Original Article

Effect of Bone Marrow-Derived Mesenchymal Stem Cells versus Probiotics in the Treatment of experimentally induced Acute Colitis in Rats: A Histological and an Immunohistochemical Study

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ABSTRACT

Introduction: Inflammatory bowel disease (IBD) is a progressive inflammatory condition of the gastrointestinal tract (GIT). Mesenchymal stem cells have immunomodulatory capabilities and probiotics may modify the pathophysiology of gut disorders and have positive effects on the gut microbiota

Aim of Work: Compare the effect of BM-MSCs versus probiotics on the treatment of acute colitis in adult male albino rats.

Material and Methods: Forty adult male albino rats weighed (200-220 gm) were equally divided into five groups. Group I (control). Group II (Acute colitis). Group III (recovery): sacrificed 14 days from induction of acute colitis (IAC). Group IV (Colitis + BM-MSCs): received a single injection of 1ml of phosphate buffer saline containing 1×106 BM-MSCs. Group V (colitis + Probiotic): received 1x109 CFU/ml/100 gm of b.w./day of Lactobacillus Plantarum orally. In groups IV and V, rats were treated three days after IAC and sacrificed 14 days after this induction.

Results: The acute colitis group showed mucosal ulceration, cellular infiltration, and decreased number of goblet cells. The area percentage of collagen and TNF-α immune stain revealed a significant increase while the area percentage of PCNA-positive nuclei immune stain significantly decreased. The recovery group showed incomplete recovery of these structural changes. The histological architecture of the colon was restored following the treatment with stem cells and probiotics.

Conclusion Treatment with stem cells or probiotics restored the integrity of the colonic mucosa in a rat model of acute colitis. Probiotics therapy was superior to stem cell therapy as evidenced by allowing rapid division and differentiation of stem cells into goblet cells that form mucous barriers.

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Key Words: Acute colitis, BM-MSCs, PCNA, probiotic, TNF- α.

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INTRODUCTION

Ulcerative colitis (UC) and Crohn's disease (CD) are the two forms of inflammatory bowel disease (IBD), that are characterized by chronic relapsing and progressive inflammation of the gastrointestinal tract (GIT)[1]. The pathological features of IBD include infiltration by inflammatory cells, and activation of nuclear factor kappa B (NF-κB) dependent proinflammatory mediators like tumor necrosis factor-alpha (TNF-α) and interleukin 6 (IL-6). Excessive generation of free radicals such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) causes depletion of the antioxidant capacity of the colon, and loss of mucosal integrity. The risk of colorectal cancers and lymphoma was rising in patients with UC, which can reveal the high mortality rates in the affected patients[2]. Because the current treatment varieties have many side effects and cause incomplete remission. So, this condition forced us to search for alternative therapies and natural dietary compounds to treat IBD[2].

Experimentally produced acute colitis (AC) in animals is comparable to UC in humans. Thus, using animal models to research the pathological mechanisms of the disease and test new drugs for their anti-ulcerative action has become essential[3]. Acetic acid (AA) is administered intrarectally to rats to create the AC model[4].

According to previous reports, mesenchymal stem cells have immunomodulatory capabilities that might hypothetically be used to treat a variety of disorders[1]. Stem cells have the ability to target the site of damage as a reaction to the chemotactic factors e.g., cytokines, adhesion molecules, and growth factors[5,6]. They are multipotent cells that can differentiate into colonic mucosal stem cells and later distinguish into colonic epithelial cells to restore the colonic mucosa[7,8]. Hayashi et al.[9] stated that bone marrow-derived stem cells can migrate to the colonic mucosa and repair the damaged colonic tissue of rats with stimulated experimental colitis.

Probiotics are live microorganisms that assist in the re-establishment and maintenance of microbial balance in case of dysbiosis[10,11]. Probiotics may modify the pathophysiology of gut disorders and have positive effects on the gut microbiota. Additionally, research suggests that

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consuming probiotics may have an anti-inflammatory effect on the digestive system[12]. One of the most well-known Lactobacillus species is Lactobacillus Plantarum (L. Plantarum), which is found in fermented foods. L. Plantarum is commonly employed as starter cultures or probiotics in the food and pharmaceutical industries. It manages the fecal bacteria composition, prevents and treats irritable bowel syndrome (IBS), and alleviates some gastrointestinal symptoms, among other health-promoting properties[13].

AIM OF THE WORK

The present work aimed to compare the role of BM-MSCs versus probiotics in the treatment of acute colitis in adult male albino rats.

MATERIALS AND METHODS

Ethical acceptance

The study protocol of the present work was approved by the Faculty of Medicine, Ain shams University Research Ethics Committee FMASU REC R 105/2022. All animal experiments comply with the ARRIVE guidelines and were carried out in accordance with the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978).

Materials

Glacial acetic acid (GAA): used for the induction of colitis. It was provided by Merck Millipore, Darmstadt, Germany.

Probiotic: Lactobacillus Plantarum (DSA 20174) was provided by the National Research Center, Cairo, Egypt.

Bone marrow-derived mesenchymal stem cells (BM-MSCs): were prepared in Histology Department (stem cell research unit), Faculty of Medicine, Ain shams university.

Methods

Probiotic preparation: L. Plantarum (LP) was prepared in a skim milk flask (100 mL), cultivated for 24 hours at 37°C in de Man, Rogosa, and Sharpe (MRS) broth, and sterilized for 10 minutes at 115°C. LP was activated and cultured in MRS broth, then anaerobically incubated for 24 hours at 37°C (Gas Generating Kit Anaerobic System, Oxoid, UK). After that 5% LP (108 CFU/ml)[12] was added to the skim milk flask and incubated at 370C for 24 hours after the initial incubation time. Utilizing MRS agar media, the number of LP was counted following the incubation period. Previously prepared skim milk flasks were serially diluted using 1 ml in 9 ml of sterile saline and 1 ml from each of the dilutions 10−7 and 10−8 was placed on a Petri plate (triplicate plates for each dilution), then MRS agar medium was poured into the previous prepared Petri dishes. The pour dishes were incubated at 37 °C for 48 h. After incubation, counting was done in the most countable dish[14]. The concentration of probiotic bacteria dose was $1x10^9$ (CFU)/mL/100 g of body weight[15].

Stem cell preparation

Stem cells were obtained from five young rats. BM-MSCs were separated in agreement with McFarlin et al.[16]. Long bones were obtained from young rats and all the adherent tissues were removed. The upper and lower ends of long bones were excised with surgical scissors. Two ml of culture media were injected from one end of the bone. The marrow exiting was received from the other end at 15 ml falcon with the disintegration of the cells by pipetting the cell suspension. The suspension was centrifuged to form a cell pellet which was suspended in Dulbecco's modified Eagle's medium (DMEM) added to 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin (obtained from Lonza company, Swiss), Afterwards, they were grown and kept at 37°C in 5% humidified CO_2 for 2–3 days. On the formation of large colonies (80~90% confluence), washing by phosphate buffer saline (PBS) was done twice, then detachment of cells was performed using 0.25% trypsin (purchased from Lonza Company, Swiss) in 1 mM EDTA for 5 min at 37º C. centrifugation of the suspension was done, then by using a hemocytometer the count of viable and non-viable cells was done. After that, the viable cells were subcultured at 4×10^3 cells/cm². Adhesiveness and fusiform shape were characteristics of BM-MSCs in culture. The stem cells used in this experiment were of the third passage.

