Intestinal Anti-Inflammatory Improvement with Fenugreek Seeds as A prebiotic and Synbiotic with *Lactobacillus acidophilus* in Rats Experimentally Infected with *Escherichia coli*

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ABSTRACT

Synergistic action of probiotics and prebiotics (synbiotic) has been suggested to be more effective than the two separate components in the prevention and treatment of many intestinal and immune diseases. The present study aimed to examine the anti-inflammatory role of Fenugreek as synbiotic with *Lactobacillus acidophilus* against *Escherichia coli*. Twenty four adult males of Wister rats aged 3-4 months and weighted 200-250 gm were used and divided into 4 groups: 1st and 2nd groups were negative and positive control (C and C++) fed with basal diet, the 3rd group (T1) fed diet with Fenugreek seeds (5%) and the 4th group (T2) fed with the synbiotic Fenugreek seeds (5%) and *L. acidophilus* (5×10^8 CFU/ml) for 45 days. After that, enteritis was induced in rats of the C++, T1, and T2 groups by administrating 1 ml (2.5×10^6 CFU/ml) of enteropathogenic *E. coli* (EPEC O125:H6). The preventive role of prebiotic and synbiotic was evaluated depending on macro and microscopic duodenum pathological changes in correlation with butyric acid production for 7 days of infection. The results of the macro and microscopic scoring of enteritis revealed that the synergistic effects of the synbiotic in preventing *E. coli* enteritis was favored by an increase in goblet cells mucin secretion. This anti-inflammatory role was significantly increased by synbiotic and correlated with the production of butyric acid. The synbiotic improved the anti-inflammatory response of intestinal mucosa adaptive immunity via elevation of the IgA from plasma cells. In conclusion, the inclusion of nutritional supplements containing fibers that constitute a source of butyric acid production, such as Fenugreek seeds, would improve intestinal resistance to inflammation by acting as anti-inflammatory through improving intestinal lymphoid tissues and increasing the production of IgA.

Keywords: Synbiotic, Fenugreek seeds, *E. coli*, Enteritis, Butyric acid

Introduction

Gut microbiota balance plays an important role in preventing intestinal pathology (1). The balance and survival of gut microbiota can be enhanced by appropriate pre and probiotic supplementation (2). Most probiotics are naturally occurring lactic acid bacteria, such as *Lactobacillus, Streptococcus, Pediococcus, Lactococcus*, and others. Many probiotics have been tested in clinical trials to address their beneficial role in pathological cases, such as gastroenteritis, antibiotic diarrhea, lactose intolerance, and constipation due to diseases caused by colonic microflora imbalance and the effect of anti-colon tumor (3, 4). Experimental trails indicated the anti-inflammatory role of *Lactobacillus acidophilus* in ulcerative colitis **(**5-7**)**. The recent definition of prebiotic according to the International Scientific Association for Probiotics and Prebiotics (ISAPP) implies that prebiotic is defined as "A substrate that is selectively utilized by host microorganisms conferring a health benefit". As the number of food

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ingredients and dietary fibers classified as prebiotic increases, the following criteria for classification must be established: "(i) resistance to gastric acidity, hydrolysis by mammalian enzymes, and gastrointestinal absorption; (ii) fermentation by intestinal microbiota; (iii) selective stimulation of the growth and/or activity of intestinal bacteria associated with health and well-being"**(**8, 9**)**. Being prebiotic is best when it alters intestinal microbiota by oligo-fructose enriched inulin **(**10) increase carbohydrate metabolism enhanced the growth of bacteria, mediated with short chain fatty-acids (SCFA) producers (11).

Intestinal anti-inflammatory response improved by SCFAs including butyrate, usually acts as an inhibitor of inflammatory cytokine expression in the intestinal mucosa as well as a catalyst for the production of mucin and antimicrobial peptide and strengthening the integrity of the epithelial barrier by increasing the expression of tight joint proteins (TJ) (12). In addition, the SCFAs have anti-microbial properties by decreasing the pH of colon microenvironment (13). Butyric acid as well as other SCFAs produced from fermentation of undigestible fiber of fenugreek seeds serve as a good prebiotic, predominantly galactomannan (14). Since the production of butyric acid results from the combination of fenugreek seeds and probiotic lactic acid bacteria (15), the intervention of synbiotics improved health benefits to the host compared to either probiotic or prebiotic supplements alone (16). Synbiotic formulation is the term describing the combination of a beneficial bacteria (Probiotic) with fiber source natural substances such plant (prebiotics) (17**).** The most important feature for the good prebiotic is the type of fibers being good and easily fermented by probiotic enhancing their growth and proliferation **(**18), along with inhibiting the pathogenic bacterial growth in the gut (19). Fenugreek is an annual plant belonging to the family *Leguminosae*, and it is one of the medical plants that has been reported for various pharmacological activities (20). The main component of fenugreek seeds is galacto-mannan, which is a polysaccharide structurally composed of a 1→4 beta-D-mannosyl backbone substituted by a single galactose unit αlinked at the C-6 oxygen (21). Consumption of the prebiotics either as a raw material diet or prepared in therapy formula and additive foods can impact health via different mechanisms including modulation of gut microbiota, immune modulation, inhibition of pathogens and enhancement of nutrients absorption (22).

Although many researchers studied the beneficial effects of synbiotic supplementation, to date no research is available about the fermentation of the prebiotic fenugreek seeds by *L. acidophilus* in intestinal homeostasis. The aim of this study was to exploring the potential anti-inflammatory role of the synbiotic composed of Fenugreek and *L. acidophilus* against *E. coli* enteritis via favoring the production of butyric acid.

Materials and Methods

Experimental Animals' Diet and Housing

Experimental male albino wister rats obtained from the animal house of College of Veterinary Medicine were housed in plastic cages with the dimensions long 50 × width 35 × height 15 cm in a fixed temperature of about 21-25 °C, and they were well ventilated daily with 12 hours photo period. The rats were fed freely with a basal diet**.**

Prebiotic and Synbiotic

The fenugreek seed powder was purchased from the local herbalist market in Najaf, Iraq. It was ground to fine powder and added to basal diet as 5% (95 gm ration + 5 gm fenugreek seed powder) (23). Synbiotic was formulated from fenugreek seeds and the reference strain of *L. acidophilus* as freeze-dried powder obtained from Feed sciences department, Agriculture College, University of Baghdad.

