Original Article

Assessment of the Prophylactic Effect of Probiotics in an Experimental Model of Ulcerative Colitis in Male Albino Rat: Histological and Immunohistochemical Study

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ABSTRACT

Introduction: The use of probiotics supplements is becoming increasingly important for the prevention and treatment of gastrointestinal illness. They play a role in maintaining homeostasis in the gut microbial ecosystem by regulating pro-inflammatory and anti-inflammatory cytokines.

Aim of the Study: To assess the effect of using probiotics in an acetic acid induced colitis. This study might shed light on the effect of probiotics when used alone after induction of colitis without any conventional treatment.

Material and Methods: Twenty four adult male albino rats were divided randomly into four groups: group I (control group), group II (Ulcerative colitis group) received 2 ml of 3% acetic acid by intra-colonic injection, in group III colitis was induced then after 24 hrs rats received probiotics for 7 days by oral gavage while rats of group IV received probiotics by oral gavage for 14 days prior to induction of colitis and 7 days thereafter. The colonic specimens were stained with H&E, alcian blue and immunohistochemical staining of α-SMA. Biochemical analysis for the detection of TNF- α, IL-17 and IL-10. Morphometric measurement and statistical analysis were done.

Results: The intake of probiotics reversed the massive damage of the colonic wall architecture induced by acetic acid. Administration of probiotics before induction of colitis resulted in significant decrease in the weight loss of rats and reduction of inflammation as evident by restoration of normal colonic wall architecture in H&E stained sections. Also restoration of number of goblet cells secretion in alcian blue stained sections, besides continuity of muscularis mucosa and reappearance of myofibroblasts as evident by positive α-sma immunostaining.

Conclusion: Using probiotics especially before and after the induction of colitis is more protective than its use after the induction of colitis only.

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Key Words: Acetic acid, probiotics, ulcerative colitis.

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INTRODUCTION

Ulcerative colitis (UC), which is one of the inflammatory bowel diseases (IBD), is a chronic lifelong condition that has a relapsing and remitting course. It's an immune-mediated inflammation of the rectum and extends proximally to affect a variable length of the colon. Except in the most severe cases, UC is characterized by persistent inflammation with superficial ulcerations primarily localized to the large intestine mucosa and submucosa[1].

The exact etiology of UC is still unknown; it is thought to be a multifactorial process. It involves genetic susceptibility factors, environmental factors and exaggerated host immune response[2]. Changes in the composition, amount, and diversity of the microbiota are indicative of altered mucosal barrier structure and function, as well as an imbalance of the intestinal microbiota[3]. There is strong evidence that normal microbiota in the GIT, such as Lactobacillus and Bifidobacteria species, is significantly reduced in UC patients[4,5].

UC patients have a reduced mucus layer thickness, impaired continuity, and a lower number of goblet cells, among other alterations in the mucus barrier[6].

Tumor Necrosis Factor-α (TNF-α) levels in the intestinal mucosa, serum, and faeces of UC patients have been found to be higher in clinical investigations. TNF-α- is known to cause apoptosis and an inflammatory response in intestinal epithelial cells, and it could have a role in the onset and progression of intestinal barrier abnormalities[7].

Due to their pro-inflammatory involvement in the mucosal immune response, a new distinct subset of T helper 17 (Th17) cells produced primarily by activated T cells and capable of generating IL-17 (also known as IL-17A) has recently been linked to IBD pathogenesis[8,9].

Probiotics are "live microorganisms that, when provided in adequate amounts, impart a health benefit on the host," according to the World Health Organization and the Food and Agriculture Organization of the United Nations[10,11].

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Probiotic bacteria can be found in a wide range of foods and supplements. Fermented dairy products and fortified foods contain the majority of live probiotics. From yoghurt to morning cereals, these bacteria are added to a variety of meals and beverages. There are also freeze-dried probiotic tablets, capsules, powders, and sachets available[12].

Strains of the genera Bifidobacterium (bifidum, longum adolescentis, animalis, and breve) and Lactobacillus (acidophilus, casei, fermentum, gasseri, johnsonii, paracasei, Plantarum, rhamnosus, and salivarius) are the most often studied and utilized bacteria as probiotics[13,14]. These bacteria are part of the gastrointestinal microflora and are especially useful in the digestion of dairy products[15].

The repair of the microbiota, enhanced adherence to the intestinal mucosa, and concomitant suppression of pathogen adhesion are all attributed to probiotics' positive effects on GI function. The competitive exclusion of pathogenic microorganisms, production of antimicrobial substances, and increased synthesis of protective intestinal mucus could also be recorded. One of the most positive effects is the improvement of the immune function barrier and the reduction of inflammatory cytokines[16].

AIM OF THE WORK

This study aimed to assess the effect of using probiotics (including the following strains L. acidophilus, B. bifidum, and B. longum) in acetic acid-induced colitis in adult male albino rats. This study could shed light on the effect of probiotics when used alone after induction of colitis without any conventional treatment.

MATERIAL AND METHODS

Drugs

Acetic acid: Prepared in the Biochemistry Department, Faculty of Medicine, Cairo University, by adding 3 ml of acetic acid to 97 ml of distilled water "3 % concentration" and mixed thoroughly.

Probiotic capsule: contains (Lactobacillus acidophilus, Bifidobacterium longum, and Bifidobacterium bifidum), 1.5 billion cells (RITE AID pharmacy, USA).

Animals

Twenty-four adult male albino rats of similar age (12 weeks) and weight (180-200 gm) were used. They were housed in hygienic stainless steel cages and kept in a clean well-ventilated room. They were fed a standard chow diet and allowed free access to water. The study was conducted at the Animal House of Kasr-Al Aini Faculty of Medicine, according to the ethical guidelines for the care and use of laboratory animals.

Experimental design

All rats were randomly divided into four groups

Group I (Control Group): included 6 rats. The control subgroups were designed in correspondence to the experimental groups. They received the solvent of the drugs by the same route of administration for the same period. Control rats were divided equally into 3 subgroups 2 rats each:

- Subgroup Ia: Rats received 2 ml of distilled water by intracolonic instillation & were sacrificed 7 days after.
- Subgroup Ib: Rats received 2 ml of distilled water by intracolonic instillation then after 24 hours rats received 2ml of distilled water by oral gavage once daily (just before meal) for 7 days then sacrificed after.
- Subgroup Ic: Rats received 2 ml of distilled water by oral gavage once daily (just before meal) for 14 days before intracolonic instillation of 2 ml of distilled water and 7 days thereafter then sacrificed.

Group II (Ulcerative Colitis Group): It included 6 rats that received 2 ml of 3% acetic acid by a single intra-colonic injection for induction of colitis.

Group III: It included 6 rats. Colitis was induced in rats as in group II then after 24 hrs, rats received 0.145 gm of probiotic dissolved in 2 ml distilled water (just before meal) for 7 days by oral gavage.

Group IV: It included 6 rats that received 0.145 gm of probiotic dissolved in 2 ml distilled water by oral gavage once daily (just before meal) for 14 days before induction of colitis and 7 days thereafter. Colitis was induced in rats as in group II[17].

All rats of all experimental groups were sacrificed 7 days after induction of colitis.

Measurement of the Body Weight (in gm.) of each rat: Baseline body weight was recorded for each rat at the beginning of the experiment for all the studied groups and the end of each experimental period. Statistical analysis was done for this measurement.