Characterization of BM-MSCs by Streptavidin-biotin immunoperoxidase technique for recognition of CD105 and CD45:

The dishes containing BM-MSCs from passage three subculture; were fixed by acetone/methanol mixture, then covered by 2 ml H_2O_2 (3%) for fifteen min to stop endogenous peroxidase. Antigen retrieval was done by immersing the tissue culture Petri dishes in a warmed citrate buffer solution (pH 6) and the dishes were heated in a microwave at two watts for 10-20 min, cooling was allowed for 20 min at ordinary temperature then washed by distilled water (DW), incubation with goat serum for 10 min was done, then were lightly blotted after that covered completely by 80-100 µl of the primary antibody overnight in the humidified chamber in the incubator, then washed well with PBS for 5minutes and blotted. The dishes were covered completely by 80-100 µl of Streptavidin horseradish peroxidase conjugate for 15 min. Then, wash the dishes with PBS. The brown color was developed using one to two drops of diaminobenzidine (DAB) for ten minutes. Then, wash the dishes with DW and blotted. Results: Positive cytoplasmic reactions appeared brown in color.

Induction of acute colitis (IAC):

For 24 hours the rats were fasted and allowed to drink water freely to empty the gut contents. Diethyl ether was used to anesthetize. The rectum was intubated up to 8 cm to inject 2 ml of 3% GAA solution. After injection, the rats stayed in upside-down position to prevent GAA leakage[2]. Observation of the rats for diarrhea or rectal bleeding was done 24 hours after the GAA injection. The previous signs indicating colitis induction.

Animals and grouping

Forty adult male albino Wister rats weighing between (200-220 gm) were used in this study. The experiment was done at the Medical Ain Shams Research Institute (MASRI). Animals were housed in cages at normal room temperature with water and food ad libitum. All animal procedures were performed in accordance with the Guide for the Care and Use of laboratory animals and approved by The Animal Ethical Committee of Ain Shams University. The rats were divided into 5 groups:

Group I (Control group): (8 rats): Rats were similarly divided into two subgroups: subgroup Ia was left without any interference. Subgroup Ib received an intracolonic injection of 2ml DW. After three days, rats received an intravenous injection of 1ml PBS. The rats of the control group were sacrificed after 14 days from the beginning of the experiment.

The rest of the rats (32rats) were subjected to induction of acute colitis; then they were randomly divided into 4 groups of 8 rats each:

Group II (Acute colitis group): The rats were sacrificed after three days of colitis induction.

Group III (Recovery group): The rats were left without any intervention and were sacrificed after 14 days of colitis induction.

Group IV (Colitis+ BM-SCs): After three days of colitis induction, rats received a single intravenous injection of 1 ml of PBS containing 1×10^6 BM-MSCs (in the tail vein). The rats were sacrificed after 14 days of colitis induction[2]. The BM-MSCs used were from the 3rd passage (after 3 subcultures).

Group V (Colitis+ Probiotic): after three days of colitis induction, 1 x 10^9 CFU/ml of L. Plantarum was given daily to the rats by gastric tube for 14 days[16]. The rats were sacrificed after 14 days of colitis induction

Sample collection for histological examination

By the last day of the experiment, the rats were cervically dislocated. The distal colon was excised through an abdominal incision. The colonic samples; each sample is about three cm long; were taken two cm above the anus. The samples were opened longitudinally and then washed carefully with saline to eliminate any residues. Longitudinal specimens were then immediately fixed in 10% buffered formalin and processed to form paraffin sections of 5 µm thickness. Slides were subjected to staining by hematoxylin and eosin (H&E) stain, Masson's trichrome stain, and combined Alcian blue-PAS Technique.

Histological scoring

Histological scoring was carried out blindly after H&E staining. The following variables were examined based on Riley score[24,25]: (1) Acute inflammatory cell infiltrate, (2) Crypt abscesses, (3) mucin depletion or deletion of goblet cells, (4) Surface epithelial integrity and (5) crypt architectural irregularities or crypt damage. for the first four parameters, the scale was 0,1,2 and 3 meaning none, mild, moderate, and severe respectively but the scale was 0,1,2,3 and 4 meaning no damage, damage of basal one-third, damage of basal two-thirds, loss of the crypts but surface epithelium is still present and loss of both crypts and surface epithelium respectively, for the last parameter.

Immunohistochemical studies

Positively charged slides containing colonic tissues were prepared for the immunohistochemical reaction using:

a. Proliferating cellular nuclear antigen (anti PCNA); a well-known marker for epithelial cell proliferation. PCNA is a (mouse-anti-human polyclonal antibody, Santa Cruz Biotechnology Dallas Texas USA). The positive reaction was nuclear, and the positive control was the epidermis of the skin (Fig. A. A1). The negative control showed no reaction (Fig. A. A2).

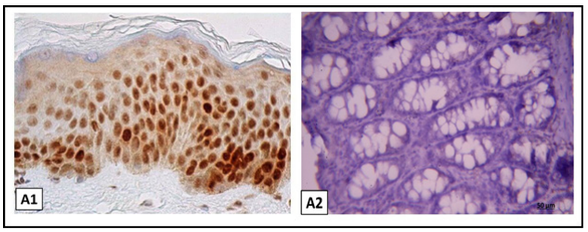

Fig. A. A1: positive control of PCNA in the epidermis of the skin (https://www.scbt.com/p/pcna-antibody-f-2?requestFrom=search), A2: negative control showing no reaction in the colon

b. Tumor necrosis factor-alpha (anti-TNF α); inflammatory cytokines; (mouse anti-human monoclonal antibody, LABVISION, USA). The positive reaction was cytoplasmic, and the positive control was the lung (Fig. B. B1). The negative control showed no reaction (Fig. B. B2).

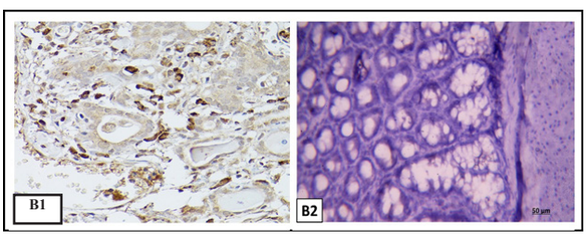

Fig. B. B1: positive control of TNF α in the lung , (https://www.thermofisher.com/antibody/primary/query/TNF/filter/tissue/Lung), **B2:** negative control showing no reaction in the colon

The addition of a secondary antibody (DAKO, Denmark) to the slides was done for half an hour then DAB solution (DAKO, Denmark) was added for 10 min to develop the reaction. Finally, counterstaining the slides using hematoxylin. After that, the slides were dehydrated, cleared, and mounted. The same steps were repeated to

prepare the negative control, but the primary antibody didn't be used.