Inducion of Intestinal Inflammation (Enteritis)

The strain enteropathogenic *E. coli* ICC 233 (EPEC O125:H6) was isolated from diarrhatic calf in the Department of Internal and Preventive Medicine/ College of Veterinary Medicine/ University of Baghdad. According to the method described by (24), the induction of experimental enteritis was done by the oral administration of 1 ml of *E. coli* (2.5×10^6 cfu/ml of suspension) to the rats through the stomach tube.

Experimental Design

A total of 24 growing rats aged 3-4 months and weighted 200-250 g were evenly assigned to 4 groups: 1st and 2nd groups were negative and positive control (C and C++) fed with basal diet, the 3rd group (T1) was fed with the prebiotic

fenugreek 5% (23), and the 4th group (T2) was fed with synbiotic formulated from 5% fenugreek seeds and *L .acidophilus* (5×10^8 CFU/ml) (25) for 45 days. After that, rats in the C++, T1 and T2 had been induced enteritis. Seven days post induction of enteritis; serum was collected and used for butyric acid and IgA analysis. Intestinal tissues (duodenum and colon) were sampled for micro and macroscopic pathological changes. This study was carried out according to the approval of the scientific committee in the Department of Physiology, Biochemistry and Pharmacology, College of Veterinary Medicine, University of Baghdad in accordance with the ethical standards of animal welfare.

1. Serum Butyric Acid

Serum butyric acid concentration (Pg/ml) was measured by using ELISA kit (AB clonal/ USA).
This assay is competitive; therefore there is an opposite relationship between the concentration of butyric acid in the sample and the absorbance measured. A graph was created with the log of the standard absorbance (y-axis) and the concentration measured (x-axis). The best fit trend line was applied through the standard points. By using this graph, sample concentrations were calculated based on their OD values.
IgA protein concentration (mg/dl) was measured by means of radial immune-diffusion plate (LTA/ Italy). The tested IgA protein in 5 µl of serum formed an immune complex with a specific antibody after the diffusion on agarose gel, and was seen as a ring surrounding the well. The diameter of the ring was directly proportional to the protein concentration analyzed according to the company instructions.

2. Assessment of Enteritis

It was assessed as follows:

a. Clinically dependent on the appearance of signs of enteritis in the experimental animals after *E. coli* administration.
b. Macroscopic score (gross lesions of colon): Enteritis (colon inflammation) was estimated through the macroscopic scoring after 7 days of infection with *E. coli*. Four animals were sacrificed from all of the groups, the distal part of colon was removed and irrigated by physiological saline solution to remove fecal remains, and then was cut longitudinally. Macroscopic scores of inflammation were recognized grossly (naked eye) depending on colonic examination by utilize an arbitrary scale extended from 0–5 according to (26).
c. Microscopic score (histopathological examination): Four animals from each group were sacrificed, the samples were collected from the distal colon, then these samples were preserved by using formalin solution (10%) for fixation until the sections of histology were ready, after that these section were stained by Hematoxylin and Eosin (H&E) stain (**27**) as well as Periodic acid–Schiff (PAS) stain. For microscopic scoring, an arbitrary scale ranging from 0–5 depending on histopathological criteria was applied (28). All tissue sections were examined by a professional histopathologist.
d. Goblet cells density in duodenum: Serial histological sections of 4 to 5 µm thickness were subjected to PAS stain. Ten random fields were selected for each sample (n=40) at 40X using a digital camera (HDCE-X5/ Japan) connected to a microscope (Olympus/ Philippines). Goblet cells density was counted in100 µm (29).

Statistical Analysis

Data were analyzed by using SAS (Statistical Analysis System, version 9.1). All data were subjected to analysis of variance (ANOVA). Least significant differences (LSD) post hoc test was performed to compare between means. $P \leq 0.05$ was considered statically significant. Data were presented as Mean±SE.

Results and Discussion

1. Serum Butyric Acid Concentration

The serum butyric acid recorded significant decrease ($P<0.05$) in the enteritis group but increase significantly in fenugreek treated groups, as compared with control (Figure 1).
The production of butyric acid resulted from the combination of fenugreek seeds and probiotic lactic acid bacteria provided not only probiotic benefits but also bioactive compounds. Zhong et al. (2019) reported that the relative abundances of the *Lactobacillaceae* family were accompanied by increased butyrate production (15). In the present study, the combination of symbiotic from *L. acidophilus* and fenugreek seeds has asynergistic effects for butyric acid production clearly than

fenugreek prebiotic. Galactomannan compound plays a significant role in modifying the intestinal flora by acting as substrate for useful microbes (14), being fermented and utilized by probiotics to produce important amount of SCFAs (30) and suppress growth of *E. coli* ATCC 25922. The fenugreek containing diet influences gut flora containing lesser *Escherichia* and greater *Lactobacillus* concentrations (9). The SCFAs are considered candidates to necrotic enteritis prevention for many activities, *in vitro* studies showed that they exert antimicrobial and antioxidant efficacy on a wide range of pathogenic bacteria (13, 31), favoring integrity of intestinal mucosal barrier (32) and modulate resistance to disease (33). Supplements containing SCFAs may modify the intestinal microbiota by acting as bacteriostatic and bactericidal (34).

