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Original Article

Reduce the Changes in Histological Features of Colon Resulting from Induced Ulcerative Colitis in Mice by Worm Antigens

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Abstract

Ulcerative colitis is one of the inflammatory bowel disease (IBD) consequences characterized by chronic inflammation of the gastrointestinal (GI) tract. The current study aims to determine the changes in colon tissue caused by induced Ulcerative Colitis and reduce these changes by worms' antigen. The study included 45 adult male albino mice (*Mus musculus*), with ages ranging between 8-10 weeks; the average weights ranged between 18-32 g. Ulcerative colitis was induced by intracolonic administration of 100 µL of 4% after they had been starved for 18 h. The animals were dissected after one week, and routine histological sections were conducted. The results of the histological study showed that in the colon of white mice treated with 4% acetic acid occurred, many histological changes were represented by the appearance of inflammatory cells in the mucous layer around the goblet cells, and the formation of vacuoles, necrosis at epithelium and intense congestion under the surface epithelium. The marked histological sections of the colon in mice with induced ulcerative colitis treated with *helminth* extract at a concentration of 0.1 mg/kg for a week, were showed that the histological changes began to decrease in the colon tissue, with the presence of a few inflammatory cells as well as little mucus secretion.

Keywords: ulcerative colitis, mice, acetic acid

1. Introduction

Ulcerative colitis is one of the inflammatory bowel disease (IBD) consequences characterized by chronic inflammation of the gastrointestinal (GI) tract. Ulcerative colitis is a chronic disease that releases inflammatory disorders of the gastrointestinal tract combined with diarrhea, weight loss and nausea, and pathological features such as a loss of mucosal integrity and inflammatory cell infiltration (1).

Many chemicals have induced experimental colitis in rats and mice (2, 3). These chemicals are included the followings: Dextran sulfate sodium (DSS), oxalone, 2,4,6-trinitrobenzene sulfonic acid (TNBS), and Dinitrobenzene sulfonic acid (DNBS) and acetic acid (4-6). Concerning the acetic acid model, several reports describe the possibility that several potential compounds can ameliorate acetic acid-induced colitis (7). Other studies also showed that many drugs and diets could worsen or improve colonic functions and symptoms according to the type and severity of colitis (8). On the other hand, in the DSS-induced colitis model, numerous vital factors, including DSS source, molecular weight, concentration, duration, mouse strain, source, age, gender, and body weight, as well as environmental factors including the hygienic condition of the vivarium have been identified as the critical factors in the severity and induced colitis outcomes (9). At the same time, TNBS and DNBS result in mechanical dysfunction of epithelial integrity, which develops spontaneous chronic inflammation in the

intestine resulting from a lack of the primary immune regulatory interleukin. In this case, the disease occurs due to a deficiency of T regulatory cells (10, 11). Previous studies indicated that acetic acid damage was practical and could increase the abnormalities in the intestinal mucosal tissues, mucosal injury, degeneration in epithelial cells of mucosa, and depletion in goblet cells. Additionally, administration with acetic acid into male mice increased the leucocytic infiltrations, abnormal changes in the intestinal crypt, and a loss of mucosal integrity. Microscopic examination of colon tissues of mice administrated with acetic acid showed an increase in the number of neutrophils and infiltration of inflammatory cells (12).

The results of previously published work revealed that the parasitic helminths could reduce the pathological inflammatory symptoms of many diseases that these worms are associated with, as their effects in inducing the immune response overlap with the effects of those diseases on the immune system, which leads to the inhibition and neutralization of those responses, and thus reducing the pathological effects of these diseases, especially inflammatory ones (12).

Therefore, the present study aimed to evaluate the changes in tissue features of ulcerative colitis induced by acetic acid.

2. Materials and Methods

2.1. Animals and Experiment Design

The experiment included 45 adult male albino mice (Balb/c), *Mus musculus*, ages 8-10 weeks, and weights ranging between 18-32 g. They were obtained from the University of Tikrit and housed in the animal house at the College of Education for Pure Sciences, University of Diyala.