Morphometric study

Samples were analyzed using an image analyzer (Leica Q win V. 3 program) connected to a microscope (Leica DM2500) (Wetzlar, Germany). The image analyzer and the microscope were in the Histology and Cell Biology Department, Faculty of Medicine, Ain Shams University. Morphometric analysis was done for all specimens from all groups. Three slides from each animal were used to obtain measurements (X40). Five haphazardly selected non-overlapping fields were examined for each slide. The following measurements were done.

1. The length of the crypt in sections stained by H & E stain.
2. The area percentage of collagen fibers in sections stained by Masson's trichrome stain,
3. The area percentage of mucin in combined Alcian blue-PAS-stained sections.
4. The Number of goblet cells in sections stained by Alcian blue PAS.
5. The area percentage of PCNA-positive nuclei.
6. The area percentage of TNF-α positive reaction in the mucosa and submucosa.

Statistical analysis

All data from histomorphometric studies were statistically analyzed using Statistical Package for the Social Sciences version 23 (SPSS Inc., Chicago, IL, USA). Results obtained from the experiment were stated as mean values and standard deviations. The significant differences among values were analyzed by using a one-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test. For all comparisons, *P-value* < 0.05 was considered significant.

RESULTS

BM-MSCs

On day one primary culture of BM-MSCs, the cells were non-adherent and rounded with variable shapes. On day three of the culture, some adherent cells were seen extending short cytoplasmic processes. Other cells were refractile and non-adherent. Colonies of rounded cells were occasionally seen (Figure 1A). on day seven of culture, the cells became fibroblast-like in appearance. They appeared confluent with multiple interdigitating long processes. They showed granular cytoplasm with vesicular nuclei. Some of which showed multiple nucleoli (Figure1B). Characterizing the cells in the third passage subculture using the streptavidin-biotin immunoperoxidase technique revealed that most of the cultured cells showed positive brownish cytoplasmic reactions for CD105 (Figure 1C) and negative reaction for CD45 immunostaining was detected (Figure 1D).

H&E-stained sections

Examination of H&E-stained sections of the control group's distal colon showed the colon's wall formed of four layers: mucosa, submucosa, muscularis externa, and serosa. The mucosa showed regularly arranged closely packed long crypts. The crypts were lined with columnar cells with basal oval nuclei and goblet cells with apical vacuolated cytoplasm. The lamina propria was occupied by the crypts. The muscularis mucosa consisted of inner circular and outer longitudinal smooth muscle fibers. The submucosa appeared beneath the mucosa and was formed of loose connective tissue containing cells, fibers, and blood vessels. The muscularis externa showed inner circular and outer longitudinal smooth muscle fibers. Nerve cells of Auerbach's plexus were also seen in the connective tissue between the two layers (Figures 2 A,B).

In group II (colitis group) loss of the colonic mucosal architecture and mucosal ulceration were frequently noticed. In some areas, the crypts were not present with the presence of extensive mononuclear inflammatory cells in the lamina propria and the submucosa. The muscularis mucosa was seen thickened and infiltrated with inflammatory cells. The site of the ulcer was sometimes seen partially covered by flattened epithelium. Dilated and congested blood vessels were also seen in the areas of the lesion. Some smooth muscle fibers of muscularis externa were seen with pyknotic nuclei (Figures 2 C,D,E).

Sections of the distal colon from the recovery group showed incomplete recovery of the colonic mucosal architecture. Some areas showed short regularly arranged closely packed crypts, other areas showed loss of the colonic mucosal architecture with lost crypts. Muscularis mucosa appeared thickened in some areas. Mononuclear cellular infiltration was seen in the lamina propria and the submucosa. Cells with vacuolated cytoplasm and pyknotic nuclei were seen in muscularis mucosa. Sometimes abscess-containing neutrophils, desquamated epithelial tissue, and goblet cells are seen in the mucosa. Submucosa was apparently thickened with the presence of dilated congested blood vessels (Figures 2 F,G,H). After treatment with stem cells (Figure 2I) and probiotics (Figures 2 J,K), sections of the distal colon showed restoration of the histological architecture of the four layers of the colon. The mucosa showed intact architecture with intact regularly arranged closely packed long crypts. The crypts were lined by continuous surface columnar epithelium and goblet cells. Some inflammatory cells were seen in the lamina propria of the probiotics group with an apparent increase in intraepithelial lymphocytes. The submucosa and muscularis externa appeared nearly like the control group. These microscopic findings were confirmed by histomorphometric results. A significant decrease in the mean length of the crypts was noticed in colitis and recovery groups in comparison to the control, stem cells, and probiotics groups. In addition, a significant increase in the mean length of the crypts was noticed in the recovery group in comparison to the colitis group (Table 1).

Histological scoring

The mean and the standard deviation of the histologic parameters involved in the score were shown in (Table 1). The acute colitis group showed a significant increase in all scoring parameters in relation to all other groups while all scoring parameters of the recovery group significantly decreased in relation to the colitis group and significantly increased in relation to other groups. There was a nonsignificant difference among the control group, the colitis + BMSCs group, and the colitis+ Probiotic group except for mucin depletion and crypt damage which showed a significant increase in the colitis + BMSCs group in relation to the control group.

Masson's trichrome

Examination of Masson's trichrome-stained sections of the control group showed few collagen fibers in the lamina propria between the crypts with bundles of collagen fibers in the submucosa (Figure 3A). The Colitis group showed a moderate quantity of collagen fibers in the lamina propria between the disturbed crypts and in the submucosa (Figure 3B). In the recovery group, there was an apparent increase in collagen fibers in the lamina propria between the crypts and in the submucosa as compared to control and colitis groups (Figure 3C). However, after treatment with stem cells and probiotics, the sections showed few amounts of collagen fibers in the lamina propria between the crypts and in the submucosa (Figures 3 D,E) respectively. In the same context, statistical analysis showed a significant increase in the mean area percentage of collagen fibers in the recovery group in comparison to all other groups. Moreover, a significant increase was noticed in the colitis group in comparison to the control, stem cells, and probiotics groups (Table 2).

Combined Alcian blue-PAS

Examination of Combined Alcian blue-PAS-stained sections of the control group showed the three types of goblet cells. Goblet cells with neutral mucin appeared magenta. While those contained acidic mucin appeared blue and were present mainly at the base of the crypts. Goblet cells with mixed mucin were stained purple (Figure 4A). In the colitis group, loss of goblet cells was seen in most sections. However, in some areas of intact crypts, few goblet cells were seen but with a marked decrease in their mucous content (Figure 4B). In sections of the recovery group, goblet cells in the lower half of the crypt appeared blue while those in the upper half were stained purple (Figure 4C). The sections of stem cells and probiotics treated groups showed an apparent increase in the number and size of goblet cells (Figures 4D,E) respectively. These histological results were confirmed with the current histomorphometric study. A significant decrease in the mean number of goblet cells and the mean area percentage of mucin was noticed in colitis and recovery groups as compared to the control, stem cells, and probiotics groups. A significant increase in the mean number of goblet cells and the mean area percentage of mucin was noticed in stem cells and probiotics groups in comparison to colitis, and recovery groups. However, a significant increase in the mean number of goblet cells in the probiotics group was noticed when compared to the stem cells group (Table 2).