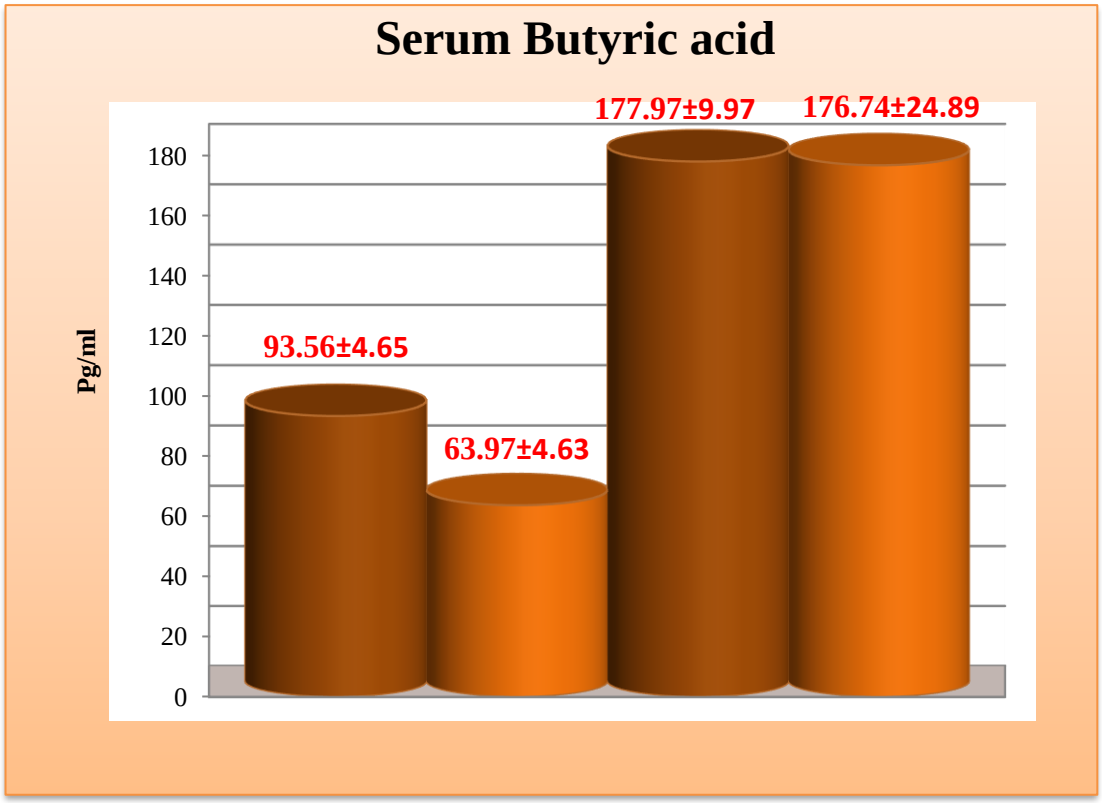

Figure 1. Protective role of synbiotic on serum butyric concentration (pg/ml) against *E. coli* infection. N=6. Mean±SE. C: control negative group. C++: control positive group. T1: Fenugreek administered to enteritis group. T2: Fenugreek and *L. acidophilus* administered to enteritis group. Least Significant Difference (LSD) of butyric acid = 40.72

2. Immunoglobulin A

The results of serum IgA (mg/dl) (Figure 2) revealed that the animals which had been fed fenugreek in the diet (T1 and T2) showed significant ($P< 0.05$) increase in IgA as compared with the control and enteritis groups (C and C++).
The IgA is the dominant antibodiy secreted by B-cells in the mucous epithelium and is therefore a critical component of mucosal immunity. Immuno-globulin A that coat epithelial cells reduces the attachment of pathogenic microbes to the surface of epithelia and may neutralize some microbial toxines. Production of IgA is positively correlated with SCFA generation in C57BL/6J mice through SCFA-dependent signaling pathways (35).
Production of the intestinal IgA is greatly influenced through the commensal gut flora colonization, as shown by the IgA low level in germ-free animals, and that is corrected after inoculation by the bacteria in the lumen (36).

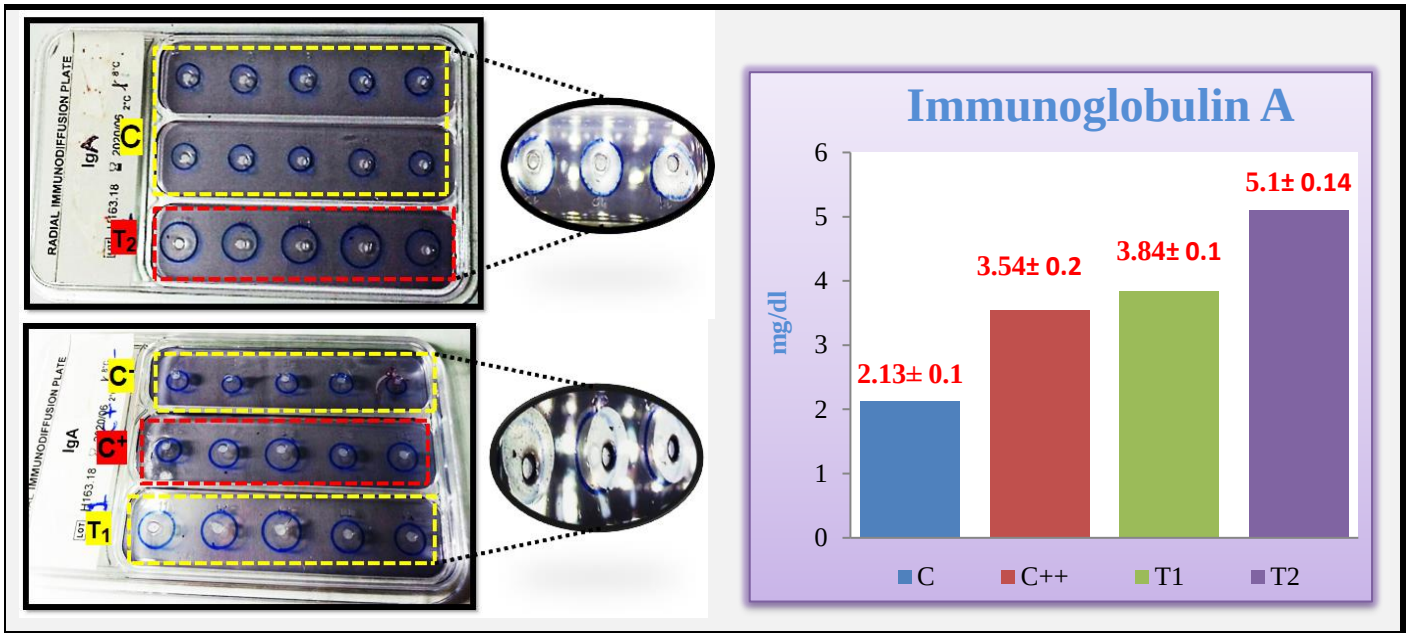

Figure 2. Protective role of synbiotic on serum IgA (mg/dl) against *E. coli* infection, N=5. Mean±SE. C: control negative group. C+: control positive group. T1: Fenugreek administered to enteritis group. T2: Fenugreek and *L. acidophilus* administered to enteritis group. Least Significant Difference (LSD) of IgA = 0.44

