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Role of *Bifidobacterium infantis* in the Treatment of Duodenal and Colon Inflammation in Induced Ulcerative Colitis

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ABSTRACT

This study aimed to investigate the potential protective role of *Bifidobacterium* (*B.*) *infantis* in alleviating induced duodenal and colon inflammation associated with ulcerative colitis (UC). Female albino Wister rats (n=24) were randomized into four experimental groups: Control Negative, acetic acid-induced colitis (AA colitis), *B. infantis*-treated (Bifido), orally gavaged with 1 mL of reference strain *B. infantis* at 10^8 CFU/mL for four weeks prior to the induction of colitis, and Bifido+AA colitis. Colitis was induced via intrarectal administration of 4% AA solution. Seven days post-colitis induction, blood samples were obtained to assess protein levels, and histopathological evaluations were conducted on duodenal and colon tissues. Additionally, immunohistochemical assessments for B-cell lymphoma 2 (Bcl-2) in colon and myeloperoxidase (MPO) in duodenum sections were performed. Results revealed that *B. infantis* treatment significantly elevated serum albumin and total protein levels in the Bifido and Bifido+AA colitis groups, approximating those in the Control Negative group. Histopathological and morphological changes of duodenum in AA colitis revealed ulceration of the mucosal epithelium, submucosal inflammatory cellular infiltration, tissue depression resulting in villus atrophy, and crypt hyperplasia. Additionally, colonic crypt gland atrophy and goblet cells depletion were observed. Most of these changes were ameliorated in the Bifido and Bifido+AA colitis groups. Immunohistochemical analysis displayed marked immunopositivity of Bcl-2 in colon and MPO in duodenum sections of the Bifido and Bifido+AA colitis groups, indicating the antiapoptotic and anti-inflammatory roles of *B. infantis*. This study demonstrates that *B. infantis* exerts a protective effect against AA-induced UC by normalizing serum protein levels, ameliorating histopathological alterations, and modulating apoptotic and inflammatory markers. These findings underscore *B. infantis* as a promising therapeutic agent for UC and warrant further research to elucidate the underlying molecular mechanisms.

Keywords: *Bifidobacterium infantis*, ulcerative colitis, myeloperoxidase, apoptosis

INTRODUCTION

Ulcerative colitis (UC) is an inflammatory bowel disease with a rising prevalence globally. The multifactorial causes of UC encompass environmental aspects such as diet, hygiene, and smoking (1). At the core of its pathophysiology is the disruption of the intestinal mucosal epithelia, which permits pathogenic bacteria to invade the submucosa. Such invasions stimulate the production of reactive oxygen species (ROS) and oxidative stress, intensifying inflammatory immune responses, consequently, extensive damage to the intestinal mucosa and submucosa due to the surge in inflammatory mediators is occurred (2, 3). Another pivotal factor in UC's

pathogenesis is the disorder of gut microbiota (4-6). Dysbiosis, an imbalance in the microbial ecosystem, exacerbates immune imbalances, thereby instigating intestinal inflammatory responses primarily via oxidative stress (7). Notably, this dysbiosis plays a potential role in the broader spectrum of irritable bowel diseases (IBD), with studies indicating more significant microbial community stability in UC than in Crohn's disease (CD) (5, 8).

Bifidobacterium as a probiotic is a Gram-positive, non-spore-forming, anaerobic bacterium. They form part of the normal flora of the gastrointestinal tract and oral cavity and are not commonly isolated from human clinical specimens (9). In addition, *Bifidobacterium* is known to be abundant in animals caring for their offspring, and several species of this genus have been reported to be among the first intestinal colonies of newborns (10). *Bifidobacterium* spp. particularly *infantis* are one of the first bacteria to colonize the human gastrointestinal tract and are believed to confer beneficial health benefits on their host (11, 12).

Increasing population of antibiotic resistant virulent pathogens has triggered the search of alternate means of pathogen combat. Photo pharmacotherapy and probiotics in combination with prebiotics has become routes of major diversion from the conventional use of antibiotics (13, 14). Furthermore, a common side effects of using antibiotics is diversity of the intestinal flora and antibiotic–associated diarrhea (15). The *Bifidobacterium infantis* (*B. infantis*) function as an immunosuppressor via cellular pathways in cell line studied (16), but the actual role of this probiotic in treatment of UC is not well studied.

Bifidobacterium not only needs to survive in GI tract, but they also need to colonize in the GI tract. An effective probiotic must reside at the preferred target sites in the GI tract long enough and at adequate concentrations to confer probiotic effects. There are currently no commercial products available that aid or increase the attachment of health-promoting bacteria to the gut mucosal surface despite the growing market for probiotics. This study aims to evaluate the potential therapeutic role of *B. infantis* in modulating duodenal and colon inflammation in an induced model of ulcerative colitis.

MATERIALS AND METHODS

Bacterial Strain

The reference strain *B. infantis* as a freeze-dried powder was obtained from the Department of Food Science, College of Agricultural Engineering Science, University of Baghdad, Baghdad, Iraq. The bacterial strain was activated using the De Man, Rogosa and Sharpe (MRS) media (CM0361, Oxoid). Approximately 1 mg of the reference strain was cultured in a screw tube and incubated in a Nuve-EN-120-Türkiye incubator for 48 h at 38 °C to establish a stock culture.