Immunohistochemical results

PCNA

Examination of immuno-histochemical stained sections for PCNA of the control group showed a positive nuclear expression in the cells lining the crypts and some cells in the lamina propria (Figure 5A), while in the colitis group, a positive PCNA reaction was seen in some cells in the deep part of the ulcerated area, and in many cells in the lamina propria (Figure 5B). Sections of recovery group showed a positive nuclear expression in the cells lining the lower half of the crypts and many cells in the lamina propria (Figure 5C). While in groups treated with stem cells and probiotics, an apparent increase in the number and intensity of PCNA positive nuclei was seen in the cells lining the crypts. Few cells in the lamina propria were seen with moderate PCNA positive nuclear reaction (Figures 5D,E). These results were confirmed by statistical analysis of the current study. A significant decrease in the mean area % of PCNA-positive nuclei was noticed in the colitis group compared to all other groups. A significant increase was also noticed in groups treated with stem cells and probiotics compared to the colitis and recovery groups (Table 2).

TNF- α

Examination of immunohistochemically - stained sections for TNF-α for the control group showed a negative reaction in most sections (Figure 6A). In the colitis group, the sections showed an apparent increase in TNF-α positive reaction in the cytoplasm of some connective tissue cells (Figure 6B). Sections of the recovery group showed relatively less expression in the lamina propria, and submucosa as compared to the colitis group (Figure 6C). Examination of the groups treated with stem cells showed a negative reaction for TNF-α, however, there was a mild reaction in the group treated with probiotics (Figures 6 D,E) respectively. A significant increase in the mean area percentage of positive reaction for TNF- α was noticed in the colitis and recovery groups in comparison to the control, stem cells, and probiotic groups (Table 2).

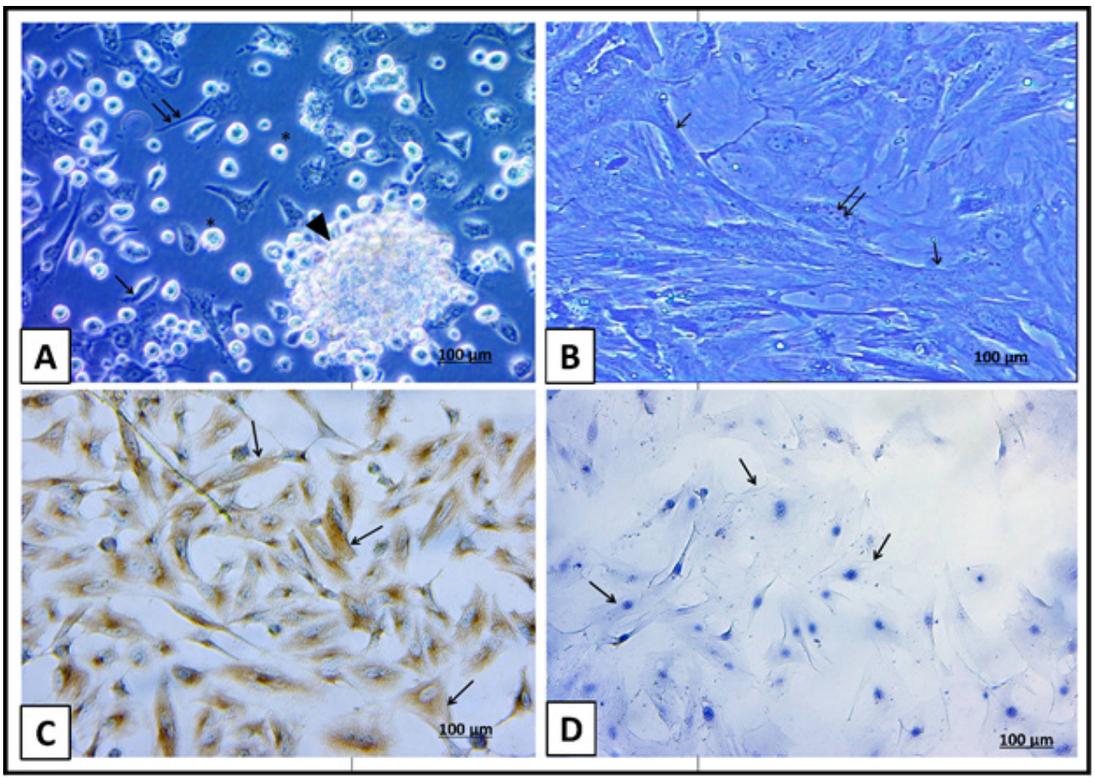

Fig. 1: Photomicrographs of BM-MSCs (Inverted microscope x200); [A] on day three of culture. The adherent cells show short (↑) and long cytoplasmic processes (↑↑). The non-adherent cells appear rounded and refractile (*). Notice a colony of rounded cells (▲). [B]: on day seven of culture showed confluent fibroblast-like cells with granular cytoplasm and long multiple interdigitating processes (↑). Multiple nucleoli appear in the nucleus of some cells (↑↑). [C, D]: Streptavidin-biotin peroxidase technique on passage three subculture of isolation and culture [C] positive brownish cytoplasmic immune reaction for CD105 (↑). [D]: negative immunostaining for CD45 (↑).