Oral Feedings

The capsule powder has 1.5 ×109 CFU per 0.2179 gm. Oral feedings required a 1 ×109 CFU dose, which contained 0.145 gm of probiotic[18].

Induction of Colitis

Before the induction of colitis, the animals were fasted overnight and given free access to water. Xylazine (10mg/kg IP) & ketamine (90mg/kg IP) was injected into each rat to sedate it. Each animal was placed in the Trendelenburg position, 2 ml of acetic acid was infused through a soft pediatric catheter 8 cm proximal to the anal verge. The rats were retained in the Trendelenburg position for 30 seconds to prevent leakage of acetic acid[19].

Sample Collection and Processing

The rats (fasting overnight) were sacrificed by an overdose of pentobarbital (300 mg/kg IP). Laparotomy was performed and the terminal 6 cm of the colon, approximately 2 cm above the anal verge, was dissected. The dissected

colonic segment was divided into 2 portions. The proximal 3 cm was stored at −80°C for further biochemical analysis and the distal 3 cm was used for histological examination.

Colonic Specimens were Processed for the Light Microscopic Study: the colonic specimens were fixed in formaldehyde (10%), embedded in paraffin at Histology Department, Faculty of Medicine, Cairo University. Paraffin blocks were cut at 5 - 7 µm thickness and subjected to the following stains:

1. Hematoxylin and Eosin (H&E)
2. Alcian Blue stain: To detect acidic mucopolysaccharides[20].
3. Immunohistochemical staining for the detection of alpha-smooth muscle actin (α-SMA) for the detection of the subepithelial (pericryptal) myofibroblasts, muscularis mucosa, and muscularis externa.

Colonic Specimens were Processed for Biochemical Analysis: The colonic specimens were stored at −80°C and were analyzed for studying the expression of Tumor necrosis factor-alpha (TNF-α), Interleukin 17 (IL-17A), and interleukin 10 (IL-10). This was done at the Biochemistry Department, Faculty of Medicine, Cairo University. Statistical analysis was done for these measurements.

Morphometric Study

Data were obtained using Leica Qwin 500 LTD image analyzer computer system (Cambridge, UK), at Histology Department, Faculty of Medicine, Cairo University. The following parameters were measured:

1. Mean submucosal thickness in H&E-stained sections: the distance from the end of muscularis mucosa to the beginning of muscularis externa
2. Mean area % of Alcian blue reaction in alcian blue-stained sections
3. Mean area % of α-SMA immunoreaction of the pericrpytal myofibroblast in α-SMA stained sections

Statistical Analysis

Data were tabulated and statistically analyzed to evaluate the difference between the groups under study as regards the various parameters. Correlations between the important researched parameters were assessed. The arithmetic mean, standard deviation, and analysis of variance were all used in the statistical study (ANOVA). Using SPSS (Statistical Package for Social Sciences), the computer instantly supplied the probability *p-value* determined from statistical tables. Windows Version 20, Chicago, USA[21].

RESULTS

General Observations and Mortality Rate

General health status of rats was observed daily throughout the experiment. Changes in the body weight, diarrhea and rectal bleeding were monitored on daily basis. The rats of group II shows poor general condition with diarrhea and rectal bleeding. Rats of group III and IV show improvement of general condition with fewer diarrheas and rectal bleeding. Mortality rate was reported as two rats in the ulcerative colitis group which were excluded from the study and replaced.

Body Weight Measurements

The mean value of the bodyweight measurement of group I (control group) was (187.68 ± 4.02 gm). There was a statistically significant decrease ($p<0.05$) in the mean value of the body weight in group II (ulcerative colitis group) to reach (106.56 ±1.78 gm) as compared to group I, group III (164.06 ± 2.25 gm), and Group IV (183.68 ± 4.51 gm). The mean value reported for group III showed a statistically significant decrease ($p<0.05$), as compared to group I and group IV. The mean value reported for group IV showed a statistically significant increase ($p<0.05$), as compared to group II and group III. However, it was statistically not significant as compared to group I (Histogram 1).

Histological Results

H&E Stained Sections

Histological examination of Group I (control group) revealed similar histological structure between Subgroup Ia, Subgroup Ib and Subgroup Ic. There were no changes noticed between all its subgroups. The examination showed normal colonic wall architecture. The mucosa was lined by continuous surface epithelium. The lamina propria was occupied by crypts of Lieberkühn which extend through the full thickness of the mucosa. The submucosa was formed of loose connective tissue containing blood vessels and sparse inflammatory cells. The muscularis externa was detected. The colon was covered externally by serosa (Figure 1A,B).

Sections in Group II (Ulcerative Colitis Group) showed disrupted colonic wall architecture. Distortion of the mucosa, in the form of erosions and desquamated surface epithelium, were seen. The lamina propria was occupied by irregularly distorted crypts, in some areas crypts were dilated with degenerated colonocytes while other areas showed patchy loss of a part of the crypt. Muscularis mucosa was interrupted in many areas with the separation of muscle fibers. Widening of the submucosa with extensive inflammatory cellular infiltration & dilated blood capillaries were observed. The muscularis externa was seen intact (Figure 2A,B).

Examination of colonic sections in Group III revealed partial restoration of the colonic wall architecture. The mucosa showed partial restoration of cryptal architecture, some crypts were uniform while others were slightly dilated. Partial restoration of the goblet cells lining the crypts was observed. Uniform & continuous muscularis mucosa was noted. A slight widening of submucosal layer thickness was observed. Some Inflammatory cellular infiltration of the submucosa and lamina propria was

observed. However, dilated blood capillaries were still found (Figure 3A,B).

Examination of colonic sections in Group IV revealed marked restoration of the colonic wall architecture. The mucosa showed intact surface epithelium which was lined by columnar and goblet cells. The crypts were regularly arranged, closely packed, parallel to each other, and extend from the luminal surface to the muscularis mucosa which was intact. The crypts were lined by numerous vacuolated goblet cells. Minimal inflammatory cells in lamina propria and submucosa with apparently normal thickness were observed (Figure 4A,B).

II. Alcian Blue Stained Sections

Colonic sections in Group I (control group) showed a widespread cytoplasmic alcian blue reaction in the numerous goblet cells lining the crypts. While in Group II (ulcerative colitis group), colonic sections showed a slight alcian blue reaction of few goblet cells lining the distorted crypts. Other crypts show a complete absence of goblets cells. Colonic sections in Group III showed a considerable distribution of alcian blue reaction in the goblet cells lining the partially restored crypts. While there is a slight reaction in few goblet cells lining the distorted crypts. Examination of Group IV sections showed a wide distribution of alcian blue reaction in the goblet cells lining the almost well-formed crypts (Figure 5A,B,C,D).

III. Alpha Smooth Muscle Actin Stained Sections

Examination of colonic sections in Group I (Control Group) showed positive α-SMA immunostaining of the muscularis mucosa and the muscularis externa (Figure 6A). Positive α-SMA immunostaining for pericryptal myofibroblast sheath closely embracing the normal intestinal crypts (Figure 7A). Group II (Ulcerative Colitis Group) sections showed interrupted α-SMA immunostaining of the muscularis mucosa with intact muscularis externa (Figure 6B). Positive α-SMA immunostaining of the pericryptal myofibroblasts remnants was detected at the base of the epithelium in few regions. Many other crypts regions were negative for α-SMA of the degenerated pericryptal myofibroblast (Figure 7B). Colonic sections in Group III showed positive α-SMA immunostaining of the intact muscularis mucosa and muscularis externa (Figure 6C). Positive α-SMA immunostaining of the pericryptal myofibroblasts was detected in many regions. A few crypts were negative for α-SMA immunostaining of myofibroblasts (Figure 7C). Examination of Group IV Colonic sections showed positive α-SMA immunostaining of the intact muscularis mucosa and muscularis externa (Figure 6D), and positive α-SMA immunostaining of regular pericryptal myofibroblasts (Figure 7D).