Animal Dose Calculation

The number of colony forming units (CFU) were determined using Miles and Misra method. Tenfold serial dilutions were prepared by mixing 1 mL of stock solution with 9 mL of physiological normal saline. To count the CFUs, 1 mL of bacterial suspension with a concentration of 1×10^8 was used. This diluted concentration of *B. infantis* was cultured by transferring 1 mL of the 10^8 dilutions into a sterile petri dish, followed by the addition of 15 mL of MRS agar. Three cultures were prepared and anaerobically incubated inverted at 37 °C for 48-72 h. The CFU value was calculated by multiplying the appropriate dilution factor by the colony count of the original sample.

Animals

All procedures used in this study were reviewed and approved by local Research Ethics Committee of the College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq in compliance with the ethical principles of animal welfare.

Female Albino Wistar rats were obtained from the animal house at the College of Veterinary Medicine, University of Baghdad, aged between 6 and 7 weeks old and weighed between 150 and 200 g. The animals were kept in standard plastic cages, each holding five animals, in a monitored environment with a temperature range of 21-25 °C and a 12-h daily photoperiod. They had unrestricted access to water and were fed a standard pellet diet. Before the experiment began, the rats were acclimatized for two weeks to their new environment.

Experimental Design

Rats were randomly and equally assigned to four groups (n=8) as follows: The control group was administered intrarectally 1 mL normal saline and received no treatment, acetic acid-induced colitis (AA colitis) group, *B. infantis* treated (Bifido) group, orally gavaged with 1 mL of reference strain *B. infantis* at 10^8 CFU/mL for four weeks prior to the induction of colitis, and Bifido+AA colitis group.

Induction of Colitis

The procedure to induce ulcerative colitis involved intrarectal (IR) administration of 1 mL of 4% acetic acid (HiMedia, India). After fasting for 24 h, rats were anesthetized with an intramuscular injection of ketamine (90 mg/kg BW, Hold Medical, Netherland) combined with xylazine (40 mg/kg BW, Rambone, Germany). A medical-grade polyurethane feeding canal (external diameter 2 mm) was inserted into the anus and positioned 8 cm proximal to the anus margin. Colitis was induced by infusing 1 mL of acetic acid (4% v/v in 0.9% saline) or saline as a control, into the colon through the cannula for 30 sec (17).

Sample Collection

Seven days post-colitis induction, blood samples (3 mL) were taken via cardiac puncture from the anesthetized rats. The samples were placed in anticoagulant-free tubes (gel tubes, Vaculab, China). After centrifugation at 3000 rpm for 15 min, the serum was transferred into Eppendorf tubes (AFME, Germany) using micropipettes and stored at -18 °C until further analysis.

After blood collection, rats were euthanized using an anesthesia overdose. Duodenal and colon samples (about 1 cm length) were dissected, separated from mesenteric tissue, and fixed in 10% formalin for 24 h. Subsequently, these tissues were embedded in paraffin wax.

Globulin, Albumen and Total protein Assessment

A commercial kit for spectrophotometric methods was used to assess the total serum protein (TP) and albumin (ALB) levels (BIOMAGHREB, Tunis). The serum globulin (GLOB) was calculated using the following formula:

$$[\text{Globulin}] = [\text{Total protein}] - [\text{Albumen}]$$

Histopathological and Histomorphology

Histopathological and histomorphology examination achieved in H&E-stained sections (4 animals/group) using light microscope (Olympus, Japan) and imaged by BX53 camera. Histomorphology measurement (depth of crypts, thickness of mucosa, and height of villi) of duodenal and gland thickness and depth of colon were measured in H&E-stained section at low power using a Winjoe ocular micrometer in 30 wells of different sections for each animal, total N= 120 (30 wells × 4 animal/experimental group).

Immunohistochemistry

The immunohistochemistry techniques used for the detection of B-cell lymphoma 2 (Bcl-2) in colon sections and myeloperoxidase (MPO) in duodenal sections were based on PathnSitu's highly sensitive and specific PolyExcel detection system protocol. Briefly, sections measuring about 4 µm in thickness were obtained from paraffin-embedded duodenum and colon samples of three rats per group. These sections were placed on charged slides and allowed to dry overnight at -60 °C. The sections were then deparaffinized, rehydrated, and subjected to HIER (heat-induced epitope retrieval) by boiling the tissue in the Pt Module using Vitro S.A EDTA buffer at pH 8.4 for 20 min at 95 °C. After completion, the sections were rinsed with 3-5 changes of distilled or deionized water and allowed to cool for 20 min. To block endogenous peroxidase activity, the sections were treated with a peroxidase solution for 10 min at room temperature. Next, the tissue sections were covered with the primary antibody and incubated for 30 min. The antibodies were diluted in an antibody diluent containing 3% bovine serum albumin in phosphate-buffered saline (PBS).

Cell positivity for BCl-2 expression was assessed under a light microscope using the Aperio Image Scope (AIS), a digital integration software (Leica Biosystem, Germany). The immunoreactivity of cells was evaluated by calculating the ratio of positive cells to the total cell count (including both positive and negative cells). This approach employs a quantitative immunostaining scoring system. Additionally, a semi-quantitative scoring method, based on an ordinal scale, was utilized to assess combined stain intensity and the percentage of positive cells. Stain intensity was categorized as follows: 1 (Weak), 2 (Moderate), and 3 (Severe). Immunoreactivity grading was determined as follows: grade 0 for less than 10% positive cells, grade 1 (+) for 10-30%, grade 2 (++) for 31-50%, grade 3 (+++) for 51-70%, and grade 4 (++++) for more than 71% of reactive cells.