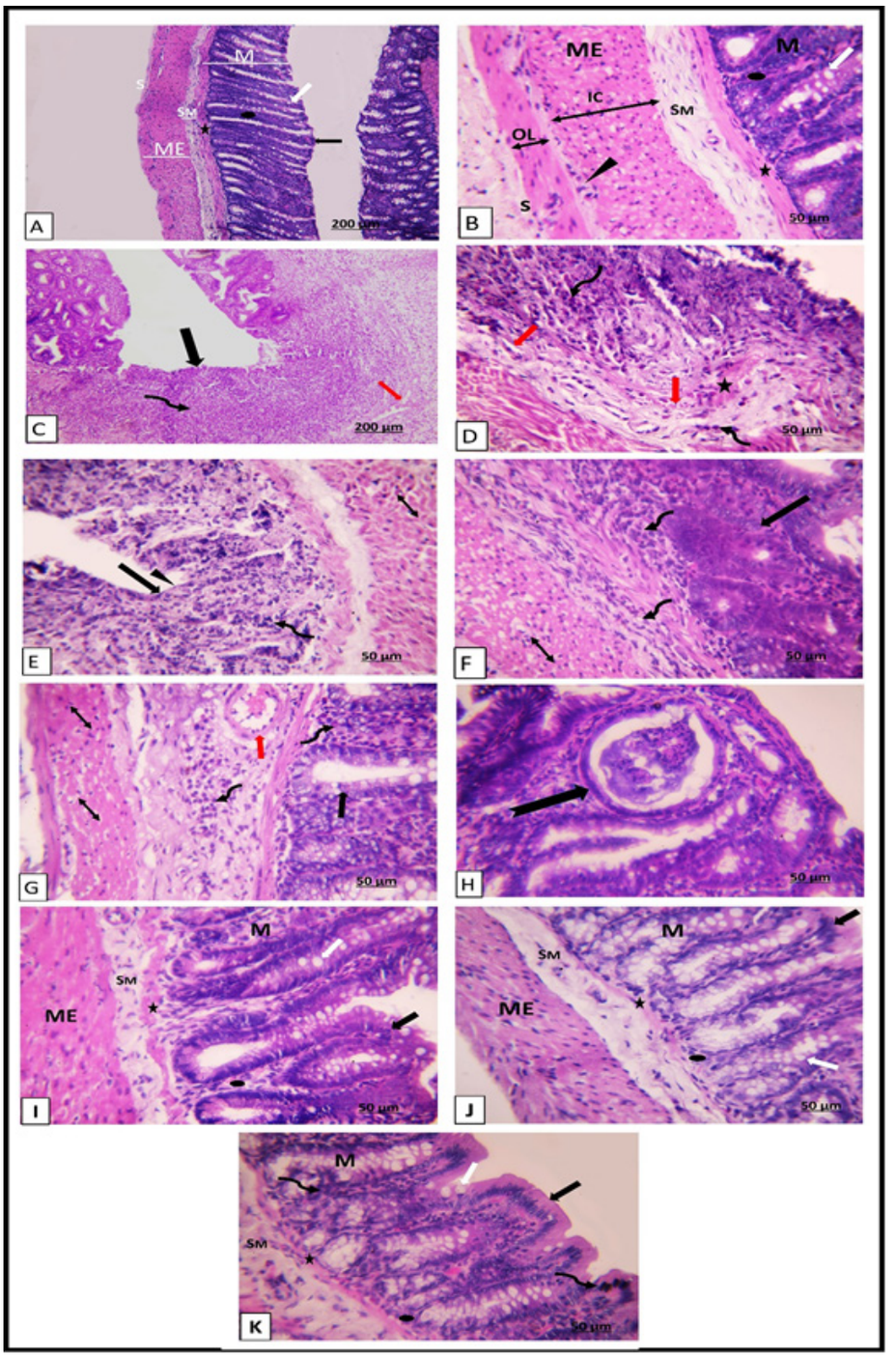

Fig. 2: Photomicrographs of H&E-stained sections of the distal colon from different groups. [A, B] control group; mucosa (M), submucosa (SM), inner circular (IC) and outer longitudinal (OL) smooth muscle fibers of muscularis externa (ME), Nerve cells of Auerbach's plexus (▲), serosa (S), columnar cells with basal oval nuclei (black arrow), goblet cells with vacuolated cytoplasm (white arrow), mononuclear cellular infiltration (●), muscularis mucosa (*). [C, D, E] Acute colitis group; mucosal ulcers (black arrow) with loss of the colonic crypts, mononuclear inflammatory cells (curved arrow), flattened epithelium covering the ulcer (▲). Dilated congested blood vessels (red arrow). Inflammatory cells in muscularis mucosa (*). Smooth muscle fibers with pyknotic nuclei (↕). [F, G, H] recovery group; loss of the colonic crypts (↑), mononuclear cellular infiltration in the lamina propria and the submucosa (curved arrows), cells with vacuolated cytoplasm and pyknotic nuclei (↕) Crypt Abscess with flattened epithelium and neutrophils in the lumen, (bifid arrow), dilated congested blood vessels (red arrow) in thickened submucosa. [I] Colitis+ BM-SCs; [J, K] Colitis+ probiotics; intact mucosa (M) with regularly arranged closely packed crypts, lamina propria (●), muscularis mucosa (*), continuous surface columnar epithelium (black arrow), goblet cells (white arrow), submucosa (SM), and muscularis externa (ME). Notice an apparent increase in intraepithelial lymphocytes (curved arrow).

Notice an apparent increase in intraepithelial lymphocytes (curved arrow). [A, C X 100, scale bar: 200 µm] [B, D, E, F, G, H, I, J, K X 400, scale bar: 50 µm]

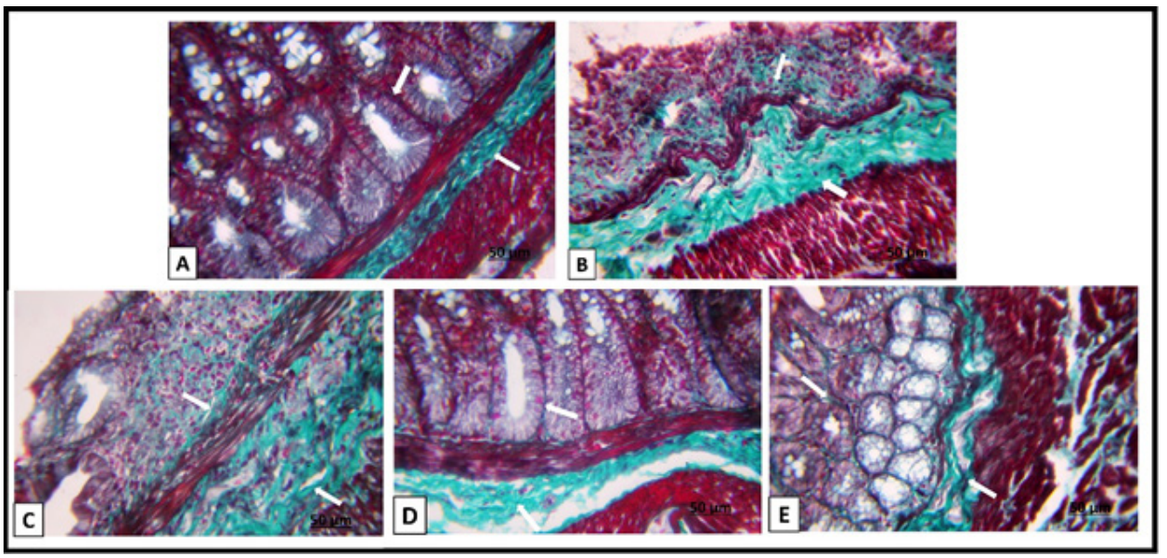

Fig. 3: Photomicrographs of Masson's trichrome stained sections of the distal colon in different groups [A] control group; few collagen fibers in lamina propria between the crypts and bundles of collagen fibers in the submucosa (white arrows). [B] Acute colitis group; a moderate amount of collagen fibers in the lamina propria between the disturbed crypts and in the submucosa (white arrows). [C] Recovery group; an apparent increase amount of collagen fibers is seen in the lamina propria, in between the crypts, and in the submucosa (white arrows). [D] Colitis + BM-SCs, and [E] Colitis+ probiotics: few amounts of collagen fibers are seen in the lamina propria between the crypts and in the submucosa (white arrows). [scale bar: 50 µm X400]