Butyrate has been shown to have effect on immune cells towards anti-inflammatory and tolerogenic phenotypes. In mice, the SCFA mixture given to each operating system increased the number of B-cells lamina propria secreting IgA, IgA expression or levels of IgA secreted in different bowel sections, and IgA and IgG levels in the circulation (37). Butyrate generated by the gut microbiota is primary to the proper functioning of the gut immunity. The deficiency in butyrate production should be restored by intestinal microbiota and dietary complements because of its association with immune activity of intestinal barrier in production of IgA normalization (38).

3. Assessments of Enteritis Caused by *E. coli*

a- Clinical Signs

Loss of appetite and depression and soft feces with frequent stools were mainly seen in experimentally infected group or control positive. Furthermore, some animals were marked by severe diarrhea with pink or yellow color. There was no observable signs in other treated groups.

b- Macroscopic Findings

E. coli administrated caused colitis with a significant ($P<0.05$) increase a in macroscopic score reported in C++ group when compared with the fenugreek (T1 and T2) groups at 7 days post *E. coli* infection.

The gross lesions and the macroscopic score images are shown in Figure 3 (A and B). The colon mucosa of control positive showed ulcer lesions as pale areas surrounded by hemorrhagic zone, with marked congestion and edema. The previous lesions were not pronounced mainly in the proximal and middle colon as compared with the distal colon. The colon mucosa of treated animals (T1) showed less marked congestion of the colon without edema. Finally, no gross pathological changes in the C and the T2 animals were seen.

c- Microscopic Score of Colitis and Histopathological Examination of Colon and Duodenum Sections

Examination of duodenal sections of control positive (C++) group in the present study showed that the majority of villi replaced by structure with less surface mass and evidence of sloughed epithelium (mainly observed at tip of villi), associated with minimum number of goblet cells and mild cellular infiltration in lamina properia. Various forms of degeneration were recorded in submucosal glands (Figure 4A). Duodenum sections of T1 group showed mild surface desquamation of villous mucosa with focal villous fusion accompanied by moderate distention of lamina properia due to MNCs infiltration, together with prominence of goblet cells that give strong reaction with PAS stain. Also, mild submucosal cellular infiltration with slight hyperactivity of

Bruner's gland associated with mucous cells hyperplasia that stained purplish with PAS stain. On the other hand, T2 duodenum section revealed elongated villi with prominence of normal goblet cells that stained purplish color with PAS stain. Together with mucosal and submucosal MNCs infiltration, also slight hyperactivity of submucosal crypt with abounded mucous cells appearance more than villi. In control negative (C) group, duodenal section had normal elongated mucosal villi with few numbers of goblet cells and well-defined glandular tissue. Figure 4B shows the synbiotic effect of fenugreek and *L. acidophilus* on intestinal immunity such as goblet cells density (cell/100 µm), in which the secretion of mucus is the front line of the innate host defensethat act on coating the epithelium of gastrointestinal tract. In the T2 group there was a significant increase ($P<0.05$) more than T1, along with decrease in enteritis was seen in C++ group compared with control.

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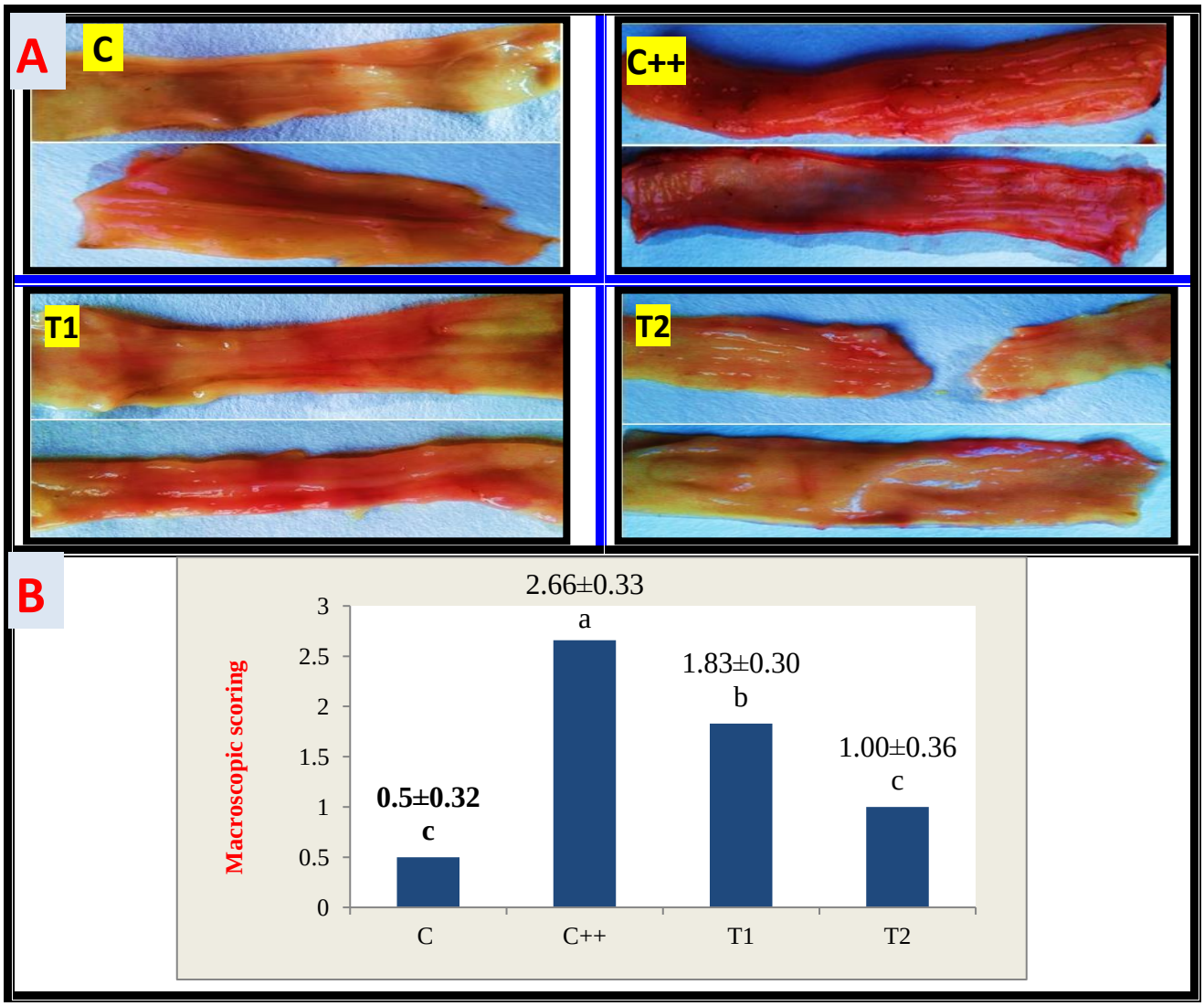