Statistical Analysis

Statistical analysis of the data was performed using SAS (Statistical Analysis System, version 9.1). Data were subjected to one-way ANOVA, and significant means were separated by the Least Significant Differences (LSD) post hoc test at $P \leq 0.05$.

RESULTS

The results of ALB, GLOB, and TP levels in different groups are presented in Table 1. The AA colitis group exhibited significantly reduced ALB levels compared to the Control Negative group. *B. infantis* treatment in the Bifido group and Bifido+AA colitis group led to significantly higher ALB levels compared to the AA colitis group. Notably, the Bifido+AA colitis group approached ALB levels similar to the Control Negative group. The AA colitis group showed a non-statistically significant marginal increase in GLOB level when compared to Control Negative and Bifido group.

Table 1. The effective role of *B. infantis* on albumin (ALB), globulins (GLOB), and total protein (TP) against the effects of acetic acid induced-colitis in female rats

Groups	ALB (g/dL)	GLOB (g/dL)	TP (g/dL)
Control Negative	3.33±0.10 [a]	2.72±0.30 [ab]	6.05±0.21 [b]
AA colitis	2.53±0.10 [b]	2.86±0.13 [a]	5.39±0.18 [c]
Bifido	3.52±0.04 [a]	3.31±0.27 [a]	6.84±0.27 [a]
Bifido+AA colitis	3.59±0.11 [a]	2.08±0.26 [b]	5.67±0.19 [bc]
P-value	<0.001	0.015	<0.001
LSD	0.28	0.75	0.65

[a-c]Means with a different letter in the same column are significantly different ($P \leq 0.05$) Control Negative: administered intrarectally 1 mL normal saline and received no treatment. AA colitis: acetic acid-induced colitis group, colitis induced via intrarectal administration of 4% acetic acid. Bifido: *B. infantis* treated group, orally gavaged with 1 mL of reference strain *B. infantis* at 10^8 CFU/mL for four weeks, Bifido+AA colitis group: combined *B. infantis* treatment and acetic acid colitis induction

In the Bifido+AA colitis group, GLOB levels were significantly reduced compared to the AA colitis group but approached the levels observed in the Control Negative group. The AA colitis group exhibited significantly reduced TP levels compared to Control Negative. *B. infantis* treatment in the Bifido group resulted in a significant

increase in TP levels compared to Control Negative, AA colitis, and Bifido+AA colitis groups. TP levels in the Bifido+AA colitis group increased compared to the AA colitis group, although this increase was not statistically significant. TP levels in this group approached those observed in the Control Negative group.

Histomorphology Measurement

The results presented in Table 2 demonstrate the effects of *B. infantis* treatment on the duodenal histomorphological parameters in the different experimental groups. In the AA colitis group, there were significant reductions in villi height compared to the Control Negative group. *B. infantis* treatment in both the Bifido group and the Bifido+AA colitis group led to a significant increase in villi height compared to the AA colitis and Control Negative groups. Notably, the Bifido group had the highest villi height, significantly higher than all other groups. Villi thickness in the AA colitis group was significantly reduced compared to the Control Negative group. Similar to villi height, the Bifido group significantly exhibited the highest villi thickness among all groups. *B. infantis* treatment in the Bifido+AA colitis group led to an increase in villi thickness compared to the AA colitis group. The AA colitis group had significantly deeper crypts when compared to Control Negative group, which had the shallowest crypt depth among other groups. Crypt depth in the Bifido group was significantly deeper than all other groups. *B. infantis* treatment in Bifido+AA colitis group resulted in reduced crypt depth compared to the AA colitis group. However, crypt depth in this group remained significantly deeper than the Control Negative group.

The results of gland morphology measurements in colon tissue of female rats with AA-induced colitis treated with *B. infantis* are depicted in Table 2. In the Control Negative group, the thickness of glands was recorded at 31.2±1.44 µm, indicating the baseline measurement for normal gland morphology. Conversely, the AA colitis group exhibited a significant reduction in gland thickness, with a measurement of 24.3±2.92 µm. This decrease likely reflects the deleterious effects of colitis on glandular structures. In contrast, the Bifido group demonstrated an increase in gland thickness, measuring 32.2±0.69 µm, suggesting that *B. infantis* treatment might promote the restoration or augmentation of glandular secretory functions. Interestingly, the Bifido+AA colitis group, which underwent *B. infantis* treatment in the context of colitis, displayed an intermediate gland thickness of 29.5±1.61 µm. The thickness of glands, an indicator of glandular secretory activity, exhibited notable variations across the experimental groups. In the Control Negative and Bifido groups, gland thickness was comparable, with means of 31.2±1.44 µm and 32.2±0.69 µm, respectively, suggesting that *B. infantis* treatment did not significantly alter gland thickness under normal conditions. However, in the AA colitis group, gland thickness decreased to 24.3±2.92 µm, indicating the impact of colitis-induced inflammation on gland morphology. Remarkably, the Bifido+AA colitis group demonstrated a moderate increase in gland thickness (29.5±1.61 µm), signifying a potential restoration of secretory functions despite the colitis challenge. The depth of glands, which is closely linked to secretory activity, followed a similar trend. In the Control Negative and Bifido groups, gland depth remained consistent at 124±2.91 µm and 121±1.19 µm, respectively. Conversely, the AA colitis group exhibited a shallower gland depth of 95.7±3.27 µm, reflecting the detrimental effects of colitis on glandular structure. Notably, the Bifido+AA colitis group displayed a depth of glands comparable to the Control Negative group (118±2.29 µm), suggesting a potential restoration of glandular depth following *B. infantis* treatment.