Quantitative Morphometric Results

Descriptive statistics of mean submucosal thickness in μm (± SD) in H&E stained colonic sections of all the studied groups: (Histogram 2)

The mean value reported for group I (control group) was (41.48 ± 9.61). There was a statistically significant increase ($p<0.05$) in the mean value in group II (ulcerative colitis group) to reach (129.26 ± 17.33) as compared to group I, group III (68.79 ± 11.36), and Group IV (53.24 ± 7.99). The mean value reported for group III was statistically significantly increased ($p<0.05$), as compared to group I and group IV. The mean value reported for group IV was statistically significantly decreased ($p<0.05$), as compared to group II and group III. However, it was statistically not significant as compared to group I.

Descriptive statistics of mean area % of alcian blue reaction (± SD) in alcian blue stained colonic sections of all the studied groups: (Histogram 3)

The mean value reported for group I (control group) was (36.42 ± 2.07). There was a statistically significant decrease ($p<0.05$) in the mean value in group II (ulcerative colitis group) to reach (5.83 ± 1.55) as compared to group I, group III (20.26 ± 3.53), and Group IV (24.62 ± 3.95). The mean value reported for group III was statistically significantly decreased ($p<0.05$), as compared to group I and group IV. The mean value reported for group IV was statistically significantly increased ($p<0.05$), as compared to group II and group III. However, it showed a statistically significant decrease ($p<0.05$), as compared to group I.

Descriptive statistics of mean area % of α -SMA immunoreaction (± SD) in α -SMA stained colonic sections of all the studied groups: (Histogram 4)

The mean value reported for group I (control group) was (12.61 ± 1.70). There was a statistically significant decrease ($p<0.05$) in the mean value in group II (ulcerative colitis group) to reach (5.88 ± 1.22) as compared to group I and group IV (10.36 ± 2.30). However, it was statistically not significant as compared to group III (7.92 ± 1.41). The mean value reported for group III showed a statistically significant decrease ($p<0.05$), as compared to group I and group IV. The mean value reported for group IV showed a statistically significant increase ($p<0.05$), as compared to group II and group III. However, it showed a statistically significant decrease ($p<0.05$) as compared to group I.

Results of Biochemical Analysis (Table 1)

TNF-α results: The mean value of TNF-α-α of group I (control group) was (37.15 ± 0.56). There was a statistically significant increase ($p<0.05$) in the mean value of TNF-α in group II (ulcerative colitis group) to reach (121.72 ± 1.0) as compared to the control group I, group III (70.02 ± 0.99), and Group IV (48.46 ± 0.81). The mean value of TNF-α reported for group III showed a statistically significant increase ($p<0.05$), as compared to group I and group IV. The mean value of TNF-α reported for group IV showed a statistically significant decrease ($p<0.05$), as compared to group II and group III. However, it showed a statistically significant increase ($p<0.05$) as compared to group I.

IL-17 results

The mean value of IL-17 reported for group I (control group) was (47.93 ± 0.72). There was a statistically

significant increase ($p<0.05$) in the mean value of IL-17 in group II (ulcerative colitis group) to reach (139.27 ± 1.0) as compared to group I, group III (61.47 ± 0.96), and Group IV (53.48 ± 0.67). The mean value of IL-17 reported for group III showed a statistically significant increase ($p<0.05$), as compared to group I and group IV. The mean value reported for IL-17 reported for group IV showed a statistically significant decrease ($p<0.05$), as compared to group II and group III. However, it showed a statistically significant increase ($p<0.05$) as compared to group I.

IL-10 results

The mean value of IL-10 reported for group I (control group) was (128.60 ± 0.9). There was a statistically significant decrease ($p<0.05$) in the mean value of IL-10 in group II (ulcerative colitis group) to reach (43.65 ± 0.88) as compared to group I, group III (97.36 ± 0.83), and Group IV (113.57 ± 1.1). The mean value of IL-10 reported for group III showed a statistically significant decrease ($p<0.05$), as compared to group I and group IV. The value reported for IL-10 reported for group IV showed a statistically significant increase ($p<0.05$), as compared to both group II and group III. However, it showed a statistically significant decrease ($p<0.05$) as compared to group I.

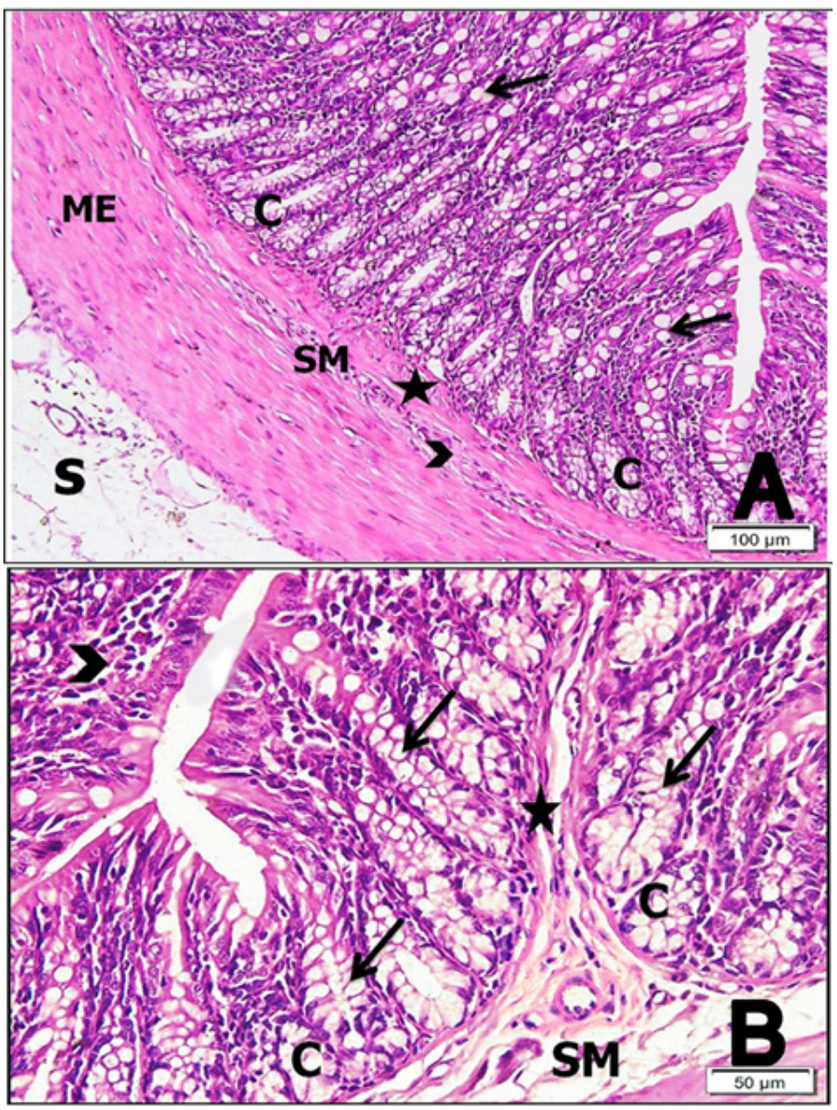


Fig. 1: Photomicrographs of a colonic section from group I (control group) showing normal colonic wall architecture formed of intact mucosa with continuous surface epithelium. The lamina propria is occupied by crypts (C) which extend through the full thickness of the mucosa. They are closely packed, parallel to each other and rest on continuous thin layer of the muscularis mucosa (star). Numerous goblet cells are detected (arrows). The submucosa (SM) is formed of loose connective tissue with sparse inflammatory cells (arrowhead). The muscularis externa is observed (ME). The colon is covered externally by serosa (S). (H&E: A × 100 & B × 200)