Figure 3. (A) Protective role of synbiotic on gross lesion of colon against *E. coli* infection. (B) Macroscopic scoring of colitis. C: control negative group. C++: control positive group showing ulcer lesions as pale areas surrounded by hemorrhagic zone, edema, severe blood congestion along the colon with the appearance of rough and marked thickening. T1: Fenugreek administered to enteritis (colitis) group showing small bleeding patches along the colon and the appearance of rough and marked thickening. T2: Fenugreek and *L. acidophilus* administered to enteritis group, the colon showed clear mucous layer and the appearance of smooth and marked thinning. LSD of macroscopic score= 0.85

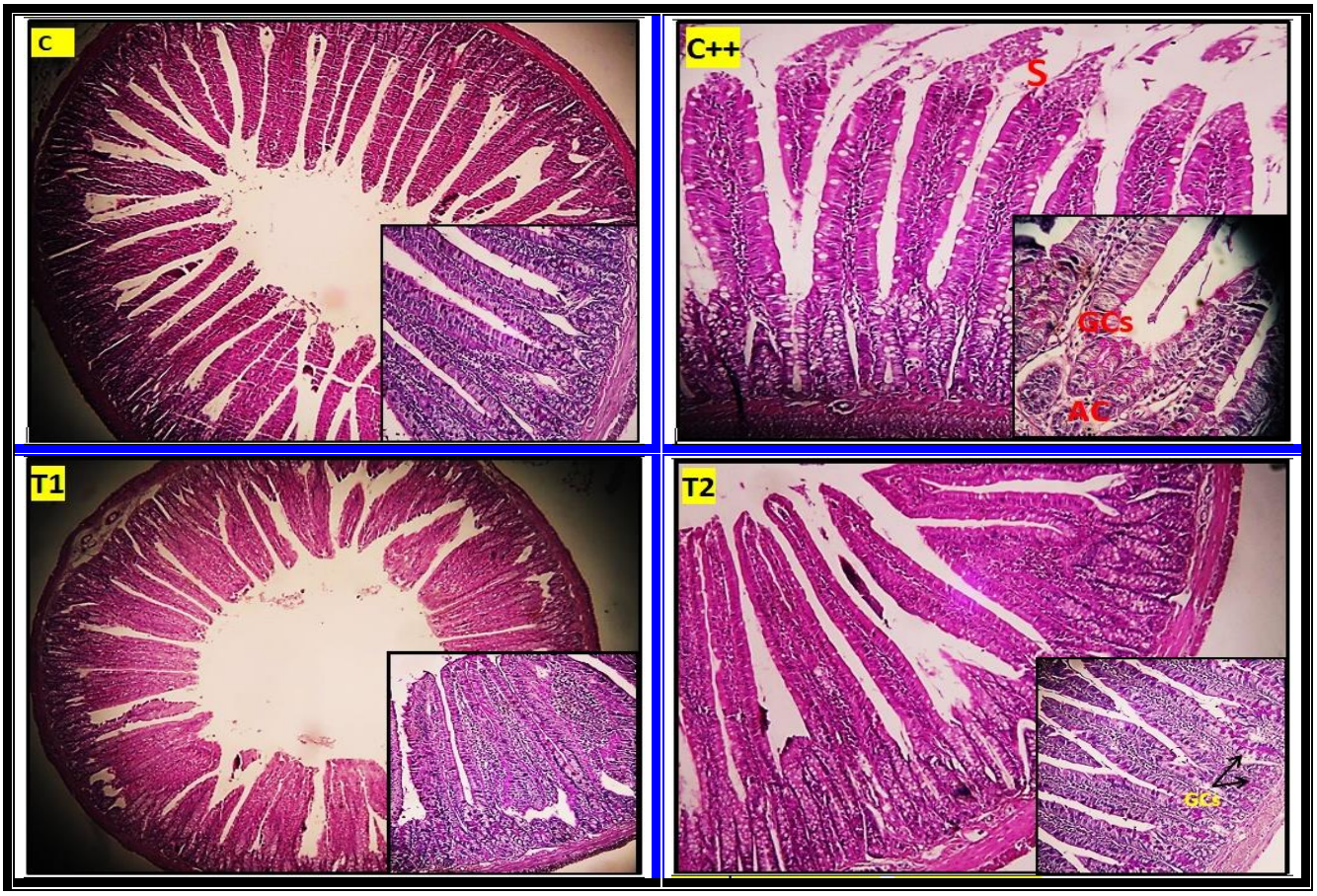

Figure 4A. Light microscopic image of duodenum sections represents the protective role of symbiotic against *E. coli* infection. N=4. Mean±SE. (GCs) secretory cells (goblet cells), (AC) Atrophy of crypt. (Pas and H&E). 100X , 200X, and 400X. C: control negative group C++: control positive group, T1; had fenugreek with enteritis, T2: had fenugreek and *L. acidophilus* with enteritis