Table 2. Histomorphological measurements (µm) in the duodenum and gland morphology measurements (µm) in the colon of female rats with acetic acid-induced colitis treated with *B. infantis*

	Duodenum			Colon	
Groups	**Villi Height**	**Villi Thickness**	**Crypt Depth**	**Thickness of glands**	**Depth of glands**
Control Negative	250.2±8.02 [c]	35.55±1.56 [b]	87.23±2.22 [d]	31.2±1.44 [a]	124±2.91 [a]
AA colitis	222.6±4.21 [d]	26.27±1.06 [c]	126.1±3.27 [b]	24.3±2.92 [b]	95.7±3.27 [b]
Bifido	379.5±8.16 [a]	49.25±0.99 [a]	150.8±1.34 [a]	32.2±0.69 [a]	121±1.19 [a]
Bifido+AA colitis	298.2±2.10 [b]	34.32±1.73 [b]	103.8±2.53 [c]	29.5±1.61 [ab]	118±2.29 [a]
P-value	<0.001	<0.001	<0.001	0.046	<0.001
LSD	18.26	4.06	7.21	5.47	7.50

[a-c]Means with a different letter in the same column are significantly different ($P \leq 0.05$) Control Negative: administered intrarectally 1 mL normal saline and received no treatment. AA colitis: acetic acid-induced colitis group, colitis induced via intrarectal administration of 4% acetic acid. Bifido: *B. infantis* treated group, orally gavaged with 1 mL of reference strain *B. infantis* at 10^8 CFU/mL for four weeks, Bifido+AA colitis group: combined *B. infantis* treatment and acetic acid colitis inductio

Histopathological Examination

In this study, the histopathological characteristics of the colon were examined using H&E staining. In the Control Negative group, colon sections displayed well-maintained, normal colonic structures characterized by a clear outer membrane, muscular layer, submucosa, and mucosa (Figure 1A, Figure 2A). In contrast, the AA colitis group exhibited severe lacerations in the colon glands, as evidenced by necrotic changes primarily in the lymphoid tissue of the submucosa and remnants of degenerated glands (Figure 1B, Figure 2B). The Bifido group (Figure 1C, Figure 2C) and the Bifido+AA colitis group (Figure 1D, Figure 2D) showed moderate inflammatory infiltration in the lamina propria of the colonic mucosa and mild mononuclear cell infiltration between the submucosal glands. In the Control Negative group (**Figure 3A**), the

duodenal tissue showed normal histological architecture with well-preserved intestinal villi. In contrast, the AA colitis group (Figure 3B) showed marked necrosis of the intestinal villi, accompanied by necrotic debris and moderate inflammatory infiltration, highlighting the damaging impact of AA-induced colitis on the duodenum. The Bifido group (Figure 3C) showed moderate elongation of the intestinal villi and only mild propria infiltration, suggesting a protective effect. Notably, the Bifido+AA colitis group (Figure 3D) displayed moderate fusion of villi along with moderate submucosal mononuclear cells infiltration.