The animals were randomly divided into three groups (n=15). In Group 1, the animals in this group received intracolonic administration of 100 µL of 4% acetic acid to induce colitis and were dissected after 14 weeks. In group 2, the animals in this group were injected with 100 µL of 4% acetic acid to induce colitis and then treated with worm antigens for 1 week (*helminth* extract at a concentration of 0.1 mg/kg for a week). Group 3 served as the control group injected with normal saline for 14 weeks. The mice were placed in plastic cages furnished with sawdust, equipped with water and ration for the duration of the study, and cleaned every day. It kept the temperature ranging from ±25°C. At the end of the experiment, for the animal's anesthesia, we used xylazine hydrochloride 50 mg/kg and ketamine hydrochloride 100 mg/kg body weight intraperitoneally to remove the distal colon (8 cm). After that, euthanasia was performed by exsanguination under anesthesia.

2.2. Induction of Ulcerative Colitis

Ulcerative colitis was induced by injecting animals after they had been starved for 18 hours with 100 µL of 4% acetic acid. Immediately after administration, the mice were held horizontally for 2 min to prevent fluid leakage. Control animals underwent a similar procedure using an equal volume of normal saline (13).

2.3. *Hymenolepis diminuta* Preparation

The worms (*Hymenolepis diminuta*) were collected from mice and rats hunted from the different local areas of Baquba city. Adult *H. diminuta* worms were flushed from the mice and rats' small intestines and rinsed (four times) in normal saline at room temperature (25°C). Then, 20 g (wet weight) of *H. diminuta* were transferred into 20 ml of sterile PBS and homogenized on the ice at maximum speed for 5 min using a Polytron PT1200 homogenizer (Kinematica, Inc., New York, NY). The homogenate was centrifuged at 4,000 rpm for 30 min at 4°C, the pelleted material was discarded, and the PBS-soluble supernatant was collected and subjected to two additional rounds of centrifugation. The supernatant was collected, and the aliquots of this "crude extract" were stored at 80-°C.

2.4. Histological Evaluations

The animals were dissected, and the colon was extracted and placed in the fixative solutions. The tissue slides were prepared to know the histological changes that occurred due to the effect of stasis. The tissue sections were examined and photographed using an optical microscope Provider with a camera.

3. Results and Discussion

Ulcerative colitis (UC) is a lifelong disease arising from an interaction between genetic and environmental factors that mainly affect the mucosal layer of the large intestine or colon. It is considered a chronic inflammatory disease characterized by a typical relapsing-remitting course. Evidence growing supports the importance of controlling the asymptomatic inflammatory activity during remission periods. Indeed, patients who achieve mucosal healing or deep remission are at lower risk of relapse and need surgery, which is an established marker of a worse prognosis (14, 15)**.**

The results of the histological study showed that the colon of mice treated with 4% acetic acid occurred, and many histological changes were represented by the appearance of inflammatory cells in the mucous layer around the goblet cells and the formation of vacuoles as in the figure 1. The current findings were consistent with previous studies that showed the effect of acetic acid that causes Colon inflammation and its effect on the tissue of the colon (12).

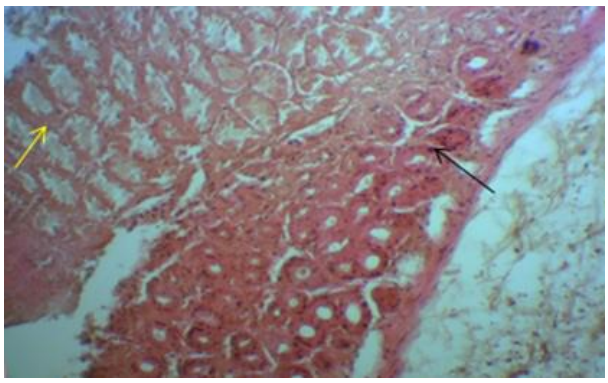

Figure 1. the histopathological appearance of the colon induced by colitis, inflammatory cells in the mucosal layer, around goblet cells (black arrow), and formed of vacuoles (yellow arrow) (H&E stain, 40×)

The examination results also showed the emergence of severe necrosis in the epithelium, which agrees with Vanlangenakker, Vanden Berghe (16), who mentions that in the necrotic process, cells and organelles swell and rupture with subsequent leakage of cellular content to the microenvironment, causing an inflammatory response. The mice in the present study also lacked Paneth cells, indicating dysregulated antimicrobial immune cell functions in the intestinal epithelium in figure 2, which agrees with Lichtenstein and Rutgeerts (17).