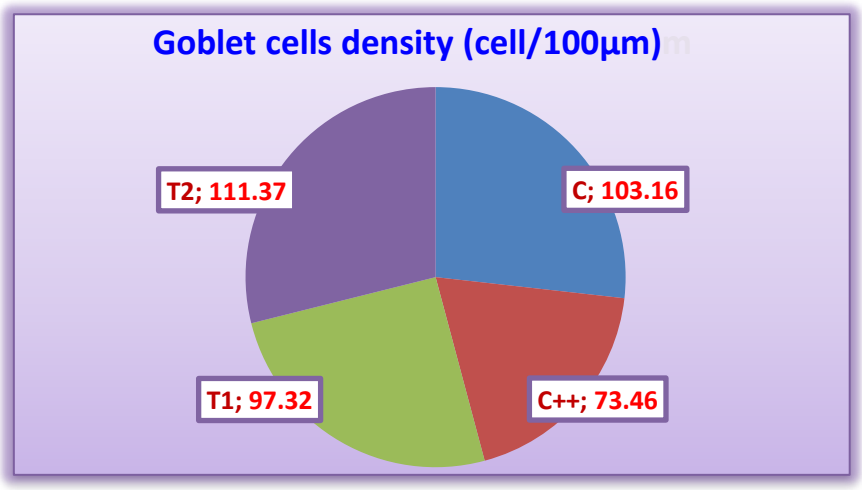

Figure 4B. Protective role of synbiotic on goblet cells density in duodenum (cell/100µm) against *E. coli* infection, N= 40 wells. Mean±SE. C: control negative group. C++: control positive group. T1: Fenugreek administered to enteritis group. T2: Fenugreek and *L. acidophilus* administered to enteritis group. Least Significant Difference (LSD) of goblet cell density = 1.07

Histopathological examination of colon sections (Figure 5A) indicated that the prominent colon findings of control positive group (C++) associated with ulcerative lesion of colon mucosa (loss of mucosa tissue and deeper submucosal); including necrotic layer with neutrophilic infiltration surrounded by fibrous zone accompanied with mild

granulation tissue, together with structural distortion of survival colon glands. Also, remnant of necrotic sloughed tissue have been noticed in the colon lumen, as well as submucosal edema mixed with diffuse MNCs infiltration resulted in pressure atrophy to the