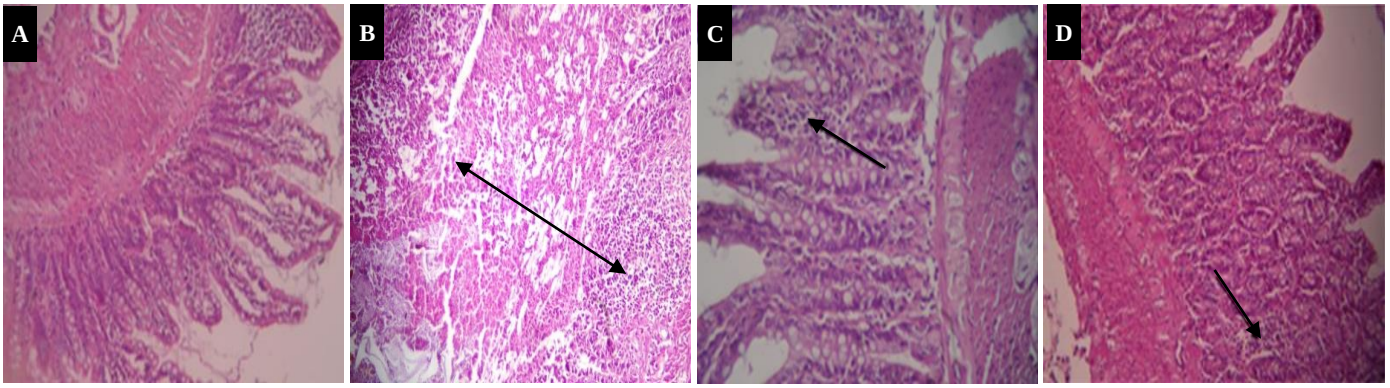

Figure 1. Histopathological analysis of colon sections in female rats. **(A)** Control Negative group displays well-preserved colonic architecture, characterized by an intact outer membrane, muscular layer, submucosa, and mucosa, **(B)** Acetic acid-induced colitis (AA colitis) group reveals severe glandular lacerations with necrotic changes in submucosal lymphoid tissue and remnants of degenerated glands (double head black arrow), **(C)** *B. infantis*-treated (Bifido) group shows moderate inflammatory infiltration in the lamina propria and mild mononuclear cell infiltration in the submucosa (black arrow), **(D)** Bifido+AA colitis group exhibits similar but ameliorated histopathological changes compared to the AA colitis group, with moderated inflammatory infiltration in the lamina propria and submucosa(black arrow). H&E, 10×

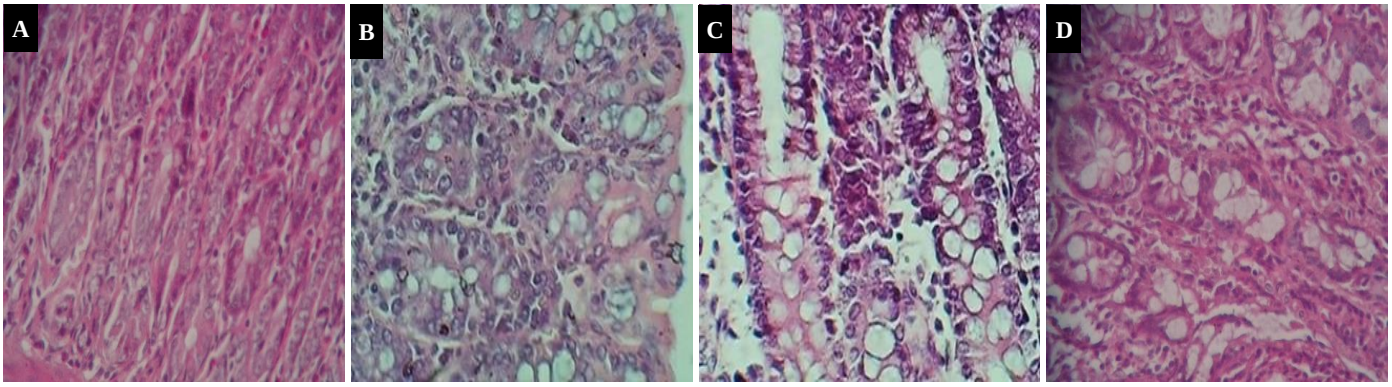

Figure 2. Light microscopic image of histopathological changes of colonic sections in **(A)** Control Negative group, **(B)** Acetic acid-induced colitis (AA colitis) group with necrotic findings of submucosal tissue mainly of lymphoid tissue with remnant of degenerated glands, **(C)** *B. infantis*-treated (Bifido) group with moderate inflammatory infiltrate in lamina propria of colonic mucosa and moderate goblet cells hyperplasia, and **(D)** Bifido+AA colitis group had acetic acid colitis with mild mononuclear cells infiltration between mucosal glands and goblet cells hyperplasia. H&E stain, 40×

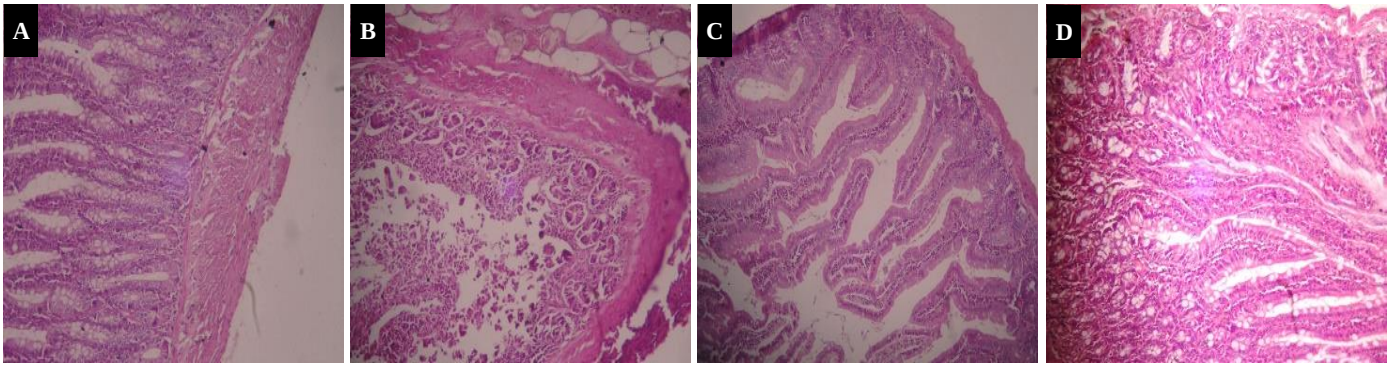

Figure 3. Histopathological analysis of duodenal sections of female rats. **(A)** Control Negative group showcases well-preserved duodenal architecture, including intact intestinal villi, **(B)** Acetic acid-induced colitis (AA colitis) group displays marked villous necrosis, necrotic debris, and moderate inflammatory infiltration in the duodenal tissue, **(C)** *B. infantis*-treated (Bifido) group exhibits moderate elongation of intestinal villi accompanied by mild lamina propria infiltration, **(D)** Bifido+AA colitis group reveals moderate fusion of intestinal villi along with moderate infiltration of mononuclear cells in the submucosa. H&E, 10×