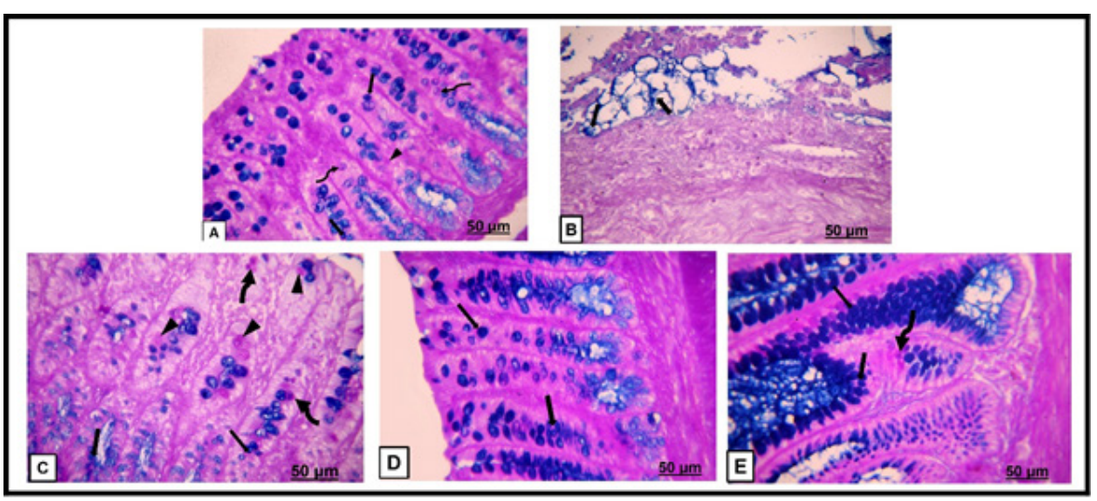

Fig. 4: Photomicrographs of combined Alcian blue-PAS-stained sections of the distal colon in different groups. [A] control group; goblet cells with acidic mucin appear blue in the base of crypts (black arrows), and those with neutral mucin are stained magenta (▲). While cells with mixed mucin appear purple (curved arrow). [B] Acute colitis group; an apparent decrease in the number, size, and content of goblet cells is seen. A little blue mucin is seen in some distorted crypts and in the lumen (black arrows). [C] Recovery group; goblet cells in the lower half of the crypts are stained blue (black arrows) while those in the upper half appear magenta (▲) and those with neutral mucin appear purple (curved arrows). [D&E] Colitis+ BM-SCs, and Colitis + probiotics respectively; an apparent increase in the number and size of goblet cells with acidic (black arrows) and mixed mucous are seen (curved arrow). [X400 scale bar: 50 µm]

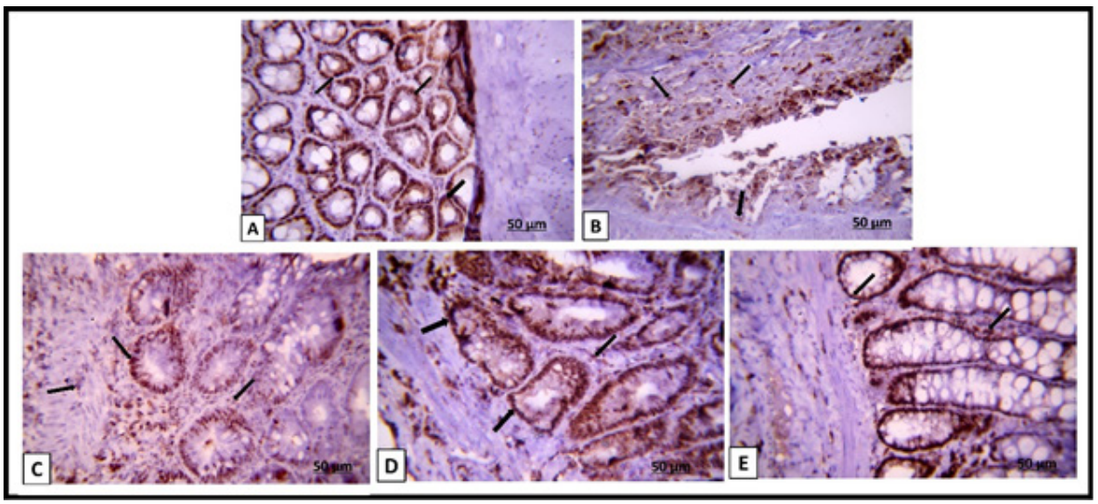

Fig. 5: Photomicrographs of PCNA-stained sections of the distal colon in different groups [A] control group; positive nuclear expression is seen in the cells lining the crypts and in a few cells in the lamina propria (↑). [B] Acute colitis group; positive nuclear expression for PCNA is seen in some cells in the deep part of the ulcerated area, and in many cells in the lamina propria (↑). [C] Recovery group; positive nuclear expression is seen in the cells lining the lower half of the crypts and many cells in the lamina propria (↑). [D&E] Colitis+ BM-SCs, and Colitis + probiotics respectively; positive nuclear expression is seen in the cells lining the crypts with minimal reaction in the lamina propria (↑). [X400 scale bar: 50 µm]

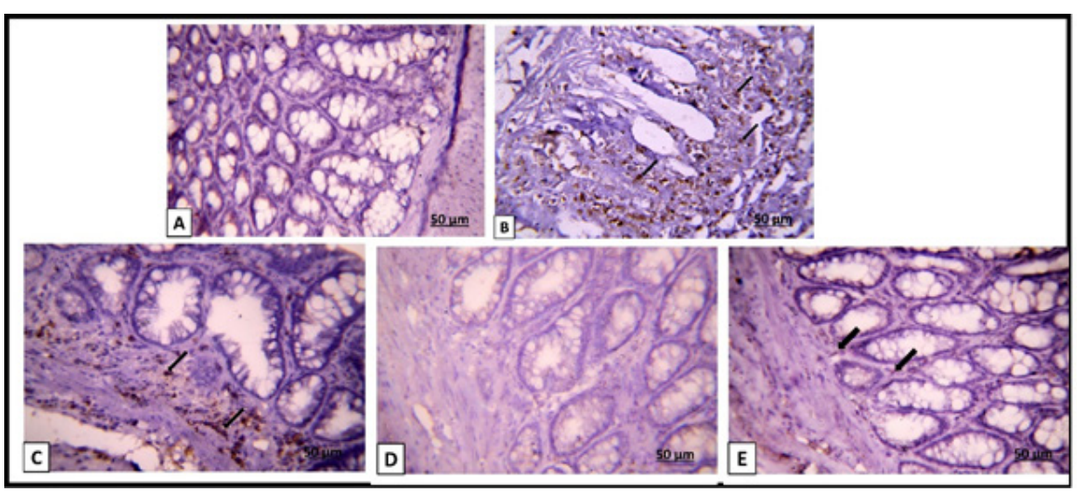

Fig. 6: Photomicrographs of TNF-α stained sections of the distal colon in different groups. [A] control group; a negative reaction is seen. [B] Acute colitis group; an apparent increase in TNF-α positive reaction is seen in the cytoplasm of some connective tissue cells (↑). [C] Recovery group; an apparently decreased expression of TNF-a is seen in the lamina propria, and submucosa (↑). [D&E] Colitis + BM-SCs, and Colitis+ probiotics respectively; the sections appear with a negative reaction for TNF-α, however, mild reaction is seen in the group treated with probiotics (↑). [X400 scale bar: 50 µm]

Table 1: Showing the mean ± SD of the acute inflammatory cell infiltrate, Crypt abscesses, mucin depletion or Deletion of goblet cells, surface epithelial integrity, and crypt architectural irregularities or crypt damage for histological scoring