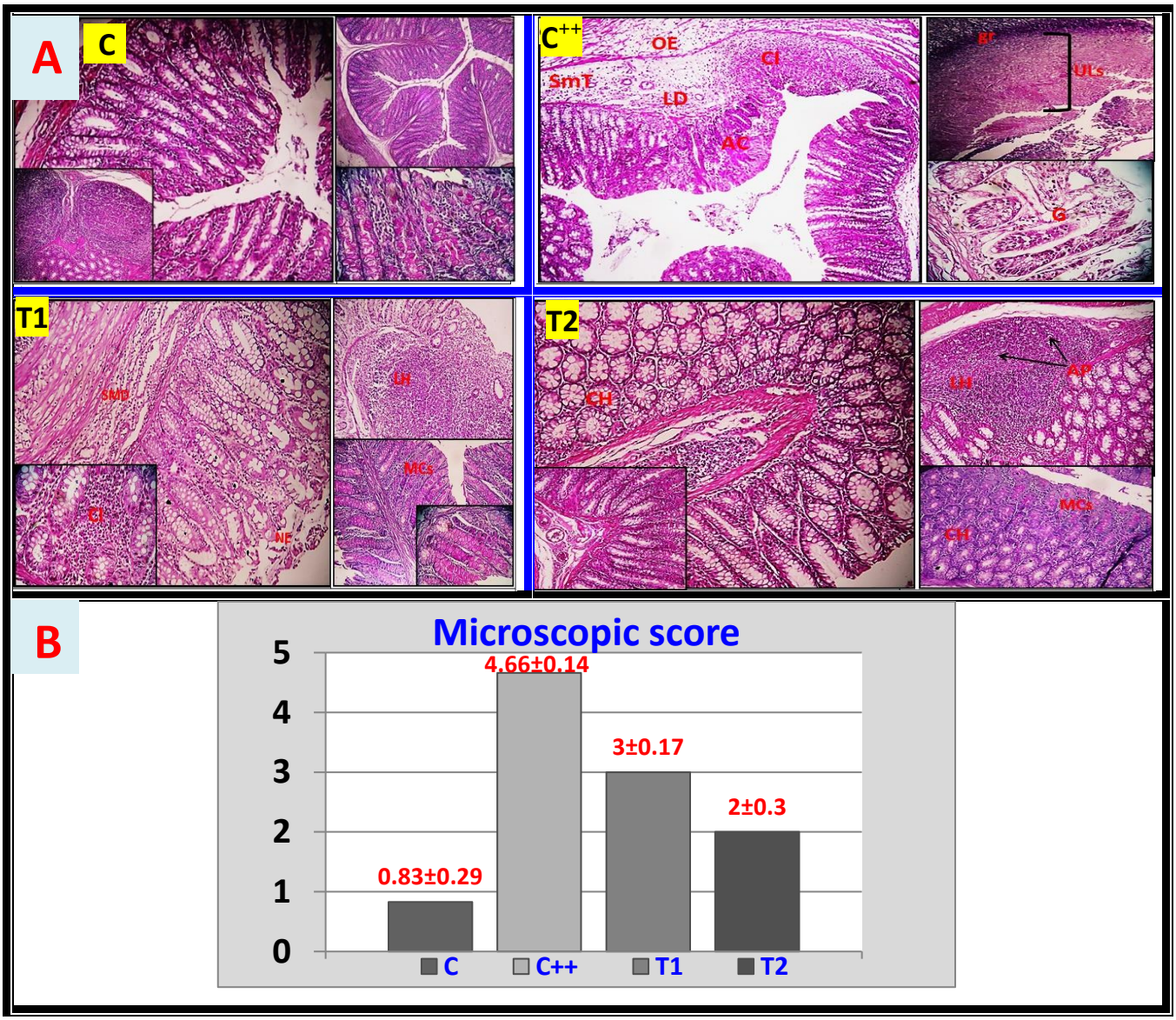

Figure 5. (A) Light microscopic images of colon sections. (B) Colon microscopic score represents th gross lesion protective role of synbiotic against *E. coli* infection. N=4. Mean±SE. (NE) necrotic, (LD) lymphoid depletion, (AC) Atrophy of crypt, (OE) odema, SmT) sub mucosal tissue, (CI) Cellular infiltration, (CH) hyperactivity of crypts, (G) gland in colon, (ULs) Ulcer layers, (gr) granulation layer, (MCs) hyperplasia of mucus cells, (SMD) Sub mucosal diffusion, (LH) Lymphoid tissue hyperplasia, (AP) Apoptosis. (Pas and H&E) 400X, 100X, and 200X. C: control negative group. C++: control positive group. T1: Fenugreek administered to enteritis group. T2: Fenugreek and *L. acidophilus* administered to enteritis group. Least Significant Difference (LSD) of microscopic score = 0.68

adjacent glands, together with lymphoid depletion of gut associated tissue. Control negative group was well-defined folded mucosa with no villous structure, normal submucosal and muscular layers. The colon of T1 group appeared with folded mucosa with prominence and hypertrophy of goblet cells that stained purplish with PAS stain. Various types of MNCs infiltration were recorded in both mucosal and submucosal tissues mainly concentrated around hyperplastic colon glands, also moderate enlargement of gut lymphoid associated tissue (hyperplasia of Payer's Patches) together with marked collagen

proliferation of submucosal muscular tissue, accompanied by moderate infiltration of plasma cell and lymphocyte with evidence of new capillaries formation (angiogenesis). Colon of the T2 group showed no clear lesion of folded mucosa lining with columnar epithelium and supported with tubular colon glands, associated with mild mucosal cellular infiltration. Hyperactivity response noticed in both colon crypts and gut lymphoid associated tissue (marked enlargement) with evidence of few apoptotic lymphocytes, also moderate thickness of submucosal tissue due to MNCs infiltration mainly around blood vessels (Perivascular MNCs aggregation).

Microscopic score of colitis is shown in Figure 5B, where the control positive group (C++) showed significantly ($P<0.05$) increased score as compared with T1 and T2 groups 7 days post *E. coli* infection. Less marked congestion without edema, as well as no gross pathological changes with no clear lesion were observed. Furthermore, there was hyperatrophy of goblet cells (increase of mucus), mild mucosal MNCs infiltration, moderate enlargement of gut lymphoid associated tissue (hyperplasia of Payer's Patches), with evidence of few apoptotic lymphocytes, moderate infiltration of plasma cell and lymphocyte. In the present study, the protective role of butyric acid production enhanced by fenugreek seeds as prebiotic and in synbiotic formulation significantly reduced the lesion score in duodenum and in colon. In the same context, the colitis microscopic score improvement in fenugreek provided in pre and synbiotic provided rats against *E. coli* infection indicated reduced apoptotic cells proportion.

Gross lesions of colon in *E. coli* administered groups indicated acute enteritis demonstrated by necrotic sloughed tissue, submucosal edema mixed with diffuse MNCs infiltration and lymphoid depletion of gut associated tissue. Oral administration of *E. coli* in the present experiment induced enteritis extended from duodenum to reach the colon, indicating the attaching and effacing (A/E) of the enteropathogenic *E. coli* (EPEC). Enteropathogenic *E. coli* are now defined as those *E. coli* strains that infect the small intestine causing acute watery diarrhea, nausea, and fever, and have the ability to cause diarrhea and produce a histopathology on the intestinal epithelia recognized as the A/E lesion (39). The EPEC bacteria bound to the apical plasma membrane of intestinal epithelial cells. The infection creates local damage or rupture (effacement) in the epithelial microvilli to form the unique A/E lesion (40). These changes combined with lower blood flow to the intestine leads to villi damage, a decreased absorptive area for nutrients and sloughing of the intestinal epithelial cells. All of these combined effects result in a loss of intestinal integrity (41). The intestinal epithelial barrier disruption is believed to increase the transport of bacteria and bacterial-associated products via the intestinal epithelium (42). The studies reported that the *E. coli* cause the tight junction proteins dissociation and destabilizeation (43).

The well-defined improveement in colon mucosa of T1 group that was administered fenugreek seeds as a prebiotic indicated the anti-inflammatory role of fenugreek seeds. In addition to the production of butyric acid, the antiinflam-matory role of fenugreek seeds could be resulted from its antioxidant and immune-logical activities (44). The biggest effect of SCFAs were the recovery of the shattered mucosa of colon, were noticed by Nakano et al. (45). Concerning the beneficial effects of SCFA treatments, they were reflected in the present study based on the values of the colitis index and the macroscopic scores. These SCFAs cause changes in the intestinal index and rise of the intestinal development-linked genes abundances, substances digestibility, and diminishing of the apoptotic cells proportion (46), and keeping the function of the intestinal barrier modifies intestinal morphological alteration, thereby increasing nutrient absorption and is useful for intestinal growth and evolution (47). The goblet cells are one of a pool of pluripotent stem cells residing at the crypts (48). The small intestine contains goblet cells in large numbers in the crypt more than on the villi, where more interspersed by enterocytes. Furthermore, they are numerous in colon and have mucus filled the large granules (49). Goblet cells are the major producer and of intestinal mucus (50). As a protective role, fermentation of fenugreek either in pre or synbiotic formulation, increased the