Immunohistochemistry

The immunohistochemical study yielded results consistent with the detection of the apoptotic marker Bcl-2 and the activity of MPO, as evidenced by specific antibody reactions that produced a brown color, indicating positive results. In the Control Negative group, colon sections displayed minimal presence of Bcl-2-immunopositive (+ve) cells (Figure 4A). In contrast, the AA colitis group showed reduced numbers of Bcl-2 +ve cells in the submucosal glands, primarily scattered within lymphoid tissue (Figure 4B). Colon sections from Bifido group showed a marked increase in Bcl-2 +ve cells in the subepithelial layer and associated lymphoid tissue (Figure 4C). Similarly, the Bifido+AA colitis group exhibited increased Bcl-2 +ve cells in both the submucosa and mucosal layers (Figure 4D).

Moreover, Bifido+AA group showed significant MPO immunopositivity both intra- and extracellularly in the intestinal epithelial and subepithelial layers (Figure 5D, Figure 6D). Meanwhile the MPO been well expressed in intestinal epithelial cells of Bifido group (Figures 5C, Figure 6C) when compared with Control Negative group (Figure 5A, Figure 6A) and AA colitis groups (Figure 5B, Figure 6B).

Statistically, the semi quantitative scoring (Ordinal scale of scores) of combined stain intensity and percent of cells positive approach revealed significant differences in the positivity of the markers between groups (Table 4).

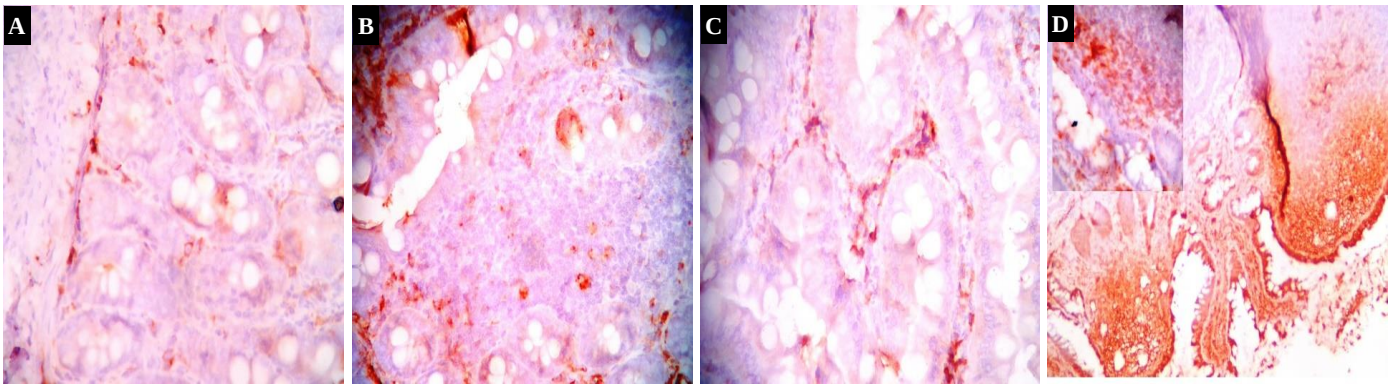

Figure 4. Immunohistochemical analysis of B-cell lymphoma 2 (Bcl-2) apoptotic marker expression in colon sections of female rats. **(A)** Control Negative group shows minimal Bcl-2-immunopositive (+ve) cells in the submucosa, **(B)** Acetic acid-induced colitis (AA colitis) group exhibits reduced Bcl-2 +ve cells, primarily scattered in lymphoid tissue, **(C)** *B. infantis*-treated (Bifido) demonstrates marked increase of Bcl-2 +ve cells in subepithelial glandular lymphoid-associated tissue, **(D)** Bifido+AA colitis group reveals increased Bcl-2 +ve cells in the submucosa and mucosa. 40×