Feature scored Score	Control group	Acute colitis group	Recovery group	colitis + BMSCs group	colitis + Probiotic group
Inflammatory cell infiltrate	0.0±0.0	2.8±0.55*&@^	2.0±0.71*#@^	0.2±0.45#$	0.4±0.55#$
Crypt abscesses	0.0±0.0	3.00±0.00*&@^	2.0±0.71*#@^	0.0±0.0#$	0.0±0.0#$
Mucin depletion or Deletion of goblet cells	0.0±0.0	2.6±0.55*&@^	2.2±0.45*#@^	0.2±0.45*#$	0.0±0.0#$
Surface epithelial integrity (Destruction of epithelium)	0.0±0.0	2.6±0.55*&@^	2.2±0.45*#@^	0.2±0.45#$	0.4±0.55#$
crypt architectural irregularities or crypt damage	0.0±0.0	3.8±0.45*&@^	3.0±0.71*#@^	0.8±0.84*#$	0.6±0.55#$

*Significant increase in relation to the control group; # Significant decrease in relation to the colitis group; & Significant increase in relation to the recovery group; $ Significant decrease in relation to the recovery group; @ Significant increase in relation to the colitis + BMSCs group; ^ Significant increase in relation to the colitis+ Probiotic group

Table 2: Showing the mean ± SD of the length of the crypts (µm), area % of collagen fibers, number of goblet cells, area % of alcian blue positive mucin, area % of PCNA positive nuclei, and area % of positive reaction for TNF- α in the different groups

	Control group	Colitis group	Recovery group	colitis + BMSCs group	colitis + Probiotic group
Mean length of the crypts	335.11 ±5.9	134.14 ±31.31*	260.01 ±24.78*#	321.33 ±39.05#$	329.78 ±37.98#$
Mean area % of collagen fibers	4.2 ± 0.87	10.29±2.23*	31.95±5.65*#	4.43±0.75#$	4.35±1.54#$
Mean number of goblet cells	275±42.3	10.5±3.78*	139.33±35.42*#	327.67±23.44*#$	430.83±78.51*#$@
Mean area % of Alcian blue positive mucin	32.89±5.6	4.52±1.31*	23.78±3.09*#	37.22±5.53#$	42.21±5.9*#$
Mean area % of PCNA positive nuclei	22.91±2.38	13.46±4.3*	18.57±4.47#	24.61±3.81#$	25.08±4.15#$
Mean area % of positive reaction for TNF- α	0.25±0.18	8.5±2.68*	3.24±1.15*#	0.26±0.19#$	0.59±0.11#$

Data are mean ± SD of 6 rats per group. *$P < 0.05$, vs. control group; #$P < 0.05$, vs. colitis group, $$P < 0.05$, vs recovery group, @ $P < 0.05$, vs colitis +stem cells group by One-way ANOVA with Tukey post hoc test

DISCUSSION

Inflammatory bowel disease; also known as CD and UC; is a long-lasting inflammatory condition that affects the intestinal tract (IT) followed by damage and disturbance of the mucosal barrier, immunological responses, and leukocyte recruitment. Although the exact origin of UC is uncertain, the excessive presence of inflammatory cells within the mucosa may lead to higher levels of inflammatory cytokines like TNF-α[17].

When intestinal epithelial cells are damaged by UC, endogenous and exogenous antigens are produced and transported through the portal circulation to promote the immune system to cause mucosal inflammation[18]. It is believed that the intestinal epithelium absorbs more antigens in the lumen because of epithelial barrier abnormalities[19].

In rats with acetic acid ulcerative colitis, probiotics have been demonstrated to alter intestinal immunity by producing short-chain fatty acids (SCFAs). The pH of the colon was lowered by SCFAs, which also stopped the growth of potentially harmful microbes. This will lead to a reduction in the production of pro-inflammatory cytokines (e.g., TNF, INF, and IL-8). Also, there was an increase in the production and secretion of anti-inflammatory cytokines (e.g., I-10 and TGFβ)[10,11].

There are many treatment options for UC, but they may have side effects and an increased risk of treatment resistance. In addition, these treatment options may not be well tolerated and stopped by patients[20]. So, in this work, we evaluated the effect of bone marrow-derived stem cells (BM-MSCs) versus probiotics on the treatment of colitis in a rat model.

In this work, the distal colon of rats of the colitis group showed loss of the colonic mucosal architecture with the presence of large ulcers. In the area of the lesion, there was a complete loss of the colonic crypts together with the presence of large areas of mononuclear inflammatory cells covering the heavily inflamed submucosa. The site of the ulcer was partially covered by flattened epithelium. Inflammatory cells were seen in the lamina propria, muscularis mucosa, and submucosa. Congestion and dilatation of the blood vessels were present in the lesion. The muscularis mucosa was seen thickened and infiltrated with inflammatory cells. Smooth muscle fibers of muscularis externa showed some smooth muscle cells with pyknotic nuclei. These findings match many previous studies[2,21,22]. Catinean et.al.[23] described similar results as they stated that rats with UC showed abnormalities in the mucosal architecture such as intestinal crypts distortion, shortening, and lowered density with depletion of the goblet cells. They also confirmed the presence of severe active inflammation.

When the rats were left for spontaneous recovery, incomplete recovery of the colonic mucosal architecture occurred. Moreover, the appearance of a cyst-like structure was noticed in the mucosa; some of them contained desquamated epithelial tissue and goblet cells. Williams et.al[24] reported atypical mucosal repair and crypt hyperplasia during the recovery phase.

In this work, rats treated with stem cells showed restoration of the histological architecture of the four layers of the colon. These results match with that of Zheng and Wang[1] who confirmed the presence of a large number of stem cells in the IT, compared to the acute colitis (AC) rat model which was done using trinitrobenzene sulfonic acid. They attributed the marked improvement caused by stem cells to the regulation of inflammatory cytokine production. The migration of MSCs to the sites of injury to replace the diseased tissues is not the only cause of their therapeutic effect but, it appears that they promote and regulate the function of immune cells like T cells, B cells, natural killer cells, dendritic cells, and macrophages during tissue repair[26]. Wang et al[27] attributed the clinical and histological improvement by MSCs in the mice model of UC to the reduction in oxidative stress. Markovic et al.,[28] also demonstrated that there was a reduction of colitis severity in a dextran sulfate sodium model of AC treated with MSCs injection. They attributed the improvement due to the formation of aggregates consisting of macrophages, B and T cells, and immunomodulatory molecules eg. IL-10, TGF-β, heme oxygenase-1, and arginase type, II in the peritoneal cavity. These aggregates improved intestinal inflammation.

In this work, the rats treated with probiotics showed nearly normal histological features, but some inflammatory cells were seen in the lamina propria with an apparent increase in intraepithelial lymphocytes. Similar findings were also reported by Gāliņa et al[29] who reported that the number of intraepithelial lymphocytes (IELs) increased by probiotics. IELs are a part of the mucosal immune system as they regulate epithelial cell turnover by destroying the infected cells and producing a keratinocyte growth factor that stimulates cell proliferation[29]. Dong et al.[18] showed the cytoprotective and anti-ulcer effects of probiotics by upregulating the expressions of Wnt ligands in the mucosa; resulting in cellular proliferation[18,30]. the potential protective mechanism of probiotics due to restoring of balance between pro- and anti-inflammatory cytokines during the development of UC[31].

