposure to 4% dilutes acid with their colonic levels.

Conclusion

The present study showed that the most similar animal model of induced colitis by acetic acid is a 10-min model of colonic exposure to 4% acetic acid (taking into account the duration of colon exposure to acid, the exact time of colitis induction, the duration to examine the pharmacological effects, and failure in colitis induction by recovery). Moreover, induced colitis was confirmed after 72 h of acid administration and the disease process was evaluated up to day 9. To complement the results, it is suggested to evaluate a time between 10 and 20 min of acid contact to the colon. Evaluation of serum inflammatory factors is also recommended to confirm the results.

Disclosure statement

No potential conflict of interest was reported by the author(s).

ORCID

Atefeh Babaei https://orcid.org/0000-0002-1140-7426

ORCID

Atefeh Babaei http://orcid.org/0000-0002-1140-7426

References

1. Low D, Nguyen DD, Mizoguchi E. Animal models of ulcerative colitis and their application in drug research. *Drug De Devel Ther*. 2013;7:1341
2. Williams KL, Fuller CR, Dieleman LA, et al. Enhanced survival and mucosal repair after dextran sodium sulfate-induced colitis in transgenic mice that overexpress growth hormone. *Gastroenterology*. 2001;120(4):925–937. doi:10.1053/gast.2001.22470.
3. Salih A, Widbom L, Hultdin J, Karling P. Smoking is associated with risk for developing inflammatory bowel disease including late onset ulcerative colitis: a prospective study. *Scand J Gastroenterol*. 2018;53(2):173–178. doi:10.1080/00365521.2017.1418904.
4. Uhlig HH. Monogenic diseases associated with intestinal inflammation: implications for the understanding of inflammatory bowel disease. *Gut*. 2013;62(12):1795–1805. doi:10.1136/gutjnl-2012-303956.
5. Ordas I, Eckmann L, Talamini M, Baumgart DC, Sandborn WJ. Ulcerative colitis. *Lancet (London, England.)*. 2012;380(9853):1606–1619. doi:10.1016/S0140-6736(12)60150-0.
6. Kiesler P, Fuss IJ, Strober W. Experimental models of inflammatory bowel diseases. *Cell Mol Gastroenterol Hepatol*. 2015;1(2):154–170. doi:10.1016/j.jcmgh.2015.01.006.
7. Randhawa PK, Singh K, Singh N, Jaggi AS. A review on chemical-induced inflammatory bowel disease models in rodents. *Korean J Physiol Pharmacol*. 2014;18(4):279–288. doi:10.4196/kjpp.2014.18.4.279.
8. Nakao K-i, Ro A, Kibayashi K. Evaluation of the morphological changes of gastric mucosa induced by a low concentration of acetic acid using a rat model. *J Forensic Leg Med*. 2014;22:99–106. doi:10.1016/j.jflm.2013.12.016.
9. Niu X, Fan T, Li W, Huang H, Zhang Y, Xing W. Protective effect of sanguinarine against acetic acid-induced ulcerative colitis in mice. *Toxicol Appl Pharmacol*. 2013;267(3):256–265. doi:10.1016/j.taap.2013.01.009.
10. Das S, Kanodia L, Mukherjee A, Hakim A. Effect of ethanolic extract of leaves of Paederia foetida Linn. on acetic acid induced colitis in albino rats. *Indian J Pharmacol*. 2013;45(5):453. doi:10.4103/0253-7613.117728.
11. Al-Rejaie SS, Abuohashish HM, Al-Enazi MM, Al-Assaf AH, Parmar MY, Ahmed MM. Protective effect of naringenin on acetic acid-induced ulcerative colitis in rats. *WJG*. 2013;19(34):5633. doi:10.3748/wjg.v19.i34.5633.
12. Low D, Tran HT, Lee IA, et al. Chitin-binding domains of Escherichia coli ChiA mediate interactions with intestinal epithelial cells in mice with colitis. *Gastroenterology*. 2013;145(3):602–612. e609. doi:10.1053/j.gastro.2013.05.017.
13. Fabia R, Willén R, Ar'Rajab A, Andersson R, Ahrén B, Bengmark S. Acetic acid-induced colitis in the rat: a reproducible experimental model for acute ulcerative colitis. *Eur Surg Res*. 1992;24(4):211–225. doi:10.1159/000129209.