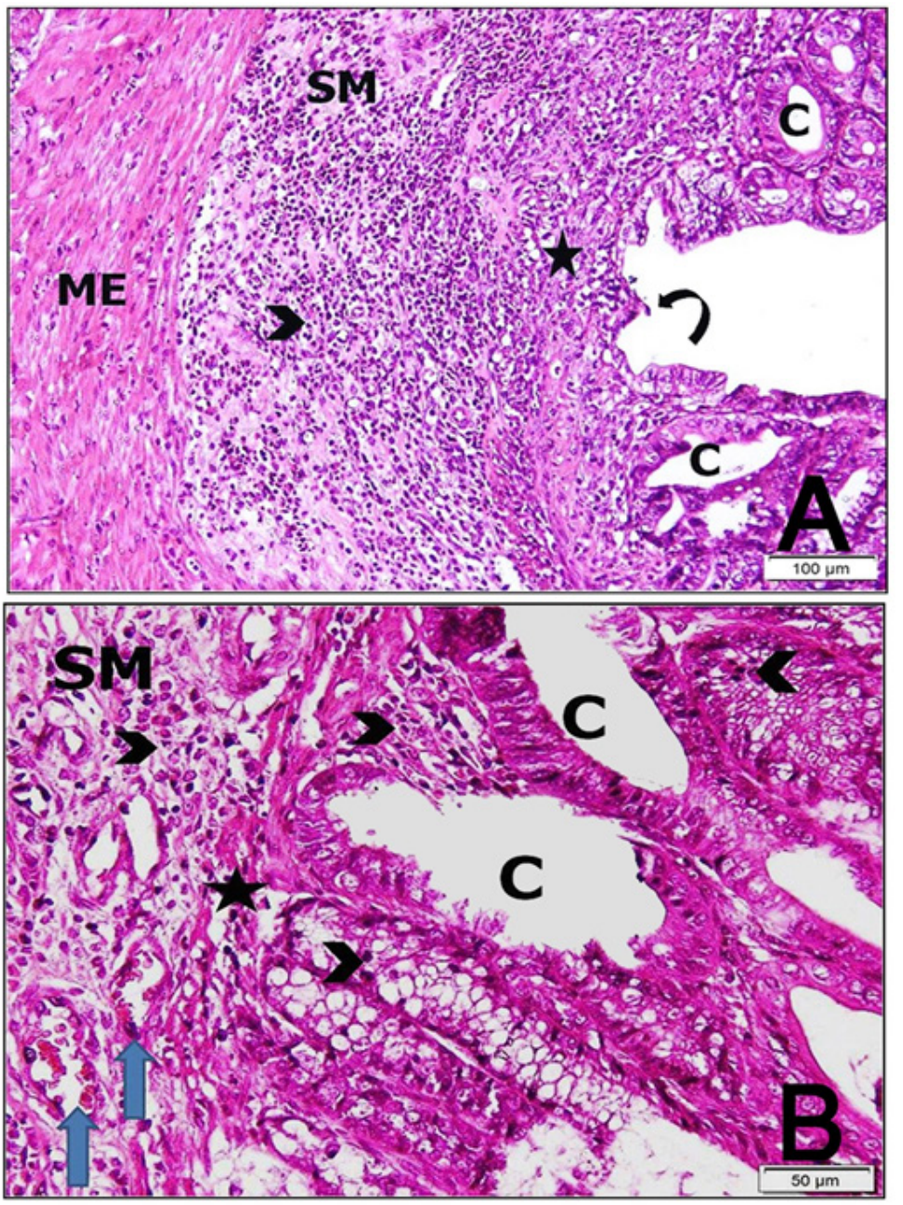


Fig. 2: Photomicrographs of a colonic section from group II (ulcerative colitis group) showing desquamated surface epithelium (curved arrow) with crypts loss in some areas. Other areas are occupied by irregular distorted crypts with degenerated colonocytes and patchy loss (C). Interrupted muscularis mucosa (star) is observed. The submucosa (SM) appears wide & occupied with extensive inflammatory cellular infiltration (arrowhead). Dilated blood capillaries (blue arrows) are demonstrated. Muscularis externa (ME) is seen intact. (H&E: A × 100 & B × 200)