The recovery group when stained by Masson's trichrome showed a significant increase in collagen fibers in comparison to the other groups indicating healing by fibrosis. A significant decrease was present in the groups treated with stem cells or probiotics compared to acute colitis (AC) and recovery groups. This indicated that the healing occurs by regeneration rather than fibrosis. Shaikh-Omar et.al[32] studied trinitrobenzene sulfonic (TNBS) acid-induced chronic colitis in rats and revealed that Masson's trichrome staining showed some large nodules in the mucosa-submucosa.

In this work, coupled Alcian blue-PAS-stain was used to examine mucin content in the goblet cells and their kinds. Acidic mucins (transmembrane mucous) were closely associated with the epithelial surface in the control group, with a few cells carrying neutral and mixed mucins. Acidic mucins act as barriers for bacteria and signaling molecules. The colonic epithelium's neutral mucins were immature. As a result, the histochemical study of mucins may be a helpful signal of damage to the colonic epithelium[33]. Combined Alcian blue-PAS-stained sections of the acute colitis group revealed loss of all types of goblet cells except for the presence of some acidic mucin in some distracted crypts and in the lumen. Similarly, Elbastawisy and Mohamed[21] confirmed the lowered number of goblet cells through the usage of Alcian blue in their study. The sections of the recovery group showed that most goblet cells in the lower half of crypts were alcian blue positive while those in the upper half were mostly (neutral mucin) PAS positive. With the treatment of colitis using stem cells or probiotics, a significant rise in the number, size, and density of goblet cells was noticed. However, a significant rise in the mean number of goblet cells in the rats treated with probiotics was noticed when compared to the rats treated with stem cells. Similar findings were observed by Gāliņa et al[29] who observed the increase in the number of goblet cells in the large intestine of piglets treated with probiotics. Also, they noted the influence of probiotics on the differentiation of goblet cells by rising the production of acid mucin in the IT. Acid mucin acts as a defense mechanism against bacteria.

Sharma et al[34] suggested that the maintenance of intestinal integrity depends on the modulation of intestinal epithelial cell proliferation, so PCNA immunohistochemical stain was used in this study to assess intestinal integrity. Examination of immuno-histochemical stained sections for PCNA in the acute colitis group showed positive nuclear expression for PCNA in some cells in the deep part of the ulcerated area and many cells in the lamina propria. This positive nuclear expression appeared to be related to inflammatory cells. The sections of the recovery group showed positive nuclear expression in the cells lining the lower half of the crypts and many cells in the lamina propria. Rats treated with stem cells and probiotics showed positive nuclear expression in the cells lining the crypts indicating restoration of the proliferative capacity of stem cells (crypt base stem cells). These results were confirmed by statistical analysis. This was agreed with previous reports[34,35,36].

In this study, the treatment with stem cells resulted in a significant increase in PCNA expression. A similar finding was also confirmed by other studies[37,38] that explained that the rise in the PCNA expression was due to the replacement of the damaged epithelial cells by the dividing stem cells and the intense ability of stem cells to induce epithelial cell proliferation. The increase in PCNA expression suggests its role in the homeostasis of epithelial cells and the improvement of colon damage[34].

Interleukin (IL) 1β, IL-6, IL17A, and IL-21, macrophage inflammatory protein (MIP)-TNF-α, and ROS are chemokines and cytokines secretions of UC[39]. In the present work, TNF-α immunohistochemical staining was used to assess the occurrence and severity of colitis.

The acute colitis group showed a positive reaction in some connective tissue cells. The recovery group showed decreased expression in the lamina propria, and submucosa as compared to the acute colitis group. Treatment with stem cells showed a negative reaction for TNF-α, however, there was a mild reaction in the rats treated with probiotics. These findings agree with the previous studies that revealed a rise of colonic[34] and serum[23] TNF- α with colitis. Catinean et.al.[23] reported that TNF-α levels in probiotics-treated rats were significantly decreased.

CONCLUSION

Both BMMSCs and probiotics improved the healing process of colitis, restored the integrity of the intestinal mucosa, decreased collagen fibers, reduced inflammation as well as increased the density of mucin secretion by goblet cells. Probiotics therapy could have better effects than stem cell therapy as evidenced by increasing the number of goblet cells acid mucin (barrier for bacteria) as well as intraepithelial lymphocytes which are considered the first line of defense and maintain the barrier and help the regeneration.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

تأثير خلايا النسيج الاوسط الجذعية المشتقة من نخاع العظم مقابل البروبيوتيك في علاج التهاب القولون الحاد المستحث تجريبياً في الجرذان: دراسة نسيجية وكيميائية مناعية

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الخلفية: يعد مرض التهاب الأمعاء حالة التهابية تقدمية في الجهاز الهضمي . تتمتع خلايا النسيج الاوسط الجذعية بقدرات تعديل مناعي . تعمل البروبيوتيك على تعديل الفسيولوجيه المرضية لاضطرابات الأمعاء ولها تأثيرات إيجابية على ميكروبيوتا الأمعاء.

الهدف من الدراسه: تقييم خلايا النسيج الاوسط الجذعية مقابل البروبيوتيك فى علاج التهاب القولون الحاد في ذكور الجرذان البيضاء.

المواد والطرق: تم تقسيم أربعين من ذكور الجرذان البالغة يتراوح وزنها بين (٢٠٠-٢٢٠ جم) بالتساوي إلى خمس مجموعات. المجموعة الأولى (االضابطه). المجموعة الثانية (التهاب القولون الحاد). المجموعة الثالثة (التعافى): تمت التضحية بها بعد ١٤ يومًا من التهاب القولون الحاد. المجموعة الرابعة (التهاب القولون + الخلايا الجذعية): تم حقنها مرة واحدة بواحد ملى يحتوى على ١ × ١٠٦ من الخلايا الجذعية. المجموعة الخامسة (التهاب القولون + البروبيوتيك): تلقت ١x١٠٩ CFU / مل / ١٠٠ جم من وزن الجسم يوميا من اكتوباكيللوس بلانتاروم عن طريق الفم. في المجموعتين الرابعة والخامسة ، تمت معالجه الفئران بعد ثلاثة أيام من التهاب القولون الحاد وتم التضحية بها بعد ١٤ يومًا.

النتائج: أظهرت مجموعة التهاب القولون الحاد تقرحًا في الغشاء المخاطي ، وارتشاحًا خلويًا ، وانخفاض عدد الخلايا الكأسية. أظهرت النسبة المئوية للمساحة من صبغة الكولاجين و TNF-α المناعية زيادة محتمله بينما انخفضت مساحه صبغة النوى المناعية الموجبة PCNA بشكل ملحوظ. أظهرت مجموعة التعافى تحسن غير كامل لهذه التغييرات الهيكلية. تمت استعادة البنية النسيجية للقولون بعد العلاج بالخلايا الجذعية والبروبيوتيك.

الخلاصة: العلاج بالخلايا الجذعية أو البروبيوتيك أعاد سلامة الغشاء المخاطي المعوي في نموذج الفئران لالتهاب القولون الحاد. كان العلاج بالبروبيوتيك متفوقًا على العلاج بالخلايا الجذعية كما يتضح من التكاثر والتمايزالسريع للخلايا الجذعية إلى خلايا كؤوس تعمل كحواجز.