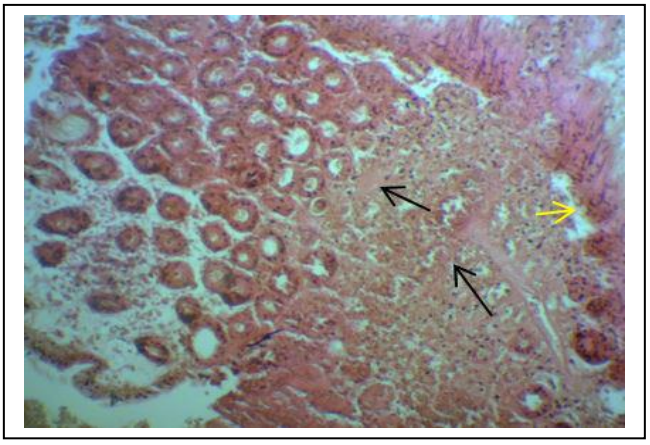

Figure 2. Histopathological appearance of the colon shows severe necrosis at epithelium, intense congestion under the surface epithelium (black arrow), epithelium granulation tissue, and intense inflammatory cells at sub-mucosa; (yellow arrow) H&E stain with low power (10×)

The marked histological sections of the colon in mice with induced ulcerative colitis treated with *helminth* extract at a concentration of 0.1 mg/kg for a week showed that the histological changes began to decrease in the colon tissue, with the presence of a few inflammatory cells as well as little mucus secretion. It was less than that was observed in the group of mice treated with Acetic acid without *helminth* treatment. The decline is due to the helminths antigen's possession of many active immune components, such as the amino acid glycan (18, 19). This corresponds to Fleming, Isaak (20), who mentioned in their studies that the *helminth* administration in multiple sclerosis reduced the inflammatory cells' responses. This came in line with the results of the current study because the *helminth* extract contained proteins that may induce an immune response that neutralized inflammatory response, as shown in figures 3 and 4.

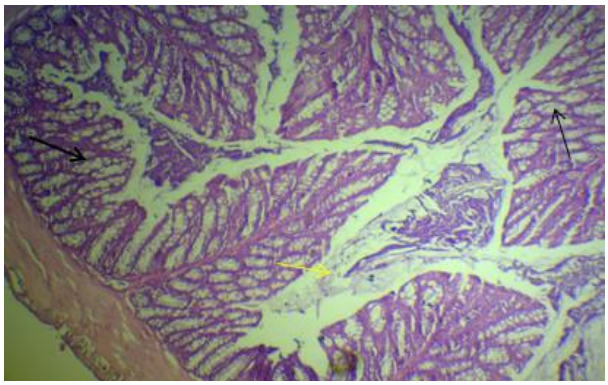

Figure 3. Histopathological appearance of colon relatively normal appearance (black arrow) with little mucus secretion in another area (yellow arrow), (H&E stain 40×)

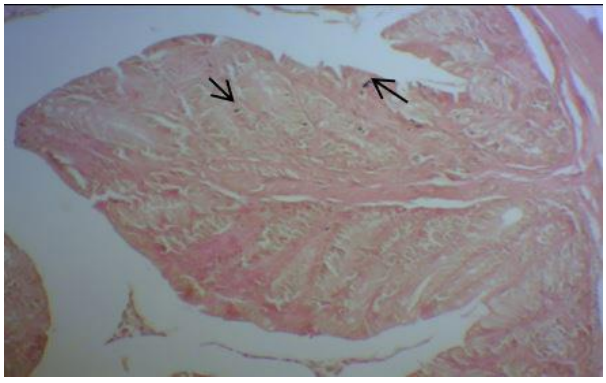

Figure 4. Histopathological appearance of colon show very a few inflammatory cells (black arrow), (H&E stain 10×)

The treatment with acetic acid caused ulcerative colitis, in addition to causing severe pathological tissue damages, represented by the occurrence of congestion, inflammatory cells in the mucosal layer, and necrosis, and it is reduced by helminths antigen, which might make it a promising future treatment.

Authors' Contribution

Study concept and design: A. D. F.
Acquisition of data: A. D. F.
Analysis and interpretation of data: A. D. F.
Drafting of the manuscript: N. Y. A.
Critical revision of the manuscript for important intellectual content: A. D. F. and N. Y. A.
Statistical analysis: N. Y. A.
Administrative, technical, and material support: N. Y. A.

Ethics

Ethical approval for this study was obtained from the Ethical Review Board of the Diyala University, Baqubah, Iraq.

Conflict of Interest

The authors declare that they have no conflict of interest.

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