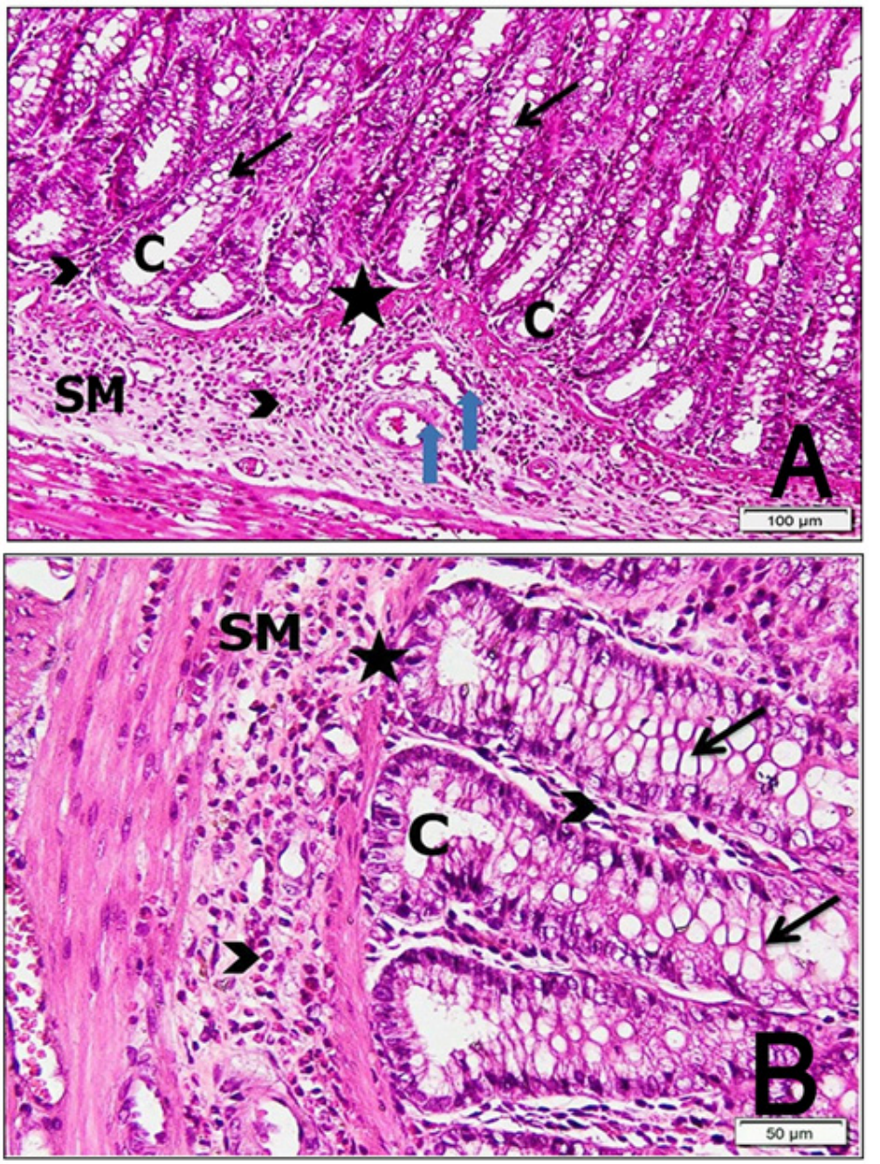


Fig. 3: Photomicrographs of a colonic section from group III showing partial restoration of cryptal (C) architecture, the crypts (C) are almost regular and parallel to each other. Some crypts were uniform while others were slightly dilated. Continuous muscularis mucosa is seen (star). Partial restoration of goblet cells (black arrows) is observed. The muscularis mucosa (star) is uniform. Slight widening of submucosal layer thickness (SM) is seen. Some inflammatory cellular infiltration is detected in the lamina propria and submucosa (arrowheads). Dilated blood capillaries are demonstrated (blue arrows). (H&E: A × 100 & B × 200)

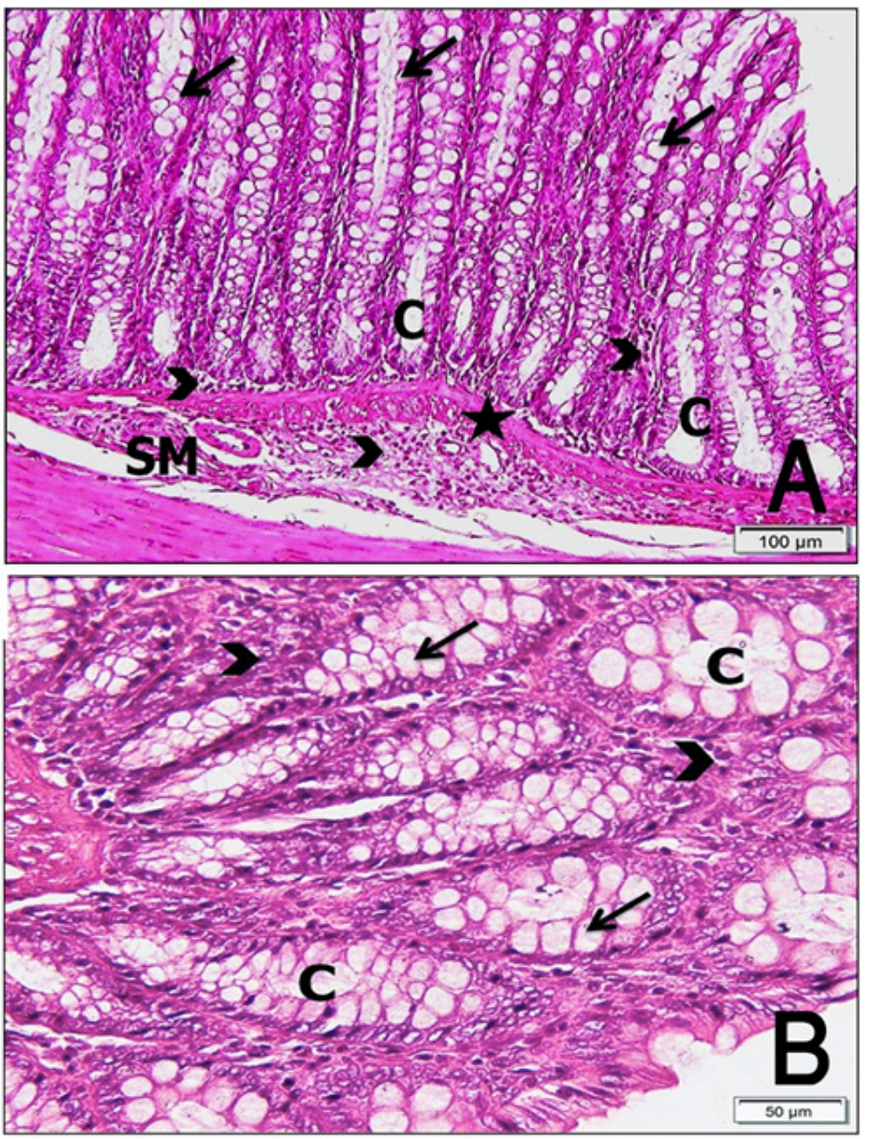


Fig. 4: Photomicrographs of a colonic section from group IV showing apparently normal colonic wall architecture with intact surface epithelium. The crypts (C) are regularly arranged, closely packed, parallel to each other and extend from the luminal surface to the muscularis mucosa (star) which is intact. Many goblet cells are observed (arrows). Minimal inflammatory cells (arrowheads) in lamina propria and submucosa (SM) are demonstrated. (H&E: A × 100 & B × 200)

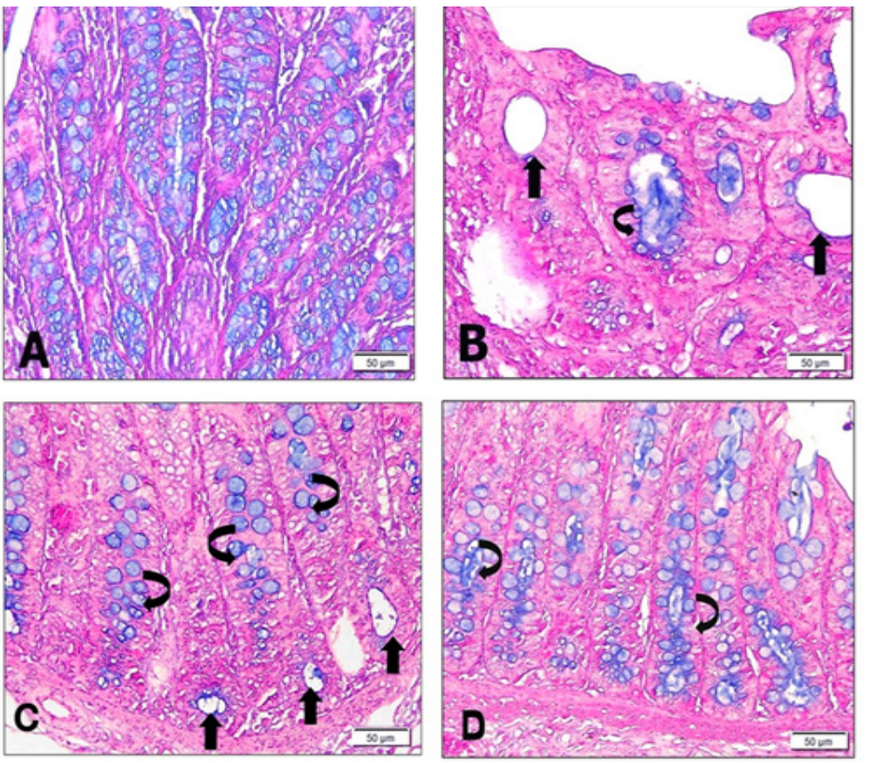


Fig. 5: Photomicrographs of colonic sections: A: Group I show a widespread cytoplasmic alcian blue reaction in the numerous goblet cells lining the crypts. B: Group II, show slight alcian blue reaction in some distorted crypts with markedly reduced goblet cells (curved arrows). Other crypts show complete absence of goblets cells (arrows). C: Group III, show considerable distribution of alcian blue reaction in the goblet cells (curved arrows) lining the partially restored crypts. While there is a slight reaction in few goblet cells (arrows) lining the distorted crypts. D: Group IV, show wide distribution of alcian blue reaction in the goblet cells (curved arrow) lining the almost well-formed crypts. (Alcian Blue × 200)