production of butyric acid. According to what has been stated by (47, 51), there is positive correlation between goblet cells activity in mucous secretion and colonic SCFAs concentration. Studies using in vitro models, stated that the SCFA stimulates MUC2 expression through mitogen-activated protein kinases (MAPK) signaling pathway, this leads to a better epithelial chemical barrier in the intestine (52, 53). Therefore, SCFA can keep intestinal chemicals and the immune barrier by controlling the generation of mucins and inflammatory cytokines (46, 47). The depleted goblet cells in *E. coli* infected rats indicated an active ulcerative colitis (UC), because mucus was thinner in these patients **(**54**)**. One manner of up-regulation is to protect the mucus by increasing the response of a Th2 cell, which in turn produce high numbers of goblet cells. This way has been therapeutically examined in IBD (55). The production of MUC2 is significant for host defense through the infections by A/E bacteria, via restricting many pathogens and commensal numbers related with the mucosal surface of the colon. This behavior restricts tissue destruction and translocation of pathogenic and commensal bacteria across the epithelium (56). The significant increase in goblet cells by pre and synbiotic supplied rats with *E. coli* demonstrated the protective role of using fenugreek seeds for enhancing butyric acid production by normal gut flora or when incorporated in synbiotic with *L. acidophilus*. As a probioitc *L. acidophilus* improved goblet cells activity in growing rats (57). The elevation of butyrate in the synbiotic is a key anti-inflammatory metabolite generated from commensal intestineal bacteria and reduced zonulin, and intestinal permeability as an indicator (58). Using the synbiotic (pre and pro-biotic) as modern treatment strategies manipulates the host microbial composition and the intestinal immune responses of the host that can induce and deal with the intestinal immune response, leading to intestinal homeostasis (59). Consequently, SCFAs are believed to possess anti-inflammatory actions. Despite the observed changes in the concentration of SCFAs in different tissues or blood, they are believed to cause disorders associated with imbalances in metabolism and immunity (60). In addition to the production of butyric acid, the anti-inflammatory role of fenugreek seeds could be resulted from its antioxidant, and immunelogical activities (61).

The current study's results distinctly indicate that the pre-enhancing intestinal mucosa supplemented with prebiotic and symbiotic significantly protect gut against *E. coli* enteritis. The results supported the anti-inflammatory potentials of fenugreek seeds as a prebiotic and the fenugreek and *L. acidophilus* symbiotic. However, the influence was known to be more favorable with synbiotic supplementation, as evidenced through its capacity to discontinue the micro and macroscopic pathological changes. Immediate standard therapies that prevent immune activation with oral immunosuppressive or biologic factors are mostly efficient treatment for IBD; including ulcerative colitis, Crohn's disease, pouchitis, and chronic intestinal inflammatory disorders mediated by immune responses dysregulation (62).

In conclusions of nutritional supplements containing fibers that constitute a source of butyric acid production, such as fenugreek seeds represent a new, effective, and inexpensive approach for avoiding and reducing the severity of digestive problems. The mechanisms that contribute to improving the intestinal resistance to inflammation by acting as anti-inflammatory could be enhanced through improving intestinal lymphoid tissues and increasing the production of IgA. Accordingly, results of the present study revealed that fenugreek seeds are good candidate for formulation of synbiotic.

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Conflict of Interest

The authors declare that they have no competing interests.

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تحسين يضاد الالتهاب المعوي مع بذور الحلبة كبريبايوتك وسايموبيوتيك مع الملبنه المحمضه في الفئران المصابة تجريبيًا بمرض الإشريكية القولونية

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الخلاصة

العمل التآزريللبريبايوتك والبروبيوتيك(سايموبيوتيك) اشار الى أكثر فعالية من المكونين المنفصلين في الوقاية والعلاج من العديد من الأمراض المعوية والمناعة. تطوير المكملات المتزامنة هو استراتيجية جديدة لتحسين العلاج والوقاية من الأمراض المتعلقة بالمناعة المعوية ، كان الهدف من هذه الدراسة هو فحص قدرة الأدوية المضادة للالتهابات للسايموبيوتيك من الحلبة والملبنة المحمضة ضد التهاب القولون المعوي عن طريق صفه إنتاج حمض البيوترك. أربعة وعشرون ذكرا منالفئرانالنامية التي تتراوح أعمارهم بين 3-4 أشهر ووزنها 200-250 جرامًا مقسمة إلى 4 مجموعات: المجموعة الأولى والثانية كانت مجموعات سيطرة سلبية وإيجابية (C و++C) تغذت على نظام غذائي أساسي ، المجموعة الثالثة (T1) تتغذى على البريبايوتك الحلبة 5٪ ، والمجموعة الرابعة (T2) تم تغذيتها بلسايموبيوتيكمن بذور الحلبة 5٪ و الملبنة المحمضة بجرعة (5 × 108 CFU / ml) لمدة 45 يومًا. بعد ذلك ، الفئران في المجاميع C++ و T1 و T2 يستحدث فيهن التهاب امعاء. توضح هذه التجربة الدور الوقائي لبذور الحلبة كبريبايوتك وفي السايموبيوتيكمع بكتريا الملبنة المحمضةلمدة 45 يومًا قبل التهاب الأمعاء تجريبيًا الذي يسببه بكتيرياالإشريكية القولونية (EPEC O125: H6). تم تقييم الدور الوقائي للبريبايوتك وسايموبيوتيك اعتمادا على التغيرات المرضية العيانيه والمجهريةللاثني عشري في علاقه مع إنتاج حمض البيوترك. كشفت نتائج التقييم العياني والمجهريللالتهاب الأمعاء عن التأثيرات التآزرية للسايموبيوتيك في الوقاية من التهاب المعوي بسبب الإشريكية القولونيةالذي يتسمبزيادة إفراز المخاطللخلايا الكأسية. دور مضاد الالتهاب مرتبط مع انتاجحمض البيوتركوالذي زاد بشكل كبيربواسطه السايموبيوتيك. علاوة على ذلك ، السايموبيوتيكتحسن الاستجابة المضادة للالتهاب للمناعة المكتسبة للغشاء المخاطي المعوي عن طريق رفع الغلوبولين المناعي IgAالناتج من خلايا البلازما. الخلاصة ، إدراج المكملات الغذائية التي تحتوي على الألياف التي تشكل مصدرًا لإنتاج حمض البيوترك ، مثل بذور الحلبة التي تساهم في تحسين مقاومة الأمعاء للالتهاب من خلال العمل كمضاد للالتهاب عن طريق تحسين الأنسجة اللمفاوية المعوية وزيادة إنتاج الغلوبولين المناعي IgA.

الكلمات المفتاحية : سايموبيوتيك ، بذور الحلبة ، الإشريكية القولونيه ، التهاب الامعاء ، حامض البيوترك .