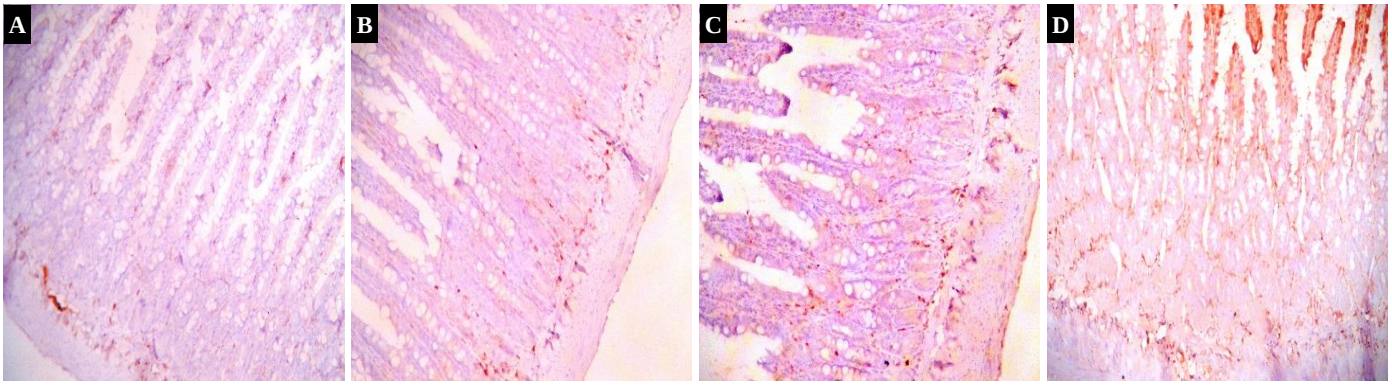

Figure 5. Light microscopic images for immunohistochemistry of duodenal sections, showing myeloperoxidase (MPO) intra and extra cellular in intestinal epithelial and subepithelial layers. **(A)** Control Negative group, **(B)** Acetic acid-induced colitis (AA colitis) group showing preseance of extracellular MPO. **(C)** *B. infantis*-treated (Bifido) group, **(D)** Bifido+AA colitis group showing marked immune +ve for MPO both intra and extra cellular in intestinal epithelial and subepithelial layers. 10×

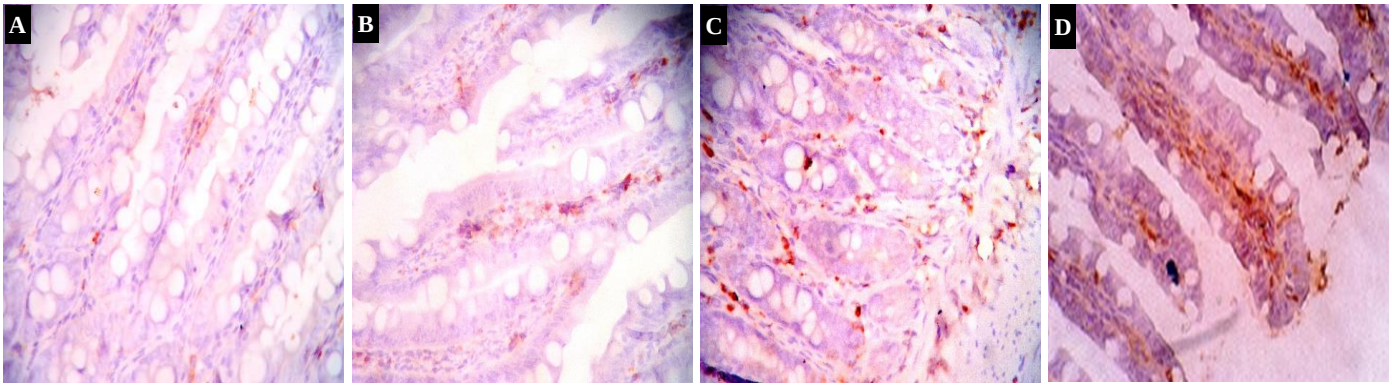

Figure 6. Light microscopic images for immunohistochemistry of duodenal sections showing myeloperoxidase (MPO) intra and extra cellular in intestinal epithelial and subepithelial layers for **(A)** Control Negative group, **(B)** Acetic acid-induced colitis (AA colitis) group, **(C)** *B. infantis*-treated (Bifido) group, **(D)** Bifido+AA colitis group. 40×

Table 4. The effective role of *B. infantis* on colonic B-cell lymphoma 2 (Bcl-2) and duodenal myeloperoxidase (MPO) expression by immunohistochemistry positivity and grading of intensity against the effects of acetic acid induced-colitis in female rats

Groups	Bcl-2	Intensity grade	MPO	Intensity grade
Control Negative	0.240 ± 0.020 [c]	+	0.114 ± 0.007 [d]	++
AA colitis	0.150 ± 0.003 [d]	+	0.140 ± 0.000 [c]	+
Bifido	0.408 ± 0.004 [a]	+++	0.303 ± 0.003 [b]	++
Bifido+AA colitis	0.348 ± 0.025 [b]	++	0.477 ± 0.007 [a]	+++
P-value	<0.001		<0.001	
LSD	0.114		0.0189	

[a-d]Means with a different letter in the same column are significantly different ($P \leq 0.05$) Control Negative: administered intrarectally 1 mL normal saline and received no treatment. AA colitis: acetic acid-induced colitis group, colitis induced via intrarectal administration of 4% acetic acid. Bifido: *B. infantis* treated group, orally gavaged with 1 mL of reference strain *B. infantis* at 10^8 CFU/mL for four weeks, Bifido+AA colitis group: combined *B. infantis* treatment and acetic acid colitis inductio

DISCUSSION

Experimentally UC induced by acetic acid which is a physical agent that disrupts the epithelial barrier when administered rectally, resulting in acute clinical symptoms (diarrhea and bloody stools), epithelial crypt loss, and inflammation. Increased colonic permeability leads to more interaction between the microbiota and the immune system, which is one of the key factors in the onset of UC and duodenal inflammation (18). Oxidative destruction is thought to be the pathogenetic factor in this scenario (19). It is assumed that the same result occurs in colitis in humans.