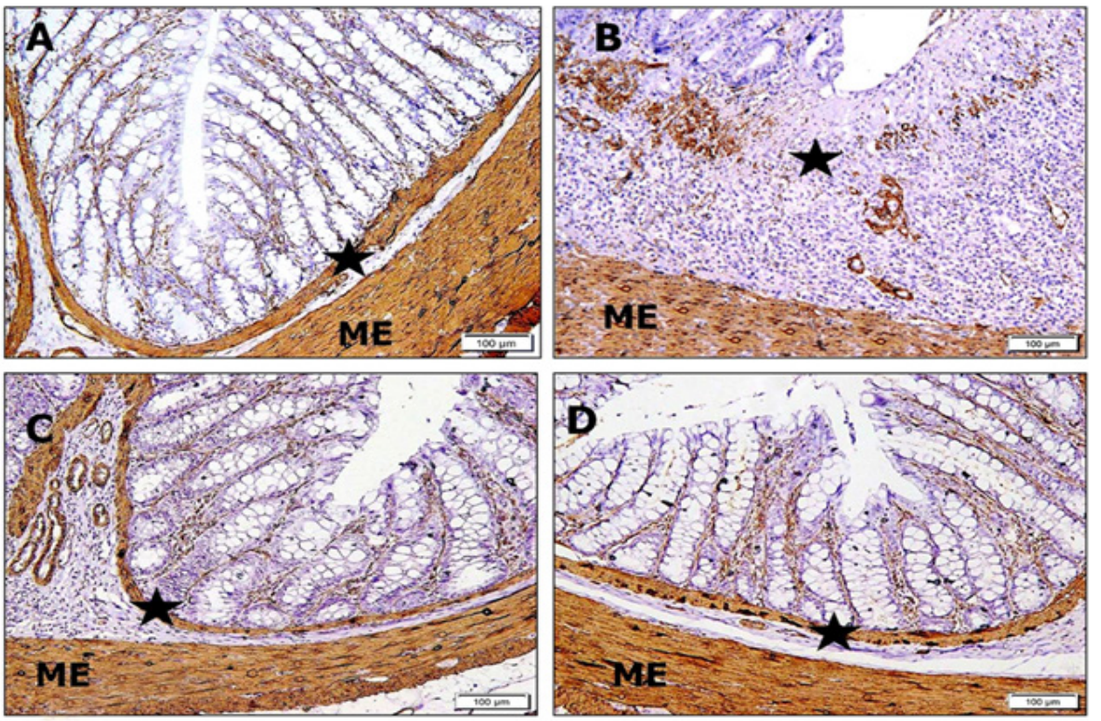


Fig. 6: Photomicrographs of colonic sections: A: Group I show positive α-SMA immunostaining of the normal thin uniform continuous muscularis mucosa (star). B: Group II shows interrupted muscularis mucosa (star). C&D: group III and IV shows positive α-SMA immunostaining of the apparently intact muscularis mucosa (star). Muscularis externa of the colon (ME) is also positively stained in all groups. (α-SMA × 100)

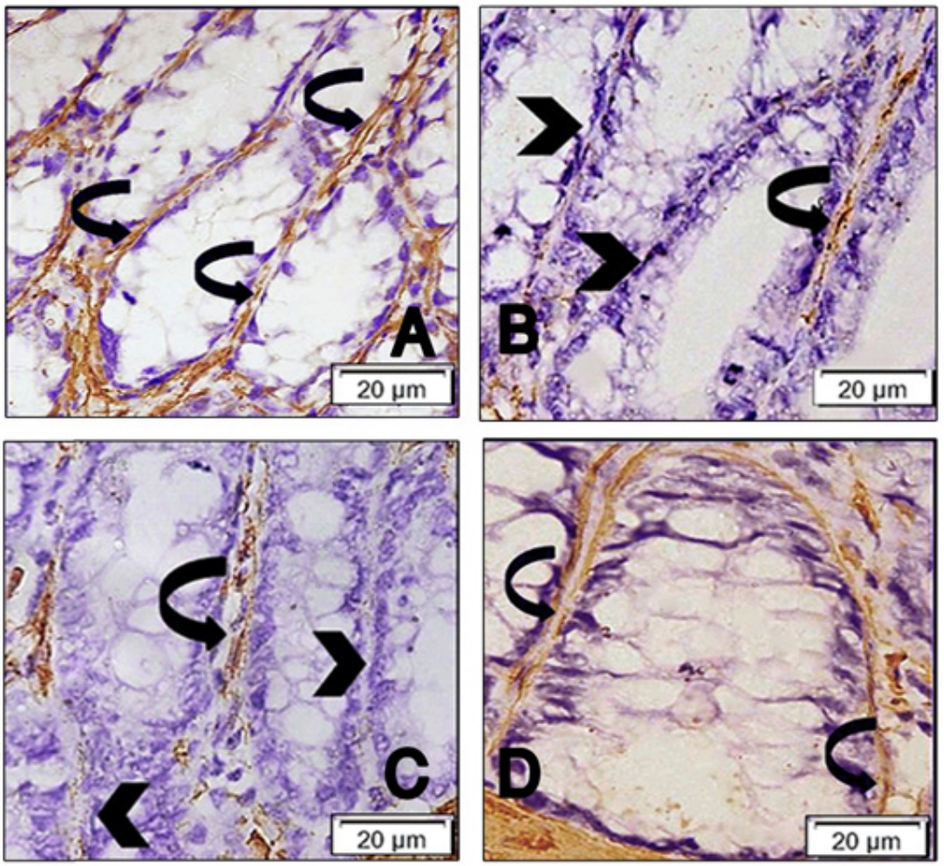
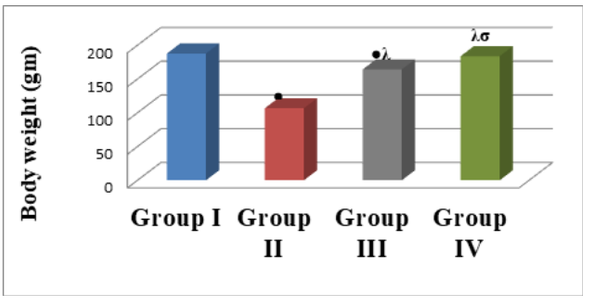