14. Ceranowicz P, Warzecha Z, Cieszkowski J, et al. Essential role of growth hormone and IGF-1 in therapeutic effect of ghrelin in the course of acetic acid-induced colitis. *IJMS*. 2017;18(6): doi:10.3390/1118.ijms.18061118.
15. Najafi A, Motaghi E, Hosseini MJ, Ghasemi-Pirbaluti M. The effect of sodium valproate on acetic acid-induced colitis in rats. *Inflammopharmacology*. 2017;25(1):137–145. doi:10.1007/s10787-016-0304-1.
16. Pastrelo MM, Ribeiro CCD, Duarte JW, et al. Effect of concentrated apple extract on experimental colitis induced by acetic acid. *Int J Mol Cell Med*. 2017;6(1):38–49. PMC5568191.
17. Sharma H, Chauhan S, Singh PP, Goel RK. The role of antioxidants and free radicals in the healing effects of Bacopa monniera on acetic acid-induced colitis in rats. *Int J Basic Clin Pharmacol*. 2017;6(10):2388–2394. doi:10.18203/2319-2003.ijbcp20174365.
18. Morris GP, Beck PL, Herridge MS, Depew WT, Szewczuk MR, Wallace JL. Hapten-induced model of chronic inflammation and ulceration in the rat colon. *Gastroenterology*. 1989;96(3):795–803. doi:10.1016/S0016-5085(89)80079-4.
19. Wallace JL, Keenan CM, Gale D, Shoupe TS. Exacerbation of experimental colitis by nonsteroidal anti-inflammatory drugs is not related to elevated leukotriene B4 synthesis. *Gastroenterology*. 1992;102(1):18–27. doi:10.1016/0016-5085(92)91779-4.
20. Gao J, Cui J, Zhong H, et al. Andrographolide sulfonate ameliorates chronic colitis induced by TNBS in mice via decreasing inflammation and fibrosis. *Int Immunopharmacol*. 2020;83: doi:10.1016/j.intimp.2020.106426.
21. Baumgart DC, Sandborn WJ. Crohn's disease. *Lancet (London, England)*. 2012;380(9853):1590–1605. doi:10.1016/S0140-6736(12)60026-9.
22. Hanauer SB. Inflammatory bowel disease: epidemiology, pathogenesis, and therapeutic opportunities. *Inflamm Bowel Dis*. 2006;12(suppl_1):S3–S9. doi:10.1097/01.mib.0000195385.19268.68.
23. Jagtap A, Shirke S, Phadke A. Effect of polyherbal formulation on experimental models of inflammatory bowel diseases. *J Ethnopharmacol*. 2004;90(2-3):195–204. doi:10.1016/j.jep.2003.09.042.
24. Branski D, Sharon P, Abrahamov A. Prostaglandins in disorders of the intestinal tract: a review. *J Pediatr Gastroenterol Nutr*. 1986;5(6):853–860. doi:10.1097/00005176-198611000-00005.
25. Tindemans I, Joosse ME, Samsom JN. Dissecting the Heterogeneity in T-Cell Mediated Inflammation in IBD. *Cells*. 2020;9(1):110. doi:10.3390/cells9010110.
26. Varnalidis I, Ioannidis O, Karamanavi E, et al. Omega 3 fatty acids supplementation has an ameliorative effect in experimental ulcerative colitis despite increased colonic neutrophil infiltration. *Rev Esp Enferm Dig*. 2011;103(10):511–518. doi:10.4321/s1130-01082011001000003.
27. Rieder F, Brenmoehl J, Leeb S, Schölmerich J, Rogler G. Wound healing and fibrosis in intestinal disease. *Gut*. 2007;56(1):130–139. doi:10.1136/gut.2006.090456.
28. Kruidenier L, Verspaget H. Review article: oxidative stress as a pathogenic factor in inflammatory bowel disease-radicals or ridiculous? *Aliment Pharmacol Ther*. 2002;16(12):1997–2015. doi:10.1046/j.1365-2036.2002.01378.x.
29. Millar A, Rampton D, Chander C, et al. Evaluating the antioxidant potential of new treatments for inflammatory bowel disease using a rat model of colitis. *Gut*. 1996;39(3):407–415. doi:10.1136/gut.39.3.407.
30. Holma R, Salmenperä P, Riutta A, Virtanen I, Korpela R, Vapaatalo H. Acute effects of the cys-leukotriene-1 receptor antagonist, montelukast, on experimental colitis in rats. *Eur J Pharmacol*. 2001;429(1-3):309–318. doi:10.1016/S0014-2999(01)01330-9.