The commensal *B. infantis* was chosen for this investigation for two reasons. First, these bacteria have been shown to have anti-inflammatory properties in some inflammatory circumstances; second, because these bacteria dwell in the gut, they maintain homeostasis there (20). *B. infantis* as a probiotic was studied in rats to see if it could alleviate the symptoms of colitis induced experimentally. Bifidobacterium is one of the most important components of the intestinal mucosal barrier and one of the body's most important probiotics (21. It has a role in digestion, absorption, nutrition, metabolism, anti-infective immunity, and, most importantly, preserving the intestinal mucosal barrier's integrity (22).

In the current study, the histological lesions were severe enough to compromise the normal intestinal functions, such as absorption, and explain the reduction in albumin and total proteins. This decrease in albumin in the colitis groups may have been caused by the direct effects of acetic acid on intestinal wall integrity and oxidative stress induction, as demonstrated by the low absorption ratio and diarrhea (23). While globulin levels are closely linked to immune and inflammatory states, even though the ratio of globulin increases in rats with colitis and decreases in rats with Bifido. Accordingly, *B. infantis* administration to rats with colitis improved intestinal wall integrity, which resulted in structural and absorptive improvements in the intestine.

Villus height and villus thickness to crypt depth ratio were both increased by *B. infantis* in the present study, indicating its role in the evolution of intestinal absorption and secretion performance. An increase in the absorptive surface area of the intestine is associated with an increase in the digestive and absorptive functions of the villus and the depth of the crypt. We found that *B. infantis* feeding reduced the severity of colitis, instillation resulted in extensive depletion of goblet cells, loss of epithelial cells and dense infiltrations of inflammatory cells. Despite this, the inflammatory infiltrates and goblet cell depletion of colitis rats fed *B. infantis* were significantly reduced, and the intestinal epithelium was restored, the exact mechanisms for overall these changes via modulating of immune response (24, 25).

The immunohistochemical detection of Bcl-2 in colon epithelia was done to elucidate the mechanism by which *B. infantis* can protect colon from acetic acid colitis. Pro- and antiapoptotic Bcl-2 family proteins have functions other than regulating apoptosis and that these functions are important for normal physiology of healthy cells (26). The pro-inflammatory cytokines such as TNF-α and IL-6, which mediated downregulation of p53 expression associated with reduced apoptosis (27) and down regulation of Bcl-2 (28). Therefore, the regulation of cell proliferation and apoptosis can be one of the main cancer treatment strategies, and probiotics are reported to be involved in the regulation of cell apoptosis and proliferation (29, 30). Colonic epithelial cells' apoptosis has been shown to be increased in patients with colitis in the past (31). Our results related to *B. infantis* effects on Bcl-2 in inflamed colon tissue indicate the cytoprotective role of this probiotic. Probiotic-treated rats down-regulation of Bcl-2, (32, 28), however, *B*. *longum* (R0175) was significantly unregulated by Bcl-2 expression (33).

There are many biochemical measurements of neutrophil infiltration, however MPO activity is a rapid, and accurate test that positively correlates with histological scoring systems to represent the extent of inflammation in intestinal tissue. Large evidence demonstrated the protective role of probiotics reducing the MPO as a mechanism for protection of duodenum (34).

Administration of *B. infantis* had a protective role against AA-induced ulcerative colitis. Our results point out that *B. infantis* treatment appears to be as effective as antibiotics alternative treatment. According to our study results, we suggest that *B. infantis* has a protective role in AA-induced colitis, but with some limitation for explain the precise mechanisms of probiotics' beneficial impacts and underline their potential therapeutic use.

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N/A

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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دور *Bifidobacterium infantis* في علاج التهاب الاثني عشري والقولون في التهاب القولون التقرحي المستحث تجريبياً

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الخلاصة

هدفت هذه الدراسة إلى دراسة التأثيرات الوقائية لبكتيريا *Bifidobacterium infantis* على التهاب القولون التقرحي المستحدث تجريبياً. تم تقسيم الجرذان البالغ عددها ٢٤ أنثى بشكل عشوائي وبالتساوي إلى أربع مجموعات ، الأولى كانت بمثابة مجموعة تحكم ، والثانية كانت مصابة بالتهاب القولون بدون علاج ، المجموعة الثالثة والرابعة كانت مصابة بالتزقيم الفموي مع ١ مل من المعلق B. التهاب القولون التقرحي بحمض الخليك. أوضحت النتائج أن البروتين الكلي والألبومينات يزدادان بواسطة *Bifidobacterium infantis* في المجموعتين الطبيعيتين والقولون. أظهرت التغيرات النسيجية المرضية للقولون في مجموعات التهاب القولون تقرحًا في الظهارة المخاطية المعوية ، وتسلل خلوي التهابي تحت المخاطي ، وانخفاض في الأنسجة مما أدى إلى ضمور الخبايا مع نضوب الخلايا الكأسية ، ومعظم هذه التغييرات من قبل مجموعة B. أظهر التحليل الكيميائي المناعي زيادة في سرطان الغدد الليمفاوية (B-cell 2 (Bcl-2 و Myeloperoxidase (MPO) في قسمي القولون والاثني عشر مما يشير إلى الدور المضاد للالتهاب والمضاد للبكتريا.

الكلمات المفتاحية: بيفيدوباكتريوم إنفانتس، التهاب القولون التقرحي، ميلوبيروكسيداز، موت الخلايا المبرمج