Fig. 7: Photomicrographs of colonic sections: A: Group I show positive α-SMA immunostaining of regular and thin pericryptal myofibroblasts (curved arrows) surrounding the crypts. B: Group II shows minimal positive α-SMA immunostaining of the pericryptal myofibroblasts (curved arrow) in few regions. While many other crypts regions were negative for α-SMA immunostaining (arrowheads). C: Group III shows positive α-SMA immunostaining of the partially restorted pericryptal myofibroblasts (curved arrows) in some regions. Other few crypts were negative for α-SMA myofibroblasts (arrowheads). D: Group IV show almost complete restoration of many positive α-SMA immunostaining of the pericryptal myofibroblasts (curved arrow). (α-SMA × 400)

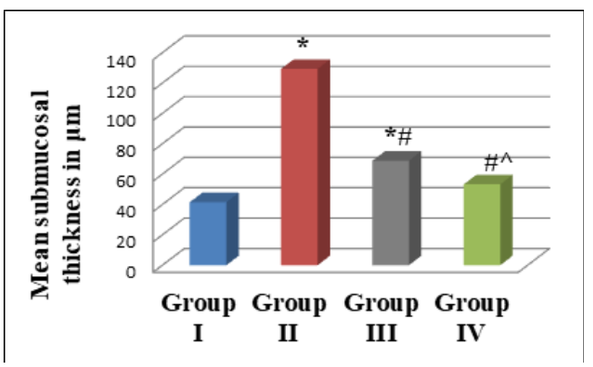
Table 1: Comparison between the mean values (± SD) of pro-inflammatory cytokines (TNF-α and IL-17) and anti-inflammatory cytokine (IL-10) in all the studied groups

	TNF-α	IL-17	IL-10
Group I	37.15 ± 0.56	47.93 ± 0.72	128.60 ± 0.98
Group II	121.72 ± 1.08*	139.27 ± 1.02*	43.65 ± 0.88$^{\bullet}$
Group III	70.02 ± 0.99$^{*\#}$	61.47 ± 0.96$^{*\#}$	97.36 ± 0.83$^{\bullet\lambda}$
Group IV	48.46 ± 0.81$^{*\#\wedge}$	53.48 ± 0.67$^{*\#\wedge}$	113.57 ± 1.12$^{\bullet\lambda\sigma}$

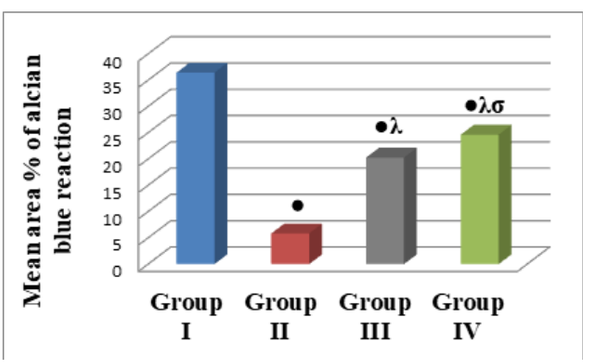
* significant increase compared to control group I .
● significant decrease compared to control group I .
significant decrease compared to group II .
λ significant increase compared to group II .
^ significant decrease compared to group III.
σ significant increase compared to group III.
p value <0.05 is statically significant.



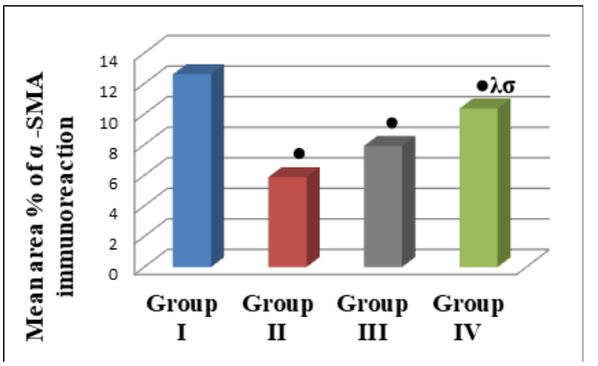
Histogram 1: Comparison between the mean values (± SD) of body weight measurement in grams in all studied groups
● significant decrease compared to control group I .
λ significant increase compared to group II .
σ significant increase compared to group III.
p value <0.05 is statically significant.



Histogram 2: Comparison between the mean submucosal thickness (± SD) in the stained colonic sections obtained from the four groups
● significant decrease compared to control group I .
λ significant increase compared to group II .
σ significant increase compared to group III.
p value <0.05 is statically significant.



Histogram 3: Comparison between the mean area % of alcian blue reaction (± SD) in the stained colonic sections obtained from the four groups
● significant decrease compared to control group I .
λ significant increase compared to group II .
σ significant increase compared to group III.
p value <0.05 is statically significant.



Histogram 4: Comparison between the mean area % of α-SMA immunoreaction (± SD) in the stained colonic sections obtained from the four groups
● significant decrease compared to control group I .
λ significant increase compared to group II .
σ significant increase compared to group III.
p value <0.05 is statically significant.

DISCUSSION

Ulcerative colitis (UC) is a chronic debilitating disease. The pathogenesis of UC is believed to involve the mucosa and submucosa of the large intestine. Aggressive bacteria such as Bacteroides, adherent/invasive E.coli, and enterococci have been observed to rise during inflammation in patients with UC, with a decrease in beneficial bifidobacteria and lactobacilli, leading to immune system dysregulation[22].

The potential value of probiotics in maintaining health and preventing disease has long been recognised. The use of probiotics in the treatment of UC is based on evidence that the intestinal microbiota has a role in the disease's aetiology[23]. Accordingly, probiotics can manipulate the intestinal microbiota and stimulate immunomodulatory mediators. Recently, Lactobacillus and Bifidobacterium species may provide a new therapy for UC[24].

Acetic acid causes significant intracellular acidification by releasing protons into the intracellular space. The epithelial barrier is first disrupted, resulting in tissue damage. As a result, the lamina propria is exposed to intestinal contents, which stimulates the production of pro-inflammatory cytokines. That initiates the inflammatory process[25,26]. The rats' general condition deteriorated after receiving an intra-rectal injection of acetic acid, with a considerable drop in body weight, diarrhoea, and rectal haemorrhage. The increased permeability of the intestinal cells causes diarrhea with excessive nutrient loss and weight reduction. While rectal bleeding is attributed to the pathological changes in the cells lining the colonic mucosa causing erosions and ulcerations[27].

However, the use of probiotics in the current study had significantly increased the body weight measurement

of rats and reduced colonic inflammation. The intake of probiotics suppresses colitis in various experimental animal models. Our findings revealed that probiotic use reduced TNF-α and IL-17A expression in the colon while increasing IL-10 production. Other earlier investigations in various experimental models of acute colitis have found similar results[28,29].

The development of ulcerative colitis has been linked to pro-inflammatory mediators and cytokines. The condition progressed due to the release of TNF-α, an important mediator of colitis[30], as well as producing marked changes in the cellular junctional proteins[31].

The expression of Th17 type cytokines in the mucosa of UC patients has been studied in previous investigations. Increased expression of IL-17A, a key cytokine that distinguishes Th17 cells, has been recorded in our research. It was discovered to play a role in the inflammatory process in UC patients, including the upregulation of other pro-inflammatory cytokines such TNF-α, IL-1, IL-6, and IL 8, the migration of inflammatory cells, and tissue damage[32,33,34].

One of the most well-known probiotics is Lactobacillus acidophilus. Its supplementation could relieve inflammatory changes in gut microbiota composition. In addition, using probiotics to reestablish gut homeostasis could be an effective way to treat inflammatory bowel disease[35].

Moreover, there is evidence that oral administration of Lactobacillus acidophilus, one of the most common natural inhabitants of the human gut, and Bifidobacterium longum suppressed the upregulation of the proinflammatory cytokine (IL-17A and TNF-α) expression in colitis. This could explain why some probiotic strains can help with the aetiology of colitis and reduce intestinal inflammation[9,36].

The lack of IL-10 signaling in colonic epithelial cells causes more severe colitis driven by increased barrier permeability. IL-10 has positive effects on the maintenance of appropriate epithelial permeability and normal function of the epithelial barrier[37,38,39]. Probiotics' capacity to increase epithelial barrier integrity, either directly by modifying junctional complexes or indirectly by regulating cytokine levels and immune cell activation, will almost certainly benefit UC patients[40].

In our study, alcian blue stained colonic sections revealed marked depletion of goblet cells in rats with colitis while there were a high number of goblet cells in rats taking probiotics. Severe depletion of goblet cells and therefore a reduction in mucus production is a specific feature of UC[41]. The continuity of the mucus layer might be impaired with a reduced number of goblet cells. These changes may be attributed to defects in mucin production[42,6]. Increased interaction between the microbiota and epithelium due to reduced mucus secretion may aggravate immunostimulation and inflammation[43].

Goblet cells secrete mucin, primarily Mucin 2 (MUC2), which keeps the mucus layers overlaying the colon epithelium in place. Mucus is divided into two layers in the colon: a densely attached inner layer and a loosely related outer layer[44].

Specific strains of probiotics were able to keep a thicker, more adhering mucus layer and goblet cells restoration. This leads to strengthening the mucus barrier, which works as physical protection against luminal bacteria[45,18]. Mucin 2 (MUC2) and Mucin 3 (MUC3) are produced by Lactobacillus species, which inhibit the adhesion of enteropathogenic Escherichia coli. Thus, the mucus layers overlying the intestinal epithelium could protect against invasion by pathogens[46].

Our study showed that α-SMA positive immunostaining of the pericryptal myofibroblast disappeared in the intestinal mucosa of rats with colitis under the effect of the pro-inflammatory conditions that prevail which disrupt the pericryptal myofibroblast.

Furthermore, there was more positive α-SMA immunostaining of the pericryptal myofibroblast in rats taking probiotics. In active inflammatory bowel illnesses with modification of the myofibroblastic sheath, pro-inflammatory cytokines target myofibroblasts in the intestine pericryptal sheath[47].

The pro-inflammatory cytokines induce apoptosis in intestinal epithelial cells and alter the expression of pericryptal myofibroblast that lead to epithelial homeostasis disturbance[48,41].

This study might throw light on the possibility of the use of probiotics to prevent the degeneration of the pericryptal myofibroblasts. This, in turn, could have an impact on therapy efficacy. Probiotics' anti-apoptotic action in epithelial intestinal cells and the pericryptal sheath may help to minimize the rupture of the colonic barrier in chemical-induced colitis[49]. As a result, future research may shed additional light on the specific role of pericryptal myofibroblasts in the immune response modulation in ulcerative colitis[50].

CONCLUSION

The results obtained in the current study support previous studies describing the efficacy of probiotics in the treatment of intestinal problems such as inflammation and diarrhea. Using an experimental model of acetic acid-induced colitis, we explored the protective effects of probiotics containing L. acidophilus, B. bifidum, and B. longum. Administration of probiotics before induction of colitis resulted in a significant decrease in the weight loss of rats and reduction of inflammation as evident by restoration of normal colonic wall architecture in H&E stained sections. Also restoration of some goblet cells secretion in alcian blue-stained sections, besides continuity of muscularis mucosa and reappearance of myofibroblasts as evident by positive α-SMA immunostaining could give a hint on the protective role of probiotics. Downregulation

of the pro-inflammatory cytokines TNF-α and IL-17A and an up-regulation of the anti-inflammatory cytokine IL-10 also support the results of this study.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

تقييم إستخدام المحفزات البيولوچية في نموذج الجرذ المصاب بالتهاب القولون: دراسة هستولوجية وهستوكيميائية مناعية

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مقدمه: أصبح استخدام مكملات البروبيوتيك مهمًا بشكل متزايد للوقاية من أمراض الجهاز الهضمي وعلاجها. تلعب هذه المكملات دورًا في الحفاظ على التوازن في النظام البيئي للميكروبات في الأمعاء من خلال تنظيم السيتوكينات المؤدية للالتهابات والمضادة للالتهابات.

هدف البحث: تقييم تأثير استخدام البروبيوتيك في التهاب القولون الناجم عن حمض الخليك. قد تلقي هذه الدراسة الضوء على تأثير البروبيوتيك عند استخدامه بمفرده بعد تحريض التهاب القولون دون أي علاج تقليدي.

مواد و أساليب العلاج: أجريت هذه الدراسة علي اربعة وعشرين ذكرا من الجرذان و تم تقسيمهم عشوائيا الي اربع مجموعات: المجموعة الأولي)المجموعة الضابطة(، المجموعة الثانية)التي استحدثت بها التهاب القولون(تم حقن الجرذان بـ٢ مل من حمض الخليك ٣٪ عن طريق الشرج. وفي المجموعة الثالثة تم اعطاء الجرذان المحفزات البيولوچية مرة واحدة في اليوم لمدة سبعة أيام عن طريق الفم بعد اربع وعشرين ساعة من إستحداث التهاب القولون بهم، بينما تم اعطاء الجرذان في المجموعة الرابعة المحفزات البيولوچية مرة واحدة في اليوم عن طريق الفم لمدة أربعة عشر يوماً قبل إستحداث التهاب القولون بهم و سبعة أيام لاحقاً. تم تشريح الجرذان في المجموعات التجريبية بعد سبعة أيام من إستحداث التهاب القولون.

تم صبغ قطاعات القولون بصبغة الهيماتوكسيلين و الإيوسين وبصبغة الألشيان الأزرق و بالصبغة الهستوكيميائية المناعية α-SMA وتم عمل التحاليل الكيميائية الحيوية لقياس قيم كل من □-TNF و١٧-IL و ١٠-IL وتم قياس الصور وعمل التحليل الاحصائي لها.

النتائج: أدى تناول البروبيوتيك إلى عكس الضرر الكبير لبنية جدار القولون الناجم عن حمض الأسيتيك. أدى إعطاء البروبيوتيك قبل تحريض التهاب القولون إلى انخفاض كبير في فقدان الوزن لدى الفئران وتقليل الالتهاب كما يتضح من استعادة بنية جدار القولون الطبيعي في القطاعات المصبوغة بصبغة الهيماتوكسلين والايوسين. أيضا استعادة عدد من إفراز الخلايا الكأسية في القطاعات المصبوغة باللون الأزرق الألسياني ، إلى جانب استمرارية الغشاء المخاطي العضلي وظهور الخلايا الليفية العضلية كما يتضح من خلال الصبغة المناعية الايجابية α- SMA

الاستنتاج: استخدام البروبيوتيك قبل وبعد استحداث التهاب القولون هو أكثر حماية من استخدامه بعد استحداث التهاب القولون فقط.